Pakistan Phytopathological Society

# ABSTRACT BOOK

## 7TH INTERNATIONAL CONFERENCE

*of*

## Pakistan Phytopathological Society

# PHYTOPATHOLOGY: CURRENT SCENARIO AND FUTURE PROSPECTS

A focused Strategy for Food Safety and Security

November 21-23, 2021


ORGANIZED BY

**Department of Plant Pathology**

University of Agriculture, Faisalabad & Ayub Agriculture Research Institute, Faisalabad

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***"Phytopathology: Current Scenario and Future Prospects"***

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Jointly organized by

**DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF AGRICULTURE FAISALABAD &**
**AYUB AGRICULTURAL RESEARCH INSTITUTE, FAISALABAD**


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# 7th International Conference of Pakistan Phytopathological Society

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The Phytopathological Society of Pakistan was established in 1983 as the first platform in the country for coordination among the renowned Plant Pathologists of Pakistan. Since then the society is working to popularize the discipline of Plant Pathology in the region. 7th International Conference of Pakistan Phytopathological Society (PPS) held at the Department of Plant Pathology, University of Agriculture Faisalabad and Ayub Agricultural Research Institute, Faisalabad during November 21-23, 2021 under the umbrella of PPS. The objective of the conference aimed at a fruitful discussion forum for national and international plant pathologists.

The conference covered the following topics;

- Climate Change and Plant Diseases
- Host-Pathogen Interactions
- Molecular Plant Pathology
- Biosecurity and Plant Quarantine
- Plant Disease Management
- Disease Resistance
- Seed and Post-Harvest Pathology
- Disease Modeling and Epidemiology
- Taxonomy and Systematics of Plant Pathogens
- Bioinformatics
- Ecology of plant pathogens
- Mycotoxins
- Natural and chemical Pesticides
- Insects in relation to Plant Diseases
- Beneficial Microorganisms

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## PAKISTAN PHYTOPATHOLOGICAL SOCIETY

The Phytopathological Society is the premier society dedicated to high–quality, innovative plant pathology research. For more than a century, members of Phytopathological Society have been making and sharing significant breakthroughs, both for the science and society. Phytopathological Society is driven by a distinctive community of scientists, whose energy and commitment ensure the global advancement of this critical science.

Development of new and innovative ways to control plant diseases is a constant challenge for plant pathologists. Plant diseases may be managed by altering the host plant, the pathogen, and/or the environment. Examples include growing resistant plant varieties, planting pathogen-free seed or stock, applying a biological control agent, modifying environmental conditions to decrease disease, and using plant medicines that inhibit or kill the pathogen without harming the plant or the environment. Plant pathology is a discipline that deals with the study of organisms that cause diseases in plants, and study of those factors in which these organisms of the induce diseases in plants and the mechanisms of controlling the diseases. Plant pathology is an interdisciplinary science that includes knowledge of botany, microbiology, crop science, soil science, ecology, genetics, biochemistry, molecular biology, and physiology.

## HISTORY OF PAKISTAN PHYTOPATHOLOGICAL SOCIETY

Late Dr. Abdul Hafiz ex Director, FAO East Regional Project on Field Food Crops on the incentive from the National Talent Pool of Ministry of Labour, Manpower and Overseas Pakistanis in 1983, conducted an extensive tour of different institutions to assess the state of affairs of Plant Pathology, its teaching and research throughout the country and gave an eye opening account reflecting on merits and handicaps of the plant pathological research underway at different universities and institutions of the country. Besides various financial and administrative problems, he emphatically pointed out the lack of dynamic research leadership for conducting meaningful collaborative research, non-availability of scientific literature and essential facilities, duplication of research activities and lack of research management and cooperation at institutional and national levels. Taking into account the growing needs of the country in plant pathology as pointed out by Dr. Hafiz, it was considered necessary to invite plant pathologists from all over the country to provide a common platform, so as to discuss various ways and means of improving the quality of research and teaching which may ultimately help in solving many national plant disease problems.

As a first step, 32 plant pathologists from the University of Agriculture, Faisalabad, Ayub Agriculture Research Institute, Nuclear Institute of Agriculture and Biology, Faisalabad and Pakistan Central Cotton Institute, Multan convened a meeting on December 20, 1983, chaired by Dr. A. H. Khan, Ex-Director, Advanced Studies and Research, University of Agriculture, Faisalabad and renowned forest pathologist. This meeting lead to the establishment of Pakistan Phytopathology Society. Late Dr. A. Sattar, the pioneer plant pathologist of Pakistan, was unanimously nominated the first president of the society and Late Dr. Abdul Hafiz was the first Chief Editor of its Journal.

After the death of Dr. A. Sattar, Dr.J. H. Mirza became the next president and Editor-in-Chief, of PJP. His tenure was from 1991 to 1997. After Dr. Mirza, Dr. S. M. Khan was elected president in 1998. While Dr. Ahmad Saleem Akhtar, our present president, took over the charged PPS in 2004. The Pakistan Journal of Phytopathology and Newsletter are official regular publications of the society. With the Grace of God Almighty and cooperation of fellow scientists, this society is not only with representatives from all over the world, the standard of Pakistan Journal of Phytopathology is not less than that of any International Journal, with International editorial board and abstracting by international abstracting agencies CABI-UK, BIOSIS-USA, AGRINDEX-FAO, Moreover, this society is good example of self-sustaining, cooperation and unity of Plant Pathologists.

## VISION OF PAKISTAN PHYTOPATHOLOGICAL SOCIETY

Our vision is to develop Plant Pathology into an integrated discipline that will develop technologies for application in resolving the plant / seed health problems and productivity.

- Healthy plants are essential to secure a safe food and energy supply for the growing world population, to sustain natural ecosystems, and to promote quality of life. The unique role of plant pathology as compared to other agricultural sciences in the scientific world is more evident in future due to increase in worldwide trade, travel and the occurrence of exotic diseases due to introduction of foreign species.
- Plant Pathologists will be an increasingly international and interdisciplinary group of scholars, practitioners and educators dedicated to the study of plant diseases and their management, and information delivery on a global and collaborative scale.
- The study of plant-microbe interactions will play a major role in building basic knowledge of biological systems operated between plants and pathogens. Through an

emphasis on plant health, plant pathologists will continue to develop broad concepts that are fundamental to many fields of science.

- Strategies for plant disease management will be based on comprehensive knowledge of cellular, organism and ecological interactions involving plants and their pathogens.
- The deep and detailed knowledge of plant-pathogen-environment interactions will be strengthened by integration across different disciplines and the adoption of new analytical tools, experimental models and information systems. Together, these will help in providing and maintaining healthy plants for the growing global population.
- The emergence of plant diseases will be continued due to pathogen evolution, international travel and trade, and alterations in climate. Through new technologies, more efficient communication tools, and broad educational backgrounds, plant pathology practitioners will be more effective in devising and disseminating practical solutions for management of plant diseases.

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**DEPARTMENT OF PLANT PATHOLOGY**
UNIVERSITY OF AGRICULTURE, FAISALABAD

With the inception of Punjab Agricultural College and Research Institute, Lyallpur, Plant Pathology was initiated as a part of Section of Botany in 1907. In 1934 Mycological Sub-Section came into existence as a part of Botanical Section. The independent Section of Plant Pathology however, came into being in April 1947, which attained the status of an independent department with the up-gradation of Punjab Agricultural College and Research Institute, Lyallpur to West Pakistan Agricultural University in November 1961. The present Department of Plant Pathology was shifted to new campus in 1976. In 2008, six research groups in the discipline of Plant Pathology viz; Plant Mycology, Plant Nematology, Plant Bacteriology, Mushroom Biotechnology, Plant Virology, Epidemiology and Integrated Disease Management, were established; each group comprises 2-4 members headed by a senior faculty member to address the research issues regarding their field of research and studies.

## OBJECTIVES

The aim of the Department of Plant Pathology is to impart the education to the students keeping in view the recent advancement in the diagnosis and management of plant diseases. Our staff is enthusiastic to encourage a strong, innovative and intellectual environment for the students where the ideas are energetically debated and rigorously tested for target/problem oriented research. As Pakistan's economy is agriculture based, so the objective of the department is to develop various disease management strategies based on the problems originating from the farmer fields, which will automatically improve the crop productivity. Faculty of the department currently consists of 3 Professors, 1 Associate Professor, 11 Assistant Professors and 2 Lecturers. All of the faculty members are well trained in their specific area and possess doctorate degree. So far the department has produced 1200 B.Sc. (Hons), 850 M.Sc (Hons) and over 60 Ph.Ds.

After completing their degrees in various programs, the students serve in various organizations such as Universities, AARI, NIAB, NIBGE, NARC, PARC, Federal Seed Certification Department, Punjab Agriculture Department, Banks, Pesticides and Fertilizers companies etc. At present more than 166 students are enrolled in Plant Pathology as Major subject in B.Sc. (Hons), 130 in M.Sc. (Hons.) and 23 in Ph.D. programs.

## VISION

- Our vision is to develop Plant Pathology into an integrated discipline that will develop technologies for application in resolving the plant / seed health problems which in turn will lead to enhancement in the crop yield.
- The unique role of Plant Pathology as compared to other Agricultural Sciences in the scientific world is more evident in future due to increase in worldwide trade, travel and the occurrence of exotic diseases due to introduction of foreign species.
- Plant Pathologists will be an increasingly international and interdisciplinary group of scholars, practitioners and educators dedicated to the study of plant diseases and their management, and information delivery on a global and collaborative scale.
- The study of plant-microbe interactions will play a major role in building basic knowledge of biological tools used by plants and pathogens against each other. Through an emphasis on plant health, plant pathologists will continue to develop broad concepts that are fundamental to many fields of science using sophisticated laboratory techniques. Moreover, the strategies for plant disease management will be based on this comprehensive knowledge.
- The deep and detailed knowledge of plant-pathogen-environment interactions will be strengthened by integration across different disciplines and the adoption of new analytical tools, experimental models and information systems. Together, these will help in providing and maintaining healthy plants for the ever-growing global population.
- Due to pathogen evolution, international travel and trade, and alterations in climate, there will be continuous emergence of plant diseases. Through new technologies, more efficient communication tools, and broad educational backgrounds, plant pathology practitioners will be more effective in devising and disseminating practical solutions for management of plant diseases.
- To find possible solution for the post-harvest disease problems faced by industry and developing interaction with them for need based research orientation of the department.
- Collaboration with national and international institutions and organization, to discuss the scientific findings and to get critical suggestions or remarks for more judicious use of available human, infrastructure and research resources.

## MISSION

The mission of Department of Plant Pathology is to produce high quality human resources in field of Plant Pathology for sustainable plant health management.

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# SECTION 1: PLANT MYCOLOGY

# SPOT BLOTCH IN SPRING BARLEY CAUSED BY BIPOLARIS SOROKINIANA SHOEM. IN SOUTHEASTERN KAZAKHSTAN

Yerlan Dutbayev[1]*, Nurlan Kuldybayev[1], Saule Daugaliyeva[2], Elvira Ismailova[2], Nadira Sultanova[3], Göksel Özer[4], Abdelfattah A. Dababat[6] and Minura Yessimbekova[5]*

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The fungal pathogen *Bipolaris sorokiniana* (telemorph: *Cochliobolus sativus*) is one of the most common worldwide pathogens and causes spot blotch and root rot in barley. The aim of this study was to characterize *B. sorokiniana* isolates obtained from barley fields for a given region. An overall disease management strategy associated with spot blotch in spring barley in the Almaty region of Kazakhstan should include the determination of the aggressiveness of the pathogen isolates. In 2020, leaves of spring barley with the symptoms of spot blotch disease were obtained from experimental plots of the Kazakh Research Institute of Agriculture and Crop Production, Almaty Region. The DNA sequencing of internal transcribed spacer regions of rDNA (ITS) was used to determine fungal species in the fungal microflora placed on barley leaves sampled. Isolates P-08 and P-15 of *B. sorokiniana* were identified as the causative agent for spot blotch of barley and *Alternaria alternata*, *A. tenuissima*, *A. infectoria, Lecanicillium aphanocladii* and *Cladosporium* sp. were also coexisted. The conidia length and width as well as the number of septa were also measured for the two *B. sorokiniana* isolates. The best radial growth of the isolates was obtained in the Saburo and Czapek media. *B. sorokiniana* isolates were severely able to infect and colonize barley seedlings. Koch's postulates were fulfilled by reisolating the pathogen from artificial inoculated plant tissues and identifying based on morphology. Further investigation is needed to understand the impact of *B. sorokiniana* on the barley production in Kazakhstan.

# ANALYSIS OF BIOLOGICAL CHARACTERISTICS AND FUNGICIDE SENSITIVITY OF *PARAMYROTHECIUM BREVISETA* CAUSING COFFEE **LEAF SPOT**

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Recently, it has been reported that *Paramyrothecium breviseta* can cause a new leaf spot of coffee. The results show that the pathogen of the disease has strong pathogenicity. *P. breviseta* is pathogenic to *Coffea canephora* is also pathogenic to *Coffea Arabica*. After being infected by the pathogen, if the control is not carried out in time, the seedling leaves will wither in 3 ~ 5 days. However, the biological characteristics and fungicide sensitivity of the pathogen are not clear. Therefore, the isolation the pathogen and analysis of its biological characteristics and fungicide sensitivity were carried out in this study. The colony of the isolate on PDA was round in shape, the mycelium was white, and the edge was neat. Dark green sticky substance appeared on the surface of the colony, forming a ring shape, and the back of the colony was light brown contain a large number of spores. The spores were rod-shaped or oblong. The spores were colourless in the middle, without septa, and length was 4.8-6.4 μm, and width was 1.6-2.6 μm. Studies on the biological characteristics of *P. breviseta* have shown that it can grow in the environment of 13-30, the optimum temperature is between 25-30$^{o}$C, the pathogen can grow in the environment of pH 3-11, the optimum pH value is 11, the slowest growth under 24 hours of total darkness, and it is conducive to mycelial growth in a neutral and weakly alkaline environment. The lethal temperature was 75 for 10 minutes. It grows best on oatmeal agar medium and grows slowest on Sabouraud dextrose agar with yeast extract medium. The growth of different carbon source media was not much different, the best carbon source for mycelium growth was soluble starch, and the lowest were sucrose and lactose. On different nitrogen sources, the best nitrogen sources for mycelium growth were beef extract, the lowest utilization rate were sodium nitrate, cysteine, ammonium sulphate, ammonium nitrate. Fluproxen and tebuconazole have the best antibacterial effect on *P. breviseta*, $EC_{50}$ value were 24.7609 g/mL and 24.3134g/mL respectively.

# EARLY INDUCTION OF FRUITING BODY IN OYSTER MUSHROOM *PLEUROTUS OSTREATUS* USING PLANT EXTRACTS

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*Pleurotus ostreatus* poses nutritional and medicinal importance as it consists of protein and other nutrients which are beneficial to human. Induction of reproductive phase of mushroom in the already prepared bed is much essential when it takes much time to initiate fruiting. The fruiting body induction of *P. ostreatus* is supported by the bio-chemical saponin. This study was aimed to evaluate the response of *P. ostreatus* towards the fruiting body induction by using plant juices contain saponin itself and cut downing the incubation period. Three different percentage (5%, 10%, and 15%) of both fenugreek seed (*Trigonella foenum*) and banana pseudostem (*Musa acuminata*) juices were accommodated in the *in vitro* and *in vivo* experiments. The *in vitro* study was designed to evaluate *P. ostreatus* mycelial response in the presence of plant juices. In *in vivo* the mushroom was cultivated in the paddy straw and on 10th day of inoculation plant juice application was done and bags were exposed to light. Control treatment was designed in both *in vitro* and *in vivo* experiments. The *in vitro* mycelial growth in control experiment was higher than the rest of the treatments. In *in vivo* the number of days taken to form pin heads, fresh weight, dry weight, biological efficiency, fruiting body diameter, stipe length and diameter and number of fruiting body 1roduced were studied. Earliest pin head formation was observed on 21st day of inoculation from both 15% juice applied treatment, and all treatments except control produced fruiting body earlier, which was statistically significant ($p < 0.05$). Among the treatments, 15% fenugreek juice application gave highly significant effect ($p<0.05$) on fresh weight yield (159.46 ± 10.39g), and biological efficiency (142.9 ± 8.92%). Among all the treatment 15% fenugreek application performed the best in early fruiting induction, yield and biological efficiency.

# NATURAL ANTIFUNGAL COMPOUNDS FROM QUINOA LEAVES AGAINST *MACROPHOMINA PHASEOLINA*

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Quinoa (*Chenopodium quinoa* Willd.), family Chenopodiaceae, is a newly introduced crop in Pakistan. Various *Chenopodium* spp. growing as weed in Pakistan possess antifungal potential against *Macrophomina phaseolina,* the cause of root diseases in hundreds species of plants. This study was done to check antifungal potential of quinoa leaves against this notorious soil-borne pathogen. The pathogen was isolated from diseased mungbean plants and identified on the basis of morphological characters and further confirmed using universal and specific primers namely ITS, β-tubulin, GADPH and MPRT. Methanolic leaf extracts (1, 2, 3, 4 and 5%) of four quinoa varieties namely V1, V2, V7 and V9 were evaluated for their antifungal activity. Various extracts suppressed fungal biomass by 36–87%. Extract of V7 exhibited the best activity, reduced fungal biomass by 73–87% and was therefore selected for further bioassays. This extract was fractionated using three organic solvents of variable polarities namely *n*-hexane, chloroform and ethyl acetate. Antifungal bioassays were conducted using 8 concentrations (1.562 to 200 mg $mL^{-1}$) of each fraction. All the concentrations of chloroform fraction completely controlled (100%) growth of the pathogen. *n*-Hexane and ethyl fractions were comparatively less inhibitory and caused 32–100% and 35–100% reduction in fungal biomass, respectively. GC-MS examination of chloroform fraction indicated that 1,2-benzedicarboxylic acid, diisooctyl ester was in the highest abundance with peak area of 29.83% followed by acetic acid,2-(2,2,6-trimethyl-7-oxa-bicyclo [4.1.0] hept-l-yl)-propenyl ester (6.75%); 9,12-octadecadienoic acid(Z,Z)-(6.46%); octadecanoic acid,9,10-dihydroxy-,methyl ester (6.44%) and octadecanoic acid, methyl ester (4.16%). It concludes that chloroform fraction of leaf extract of V7 possess potent antifungal compounds against *M. phaseolina.*

# FUNCTIONAL ANALYSIS OF AUTOPHAGY RELATED GENE *ATG12* IN POTATO DRY ROT FUNGUS *FUSARIUM OXYSPORUM.*

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Autophagy is an intracellular process in all eukaryotes which is responsible for degradation of cytoplasmic constituents, recycling of organelles, and proteins. It is an important cellular process responsible for effective virulence of several pathogenic plant fungal strains having critical impacts on important crop plants including potatoes. However, the detailed physiological mechanisms of autophagy involved in infection biology of soil borne pathogens still of potato crop needs to be investigated further. Here, we have investigated the autophagy related gene, *FoATG12* in potato dry rot fungus *Fusarium oxysporum* by means of target gene replacement and overexpression. The deletion mutant Δ*FoATG12* showed reduction in conidial formation and exhibited impaired aerial hyphae. The *FoATG12* affected the expression of the genes involved in pathogenicity, vegetative growth as well as on morphology features of colony under stresses. It was found that the disease symptoms were delayed upon inoculated by deletion mutant of *FoATG12*compared to WT and OE while, the deletion mutant showed the disease symptoms on tomato plants. Results confirm the significant role of autophagy related *ATG12* gene in the production of aerial hyphae and effective virulence of *F. oxysporum* in potato crop. Current findings have provided an enhanced gene level understanding of the autophagy related virulence of *F. oxysporum* which can be helpful in pathogen control research and have vital impacts on Potato crop.

# DISTRIBUTIONAL VARIABILITY OF BACTERIAL WILT OF CHILI INCITED BY RALSTONIA SOLANACEARUM IN EIGHT AGRO-ECOLOGICAL ZONES OF PAKISTAN

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Bacterial wilt caused by *Ralstonia solanacearum* is one of the major constraints in the production of chilies in Pakistan. As the information regarding distribution and prevalence of *R. solanacearum* is exiguous, the present studies were conducted to determine the incidence and prevalence of *R. solanacearum* in the major chili growing areas from different agro-ecological zones of Pakistan. Variations in incidence and prevalence of *R. solanacearum* were recorded throughout the country. The overall incidence and prevalence of *R. solanacearum* in the country was found to be 10% and 76% respectively. Of the four provinces, maximum disease incidence of 16.4% was recorded in Sindh province followed by Punjab and Khyber Pakhtoonkhwa showing 11.4% and 7% disease incidences respectively and the minimum incidence of 4.9% was observed in the province of Baluchistan. As regards prevalence, the same pattern was observed. Out of 8 agro-ecological zones the maximum disease incidence of 19.5% was observed in Indus delta followed by Sandy deserts (14.1%) while the minimum disease incidence of 5% was found in Western dry mountains. The disease incidence in other zones ranged between 5.4 and 14.1%. Similar trend was noticed regarding prevalence being the maximum in Indus delta (100%) followed by Southern irrigated plains (90%). Out of 114 *R. solanacearum* isolaes, 92 (81%) were identified as Biovar 3 while the remaining 22 (19%) were recognized as Biovar 4. Biovar 3 was recorded from all the four provinces and was found to be predominant in all the provinces while Biovar 4 found in the Punjab and Sindh provinces only. Similarly, biovar 3 was observed from all the eight agro-ecological zones and found to be predominant. On the other hand, biovar 4 was recorded from four agro-ecological zones. It is concluded that bacterial wilt caused by *R. solanacearum* is prevalent throughout the country in all the agro- ecological zones with varying intensities warranting stringent surveillance and control measures.

# EVALUATION OF BIOCONTROL POTENTIAL OF SEVEN INDIGENOUS *TRICHODERMA* SPECIES AGAINST CHARCOAL ROT CAUSING FUNGUS, *MACROPHOMINA PHASEOLINA*

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Charcoal rot incited by *Macrophomina phaseolina* is one of the major diseases of green gram and black gram in Pakistan reducing yields up to 40%. As there are no long-term control strategies for this seed- and soil-borne pathogen, therefore, in the present study, seven indigenous species of *Trichoderma* were evaluated for their *in vitro* and *in vivo* effectiveness against *M. phaseolina* with the objective to identify alternatives to pernicious fungicides. All seven species of *Trichoderma* significantly retarded the growth of *M. phaseolina in vitro*. Maximum reduction (79.63%) was observed with *T. harzianum* followed by *T. hamatum* (76.3%) while *T. pseudokoningii* caused the minimum decrease (58.14%) in growth of the fungus. Similarly, *Trichoderma* species had significant effects on number and size of sclerotia.*M. phaseolina* produced the minimum number of sclerotia in the presence of *T. hamatum* followed by *T. harzianum* causing reductions of 69.5 and 66.84% over control, respectively. The maximum reduction in size of sclerotia was caused by *T. harzianum*. The maximum plant survival of green and black gram was obtained with *T. harzianum* followed by *T. hamatum* and *T. viride*. The maximum individual germination of 86.67% was achieved with *T. harzianum* at a concentration of $2\times 10^8$ (propagules/ml), while the minimum (33.33%) was recorded with *T. pseudokoningii* at $2\times 10^4$. *Trichoderma* concentrations also had significant effects on plant survival, being the maximum at the highest concentration. The plant survival decreased as the concentrations of the antagonists decreased showing a direct relationship between plant survival and concentrations.

# COMPARATIVE EFFICACY OF COMMERCIAL FUNGICIDES AND *TRICHODERMA HARZIANUM* AGAINST FUSARIUM WILT IN STRAWBERRY

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Cultivation of strawberry (*Fragaria ananassa*) is surging among Pakistani farmers due to its profit ratio compared to other traditional crops. This crop is attacked by various pathogens responsible for low yield. The current study's objective was to identify a better approach to managing Fusarium wilt caused by *Fusarium oxysporum* f.sp. *fragariae* (Schlecht) in the field. Firstly efficacy of eight fungicides was evaluated by the poison agar technique against colony growth of *F. oxysporum* f.sp *fragariae in-vitro*. Among tested chemicals, four (Score, Topsin-M, Avito and Carbendazim) were found to inhibit pathogen growth compared with control significantly. Later, the effectiveness of four different isolates of *Trichoderma harzianum* was also evaluated through dual culture technique against *F. oxysporum* f.sp *fragaria*e. However, only one isolate of *T. harzianum* was found to suppress pathogen growth. Subsequently, four fungicides and an isolate of *T. harzianum* were evaluated in the greenhouse. Later, three chemicals (Score, Topsin-M and Carbendazim) and biocontrol isolate were subjected to field evaluation using artificial inoculation in a randomized complete block design (RCBD). All three chemicals, controlled wilt disease with varying degree of success. The mean results of field trials indicated that Topsin-M gave 88.0%, *T. harzianum* 80.0%, while Carbendazim and Score showed 72.0% protection in the filed after 42 days. Topsin-M was most effective, followed by T. harzianum and can be applied to manage Fusarium wilt of strawberry confidently.

# A SURVEY FOR CITRUS PATHOLOGY IN SARGODHA (PAKISTAN)

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Citrus is the second most significant natural product harvest of the world. It is a significant product of tropical and subtropical territories. Incidence and severity of citrus disease was evaluated in 7 Tehsils of Sargodha, which shares half of the creation of citrus industry in Pakistan. The Surveys was led to consider the impact of the presence of significant diseases (Citrus withertip, Citrus canker, Citrus greening and Citrus tristeza infection) causing decrease in the citrus fields. Infection rate and seriousness on leaves, stems and natural products were noticed and recorded. In disease severity evaluate that the Citrus Withertip result showed that Bhalwal had the most noteworthy level of Severity which was 10.3% and Silanwali had the least seriousness 2.76%. The general high occurrence was recorded in Citrus greening disease with rate 53.33% at the zone of Bhalwal and low frequency was recorded in Citrus withertip infection with rate 13.33% at the territory of Silanwali and most elevated severity was recorded in Citrus greening sickness with rate 21.07% at the region of Bhalwal and low severity was recorded in Citrus tristeza infection with rate 2.5% at the region of Silanwali.

# *PENICILLIUM* SPP. AS POTENTIAL BIOCONTROL AGENTS AGAINST *MACROPHOMINA PHASEOLINA*

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In the present study, five *Penicillium* species namely *P. italicum*, *P. expansum*, *P. simplisimum*, *P. oxalicum* and *P. citrinum* were identified using ITS (internal transcribed spacer) and β-tubulin markers and screened for their *in vitro* antagonistic potential against a soil-borne fungal pathogen *Macrophomina phaseolina* in a dual culture plate assay. Among all the tested strains, *P. italicum* showed the highest antagonistic potential against *M. phaseolina* by reducing its growth up to 57% over control followed by *P. citrinum* (42%), *P. simplisimum* (21%), *P. expansum* (11%) and *P. oxalicum* (9%). In order to find out the mechanism of action of *P. italicum,* genomic DNA of *M. phaseolina* was exposed to *P. italicum* secondary metabolites. The findings showed that these metabolites completely degraded the fungal DNA after the 48 h incubation period. To further explore the antifungal mechanism of action of *P. italicum,* chloroform and ethyl acetate fractions of its metabolites were subjected to GC-MS analyses. The major compounds identified in these fractions were 9,12-octadecadienoic acid (Z,Z)- (25.19%), decane (19.72%), dodecane (18.05%), Benzene, nitro- (14.62%), benzene, 1,3,5-trimethyl (14.37%), benzene, 1,4-diethyl (11.62%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (9.02%), and 1-nonadecene (8.99%), which could be responsible for control of *M. phaseolina* growth.

# RE-EMERGENCE OF SCLEROTINIA SPP. A WINTER FUNGAL PATHOGEN AND ITS IMPACT ON FODDER CROP PRODUCTION IN CENTRAL PUNJAB PAKISTAN

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Sclerotinia spp is a ubiquitous fungus belongs to Phylum Ascomycota class Discomycetes. It is a cool climate fungus loves to grow between 12-16 °C (optimum temperature). Under unfavorable conditions produce sclerotia which remains viable for years in soil and on diseased plant debris. Owing to climate change and severe winter for the last 5-6 years, this fungus has been reported on berseem, Lucerne, chickpea, canola, carrot, eggplant squash gourd, strawberry, and cucumber. Berseem is our very important fodder crop is affected severely by Sclerotinia in central region of Punjab Pakistan. Twelve different varieties (SB-III, S-G-07, SB-2-14, SB-10, SG-2-07, SB-3-14, SG-12, SB-1-14, SB-4-14, SB-112, Agaiti, SB-8SB-11 and Pakistani) of berseem were evaluated/screened out against Sclerotinia fungus. Pakistani was planted as check in filed condition. Maximum disease incidence (60-70%) was recorded on varieties (SB-3-14, SB-12, SB-112, SB-8) whereas least disease (10-20%) incidence was recorded on (SB-III). Integrated approaches were followed and is an effective way to get rid of this fungus, for this deep ploughing before sowing followed by flooding to drain out sclerotia from field., light irrigation during growth stages is recommended instead of heavy irrigation which favours microclimate for fungus growth., application of fungicides (Score, Native, Thiophanate methyl) is recommended before sclerotia formation.

# INVITRO TESTING OF NANOPESTICIDES AGAINST *COLLETOTRICHUM FALCATUM* CAUSING RED ROT OF SUGARCANE

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Sugarcane *(Saccharum officinarum)* is a major cash crop cultivated in tropical and sub-tropical regions. Among leading producing countries, Pakistan ranks 5th in area and 11th in sugarcane production. In Pakistan, it is cultivated on 1341.8 hectares with total production of 0.083 million tons. Sugarcane in Pakistan is mainly cultivated in Punjab followed by Sindh and KPK. It is abundant in carbohydrates and used as food for humans and fodder for animals. It is a key crop in biofactory progress because it produces high yield of valuable products like sugar, cane syrup, rum, biofibers, waxes, bio plastic, organic fertilizers and bio fuel. Sugarcane is attacked by several diseases including red rot, whip smut, sugarcane mosaic virus and red stripe. Among these diseases, red rot caused by *Colleotrichum falcatum* is the most destructive disease for hindering its successful production. It results in yield reduction up to 5-10 percent. The disease can be managed by certain management strategies including chemical control, cultural control and biological control. Silver nanoparticles (AgNPs) and Zinc nanoparticles (ZnNPs) are known as potential antimicrobials and are reported to possess antifungal activity against *C. falcatum*. In the present study, nanoformulated Carbendazim and Mancozeb were tested for their effectiveness against *C. falcatum* in the lab conditions. Use of chemicals has some hazardous effects on environment and there is a chance of cross resistance in pathogen against those chemicals. Botanicals (phyto-extracts) were also evaluated against the pathogen in lab conditions. Among the botanicals, Nanoformulation of Neem and Turmeric were tested for their effectiveness. Nanoparticles of all chemicals and botanicals were prepared by using silver nitrate and zinc nitrate and they were tested for the effective control of *C. falcatum*. The combination of nanoformulated Neem phytoextract and Carbendazim fungicide (Carbendazim + $AgNO_3$ + *A.indica*) gave minimum fungal growth of 16.519 mm followed by combination of nanoformulated fungicides (Carbendazim NPs+ Mancozeb NPs) with fungal growth of 18.244 mm and nanformulated phyto-extracts (*A. indica* NPs + *C.longa* NPs) with fungal growth of 25.311 mm. The ultrastructure imaging through scanning electron microscopy (SEM) with EDX analysis confirmed the morphology and size of AgNPs and ZnNPs as mono-dispersed spheres. Nanoparticles (NPs) could be an alternative for plant disease management and have shown to be highly efficient in inhibiting *C. falcatum* as compared to non-nano-formulated botanicals and commercial fungicides.

# ASSESSMENT OF PATHOGENIC FREQUENCY OF SEED MYCOFLORA ASSOCIATED WITH COTTON (*GOSSYPIUM HIRSUTUM*, L.) SEEDS AND THEIR MANAGEMENT

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Cotton (*Gossypium hirsutum*) is one of the most important cash crop of Pakistan. At the moment it has been facing many threats regarding supply of quality seeds to the farmer. There are a number of mycoflora that associated with stored seeds of cotton. So it was direly needed to conduct a comprehensive study for the assessment of seed borne mycoflora associated with cotton seeds and their management by some effective seed treatments. For this purpose, a survey of different location in Nankana Sahib, Multan and Khanewal district was conducted. On the basis of visual observations three samples were collected from unhealthy seed lots of ten varieties (MNH-886, FH-114, FH-Lalazar, MM-58, MNH-992, MNH-988, BS-52, IUB-13, FH-142 and VH-259). Collected cotton seeds were examined for mycoflora prevalence adopting blotter paper method and PDA medium method. Five different fungi namely *Aspergillus niger, A. flavus, Fusarium oxysporum*, *Alternaria alternata* and *Penicillium* spp. were predominantly detected. Identification was carried out on the basis of morphological characteristics and standardized protocols. Infection frequency of associated pathogens was calculated. On PDA medium 100% mycoflora frequency was observed for each variety. Amongst the all mycoflora isolated on PDA medium, the infection frequency of *F. oxysporum* was recorded highest (31.33%) followed by *A. flavus* (23.066%) and minimum isolated fungi was *Penicillium* spp. (7.999%) on an average in all varieties. On the other hand, on blotter paper maximum frequency of seed associated mycoflora was found in samples of variety # 03 (i.e 93.75%) and minimum in variety # 2 (i.e 76.25%). Whilst amongst the all mycoflora isolated on blotter paper, the averaged infection frequency of *F. oxysporum* was recorded highest (29.75%) followed by *A. flavus* (21.25%) and minimum isolated fungi was *Penicillium* spp. (6.875%) on an average in all varieties. Seedling vigor index for untreated seeds was observed maximum for variety # 04 (i.e.128.025) while it was minimum for variety # 05 (i.e. 10.125). Seedling vigor index for treated seeds was observed Maximum for variety # 02 (i.e.906.95) while it was minimum for variety # 04 (i.e. 750.00). For the management purpose, the cotton seeds were dipped for 6 hours in the solutions of different fungicide prepared at 200ppm concentrations. Treated seed showed improved seedling vigour index as the root length, shoot length and germination rate was positively affected by fungicides. Mencozeb remained very effective to restrict the growth of seed associated mycoflora followed by Curzate M-8 while the minimum effectiveness was shown by Hombre.

# A COMPARISON OF SPATIAL AND BIOCHEMICAL STUDIES OF CITRUS GUMMOSIS DISEASE IN SARGODHA (PAKISTAN)

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Citrus is the most valuable fruit all over the world as well as Pakistan due to its taste, nutritional and medicinal qualities. Being a soil borne, water loving pathogen *Phytophthora* species are common and present in all citrus producing areas. Gummosis is the major disease which destroying the backbone of citrus industry. The infected tree shows decline symptom in conjunction with leaf chlorosis, twigs dieback, discoloured or poor coloured fruits, wilting tips and leaves withering. The objective of this is to identify citrus gummosis disease in the Sargodha region and to check its nutritional status by comparing diseased and healthy citrus samples. Surveys were conducted in 3 citrus growing areas of Sargodha. In each orchard, 15 trees were selected and totally 135 trees were assessed for gummosis incidence. A tree decline was scored on a 0-4 scale where 0=free of any decline symptom, 4=complete drying of the plant. A tree was defined and recorded as cankered when it had any of the following symptoms: discoloration of the bark surface, discoloration of the underlying tissues, dieback, dried the whole part of the plant and exudation of gum from infected tissues. 15 trees were randomly selected and observed for disease symptoms in an orchard to assess disease incidence. Disease incidence was calculated simply by dividing the number of trees showing disease symptoms over total number of trees observed and multiplied by one hundred. The results of total soluble phenols in leaf were higher than in bark and healthy leaf & bark show higher amount of phenol than diseased leaf & bark, the results of sugar analysis was fruit have higher amount of sugar than leaf and bark, the results of enzymes was CAT shows higher quantity after this SOD is in second number and last was POD. The results of enzymes were higher in diseased plants when compare with healthy plant. The status of minerals and heavy metals were iron was most abundant in present study in Sargodha region after iron zinc, manganese and copper was present respectively in the current study in both healthy and diseased plants. In conclusion, this study is expected to enable farmers to identify and predict the timing and severity of citrus gummosis disease. Nutrient loss, loss of immunity, reduction of fruit and taste, change of plant metabolism, all their factor reducing yield thereby, achieving yield improvement and fruits quality.

# CHEMICAL MANAGEMENT OF FICUS DIEBACK DISEASE: SEVERE THREAT TO *FICUS* SPP. IN PAKISTAN

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*Ficus* species are frequently planted and preferred for ornamental purposes. Recently *Ficus* spp. were found affected with Dieback disease in Pakistan. A study was carried to find out the effective management of Ficus dieback in Pakistan. According to the results, the incidence of Ficus dieback in Pakistan is up to **62%**. Among the isolated fungi, *Fusarium proliferatum* was found to be the principal cause of dieback. *Ficus benjamina* was identified as the most susceptible species to dieback disease. Various fungicides including, Carbendazim, Topsin-M, Nativo, Difenoconazole, Alliete, were used in vitro to assess their inhibitory effects on *F. proliferatum* by poisoned food techniques. Colony diameter was recorded in centimeters (cm). Carbendazim was evaluated as the best fungicide against *F. proliferatum*; hence, it was used in greenhouse evaluation for optimized performance evaluation. The best dose ($1.5\times10^2$ ppm) was applied by drench and foliar sprays on the one-year-old plants sown in 30 cm diameter earthen pots in the artificially prepared sick soil with *F. proliferatum*. Fungicide was applied every week, and disease severity was recorded during the same time. Control plants were transplanted in sick soil in earthen pots without treatment (sprayed with distilled water). Results indicated that Carbendazim provided the best control from dieback in *Ficus benjamina*. Disease progress was slight or non-significant.

# CURRENT STATUS OF FUNGAL DISEASES OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN DIFFERENT AREAS OF PUNJAB

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Sunflower (*Helianthus annuus* L.) is a useful oil seed crop which is fit in agricultural framework of Pakistan. Different fungi, bacteria, virus, bugs and nematodes attack the sunflower crop and cause harm which results in loss of yield of sunflower. Fungal diseases in sunflower are rust, downy mildew, powdery mildew, verticillium wilt, sclerotinia stalk rot, head rot, charcoal decay, leaf blight, leaf spot, botrytis head rot, damping off, Fusarium stalk rot, leaf smut, phoma black stem, phomopsis stem canker, rhizopus head rot, septoria leaf spot and blight, sclerotium blight, verticillium leaf wilt and white rust. The objectives of the study were to report the fungal diseases which are present in different areas of Punjab and to record the incidence of fungal diseases which are present in different areas of Punjab. These diseases reduce the production of sunflower crop when become severe. For this purpose, surveys of both summer and winter crop of sunflower fields were conducted in different areas of Punjab (Chakwal, Faisalabad and Sargodha), Pakistan, during October 2020 to April 2021 for the collection of data. Disease incidence was calculated by counting the number of plants infected by its total and disease severity was calculated by using disease rating scales 0-5, where: 0 = no disease, 1= 1-10% plant affected and 5= 75% and above plants affected. Leaf spot and leaf blight were with high incidence and widely distributed in three regions of the Punjab than remaining other fungal diseases. The other diseases observed in these areas were charcoal rot, phoma black stem, rust, verticillium wilt, downy mildew, powdery mildew and phomopisis stem canker. Significant difference in the incidence of fungal diseases was also observed on different locations. The varieties and hybrids showed different responses against these fungal diseases on different locations. During surveys, susceptible, moderately susceptible, resistant and moderately resistant responses were observed on different varieties and hybrids. However, most of the response of varieties and hybrids was resistant to moderately resistant. The current study provides baseline information about the fungal diseases prevalence in different areas of Punjab and will help designing new studies regarding these diseases, so that, the future epidemics of these fungal diseases may be avoided.

# CURRENT STATUS OF ALMOND DISEASES IN DISTRICT LORALAI AND ZIARAT

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The main objective of current research was to check the disease incidence (DI) of almond diseases in different areas of district Loralai and Ziarat of province Balochistan. The villages of district Ziarat in which surveys were conducted included Inzer-gut, Short-cut, Baghao, Pasra, Regorha, Kanobai and Aghberga, while the villages of district Loralai included Pathan kot, Shah karez, Durgai, Zingi wala, Shah bozai, Mehwala, Marga and kibzai. Fifteen diseases were identified in district Ziarat and Loralai. Three surveys were conducted to record the DI of different diseases. These surveys were conducted at the time of blossoming, pre-harvest and post-harvest stages from May 2020 to April 2021. In the current research, we have presented the DI of different diseases in the form of percentage. DI of anthracnose of almond was significantly high 35% in Ziarat and 30% in Loralai. The second highest DI was of almond leaf scorch 19% in Loralai and 11% in Ziarat. The DI of leaf rust of almond in Ziarat was 18% and in Loralai 12%, respectively. The DI of Armillaria root rot in Ziarat was 13%, and also 13% in Loralai. Almond leaf curl was 8% in Loralai, whereas in Ziarat it was 4%. Alternaria leaf spot in Loralai was 9% and 3% in Ziarat; powdery mildew in Ziarat 7% and in Loralai 5%; phytophthora root rot in Ziarat 6% and in Loralai 4%; crown gall in Ziarat 2% and in loralai 3%; bacterial leaf blast in Loralai 3% and also 3% in Ziarat; shot-hole in Ziarat 3% and in Loralai 4%; green fruit rot in Ziarat 1% and Loralai it was also 1%; and red leaf blotch in Loralai was 10% and in Ziarat 9%. The present study revealed that the almond trees cultivated in Ziarat and Loralai are attacked by several diseases, and these diseases are hindrance in getting potential yield of almond in these two districts of Balochistan.

# EFFECT OF SELECTED FUNGICIDES AND PLANT EXTRACTS ON THE CONTROL OF EARLY BLIGHT OF TOMATO CAUSED BY *ALTERNARIA SOLANI*

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Tomato (*Solanum lycopersicum* L.) is the second important vegetable crop world widely. *Alternaria solani* is causal agent of early blight of tomato which reduced 79 % tomato crop yield. Current study was conducted to study the antifungal potential of selected fungicides and plant extracts against *A. solani.* Two fungal isolates of *A. solani* were subjected to morphological characterization. The results of pathogenicity assay revealed high virulence response of (AS-1) fungal strain. The results of in-vitro study demonstrated that 25 % ginger extract significantly inhibited mycelial growth of *A. solani.* Pot experiment results showed that among plant extract (ginger) substantially suppressed *A. solani* while in case of fungicides Miravis duo was most effective. Correspondingly, the results of secondary metabolites detection indicated the presence of tannins, terpenoids, flavonoids, alkaloids, reducing sugars, anthraquinones. DDPH scavenging results of ginger shown (64.9 ± 1.85) scavenging potential. The results of GC-MS analysis of ginger and black seed indicated the presence of 13 and 16 bioactive compounds respectively which include Ethanol, Acetone, 2-Butanone, Trichloromethane, Propane,2,2-dimethoxy-2, 2-Dimethoxybutane, hexanal, 2-Pentanone, 4-hydroxy-4-methyl-,2-Butanone,4-(4-hydroxy-3-methoxyphenyl)-,Gingerol,1, 2-Benzenedicarboxylic acid, diisocotyl ester, Hexadecanoic acid, methyl ester , n-Hexadecanoic acid, Methyl 9-cis,11-trans-octadeca dienoate ,9-octadecadienoic acid(Z)-methyl ester ,9,12-octadecadienoic acid(Z,Z)- Cis-Vaccenic acid, 9,12-octadecadienoic acid (z,z)-,1-Monolinoleoylglycerol trimethylsilyl ether, and 9,12,15-Octadecatrienoic acid. FTIR analysis of ginger and black seeds demonstrated 12 and 13 peak values correspondingly; NMR of ginger explored the presence of 4 compounds. It is needed to explore the disease suppressive activity of plant extracts under field conditions.

# ANTI-PHYTOPATHOGENIC POTENTIAL OF CURCUMIN- CHITOSAN FUSED NANOPARTICLES AGAINST PHYTO-PATHOGENS

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Chitosan and Curcumin, both novel molecules are of great in today's era. These molecules are having significant antibacterial, antifungal, antiviral, insecticidal properties. Moreover, these molecules are natural, biodegradable, and biocompatible and thus can be easily utilized in novel formulations related to medicine, health and agriculture. Chitosan is nowadays used as a significant agent in micro and nano-capsulations and in drug delivery. These molecules are of great interest for nano-medicine, biomedical engineering and development of new therapeutic drug release systems with improved bioavailability, increased specificity and sensitivity, and reduced pharmacological toxicity. The present study was performed in order to prepare the nano-formulations of chitosan blended with curcumin. The chitosan was extracted and purified from exoskeleton of fresh water crustaceans while curcumin was extracted via solvent extraction method from fresh rhizomes of Curcuma longa (Haldi). These nano-formulations were further screened to evaluate the phyto-pathogenic (antifungal) potential against *Colletotrichum gelopsporides, Phytophthora, Sclerotina sclerotiorum* and *Fusarium oxysporum*. The results showed the remarkable and significant antifungal potential against the pathogens studied as determined in triplicates. The results were observed in vitro against the mentioned cultures by the modified method of well puncture method and radial axis inhibition of the respective mycelium. The studies will be replicated on crops having fungal attack in order to validate the studies.

# MANAGEMENT OF CHILLI LEAF CURL DISEASE THROUGH RESISTANT GERMPLASM AND NUTRIENTS IN RELATION TO ENVIRONMENTAL FACTORS

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Chilli (*Capsicum annuum* L.) is an important and commercial spice crop that is cultivated in many countries all over the world. It is flowering plant that belongs to the genus capsicum under the Solanaceae family with different names such as hot pepper and Chilli pepper. Leaf curl disease of chilli is caused by Chilli leaf curl virus (ChiLCV) that is the most devastating Begomovirus. Six varieties of Chilli i.e. Zinia $F_1$, Desi (Jalalpuri), Desi (Jalalpuri 2), Wonder Hot, Summer Queen $F_1$ and Faisalabad Selection were evaluated against the Chilli leaf curl disease for resistance source. Out of all varieties, two varieties i.e. Wonder Hot and Summer Queen $F_1$ showed moderately resistant (MR) response while three varieties including Zinia $F_1$, Desi (Jalalpuri) and Desi (Jalalpuri 2) expressed moderately susceptible (MS) response and only one variety namely Faisalabad Selection expressed resistant (R) response. Four varieties including Zinia $F_1$, Desi (Jalalpuri), Desi (Jalalpuri 2) and Wonder Hot were used for management purpose. Four nutrients including Zinc Sulphate, Copper Sulphate, Manganese Sulphate and Boric Acid were used in different combinations i.e. ($ZnSO_4$ + $CuSO_4$), ($MnSO_4$ + $CuSO_4$), (Boric Acid + $CuSO_4$) and ($ZnSO_4$ + $CuSO_4$ + $MnSO_4$ + Boric Acid). Disease incidence and disease severity was recorded with 15 days interval. Out of all treatments, ($ZnSO_4$ + $CuSO_4$ + $MnSO_4$ + Boric Acid) showed minimum disease severity (11.63%) as compared to control (58.14%). In case of disease incidence, ($ZnSO_4$ + $CuSO_4$ + $MnSO_4$ + Boric Acid) has given better results with minimum disease incidence (35.65%) as compared to control (92.59%). Effect of treatments on plant parameters was also observed and the analysis showed positive effects on plant parameters as compared to control treatment. The maximum averaged shoot length (27.00cm), root length (14.13cm), fresh weight (10.00g) and dry weight (2.79g) were observed on plants treated with the combination of ZS+CS+MnS+BA. Effect of environmental conditions such as maximum and minimum temperature, relative humidity and wind speed on the disease severity of 4 varieties (Zinia $F_1$, Desi (Jalalpuri), Desi (Jalalpuri 2) and Wonder Hot) was also observed. There was significant ($p<0.05$) and positive correlation between wind speed and disease severity while the relationship of disease severity with maximum temperature, minimum temperature and relative humidity was strongly negative and significant.

# ASSESSMENT AND BIOLOGICAL MANAGEMENT OF POSTHARVEST SOUR ROT (*GEOTRICHUMCITRI AURANTII*) OF CITRUS USING **ANTAGONISTIC YEAST**

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Sour rot disease of citrus engendered by *Geotrichum citriaurantii* is a significant disease of postharvest that disrupts all species of Genus *citri* cultivated all over the world. Imazalil, thiabendazole, as well as azoxystrobin, fludioxonil, and pyrimethanil, are currently listed to control *Penicillium digitatum* caused green mould, but they are ineffective against *G. citriaurantii* causing Sour rot disease of oranges. The constraint of the use of chemical products due to phytotoxicity, residual effects, action spectrum, attaining of resistant strains from pathogen and its harmful effects have stimulated the reduction of its use and the implementation of alternative methods. For the management of sour rot of citrus, after harvesting of Citrus fruit, antagonistic yeast (also known as a biocontrol agent) is a promising alternative to chemical fungicides. Due to their prevalent dissemination, environmentally safe, and non-toxic for human health, antagonistic abilities such as nutrient and space competition, mycoparasitism and inhibit the growth, development, reproduction, or action of phytopathogen. Antagonistic yeast isolated from fruits and leaves of citrus plants (orange, lemon, and grapefruit) will be collected from the citrus orchard. Research has been planned to evaluate the antifungal effect of antagonistic yeast on *G. citriaurantii.* The activity of peroxidase and superoxide dismutase was altered in the fruit inoculated with the two yeasts. *Cryptococcus laurentii*, in particular, was able to elicit a strong response in citrus fruits that had been treated.

# SUPPRESSIVE EFFECT OF SALICYLIC ACID AND JASMONIC ACID ON BLUE AND GREEN MOLD OF CITRUS BY MODULATING THE ACTIVITY OF PEROXIDASE AND POLYPHENOL OXIDASE

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Green and blue mold is a major post-harvest threat to Citrus that causes the spoilage of fruit during storage. The effect of salicylic acid (SA) and jasmonic acid (JA) to reduce the disease incidence and severity of green and blue mold on 3 *Citrus* species viz, species *Citrus reticulata* cv. 'Kinnow', *C. limon* cv. 'Meyer Lemon', and *C. limetta* cv. 'Mosambi' was studied at different doses. The fruit were subjected to dip treatment with resistance inducers at different concentrations SA (0, 2, 4, 6, 8 mM) and JA (0, 1, 2, 3, 4 mM). Both SA and JA reduced the disease severity significantly compared to infected control treatment. The suppression of the disease was significantly increased in a dose dependent manner, with highest suppression at the highest dose of the resistance inducer. To reveal the mechanisms underlying the disease suppression the activity of peroxidase (POD) and polyphenol oxidase (PPO) was evaluated in the treated and untreated fruit. The activity of POD and PPO was increased with the increasing dose of resistance inducer. The highest activity of PPO and POD was recorded in *C. reticulata* 'Kinnow' and the lowest in *C. limon* 'Meyer Lemon' fruit. Conclusively, SA and JA suppressed the development of green and blue mold by inducing the activity of defence enzymes PPO and POD.

# PATHOGENICITY AND *INVITRO* CONTROL OF BRINJAL LEAF BLIGHT CAUSED BY *BIPOLARIS BICOLOR*

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Brinjal or eggplant is an important vegetable crop of tropical and subtropical regions of the world. Leaves of the crop were found severely affected with spots or blight symptoms at different locations of Hyderabad district. In order to confirm the etiology of the disease, isolation was performed from 250 collected samples. Out of 250 tissues, 180 colonies were diagnosed with *Bipolaris bicolor* followed by *Alternaria* spp, *Cercospora* spp and *Aspergillus* spp. The pathogenic nature of *B. bicolor* was confirmed by following Koch's postulates. Conidial suspension was used for artificial inoculation of leaf by spraying method. Five fungicides (Shincar, Kocide, Antracol, Melody dew and Pyrazole) were selected and applied under *in-vitro* condition with different dosages. Among them Shincar, Kocide, Antracol and Melody dew, The Pyrazole had a great efficacy and was observed the most effective to inhibit the colony growth of *B. bicolor* followed by Kocide and Melody dew. Shincar and Antracol were very less effective to inhibit the mycelial growth of *B. bicolor.*

# INCIDENCE OF LATE BLIGHT OF TOMATO IN DISTRICT FAISALABAD

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*Phytophthrora infestans* is the casual organism of the late blight of tomato. In 2009, it was firstly reported in Wisconsin. Disease spread when minor temperature corresponds with humid condition, it can spread upto 40 miles and infected plant dies in 7 to 10 days. Infection appears in ten hours when humidity is more than 90% and temperature is 60˚-78˚ F. Young small leaf lesions rapidly expand with white mold appearance. In fourteen days, complete defoliation occurs. Incidence of late blight was assessed through sampling of different tomato growing areas in District Faisalabad. Infected samples were collected and processed in laboratory. The infected leaves were disinfected with Sodium hypochlorite and washed subsequently with distilled water to process it for isolation. In Petri plates these infected pieces of leaves were placed on the blotter paper and incubated for cultural growth. Then the Growth on blotter paper was transferred in PARP media and incubated. These fungal colonies showed microscopic asexual spores known as sporangia. These are in lemon shape, hyaline and long 20 to 40 um. At high humidity when it placed in water, sporangial cytoplasm distributed and from each sporangium many zoospores emerged.

# BIOLOGICAL AND CHEMICAL CONTROL OF CITRUS GUMMOSIS DISEASE

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Phytophthora spp. cause economically important diseases in citrus. Root rot, gummosis, and brown rot of fruit are the most common diseases produced by Phytophthora spp. in citrus. From the species of Phytophthora, associated with citrus, P. citropathora has been reported the cause of citrus gummosis. The objective of this study was to check the morphological characteristics of citrus plants infected with gummosis and the use of Bio-control agents and chemicals to manage the disease. For this purpose, surveys of different citrus orchards of Sargodha district (Bhalwal, Sargodha, and Kotmomin) were conducted to check the leaf area and trunk size of diseased trees along with healthy trees and collect the samples of roots, soil and bark of infected trees. 25 fields were surveyed in each tehsil for this purpose. The disease was observed in almost all the citrus orchards. After recording the whole readings of different villages orchards, Sargodha tehsil had recorded the highest leaf area in average of about 2685.368 $mm^2$ followed by bhalwal 2548.428 $mm^2$. Although in the case of trunk size there was a wide difference between healthy and severely infected tree trunk. The highest average healthy trunk size was recorded in kotmomin tehsil having 101.56 cm and diseased trunk in Bhalwal having 88.56 cm in size. Healthy leaf area and trunk size was not significantly different from tehsil to tehsil but diseased trees are significantly different from each other having $p<0.05$. For isolation of Phytophthora spp., PARP media was used. The fungal species were identified on genus level on the basis of macroscopic and microscopic characteristics. Dual culture technique was used for the treatments of bio-control and food poisoning technique was used for the evaluation of five different fungicides against P. citropathora. Three concentrations 100, 200 and 300 ppm were used for each fungicide. For each treatment, three replicates were used. Results showed that all the bio-controls and fungicides, significantly inhibited mycelial growth of P. citrophthora at 3rd, 5th and 7th day ($P<0.05$). In fungal bio-control, highest percent inhibition was 86%, 89% and 90%, given by Trichoderma harzianum followed by T. atroviridae was 75%, 80% and 81% at 3rd, 5th and 7th day respectively. Lowest percent inhibition 64%, 73%, 72% was given by T.viridae, while in case of bacterial bio-control B09 gave the maximum percent inhibition followed by B31, which was 78% and 71 % respectively at 7th day. B62 gave the least percent inhibition of 19% at 7th day. In chemicals higher percent inhibition was given by tuberconazole78% at 300 ppm on 7th day of the treatment followed by alliete 72% at 100 ppm on 5th day and then Topsin-M 66% at 300ppm on 7th day of the treatment All plant extracts, inhibited the mycelial growth of P.citrophthora at 3rd, 5th and 7th day ($P <0.05$). Maximum percent inhibition was given by Eucalyptus, 88 %, 91% and 91%, followed by mint was 69 %, 61 % and 61% at 3rd, 5th and 7th day respectively. Neem and garlic gave low percent inhibition as compared to eucalyptus and mint. Garlic gave up to 31 % maximum percent inhibition at 3rd day and neem was 49% at 7th day.

# POST-HARVEST MANAGEMENT OF *BOTRYTIS CINEREA* CAUSING GRAY MOLD IN POMEGRANATE THROUGH ESSENTIAL OILS

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Pomegranate is one of the oldest edible fruit and its cultivars are wonderful with sweet taste and flavored juices. The fruit appears as white yellow pink and deep red color tones depending upon the variety and stage of ripening. Under favorable conditions, various pathogens attack the fruits causing economic losses. Gray mold disease caused by *Botrytis cinerea* is economically the most important postharvest disease in pomegranate fruits. This study is aimed at using bio control agents for managing gray mold diseases in pomegranate. In *vitro* results indicate that the essential oils suppress the radial growth of *B. cinerea*. In this study, the essential oil from five medicinal plants were evaluated and compared for their fungal activities against *Botrytis cinerea*, which is a postharvest pathogen on a wide range of fruits. According to a modified in *vitro* test, of coconut (*Cocos nucifera*), rose (*Rosa sp.*), sesame (*Sesamum indicum*), clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) exhibited strong effect on *B. cinerea*. All these oils possess the fungicidal effects on the pathogens through contact and volatile phases. These phases have been tested for their affect upon different concentrations to inhibit the fungal growth of *Botrytis cinerea* and might be utilized for the control of fungal disease on postharvest organic fruits.

# INVITRO EVALUATION OF DIFFERENT FUNGICIDES TO CONTROL *PUCCINIA RECONDITA* F. SP. *TRITICI*

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Wheat (*Triticum aestivum* L.) is one of the most important food crop around the globe and is staple food of Pakistan. Number of diseases attack on wheat, among which leaf rust is most devastating disease. Present study was planned to evaluate the antifungal efficacy of different fungicides invitro conditions. Different fungicides such as Mancozeb, Triadimefon, Propiconazole and Hexaconazole were evaluated. Three concentrations of each fungicides i.e 0.1%, 0.05% and 0.025% were prepared. All fungicides inhibited the growth of fungus. Propiconazole was best at all concentrations followed by hexaconazole, mancozeb and triadimefone. It was observed that growth inhibition increased by increasing the concentration of fungicide.

# MANAGEMENT OF EARLY BLIGHT OF TOMATO THROUGH RESISTANT GERMPLASM AND NUTRIENTS IN RELATION TO ENVIRONMENTAL FACTORS

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Tomato *(Lycopersicon esculentum* Mill.) is one of the most important vegetable crops around the world as well as in Pakistan and it belongs to family Solanaceae. Early blight caused by the fungus *Alternaria solani*, is perhaps the most common foliar disease of tomato in world leading to a reduction of the economic fruit yield. To manage this disease under field condition the current study was planned with certain objectives of resistance source evaluations and management through different nutrients and their combinations. For resistance source, six varieties (Nadir F1, Naqeeb F1, Johir, Gohar, Walter Gaint FM9 and Money Maker) were screened out against *A. solani*. Out of six varieties, one variety Johir expressed resistant (R) response, two varieties Nadir F1 and Gohar showed tolerate (T) response and only one variety Walter Gaint FM9 showed susceptible (S) response with disease severity. While two varieties Naqeeb F1 and Money Maker showed highly susceptible (HS) response. Four varieties including Nadir F1, Naqeeb F1, Johir and Gohar were used for management experiment. Four nutrients including Zinc Sulphate, Copper Sulphate, Manganese Sulphate and Boric Acid were used in different 4 combinations i.e, ($ZnSO_4$+$CuSO_4$), ($MnSO_4$+$CuSO_4$), (Boric Acid+$CuSO_4$) and ($ZnSO_4$+$MnSO_4$+$CuSO_4$+Boric Acid). Disease severity and disease incidence was recorded with 15 days interval. Out of all treatments, combination of Zinc sulphate, Copper sulphate, Manganese sulphate and Boric acid showed minimum disease severity (5.85%) as compared to control (28.59%). In case of disease incidence combination of Zinc sulphate, Copper sulphate, Manganese sulphate and Boric acid also gave best result with minimum disease incidence (23.15%) as compared to control (80.09%). Effect of treatments was also observed on plant parameters and the analysis showed positive and increasing effects on plant parameters as compared to control treatment. The maximum averaged shoot length (104.50cm), root length (41.75cm), fresh weight (191.60g) and dry weight (53.82g) were observed on plants treated with the combination of $ZnSO_4$+$MnSO_4$+$CuSO_4$+Boric Acid. Effect of environmental factors such as maximum and minimum temperature, relative humidity, wind speed and rainfall on the disease severity of four varieties (Nadir F1, Naqeeb F1, Johir and Gohar) was also observed. Wind speed and rainfall were positively correlated with disease severity for early blight of tomato. Wind speed$(r = 0.97)$and rainfall$(R = 0.037)$ were highly correlated with the disease severity of Nadir F1 while a strong negative inter-relationship of disease severity was observed with maximum temperature, minimum temperature and relative humidity.

# SYNERGETIC EFFECT OF ANTAGONISTS IN COMBINATION WITH BIO-PESTICIDES FOR THE MANAGEMENT OF TWIG AND STEM BLIGHT (*BOTRYODIPLODIA THEOBROMAE*) OF COTTON

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Cotton is an important cash crop of Pakistan. It is widely cultivated as a fiber crop throughout the world. Cotton considered as the backbone of economy of Pakistan. Cotton is infected by different types of pathogen including Fungi, Viruses, Bacteria and Nematodes. Now days, the twig and stem blight disease of cotton caused by *Botryodiplodia theobromae* is an emerging threat to the cotton production in Pakistan. The incidence of twig and stem blight was recorded 25% and Disease Prevalence was 80% in the southern areas of Punjab, Pakistan. Dark brown stem and necrotic lesions and Pycnidia were observed on the branches. Plant infected by *B. theobroame* shows dieback symptoms. The research was done to manage the twig and stem blight disease by antagonistic fungi in combination with bio-pesticides. *Trichoderm harzianum* (TH), *T. viride* (TV), *Bacillus* spp. (BC), *Pseudomonas inflouresence* and Nativo (NA) was used by making their different combinations. Data was recorded by the infected growth parameters. In-vitro evaluation of these combinations was done by making 40, 50, 60 ppm. These combinations were also checked under greenhouse and field conditions. All the PGPR's showed effective results alone and in combination of two. In both cases in-vitro and in-vivo *T. Harzianum* in Combination with Nativo showed best results against pathogen inhibition. Data was analyzed by standard statistical techniques.

# SCREENING OF SUNFLOWER HYBRIDS AGAINST HEAD ROT DISEASE (*RHIZOPUS ARRHIZUS*) AND IN VIVO EVALUATION OF VARIOUS FUNGICIDES

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Rhizopus head rot is an important disease in Sunflower causing considerable losses both qualitatively and quantitatively. Rhizopus is a widespread fungus that can be found in soils and in the air as a spore. Rhizopus head rot was first reported in Pakistan in 1983 until that this disease is prevalent in the field at varying intensity. Under favorable conditions during the cropping season, it can cause major losses in certain fields and can also be in epidemic form. Wounds caused by hail, birds, or insects are the first source of infection. In-vitro condition, isolation, purification and mass culturing were done to prepare the inoculum, for the screening of sunflower germplasm against head rot disease in the field. Out of 20 collected hybrids from National Agriculture Research Center (NARC), 3 were screened as susceptible while 17 were highly susceptible. Management studies were carried out on three susceptible varieties using systematic fungicides includes Azoxystrobin, Trifloxystrobin+ Tubiconazole, Benzimedazole while, non-systematic fungicides include Topsin-M, Mancozeb, Carbendazim. All of the fungicides were sprayed on the susceptible varieties including their replications with different concentrations after regular interval of days 15, 30 and 45. According to the results, Azoxystrobin (systematic fungicide) and Carbendazim (non-systematic fungicide) gave the best results with the minimum disease severity percentage after 45 days while Mancozeb and Benzimedazole showed the least impact after 45 days on the susceptible hybrids FH-516, FH-748 and FH-749 with maximum disease severity.

# EXPRESSION OF ADULT PLANT RESISTANCE BY WHEAT *(TRITICUM AESTIVUM* L.) GERMPLASM AGAINST YELLOW RUST *(PUCCINIA STRIIFORMIS* F. SP. *TRITICI*) UNDER RAWALAKOT CONDITIONS

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Wheat (*Triticum aestivum* L.) is most important staple food crops of Pakistan. Regular and occasional yellow rust threats resulted in huge economic losses to country and farming community, posing a food security threat to Pakistani people. The disease can reduce yield up to 80-90% under conducive environmental conditions. National trap nursery prepared by the Crop Diseases Research Institute (CDRI) NARC; Islamabad was used in this study. Nursery was planted in the farm area of University of the Poonch Rawalakot at Chota Gala. Two hundred and sixty-five wheat genotypes were screened against stripe rust. The disease progress was monitored on weekly basis on all sown genotypes. Most of the genotypes showed reaction to this disease. For the development of disease natural inoculum was relied for infection on control in the environmental conditions of Rawalakot. Plants were monitored for initiation of yellow rust infection on control (Morocco) and final data was taken when control covered 80% of flag leaves with disease. Field screening results showed that 45 varieties were found resistant (R) which is 16%, 116 were moderately resistant (MR), 56 were moderately susceptible (MS) and 46 was susceptible (S) which contribute 41%, 20% and 16% respectively. It was concluded that screening and identifying the virulence pattern of yellow rust on wheat germplasm and utilizing these virulence genes on advanced lines may be helpful to produce for rust resistance in wheat to get maximum production.

# *IN VITRO* EVALUATION OF CHEMICALS AGAINST FUNGAL PATHOGENS OF ORNAMENTAL PLANTS

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During study six pathogens viz. *Diplocarpon rosae* (Rose), *Alternaria alternata* (Rose), *Stagonospora curtisii* (Amarylis), *Fusarium oxysoprum gladioli* (Gladiolus), *Cercospora ruscicola* (*Ruscus hypophyllum*), *Cercospora fici* (*Ficus elastica*) were isolated and characterized. *Trichoderma* sp. showing antagonistic activity on media plate in soil sprinkling method was purified for *invitro* assays. Dual culture was carried out to see the antagonistic activity of *Trichoderma* sp. against pathogens of ornamental plant. Percent growth of inhibition (PGI) of *Trichoderma* sp. was calculated by using formula. Data of study revealed that maximum PGI was found against *Alternaria alternata* (80.38%). PGI also found in other pathogens viz. *F. oxysoprum gladioli* (46.56%), *D. rosae* (51.61%), *C. ruscicola* (58.36 %), *C. fici* (60.36%), *S. curtisii* (61.53%). Food poisoned technique was used to study effectiveness of fungicides (Topsin-M, Dithane M-45) and chemicals (Copper sulphate) against ornamental plants. Well discs were put on media plates containing respective chemicals. Media plates without chemicals were uses as positive control. PGI data showed that both fungicides and copper sulphate reduced growth but effectiveness vary from pathogen to pathogen. Topsin- M was found most effective against *C. fici* (79.06%). Dithane-M was found most effective against *C. ruscicola* (62.79%), *C. fici* (62.78%). Copper sulphate found best against *A. alternata* (80%) followed by *C. fici* (72.09%). It is plausible to mention that among chemicals copper sulphate gave best results against all pathogens and may be incorporated in disease management of ornamental plants.

# GENETIC VARIABILITY OF *FUSARIUM OXYSPORUM* ASSOCIATED WITH CHICKPEA WILT

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Chickpea (*Cicer arietinum* L.*)* ranks third among legumes and contributes 70% share in pulses. Productivity is limits by different factors which include biotic and abiotic factors; among these chickpea wilts is a major factor which causes 29-57% yield reduction. The disease is soilborne and appears at every level of plant growth. Symptoms appear at both vegetative and reproductive stage of plant. Chickpea wilt pathogen has been studied along with its pathogenicity on different chickpea varieties. The present study is aimed the molecular characterization and genetic variability of *Fusarium oxysporum* f. sp. *ciceris*. The samples were collected from different chickpea growing areas of Punjab on the basis of disease prevalence and disease incidence. Significant results for disease incidence were obtained after survey followed by isolation and pure culturing of the pathogen. After preliminary morphological identification of the *F. oxysporum* f. sp. *ciceris*, the pure culture of the pathogen was subjected to total genomic DNA extraction for nucleic acid-based amplification of ITS (Internal transcribed spacer) and EF (Enhance Factor) genomic regions of the pathogen. The Amplicons was TA-cloned and subsequently sequenced for analysis. The sequence was being assembled and aligned for pairwise sequence comparisons for similarity matrix and phylogenetic tree construction. Current study provides valuable scientific information about the prevailing races of *F. oxysporum* f. sp. *ciceris* in chickpea growing fields.

# CHEMOTHERAPEUTIC MANAGEMENT OF BROWN LEAF SPOT CITRUS CAUSED BY *ALTERNARIA CITRI*

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Alternaria brown spot is very important disease of mandarins and its hybrids which is caused by *Alternaria citri.* This disease is most prevalent in humid subtropical citrus producing areas. Present study gives the new information about the selection of fungicides for this disease. Seven fungicides (Hexaconazole+ captan, mancozeb+ metalexyle, difenoconazole, azoxystrobin+ flutriafol, foestyle aluminium and metiram) were evaluated against pathogen under in vitro conditions in Department of Plant Pathology, University of Agriculture Faisalabad. Three concentrations of each treatment as (250, 500, and 750) ppm were applied by poisoned food technique. Out of these fungicides difenoconazole completely inhibited mycelium of *A. alternata* and expressed minimum growth (0.00) mm followed by hexaconzole+ captan, azoxystrobin+ flutriafol, thiophenate methyl, mancozeb+ metalexyle, metiram and fosetyle aluminium (1.42, 1.93, 7.30, 7.52, 7.65, 7.67)mm in comparison to control dishes (15.22mm). Difenoconazole gave 100% results at all concentrations with 0.00 mm growth, also hexaconazole+ captan and azoxystrobin+ flutriafol at 500 ppm and 750 ppm inhibited the mycelium and expressed 0.00 mm growth. From the lab experiments top five chemicals (thiophenate methyl, difenoconazole, metiram, hexaconzole+ captan and azoxystrobin+ flutriafol) were selected and three concentrations (0.25, 0.50, 0.75) % of each treatment were sprayed on one-year-old infected plants under RCBD. Similarly difenoconazole significantly reduced the disease incidence (23.0%), hexaconzole+ captan, and azoxystrobin+ flutriafol expressed disease incidence (29.70, 30.00) % respectivle, thiophenate and metiram proved average by expressing disease incidence (38.90, 47.70)% repectively as compared to control (72)%. Difenoconazole is recommended to the growers of citrus for the management of ABS disease.

# *PEBB1*, A NOVEL PROTEIN ELICITOR DERIVED FROM *BEAUVERIA BASSIANA* ARSEF 2860 STRAIN, INDUCES SYSTEMIC RESISTANCE IN *BRASSICA RAPA* SSP. *PEKINENSIS* AGAINST *MYZUS PERSICAE* (HOMOPTERA: APHIDIDAE)

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This study reports the characterization of a novel protein elicitor PeBb1 derived from entomopathogenic fungus *Beauveria bassiana* ARSEF-2860 strain and its putative role in induced systemic resistance in *Brassica rapa* ssp. *pekinensis* against green peach aphid *Myzus persicae*. The sequence of purified elicitor protein was matched with the genomic sequence of a hypothetical protein BBA_10269 from *B. bassiana* ARSEF-2860 (GenBank Accession No. XP_008603588.1). The protein-encoding gene PeBb1 contained 534 bp cDNA encoding a polypeptide of 177 amino acids with a molecular mass of 19 kDa. The recombinant elicitor protein was expressed in *Escherichia coli* using pET-28a (+) expression vector and induced necrosis in the leaves of tobacco. The sublethal effects of elicitor protein on aphid *M. persicae* was determined by applying three different concentrations of PeBb1 (*i.e.* 26, 35, 53 µM) on *B. rapa* plants at 4-leaf stage and the treated plants were exposed to newly emerged (0–6 h old) apterous adult aphids. Bioassay results revealed that the exogenous application of PeBb1 elicitor exhibited significant ($p < 0.05$) sub-lethal effects on *M. persicae*. Moreover, the RT-qPCR gene expression analyses showed a significant up-regulation of most of the key genes associated with jasmonic acid (JA) and ethylene (ET) associated plant defense pathways in elicitor-treated plants. These results not only suggest the potential utilization of PeBb1 elicitor protein in future biological management strategies against phloem-feeding insect pests such as *M. persicae*, but also help in better comprehension of the mechanisms through which beneficial fungi trigger the induced plant resistance.

# *IN VITRO* PATHOGENICITY OF *LECANICILLIUM LECANII* AND *BEAUVERIA BASSIANA* AGAINST COTTON APHID *APHIS GOSSYPII* (HEMIPTERA: APHIDIDAE)

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Cotton aphid *Aphis gossypii* (Hemiptera: Aphididae), is an economically significant pest of different crops. *A. gossypii* damages crop plants by sucking the cell sap of host plant, as a result, the leaves often curl downwards and become wrinkled. Also, they have ability to transmit plant viruses. The current study was conducted to evaluate in-vitro pathogenicity of two *Lecanicillium lecanii* (V-3 and V-5) strains and one *Beauveria bassiana* (BB-76) strain against *A. gossypii*. Three divergent bioassays were performed encompassed of filtrates of fungus and concentrations of conidia of V-3, V-5 and BB-76 strains of fungal and their binary combinations by using leaf dip method. Three mL filtrate in the filtrate bioassays while three diverse concentrations ($1 \times 10^6$, $1 \times 10^7$ and $1 \times 10^8$ conidia $mL^{-1}$) in conidia bioassay were used for each fungal strain, whereas in the bioassays of binary combination, $LC_{50}$ and $LC_{33}$ of each strain of fungus were assessed. As per the outcomes, the maximum virulence against *A. gossypii* was observed at 9th day for all the treatments. Both the strains of *L. lecanii* (V-3 and V-5) revealed higher aphid mortality (up to 93 and 89%, respectively) than *B. bassiana* (BB-76) strain (85%) in all three bioassays. Furthermore, binary combinations of V-3 and V-5 strains exhibited considerably higher mortality of aphid (91%) compared to other binary combinations. Mortality reduced in BB-76 strain of binary combinations probably due to antagonistic effect of strain of *B. bassiana* with both strains of *L. lecanii*. Hence, combined in-vitro pathogenicity of the *L. lecanii* (i.e., V-3 and V-5) fungal strains was comparatively high and both of these strains can be used together as a novel biological control agent against *A. gossypii*.

# YIELD POTENTIAL OF PLEUROTUS SAPIDUS USING VARIOUS LIGNOCELLULOSIC WASTE MATERIAL (KIKAR (ACACIA NILOTICA), TALI (DALBERGIA SISSOO), COTTON WASTE AND WHEAT STRAW)

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Oyster mushroom (*Pleurotus sapidus)* is a marvellous mushroom having an unwavering cap and twig. It is a vital nutrition element regarding human health, nourishment and etc. In addition to having various nutritional standards, they are also convenient in waste administration. The research experiment was implemented to inspect the production of oyster mushroom on agricultural scraps like wheat straw, cotton waste, and sawdust (Kikar and Tali) mixed in a different ratio. In this study, the result of cotton waste, wheat straw, and sawdust growth of *P. sapidus* along with spawn running, fruiting body, and yield were examined. The trials were conceded at the mushroom lab in the Institute of Horticulture Sciences, University of Agriculture, Faisalabad under a completely randomized design (CRD). The experiment was planned with five replications and eight different treatments in such a ways T1 **(**100% cotton waste), T2 (wheat straw), T3 (kikar sawdust), T4 (100% tali sawdust), T5 ( 50% Cotton waste+50% Wheat Straw), T6 (50% Kikar sawdust+50% Tali sawdust ), T7 [25% kikar sawdust+25% tali+50% Wheat Straw],T8 [ 25% kikar sawdust+25% tali+50% Cotton waste] ) to check the period (number of days) to mycelium development, pinhead emergence, , fruiting bodies formation in number of days, yield assessment on all three flushes and biological efficiency. Results shown that the maximum number of days taken for mycelium growth in treatment T4 was (47 no. of days) as compared to treatment T1 took minimum days was (18 no. of days) the other treatments T2, T3, T5, T6, T7 and T8 took 23,28,32,41,37,35 number of days respectively. The maximum number of days in fruiting bodies emergence taken by the treatment T4 was (18.9 no. of days) and the minimum was observed in treatment T1 (6.5 no. of days), all other treatments T2, T3, T5, T6, T7 and T9 took number of days 8.4,9.8,10,14,12.5,11 respectively. The highest yield of mushroom was gained in the treatment T1 was (954 g) and the lowest yield was recorded in T 4 was (253g) compared with others treatments T2, T3, T5, T6, T7 and T8 were 751,739,716,414,497,610 grams respectively. It was concluded that the integration of all these cotton wastes gave the maximum yield production in early days that was observed by treatment T1. Whereas tali sawdust alone gave the lowest yields alone which was in treatment T4 but gave favourable yields along with combination of other substrates.

# IDENTIFICATION OF PATHOGENS AFFECTING THE HEALTH STATUS OF SAFFRON IN MOROCCO

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The production of saffron in Morocco is located in the mountainous area of the Moroccan Atlas at the junction of the High Atlas massifs. It is one of the specialties of the region of Taliouine and Taznakht. The extension of the area of saffron in the two main production areas: Taliouine and Taznakht, requires the availability of good quality corms in sufficient quantity. Unfortunately, the current system of self-production of corms is rarely accompanied by selection aimed at the quality of the saffron produced and the suitable size and sanitary condition, essential for the success of new saffron crops. In order to overcome this problem, we proceeded to analyse the health status of a collection of corms and soil from saffron fields which suffered from several anomalies. After isolation and purification, macroscopic and microscopic identification of the fungi causing the anomaly was performed. Indeed, this study revealed the presence of *Penicillium cyclopium*, causal agent of the Tacon disease, of the Rhizoctonia violet caused by *Rhizoctonia violacea*. It is the most serious disease that causes corm death. Other fungi have been identified including *Penicilium sp, Rhizopus, Monacrospium sp, Thrycophyton sp* and *Fusarium oxysporum.*

# SEED BORNE MYCOBIOTA ASSOCIATED WITH *CYAMOPSIS TETRAGONOLOBA* L. ACCOMPANIED WITH ITS CHEMICAL CONTROL

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Seed borne mycobiota is a major cause of crop yield losses in the world. Therefore, this study was designed to determine the mycobiota associated with the seeds of different varieties of drought resistant multipurpose crop Guar (cluster bean, *Cyamopsis tetragonoloba*). Further, different Phenolic compounds were used to control the seed-borne mycobiota of selected varieties. Fifteen samples comprising a total of 21 fungal species corresponding to 10 genera were isolated by ISTA (International Seed Testing Association) techniques. The most common fungus found in guar seeds were *Aspergillus niger*, *A. flavus*, and *Rhizopus stolonifer*. Highest mean percent incidence was recorded in non-sterilized seeds from blotter method in which *Aspergillus flavus* showed (19.0%). The agar plate method was shown to be the most effective for isolating seed-borne mycobiota. While Principal Component Analysis (PCA) and Alpha Diversity indices were also performed, these showed a significant difference between the varieties infected by fungal species. Phenolic compounds (qurecitin and salicylic acid) at different concentrations (0, 1000 and 5000 ppm) were used to treat the seeds of three most susceptible guar varieties (Br-2/1, Br-90, and Br-99) seed germination, vigour index, and seed borne mycobiota which were recorded at 0, 15, 30, and 45 days of storage at room temperature. After treatment, seeds were found to be associated with five saprophytic fungi, including *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, Mucor sp.*, and one root rotting fungus (*Fusarium solani*). These treatments shown significant effect on seed-borne mycobiota, seed germination rate and lowered the number of fungal species.

# ASSESSMENT OF DIFFERENT CHEMICALS AND THEIR COMBINATIONS AGAINST *ALTERNARIA ALTERNATA,* CAUSING LEAF SPOT OF SPINACH

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Spinach (*Spinacia oleracea* L.) is an important vegetable crop grown and consumed in all over the world. There are different diseases threatens the production of spinach, leaf spot caused by *Alternaria alternata* is also one of them causing low yields and poor quality of crop. Current study was also design to assess chemicals with different combinations and concentrations against *A. alternata*. Five chemicals i.e Tilt, Score, Antracol, Bloom and Nanok were used for this experiment with three concentrations (100, 300 and 500 ppm) and ten combinations (Tilt+Score, Tilt+Antracol, Tilt+Bloom, Tilt+Nanok, Score+Antracol, Score+Bloom, Score+Nanok, Antracol+Bloom, Antracol+Nanok and Bloom+Nanok) at maximum concentration 500 ppm of each *in vitro* under CRD by using poisoned food technique. While, the best performing three chemicals (Tilt, Score and Antracol) and their combinations (Tilt+Score, Tilt+Antracol and Score+Antracol) under lab conditions were analyzed for green house and field trials. The results revealed that all the chemicals and their mixtures have a significant growth inhibition effect on *A. alternata*. Therefore, among alone treatments Tilt at 500 ppm concentration was found most effective as it inhibited 89% mycelial growth followed by Score, Antracol, Bloom and Nanok (81, 78, 71 and 67%) and among the combinations Tilt+Score inhibited 93% fungal growth followed by Tilt+Antracol and Score+Antracol (90 and 87%) as compared to control treatment. Findings from green house and field trials expressed minimum disease incidence by Tilt+score (17% and 21%) followed by Tilt+Antracol (22% and 29%), Score+Antracol (25% and 33%) and among solo treatments Tilt expressed minimum DI (27% and 37%) as compare to Score and Antracol (31% and 39%; 33% and 41%), while highest disease incidence (91% and 93%) was recorded from the control treatment.

# *IN VITRO* EFFECT OF FUNGICIDES AGAINST FRUIT ROT OF GRAPES CAUSED BY *ALTERNARIA ALTERNATA*

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Grapes (*Vitis vinifera*) belongs to family *vitaceae* is a temperate fruit consumed vigorously around the globe, due to its flavor and nutritional values. It is grown in tropical and subtropical climatic regions of the world. Fruit rot of grapes by *Alternaria alternata* is one of the destructive diseases causing huge economic losses. The present study was conducted in order to evaluate the efficacy of fungicides against *A. alternate*. Isolation of pathogen on PDA media was done, from grapes showing disease symptoms, collected from local fruit market of Faisalabad. To fulfil Koch's postulates pathogenicity test was carried out on fresh grapes in the Phyto-pathological Laboratory, Department of Plant Pathology, UAF. Three different fungicides i.e. Evito, Bloom and Kasumin were used with three concentrations 200, 400 and 600 ppm, to check their efficacy against isolated pathogen under lab conditions by using poisoned food technique. The experiment was done under complete randomized design (CRD). Results expressed that all three fungicides have excellent effect on growth of *A. alternata* at different levels, it was concluded that maximum fungal growth inhibition was recorded by Evito fungicide showed least fungal growth (7.3 mm) at 600 ppm concentration followed by Kasumin and Bloom (9.5 and 13 mm).

# ESTIMATING THE EFFICACY OF CHEMICALS AND BIOCONTROL AGENTS IN VITRO AGAINST PLANT FUNGAL PATHOGEN *BOTRYTIS CINEREA*

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*Botrytis cinerea* is a plant fungal pathogen causing the grey mold disease of rose (*Rosa indica* L.). Finding new and alternative environment friendly control strategies than hazardous chemicals on different crop diseases is crucial and a healthy step to cope with current climate change challenges. Therefore, this study aimed to evaluate *in vitro* different botanicals and biocontrol agents along with different fungicides against *Botrytis cinerea*. Three different concentrations of 100, 200 and 300 ppm of five fungicides were applying, and also botanicals of eight plants were also applied at three different doses of 5, 10, and 15%. Eleven biocontrol fungal agents were using as antagonistic *in vitro*. The survey of disease incidence% of grey mold on rose crop in the region, was showing high difference between the Hyderabad region shown significantly maximum disease incidence (60%) as compared to Tandojam region (40%). Among fungicides, cabrio top exhibited significantly reduced linear colony growth (31 mm) of *B. cinerea* at 300 ppm concentration. Extracting of plant neem exhibited significantly lowest colony growth (23.33 mm) followed by plant ginger (25 mm) and plant Dhatura (26 mm). The higher concentration of fungicides and higher doses of botanicals appeared significantly efficient to control the pathogen. *Fusarium solani* appeared prominent in reducing colony growth (25.16 mm) of the pathogen but the difference was not significant with most of the tested biocontrol agents. The results showed the high ability of botanicals and biocontrol agents in reducing growth of plant fungal *in vitro* that potential consider using them instead of synthetic fungicides in synthetic fungicides and more safety for ecosystem.

# FIELD BASED ASSESSMENT OF GENETIC DIVERSITY FOR LEAF RUST RESISTANCE AND YIELD ATTRIBUTES IN THE NATIONAL COLLECTION OF WHEAT GERMPLASM USING MULTIVARIATE ANALYSIS

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Brown leaf rust severely affects the production of wheat crop and cause economic losses worldwide. In the present study, screening of the national germplasm collection consisting of 133 varieties and lines was done to assess local diversity against leaf rust disease and yield attributes under natural conditions in Faisalabad. For this purpose, a field trial was conducted in Faisalabad, Pakistan under randomized complete block design (RCBD) in 2018. Various multivariate analyses were carried out including analysis of variance (ANOVA) correlation analysis, principal component anaylsis and cluster analysis to assess varietal response under rust diseased conditions. The results showed that, 26 cultivars have an R type response while MR, MRMS, MS and S response was demonstrated by 47, 16, 16, and 28 germplasm accessions respectively. Analysis of variance indicated; all the verities have highly significant difference for all the characters. The pearsons coefficient correlation revealed that grain yield per plant was positively correlated with most of attributes, but negatively correlated with disease incidence. Principal component analysis indicated that five PCs having an eigenvalue >1 that contributed 78.4% of the total variability while 6 attributes contributed (21.6%) between wheat cultivars evaluated for different morph-physiological characters. Cluster analysis categorized the 133 genotypes into five different clusters. The members of the 2nd and 3rd clusters showed the sufficient degree of diversity with those of cluster 1. On the other hand, members of cluster 4 have maximum diversity with cluster 3, while the 1st and 2nd clusters showed the maximum similarities. Scatter plot showed that the morpho-physiological variation in the studied germplasm accessions and this diversity can be used for breeding programs aimed at improvement of disease resistance in the wheat germplasm. The present study, clearly emphasize the importance of resistant varieties which are great edge to the farmers in order to prevent yield losses.

# MACROFUNGI BELONGING TO ORDER RUSSULALES, POLYPORALES AND TRAMELALES RECORDED IN POONCH DISTRICT, AJK

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Macrofungi play a crucial role in natural ecosystem such as decomposers as well pathogens of higher plants. Many of these fungi are edible and also source of drugs used against diseases such as cancer. Therefore, this study was planned to record the presence of macrofungi in district Poonch AJK. Seven species belonging to three orders viz. Russulales (*Russula delica*, *Lactarius phillidus*), Polyporales (*Polyporus versicolor*, *Laetiporus sulphurens*, *Phaseolus scheiweinitzii*), Tremalales (*Tramella folliaceae*) were collected from Poonch district. Morphological features such as pileus, stipe and spore size along spore print colour was also recorded. Maximum pileus (14cm) and stipe (13cm) was found in *Lactarius phillidus* while no stipe was found in case of *Polyporus versicolor*, *L. sulphurens* and *T. folliaceae*. Spore print was variable ranging white (*L. phillidus*, *L. sulphurens*, *P. scheiweinitzii*), pale (*R. delica*, *T. folliaceae*) and brown (*P. versicolor*). There was subtle variation in spore size among recorded species was also found. The macro fungi found during study are of great importance in terms of nutrition and medicine and further needed to be explored at large scale.

# SYNERGISTIC EFFECT OF CHITOSAN WITH SELECTED PLANT ESSENTIAL OIL TO INHIBIT THE *ASPERGILLUS NIGER* DEVELOPMENT IN APRICOT (*PRUNUS ARMENIACA* L.)

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This study assessed the effectiveness of chitosan (CHI) and selected plant essential oil (EO) alone and in combination to control the mycelial growth of *Aspergillus niger* identified as a potential causing agent of black rot (BR) in Apricot (*Prunus armeniaca* L.). The efficacy of chitosan (CHI) (6, 9 and 12 mg/ mL) and moringa essential oil (MEO) at 0.9, 1.2 and 1.5 mg/ml concentrations were evaluated. Results revealed that mixtures of CHI (12 mg/mL) and MEO (1.5 mg/ml) strongly inhibited mycelial growth (98.7%) and showed synergistic inhibitory effect on the tested *A. niger* under lab condition. The coating application of CHI (12 mg/mL) and MEO (1.5 mg/ml) mixtures that presented synergistic interactions decreased the decay percentage (DP) and weight loss (%) of apricot cv. Skardu which artificially inoculated with *A.niger* inoculum over 6 days of storage at room temperature (25 $^{o}$C) in different packaging boxes. It is concluded that coating application with mixtures of CHI and MEO is an effective management strategy for controlling the post-harvest development of black rot in apricot. Furthermore, this combined application did not negatively affect the physical and physicochemical aspects of the apricot. These findings reveal the potential of the combined application of CHI and MEO at maximum inhibitory concentration to control the growth and survival of pathogenic fungi in fruits, which may be an alternative to synthetic antifungal agents.

# CHEMICAL MANAGEMENT OF CITRUS GUMMOSIS CAUSED BY *PHYTOPHTHORA NICOTIANA*

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Citrus belongs to family Rutaceae, is a high demanding fruit around the globe due to its economic and nutritious values. In Pakistan, citrus fruit is considered as an important source of foreign exchange from a long time. There are different biotic and abiotic stresses responsible for yield losses in citrus production, *Phytophthora nicotiana* is also one of them causing a serious threat to quantity and quality of fruit production. In the current study, different chemicals with multiple concentrations were evaluated against *Phytophthora nicotiana*. For this purpose, ten chemicals i.e Topsin-M, Ridomil gold, Aliette, Nativo, Score, Novice ultra, Kocide 3000, Cymoxanil + Mancozeb, Excel and Diathane M-45 with three concentrations (150, 250 and 500 ppm) were used in in-vitro conditions under CRD by using poisoned food technique. Whereas, the best performed two chemicals (Topsin-M and Ridomil gold) and their combination were assessed in in-vivo conditions by using soil drenching method under RCBD design. Results of this study revealed that Topsin-M was found most effective against the isolated pathogen with minimum mycelium growth (4.94 mm) followed by Ridomil gold (7.65 mm) under lab conditions. While under green house and field experiment the mixture of Topsin-M and Ridomil gold performed significantly with minimum disease incidence (17 and 21%) followed by Topsin-M and Ridomil gold as compared to control treatments (79%).

# IDENTIFICATION OF RESISTANCE SOURCE IN WHEAT GERMPLASM AGAINST BROWN AND YELLOW RUST DISEASES OF WHEAT AND THEIR MANAGEMENT THROUGH PLANT EXTRACTS AND FUNGICIDES

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Wheat (*Triticum aestivum* L.*)* is the major cereal crop which is extensively grown all over the world and contributing about 40 % of the world food requirements. In Pakistan, wheat is mainly attacked by different fungi including rusts, smuts and bunts which are considered to be the most devastating diseases of wheat crop. During current study, 406 varieties/lines of wheat (*T. aestivum* L.) were screened against stripe rust and leaf rust of wheat caused by *Puccinia striiformis* f. sp. *tritici* and *P. triticina* respectively, deploying randomized complete block design (RCBD). The results revealed that 41 genotypes were highly resistant against, 26 were resistant, 46 highly vulnerable, 11 vulnerable were mesotheic against yellow rust and 39 were highly resistant, 70 were resistant, 56 highly vulnerable and 14 vulnerable were mesotheic against leaf rust. For economical and environmentally safe management of wheat leaf and stripe rust, six plant extracts; Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Pepper (*Capsicum annuum*) Pomegranate (*Punica granatum*) and Mint (Mentha piprata) was evaluated at different concentrations in pot experiment and disease severity data was recorded at weekly interval. Minimum disease severity was recorded with (Neem) against yellow rust (20) and leaf rust (21.33). Furthermore, effect of two fungicides (Ellectus ultra and Tilt) with plant extracts was evaluated against wheat rusts through foliar spray. Minimum disease severity was recorded with the application of (Neem 10% and tilt) against yellow rust and leaf rust. Genotype, with immune and resistant response against rust disease was recommended for future varietal development program. Environmental factors were significantly correlated with disease development. Maximum temperature was negatively correlated with disease incidence and minimum temperature, wind speed, relative humidity and rainfall showed positive correlation with disease. The results of the present study could be used for future wheat breeding program and genotypes that gave tolerant response could be recommended to farmers with application of neem along with tilt in order to avoid the heavy losses caused by Rust diseases of wheat.

# BIOLOGICAL CONTROL OF EARLY BLIGHT DISEASE OF TOMATO BY USING *TRICHODERMA ASPERELLUM*

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Twenty-one isolates of *Trichoderma* were collected from the rhizosphere soil of healthy tomato plants from different areas of Punjab and investigated for their antagonistic activities against early blight of tomato produced by *Alternaria solani*. The dual culture assay showed their mycoparasitic activity and competitive capability against the mycelial growth of *A. solani*. Morphologically, *Trichoderma* isolates demonstrated the overgrowth upon the mycelia of *A. solani* in the Petri plates. Five *Trichoderma* strains i. e. TH 5, THM, TV, AT 3, RHZ showed 67%, 60%, 59%, 37% and 42% mycelial growth inhibition of pathogen and found the most among 21 isolates. These five isolates were also tested using detached leaf assay and in greenhouse on planta. The *Trichoderma* strain TH 5 the best result in detached leaf assay by reducing the disease to 52.3% and in greenhouse to 65%. The isolate that showed high potential in reduction of *A. solani* in all experiments was identified on molecular basis using ITS region of rDNA. Potential isolate was identified as *T. asperellum.* This study highlights the possibility of using that *T. asperellum* in IPM programs as an effective biological agent against early blight of tomato.

# MOLECULAR CHARACTERIZATION OF *MAGNAPORTHE ORYZAE*: THE RICE BLAST FUNGUS IN PUNJAB PAKISTAN

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*Magnaporthe oryzae* (Anamorph: *Pyricularia oryzae*), the cause of rice blast found to be the most important disease of rice globally but has received little attention in Pakistan. To investigate the pathogen diversity, 125 *M. oryzae* isolates were collected from diseased rice plants in different geographical locations in Punjab Province, Pakistan, in 2016 and 2017. Based on morphological characterization, four distinct sub-specific groups were found. Pathogenicity assays has showed that one of these groups (G-I) was highly pathogenic, with disease severity of 44% four weeks after inoculation. DNA sequence analysis of the internal transcribed spacer (ITS) region conducted with MUSCLE alignment found an identical single-base-pair substitution in thirty-five isolates. Phylogenetic analysis of the ITS region, ß-tubulin, actin and calmodulin genes using maximum parsimony (MP) with tree bisection reconnection (TBR) confirmed the identification of all the isolates as *M. oryzae* when compared with the available data set of *M. oryzae* on NCBI with 100% bootstrap value. The variated isolates of *M. oryzae* collected from Punjab, Pakistan were existed as a sub-clade under the main clade of *M. oryzae* with 95-100% bootstrap values. We concluded that a new ecotype of *M. oryzae* was prevailed in different geographical areas of Punjab, Pakistan.

# MANAGEMENT OF CITRUS BLUE MOLD THROUGH ESSENTIAL OILS

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Citrus is the leading fruit after mango, banana, grapes and apple produced in Pakistan. Citrus contains high amount of vitamin C. Post-harvest losses occur due to many postharvest diseases in citrus. Blue mold incited by *Penicillium italicum* is a major destructive disease which leads to great economic losses in citrus production. In recent study infected fruits and healthy fruits (Musambi, Sweet range, Grape fruit, Lemon and Fruiter early) were collected. Healthy fruits were coated with four different essentials oils namely Caster, Eucalyptus, Clove and Neem were applied at 10%, 20% and 30% concentrations after inoculation with *Penicillium italicum* isolated from the diseased fruits and then stored in polythene bags for 2, 4 and 6 days separately. This trial was carried out in CRD-factorial contained three replications in each treatment. Oil coating with clove oil at all concentrations proved to be most effective (0.25-16.19 mm) against *P. digitatum* growth as compared to control (7- 30.37mm) and other oil coatings after 2, 4 and 6 days respectively. It is very cheap and effective method to control this disease.

# CONTROL OF CITRUS GUMMOSIS CAUSED BY *PHYTOPHTHORA NICOTIANA* THROUGH PHYTOEXTRACTS

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Citrus is one of the most edible fruits in the world nowadays and clutches an exclusive position in the plant kingdom. From years' citrus is valued as a health and nutrition-rich. There are multiple diseases that are causing serious damage to citrus, citrus gummosis caused by *Phytophthora nicotianae* is one of the serious threats that cause sudden death of citrus fields. Mostly fungicides are used to quickly control the disease but they are not environment friendly. So, current study is design to evaluate different Phytoextracts with concentrations in the green house and field conditions. Pathogen was isolated and purified mainly from roots of citrus and identification was done on morphological basis. *D. stramonium* (Dhatura), *A. catechu* (Acacia), *L. camera* (Lantana), *L. inermis* (Mehendi) and *G. maculate* (Glyricidia) were the five Phytoextracts which were evaluated with the concentration of (0.25, 0.5, 0.75%) in green house and their combinations were used in the field as well. The results showed that all the Phytoextracts have significant effect on controlling disease but *D. stramonium* showed the best results as by using this disease incidence was 9.54 % at 0.75% Concentration followed by *G. maculate, L. camera, A. catechu and L. inermis* (15.28, 21.5, 30.55, 33.87 %) as compared to control. Results from the field trials showed that combination of *D. stramonium* + *A. catechu*+ *L. camera* showed only 1.53% disease incidence.

# EVALUATION OF TOLERANCE AND SUSCEPTIBILITY LEVEL OF NATIVE MANGO CULTIVAR KHANEWAL 250 AGAINST MANGO QUICK WILT PATHOGEN *CERATOCYSTIS MANGINECANS*

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Mango quick wilt disease caused by *Ceratocystis manginecans* is considered most serious threat to mango industry in Pakistan. Research was carried out at Department of Plant Pathology, University of Agriculture, Faisalabad to evaluate the tolerance and susceptibility level of native mango cultivar Khanewal 250. Survey was conducted in three districts of Sindh Province for the estimation of disease incidence and collection of diseased samples having typical symptoms of mango quick wilt disease. Maximum disease incidence was recorded in Hyderabad (75%) followed by Tando Jam Muhammad (66%) whereas least disease incidence was recorded in Tando Allah Yar (43.75%). Isolation, purification and identification of the pathogen were done associated with infected mango samples under sterilized conditions. Screening of germplasm for management of mango quick wilt disease was done followed by pathogenicity on twelve-month old mango seedlings of native germplasm that was inoculated artificially with *C. manginecans* in the screen house at Institute of Horticultural Sciences, UAF. Data of disease progression were taken after 30 days after inoculation. Logistic regression studies showed that there was no significant difference in upward and downward discoloration. Disease severity and disease incidence were calculated, Khanewal 250 was found to be resistant against the disease. *In-vitro* management of *C. manginecans* was done by using different fungicides Alliete®, Curzate®, Nativo®, Flumax® and Melody Duo® at four concentrations 50ppm, 100ppm, 150ppm and 200ppm with one control treatment. Alliete® was found most effective against *C. manginecans* that inhibited mycelia growth 1.10 cm, 1.46 cm, 1.41 cm, 0.96 cm while Melody Duo® was found least effective at all concentrations.

# *IN VITRO* CHEMICAL CONTROL OF *PENICILLIUM EXPANSUM,* THE PATHOGEN OF BLUE MOLD IN APPLE

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*Penicillium expansum* is a common soil-borne fungus occurring worldwide and causes post-harvest diseases in a wide range of plant species especially in apple. Three fungicides namely metalaxyl+mencozeb 72% WP, thiophenate methyl 70% WP and fosetyl-Al 80% WP were evaluated *in vitro* against this fungal pathogen. Five concentrations of each fungicide *viz*. 50, 100, 150, 200 and 250 ppm, were tested by food poisoning technique using malt extract agar as a growth medium. All the three fungicides significantly reduced the pathogen with variable extents. Among these, thiophenate methyl was the most effective one causing 65–90% reduction in fungal growth. Metalaxyl+mencozeb also showed a very similar effect resulting in 73–88% suppression in the fungal growth. Fosetyl-Al was comparatively less effective fungicide and reduced fungal growth by 73–88%, over control. This study concludes that thiophenate methyl and metalaxyl+mencozeb are highly effective fungicides against *P. expansum,* whose 50 ppm concentration can control fungal growth by 65 and 73%, respectively.

# FUSARIUM WILT: A MAJOR DEVIATOR OF CHILLI PRODUCTION-A REVIEW

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Fusarium wilt which is caused by *Fusarium oxysporum* f.sp. *Capsici* is counted in list of most devastating phytopathological issue in chilli production. Pakistan was 5th largest exporter of chilli pepper in past years, but due to some pathological issues, among them fusarium wilt is most destructive one, now it ran out from the list of major exporters. It is responsible upto 40% yield losses in Pakistan. The pathogen is a soil borne fungus which remain persistent for long times in the form of thick walled chlamydospores. Various management strategies have been practiced to manage this disease. It is very hard to manage this disease by cultural practices because of harsh nature of pathogen. Injudicious use of synthetic chemicals produces chemical toxicity in chilli which restricts its export. Induction of resistance by nutrients and plant defense activators can by a milestone to manage this crucial issue. This review covers all integrated management approaches by which the production of chilli pepper can be improved by the management of fusarium wilt.

# IN-VITRO MANAGEMENT OF ALTERNARIA ALTERNATA FUNGAL PATHOGEN ASSOCIATED WITH EARLY FRUIT DROP IN CITRUS

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Citrus brown spot caused by *A. alternata*, mainly affects plant branches, leaves and citrus fruits. *Alternaria* is a ubiquitous fungus found on plants, soil and animals. *Alternaria* species cause lesions on leaves and twigs, they also produce a specific toxin in the host. Symptoms include brown to black necrotic patches. Pathogenic lesions appear on the surface of the plant which spread through the air or rain. Diseased samples were taken from the usual symptoms of necrotic spots, followed by yellow halos from various citrus orchards. After collecting the samples, they were taken to the plant diagnostic laboratory for further isolation, purification and identification of various pathogens associated with Alternaria brown spot. Laboratory evaluation of various fungicides such as hexaconazole, mancozeb, topsin M and fosetyl aluminum was performed using various concentrations (100, 200 and 400 ppm) against isolated pathogens using the poisoned food technique. Hexaconazole was very effective against A. alternata followed by mancozeb, topsin M and fosetyl aluminum at various concentrations after 3, 5 and 7 days. While, fosetyl aluminum was the least effective against mycelium growth of A. alternata.

# ISOLATION, IDENTIFICATION AND MANAGEMENT OF FUNGAL PATHOGEN RESPONSIBLE FOR CHILI LEAF SPOT DISEASE

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Chili (*Capsicum annuum L.*) is an economically important crop in the world. Chili is a rich source of vitamin A and C. Chili is cultivated in almost 38.4 thousand hectares' area of Pakistan and it is used three times in meal daily in fresh form or dried powder. Production of chilli in thousand tons is 53.7. Many biotic and abiotic factors cause reduction in yield. In biotic factors the most devastating pathogens are fungi. Leaf spot is an important yield reducing fungal disease of chili caused by *Alternaria alternata*. In the current experiment the isolation, identification and management of *A. alternata* was done both under in-*vitro* and *in-vivo* conditions by using various biocontrol agents i.e. META (*Metarahizum*), *Trichoderma harzianum,* LEC (*Lecanicillium lecani*) and Siderophore *producing Pseudomonas* bacteria. In field trial the effectiveness of various biocontrol agents to manage the leaf spot disease against six common cultivated varieties (CH121, 900F1, Red wing, SAYBAN, AAHP-I, Priya chili) was carried. Isolation, identification, and purification of the pathogen causing leaf spot of chili was carried out after the sampling from the disease fields of Horticulture Department, University of Agriculture, Faisalabad and from Chak 597 TDA. For management purpose, all the varieties were inoculated with *A. alternata* inoculum by spraying and drenching method and the results were assessed on the basis of development of the disease. In the lab experiment of antagonistic tests of fungi with BCA were conducted and the results were assessed by checking the development of inhibition zone. By using SPSS 19 software for statistical analysis the data were analyzed. Under in-*vitro T. harzianum* and *Metarhizium* were found more efficient in suppressing the growth of *A. Alternaria* while in field and pot trials only the former was efficient to check the disease.

# RESPONSE OF CHILI VARIETIES AGAINST DAMPING-OFF CAUSED BY *PYTHIUM APHANIDERMATUM* AND ITS MANAGEMENT

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Chili (*Capsicum annum* L.) is very significant and valuable crop all over the world. It belongs to family Solanaceae. Sindh is the leading producer of chili in Pakistan. Damping off disease in chili caused by *Pythium spp.* is responsible for a potentially serious threat causing more than 60% seedling mortality in both nurseries and field grown crops. *Pythium spp*. is bad fungal-like agents that can lead up to 90% plant death. Pythium damping off management has become a major concern. Identification of resistant sources within available germplasm against Damping-off disease is very necessary. Seventeen varieties were collected and were evaluated in sick field under Randomized Complete Block Design. Seventeen varieties, V1 10549, V2 8127, V3 2125, V4 10938, V5 4728, V6 10544, V7 10554, V8 10468, V9 10551, V10 10466, V11 1788, V12 Ghootki, V13 Nageena, V14 NARC-4, V15 4828, V16 10559, V17 Desi were collected from Ayub Agriculture research institute (AARI), Faisalabad, which were used as research material to screen resistant germplasm against Damping-off disease. Out of seventeen varieties V1 10549 highly show resistance response, V2 8127 and V3 2125 exhibited resistance response, V4 10938, V5 4728, V6 10544, V7 10554, V8 10468 and V9 10551 show moderately resistance, V10 10466, V11 1788, V12 Ghootki, V13 Nageena, V14 NARC-4 and V15 4828 show moderately susceptible response, V16 10559 and V17 Desi show highly susceptible response toward Damping-off disease. For the management of Damping-off disease of chili an experiment was designed with three replications of each treatment with three different concentration of chemicals (Contaf plus, Flint max, score, Ellectus super, Fossil, Topsin M) and Phyto-extracts (Kalonji (*Nigella sativa)*, Kari patta (*Murraya koenigii)*, Ajwain (*Trachyspermum ammi)*, Kasni (*Cichorium incubus)*, Gajar boti (*Parthenium hysterophorus)* and Alsi (*Linum usitatissimum*) under Completely Randomized Block design (CRD), separately. Among the Chemicals Ellectus super expressed the best results to manage the Damping-off disease. Similarly, among different Phyto extracts Kasni showed the best results to manage the disease. For the management of Damping-off disease an experiment was designed with three replications of best Phyto-extract and chemical that are evaluated in *In-vitro* condition alone or in combination with two different concentrations (T1(Kasni), T2 (Ellectus super), T3 (Kasni+ Ellectus super) under Randomized Complete Block Design (RCBD). Among these Treatment Kasni+ Ellectus super in combination showed the best result to manage the Damping-off disease.

# ASSESSMEMNT OF VARIOUS FUNGAL DISEASES IN FOREST NURSERIES AT DIFFERENT LOCATION OF FAISALABAD

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Forests are very beneficial economically as well as from the environment point of view. They help mankind in a variety of ways by providing the basic necessities of life such as; food, fuel wood and timber. Forests are known as "the lungs of Earth" as they clean the air, help to maintain our climate moderate and are suitable for living. But a number of factors are causing decrease in the forest cover around the globe. Among those factors, fungal diseases are a principle cause of deforestation. The fungal species causing different diseases needs to be assessed for proper preventive measures to reduce their outbreak in the forests. Keeping in view the need of the time, present study is designed to assess the different fungal strains affecting the forest seedling at different forest nurseries in the locality of Faisalabad. For this purpose, a survey was conducted to assess disease across different nurseries and diseased samples were collected and brought into the laboratory for proper testing. Samples were observed under compound and dissecting microscope to have an idea of the fungal species which are causing diseases in the forest nurseries and result shown that disease percentage of tree seedlings of *Leucaena leucocephla* was 22.50% and *Tamarix aphylla* was 22.40% followed by *Celtis australis* 20.56%. *Ficus infectoria* disease incidence was 18.13%, *Moringa oleifera* 13.18%, *Morus alba* 12.3% and *Ficus religiosa* 10.46%. *Erythrina suberosa* affected under disease incidence 1.50%, *Tamarix aphylla* tree seedlings under disease incidence was 1.30%, and Morous alba 1.25%. Disease incidence of *Euclyptus camaldulensis* was 1.17%. The maximum disease incidence was found in DFO Samundri road nursery followed by PFRI Gutwala, UAF Forest Nursery, Fateh Kashmir Nursery whereas the minimum disease incidence was recorded for Qadir Baksh Nursery Farm.

# GENETIC DIVERSITY OF NEW ADVANCED SUNFLOWER HYBRID AGAINST CHARCOAL ROT (*MACROPHOMINA PHASEOLINA*) DISEASE AND ITS IN-VIVO MANAGEMENT THROUGH EVALUATION OF VARIOUS FUNGICIDES

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Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops and potentially fit in agricultural system and oil production sector of Pakistan. Its seed contains 45% oil content, 40% protein, 25-36% carbohydrate and lipids. Sunflower crop is affected by many biotic and abiotic factors that cause economic losses in production. Sunflower plant is attacked by different pathogens from sowing to harvesting in which *M. phaseolina* is most damaging pathogen that cause huge losses in sunflower crop worldwide. *M. phaseolina* generally cause a progressive type of wilting, flagging, defoliation of mature plants and drying of plants in the field occur suddenly and blacking of stem at collar region. Present studies was conducted to screening of new advanced sunflower hybrids against charcoal rot (*M. phaseolina*) disease under field condition in Ayub Agriculture Research Institute, Faisalabad in randomize complete block design. Isolation, purification, and identification of causal agent was done on susceptible plant. In vitro management of the fungus (*M. phasseolina*) was done by using different fungicides like Topsin-M, Azoxystrobin, Mancozeb, Carbendazim, Propineb and Nativo (Tebuconazole and Trifloxystrobin) in the field condition. After that, according to the results Carbendazim and Azoxystrobin gave the best results by giving maximum effects with least disease severity percentage followed by Topsin-M, Propineb and Nativo while Mancozeb showed minimum effects by giving maximum disease severity percentage.

# SCREENING OF WHEAT GERMPLASM AGAINST LEAF RUST AND **ITS MANAGEMENT**

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In Pakistan, leaf rust *(Puccinia triticina)* is the most significant disease of wheat and it affects both quality and quantity of crop. The disease is caused by an airborne fungus which lands on the leaves of wheat plants and initiates the disease on susceptible wheat varieties. In this study, brown rust of wheat was investigated on different lines/varieties under field conditions. Experimental trial was conducted in the field in Randomized Complete Block Design layout. In this experiment, 40 lines/varieties of wheat were evaluated for resistance against leaf rust caused by *Puccinia triticina*. The plants were grown under field conditions and varieties were assessed for resistance to leaf rust on the basis of symptoms development. The effects of environmental parameters including maximum and minimum temperature, rainfall, relative humidity, and wind speed for leaf rust disease development were studied and data was subjected to regression analysis. The relationship between leaf rust and environmental parameters was studied. For the management purpose, two fungicides Difenoconazole and Top-Guard and two plants extracts of garlic and neem were applied for the management of leaf rust under field conditions. The data obtained was statistically analysed. The results of the study showed that different wheat varieties react differently to leaf rust under field conditions. Out of 40 lines/varieties, 4 varieties were categorized as resistant and four varieties were categorized as moderately resistant. However most of the lines/varieties were found susceptible to leaf rust disease under natural field conditions. Environmental parameters were related to leaf rust development on the wheat plants and they strongly affected leaf rust development on wheat plants. For better management of disease, difenoconazol was found effective followed by Top-Guard however, among plant extracts used for the management of disease neem extract was found as most effective.

# CHARACTERIZATION AND INTEGRATED MANAGEMENT OF *FUSARIUM* SPP. ASSOCIATED WITH GRAM WILT IN THAL REGION OF PUNJAB, PAKISTAN

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Chickpea (*Cicer arietinum* L.) is a major source of human and animal food and the world's third most important pulse crop after beans and peas. In Pakistan, it is one of the most prominent rabi crop mostly cultivated in Thal region of Punjab. Gram wilt disease severely affects the crop production and livelihood of these areas. Considering the current situation, it was necessary to find the exact cause of Gram wilt and to develop sustainable management strategies. To achieve the goal of this study, a comprehensive survey of Thal areas including districts of Layyah, Jhang, Bhakkar, Khushab and Mianwali was conducted. Maximum disease incidence (83.88±4.14) and disease severity (7.12±0.51) was observed in district Layyah which was followed district Bhakkar showed disease incidence (81.06±3.52) and disease severity (6.52±0.57). Intermediate result of district Khushab was observed in which DI and DS was (79.58±4.54) and (6.3±0.64) respectively. District Jhang showed DI (76.24±3.12) and DS (5.26±0.71). Minimum disease incidence (73.34±4.12) and disease severity (4.44±0.55) was observed in district Mianwali. Pathogenicity test demonstrated the most virulent isolates for further studied. Morphological studies showed purplish white to brownish white and fluffy and cottony texture of *Fusarium* spp. Physiological studies showed optimum pH 7.0, optimum temperature 30$^{o}$C gave maxium growth of fungus. Molecular characterization of fungal isolate(s) was done in which *Fusarium oxysporum, Fusarium solani* and *Gibberella moniliformis* were confirmed from these locations. Three fungal antagonists (*Trichoderma harzianum, Trichoderma viride* and *Trichoderma virens*) were evaluated against virulent pathogens in which *Trichoderma harzianum* showed maximum inhibition under lab and field conditions. Ten fungicides (Score, Topsin M, Baviston, Cholorothalonil, Stump, Big Time, Champion, Velvet, Curzate M8 and Co-oxy) were evaluated against virulent pathogens in which Score showed maximum inhibition of isolates of *Fusarium spp.* which were followed by Topsin M and Baviston under lab conditions while on the other hand Score showed maximum disease reduction under field conditions.

# ETIOLOGY AND BIOCHEMICAL MANAGEMENT OF WHITE MOLD DISEASE PROBLEM OF CABBAGE (BRASSICA OLERACEA VAR, CAPITATA)

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The cabbage (*Brassica oleracea* var. *capitata*) is a herbaceous annual or biennial vegetable in the family Brassicaceae grown for its edible head. Cabbage is facing lots of losses due to attack of different diseases. White mold is one of the destructive diseases prevalent through worldwide. The disease can be found on crops in the field, transit and storage on during marketing; resulting in great economic losses. The problem gets aggregated due to respiration of the living cells and poor transport technology. Present investigation was carried out to explore the status and actual cause of the white mold in cabbage and its management through various Biochemical and plant extract to reduce the losses. Research was conducted under randomized complete block design (RCBD) in the research area of Plant Pathology, University of Agriculture, Faisalabad. Sampling was done from the Ayub Agricultural Research Institute (AARI), Faisalabad. Pathogen isolation and its purification was done under the sterilized condition. After conformed the link between the isolated fungus and disease an experiment was conducted by using Koch's postulates. Same symptoms were produced on plant and subsequently, the causal agent of the disease was verified as *Sclerotinia sclerotiorum.* Morphological and Physiological study was conducted to check the effect of different level of temperature and pH on the fungus development. Results showed that fast growth of fungus was assisted by 20-25 $^{o}$C whilst the minimum growth was observed at 10 $^{o}$C and maximum dry weight was recoded at pH 5.0 followed by pH 5.5 whilst the minimum average dry weight was observed at pH 7.5. The statistical analysis showed that pH 5.0 – 5.5 is the best pH level for the growth of fungus. The efficacy of four (04) fungicides was evaluated against the pathogen (*S. sclerotiorum*) on PDA medium through Poisoned food technique. Four fungicides viz. Raydar, Crest, Curzate and Mancozeb were evaluated against *S. sclerotiorum* mycelial growth at three different concentrations i.e., 50 ppm, 100ppm and 200ppm. Five replications for each treatment were made to minimize the chances of within group error. The data was recorded after 48 hours, 72 hours and 96 hours. According to the results fungal mycelial growth inhibition ranged from 92.91% to 34.69% after 96 hours. The results of current studies showed that the chemicals and fungicides performed better in relation to control fungal growth. Application of chemical fungicides are often cost prohibitive, impractical and hazardous to environment and human health. Keeping this in view, the need was felt for an alternative method to manage this disease in eco-friendly manner by using microbial bioagents and plant extracts. After 96 hours the comparison of treatments with different concentrations levels exhibited that Garlic with S concentration performed best amongst all the treatments applied Garlic with S concentration inhibited the fungal growth up to 70.58% followed by Garlic S/25 whilst Sufeda leaf extracts showed very poor response for all the concentrations and exhibited minimum inhibition.

# ISOLATION, PURIFICATION, IDENTIFICATION AND CHEMICAL MANAGEMENT OF IMPORTANT FUNGAL PATHOGEN OF MAIZE SEED

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Maize is very important crop. Its commercial value is evident from its use from human to animal feed. It is also a vital component of poultry feed. Maize is grown on vast area in Pakistan. Maize with high yield per unit area it is critical crop in Pakistan. It is also the world's leading crop. After wheat and rice, maize is Pakistan's third most important cereal. Cultivation area expanded from 1251 thousand hectares in 2017-2018 to 1418 thousand hectares in 2020-2021.thus registering a 13.3% increase in overall area. Similarly, maize production witnessed a 43.4% increase in 2017-2018 and 2020-2021. In the field, there a number of diseases which reduce the yield of maize. Important diseases include anthracnose, kernel rot, smut and wilt. A study was carried out to detect and characterize seed-borne fungal pathogens linked to maize under storage. Seed borne fungal pathogenic infections of maize were investigated using seed samples obtained from various locations across Faisalabad. Samples were collected and surface sterilized with 70% ethanol followed by brief washing in autoclaved double distilled water. The samples were placed on PDA media plates and were incubated at 28 $\pm 2^0$C. After appearance of fungal growth around the samples, hyphal tip method was used to identify fungal pathogen. *Colletotrichum* and *Fusarium* were isolated in the initial isolation of fungal pathogens from the seed. Further, more attempts will be undertaken to isolate other seed associated bacterial and fungal pathogens from maize to make diversity analysis by using sophisticated bioinformatics and biotechnology techniques.

# ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGEN ASSOCIATED WITH LEAF SPOT DISEASE OF CAULIFLOWER

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Cauliflower is one the important vegetables in the species *Brassica oleracea* in the genus *Brassica*, is the main member of *Brassicaeae* family. It is an annual plant which is being grown all over Punjab Province. It is propagated through seed. Pakistan included in main ten cauliflower producing countries in the world, having 11420 hectares area with an average 209010 tons of production in 2013. The important diseases of cauliflower are leaf spot, root rot, bacterial soft Rot, Blackleg, rot, Damping Off, powdery mildew and Downy Mildew. The most common disease is leaf spot caused by bacterial pathogen having complex with *Alternaria brassicicola.* These two pathogens have a major impact on the yield of this important vegetable. In the current work, a survey was in the district Faisalabad to collect infected plant samples. Then samples were treated with 70% ethanol to remove surface bacterial and fungal spores. Then samples were treated with autoclaved double distilled water and were left to dry. Samples were placed on Nutrient Agar media and then for placed for incubation at 28$^{o}$C for 24h. Next day bacteria ooze was observed around samples. With fine autoclaved spatula, the bacteria were streaked on the NA plates. Next day fine colonies were observed. From a single colony, liquid culture was prepared. 80% glycerol was used for cryo-preservation of pure bacteria. In future, infection test corn plant will conducted and pathogen will be characterized through molecular techniques.

# EXPLORING THE PATHOGEN COMPLEX INVOLVED IN THE WILT DISEASE OF CULTIVATED PEAS

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Pea (*Pisum sativum*) belongs to important legume plant family. Pea is important because it is used as food for human and also a good source of nutrition for animals and poultry. Pea is a winter crop which require cool and humid environment. Pea vegetable is rich in nutrition. It contains vitamins and antioxidants. One grain of pea has 42.65% carbohydrate, 27.8% protein and other nutrients essential for human body. Green peas are rich source of many Vitamins like ascorbic acid. Peas have the compounds which lower the cholesterol level in human body. Pea is cultivated on an area of 2589.1 thousand hectares with production of 19877.3 thousand tons throughout the world. Pea crop is affected by many microbial pathogens during the entire life cycle like bacteria and fungi. Peas are majorly affected by fungal pathogen and cause root rots, wilting, drooping, powdery mildew and downy mildew. In current study Fusarium fungus which causes wilt disease of pea was isolated from infected tissues. The fungus was identified as *Fusarium oxysprum f. sp. pisi.* In future, different genotypes of peas will be used for screening purpose against this pathogen. Later, various BCAs will be applied to manage the wilt disease at various plant growth stages under the stress of the fungus. Data will be analysed through SPSS 19 statistical software.

# ISOLATION, PURIFICATION, IDENTIFICATION AND CHEMICAL MANAGEMENT OF MICROBIAL PATHOGEN ASSOCIATED WITH LEAF SPOT DISEASE OF CAULIFLOWER

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Spinach (*Spinacia oleracea*) is an important vegetable crop cultivated in almost every region in Pakistan. As for its nutritional values Spinach contain a decent amount of B6 vitamin it is also a great source of other vitamin like A, C, K, E, Riboflavin, Potassium, magnesium, dietary fiber and calcium. As a rich source of iron spinach is used for the treatment of disease which is caused by low iron in body like osteoporosis and anemia. In Pakistan the production of Spinach was 111215 tons in 2019. The important disease of Spinach includes Downy mildew, White rust, Leaf spot diseases and soilborne diseases. Leaf spot of Spinach is caused by *Alternaria alternata*. The current work was conducted in the Department of Plant Pathology, UAF. In the current study, a survey was undertaken in the various locations of district Faisalabad to collect leaf spot disease samples of spinach vegetable. Samples were collected from the selected locations and were brought to laboratory. Then were, surface sterilized with 70% ethanol followed by washing in autoclaved double distilled water for 20 seconds to remove the surface contaminants. PDA media was prepared and the samples were placed on autoclaved PDA media plates (+100 µg/ml ampicillin) and incubated at 28±2$^{o}$C for 48h. Fungal mycelia were observed around the samples after incubation. Fungus was purified by using hyphal tip technique. The fungus was identified as *A. alternata* on the basis of morphology, spore shape and colour of the plate and other microscopic attributes. Further, DNA will be isolated from the fungal samples; will be subjected to ITS based identification through sequencing and phylogenetic analysis.

# MANAGEMENT OF CHILI DAMPING OFF CAUSED BY *PYTHIUM MYRIOTYLIUM* THROUGH BIOLOGICAL AGENTS

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A well-known soil-born oomycetes *Pythium myriotylium* produced post-emergence damping off in chili peppers. Numerous disease control measures, such as the usage of PGPR, were employed in the prevention of illness and the promotion of plant development. A research was conducted in Pakistan to identify and characterize PGPR in the chili rizosphere. Discrete bacterial isolates and antagonists were utilized to reduce colony expansion. In a dual culture experiment, 8 bacterial isolates were employed to limit the development of *P. myriotylum*, including 4a2, JHL -8, JHL12, IC2, Rh-24, ID, 5C, RH -87. *Pythium* sp. in damping off chili paper caused the death of germinating seeds and developing seedlings at the pre- or post-emergence growth stage. This illness was estimated to cause a yield loss of 5 to 80%. Young symptomatic seedlings with *Pythium* infection displayed damping off reduced growth, brown root rot, and brown discolouration. 13 *Pythium* isolates were recovered from these infections on corn meal agar medium supplemented with ampicillin and rifampicin. Growth promotion and disease suppression are important factors in attaining high-quality product. The researchers looked for rhizobacterial isolates that might be used as *Pythium* inoculums and promote seedling germination without causing phytotoxicity or pathogenic effects on young seedlings in vitro. Bacterial isolates showed varying degrees of resistance and susceptibility to various antibiotics, as well as non-pathogenic to chili seeds and increased percentage seed germination, plumule, and radical length in uninoculated controls.

# PLANT DISEASE RESISTANCE CONFERRED BY FUNGAL ENDOPHYTES IN CHANGING CLIMATE

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The global food security, on account of a blazing intimidation of climatic change, is highly distressed by the capricious plant pathogenic diseases and drought which will surge in severity and frequency in the future leading to a credible shrink in food production. Endophytes are valuable microorganisms and naturally safe for plants to induce tolerance. Therefore, the current study was executed to assess the endophytic potential in wheat against rust diseases and drought stress. The fungal endophytes were isolated from the desert plants samples, cultured on different media and identified by molecular, immunological and biochemical analytical methods. Efficient fugal endophytes were inoculated by dipping the wheat seeds in fungal spores' suspension. Rust and drought stresses were applied to the rust susceptible and drought sensitive wheat genotypes respectively by skipping the irrigation at anthesis stage and by applying disease inoculums. Wheat genotypes inoculated with endophytes revealed the tolerance against diseases and drought.

# RESISTANCE POTENTIAL OF WHEAT GERMPLASM AGAINST LEAF RUST IN RELATION TO EPIDEMIOLOGICAL FACTORS AND ITS MANAGEMENT

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Brown rust is one of the most important disease of wheat in Pakistan. It causes significant yield and quality losses. In order to feed the increasing population, two times increase in the present wheat production is required. Use of resistant varieties is affordable and effective solution to manage leaf rust of wheat. Usually screening associated with wheat germplasm with regard to resistance is carried out in the field. In this experiment, 44 varieties/lines were evaluated for resistance against leaf rust by RCBD with three replications. Out of these 44 wheat lines/verities five (05) lines/verities showed resistant response against leaf rust of wheat, eleven (11) lines/verities showed moderately resistant response against leaf rust of wheat, twenty (20) lines/verities showed moderately susceptible response and eight (08) lines/verities showed susceptible response against leaf rust of wheat. For management, two chemicals [Difenoconazole (Diafenoconazole @ 250g/L) and Top Guard (Flutriafol @ 75g/L, Tebuconazole @ 225g/L)] were used at standard concentration, two plant extracts (*Azadirachta* and *Eucalyptus*) and a bio-control agent (*Trichoderma*) were used for the suppression of disease. Difenoconazole showed best results to manage leaf rust of wheat leading to Top Guard, *Azadirachta* and *Eucalyptus. Trichoderma* also showed significant results to manage the disease when it was applied to the foliar parts of the wheat plant. Epidemiological factors also enhance the disease spread significantly. All the epidemiological factors showed positive correlation (increase in epidemiological factors results increase in the disease spread) with rust development.

# EFFICACY OF DIFFERENT PLANT EXTRACTS FOR THE MANAGEMENT OF FUNGAL PATHOGENS ASSOCIATED WITH STEM AND ROT DISEASE

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Mango (*Mangifera indica*) is the most vital fruit crop cultivated widely all over the world. Regarding mango production, Pakistan stands on fifth rank in the world. Several diseases attack the fruit crop during all of its life stages. In Pakistan, stem end rot (*Lasiodiplodia theobromae*) is one of most prevalent post-harvest mango diseases. Some other pathogens including *Alternaria alternata, Botrytis cinearia, Colletotrichum gloeosporiiodes* and *Aspergillus niger* are also the cause of stem end rot disease. This disease leads to heavy storage and market losses. The study was conducted for Sindhri cultivar. The samples of mature fruit were collected and stored at temperature 25°C (ambient condition) and 12°C (cold storage). The pathogen was isolated, purified and identified from infected mango fruits. *L. theobromae* is found to be the most prevalent pathogen infecting the fruits. The disease frequency both at normal conditions and cold storage was maximum by *L. theobromae* (48.31% and 50.25%), other pathogens *C. gloeosporiiodes* (19.84% and 20.50%), *A. niger* (16.04% and 5.23%), *B. cinearia* (12% and 13.03%) and *A. alternata* (7.81% and 10.89%) respectively showed low frequency. Application of Nativo and Cabrio Top fungicides @ 250 µg/ml significantly suppressed the fungal growth of *C. gloeosporioides* and *L. theobromae* and *A. alternata* was effectively at lower concentration i.e., 150 µg/ml of Nativo and Cabrio Top but this fungicide showed low efficacy for the suppression of B. cinerea which was significantly reduced by Scholar applied @ 250 µg/ml.

# BACILLUS STRAINS TRX-1 AND TRX-3 REVEALED AS POTENTIAL BIOLOGICAL CONTROL AGAINST *ALTERNARIA BRASSICAE* IN **DUAL CULTURE**

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Alternaria blight also known as black spot is the most critical disease of Rapeseed. Alternaria blight caused by *Alternaria brassicae (A. brassicae)* is responsible for huge yield loss annually. The use of chemicals for plant pathogen control leaves its toxic residual effects that can cause serious health hazards to human and animal. However, biological control using rhizospheric beneficial bacteria is a safe, effective, and an alternative method to protect plants from potential pathogens that ultimately enhance crop production. Among Rhizospheric bacteria, *Bacillus* spp., are the most widely used biological control agents. *Bacillus* spp. are gram-positive and spore-forming bacteria that are present almost everywhere. The inhibitory effect of rhizospheric bacterial strains on mycelial growth of *A. brassica* has been tested in this study *via* dual culture assays. Only pathogen inoculated plates have been used as positive control. Co-inoculation of three Bacillus strains i.e., Bacillus TRX-1, Bacillus TRX-2, Bacillus TRX-3 showed varied effect on the pathogen growth on the PDA plate in dual culture assays. The results conclude that Bacillus TRX-2 did not show any significant control of *A. brassica* as compared to the control. Bacillus TRX-3 showed 1.1cm inhibition zone against *A. brassica*. However, Bacillus TRX-1 strain significantly controls *A. brassica* as it showed 1.4cm inhibition zone. Hence, this shows that Bacillus TRX-1 and TRX-3 could be further explored to unleash their biocontrol potential *in planta*. Furthermore, the antimicrobial compounds of this *Bacillus* spp. should be identified through MALDI-TOF or LC-MS that will explore their potential as bio-fungicide at commercial level against *A. brassicae*.

# THE INFLUENCE OF TEMPERATURE ON RICE LEAF BLAST PATHOGEN *MAGNAPORTHE ORYZAE* GROWTH AND SPORULATION

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Rice (*Oryza sativa*) is a staple cereal grain that should account for up to 20% of total calorie intake and is the primary source of nutrition for more than half of the world's population. *Magnaporthe oryzae*, the pathogen that causes rice blast, is a well-known pathogen that has reduced global rice production by 8% every year. According to results, rice blast outbreaks are a major and reoccurring concern in all rice-growing regions around the world. Because of its ideal climatic conditions during the growing season, rice blast is a serious issue. The temperature has an impact on the appearance, proliferation, and transmission of blast fungus. At any crop growth stage of hypersensitive varieties, blast epidemics demand a minimum night temperature of 20°–26°C, as well as >90% high humidity, dew drop, prolonged leaf wetness time (> 10 h), and foggy rainy precipitation. Exchanges within sensitive host plants, damaging pathogens, and the environment all contribute to the spread of plant illnesses. When temperatures rise as a result of climate change, the pathogen's virulence is likely to fluctuate. Temperature influences both pathogen growth and sporulation in rice leaf blast. Both growth and sporulation were elevated to a temperature (27°C) in response to increased or decreased temperature, but then simply declined further. Temperatures (32°C and 22°C) have been lowered. Increase and overall growth in Oat meal agar media, lesion development and rate of lesion progression in susceptible variety Basmati 2000, pollen tube growth and rate of sporulation in Oat meal agar media, and especially vulnerable variety Basmati 2000 were all highest at 27°C (optimal temperature), compared to conceptually flawed (22°C) and supraoptimal (32°C). Increased weather produces an increase in growth and sporulation of *M. oryzae* in tropical and subtropical regions of the world, as well as assertiveness in the outcome of the tropical rice blast pandemic. As a result of the kinetics on infection components, crop loss assessment models, rice blast prediction models, and genotype appraisals for future plant disease control mechanisms will be developed.

# VARIETAL SCREENING OF TOMATO GERMPLASM AGAINST LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS*

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Tomato (*Lycopersicon esculentum* L.) is most consumed vegetable crop and also important for its genetic studies. Diversity of traits in cultivars makes tomato best fit for breeding and genetic studies. Tomato cultivation is facing a lot of problems including conventional genome. In Pakistan major threat to tomato cultivation is fungal disease late blight caused by *Phytophthora infestans* which causes severe loses in yield. In this study twenty-one genotypes which collected from different countris were screened for resistant genotypes. Research was performed in research area of College of Agriculture, BZU Bahadur Sub Campus Layyah in year 2018-19. Completely Randomized Design was used with three replications. Screening results shows some varieties were moderately resistant West Virginia 63, Floradel) and (F4T5 (ISL), while some show less resistance to late blight (Nunhem's Tuckqueen, Tres Cantos Fito, Robar, Muchamiel, Fortuna and Centennial, M.O.G. 10, Cromco,). The remaining (Dwarf Moneymaker, Balady, Pusa Ruby, ZhongShuy 4, ZhongShuy 5, 8 A II, Jaguar F1 and Rio Grande) were highly susceptible to late blight over control. Thus none of the variety show resistance however (F4T5 (ISL) can be used for cultivation if proper management applied.

# VARIETAL DIVERSITY OF WHEAT GERMPLASM FOR METRIC TRAITS AND LEAF RUST RESISTANCE

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Wheat is a major cereal crop in Pakistan. About 80-90% of the daily food of every individual in Pakistan is met by bread wheat. Different pathogens cause diseases of wheat crop including fungi, bacteria, viruses, and nematodes. All fungal pathogens cause significant losses, but rust is the most significant and widespread disease in Pakistan and around the world which cause great losses to the quality and yield of wheat. Leaf rust caused by *Puccinia triticina* is distinguished in susceptible plants by small to large reddish-brown pustules which arise from the upper epidermis of the leaves. In current study, different varieties of wheat commonly cultivated in Pakistan have been screened against leaf rust of wheat and metric traits of wheat have been assessed. A trial was conducted in the experimental area of Department of Plant Pathology at University of Agriculture Faisalabad using randomized complete block design to conduct this work. Different morphological parameters of wheat such as number of tillers, plant height, spike length, number of spikelets, number of grains per spike, spike weight, grain yield per plant have been calculated. A field experiment was conducted by using forty-one varieties of wheat that were cultivated in three replications and Morocco as a spreader around the field. The result showed that the variety SA-42 revealed the maximum value of disease severity, while the mean comparison of grain yield per plant the variety C-250 showed the maximum value of grain yield per plant. Correlation analysis displayed that spike length had positive correlation with number of grains per spike and grain yield per plant. Cluster analysis showed that cluster 1 comprised of 17 genotypes, cluster 2 contained 14 members and cluster 3 contained 10 genotypes respectively. Thus, it could be concluded that leaf rust of wheat affects the morph-physiological traits of wheat plants and reduce the yield as well.

# ETIOLOGY AND MANAGEMENT OF WILT IN DIOON PALM (*DIOON SPINULOSUM*) IN PAKISTAN

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Dioon palm (*Dioon spinulosum*) is a cycad belongs to family Zamiaceae and known as an ornamental plant suffering from wilting/declining problem in Pakistan. Survey was conducted for the detection of mycoflora from nurseries, green parks and educational centers of district Faisalabad. Samples were collected from soil, root, and leaves as well as from the healthy/diseased plants of Dioon palm. Isolation, identification and mass culturing of pathogenic fungi were done to detect the most frequent fungal pathogen causing wilt. The most frequently isolated pathogen was *Fusarium oxysporum*. For the confirmation of pathogen on host plant, pathogenicity test was performed following by Koch's postulates. For present study a suitable integrated management against wilt of Dioon palm was done by using different fungicides and antagonistic fungi. Aliette showed more inhibition percentage @ 200 ppm against *Fusarium oxysporum* than other fungicides. Fungi play a significant role as biocontrol agent against diseases. As an antagonistic agent *Trichoderma viridae* was used to inhibit the growth of *Fusarium oxysporum*, it showed more inhibition percentage on day7. A significant disease resistance was noted in disease severity by using SAR chemical (salicylic acid). Data relating to the biological control was recorded and statistically analysed by using Fisher's analysis of variance (ANOVA).

# ROLE OF ENVIRONMENTAL FACTORS IN THE EXPANSION OF LEAF RUST OF WHEAT CAUSED BY *PUCCINIA TRITICINA*

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Leaf rust is one to the most devastating disease of wheat in Pakistan since last few years. It is a potential threat in wheat production which reduces its quality and quantity as well. Epidemiological factors play their vital role in its epidemic which leads to the reduction of its production. In order to feed the increasing population, two times increase in the present wheat production is required. Current study was carried out with the objective to find out most auspicious environmental factors for the development of leaf rust disease. The relation of maximum & minimum temperature ($^{o}$C), rain fall (mm), wind speed ($ms^{-1}$) and relative humidity (%) with leaf rust development was studied on five different varieties of wheat. It was observed that the first onset of disease was significantly influenced by high relative humidity and low temperatures at night. All above mentioned epidemiological factors showed positive correlation (increase in epidemiological factors results increase in the disease spread) with disease development.

# THE EFFICACY OF VARIOUS FUNGICIDES FOR THE MANAGEMENT OF BLACK SPOT OF ROSE

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Rose (Rosa indica L.) falls in Rosaceae family, it is extensively used for welcome events, celebrations, ornamental and also a food tonic supplement all over the world. Cercospora leaf spot, Powdery mildew, *Agrobacterium tumefaciens*, black spot, Botrytis Blight, and rust are all fungal and bacterial diseases that can affect roses at any stage of its growth. Black spot disease is the most severe, prevalent, and widespread among all of these diseases. The present study was conducted at the College of Agriculture, Bahadur sub-campus, Layyah, Punjab, Pakistan, in RCBD with three replications in a factorial design. The research was conducted to test and verify the effectiveness of five fungicides. (Amistar Top, Antracol, Acrobat MZ, Myclobutanil and Companion) to control the disease at various concentration and days. Results indicate that disease is maximum controlled by Antracol (18.03%) followed by Amistar Top (44.17%) Acrobat MZ (25.25%), Myclobutanil (35.87%) while companion (47.98%) indicated less effectiveness as compared to control (55.96%). So, Black spot of roses is effectively control by Antracol.

# EVALUATION OF PEA TEST LINES/ COMMERCIAL VARIETIES AGAINST POWDERY MILDEW OF PEA (ELYSIPHAE POLYGONI) UNDER FIELD CONDITION

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In Pakistan, pea is an important crop which plays an important role in farmer's economy. It is the most common crop and enjoys a great commercial demand due to its nutritional value. It is cultivated during winter in plains and during summer in highlands. Forty-seven test lines/varieties of pea received from vegetable research institute, Faisalabad were screened out in vivo trial to identify the best source of resistance for breeding program against powdery mildew of pea at Plant Pathology Research institute, Faisalabad during three Rabi seasons i.e. 2018-2021. A susceptible check METEOR-1 was also planted after three entries. Layout design was RCBD and spore suspension spray of inoculum was also done to produce maximum disease intensity. Among the test lines/varieties, fourteen were found resistant, eight varieties/lines are moderately resistant, fifteen are moderately susceptible, eight are susceptible and two of them are highly susceptible.

# EVALUATION OF ONION GERMPLASM AGAINST STEMPHYLIUM BLIGHT CAUSED BY S. BOTRYOSUM

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Onion (*Allium cepa L*) is one of the important condiments widely used in all households all the year round. The green leaves and immature and mature bulbs are eaten raw or used in preparation of vegetables. Twenty-five test lines/varieties of Onion received from vegetable research institute, Faisalabad were screened out in vivo trial to identify the best source of resistance for breeding program against *Stemphylium blight* caused by S. *botryosum* at Plant Pathology Research institute, Faisalabad during Rabi seasons, 2019-20 and 2020-21. Layout design was RCBD and spore suspension spray of inoculum were also done to produce maximum disease intensity. Among the test lines/ Varieties four was found moderately resistant, twenty varieties/ lines are found moderately susceptible and one of them are Highly Susceptible. Disease data was recorded according to the disease rating scale described by (Sharma,1986).

# EVALUATION OF DIFFERENT FUNGICIDES AGAINST STEMPHYLIUM LEAF BLIGHT AND PURPLE LEAF BLOTCH OF ONION SEED CROP UNDER **FIELD CONDITIONS**

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Onion (*Allium cepa*) is one of the most important crops grown all over the world. Diseases in onions are caused by many factors i.e., fungi, bacteria, viruses, nematodes and abiotic factors. But fungal diseases stemphylium leaf blight and purple blotch are most serious as these limit the quality and quantity of the bulb and seed of onion. This year an experiment for the management of stemphylium leaf blight and purple blotch of onion was carried out at Vegetable Research Institute AARI Faisalabad. Experiment included various treatments and among those the treatment (T26) NANOK was found most effective in controlling the disease of purple blotch followed by THRILL, STARK, TOPSIN M, DEFENATE, PYRAZOLE having rapid effect in controlling the disease but lesser than (T26) NANOK and rest of the fungicides CURZATE M, ALLIETE, COPPER OXYCHLORIDE, FLUMAX, MIRAVEOUSDU, SULPHER, CABRIO TOP, JALWA, FORUMTOP, STROLE, WISDOM, STEROLEPLUS, SHINKAR, TOPPAS, SUCCESS, RALLY, DIATHENE M, RIDOMIL GOLD, KASUMIN were not effective in controlling the pathogens of the purple blotch disease. Also, another experiment was carried to manage the stemphilium leaf blight disease by applying same treatments. This experiment also involved application of various treatments and among those the treatment (T20) AMISTAR TOP was found most effective in controlling the disease of stemphylium leaf blight followed by STARK, COPRIDE, NANOK having rapid effect in controlling the disease but lesser than (T20) AMISTAR TOP and rest of the fungicides CURZATE M, DEFENATE, PYRAZOLE, ALLIETE, COPPER OXYCHLORIDE, FLUMAX, MIRAVEOUS DU, SULPHER, CABRIO TOP, JALWA, TOPSIN M, FORUM TOP, THRILL, STROLE, WISDOM, STEROLEPLUS, SHINKAR, TOPPAS, SUCCESS, RALLY, DIATHENE M, RIDOMIL GOLD, KASUMIN were not effective in controlling the pathogens of the stemphylium leaf blight disease. Therefore, it is concluded that the fungicides (T26) NANOK and (T20) AMISTAR TOP should be used in field for controlling of purple blotch and stemphylium leaf blight respectively.

# INVESTIGATION ON APPLE POSTHARVEST ROTS AND THEIR MANAGEMENT

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Apple (*Malus domestica* L.) a member of *Rosaceae* family is an important deciduous orchard fruit, produced worldwide in cool climate with high altitude. Apple has significant market and nutritional value, being succulent is subjected to various diseases caused by abiotic as well as abiotic factors, among biotic factors several pathogens especially rotting postharvest fungi i.e., *Penicillium expansum*, *Botrytis cinerea*, and *Alternaria alternata* causes considerable losses during transit and storage. Present study was conducted in the plant disease diagnostic laboratory, department of plant pathology, University of Agriculture Faisalabad. The infected apple showing typical symptoms were collected from main fruit market of district Faisalabad, for isolation, identification, and purification of associated postharvest fungi of apple. Potential of different fungicides with different concentrations were evaluated against associated fungi by poison food technique. The result showed that against *Botrytis cinerea,* Nativo at the rate of 200 ppm showed maximum mycelium inhibition. Flumax significantly retarded the mycelial growth of *Penicillium expansum* at the rated of 200 ppm while in case of *Alternaria alternata* Gemstar significantly retarded the mycelial growth at the rate of 200 ppm after 3, 5, and 7 days. The experiment was executed under Complete Randomized Design (CRD) and data were statistically analyzed using Least Significant Difference (LSD) test.

# EFFECT OF DIFFERENT CHEMICALS AND FUNGICIDES TO INDUCE FLOWERING AND CONTROL OF INFLORESCENCE DISEASES IN MANGO CULTIVAR-CHAUNSA SUMMER BAHISHT

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Mangoes (*Mangifera indica* L.) are universally regarded as superb fruit and one of significant fruit crop in tropical and subtropical areas of the world. Mango is prone to various diseases throughout its development stages but diseases at flowering stages of mango cause more losses and directly affect the productivity. Flowering in mango from one season to the next is unreliable because the environmental signals for flower initiation are often inconsistent. During the current research we find out the most promising synthetic chemical of laboratory grade, chemical fungicide or their possible combination which not only promote the flower development but also induce flowering and to combat inflorescence diseases which are the main reason to lessen the yield. The filed trail was performed for this research with various treatments. maximum flowering/ flower emergence was observed with the treatment $KNO_3$ (8.67) followed by $CaNO_3$ (7.33) and $KNO_3$ with the combination of champion (6.66) and $KNO_3$with the combination of cabrio top (5.33) was calculated as compared to control. Minimum flowering occurred on the plants treated with the fungicides alone i.e., Contaf plus with 3.33 as compared to control which was recorded only 1.33. Among all the chemicals Contaf plus gave best control for all the inflorescence disease in the field. Maximum disease incidence was 42.90% of BB followed by 39.00% with AN, 17.70% with MM and minimum 0.40% was recorded with powdery mildew. To induce flowering and flower setting $KNO_3$+ Cu $(OH)_2$treatment showed best response in the field on mango inflorescences when used in combination while $KNO_3$ and $CaNO_3$also proved to be the best for the induction of flowers when applied separately on the mango trees. current research is amazing and tremendous regarding the induction of flowering and suppression of flower disease by the application of the chemicals either singly or in combination with one another.

# A COMPARATIVE STUDY ON ANTIFUNGAL EFFECTS OF MEDICINAL PLANTS ON ANTHRACNOSE OF MANGO

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Mango Anthracnose (*Colletotrichum gloeosporioides*) is the most divesting disease of Mango orchard and at storage in Pakistan. The aim of the present study is to investigate the effectivity and antifungal activity of different plant extracts against the Mango Anthracnose (*C. gloeosporioides*) in vitro condition by using food poising method. The virulence of isolated pathogen (*C. gloeosporioides*) was confirmed by whole fruit technique where the associated pathogen produces the characteristic symptoms of mango anthracnose. Six plant extracts such as Garlic bulb, Eucalyptus (Leaves), Ginger (peel), Akk (leaves), Datura (Leaves), and Onion (peel) were tested at three concentrations i.e., 15%, 25% and 40% while fungicide and distilled water was served as a positive and negative control respectively. The results of present study showed that the akk is the most effective plant extract against *Colletotrichum gloeosporioides* followed by other plant extracts. The maximum inhibition was recorded by akk is 84.56 percent as compared to eucalyptus 83.07 percent, onion 76.25 percent, garlic 74.90 percent, datura 68.51 and, ginger was recorded 68.37 percent respectively. Hence, use of plant extracts proved basis for the establishment of sustainable environment, long lasting and efficient system of biocontrol for the control of anthracnose disease of mango.

# CHARACTERIZATION OF SUGARCANE CULTIVARS BY CONVENTIONAL AND MOLECULAR SCREENING METHODS AGAINST WHIP SMUT *SPORISORIUM SCITAMINEUM*

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Mango Anthracnose (*Colletotrichum gloeosporioides*) is the most divesting disease of Mango orchard and at storage in Pakistan. The aim of the present study is to investigate the effectivity and antifungal activity of different plant extracts against the Mango Anthracnose (*C. gloeosporioides*) in vitro condition by using food poising method. The virulence of isolated pathogen (*C. gloeosporioides*) was confirmed by whole fruit technique where the associated pathogen produces the characteristic symptoms of mango anthracnose. Six plant extracts such as Garlic bulb, Eucalyptus (Leaves), Ginger (peel), Akk (leaves), Datura (Leaves), and Onion (peel) were tested at three concentrations i.e., 15%, 25% and 40% while fungicide and distilled water was served as a positive and negative control respectively. The results of present study showed that the akk is the most effective plant extract against *Colletotrichum gloeosporioides* followed by other plant extracts. The maximum inhibition was recorded by akk is 84.56 percent as compared to eucalyptus 83.07 percent, onion 76.25 percent, garlic 74.90 percent, datura 68.51 and, ginger was recorded 68.37 percent respectively. Hence, use of plant extracts proved basis for the establishment of sustainable environment, long lasting and efficient system of biocontrol for the control of anthracnose disease of mango.

# GUAVA FRUIT CANKER: AN EMERGING THREAT TO THE GUAVA ORCHARDS IN PAKISTAN

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During the field surveys of guava growing regions of Punjab (2018-19) documented an emerging disease in the orchards, identified as guava fruit canker. The disease incidence was recorded up to 52%. Diseased samples were collected and processed for the isolation of associated mycoflora. The isolated fungi were Neopestalotiopsis *spp. Alternaria spp. Rhizopus spp. Aspergillus niger, Colletotrichum spp.* However, *Neopestalotiopsis spp*. was isolated with the highest frequency, .i.e., 89% from the canker affected fruits. Eight isolates of *Neopestalotiopsis spp* were purified and deposited to Fungal Molecular Biology Laboratory Culture Collection University of Agriculture Faisalabad (FMB CC UAF) with accession numbers FMB 0013, FMB 0026, FMB 0027, FMB 0028, FMB 0127, FMB 0128, FMB 0129, and FMB 0130. These eight isolates of *Neopestalotiopsis spp.* were further identified molecularly, submitted the sequences to Genbank, and assigned the accession numbers MF783083, MF783085, MF783084 MF783082, MH209246, MH209077, MH208973, and MH209245, respectively. The phylogenetic tree was constructed to assess the relationship of queried sequences with other sequences of *Neopestalotiopsis* (on a homology basis) obtained as a result of the BLAST search. Koch's postulates were followed to confirm the pathogenicity of fruit canker. The isolate (Genbank accession No. MH209246 and FMB-CC-UAF accession No. 0128) was used for the Koch's postulates completion because it was the isolate predominantly isolated from canker affected fruit of guava.

# CHARACTERIZATION OF *FUSARIUM* ISOLATES COLLECTED FROM THAL REGION AND WILT SCREENING FOR RESISTANT PLANT TYPES

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Chickpea (*Cicer arietinum* L.) is an important and valuable crop across the world. It is a widely grown legume crop in tropical, sub-tropical, and temperate regions, including India, Pakistan, Turkey, Myanmar, and Ethiopia. Its high protein contects, minerals, unsaturated fatty acids, fiber, and β-carotene contents distinguish it from other pulses around the world. Many destructive fungal diseases threaten its production. Due to low water needs, the crop is preferred in the rain-fed areas of Thal regions of Punjab province having marginal lands. The crop is exposed to various biotic and abiotic stresses. Fungal, bacterial, and viral diseases are important in chickpea. Chickpea wilt, caused by *Fusarium*, is the most common soil-borne fungus that affects chickpeas around the world. *Fusarium* wilt disease can destroy the crops, causing up to a 100% loss in heavily infested fields under favorable conditions. Surveys were made at the seedling and pod formation stage for the collection of wilted plant samples in the district Layyah, Jhang, and Bhakkar which are contributing almost 80% of the national yield. Fungal pathogen associated with wilt was isolated on water agar and PDA media plates. DNA was isolated from pure isolates and was further subjected to18S ribosomal gene amplification for diversity determination. ITS1 and ITS4 primer pair was used for the amplification of targeted sequence. Seed of 33 genotypes of chickpea was collected from CAS (Center for Advanced Studies) the University of Agriculture Faisalabad was sown in the field by following RCBD design. Data was analyzed by using MS excel and statistical software SPSS.19.

# EVALUATION OF APPLICATION METHODS OF RHIZOBACTERIA FOR THE MANAGEMENT OF ANTHRACNOSE OF CHILLIES

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The present study was aimed to evaluate plant growth promoting rhizobacteria (PGPR) for their effect on chilli (*Capsicum annum* L.) against anthracnose disease. A total of 25 PGPR were evaluated for their antagonistic activity against *Colletotrichum truncatum* and only five PGPR isolates were able to inhibit the mycelial growth of the pathogen. The pathogenicity and root colonization of the selected PGPR showed that out of five isolates only two isolates of Isolate -2 and Isolate-4 were able to be both non-pathogenic and possessed the ability to colonize the host plant. Among the PGPR isolates, seed treatment with Isolate-2 resulted in maximum enhancement of seed germination (82.75%),along with an increase in vegetative growth parameters. Significant disease protection of 72% against anthracnose disease was observed in plants pretreated with Isolate-2 followed by Isolate-4, under greenhouse conditions.The results evidenced that the Isolate-2 of PGPR possesses immense potential to increase the plant growth apart from suppressing anthracnose disease in chilli.

# EVALUATION OF DETOXIFICATION EFFECT OF FRESH LEAVES OF *MORINGA OLEIFERA* ON AFLATOXIN B1 PRODUCTION

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International Agency for Research on Cancer (IARC) have been classified the Aflatoxins as class IA carcinogen. According to a FAO report, about 25% of crops are contaminated by mycotoxins worldwide. Climatic conditions of Pakistan are favorable for rapid growth and proliferation of various toxigenic fungi i.e. *Aspergillus sp., Penicillium sp.* etc. Different methods (chemical, physical and biological methods) are adopted for the control of fungal growth and their toxins. Recent studies revealed the inhibitory effects of biological methods against aflatoxins. Bioactive compounds of plants are active source of antimicrobial compounds. They are safe to environment and consumer and are useful to control different diseases, being an excellent alternative source of synthetic herbicide in agriculture. In view of this, in present study, fresh plant leaves were used to assess the detoxification/ inhibitory effect against Aflatoxin B1. *In vitro* study plant leaves were used for detoxification purpose at different incubation times (0- hr, 1-hr, 3-hrs, 6-hrs and 24-hrs). Findings of present study revealed encouraging results. Aqueous extract of *Moringa oleifera* shows 94% reduction of Aflatoxin B1 after 24-hrs of incubation time. Phytochemical analyses of selected plant revealed that saponins were absent, whereas *Moringa oleifera* contain the higher concentration of tannins, volatile oils, and phenols.

# POST-HARVEST MANAGEMENT OF CROWN ROT OF BANANA CAUSED BY *FUSARIUM SEMITECTUM* THROUGH *SACCHAROMYCES CEREVISIAE*

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Banana is one of the most important fruit throughout the world including Pakistan. It is being affected by various post-harvest diseases, among them; crown rot of Banana is adversely affecting the quantity and quality of the fruits for consumption, utilization and value addition. Post-harvest diseases causing heavy losses. For this study banana fruits were collected from the local markets of Hyderabad, TandoAllahyar and Quetta, They were found suffered with different types of rots such as crown rot, anthracnose rot, fruit rot and stem end rot. Among them, crown rot of banana was found predominantly followed by anthracnose rot and other fruit rots. Incidence and severity varied with the markets and locations. *Fusarium semitectum* was found predominately associated fugus, affected fruits showing crown rot symptoms. Antifungal potential of yeast (*Saccharomyces cerevisiae*) is one of the strategies scientists being using for the control of postharvest diseases of banana which reduced risk of chemicals or alternative of chemical control method in commercial pack houses. So yeast (*Saccharomyces cerevisiae*) was evaluated against *F. semitectum* under *in-vitro* conditions, It was also tested against crown rot disease development of banana fruit. The results of our study showed that *Saccharomyces cerevisiae* by the dual culture technique has brought 93% reduction in the mycellial colony growth of *F. semitectum* as compare to control. Yeast was also tested against crown rot disease development on banana fruits by direct contact method. Yeast treatment brought more than 50% reduction in crown rot disease development as compared to the untreated fruits. The present study indicates that active yeast has the potential to manage crown rot of banana disease and thus could provide an alternate, reliable and eco-friendly control of crown rot disease.

# MULTIVARIATE ANALYSIS OF MORPHO-PHYSIOLOGICAL AND GRAIN YIELD TRAITS IN ADVANCE LINES OF BREAD WHEAT UNDER DIFFERENT LEAF RUST DISEASE REGIMES

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Leaf rust disease affects the wheat crop in several countries around the globe and it can influence the grain yield and its components. This research was carried out using 35 wheat genotypes under diverse rust disease regimes. The statistical techniques used for the multivariate analyses include the calculations of correlations, principal components and clusters. The data about various physiological traits, grain yield components and disease severity were recorded under high disease pressure and maximum disease control conditions. Correlation analysis showed highly significant positive relationship of disease severity with relative membrane permeability but negative association with chlorophyll contents in the flag leaf and yield attributes. In control conditions, the relative water content exhibited a highly significant positive relationship with the flag leaf area while highly significant negative relationship was observed with specific flag leaf weigh under disease condition. Principal component analysis demonstrated that first 4 PCA having eigenvalue >1 elucidating (73.578%) and (82.80%) of the total variation under diseased and control conditions respectively. Cluster analysis grouped 35 wheat genotypes among four different clusters. The members of cluster 1 revealed the maximum disease severity under control condition, but the disease severity was maximum for cluster 4 under disease condition. Scatter plot explained that, not a single cluster exposed clear separation. The tree diagram also indicated that diversity in different disease conditions is controlled by genotypes. The results concluded that this genetic diversity could be helpful to the researchers and breeder for future objectives to enhance the food production and leaf resistance in bread wheat.

# POST-HARVEST MANAGEMENT OF CROWN ROT OF BANANA CAUSED BY FUSARIUM SPECIES THROUGH ESSENTIAL OILS

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Banana is being widely cultivated; crown rot of banana is a major factor affecting adversely the quantity and quality of the fruits for consumption and utilization. In commercial pack houses, crown rot of banana usually managed with the treatment of systemic fungicides. However, excessive or injudicious use of fungicide causing health hazards. Emphasis must be given to exploring reduced risk of chemicals or alternative of chemical control methods. In this connection, antifungal potential of essential oils is one of the strategies scientists being using for the control of postharvest diseases of banana. In present comparative studies eight different essential oils *viz*., tarpeen (*Pistacia Terebinthus*), rose (*Rosa* sp.), sesame (*Sesamum indicum*), clove (*Syzygium aromaticum*), neem (*Azadirachta indica*), kalonji (*Nigella sativa*), cinnamon (*Cinnamomum zeylanicum*) and caster (*Ricinus communis*) were evaluated against *F. semitectum* under *in-vitro* conditions. These essential oils were tested against crown rot disease development of banana fruit. These tests were carried out by two different methods. In first method, volatiles compound produced by the oils were evaluated, while in second method effect of total metabolites by the amendments of oils in culture media. In both methods, all doses of clove appeared remarkably effective and brought 100% inhibition of *F. semitectum*. Highest dose of tarpeen brought 60-100% and 87-100% inhibition by volatile compounds (paper strip method) and direct amendment of oils in media, respectively. Clove oil, tarpeen oil were tested against crown rot disease development on banana fruits by volatile method and direct contact or dip method. Fruit dip method appeared more effective than volatile method in controlling the crown rot disease on treated banana fruits.

# ASSESSMENT OF ANTAGONISTIC POTENTIAL OF *TRICHODERMA SPP.* AGAINST *NEOPESTALOTIOPSIS SPP.* CAUSING GUAVA SCAB IN *PSIDIUM GUAJAVA* L. AND THE DEVELOPMENT OF *TRICHODERMA* FORMULATIONS FOR ITS MANAGEMENT

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Common guava is a fruit tree of economic importance all over the world. Its production is limited by a fungal pathogen in the *Neopestalotiopsis* genus causing guava scab disease. In the present study, a research was conducted to evaluate the antagonistic potential of *Trichoderma spp*. against the pathogenic *Neopestalotiopsis spp*. Fungal isolates purified from samples were subjected to morphological, molecular characterization and pathogenicity tests. *Trichoderma* isolate Td2 exhibited faster growth as compared to Td6 whereas the pathogenic *Neopestalotiopsis* isolate B4 exhibited faster growth than MB isolate. Td2 isolate exhibited 87.51 % and 78.83% inhibition percentage against MB and M4 isolates and Td6 isolate exhibited inhibition percentage of 85.68 % and 98.31 % against MB and B4 isolates upon simultaneous inoculation. Staggered inoculation resulted in 65.49 % and 81.87 % inhibition percentage by Td2 against pathogenic MB and B4 isolates. Whereas, Td6 isolate exhibited inhibition percentage of 73.75 % and 89.56 % against MB and B4 isolates with staggered plating. In *Trichoderma* formulations, about half of the conidia lost their viability after six months. Also 74 % reduction in disease incidence was observed with soil treatment. Application of *Trichoderma* based formulations have exhibited a substantial increase in the guava biomass as well. The maximum colonization of the guava plants by Trichoderma species was exhibited by fly ash + lignite and lignite based bioformulation. Besides that, the *Trichoderma* population also exhibited a stable increase up to 28 days.

# EXPLORATION OF RESISTANCE AND SUSCEPTIBILITY IN CHILLI VARIETIES/ADVANCED LINES AGAINST FUSARIUM WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CAPSICI* (*FOC*)

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Chilli (*capsicum annum* L.) is an important edible spice crop grown in tropical areas of world due to its richness of nutrients like carotinoides, fibers, mineral components, oils, proteins and vitamins. A number of biotic and abiotic factors are challenging devastatingly the successful production of chilli. Among all of these factors, Fusarium wilt caused by *Fusarium oxysporum* f.sp.*capsici* (*Foc*) is a potential risk of declining its yield every year. Among all management approaches, use of resistant varieties is the best option towards *Foc*. For this purpose in contemporary study twenty five varieties/advanced lines of chilli were evaluated against Fusarium wilt under natural field conditions in research area, Department of Plant Pathology, University of Agriculture Faisalabad for two years 2017-18 and 2018-19 under randomized complete block design (RCBD). Results exhibited that none of varieties/advanced line expressed immune response against the disease. Only one variety (BPVLC 14-1) was resistant with 18.76% disease incidence. Uttal, fengaio, Glaxy-2, Big daddy, GHHP 01, PH-275, Super sky AB, HPO33 and Super king were found moderately resistant (MR) with 21-40% disease incidence. Four varieties/advanced lines Hot-701. Hot shot, Omega and Silkey Red showed moderately susceptible response (MS) with 41-50% and Four (Super hot, Patyala F1, Angel F1 and Green king) were susceptible (S) with 51-70% incidence of Fusarium wilt. Seven (Tejal, BSS-410, Big Red AB, SB 6864-HM, Glory F1, Revival and Amber F1) varieties / advanced lines exhibited highly susceptible (HS) response.

# MANAGEMENT OF FUSARIUM WILT OF CHILLI (*FOC*) THROUGH PLANT DEFENSE ACTIVATORS

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Natural plant defense activators are known to have anti-fungal potential. Their impact on plant and pathogen are poorly described and understood. Considering this, the present study use five different plant defense activators; salicylic acid (SA), benzoic acid (BA), di-potassium hydrogen phosphate ($K_2HPO_4$), potassium di-hydrogen phosphate ($KH_2PO_4$) and citric acid (CA) on an important chilli wilt pathogen *Fusarium oxysporum* f.sp. *capsici* (*foc*) based on their three (0.25, 0.50, 0.75%) effective doses. Among all the tested defence activators, SA with (14.65%) followed by BA with (20.85%) disease incidence were tested as strongest defense activator at their third foliar spray with 0.75% concentration in the glass house experiment. These best performed activators (SA, BA) and their combination (SA+BA) were selected to apply in the field experiment with replicated sprays. Result of field experiment showed that combined effect of both defense activators (SA+BA) is more significant with (19.51%) disease incidence as compare to individual spray of each SA (23.11%) and BA (28.55%). They activate the defence related genes to produce low molecular weight compounds and reactive oxygen species which inhibit the plant pathogenic fungi growth *in vitro*. The defense was inducted through oxidative burst and salicylic acid dependent pathway. These all defense responses lead the plat to an efficient protection against *foc* which is comparable to the effect of fungicides. SA inhibited the colonization of *F. oxysporum* f.sp. *capsici* inside the chilli plant and activated the SA dependant immune mechanism pathway, production of fungal degradative enzymes and altered level of antifungal secondary metabolites without any direct antifungal effect. Therefore, the study shows the ability of defense activators to trigger the plant immune system and manipulate the plant biosynthetic pathway giving the altered biochemical profile that improves plant disease resistance status, growth, yield and other horticultural attributes. So, these defense activators have great potential of anti-pathogenic activity to enhance the disease resistance for commercial purposes.

# IN VITRO EFFECT OF SILICON ON GROWTH SUPPRESSION OF DIFFERENT PHYTO-PATHOGENIC FUNGI

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Plant diseases are a major threat to agricultural production as they cause serious loss of crop yield and quality. Amid a number of fungicides, botanicals and plant nutrition boosters, Silicon (Si) is gaining popularity as a multi-faceted approach for both plant strengthening and controlling diseases caused by plant pathogens. The aim of this study is to examine the effect of Si on the growth suppression of phyto-pathogenic fungi along with one bio-control agent *Trichoderma. sp*. Different concentrations of sodium silicate ($Na_2Sio_3$) 1000ppm, 100ppm, 10ppm and 1ppm were examined against *Macrofomina phaseolina, Sclerotium Rolfsii, Rhizoctonia solani,* and *Trichoderma sp.* under in-vitro conditions by using food poisoning and agar well method. The present study indicates Silicon to be effective in suppressing fungal growth of all tested species. It showed most effective suppression on *Macrophomina* in which 10 ppm, 100 ppm and 1000ppm Silicon significantly ($p<0.01$) reduced the fungal growth. In case of *Rhizoctonia sp, Trichoderma sp* and *Sclerotium rolfsii sp* 100 ppm and 1000ppm Silicon significantly ($p<0.01$) reduced the fungal growth while 10 ppm found to be ineffective on fungal growth suppression. 1 ppm silicon treatment exhibited non -significant reduction on all tested fungi.

# NOVEL STUDIES ON THE EFFICACY OF SYNTHETIC FUNGICIDES AGAINST FUSARIUM WILT OF TOMATO UNDER TUNNEL FARMING CONDITION

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Tomato (*Lycopersicon esculentum* Mill.) is the most important solanaceous vegetable crop and is one of the most vital vegetables economically in the world including Pakistan. It is susceptible to various diseases in tunnel farming conditions which bring huge losses to its production, among them; fusarium wilt is the most destructive disease. This study was conducted to test the efficacy of synthetic fungicides *viz.* Metalaxyl+Mancozeb, Copper oxychloride, Benalaxyl+Mancozeb, Carbendazim and Mancozeb at different concentrations (2, 2.5, 3, 3.5, 4 g/litre water) through soil drench method against fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* and also to observe the impacts of fungicides on plant height and yield under the tunnel farming condition. The result revealed that Copper oxychloride was significantly effective in all its doses to control the fusarium wilt of tomato as compared to control treatments, the most effective dose was 3 g/l where the disease severity was recorded 6.2% only, followed by Metalaxyl+Mancozeb (4g/l) in which the disease severity was recorded 9.6% as compared to control (76.6%), other fungicides also showed good results but Mancozeb alone was not effective, however it had synergistic effect and could be used as basis with the other product to control the fusarium wilt. Two of the fungicides proved to be the less effective fungicides having no significant effects on plant height and overall yield. The highest plant height was recorded 10.96 and 9.38 feet whereas the highest yield per plant was recorded 3.97 and 3.67 kg in case of Copper oxychloride and Metalaxyl+Mancozeb respectively. It is concluded that Copper oxychloride was the most effective fungicide against the fusarium wilt disease among the five tested fungicides under tunnel farming conditions, so should be suggested to be used against this devastating disease.

# CHARACTERIZATION OF *CANDIDATUS* LIBERIBACTER ASIATICUS FROM PAKISTAN BY DIFFERENT MOLECULAR METHODS

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*Candidatus* Liberibacter asiaticus (CLas) is devastating pathogen of citrus causing Huanglongbing (HLB), previously known as citrus greening disease. This disease is an important disease in Asia and has destroyed 100 million citrus trees worldwide during the last century. In recent years the presence of HLB was suspected in different citrus growing regions of Pakistan. Surveys conducted in citrus groves of Punjab, Pakistan, citrus samples exhibiting symptoms of HLB were collected. The aim of this study was to investigate the presence and the genetic diversity of CLas in Pakistan. A PCR method using specific primers allowed the detection of CLas in citrus leaves by amplification of a 1160-bp fragment from the 16S rDNA. These samples were also tested by q-PCR. Both of them showed similar results, the results of sequenced and NCBI BLAST drawn accordingly verify that these samples were related with other sequences from the rest of the world with varying degree of similarity. This reports the characterization of HLB from South Punjab region of Pakistan using different molecular method.

# EVALUATION OF STEPWISE REGRESSION MODELS BASED UPON MONTHLY ENVIRONMENTAL CONDITIONS TO PREDICT LEAF RUST ON WHEAT

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Wheat (*Triticum aestivum* L.) is an important cereal crop for the majority of world's populations and main staple food crop of the people of the Pakistan. Current status of wheat is threatened by a number of biotic and abiotic factors in the country. Among biotic factors the most serious epidemic in past decades has been the presence of rust diseases, namely stem rust (*Puccinia graminis* Pers. f. sp. *tritici*), leaf rust (*Puccinia recondita* Rob. Ex. Desm. f. sp, *tritici*) and stripe or yellow rust (*Puccinia striiformis* Westend f. sp. *tritici*). The genetic potential of wheat genotypes was screened in the light of favorable epidemiological factors against leaf rust and out of 85 varieties/lines 37 varieties/lines showed resistance against leaf rust epidemic. The disease severity data was also correlated with various meteorological variables and minimum temperature showed the highest correlation coefficients. Five varieties/lines (V-49, V-54, V-55, V-60 and V-63) with best correlation coefficient values were optimized to develop various regression model equations. The best suited models were validated by contrasting the predicted disease severity with the actual data. The model selected was one variable model showing minimum temperature as the most conducive factor for leaf rust development with 65% value of coefficient of determination ($R^2$). This one variable model can be used by ordinary wheat growers to take management decision regarding protectant spray of fungicide against leaf rust on wheat.

# MANAGEMENT OF LATE BLIGHT OF POTATO BY NEW CHEMISTRY FUNGICIDES

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Potato (*Solanum tuberosum* L.) is important vegetable crop of the world and in Pakistan potato is highly demanded crop. This crop is attacked by many pathogens which may cause diseases. A fungus *Phytophthora infestans* causes an important disease late blight to potato. Human being is going advance in production technology day by day but a major portion of the field crop is lost every year due to late blight. The objective of this research was to evaluate newly introduced fungicides hiten (fentin hydroxide), defeter (flumorph) and defeter plus (flumorph + fosetyl almunium) against late blight o potato. Foliar application of fungicides were done after appearance of disease incidence. Hiten showed excellent results as compared to defeter plus and defeter in disease incidence.

# IDENTIFICATION OF RESISTANT SOURCE IN LENTILL GERMPLASM AGAINST FUSARIUM WILT IN RELATION TO EPIDEMIOLOGICAL FACTORS

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Lentil has become an important food legume crop in the farming and food systems of many countries globally. It is a valuable human food and one of the oldest known protein rich food legumes. Lentil is also called poor men's meat. Globally, lentil shares only 5% of the total area under pulses. It is recognized as one of the most nutritious pulse crop ranking next to chickpea amongst Rabi pulses and second major Rabi pulse crop in Pakistan after chickpea. Owing to biotic and abiotic stresses, the crop yield is below attainable levels. Among the biotic factors diseases are serious threat to lentil production in many parts of the world. This declining lentil production is attributed to various agronomic, environmental and pathological factors. Regarding pathological factors, lentil wilt caused by *Fusarium oxysporum* is the important disease that affects the production of lentil in Pakistan that can cause complete crop failure, especially in a warm spring and dry, hot summer. Current studies were designed to screen the available lentil germplasm against the lentil wilt disease and its epidemiological studies. In the screening experiment, 28 lines were evaluated under field conditions. There was no line found to exhibit immunity against lentil wilt. Twenty-two (22) lines, i.e., 10501, 10502, 10503, 10504, 10506, 10507, 10509, 10510, 10511, 10512, 10513, 10514, 10503, 10505, 10506, 10507, 10508, 10509, 10510, 10511, 10512, 10513, were highly susceptible against lentil wilt. No line was found resistant against *Fusarium* wilt. In epidemiological studies, maximum disease incidence was recorded at maximum and minimum temperature ranging from 17-18$^0$C respectively. At this temperature range disease incidence continuously increased. This relationship was explained by linear regression models in all varieties indicated by high value of r=0.95 and r=0.74 respectively. Rainfall was positively correlated with disease with most advance lines. This relationship was elaborated by linear regression models and maximum disease developed by 0-2 mm, when the rainfall increased disease incidence also increased. Relative humidity (RH) had positive correlation with disease incidence; this relationship was explained by linear regression models in different varieties and maximum disease developed at 60-78% (RH). The data were subjected to stepwise regression to screen environmental variables not having a significant influence on lentil wilt development. Stepwise regression employed to predict lentil wilt incidence, the model developed significantly fit to the data and the predicated values of lentil wilt incidence were in close conformity with the observed values.

# IMPACT OF FUNGAL DISEASES ON COTTON PRODUCTION IN PAKISTAN

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Cotton belonging to Malvaceae family is an important cash crop in Pakistan. It is native to subtropical and tropical regions of the world. In Pakistan it is also referred as king cotton and white gold. Its importance is always on rise for being source of economy of the country and raw material for industries. Pakistan ranked 4th in the production of cotton but it is the 3rd largest exporter of the world. The production of the cotton and its products subsidize almost 10% to GDP and bring in 55% foreign exchange. Now a days cotton production is on decline due to fungal diseases, bacteria and nematode and viruses that reduce the yield drastically. There is increasing difficulty in the production of cotton. The reasons are various biotic and abiotic factors but the major threats are fungal diseases. Climatic conditions of Pakistan are benevolent for fungal diseases and their further interaction results in crop failure. About 85% of diseases in cotton are caused by the microorganisms which serves fungi in general. Fungal diseases are leaf spot, caused by *Alternaria macrospora,* charcoal rot of *Macrophomina phaseolina,* black root rot caused by *Thielaviopsis basicola,* Fusarium wilt caused by *fusarium oxysporium,* lint contamination caused by *Nigrospora oryzae,* cotton rust caused by *Puccinia cacabata,* seedling disease complex caused by *Colletotrichum gossypii*, *Fusarium spp, Pythium spp,* Boll rot of disease which is caused by *Lasiodiplodia theobrome* and *Rhizoctonia solani,* Powdery mildew caused by *Leveillula taurica.* Though cultural practices can help to reduce the potential of diseases but some fungicides also play an effective role while using the combination of fungicides. The most effective way of control the cotton diseases is through the bio control agents e.g., Alternaria leaf spot is controlled by two strains of Pseudomonas fluorescens and by two strains of Trichoderma species so these can be used as the components of integrated disease management.

# ANALYSIS OF CHARCOAL ROT INFECTION CAUSED BY *MACROPHOMINA PHASEOLINA* TASSI(GOID) IN SESAME (*SESAMUMINDICUM* L.)

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Dry and humid weather condition serves as favouring elements of Charcoal rot infection in sesame. Generally, the disease is identified by the symptoms appeared in adult plants which includes blackening of stem and root tissue disintegration because of presence of numerous microsclerotia. The population of viable sclerotia in the soil determines the severity of the infection. The present study investigated charcoal rot infection in sesame. Experimental assay was arranged in Earthen pots filled with sterilize soil and fungal inoculum. Surface sterilized sesame seeds were sown roots and stem pieces from both healthy and disease plants were subjected to histopathological studies after 45 days. Infected tissue showed invasive hyphal structure through cortex region of plant tissue that results in tissue browning, disintegration and formation of large spaces. Large no of swelled and lightly melanized structure were also observed intercellularly, which blocks the nutrients transport and caused wilting and death of the plant.

# MANAGEMENT OF CHILI DAMPING OFF CAUSED BY *PYTHIUM MYRIOTYLIUM* THROUGHBIOLOGICAL AGENTS

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A well-known soil-born oomycetes *Pythium myriotylium* produced post-emergence damping off in chili peppers. Numerous disease control measures, such as the usage of PGPR, were employed in the prevention of illness and the promotion of plant development. A research was conducted inPakistan to identify and characterize PGPR in the chili rizosphere. Discrete bacterial isolates and antagonists were utilized to reduce colony expansion. In a dual culture experiment, 8 bacterial isolates were employed to limit the development of *P.myriotylum*, including 4a2, JHL -8, JHL12,IC2, Rh-24, ID, 5C, RH -87. *Pythium sp.* in damping off chili paper caused the death ofgerminating seeds and developing seedlings at the pre- or post-emergence growth stage. This illness was estimated to cause a yield loss of 5 to 80 percent. Young symptomatic seedlings with *Pythium* infection displayed damping off reduced growth, brown root rot, and brown discolouration. 13 *pythium* isolates were recovered from these infections on corn meal agar medium supplemented with ampicillin and rifampicin. Growth promotion and disease suppression are important factors in attaining high-quality product. The researchers looked for rhizobacterial isolates that might be used as *Pythium* inoculums and promote seedling germination without causing phytotoxicity or pathogenic effects on young seedlings in vitro. Bacterial isolates showedvarying degrees of resistance and susceptibility to various antibiotics, as well as non-pathogenic to chili seeds and increased percentage seed germination, plumule, and radical length in uninoculated controls.

# ISOLATION, PURIFICATION, IDENTIFICATION AND CHEMICAL MANAGEMENT OF MICROBIAL PATHOGEN ASSOCIATED WITH LEAF SPOT DISEASE OF CAULIFLOWER

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Cauliflower is one of the several vegetables in the species *Brassica oleracea* in the genus *Brassica*, which from the mustard or *Brassicaeae* family. It is an annual plant, propagate through seed. The cauliflower head is composed of a white inflorescence meristem. Cauliflower is is high in fiber and Vitamin B. It provides antioxidants and phytonutrients that contain anti cancerous properties. Pakistan included in main ten cauliflower producing countries in the world, having 11420 hectares area with an average 209010 tons of production in 2013. The common diseases of cauliflower are *Alternaria* leaf spot, bacterial soft Rot, Blackleg, Black rot, club Rot, Damping Off and Downy Mildew. The most common disease is leaf spot caused by *Alternaria brassicicola* which has a major impact on the yield of this important vegetable. In the current work, a comprehensive survey was made in the different locations of district Faisalabad for the collection of diseased samples. Samples were collected and were surface sterilized with 70% ethanol followed by brief washing in autoclaved double distilled water. The samples were placed on PDA media plates (+50 µg/ml kanamycin) and were incubated at 28±2°C for 24-48 hours. After appearance of fungal growth around the samples, hyphal tip method was used to purify the fungal pathogen involved in leaf spot disease. The fungus will be subjected to DNA isolation and amplification of ITS regions. Fungus will also be applied to the cauliflower plants grown under controlled conditions to confirm the pathogenicity. Different physiological determinants will be measured to check the possible effects of fungus on the physiology and yield of the plant. Data will be analysed through SPSS 19 statistical software.

# COMBINED IMPACT OF DIFFERENT ORGANIC WASTES FOR THE CULTIVATION OF OYSTER MUSHROOM (*PLEUROTUS SAPIDUS*) TO ASSESS THE YIELD POTENTIAL

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The mushroom cultivation is a tremendously expert mode of nutritious rich food through the reprocessing of accumulated agricultural waste residues. The mushroom (*Pleurotus sapidus*) has huge extent of protein, amino acid, vitamins and diminutive level of cholesterols and fats. The present experiment was designed for the evaluation the different organic waste such as Paddy straw, Wood shaving, Bermuda grass, Card board in different proportions under the completely randomized design (CRD). The experiment was planned with four replications and nine different treatments in such a ways T1 (100% P.S), T2 (50% P.S+50% W.S), T3 (50% P.S+50% B.G), T4 (50% P.S+50% C.B), T5 [50% P.S+ (50% W.S+ B.G+C.B)], T6 [75% P.S+ (25% W.S+ B.G+ C.B)],T7 [25% P.S+ (75% W.S+ B.G + C.B)] and T8 [25% W.S + 25% B.G+ 25% C.B] to check the period (number of days) to mycelium development, pinhead emergence, total number of pinhead, fruiting bodies formation in number of days, yield assessment. Results shown that the maximum number of days taken for mycelium growth in treatment $T_6$ was (31 no. of days) as compared to treatment T8 took minimum days was (15 no. of days) the other treatments T1, T2, T3, T4, T5, and T7 took 21,23,28,22,19,29 number of days respectively. The maximum number of days in fruiting bodies emergence taken by the treatment $T_6$ was (42 no. of days) and the minimum was observed in treatment $T_8$ (26 no. of days), all other treatments T1, T2, T3, T4, T5 and T7 took number of days 31,32,38,34,29 and 40 respectively. The highest yield of mushroom was gained in the treatment $T_8$ was (780 g) and the lowest yield was recorded in $T_6$ was (290g) related with others treatments T1, T2, T3, T4, T5, and T7 were 615,595,410,525,695,360 grams respectively. It was concluded that the treatment T8 is the best way to obtain highest yield of Oyster mushroom.

# EVALUATION OF DIFFERENT FUNGICIDES AGAINST STALK ROT OF MAIZE CAUSED BY *FUSARIUM MONILIFORME*

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Maize is the world's leading crop and is widely cultivated as cereal grain, it is the only food cereal crop that can be grown in diverse seasons, ecologies and uses. Maize yields in Pakistan are very poor due to insect pests and diseases. Among them, the stalk rot of maize caused by *Fusarium moniliforme* is very important. Keeping in view the huge losses caused by the stalk rot of maize, the present study was conducted to evaluate the efficacy of selected fungicides against the disease development under field conditions and on the plant growth and yield parameters. A total of six fungicides were used such as Scoure, Plyrem, Topguard, Ridomil, Fenatac M, and Cobox with three different concentrations *i.e.*, (50, 100 and 200 ppm). The effect on disease incidence and effect on the growth and yield parameters such as plant height, root length, root weight, leaf weight per plant, grain weight, and biological yield (t acre$^{-1}$) were recorded. The results showed that all applied fungicides significantly affect against stalk rot of maize caused by *F. moniliforme.* The maximum plant height, root length, root weight, leaf weight per plant, grain weight, and biological yield (t acre$^{-1}$) was noted under Ridomil and Scoure fungicides, followed by Topguard, Cobox, Plyrem and Fenatac M. Whereas, the minimum plant height, root length, root weight, leaf weight per plant, grain weight, and biological yield (t acre$^{-1}$) was observed under control plants where no fungicides were applied. It is concluded that different fungicides significantly affect the stalk rot of maize caused by *F. moniliforme*, it is also noted that Ridomil fungicide at 200 ppm dose performed better throughout the experiment and produced maximum in all parameters of maize growth, development, and yield, so it is suggested that timely use of these fungicides will improve overall yield.

# QUANTIFICATION OF DISEASE INCIDENCE AND PREVALENCE OF BLACK SCURF OF POTATO CAUSED BY *RHIZOCTONIA SOLANI* IN PUNJAB, PAKISTAN

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Potato is ranked fourth important economically important crop in the world after wheat, rice and maize. It is nutritionally rich in minerals such as (phosphorus, potassium and magnesium) also vitamins (B1, B2 and B6). *Rhizoctonia solani* is an infectious fungal pathogen responsible for a disease known as black scurf of potato is associated with lowering the quality and market value of Potato. The disease has been reported in Punjab. A survey was conducted to quantify the disease risk in major potato growing areas of Punjab including Sialkot, Pakpattan, Narowal, Kasur, Sheikhupura, Jhang Lahore, Toba Tek Singh, Bahawalpur Bahawalnagar, Islamabad and Gujranwala. Disease samples were collected from farms as well as markets and morphological and molecular characterization of the pathogen was done and pathogenicity tests were performed afterwards to confirm the pathogen. Results of the survey indicate that the disease is prevalent in Punjab with varying degrees of incidence and percentage severity in different regions. The maximum disease incidence was found in Gujranwala with 23.11% disease incidence and the lowest disease incidence i.e 11.23% was observed in the Bahawalnagar region.

# POTENTIAL APPLICATION OF PGPR AS AN ALTERNATIVE TO AGROCHEMICALS FOR DISEASE SUPPRESSION AND EXPONENTIAL CROP PRODUCTION

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Agriculture produce is under the attack of a wide variety of phytopathogens which substantially lower crop yield. Pathogenic attacks on major crops have arrested the attention of the scientific world, and lots of efforts have been made to defeat pathogens since their evidence. Methods of disease control are still under consideration because this area of research needs further advancement due to environmental and human health concerns. In the 1960s scientists used chemical pesticides on a very large scale and were succeeded in controlling plant population from noxious pathogens at some instances but on the other side, these agrochemicals negatively affected these crops and ecosystem. Agrochemicals intensively increased crop production but also disturbed the soil structure, water quality, and create issues like insect resistance, genetic modifications in the plant, and serious human disorders. The world trend has greatly shifted towards using beneficial microbes in place of agrochemicals. These effective microbes suppress the phytopathogens by employing several mechanisms that also enhance the plant growth traits. In addition, beneficial microbes such as PGPR, greatly promote crop yield by promoting the rate of photosynthesis, production of nucleic acids, sugars, and other bioactive substances. At last, but not least, PGPR because of multifaceted impacts could be efficiently employed in suppressing the phytopathogens and promoting plant growth.

# Efficacy of different Plant Extract and Essential Oils against *Rhizopus stolonifer* and *Aspergillus niger* causing Post Harvest decaying in Grapes

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Grapes (*Vitis vinifera* L) are widely consumed fruit in the world. In Pakistan, from an area of 78.30 thousand hectares about 66 thousand tons of grapes are produced annually. While transporting, storage, marketing and for common consumption Grapes are highly perishable fruit. Post-harvest losses of Grapes are up to 16- 23%. Several abiotic and biotic factors reduce the production and quality of grapes. In present study, samples of rotten grapes were collected from different fruit markets of district Pishin (Balochistan) and Faisalabad (Punjab). The samples were subjected to isolate the fungal pathogens, responsible for the post-harvest decay of fruits. The fungal pathogen isolated was identified as *Aspergillus niger* and *Rhizopus stolonifer* causing post-harvest decay of grape berries. The pathogenicity of the two isolated pathogens was evaluated on different temperatures (5, 20, 30& 40 °C) on Thompson seedless variety of grapes. The temperature 40°C was found conducive for infection and proliferation of fungi. Five organic origin chemicals namely Neem, cinnamon and Clove oils, with concentrations of (500, 1000, 2000 ppm) for each oil and Neem and Marigold Extracts with concentrations of (12.5, 25, 50%) of both extracts were used as antifungal agents. Among the tested organic compounds, Cinnamon oil @ 2000 ppm was found better in delaying the decay process of both fungal pathogens. The decay was reduced up to 50% as compared to control. This study indicates that post-harvest application of essential oils can potentially enhance the storage life of grapes prior to marketing.

# EVALUATION OF CHEMICALS TO CONTROL THE ROOT / COLLAR ROT DISEASE OF BELL PEPPER IN GB

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A study was conducted on bell pepper (*Capsicum annum* L) to investigate the chemical control of the root and collar rot (*Phytophthora capsici* L.) disease by nursery dip method (*in-vivo*), drench method and application of 03 fungicides in field at the Agriculture Research station Skardu during the year 2020-21. The experiment was laid out in randomized complete block Design with three replications. It was consisted of five treatments in drench and dip methods T1(control inoculated, untreated), T2(Red cell, T3(Megma), T4(Ridomil Gold), T5 (control uninoculated, untreated) and four treatments T1(control), T2(Red cell), T3(Ridomil Gold) in field application. The data was recorded percent mortality after 10, 20, 25 days after the application of fungicides, each replication consist of four plants. In nursery dip methods ridomil gold (0.2%) showed good result in disease control after 10, 20, 25 days (o.5, 10, 54.77 percent). While Megma showed 5.5 and 32.5 percent mortality rate in 10 and 20 days interval. In drench method ridomil gold also exhibit significant result in disease incidence 15,20,and 45 days interval with mortality rate of 0.6,0.033.32 %.in the field by application of fungicides Ridomil gold also proved to be best chemical 8.2 percent control over disease It was concluded that Ridomil gold was the best chemical to control the root/collar rot disease of bell pepper fallowed by Megma and red cell in drench methods.

# EVALUATION OF *ALTERNARIA ALTERNATA* FOR BIOLOGICAL CONTROL OF WEED *CONVOLVULUS ARVENSIS* L. IN WHEAT CROP

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Lehli (*Convolvulus arvensis* L.) is one of the most invasive weeds in wheat, barley, Sugarcane, onions, vegetables, and many other crops around the world and caused severe loss to agricultural yield. This technology has gained much importance, due to less human health risks, pollution free, environment friendly approach. Diseased samples of weeds with typical symptoms of spot and necrosis were collected from different localities of the University of Agriculture Faisalabad and pathogen was isolated, purified and identified based on symptomology as well morphologically as *Alternaria alternata* in Plant diseased diagnostic laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad. Five different isolates of *A. alternata* was isolated and evaluated on different weeds as a biological control of weeds. *Alternaria alternata* pathogen significantly inhibits the growth of *Convolvulus arvensis* weed. *Alternaria alternata* was isolated from lehli weed and make different concentrations were used to determine the myco-herbicidal potential of *Alternaria alternata* on lehli weed. Followed by treatments $T_1$ ($1\times10^4$), $T_2$ ($1\times10^6$), $T_3$ ($1\times10^8$) and $T_4$($1\times10^{10}$) that treatments are used *in vivo* and *in-vitro* condition. Maximum disease severity was found on $T_4$ ($1\times10^{10}$) in-vitro and in-vivo. Pathogenicity of weed was also confirmed through detached leaf bioassay as well as whole plant bioassay, separately. So, keeping in view the present study, it was suggested that *Alternaria alternata* can be a potential biocontrol agent that can be used as a potential myco-herbicidal agent against weeds.

# MANAGEMENT OF CERCOSPORA LEAF SPOT OF MUNG THROUGH RESISTANT GERMPLASM AND DIFFERENT CHEMICALS IN RELATION TO EPIDEMIOLOGICAL FACTORS

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Mung bean (*Vigna radiata* (L.) is a widely grown legume crop in tropical, sub-tropical and temperate regions including India, Pakistan, Indonesia and China. It is the most valuable nutritious crop as it is rich in protein, minerals and vitamins. Many catastrophic fungal diseases effect its production. Cercospora leaf spot disease is the main threat that is caused by fungus *Cercospora canescens*, belongs to order Capnodiales and Mycosphaerellaceae family. Disease can destroy the crop, causing more than 90% loss in heavily infested fields under favorable conditions. Plants with Cercospora symptoms exhibit small spots of grayish to brown in color present on all parts of plant. Considering the above facts, the current research work was conceived with the aim of management. To evaluate the resistance source, seeds of eleven varieties i.e., NM-54, NM-51, NM-28, NM 121-25, NM-98, NM 20-21, NM 19-19, NM 31-1, NM-201, Kabuli mung, NM-2016 were collected and sown for screening against Cercospora leaf spot disease. From these, two lines were resistant (NM-54 and NM-51), three were moderately resistant (NM-201, NM-13-10 and NM-121-25), three were susceptible (Kabuli mung, NM-20-21 and MN-28, three were highly susceptible (NM-19-19, NM-2016 and NM-98). Four varieties were used for management purpose (NM-54, NM-121-25, Kabuli mung and NM-28). Four treatments, comprise of Boric acid, $ZnSO_4$, $MgSO_4$ and all three combined (Boric acid +$ZnSO_4$ +$MgSO_4$) were applied by foliar spray. Among all of the treatments, the combined treatment (Boric acid + $ZnSO_4$+$MgSO_4$) showed most effective results with minimum disease severity of 29.00% on the plants of NM-54 as compare to control (water) which showed maximum disease severity (97.37%) on Kabli Mung variety. Effect of treatments on plant parameters was also observed and the analysis showed positive effects on plant parameters as compared to control treatment. The maximum averaged plant height (58.27cm), root length (28.58cm), fresh weight (107.75g) and dry weight (10.42g) were observed on plants treated with the combination of Boric acid + $ZnSO_4$+$MgSO_4$. Environmental data were collected and statistically analyzed. The results revealed that as maximum temperature increase by one degree there was a 3.42% decrease in severity of diseases and one degree increase in minimum temperature there was a 4.30% decrease in disease severity. As relative humidity, wind speed and rainfall increase by one unit, the severity of disease also increase up to (2.40%, 08.84%and 508.79%) respectively.

# SCREENING OF CHICKPEA GERMPLASM AGAINST FUSARIUM WILT AND ITS MANAGEMENT THROUGH NUTRIENTS AND CHEMICALS

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Chickpea (*Cicer arietinum* L.) is a widely grown legume crop in tropical, sub-tropical, and temperate regions, including India, Pakistan, Turkey, Myanmar, and Ethiopia. The contents of this legume such as high protein, minerals, unsaturated fatty acids, fiber, and β-carotene, distinguish it from other pulses around the world. Many destructive fungal diseases has been threatening its production around the world. Chickpea wilt, caused by *Fusarium oxysporum*, is the most common soil-borne fungus that causes up to 100% loss in heavily infested fields under favorable conditions. To manage this pathogen under field condition following study was arranged. By this study, the effects of micronutrient along with the fungicides were evaluated. To fulfill the objective of research, the seeds of twelve varieties i.e., Bittal-110, Noor-2013, Noor-2019, $NF_1$-17339, $NF_1$-17336, $NF_1$-17333, $NF_1$-17342, $NF_1$-17367, $NF_1$-17329, $NF_1$-17345, AD-4 and AD-8 were sown for screening. Among these, three lines were resistant (AD-08, $NF_1$-17367 and $NF_1$-17329), four were moderately resistant (Noor-2019, $NF_1$-17342, AD-04 and $NF_1$-17345), three were susceptible (Bittal-110, $NF_1$-17339 and Noor-2013), while two lines were highly susceptible ($NF_1$-17336 and $NF_1$-17333) against Fusarium wilt of Chickpea. Three varieties (viz. Bittal-110, Noor-2013 and Noor-2019) were selected for the management experiment under field conditions. Three treatments each having nutrients and chemicals ($ZnSO_4$+ $MnSO_4$+Boric acid+ Capnazole), ($ZnSO_4$+ $MnSO_4$+Boric acid+ Flumax) and (ZnSO4+ $MnSO_4$+Boric acid+ Mixtin) were applied by foliar spray. Among them, $ZnSO_4$+ $MnSO_4$+Boric acid+ Capnazole was most effective against disease. Minimum disease incidence was expressed by the ZS+MS+BA+Capnazol x Noor-2019 (36.667%) and maximum by the Control x Noor-2013 (96.667%). Application of treatments also had positive effects on plant parameters as compared to control plants. The maximum averaged shoot length (55.78cm), root length (15.18cm), fresh weight (37.36g), dry weight (18.69g) and yield per plant (22.77g) were observed on plants treated with $ZnSO_4$+ $MnSO_4$+Boric acid+ Capnazole. Environmental data was also collected and statistically analyzed. There was significant ($p<0.05$) but positive correlation between maximum temperature, minimum temperature, wind speed, rain fall and disease incidence. The results revealed that as maximum temperature increase one degree there was a (3.77%) increase in disease incidence and one degree increase in minimum temperature there was (5.25%) increase in disease incidence. As wind speed and rainfall increase one percent incidence of disease also increase up to (5.77%, and 21.75%) respectively.

# EVALUATION OF TURNIP GERMPLASM AGAINST ALTERNARIA LEAF SPOT DISEASE AND ITS MANAGEMENT THROUGH NUTRIENTS

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Turnip (*Brassica rapa* L.) is an important and valuable vegetable belongs to the family of *Brassicaceae*, cultivated for its fleshy roots and tender growing tops. Turnip provides cure against the hepatic injury caused by high antioxidant activity and diabetes. It has enormous medicinal as well as nutritional values. Many destructive fungal diseases threaten its production. One of the most important diseases of this crop is *Alternaria* leaf spot disease caused by *Alternaria brassicicola* which made circular spots with target board appearance like, concentric rings on the turnip leaf. Turnip *Alternaria* leaf spot disease can destroy the crops, causing up to 100% loss in heavily infested fields under favorable conditions. Seeds of four varieties i.e., Nankana red, Purple red, Golden Ball and PTWG were sown for the evaluation of resistance source. From these four varieties, one variety was found resistant (Nankana red), one was moderately resistant (Golden Ball), one was susceptible (PTWG), while one variety was highly susceptible (Purple red). Four varieties were used in management experiment under field conditions (Nankana red, Purple red, Golden Ball and PTWG). For this purpose, six treatments developed by the different combination of micro-nutrients ($ZnSO_4$+$CuSO_4$, Boric-acid+$CuSO_4$, $MnSO_4$+$ZnSO_4$+$CuSO_4$, $CuSO_4$+$ZnSO_4$+Boric-acid, $MnSO_4$+$ZnSO_4$+Boric-acid+$CuSO_4$, $MnSO_4$+Boric-acid+$CuSO_4$) were applied by foliar spray. Minimum disease severity was expressed by the $MnSO_4$+$ZnSO_4$ +Boric acid+$CuSO_4$ x Nankana red (15.667 %) and maximum by the Control on Purple red (85.833%). Effect of treatments was also observed on plant parameters and the analysis showed positive effects on plant parameters as compared to control treatment. The maximum averaged Plant height (82.97cm), shoot length (75.41cm), root length (18.00cm), fresh weight (24.99g) and dry weight (16.82g) were observed on plants treated with $MnSO_4$+$ZnSO_4$ +Boric-acid+$CuSO_4$. Effect of environmental conditions such as maximum temperature, minimum temperature, wind speed and relative humidity on the disease severity of 4 varieties (Nankana red, Purple red, Golden Ball and PTWG) was also observed. The results revealed that as maximum temperature increase one degree there was a (0.94%) increase in severity of diseases and one degree increase in minimum temperature there was (0.95%) increase in disease severity. As relative humidity increase one degree there was a (0.80%) decrease in severity of disease. As wind speed increase one percent severity of disease also increase up to (0.96%). There was significant ($p<0.05$) but positive correlation between maximum temperature, minimum temperature, wind speed and disease severity. There was negative correlation between relative humidity and disease severity.

# SCREENING OF CHILLI GERMPLASM AGAINST *Alternaria alternata* AND ITS MANAGEMENT THROUGH NUTRIENTS AND CHEMICALS

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Chilli (*Capsicum annum* L.), which is also known as pepper in Indo-Pak region, is an important vegetable crop cultivated in Pakistan. Chilli is cultivated in tropical and subtropical regions. Chilli crop can come under biotic as well as abiotic stresses. Certain pathogens cause both quantitative as well as qualitative losses in yield of Chilli crop. One of the most important diseases of this crop is Leaf Spot disease caused by *Alternaria alternate*. This pathogen produces lesions on leaf, begin as circular water soaked spots. To find out the resistance source from chili germ-plasm and also to manage this disease under field condition, the current study was planned. For resistance source, seeds of six varieties (CH-121, Chilli-900 $F_1$, Red Wing, SAYBAN, AAHP-1, and Diamond CH-121) were sown under field condition. From these, only 2 varieties (CH-121 and Chilli-900-F1) were moderately resistant, 2 were susceptible (Diamond CH-121 and Red Wing) and 2 were highly susceptible (SAYBAN and AAHP-1) to disease. Three varieties were used for management experiment (CH-121, Red Wing and Chilli-900-F1). Developed treatments were the combination of nutrients and fungicides. These developed treatments were ($ZnSO_4$+ $MnSO_4$+Boric acid+ Topsin M), ($ZnSO_4$+ $MnSO_4$+Boric acid +Mancozeb) and (ZnSO4+$MnSO_4$+Boric acid+ Vitavax). Among them, (ZnSO4+$MnSO_4$+Boric acid+ Vitavax) was most effective against the disease. Minimum disease severity was expressed by the ZS+MS+BA+Vitavax x CH-121 (15.00%) and maximum disease severity was expressed by the Control on Red Wing cultivar (93.66%). Effect of treatments was also observed on plant parameters and the analysis showed positive effects on plant parameters as compared to control treatment. The maximum averaged shoot length (76.01cm), root length (16.77cm), fresh weight (352.46g) and dry weight (146.89g) were observed on plants treated with ZS+MS+BA+Vitavax. Environmental data was collected and statistically analyzed. The results revealed that as maximum temperature increase one degree there was a (0.98%) increase in severity of diseases and one degree increase in minimum temperature there was (0.95%) increase in disease severity. As relative humidity increased one degree there was a (0.96%) decrease in severity of disease. As wind speed and rainfall increase one percent severity of disease also increase up to (0.62%, and 0.24%). There was significant ($p<0.05$) but positive correlation between maximum temperature, minimum temperature, wind speed, rain fall and disease severity. There is negative correlation between relative humidity and disease severity.

# ETIOLOGY AND *IN VITRO* CHEMICAL CONTROL OF EYE SPOT OF SUGARCANE CAUSED BY *BIPOLARIS SPICIFERA*

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Sugarcane is an important industrial and cash crop. Diseases are the main constraints reducing its yield. Under favorable conditions minor diseases such as eye spot of sugarcane causes considerable damage to leaf by causing necrotic lesions. During survey of sugarcane fields district Hyderabad, Sindh, Pakistan, the sugarcane crop was found affected with typical symptoms of eye spot disease, with 10 to 15% disease incidence. Symptoms of the disease includes elliptical spots on the leaf blade as radish brown to black colour margins with light brown inner portion that resembles to eye shape. Disease reduces the area of leaf for photosynthesis by causing necrotic spots which collapse and merged into leaf blight. *Bipolaris spicifera* were isolated predominantly and identified based on conidial morphology. The pathogenicity test was performed through three different methods. Among them pinning and spraying of conidial suspension method was remained the most favorable to induce the symptoms, whereas soil drenching and spray methods were remained less favorable for disease development. Efficacy of various recommended fungicides for foliar diseases are checked against the pathogen under *in vitro* conditions. Pyrazole remain as most effective to inhibit the colony growth of *B. spicifera.* followed by Shincar, Kocide, Antracol and Melody dew.

# CURRENT STATUS OF FUSARIUM WILT DISEASE OF BANANA IN SINDH, PAKISTAN

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Fusarium wilt of banana, popularly called as Panama wilt disease caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) is a lethal disease, which is already prevailed in major part of the world. Dwarf Cavendish variety is most susceptible to TR4 race of *Foc*. Basrai (Dwarf Cavendish) is the major cultivated variety in Pakistan, therefore banana cultivation is at high risk, as the Panama wilt is reported to be affected by *Foc* tropical race 4 (TR4) in certain areas of Sindh province. The survey was conducted to assess the incidence and severity of Panama wilt disease in banana growing areas of Sindh province, in order to generate distribution and incidence map. The disease incidence and severity was assessed at randomly selected five different fields of each district with a total of 55 different fields of 11 districts, namely, Thatta, Hyderabad, Tando Allahyar, Mirpurkhas, Sanghar, Tando Mohammad Khan, Badin, Matiari, Shaheed Benazirabad, Naushahro Feroz and Khairpur. Prevalence of Panama wilt disease was found at all banana growing areas of each district surveyed with average disease incidence ranging from 10-70% and severity ranging from 20-80%. The highest average disease incidence (70%) and severity (80%) was recorded from district Thatta whereas the lowest average disease incidence (10%) and severity (20%) was recorded from district Khairpur. Results of the study demanded the immediate and serious management action to prevent the further spread of this devastating disease of banana.

# DETERMINATION OF PATHOGENICITY OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* USING DIFFERENT PHENOTYPING ASSAYS

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*Fusarium oxysporum* f. sp. *cubense* (*Foc*) is considered one of the most devastating soil borne fungal pathogens of banana worldwide causing Panama wilt disease, which is one of the major constraints in global production of banana. Determination of pathogenicity of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) was carried out on tissue cultured banana plants of cultivar NIGAB-1 and Grand Nain in green house by three different inoculation methods, namely, pouring method (PM), chlamydospore method (CM) and kernel method (KM). The latency period in all tested methods was between 2 and 3 weeks after inoculation. Plants inoculated by chlamydospore method showed symptoms earlier than the pouring and kernel method. External and internal disease evaluation revealed significant difference in final disease ratings. Pouring method proved more effective as compared to other two methods of inoculation as it gave consistent, reproducible results and can be used for determination of pathogenicity or screening of large number of banana plants for resistance against Panama wilt disease.

# MUSHROOM MYCOFLORA FOUND IN PAKISTAN

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Mushroom is an edible fungus from Phylum Basidiomycota or Ascomycota of kingdom Fungi. Oyester, Button and Chinese mushrooms are commercially grown in Pakistan. *Podaxis pistillaris* is a common edible mushroom found in desert or under trees on sandy soil. This type of mushroom found in southern desert of Iraq, Saudi Arabia, Yemen, Mexico, India (Haryana) and Thar (Sindh) desert of Pakistan. This is used as food, medicine and probiotic. Fifty-six species of mushrooms are found in Pakistan. A desert mushroom, in Sindh province *Podaxis pistillaris* locally known as khumbi grows after rain. Many people collect this edible mushroom and used as a food or sell it to market. Mushroom farming is potential source of creating livelihood for hundreds of unemployed youths. Desert mushroom can be cultivated easily as that of other mushrooms like button/oyster. It does not need tissue culture or artificially prepared spawn. Only mature spores can be sown directly on small beds with 9-12 inches layer of sandy loam soil under shady places. One soaking dose of irrigation should be applied and after a day matured mushroom spores (khumbi) broadcasted on prepared bed.

# STANDARDIZATION OF INOCULATION METHODS FOR INDUCTION OF *SPORISORIUM SCITAMINEUM* INFECTION IN SUGARCANE AND EFFICACY OF DIFFERENT FUNGICIDES FOR ITS CONTROL

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Whip smut intensity could effectively be minimized by planting smut resistant cultivars. For evaluating a large number of propagative materials; standardized germplasm screening protocols as well as optimized inoculation techniques are required. During present study, six different inoculation methods, i.e., dipping, paste, wound+paste, soil infestation, spraying and injection methods for the establishment of smut disease. Injection method followed by wound+paste method and dipping method produced maximum disease incidence, highest number of whips, more tillering and less germination. Significant positive correlations were found among the disease incidence and other traits as well as between numbers of whips and tillers. Fungicides not only eradicate smut from the planting material, but also prevent re-infection when they are used as a pre-plant treatment of setts. During the present investigation 11 fungicides were evaluated either in hot water (52°C) or ambient. Most of the fungicide treatments brought significant increment in sett germination and greatly checked the smut development. For most of the evaluating parameters Bayleton, Bavistan and Tilt performed well than other fungicides. The performance of these fungicides in inhibiting the pathogen infection in artificially inoculated planting material was much better than hot water treatment alone. However, their efficacy was marginally increased when they applied as hot fungicidal dip than ambient. These three fungicides also provide maximum germination, higher yield as well as CCS% from treated plants.

# CHEMICAL CONTROL OF SOIL-BORNE ROOT INFECTING PATHOGENS OF CHILLI

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Chilli (*Capsicum annum* L.) is one of the popular Solanaceae vegetable in tropical and subtropical region. It is subjected to attack of many plant pathogens especially soil borne fungi. The soil borne pathogen in chili crop caused huge losses in chili production worldwide every year. In areas of disease hot spots, the most effective and reliable control can only be achieved through chemical fungicides. Therefore, during the present studies ten fungicides namely, Evito, Shincar, Kocide, Ridomil Gold, Moncut, Nativo, Alliete, Antracol, Systhane and Topsin M were evaluated against ten frequently isolated soil borne fungi such as, *Fusarium oxysporum* f. sp. *capsici. F. solani, F. acutatum, F. semitectum, Microdochium nivale, Rhizoctonia solani, Macrophomina phaseolina, Pythium aphanidermatum, P. deliense* and *Phytophthora capsici* under *in vitro* conditions. Each fungicide was tested at five distinct concentrations (200, 400, 600, 800 and 1000 ppm) against each isolated fungi separately. The results revealed the different degree of efficacy of tested fungicides against different pathogens. Shincar, Ridomil Gold and Topsin M were the most effective followed by Systhane, Kocide, Moncut, Nativo, Evito, Alliete and Antracol. However, the maximum inhibition of fungi was obtained at 800 and 1000 ppm concentrations of Shincar, where growth of all tested pathogens were completely checked.The radial colony growth of *M. phaseolina* was completely checked by Systhane, Moncut, Shincar, Nativo, Antracol, and Topsin M at 800 And 1000 ppm. Out of ten fungicide four fungicide *viz*., Systhane, Ridomil Gold, Shincar and Nativo were highly effective against *P. aphanidermatum*, *P. dilense, M. nivale, F. oxysporum* and *F. solani*. Complete inhibition of mycelial growth of *P. capsici* was recorded with Kocide, Ridomil Gold, Shincar and Nativo. The Shincar fungicide showed best performance against *F. acutatum* and *F. semitectum* as compared to others; while against R. solani the Kocide and Nativo appeared highly effective.

# INFLUENCE OF PLANT AGE ON THE SUSCEPTIBILITY OF CHILLI PLANT TO DIFFERENT WILTING PATHOGENS AND THEIR CONTROL

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Recently, chilli plants growing in Hyderabad, TandoAllayar and other areas were found to suffer with wilt disease. The disease spread very quickly and causes complete destruction of the field at some places. Therefore, the present study was carried out to determine and characterized the fungal pathogens associated with the recent outbreak of chilli wilt and to evaluate the relationship between chilli plant age and pathogenicity of fungal root infecting pathogens. Efficacy of different fungicides for the management of chilli wilt was studied. During the present study chilli fields of Hyderabad, TandoAllahyar and Tando Muhammad Khan were visited. Partial and complete dead mature/fully grown chilli plants were collected with roots. Isolation was made from the roots of affected chilli plants revealed the consistence association of *Fusarium oxysporum* Schlecht., *Fusarium solani* (Mart.) Sacc. (Teleomorph = *Nectria haematococca* (Berk. & Br.), *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Khun. (Telemorph: *Thanatephorus cucumeris* (Frank) Donk. and *Pythium aphanidermatum* (Edson) Fitzp. with the roots of affected chilli plants. Pathogenicity studies conducted on cv. Ghotki and seedlings of different ages i.e., 1, 2 and 3 months old were inoculated with five isolated pathogens separately such as *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Pythium aphanidermatum*. The chilli plants of different age show mixed response to different pathogens. Some pathogens caused more reduction in one group of plants while others caused another group of plants and so on. Generally, in terms of plant length reduction, pathogens effects were more pronounced on 1-2 months old plants than 3 month old plants. *F. solani* caused least reduction in all age groups of plants. Reduction in plant length ranged from 31-38, 29-37 and 19-30.76% in one, two and three month old plants. All pathogens also caused a remarkable reduction in plant weight of different aged chilli plants, ranged from 50-85%. Pathogens inoculation caused more reduction in weight of 2 and 3 months plants than in 1 month old plants. Fungicides *viz*., Shincar, Miravis Duo, Systhane Antracol, Nativo and Moncut appeared highly effective against *F. oxysporum* and *F. solani*. Three fungicides *viz*., Miravis Duo, Nativo and Evito were found most effective and resulted in complete inhibition of mycelial growth of *R. solani*. Complete inhibition of mycelial growth of *M. phaseolina* and *P. aphanidermatum* was recorded with Systhane, Shincar, Nativo Antracol and Evito. *T. harzianum* and *T. longisporum* followed by *P. variotii* also significantly checked the colony growth of tested pathogens.

# ROOT INFECTING PATHOGENS OF CHICKPEA AND THEIR MANAGEMENT THROUGH DIFFERENT PRIMING METHODS

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Chickpea (*Cicer arietinum L.*) is cultivated worldwide. It is Important in Pakistan just because it is said to be poor's meal and due to its quality protein. Only Punjab province produces 80% of chickpea out of which 90% is grown in Rainfed areas. In recent years, chickpea production shows negative trend that is because of drought stress and pathogens that cause severe diseases. Among pathogens Root borne pathogens are more damaging. *F. oxysporum f.sp. ciceri (Foc), M. phaseolina, Verticillium spp., Sclerotium rolfsii* and *R. solani* were seen to be devastating, their incidence and Severity is reviewed in this article. Moreover, management through seed priming with deferent methods of leguminous (Chickpea) and several non-leguminous crops shows significant results.

# MANAGEMENT OF LEAF SPOT OF ALOE VERA CAUSED BY *ALTERNARIA ALTERNATA* THROUGH CHEMICALS AND ESSENTIAL OILS

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Leaf spot disease caused by *Alternaria alternata* affected the aloe vera plant in previous few years and caused heavy losses to aloe vera production in Pakistan. The research was conducted at Department of Plant Pathology, University of Agriculture Faisalabad. Infected leaf samples of aloe vera showing the characteristic symptoms of leaf spot were collected from different nurseries of Pattoki and ornamental areas of Faisalabad. Under aseptic condition isolation and purification of the pathogen associated with infected samples was done and morphologically characterized. Pathogenicity tests were performed by Koch's postulates and *A. alternata* was confirmed the pathogen caused leaf spot of aloe vera. For the management of leaf spot disease of aloe vera four fungicides (Curzate, Nativo, Alliete and Flumax)® and essential oils (Clove, Castor, Fennel and Cardamom) were evaluated at four concentrations 50ppm, 100ppm, 150ppm and 200ppm with one control treatment by using three replications against *A. alternata* under in-vitro condition. Fluazinam+Metalaxyl-M (Flumax)® was found effective at all concentrations with the values of 1.11cm, 1.00cm, 1.02cm, 1.05cm respectively and Cymoxanil+Mancozeb (Curzate)® at 150 ppm with the value of 2.49cm was most effective to inhibit the growth of *A. alternata*. Among essential oils all concentrations of castor oil with the values of 1.53cm, 1.50cm, 1.02cm, 1.84cm respectively and 150ppm concentration of fennel oil with the value of 2.5cm was most effective to inhibit the growth of *A. alternata* pathogen of leaf spot of aloe vera.

# INVESTIGATION AND *IN-VITRO* MANAGEMENT OF PREMATURE FRUIT DROP IN CITRUS CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES*

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Citrus is the most important fruit grown worldwide in the past 4,000 years. Citrus fruits are considered a flowering tree and shrub in the Rutaceae family. All varieties of citrus fruits are of great commercial, food, nutritional, medicinal and aromatic value. Citrus (Kinno) is a treasured fruit in Pakistan, where it ranks first in both area and production among all fruits. The citrus industry faces many challenges. These factors include biotic and abiotic agents. *Colletotrichum gloeosporioides*, the most important microbial pathogen causing severe destruction of citrus fruits in citrus orchards. In the present study, experiment was conducted to check the Cultural and morphological features of *Colletotrichum gloeosporioides* and Evaluation of different fungicides for the management of *Colletotrichum gloeosporioides* associated with citrus wither tip disease. Samples of infected citrus tips from different fields of Faisalabad were collected and then the samples were transferred to the plant disease diagnosis laboratory. Isolation of specific pathogens was performed in an artificial medium. Cleaning was performed using the Hyphal tip method. Additional refined culture was then identified morphologically under a microscope (NikonY100). *Colletotrichum gloeosporioides* mycelium is white to cream on PDA plates and turns dark brown or black after 14 days. The growth of pathogenic mycelium was revealed by a ring pattern with a fluffy appearance on PDA plates. Conidia average about 15-16 µm in length and 4.5-5.5 µm in width, which have been reported for pathogenic isolates of *Colletotrichum gloeosporioides*. There were four different fungicides (Topsin M, Metalaxyl + Mancozeb, Aliette and copper oxychloride) along with a control formula evaluated at different concentrations of 100, 200 and 300 ppm. After 21 days, topsin M compared to the control radial growth of 4.20 cm. Topsin M showed the highest inhibition by *Colletotrichum gloeosporioides*, while copper oxychloride had the lowest effect.

# ACTIVATION OF DEFENCE SYSTEM OF CITRUS AGAINST BROWN LEAF SPOT THROUGH PLANT ACTIVATORS

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Alternaria brown leaf spot (caused by *Alternaria alternata*) is major disease of citrus in subtropical citrus cultivated areas. Resistance in plants against invading pathogens is enhanced through treatment with plant activators, which act on various points in the signalling pathways involved in disease resistance. One year old mandarin plants were established at the research area of University of Agriculture Faisalabad. Five plant activators (Salicylic acid, $KH_2PO_4$, citric acid, $K_2HPO_4$ and benzoic acid were tested against disease at three concentrations (0.25, 0.5 and 0.75%). Plant activators were directly sprayed on plants early in morning when stomata are opened and treatments were repeated after one week of first treatment for good results. Salicylic acid proved best with disease incidence of (32.8%) followed by citric acid, $KH_2PO_4$, $K_2HPO_4$ and benzoic acid (38.70, 42.50, 48.41, 53.41)% respectively. These all treatments were also tested in green house conditions and they gave better results than field conditions and salicylic acid was on top with the disease incidence (27.17%). SA is recommended to farmers as non-chemical control for ABS disease.

# NEW DECLINE EXPRESSIONS IN MANGO: A CASE STUDY IN MULTAN REGION

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Mango (Mangifera *indica L.)* is the second major fruit crop of Pakistan and main mango growing districts in Punjab province are Multan, Bahawalpur, Khanewal, Muzaffargarh and Rahim Yar Khan. Although soil and climatic conditions of Pakistan are suitable for mango production yet some diseases are of great significance leading to its low production and New Decline in Mango is becoming the most serious one in Multan region in Punjab. Field investigations of Multan region showed that infected plants were presenting symptom viz., defoliation, drying of twigs/branches, gummosis, bark splitting, nutritional stress, withering of leaves, necrosis, wilting and salt injuries collectively counted as diseased irrespective of the stage of pathogenesis. Maximum disease incidence of the new decline in mango was recorded as 20.99% on West side followed by 18.99% on east in Multan while the least incidence 13.99% was observed on north side. Different predisposing factors such as fertilizer and inter-cropping, continuous spray from Triazol group were also recorded in the survey and similarly, the four fertilizer sources (NPK, FYM, Gypsum and micronutrients) were also kept as main keys of nutrient to find out the effect of application in each garden and to bring into light its relationship with disease incidence. Maximum disease incidence 21.56% was noted in the orchard where only F.Y.M application was mostly found in practice while minimum disease incidence (4.78%) was observed in the orchard where micronutrients application was done regularly. *Alternaria* spp. were mostly isolated from flower and leaves while *Lasiodiplodia theobromae* was isolated from roots, twigs and branches, *Fusarium* spp. were isolated from leaves, twigs and roots, *Rhizoctonia spp.* were isolated from root samples. *L. theobromae* was dominant fungus causing the 41.66-75% infection frequency. Evolution of fungicides under in-vitro with 03 fungicides viz., Topsin M, Ellactus Ultra and Nativo with four different concentrations viz. 25,50,75 and 100 ppm by using food poisoning technique. Nativo showed 90.53% decrease over control at 100 ppm. Management of affected plants in field was also conducted during this study with 06 treatments including pruning and three foliar sprays and soil amendment using Topsin-M, Ellactus Ultra and Nativo. It was found that maximum decrease in disease over control (72.92%) was noted where soil amendment was done with application of Nativo was done in the soil.

# SCREENING OF POLYEMBRYONIC VARIETIES OF MANGO AGAINST CERATOCYSTIS MANGINECANS, A CASE OF SUDDEN DEATH OF MANGO

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The available seedlings were successfully grafted with the scion woods of the desired polyembryonic variety through the wedge grafting. The microscopic study of the isolated colonies under compound microscope revealed the association of 4 fungi viz., *Ceratocystis mangnicans, Fusarium spp, Nattrassia mangiferae, Cladosporium spp,* and *Penicilium spp*. significantly highest frequency of *Ceratocystis mangnicans* was observed in all the inoculated petri dishes followed by *Nattrassia mangiferae*. While, *Cladosporium* and *Penicilium* were recorded with the lowest frequency. The findings of the pathogenicity of the fifteen varieties against *Ceratocystis mangnicans* and *Natrassia mangiferae* showed varying response of the polyembryonic germplasm towards the symptoms development and its resistance or susceptibility. Two varieties Bullock heart and Rock dale Siagon were found to be the resistant varieties and showed minimum of their symptoms development of MSD during the due course of time. While all the other varieties showed susceptible response towards the *Ceratocystis mangnicans* and *Natrassia mangiferae*. All the symptoms viz., Necrosis, withering, yellowing drying of leaves, leathering, gummosis, canker, dieback, wilting and drying were observed on all the polyembryonic varieties but with varying intensity. Yellowing of leaves, leathering and wilting were the most common disease symptoms observed on different varieties. For the confirmation of the associated fungi with the mango sudden death disease symptoms on various mango polyembryonic germplasm, it was concluded that the same fungi were re-isolated from the inoculated plants with varying frequency. Bullock heart and Rock dale Siagon were found to be resistant varieties against the MSD pathogens by both methods.

# CHEMICAL CONTROL OF *CURVULARIA LUNATA*

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*Curvularia lunata* is a fungal pathogen that causes leaf spot, leaf blight and root rot diseases in plants, and sinusitis, mycotic keratitis, asthma and conjunctivitis in human. This *in vitro* study was conducted to assess the potential of three synthetic fungicides to control *C. linata*. The fungicides evaluated in this study were thiophenate methyl 70% WP, metalaxyl+mencozeb 72% WP and fosetyl-Al 80% WP. The concentrations of these fungicides used in food poisoning technique were 50, 100, 150, 200 and 250 ppm, which were compared with a control having 0 ppm concentration. Metalaxyl+mencozeb proved the best fungicide in inhibiting the fungal growth by 26–67% over control. Thiophenate methyl and fosetyl-Al showed poor activity against the targeted pathogen by suppressing its radial growth by just 15–26% and 8–12%, respectively.

# *IN VITRO* CHEMICAL CONTROL OF *PENICILLIUM EXPANSUM,* THE PATHOGEN OF BLUE MOLD IN APPLE

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*Penicillium expansum* is a common soil-borne fungus occurring worldwide and causes post harvest diseases in a wide range of plant species especially in apple. Three fungicides namely metalaxyl+mencozeb 72% WP, thiophenate methyl 70% WP and fosetyl-Al 80% WP were evaluated *in vitro* against this fungal pathogen. Five concentrations of each fungicide *viz*. 50, 100, 150, 200 and 250 ppm, were tested by food poisoning technique using malt extract agar as a growth medium. All the three fungicides significantly reduced the pathogen with variable extents. Among these, thiophenate methyl was the most effective one causing 65–90% reduction in fungal growth. Metalaxyl+mencozeb also showed a very similar effect resulting in 73–88% suppression in the fungal growth. Fosetyl-Al was comparatively less effective fungicide and reduced fungal growth by 73–88%, over control. This study concludes that thiophenate methyl and metalaxyl+mencozeb are highly effective fungicides against *P. expansum,* whose 50 ppm concentration can control fungal growth by 65 and 73%, respectively.

# SURVEY OF ROOT ROT OF GUAR CROP IN SINDH

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A Survey was conducted during July 2014 to August 2015 to record disease incidence of root rot in Guar. The study area was based on 52 sites of 13 places from five districts of Sindh. i.e. Mirpurkhas, Hyderabad, Tando Allahyar, Sanghar and Karachi. *Fusarium* spp. was the most common destructive root rot fungi found in all survey areas. The colonization percentage of *Fusarium* spp. was recorded to be between 55 - 100%. Four different species of *Fusarium*; namely *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *F. semitectum* were isolated from the sampled areas. Another root infecting fungi *Macrophomina phaseolina*, was found that ranged between 0 – 45 %. The 3rd tested root rot pathogen, *Rhizoctonia solani* was found at its lowest distribution i.e. 0 - 30 % in all governorates on the diseased, survey area. These results showed that *Fusarium* spp. is the most prevalent in the soil of the agricultural field and responsible for the decline of guar crop. In this study, physicochemical properties of soil i.e., soil organic matter, Ec, cation and an-ion showed significant variation and $K^+$ deficiency was recorded in all sampled areas.

# IDENTIFICATION OF ANTHRACNOSE RESISTANCE IN LENTIL

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Lentil (*Lens culinaris* Medikus) anthracnose caused by fungus *Colletotrichum lentis* Damm is one of the most significant threats to lentil cultivation globally. Lots of information regarding identification of lentil anthracnose resistance has been published all over the world, however no comprehensive data prior to this work was available in Pakistan. Current work encompasses status of available lentil germplasm accessions towards this disease. For this purpose, screening of 31 available lentil accessions were conducted against the known highly virulent *Colletotrichum lentis* isolate CLK-63 at seedling and flowering stage. Accession Masoor-85 served as check/spreader in the investigation. At seedling stage, most of accessions; 20 (64.51%) were found susceptible (11, 35.48%) to highly susceptible (9, 29.03%) to isolate CLK-63; whereas three (9.67%) moderately resistant and eight (25.80%) resistant to anthracnose. At adult stage, like seedling stage, the maximum accessions 19 (61.29%) were found su Mujahid Rasoolsceptible to highly susceptible to isolate CLK-63 followed by eight (25.80%) resistant and four (12.90%) moderately resistant to anthracnose. Thus, almost all the accessions responded in the same manner towards isolate CLK-63 at seedling and adult stage. Accessions, specifically Masoor-89, Masoor-86, Masoor-89 bold seeded, Masoor-2004, NARC- 11-1, 08505, Punjab Massor-2009 and Shiraz-96 were found resistant, while accessions 12-308-Lentil, NL-2 and 03501 were found moderately resistant to disease at both the stages of growth.

# IMPLICATION OF DROUGHT STRESS ON SHISHAM DIEBACK UNDER *FUSARIUM SOLANI* INFECTION

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*Dalbergia sissoo* is a native timber-producing species in Pakistan that is used in the agroforestry, fuelwood, and furniture industries. Dieback has negatively impacted *D. sissoo* in Pakistan. Field observations suggested a positive correlation between drought and dieback however practical demonstration of this idea was missing. The main objective of this research was to evaluate the association of *Fusarium solani* that has been most commonly associated with dieback under water stress on shisham dieback. Five fungi, i.e. *Fusarium oxysporum*, *Curvularia lunata*, *Cladophialophora carrionii, Alternaria alternaria,* and *Fusarium solani*, were identified based on ITS sequence homology from the dieback infected tissues. *D. sissoo* seedlings showed dieback symptoms only when subjected to water stress. It seems water stress is the primary factor and fungi are secondary agents in developing shisham dieback. Stressed plants responded to stress by decreasing stomatal conductance, transpiration rate, and photosynthetic activity as a result of low water potential. Dieback-affected plants had increased production of CAT and POD enzymes. Shisham dieback occurs after abiotic stress, such as drought, predisposes the plant to fungal infection and disease. This study emphasizes the importance of exploring the interaction of different abiotic factors and fungal pathogens on the growth of *D. sissoo* for sustained production and management of shisham dieback in the future.

# YIELD PRODUCTION OF *PLEUROTUS OSTREATUS* - P1 USING DIFFERENT SUBSTRATES ON APPLE POMACE AS HORTICULTURAL WASTE AND WITH DATE PALM LEAVES, BANANA LEAVES)

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Apple pomace is a byproduct from apple industry and considered also horticultural waste and has the potential to support the growth of microorganisms. In this study, the effect of apple pomace on the growth rate of *Pleurotus ostreatus* was investigated. *Pleurotus Spp.* is an important mushroom group and extensively used in food handling industries and has pharmaceutical uses. Its capability to produce in a varied range of temperatures and to consume existing lingo cellulosic materials is reason to be reflected as a extremely fascinating mushroom for manufacture. Different substrates such as cereal straws, corncobs, coffee grounds and urea are used in greater part to create mushroom. To check the influence of various substrates such as apple pomace, date palm leaves and banana leaves proceeding the cultivation and production of Oyster mushroom, On the basis of growth of mycelia, primordial appearance time, yield of mushroom, colonization time, fresh weight and biological efficiency were analyzed. Different treatments were used and significant results indicated that among all the treatments used best growth of mycelia, earlier formation of pin head, better yield and days to fruiting were observed in T3 (50:50%(Date palm leaves + Banana leaves) with 5% apple pomace,T5 (25:75%(Date palm leaves +Banana leaves) with 5% apple pomace, 50:50%(Date palm leaves + Banana leaves), T2 (50:50%(Date palm leaves +Banana leaves) with 2% apple pomace, T4 25:75%(Date palm leaves +Banana leaves) with 2% apple pomace, T1 (50:50%(Date palm leaves + Banana leaves). By using standard statistical method, results were evaluated. Maximum yield (439.3) and maximum biological efficiency (89.1) was obtained from treatment T3 (50:50%(Date palm leaves + Banana leaves) with 5% apple pomace .Above findings showed that treatment T3 was the best substrate as compared to all others for the production of mushroom respectively.

# USE OF TEA WASTE (LIPTON) WITH COMBINATION OF VARIOUS MIXTURE OF AGRICULTURAL WASTE MATERIALS (WHEAT STRAW, COTTON WASTE, CORN MEAL, THATCH GRASS) FOR CULTIVATION OF *PLEUROTUS PULMONAIOUS*

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The waste of agriculture was used for mushroom cultivation was good for the elimination of ecological pollution and had better impact on enviourmental and friendly attitude. Mushroom comprised a filthy extent of carbohydrate, protein, lipids and fibres. Tea waste (lipton)latterly along by agriculture unused substantial (straw of wheat, corn meal, cotton waste and thatch grass) were applied to make compost by means of substrate for the rearing of mushroom (*Pleurotus Pulmonarius*). Gypsum and wheat bran were applied to be pre-owned as auxiliary stuff estimated 5 % of the substrate dehydrated mass in entire conducts. Complete randomized design (CRD) was applied to the experiment having arranged five different ratios (100% tea waste, 75% tea waste+ 25% agri. waste, 50% tea waste+ 50% agri. waste, 25% tea waste+ 75% agri. waste, 0% tea waste+ 100% agri. waste). Substrates arranged by standard strategies and put into the autoclave plastic packs, can sack immunized by 10% bring forth. Bring forth run pinhead development first gathering time, absolute yield, organic effectiveness (%) and nature of mushroom was watched. The time taken for 100% produce run were run to fluctuate between 19-36 days and time for pinhead arrangement was in the scope of (54.5 to 67.4) days. in spite of the fact that were no factually huge yield contrast related with the forced medicines, the mean most noteworthy yield was found in , 50% tea waste+ 50% agri. waste (410.14 g), Trailed by medications 25% tea waste+ 75% agri (379.98 g), 0% tea waste+ 100% agri. waste (338.88 g), 75% tea waste+ 25% agri. waste (291.22 g), 100% tea waste (249.46 g), The efficiencies of different blend were somewhere in the range of 83.8% and 52.08%.As a conclusion that the leaves of Tea waste used as substrate are appropriate and new organic material for production of Oyster mushroom (*Pleurotus pulmonarius*) when mixed with waste of agriculture material like wheat straw, cotton waste, corn meal and thatch grass. This experiment has given us very promising outcomes that the Tea waste plentifully present, can be monetarily when utilized in blends with Agri. waste or other cellulosic material for mushroom development, in this way its utilization is prescribed to farmers in this nation where huge volumes of tea waste are disposed of as agricultural waste material.

# ETIOLOGY, PHYSIOLOGY AND MANAGEMENT OF STEM ROT DISEASE OF CANOLA (*BRASSICA NAPUS* L.)

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Canola (*Brassica napus* L.) is one of the leading oilseed crops in the world and as well as in Pakistan which fulfil the edible oil needs. A major factor impacting canola production in the world is canola diseases. Stem rot of canola is the most damaging disease of canola after Black leg. This research was designed by keeping in view the economic importance of the losses caused by this disease to develop some novel strategies to control Stem Rot of canola. The pathogen associated with the stem rot disease was identified as *Sclerotinia Sclerotiorum* on the basis of the morphological studies. Pathogenicity test was performed that confirmed the association of the isolated fungus with the disease. Physiological studies of the pathogen exhibited that the temperature 20-25°C and pH 7.5 to 8.0 was most suitable for the growth and sclerotial formation by *Sclerotinia sclerotiorum*. Four plant extracts namely Garlic (*Allium sativum*), Safeda (*Eucalyptus comaldulances*), Neem (*Azadirachata indica*) and Datura (*Datura strumarium*) at three concentration levels (S, S/25, S/50%) were evaluated against *S. sclerotiorum* through poisoned food technique on PDA. According to the results of the experiments, Garlic proved to be the most effective, gave maximum inhibition at all concentration levels (49.54, 58.67 and 73.90%), followed by Neem. Safeda gave the least inhibition percentage (13.61%) at S/50 % concentration and was found least effective against *S. sclerotiorum.* Among the four evaluated fungicides (Aerosal, Score, Capnazole and Nanok) @ 5 ppm, 10ppm and 20ppm concentrations. Aerosal gave the most promising results by inhibiting the fungus growth 92.11% @ 20ppm after seven days, followed by Capnazole @ 20ppm (73.11%) and Score @ 20ppm (60.114%). The least growth inhibition (34.69%) was recorded by Nanok @ 5ppm.

# ASSESSMENT OF COMMON CULTIVARS OF POTATO FOR INCIDENCE OF SCAB DISEASE UNDER FIELD CONDITIONS AND EFFECTIVE MANAGEMENT MEASURES

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To increase the quality and yield of Rhodes grass through management techniques an experiment was study at University of Agriculture, Faisalabad. Experimental treatments was consist of sowing dates ( 1st week of July,2nd week of July,3rd week of July) and sowing methods (broad cast , line sowing 30cm apart ,line sowing 45 cm apart) .The randomized complete Block design (RCBD) with split plot Arrangement was used in experiment. The field trial consist of three replications. Gross plot size is 7m×3.6m.Sowing dates was kept in main plot whereas sowing method was in subplot .In field trail all cultural practices during the growing season was remain normal and uniform. The findings regarding emergence count, number of leaves per plant ,plant height, fresh weight per plant , dry weight per plant, dry matter percentage, leaf area index, leaf area per plant, fresh forage yield, crude fiber content, crude proteins content, total dry matter yield and total ash content was determine . By using Fisher's analysis of variance techniques all collected sample were statistically analyzed and least significance (LSD) test with 5 % probability was used to analyze the treatment means. Rhodes grass performed well and providing promising results in different sowing methods but not affected by different sowing dates .Therefore, different sowing methods drive for batter management to avoid shortage of productivity.

# BIOCHAR INDUCED RESISTANCE AGAINST EARLY BLIGHT (ALTERNARIA SOLANI) IN TOMATO

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Sustainable agriculture deals with the efficient use of resources for the benefit of human and its environment. By this we can fulfill the requirement of food, feed and fiber efficiently. Use of heavy doses of chemicals like fertilizers or pesticides is not suitable for sustainable agriculture. Biochar usage is alternative way to control diseases and enhance the growth of plants. This study emphases on the effective use of different concentrations of biochars and composts to promote the growth of plant biomass and nutrients availability like N, P and K in the presence and absence of Alternaria solani. Two types of biochars (Wood biochar and Green waste biochar in 3% and 6% concentrations) and compost were used as a soil amendment. All the different concentrations has very effective control on disease reduction and enhancement of plant biomass but 6% Wood biochar has better effect from all of them. It also enhanced the growth of stem and root. Biochar also reduced the disease incidence and severity in plants. Under stress conditions Nitrogen and Phosphorus contents were increased in tomato. It was observed that Nitrogen contents were increased to 12.44% and Phosphorus contents were increased to 55.17%. Disease incidence was 90% in tomato plants when they were grown in soil and it was reduced to 40% when they were grown into 6% Wood biochar combination. However, it was observed that disease severity was reduced to 47% in 6%wood biochar. In conclusion, Biochar has positive impact in reduction of early blight in tomato by reducing the incidence of disease and enhancing the plant growth parameters. Biochars can play an important role in sustainable agriculture.

# SOIL-BORNE FUNGI ISOLATED FROM POTATO FIELDS OF DISTRICTS GILGIT, HUNZA AND NAGAR OF GILGIT-BALTISTAN

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A survey of potato (*Solanum tuberosum* L.) cultivated fields at different locations of three districts *viz.,* Gilgit, Hunza and Nagar of Gilgit-Baltistan was carried out to study the distribution of soil-borne fungi. A total of 27 fungal species belonging to 16 genera *viz., Alternaria, Aspergillus*, *Curvularia, Exserohilum, Fusarium, Gibberella, Globisporangium, Macrophomina, Neocosmospora, Nigrospora, Paecilomyces, Penicillium, Pythium, Rhizoctonia, Rhizopus,* and *Syncephalastrum* were isolated. All these 27 species appeared to be new record from districts Gilgit, Hunza and Nagar of Gilgit-Baltistan. Species of *Alternaria*, *Aspergillus*, *Curvularia*, *Fusarium*, *Penicillium*, *Pythium*, *Rhizoctonia* and *Rhizopus* were more prevalent as compared to other fungi.

# COMPARATIVE EFFICACY OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST *Rhizoctonia solani,* THE CAUSAL AGENT OF ROOT ROT DISEASE IN COMMON BEANS

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Common bean (*Phaseolus vulgaris* L.) is an important pulse crop. Of the various factors affecting crop yield and quality, soil-borne plant pathogens are one of the major problems. Root rot disease of common bean is cause by many plant pathogens including species of *Fusarium, Pythium* and *Rhizoctonia.* Root-associated fungi were isolated by using agar plate methods and baiting techniques. These fungi were identified morphologically through light microscopy. *Rhizoctonia solani* was associated with 100% plants. *In-vitro* pathogenicity test was done by radical assay in Petri dishes. Chemical fungicides and bio-control agents were evaluated *in-vitro* against *Rhizoctonia solani.* Among the tested biocontrol agents, the highest growth inhibition was produced by *Trichoderma longibrachiatum* (66.66%) followed by *T. harzianum* (60.44%), *Gliocladium virens* (47.11%), *G. roseum* (24.44%), *Paecilomyces variotti* (37.33%) and *P. lilacinus* (21.77%). Fungicides Nativo (Tebuconazole & Trifloxystrobin) at 10 ppm caused highest growth suppression (81%) followed by Ready Super (Pyraclostrobin and Tebuconazole) at 10ppm (72%), Score (Difenoconazole) at 100ppm (52%). Aliette (Fosetyl-Aluminium) at 1000ppm (46%), Antracol (Propineb) at 1000ppm (65%) and Zaver (Sulphur) at 1000ppm (40%).

# DETECTION OF GRASSY SHOOT DISEASE OF SUGARCANE IN SINDH, PAKISTAN

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Sugarcane (*Saccharum officinarum* L.) is an important crop of Pakistan. In recent past, symptoms similar to the Grassy shoot disease (SCGS) caused by phytoplasma were observed in major sugarcane fields of Sindh. Leaf samples of sugarcane plants showing symptoms of SCGS were collected from Tando Allah Yar area of Sindh, Pakistan. Conserved region of the 16S rRNA was amplified from extracted DNA of diseased leaf samples. Sequence information of amplified sequence revealed that the samples are identical to many isolates of SCGS reported earlier from different regions of South Asia. This appears to be the first authentic confirmation of occurrence of SCGS in Sindh, Pakistan.

# ENDOPHYTIC FUNGI ASSOCIATED WITH *Murraya koenigii* AND THEIR BIOCONTROL POTENTIAL AGAINST *Macrophomina phaseolina*

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Endophytic mycoflora in medicinal plants produces bioactive chemical compounds that protect host plants from diseases and provide resilience to poor environmental conditions. Mycodiversity of endophytes and the ability of these fungi to produce natural secondary metabolites have been discovered in several plants. As a result, there is a need to investigate the diversity of endophytic fungi associated with various plants, as well as their ability to create novel chemicals for medicinal application. The current study was initiated to screen endophytic fungi associated with *Murraya koenigii, commonly known as Curry-patta.* Twenty-five endophytic species, representing 15 genera were isolated from healthy, surface-sanitized leaf and stem segments of *M. koenigii* placed on Potato Sucrose Agar (PSA), Malt Extract Agar (MEA), and Czapek's Dox Agar (CzDA). Majority of the isolates grew well on PSA. Isolation of fungal endophytes was less from stems as compared to leaves. Using dual culture plate assay, all the isolates were tested for antagonistic activity against the charcoal rot fungus, *Macrophomina phaseolina*. Only *Aspergillus candidus* and *Aspergillus quadrilineatus* suppressed the pathogen and formed noticeable inhibition zones.

# COST BENEFIT ANALYSIS OF TOMATO YIELD AFTER SOIL AMENDMENTS WITH *Trichoderma harzianum* AND ORGANIC FERTILIZERS

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A rooftop pot experiment was conducted to study the effect of different soil amendments viz., biochar (BC), composted farm yard manure (FYM), green manure (dried alfalfa; GM), and *Trichoderma harzianum* (TH) either alone or in various combinations. Soil without any amendment served as control. In a comparable set of treatments, soil was also amended with Humic acid (HA) to see its effect on the efficacy of soil amendments. The experiment was carried out in Randomized Complete Block Design (RCBD) with three replicates for each treatment. The main results showed that T-12 (FYM+GM+TH+BC+HA) effectively promoted plant growth including number of branches, flowers and fruits per plant as compared with other treatments, while the T-7 (FYM+GM+TH+BC) produced highest average fruit weight 150g and maximum marketable yield were recorded 3.5 Kg/plant in contrast to remaining treatments. The benefit cost ratio with treatment having T7 followed by T12 was recorded 2.5 and 2.0 respectively. Other treatments gave non-significant results for benefit to cost ratio as compared to control.

# EFFECT OF DIFFERENT PLANT EXTRACTS ON COLONY GROWTH OF SOIL-BORNE PATHOGENS

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Extracts of *Azadirachta indica*, *Eucalyptus* sp., *Moringa* sp., *Conocarpus erectus*, *Murraya koenigii* were investigated for their inhibitory effect on colony growth of soil-borne pathogens *viz*., *Sclerotium rolfsii*, *Rhizoctonia solani* and *Macrophomina phaseolina*. *Azadirachta indica* extract showed the highest inhibition in growth of *Sclerotium rolfsii* (100%) followed by *Eucalyptus* sp. (54.81%), *Conocarpus erectus* (47.78%), *Moringa* sp. (28.15%) and *Murraya koenigii* (15.19%). In case of R. *solani, Azadirachta indica* extract showed highest inhibition percentage (100%) followed by *Conocarpus erectus* (40%), *Moringa* sp. (18.89%) and *Murraya koenigii* (4.07%), whereas, *Eucalyptus* plant extract showed no growth inhibition. However, only *Eucalyptus* plant extract showed 57.04% inhibition in growth of *M. phaseolina* whereas all the other tested extracts were found ineffective.

# IN VITRO EVALUATION OF ANTIFUNGAL ACTIVITY OF HERBICIDES AGAINST SOIL-BORNE PATHOGENS

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Weedicides are the chemicals used to control the broad or narrow leaf weeds in agricultural fields. The aim of the present study was to check the toxicity of weedicides Glyphosate, Paraquat, Buctril Super and Walter Super towards the soil-borne pathogens *viz*., *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Rhizoctonia solani* by using Poison Food Technique. Among the tested weedicides, Buctril super @ 10 ppm showed the significantly highest growth inhibition (100%) against *S. rolfsii* (100%), *M. phaseolina* (47%) and *R. solani* (73%). Walter super was the second most effective weedicide that gave 78, 57 and 35% inhibition in growth of *S. rolfsii*, *M. phaseolina* and *R. solani,* respectively, when used @ 1000 ppm. Paraquat used @ 1000 ppm gave 33, 100 and 67% inhibition in growth of *S. rolfsii*, *M. phaseolina* and *R. solani,* respectively. Similarly, Glyphosate @ 1000 ppm resulted in 18, 24 and 13% reduction in growth of *S. rolfsii*, *M. phaseolina* and *R. solani,* respectively.

# AFLATOXIN CONTAMINATION IN GROUNDNUT- MANAGEMENT STRATEGIES

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Aflatoxins are poisonous, carcinogenic by-products of *Aspergillus flavus* and *Aspergillus parasiticus*, and these are the most studied and widely known mycotoxins. There are four major types of aflatoxins: B1, B2, G1 and G2. Aflatoxin M1, a metabolite of Aflatoxin B1 in mammals, may be found in the milk of animals eating feeds contaminated by Aflatoxin B1. Suitable temperature and moisture contents play critical role in toxin production during field, harvest and storage so cultural practices such as soil amendments of farm yard manure, wheat straw and gypsum in single or in combination at various growth stages were applied to study the chances of reduced toxin production under medium and high rainfall environments. Pod drying by inverting plants or hanging over tight rope or making heap in field or spreading produce on flour or on netted plate forms were tested for aflatoxin production. Storage of groundnut produce in gunny gags, cotton bags or in polyprelene bags for various durations were compared for aflatoxin production during storage.Toxin removal has great significance for table purpose groundnut. Three year old groundnut samples were used to determine influence of various toxin removal methods e.g, fresh lemon juice, citric acid, hydrogen peroxide or fresh water heated under microwave oven for ten minutes and compared with untreated samples.

# BIOCONTROL AND THEIR MECHANISM IN PLANT DISEASE MANAGEMENT

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Environmental pollution caused by heavy use and misuse of agrochemicals as well as fear-mongering by some opponent of pesticides has responsible for considerable changes in people behaviour towards the use of pesticides in agriculture. So, pest management researcher has tried their efforts on developing the alternative inputs to synthetic chemicals for controlling pest and diseases. These alternatives referred to as biological control. Biocontrol of plant pathogens provides practices compatible with the goals of a sustainable agriculture system. Biocontrol mechanism containing antibiosis, competition, mycoparasitism, cell wall degrading enzyme and induced resistance. These mechanisms are might be never mutually exclusive. These terms are meant to organize the examples into general groups to facilitate the comparisons. Numerous biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people and the environment.

# EVALUATION OF PLANT EXTRACTS USED TO CONTROL THE SEED BORN FUNGAL CONTAMINATION AND ANTIFUNGAL POTENTIAL OF TANNINS EXTRACTED FROM CALLUS CULTURES OF *Achyranthes aspera* L.

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Seed borne fungi are problematic in various ways including *in vitro* cultures and reducing the productivity of plants. Fungicides used to control the seed borne diseases are not ecofriendly. In present study, aimed to test antifungal potential of callus cultures of *Achyranthes aspera* seed born fungal contamination was problematic for seed germination under aseptic environment. Control of seed born fungal contamination by using synthetic fungicide and natural extracts of Ginger and Neem showed in the following descending order; Fungicide ≥ Ginger ≥ Neem. Antifungal potential of tannin content of extracts of seedling leaf, stem and root callus cultures of *A. aspera* were tested against *A. niger* and *F. saloni*. Methanol and petroleum ether extracts of tannin content of leaf and root callus culture grown on medium MSDN (2.0 mg/l 2,4-D +4.0 mg/l NAA) and MSDI (2.0 mg/l NAA+2.0 mg/l) of *A. aspera* showed the maximum and minimum zone of inhibition *i.e.* 34 mm and 2 mm against *A. niger* and *F. salani* respectively. The positive correlation existed between tannin content and inhibitory concentration i.e. MIC. Highest total antifungal activity was exhibited by methanol extract of tannin content of leaf callus culture grown on medium MSDN (2.0 mg/l 2,4-D +4.0 mg/l NAA) i.e. 7500 mg/g while methanol extract of root callus culture on medium MSDI (2.0 mg/l NAA+2.0 mg/l BAP) showed lowest activity i.e. 41.2 ml/g in antifungal assay. The *in vitro* research on *A. aspera* plant may provide alternative use of callus cultures as source material for extraction of tannins at preclinical level.

# EXPLORING THE POTENTIAL OF PLANT DERIVED PRODUCTS ON THE CONTROL OF MYCOTOXINS PRODUCED BY MYCOTOXIGENIC FUNGI

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Mycotoxin contamination is a major problem that causes significant losses in the yield and quality of field crops worldwide. This contamination rises adverse health effects that pose serious threats to both humans and livestock. Mycotoxins are the toxic secondary metabolites that are produced by mycotoxigenic fungi. Members of three fungal genera, *Aspergillus*, *Fusarium*, and *Penicillium*, are the major mycotoxin producers. Mycotoxins such as Aflatoxins, ochratoxins, zearalenone, etc. are unavoidable as they enter our food chain either directly or indirectly and can accumulate in human and animal tissues. Keeping in view the above mentioned circumstances, various strategies have been adapted to exclude mycotoxin contamination. But the use of synthetic chemicals can lead to additional health problems such as the application of fungicides could lead to the development of fungal resistance. By screening all these circumstances, research has been conducted on the management of mycotoxins by using plant-derived products or botanicals. Experimental results have shown that plant-derived products such as neem materials, leaves, seeds or kernal powder, and oil comprised of various bioactive components called phytochemicals that can effectively control mold growth. Phytofungicides can be formulated from the leaves, seeds, stem bark, or roots of plants and can be applied in the form of extracts, powders, and cakes, or as plant exudates. In particular, these plant-based bioactive compounds are efficiently used to inhibit toxigenic fungi and decontaminate mycotoxins in food without leaving any health and environmental hazards to the consumers. There is a strong need to explore the potential phytofungicides to control the mycotoxin producing fungi.

# POTENTIAL ANTIBACTERIAL AND ANTIFUNGAL CONSTITUENTS OF *Plumeria rubra* FLOWERS

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This study reports the antibacterial and antifungal activities of flower extracts of famous gardens and parks plant *Plumeria rubra* L., and identification of the possible antimicrobial compounds through GC-MS analysis. Petals were extracted in methanol and the effect of different concentrations (5, 10, 20, 50, 100, 500 and 1000 ppm) of this extract was checked against five bacterial species viz. *Salmonella* sp., *Escherichia coli, Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp., and five fungal species namely *Trichoderma harzianum, T. viride, T. hamatum, T. koningii* and *T. reesei.* All the extract concentrations significantly reduced growths of all the target microorganisms. There was 5–90%, 13–88%, 5–80%, 6–74% and 4–70% reduction in growth of *E. coli*, *Pseudomonas* sp., *Salmonella* sp., *Staphylococcus* sp. and *Bacillus* sp., respectively. Similarly, different concentrations of the extract suppressed growths of *T. hamatum*, *T. koningii*, *T. harzianum*, *T. reesei* and *T. viride* by 32–98%, 28–98%, 26–94%, 24–93% and 16–90%, respectively. The extract was analyzed by GC-MS that revealed the presence of 25 compounds with 9-octadecyne (22.41%) as the predominant constituent followed by benzofuran, 2,3-dihydro- (21.39%). Other prominent compounds were 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (12.59%), 2-cyclopenten-1-one, 2-hydroxy- (7.81%), cyclooctene, 1,2-dimethyl- (7.54%), cyclononanone (2.60%), and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (2.29%).

# ENDOPHYTIC FUNGI, A SUSTAINABLE SOLUTION FOR PLANT DISEASE MANAGEMENT

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Endophytic fungi (EF) are the microscopic organism that present in healthy plant tissues for all or part of their life cycle without causing any diseases. The study of EF is now a valuable impact because of their substantial role between explored and unexplored fungal diversity and their high potential for producing unique natural products inside plants. EF produced products have a big role in the development of resistance to biotic and abiotic stresses predominantly in agriculture crops. Fungal endophytes play a pivotal role in the growth promotion and inducing resistance to various biotic and abiotic stresses in plants. Different types of EF isolated mostly from woody plants were enormously active against *Phytophthora infestans* in tomato plants while endophytic *Fusarium oxysporum* proved effective against Fusarium wilt in tomato plants. The plant viruses are mostly transmitted by Whitefly and Aphids which have sucking mouth parts. Endophytic fungi are known to produce a large number of insecticidal metabolites such as destruxins, ibotenic acid, pantherine, tricholomic acid, etc which make plants undesirable for sucking insects limiting the dissemination of plant viruses which is an highly effective management strategy for plant viral diseases. The studies have revealed that some endophytic-infected *Festuca arundinaceum* seem to be more resistant to *Barley yellow dwarf virus* (BYDV). The EF also produce some compounds which are toxic to nematodes and demonstrated in control of *Pratylenchus scribneri*. by *Fusarium arundinacea*. The endophytic fungi have good potential for utilization as safe and cost-effective alternative to chemical pesticides which is an eco-friendly approach towards sustainable agriculture.

# CONTROL OF *SCLEROTIUM ROLFII* BY STEM EXTRACT OF *DATURA METEL*

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The present study aimed to investigate the antifungal activity of methanolic stem extract of *Datura metel* L. and its different organic solvent fractions against *Sclerotium rolfii* Sacc., the cause of collar rot disease of bell pepper (*Capsicum annuum* L.). In a laboratory bioassay, different concentrations (0.5–4.0% w/v) of methanolic stem extract significantly reduced fungal biomass by 69% to 86%. Methanolic stem extract was fractionated sequentially with four organic solvents namely *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Chloroform fraction exhibited the highest antifungal activity. Different concentrations (3.125–200 mg $mL^{-1}$) of this fraction significantly reduced fungal biomass by 50–71% over control. GC-MS analysis of chloroform fraction reveled 6 compounds in this fraction namely oleic acid; hexadecanoic acids; 1-octadecanol; 1-heptadecene; 1,6,10- farnesatrien-3-ol and 1-pentadecanol.

# EVALUATION OF GERMPLASM AGAINST LEAF RUST AND ITS IMPACT ON YIELD AND YIELD COMPONENTS OF WHEAT

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Rusts of wheat (*Triticum aestivum* L.) are common diseases affecting wheat in all parts of the world where wheat is sown. There are three kinds of rust that are destructive to wheat which is caused by *Puccinia triticina*, wheat stem rust (*Puccinia graminis f. sp. Tritici*) and wheat stripe rust (*Puccinia striiformis f. sp. Tritici*). Rust fungi are obligate biotrophs or parasite which requires living host's tissues to survive and reproduce.In order to evaluation of germplasm against leaf rust and its impact on yield & yield components of wheat crop. A total of 36 wheat lines including susceptible check highly susceptible variety were sown at Nuclear Institute of Agriculture (NIA) Tandojam Sindh to observe their adaptability and performance in Agro climatic condition of this area. The correlation coefficient (0.1610) depicted highly significant effect of leaf rust on lowering 100 grain weight, ultimately the wheat yield. Variation in resistance level was also observed among different wheat genotypes. The extensively cultivated wheat genotype, 11 and 1 proved to exhibit minimum (0.12 g) and (0.21 g)grain weight loss followed by 33, 28, 26, 18, 14 and 25, (4.26 g), (4.24 g), (4.22 g), (4.14 g), (4.14 g) and (4.11 g). The 5, 32 and 24 however, expressed as the most susceptible from all the genotypes with maximum grain weight loss (5.00 g), (5.23 g) and (5.21 g). Evaluation of disease resistance revealed that wheat genotypes33, 28, 26, 18, 14 and 25 proved to be the moderately susceptible whereas 5, 32 and 24 ranked as susceptible to *Puccinia triticina*. Among the 34 wheat genotypes grown to assess the yield loss, sowing of 5, 32 and 24 was recommended because of their potential to withstand heavy yield losses inflicted by the leaf rust. It is concluded from this study that among all the varieties tested 1 and 11 genotypes were found highly resistant which should be for multiplication.

# SEED BORNE MYCOFLORA OF SOME CEREAL CROPS (WHEAT, BARELY AND SORGHUM) FROM FIVE REGIONS OF MAKIRAS /YEMEN

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The present study aimed to survey fungi on seeds wheat, Barley and sorghum from 5 four Regions of makiras /yemen. At least eight fungal genera were recovered from Seeds of wheat: (Aspergillus ، Alternaria ، Fusarium ، Helmenthosporium ، Chaetomium ، Curvularia ، Rizopus ، Pencillium). The most frequently isolated fungi were Aspergillus *niger* (32%), *Helmenthosporium sativum* (15%), and the low frequently isolated fungi were Pencillium *sp*(9%) , *Chaetomium globosum* (7%) . the fungal genera were recovered from Seeds of Barely : (Aspergillus ، Alternaria ، Fusarium ، Helmenthosporium ، Curvularia ، Rhizopus ، Pencillium ، Cladosporium ، Chaetomium ، Rhizoctonia ، Macrophomina. The most frequently isolated fungi were *A. niger* (29 %), *A. alternata* (15%), and the low frequently isolated fungi were Cladosporium *sp* (5.5%) , *M. phasolina* ( (4%) . The fungal genera were recovered from Seeds of sorghum : ( Aspergillus ، Alternaria ، Fusarium ، Drechslera ,Curvularia، Cercospor, Rhizopus and Pencillium ) The most frequently isolated fungi were *A. niger* (28 %) , A.flavus (13 %). the low frequently isolated fungi were Pencillium sp (7 %) , *F.moniliforme* (7%). The Seedling Symptoms Test the results showing that , a wheat of seeds ( mikearas variety) , were infected by seed borne mycoflora 32.2 % while were least infected by seed borne fungi , A pathogen on wheat seedling was most prevalent fungus on the seeds of all Regions. The isolated fungi were *C.lunata, F.oxysporum, H. sativum , A. alternata.* A barely of seeds were infected by seed borne mycoflora more than 22.4 % while were least infected by seed borne fungi (15 %) .The isolated fungi were *C. lunata , F.oxysporum, H. sativum A.alternata. Cladosporium sp and polymyxa graminis.* The seeds of sorghum were infected by seed borne mycoflora more than 18.8 % while were least infected by seed borne fungi (12 %). The isolated fungi were C. *lunata , F. oxysporum, Drechslera sorghicola , A.alternata. Cladosporium sp and polymyxa graminis.* The fungi associated in wheat, barely and sorghum. reducing the germination capacity. Samples were collected from plants were checked under microscope. Some roots of barley and wheat were detected to be infected by *Polymyxa graminis*. This is the first report of *P. graminis* in yemen.

# RESPONSE OF PHYTOEXTRACTS AND CHEMICALS AGAINST ROOT ROT OF CARROT CAUSED BY *Phoma* spp.

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Carrot (*Daucus carota* L.) is a biennial herbaceous species and belongs to *Apiaceae* family. Root rot of carrot caused by *Phoma* spp. is the most destructive disease of carrot and cause huge economic losses. The present study was conducted in order to evaluate response of phytoextracts and chemicals against *Phoma* spp. Infected carrots with typical symptoms were collected from the local fruit market of Faisalabad. These samples were further processed in the Phyto-pathological Laboratory, Department of Plant Pathology, UAF, for the isolation and purification of the suspected pathogen. Three phytoextracts named as turmeric (*Curcuma longo*), cloves (*Eugenia caryophyllata*) and black pepper (*Piper nigrum*) were used with three concentrations 10%, 20% and 25% to check their efficacy against *Phoma* spp. under lab conditions by using poisoned food technique. The experiment was done under complete randomized design (CRD). Results expressed that among three phytoextracts black pepper expressed lowest mycelial growth at concentration of 10% (14.139), 20% (12.889) and 25% (10.417). Three different fungicides named as Nanok (Flutrifol + Azoxystrobin), Novice (Azoxystrobin + Difenoconazole) and Bloom (Myclobutanil) were used with three 400,600 and 800 ppm concentrations against *Phoma* spp. Bloom expressed minimum fungal growth at concentration of 400ppm (13.250), 600ppm (7.556) and 800ppm (5.994). Bloom (Myclobutanil) fungicide, black pepper (*Piper nigrum*) extract and their combination as Bloom (Myclobutanil) + black pepper (*Piper nigrum*) extract were evaluated against *Phoma* spp. under field conditions. The experiment was done under complete randomized Block design (CRBD). Bloom + *P. nigrum* showed a substantial reduction in disease incidence (5.833%).

# INTEGRATED MANAGEMENT OF FUSARIUM WILT OF CHILI CAUSED BY *Fusarium oxysporum* f. sp. *capsici* THROUGH DIFFERENT MANAGEMENT APPROACHES

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Chili (*Capsicum annuum* L.) is a solanaceous vegetable crop. Chili is an important cash crop of Pakistan, cultivated in various parts of country for local consumption and export purpose. *Fusarium* wilt is most destructive disease of chili which potentially reduce the yield of crop. For the management of *Fusarium* wilt of chili six different plant activators were observed under field condition with three concentrations 0.5, 0.75 and 1% respectively, the minimum disease incidence expressed by Salicylic acid followed by benzoic acid, citric acid, $K_2HPO_4$, $KH_2PO_4$ and ascorbic acid. There were five plant extracts (*Moringa oleifera, Zingiber officinale*, *Azadirachta indica*, *Aloe vera*, and *Allium sativum*) and four chemicals (carbendazim, Topsin-M, Capnazol and Alliete) observed by poisoned food technique at the rate of 10%, 15% and 300 ppm, 500 ppm respectively. Among all treatments, *M. Oleifera* and Carbendazim showed maximum reduction in mycelial growth as 6.41 mm and 4.26 mm. After that another *in-vivo* assessment through integrated management approach, *M. oleifera,* Salicylic acid and Carbendazim were assessed under field conditions against wilt disease of chili caused by *F. oxysporum* f. sp. *Capsici* the minimum disease incidence was expressed by Carbendazim (19 %) followed by Salicylic acid (25%) and *M. oleifera* alone (33%) and in combination, minimum disease incidence expressed by Salicylic acid + *M. oleifera* + Carbendazim (13%) followed by Salicylic acid + Carbendazim (15%), *M. oleifera* + Carbendazim (15%), and Salicylic acid + *M. oleifera* (20%) as compared to control. All treatments showed significant results but the minimum disease incidence was exhibited by Salicylic acid + *M. oleifera* + Carbendazim through integrate management (11%).

# STUDY OF EPIDEMIOLOGICAL FACTORS FOR THE DEVELOPMENT OF FUSARIUM WILT OF CHILLI CAUSED BY *Fusarium oxysporum* f. sp. *capcici* UNDER FILED CONDITION

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Environmental factors are considered to be the most important for establishment and development of plant diseases. Fusarium wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici* is one of the most devastating chilli pepper diseases. The disease development is favoured by some typical environmental factors which were studied in this study. For current study the chilli crop was grown at research area of department of Plant Pathology, UAF. Before transplantation of chilli nursery soil were artificially infested with 7-10 old pure culture of *Foc* suspension of $1x10^6$ spores/ml of water. Soil moisture was measured by soil moisture meter while soil temperature was measured by soil thermometer. Environmental factors including maximum and minimum air temperature, relative humidity (R.H), evaporation, rainfall, sun shine and wind speed were examined under natural field conditions. Maximum and minimum air temperature, evaporation, sun shine, maximum and minimum soil temperature and soil moisture expressed positively significant correlation, relative humidity expressed negatively significant correlation, while rain fall and wind speed expressed non-significant correlation ($P \leq 0.05$) with disease incidence on all lines/varieties of chilli. Maximum disease incidence (65-78%) was recorded at maximum (21-38$^o$C) and minimum (9-21$^o$C) air temperature, relative humidity (21-61%), evaporation (3-7%), sun shine (6-10 h), wind speed (3.3-6.4 km/h), maximum (21-36$^o$C) and minimum (8-14$^o$C) soil temperature and soil moisture (21-31%).

# ASSESSMENT OF ANTIFUNGAL ACTIVITY OF ORGANIC FRACTIONS OF *Azadirachta indica AND PHYTOCHEMICAL ANALYSIS WITH GC-MS*

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Medicinal plants are great source of bioactive compounds that have tremendous applications in agriculture. *Azadirachta indica A. Juss.,* member of family *Meliaceae* is well known for its fungicidal effect and used to control many plant pathogens. The aim of the present study is to investigate the antimicrobial potency of methanolic leaves extract and its *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions by poisoned food technique against *Alternaria tenuissima.* Results indicated that highest concentration of these fractions gave the inhibition of 60.14 %, 49.96%, 59.77%, 51.86%, 98.34%, and 56.65% respectively. Best result oriented *n*-butanol fraction was kept under Gas chromatography–mass spectrometry (GC-MS) analysis to identify the phytochemicals present in its. Results revealed the presence of valuable bioactive compounds such as Dodecanoic acid; 2H-Indeno[1,2-b] furan-2-one,3,3a,4,5,6,7,8,8b- octahydro-8,8-dimethyle; 10-octadecenoic acid,methyl ester; 9,12- Octadecadienoic acid(z,z)-,2,3- dihydrooxypropyl ester which might be responsible for its antifungal activities. Hence, it can be concluded that all organic crude leaf extracts of *Azadirachta indica* could possibly be used as antifungal agents.

# EVALUATION OF DIFFERENT CHEMICAL ELICITORS AGAINST FUSARIUM WILT OF CHILIES CAUSED BY *Fusarium oxysporum*

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Chili is one of the most important spice crops in the world, it is grown both for national consumption and export purposes, also having nutritive value especially rich in vitamin C and also valued for its pungency which is imparted by an alkaloid, capsaicin, and red pigments viz., capsanthin, capsorubin, and capxanthin. Amongst the diseases of chili, Fusarium wilt has become a serious problem in recent years in all chili growing irrigated zones of Pakistan. The pathogen (*Fusarium oxysporum*) is known to cause wilting disease in chilies ultimately leading to poor growth and low yield. This pathogen is generally controlled by using commercial-scale synthetic fungicides. The excessive and indiscriminate use of these synthetic chemicals is polluting the environment along with rising concerns for human health. The different elicitors include salicylic acid (SA), magnesium chloride (MgCl2), and acibenzolar-5-methyl (Bion) were tested against *F. oxysporum*. The results, SA significantly inhibited the disease severity (34%) followed by BION (43.14%) and control (60.22%). Similar outcomes were recorded for minimum mortality rate (1.83%) in SA-treated plants as compared with control plants exhibiting 2.47% mortality. While SA application also enhanced the vegetative growth parameters such as diameter (1.2 mm), stem fresh weight (3.5g), stem dry weight (0.106 g), stem length (26 cm), root length (8.3 cm) as compared with BION (1.01 mm, 3.49 g, 0.102 g, 21.08 cm, 7.26 cm) and MgCl2 (0.73 mm, 3.36 g, 0.98 g, 19.40 cm, 6.64 cm) respectively. Conclusively, these elicitors showed improved growth in the *F. oxysporum* affected chilies plant by inducing better resistance against the disease of fusarium wilt. Therefore, these elicitors could use to induce broad disease resistance and offer an alternative option to farmers to overcome the use of fungicides and it will also be beneficial for human health.

# ASSESSMENT OF WHEAT LEAF RUST FROM LOWER SINDH AND IT'S MANAGEMENT THROUGH DIFFERENT FUNGICIDES

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Several fungicides can effectively control the leaf rust of wheat. In most cases, however, 4 to 10 applications per season are required for complete control of the rust because of the low income return per acre of wheat; such a control program is not economically practical. Two applications of some fungicides, coordinated with forecasts of weather conditions favoring rust epidemics, may reduce damage from leaf rust by as much as 75%. These chemicals, which have both protective and eradicative properties, and therefore even two sprays, one at trace to 5% leaf rust prevalence and the second 10 to 14 days later, can give an economically rewarding control of rust. In order to assessment of wheat leaf rust from lower Sindh and it's management through different fungicides in field of the farmer fields were surveyed i.e. Hyderabad, Tando Muhammad khan, Thatha & Badin. From each district samples were collected after each ten km. The analysis of data showed that effects of different fungicides were significant ($P<0.05$). The results indicated that (Tilt®250 E.C 10ml/4liter of water). Fungicide maximizing result recoded under Plant heightcm, Pedicle lengthcm, Awn lengthcm, number of plants ($ft^2$), Numbers of spike $plant^{-1}$, numbers of internodes $plant^{-1}$, Grains weight g, 100 grains and yield followed by (Mancozeb 250%W.P 7gram/ 4 liter of water) and (Sulphur 80%W.G4 gram/4liter of water) was observed under Plant height cm, Pedicle length cm, Awn length cm, number of plants ($ft^2$), numbers of spike $plant^{-1}$, numbers of internodes $plant^{-1}$, grains weight g, 100 grains and yield. However the minimum results were recorded under (Bloom 25% E.C, 2ml/4liter of water) and (Topsin M 100% E.C 7ml / 4 litre of water. It is concluded from the results that the fungicide (Tilt®250 E.C 10ml/4liter of water) performance best in all agronomical traits including yield which should be recommended and used against leaf rust of wheat.

# OYSTER MUSHROOM (*Pleurotus pulmonarius*) CULTIVATION USING LOW- COST RESIDUES (WASTEPAPER, DATE PALM LEAVES, CORN COBS AND COTTON WASTE)

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Different agro -wastes contain minerals, cellulose and lignin which promote the growth of oyster mushroom. Oyster mushroom (*Pleurotus spp*.) is one of the very attractive crops successfully cultivated in most developing countries due to its easy cultivation technology. High yield of Oyster mushroom cultivation can be achieved through the usage of these low- cost agricultural wastes. In this study autoclaved sterilized substrates such as date palm leaves, corncobs, wastepaper and cotton waste were used for the analysis of yield and growth performance of oyster mushroom (*Pleurotus pulmonarius*). Under complete randomized design, combinations of these substrates were assessed on the basis of parameters i.e mycelial growth, days to pinhead formation, no. of pinhead formation, days to fruiting bodies , yield of three flushes, fresh weight, total yield and B.E. Significant ($P<0.05$) results declared that compared to all other substrate combinations, T5 (25% date palm leaves+ 25% corncobs+ 25% wastepaper+ 25% cotton waste) proved to be best as it gave peak values of mycelial growth , lowest days to pinhead formation (52 days), no. of pinhead formation (53), took minimum days to fruiting bodies (6 days) , yield of three flushes (158 g),(148 g and 128 g), fresh weight (434 g), highest B.E (87%) and 434.8 g yield. So according to results, T5 could be aggressive growing option for farmers to cultivate maximum *Pleurotus pulmonarius* including exported values.

# IDENTIFICATION OF POSSIBLE ANTIFUNGAL CONSTITUENTS IN LEAVES OF *KOCHIA INDICA* FOR CONTROL OF *MACROPHOMINA PHASEOLINA*

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This study was aimed to analyze antifungal potential of leaf extract of *Kochia indica* (Wight) Scott, a weed of Chenopodiaceae, against *Macrophomina phaseolina* and identification of phytoconstituents through GC-MS analysis. Different concentrations of methanolic leaf extract (1 to 5%) reduced biomass of the fungus by 66 to 80%. The extract was partitioned using various organic solvents. Among these, different concentrations (1.56 to 200 mg $mL^{-1}$) of *n*-hexane, chloroform and ethyl acetate fractions caused 44–89%, 77–92% and 84–93% decline in *M. phaseolina* biomass over control, respectively. GC-MS analysis of these fractions showed presence of 4-pyrimidinecarboxylic acid; sitosterol; 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl)ester; di-*n*-octyl-phthalate; acetic acid, butyl ester as major constituents, which may be responsible for decrease in growth of *M. phaseolina*.

# COMBINE APPLICATION OF ESSENTIAL OILS AND GUM ARABIC COATING INFLUENCES POSTHARVEST STEM END ROT AND QUALITY OF MANGO FRUIT

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*Lasiodiplodia pseudotheobromae* is one of the most emerging postharvest pathogens responsible for stem end rot (SER) of mango fruit in Pakistan that results in major economic losses. To determine the effect of gum arabic (GA), clove oil (CLO), cinnamon oil (CMO) and their combinations on control of *L. pseudotheobromae in vitro* as well as *in vivo* and maintenance of fruit quality during storage, mango fruit treated with 8% GA, 0.50% CLO, 0.25% CMO and their combinations CLO+GA and CMO+GA were stored at 12°C for 28 days. Data regarding antifungal assays and fruit quality were taken at 7 days interval. Fruit treated with GA alone did not show any promising results while the combination of 8 % GA and 0.50% CLO exhibited highest reduction in mycelia growth (78%), SER disease development (82%) and also delayed the reduction of weight loss, fruit firmness, soluble solid contents, titratable acidity and ascorbic acid contents of mango fruit as compared to other treatments and control. The results suggest the possibility of using 8 % GA and 0.50% CLO as a new tool for management of postharvest SER disease as well as maintaining quality of mango fruit.

# IDENTIFYING *COLLECTOTRICHUM FALCATUM* WENT. CAUSING RED ROT OF SUGARCANE USING TISSUE-BLOT IMMUNOASSAY (TBIA)

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Red rot of sugarcane caused by *Colletotrichum falcatum* Went is one of the most destructive diseases of sugarcane (*Saccharum officinarum* L.) in Pakistan. In order to develop immunological method for detection of *C. falcatum*, its mycelium culture was purified from the susceptible clone SPF-234 and used as antigen source to produce polyclonal antibodies in albino rabbits. These antibodies were tested to detect *C. falcatum* by tissue-blot immunoassay (TBIA). A total of 26 sugarcane clones each with 10 replications of plants were tested simultaneously by serological and symptom expression following plug inoculation. The plug inoculation method tests showed that 13 of 26 tested sugarcane clones with symptoms indicating infection by *C. falcatum* spread and 13 clones with no symptoms of infection; whereas, in duplicate samples tested using TBIA to assay the internode above the inoculation site only 6 samples were negative for *C. falcatum*. The TBIA membranes showed dark blue stain color in the 20 samples, which was positive for *C. falcatum* and the other six were negative. The TBIA results exhibited that the developed polyclonal antibodies were highly reactive to *C. falcatum*. The high specific reactivity and sensitivity of the antisera indicate its potential suitability for TBIA-based detection of *C. falcatum*. Comparison of artificial inoculation of *C. falcatum* symptoms through plug method and TBIA detection of the pathogen indicated that TBIA can be used to complement the conventional inoculation method for early detection of red rot susceptible clones.

# SEED TO PLANT TO SEED TRANSMISSION OF WHEAT BLAST PATHOGEN *Magnaporthe oryzae* TRITICUM

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Experiments were conducted to investigate the possibility and the extent of infection of blast pathogen in wheat seeds in a seed lot and transmission of the pathogen from seed to plant for selection of healthy seeds. Seed samples of wheat variety BARI Gom23 were collected from the farmers of three upazila of Meherpur district such as Meherpur sadar, Mujibnagar and Gangni. Seed samples were representative of 2019 harvest. Seeds were incubated in moist blotter in room temperature (30 C ± 1) and were examined under stereo-binocular microscope for seed infection. Any growth of *Magnaporthe oryzae pv* Triticum (MoT) seen under stereo-binocular microscope was confirmed through microscopic slide preparation. In seed samples, seed infection of MoT ranged 0-22% were grouped into four treatments (0, 1-10, 11-20 and >20%) which were later sown in pots. Seed germination ranged from 83 to 87% for the four seed categories. The plants grown out of four category seeds were kept under observation for blast infection and its severity on the four treatments under natural conditions. Seeds and plant parts (root, stem, rachis) were processed for DNA extraction, PCR was run with primer MoT3. For anatomical study, TS of root, stem and rachis was made. Plants grown out of the four groups of seeds all except healthy seeds carried blast infection showing different levels of bleached spikes. Agarose (1.5%) gel electrophoresis produced monomorphic bands of 361bp in all categories of seeds and plant parts except those from healthy seeds. TS of plant parts displayed both intra- and inter-cellular mycelium of MoT in the ground tissues and xylem bundles of all the parts (root, stem and rachis) of plants. The results of this study confirmed the seed infection as observed in moist blotter was of *Magnaporthe oryzae* pv Triticum and also confirmed the transmission of pathogen from seed to plant to seed through vascular bundle. It may be concluded that MoT is both seed-borne and seed transmitted.

# SECTION 2: PLANT BACTERIOLOGY

# BIOLOGICAL NANOPARTICLES BASED ON NATURAL MOLECULES AND METABOLITES: SIGNIFICANT GROWTH PROMOTER AND DEFENSIVE AGENTS OF CROPS

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Microorganisms utilized in the form of Plant growth promoting rhizobacteria (PGPRs) play a vital role in maintaining soil fertility and plant health. The microbes residing in the soil are beneficial for the growth of crops in terms of vegetative and reproductive growth are known as plant growth promoting rhizobacteria (PGPRs). They can act as biofertilizers, pesticidal and insecticidal agents. Simultaneously, the PGPRs increase the resistance to biotic and abiotic stress. These are effective growth modulators for the crop as they secrete novel metabolites and growth molecules that enable the crop to sustain in adverse and stress conditions. These molecules also induce systemic resistance and anti- pathogenic effect against the soil borne infections. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirect promotion of growth in many plants, such as siderophores and antibiotics. The PGPRs (growth promoting action, pesticidal and insecticidal action) were found to have positive PGPR traits viz. IAA production, siderophores production, Cytokinin and HCN production. The molecules and secondary metabolites secreted by such PGPRs are beneficial in terms of growth promotion and defensive properties. These metabolites can be utilized further to synthesize nanoparticles after combination with in organic metallic ion. These fused nanoparticles are found to have more significant and beneficial aspects in agriculture. Nanoparticles are 100 mm or less, nanoparticles synthesize by a biogenic enzymatic process. Microorganisms such as bacteria, actinomycetes, and fungi play an important role in the treatment of toxic metal through reduction of metal ions and are considered as potential Nano factories. These microbes and their secondary metabolites are being used for the preparation of biogenic nanoparticles. These nanoparticles are very cost effective and are significant in terms of application potential. The present study will reveal the preparation; formulation and application of biological nanoparticles based on microbes and their metabolites for their growth promoter and defensive action properties on selected crops for sustainable agricultural productivity.

# ROLE OF NONCODING RNAS DURING PATHOGENESIS OF RICE BY *ACIDOVOREX AVINAE*

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Non-coding RNAs (ncRNAs) that are usually 50–500 nt in length have been attracting an increasing amount of attention due to their regulatory roles in various cellular processes in bacteria. With the 'explosion' in the publication of genomic sequences over the past decade, a large number of ncRNAs have been predicted in a variety of bacteria, which includes pathogens of humans, animals and plants. Yet, the function of ncRNAs and their regulatory targets are largely unknown, even though, few ncRNAs are found to be associated with bacterial virulence. In our latest study, genomewide screening and subsequent analysis a high level of condition-dependent differential expression of ncRNA in *Acidovorax oryzae* (Ao) RS-1, which is bacterial brown stripe pathogen of rice. About 66 differentially expressed ncRNAs wer experimentally validated by using an integrative analysis of conservative genome sequences and transcriptomic data during *in vivo* interaction of the bacterial pathogen with the rice plant. To test the relevance of the differentially expressed ncRNAs, four with different positions within the genome, and with different secondary structures and promoter activities were chosen. The results showed that the overexpression of the four ncRNAs caused a significant change in virulence-related phenotypes, resistance to various environmental stresses, expression of secretion systems and effector proteins, while changing the expression of ncRNA putative target genes. We can conclude that these ncRNAs are examples for the inherent regulatory roles for many of the observed ncRNAs in response to changing conditions such as host interaction or environmental adaption.

# ROLE OF QUORUM QUENCHING BACTERIAL CONSORTIA IN TACKLING *PECTOBACTERIUM*; A QUORUM SENSING PATHOGEN

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Blackleg, a bacterial disease caused by *Pectobacterium atrosepticum* (Pba) inflicts incredible losses to potato crop in fields. In *Pectobacteria,* pathogenicity and virulence are regulated through quorum sensing (QS) in a density dependent manner utilizing N-acyl homoserine lactones (NAHLs) as QS signalling molecules. Interference with QS through Quorum quenching (natural or engineered) is a demonstrated biocontrol tool. In this study, potato plants with characteristic blackleg symptoms from Okara, Chiniot and Faisalabad districts of Punjab were investigated and the pathogen responsible for the disease was identified as Pba through pathogenicity, biochemical (Crystal Violet Pectate) and tuber assays. PCR analysis of *Pectobacterium* was also done by using gene specific primers. NAHLs produced by the local Pba strains were determined and evaluated through biosensor-based plate assays using *Chromobacterium violaceum* (Cv026) and *Agrobacterium tumefaciens* (NTLR-4) biosensors. Also, several NAHL degrading strains were sorted out from rhizosphere soil and their ability to degrade exogenous NAHLs was evaluated. Out of 36 bacterial isolates, nine effective quenchers were then trialed against *Pectobacterium* under plate and tuber assays that effectively reduced *Pectobacterium* based infections in potatoes.

# EPIDEMIOLOGICAL AND BIOCHEMICAL FACTORS FOR THE DEVELOPMENT OF BACTERIAL BLIGHT OF POMEGRANATE

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Pomegranate *(Punica granatum*), is a delight and "heaven fruit" which has grown worldwide. The present research comprises studies on epidemiological and biochemical characterization and alteration in ionic profiling in diseased and healthy leaves of pomegranate. Different isolates of the bacterial blight pathogen were isolated from infected leaves, twigs and fruits and grown on YDCA and NA medium. Disease incidence and severity were measured from five different locations (Ali pur, Multan, Shujabad, Layyah, and D.G khan) of southern Punjab. Maximum disease incidence and severity was observed in Multan D.G khan and Ali pur while the minimum was observed in Shujabad and Layyah. Epidemiological factors such as maximum and minimum temperature, humidity, rainfall and wind speed play a key role in the spread and development of disease. The pathogen spreads very fast at high temperatures and low moisture. Temperature, rainfall and wind speed showed a positive correlation to disease development while relative humidity showed a negative correlation. Different biochemical tests (Gram staining, KOH, catalase, oxidase, starch hydrolysis, gelatin liquefication) were performed for the identification of the bacteria. The results of all tests confirmed the presence of *Xanthomonas axonopodis* pv *punicae*. Pathogenicity was done in the greenhouse by using three different methods (Pin-pricked, infiltration and spray method) and symptoms appeared in 8 days and the infiltration method showed early symptoms as compared to the spray and pinprick method. Ionic contents (Nitrogen, Potassium, Phosphorus, Magnesium, Calcium, Iron, Boron, Zinc and Copper) reduced the growth, multiplication and survival of pathogenic bacteria. Proper application of these nutrients helps the host plant to improve its physiological and biochemical development and ultimately helps to improve resistance to bacterial blight disease in pomegranate.

# IMPACT OF CLIMATE CHANGE ON POTENTIAL DISTRIBUTION OF BACTERIAL STALK ROT OF MAIZE DISEASE IN SIALKOT DISTRICT, PAKISTAN

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Maize (*Zea mays* L.) is the 3rd most influential crop by its bulk production all over the world. It majorly contributes towards food security in several developing countries of Asia and Africa. However, the maize production is affected by the invasion of many phytopathogens resulting into many diseases at various growth stages and at various hotspot regions. Of these diseases, bacterial stalk rot of maize caused by *Erwinia* sp., result into significant crop yield reduction, thus need to be addressed. The aim of the study was to predict the potential distribution of bacterial stalk rot of maize disease in Sialkot region, Pakistan and produce epidemiological evidence for the management of the disease outbreaks in the region. An extensive survey revealed that out of 266 visited areas, high disease incidence was observed in Bhoopalwala (78.5 %) followed by Bangla Chowk (76 %), Suraj (74 %), Bakhray wali (68.5 %), Wasar key (67.5 %) and Bopal wala (66.5 %) while lowest disease incidence was recorded from Pasrur (20 %), Chawinda (15 %) and Head marala (9 %). The Maxent algorithm modelling was used to predict areas at risk of disease outbreaks based on the occurrence and environmental data in Sialkot regions; the model was later used to predict or forecast the disease outbreaks in coming years. Among the nineteen bioclimatic/environmental variables, four factors including Temperature seasonality (standard deviation*100) (bio-4), Mean temperature of wettest quarter (bio-8), Annual precipitation (bio-12) and Precipitation of driest month (bio-14) were found to be the most critical factors influencing the disease distribution in current and coming years. It is predicted that high risk area and disease distribution will increase across the four districts of Sialkot Pakistan over the years in 2050 and 2070. These results are important for policymakers and researchers to effectively take disease control measures for bacterial stalk rot of maize disease outbreaks in the Sialkot region.

# ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN PLANT GROWTH PROMOTION AND DISEASE MANAGEMENT

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Crop losses due to pathogens are a persistent issue in the world and the present situation reflects a serious threat to food security. To avoid this loss, various strategies are in use for disease management like biological methods, cultural and chemical control. Use of chemical fertilizers promotes crop production but this approach is not eco-friendly resulting in deleterious effects on the environment and biota of this planet. Apart from this, they are disrupting the numbers of beneficial microbes present in the soil that are capable to enhance soil fertility. Thus, there is a need to find alternative strategies to minimize the dependency on harmful chemicals. PGPR, due to low cost and easy applicability gained much attention in the agricultural sector. PGPR are directly involved in promoting plant growth by the secretion of phytohormones, fixation of atmospheric nitrogen, siderophore production, etc. Indirectly, PGPR act as a bioprotectant, produce antibiotics and provide protection to the plants against harmful phytopathogens. Many researches have reported the efficacy of PGPR as a single strain or in the consortia form to suppress the notorious plant pathogens and plant growth promotion. PGPR application initiates a cascade of reactions that helps in plant growth promotion and disease suppression. Being cost-effective, eco-friendly, and naturally occurring microbes, PGPR can be effectively employed in controlling the phytopathogens and plant growth promotion under the sustainable agriculture system.

# ASSESSMENT OF ANTIBIOTICS AGAINST *ACIDOVORAX AVENAE* CAUSING RED STRIPE DISEASE IN SUGARCANE

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Red stripe is a bacterial disease of sugarcane causing important economic losses in Pakistan that affects 30 % of the milling stems and consequently the juice quality. In this study, sugarcane leaves exhibiting red stripe symptoms were sampled in the 2020–21 growing season from District Chiniot. Isolation of bacteria was done from these sampled leaves. Conformation of *Acidovorax avenae* subsp. *Avenae* was done through pathogenicity test. In Molecular Phytopathology laboratory experiment was conducted using CRD to study the efficacy of 5 antibiotics viz. Neflox (Florfenicol 65%), Trisulpha (Sulphamerazine 55%), Velosef (Cephradine 75%), Rethmo (Clarithromycin25%), Inocef (Ceftriaxone sodium 45%) through inhibition zone technique were evaluated. It was concluded that Inocef showed best results followed by Velosef, Trisulpha, Rethmo and Neflox. Through antibiotics management is easy, direct, rapid action and helped to solve disease problems.

# COMPARATIVE STUDIES ON INFECTION FREQUENCY OF APICAL NECROSIS OF MANGO-AN EMERGING THREAT TO MANGO ORCHARD

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Mango (*Mangifera indica* L.) is an important fruit of warm and humid climatic areas. Mango is one of the most cultivated fruit in tropical and sub-tropical areas. Mango belongs to family "Anacardiaceae". It occupies 2nd position in Pakistan amongst fruits but it is prone to several diseases caused by biotic and abiotic factors. Due to these diseases the production of mango fruit is comparatively low in Pakistan as the area is increasing every year but the production has not been increasing accordingly. At the moment it is facing a disease that shows necrosis and blackening of floral buds which is spreading more and more as the time is passing. The disease is known as apical necrosis of mango caused by *Pseudomonas syringae*. Now, it is expected that in near future this disease may become an epidemic. So, during 2019 the current study was planned to get an idea about the infection frequency of causal organism. To calculate the infection frequency, isolation were made on King's B agar medium from floral buds at each stage (from stage A to stage K) by following standardized protocols. Results revealed that maximum infection frequency of *Pseudomonas syringae* was observed at stage A (Dormant Bud), i.e., 95.67%. The 2nd maximum infection frequency (91.00%) was noted at stage B (Bud Swell) followed by 89.66% at stage C (Bud Break). Whilst, the least infection frequency (58.67%) was noted at stage J (Fruit Set) just before the fruit development. The same experiment was conducted during 2015 and the results of that experiment revealed that maximum infection frequency of *Pseudomonas syringae* was observed at stage A by 80.22%. At this stage minimum temperature (16.25°C) and the maximum temperature were recorded as 29 °C. while average relative humidity at stage A was 89.25% followed by 69.33% which was noted at B stage. At C stage 20°C temperature as minimum while the maximum temperature (33.25 °C) were recorded with the average relative humidity of 80%. The least infection frequency was noted at J stage of flowering by 2.22%. The comparison of both data revealed that during the year of 2015 the infection frequency was less as compared to the infection frequency calculated during the year of 2019. This also revealed that with the passage of time the disease is increasing with great extent and if the disease may not managed properly then the there will be no ambiguity that apical necrosis of mango become an epidemic to the mango orchards.

# DEVELOPMENT OF DISEASE FORECASTING MODEL FOR BACTERIAL BLIGHT OF COTTON BASED ON WEATHER PARAMETERS WITH RESPECT TO DIFFERENT SOWING DATES

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Cotton is an important fiber cash crop extensively sown in tropical and sub-tropical areas around the globe. Bacterial blight of cotton is a re-emerging threat on cotton crop in Pakistan under climate change scenario. The present study was conducted to develop a disease forecasting model based on weather parameters according to different sowing dates. Seeds of fifteen cotton varieties/lines were sown at experimental are of Plant Pathology Research Institute, Faisalabad. Three different sowings were done at 15 days intervals. Maximum and minimum temperature, relative humidity, rainfall, and wind speed data were recorded from a mini weather station. Disease severity data were recorded using Brinkerhoff's disease rating scale at 15 days intervals after the appearance of the disease. The relative impact of different sowing dates was evaluated using analysis of variance (ANOVA). Pearson's Correlation was used to find the correlation between disease severity and weather parameters. Stepwise forward regression was used, and the model was selected based on high $R^2$, low Mallow's CP and low Mean Square Error (MSE). A significant difference in disease severity was recorded among the three sowing dates. Maximum and minimum air temperature, rainfall and relative humidity played a significant role in disease severity in all three sowings. A strong positive correlation was observed between disease severity and maximum air temperature (0.81), relative humidity (0.83), rainfall (0.71) was observed. A negative weak correlation (-0.48) was recorded between minimum air temperature and disease severity. The present study is beneficial to adopt the prophylactic measures to manage the disease according to different sowing dates.

# EPIDEMIOLOGICAL MARKERS AGAINST XANTHOMONAS CITRI PV. CITRI: A POTENTIAL THREAT TO GLOBAL CITRUS INDUSTRY

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Citrus canker caused by a notorious bacterium *Xanthomonas citri* pv. *citri* (*Xcc*) is one of the major threats being faced by citrus industry. Ecosystem of crops changing in most of the world due to the climatic effect. In present study, impact of environmental parameters (Max. and min. temperature, rainfall, relative humidity and wind speed) were examined in the major citrus growing areas of Punjab, Pakistan. Significant positive correlation was observed on all varieties between environmental variables and citrus canker. A multiple regression model ($Y = +24.02 + 0.5585\ X_1 + 0.2997\ X_2 + 0.3534\ X_3 + 3.590\ X_4 + 1.639\ X_{5)}$) was also developed to find out the relationship between environmental parameters and disease projection. Goodness of model on statistical ground is indicated by coefficient determination value (97.5%). On conclusion, it was established that all the environmental factors like max. temperature (37 $^0$C), min. temperature (27 $^0$C), relative humidity > 55%, rainfall (4.7-7.1 mm) and wind speed 8 km/h were the conducive for the development of citrus canker.

# EXPLOITATION OF NUTRIENTS AND PHENOLIC ANTIOXIDANTS IN CONTROLLING CITRUS CANKER

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Citrus belongs to family *Rutaceae* have high economic, nutritional and medicinal value. It is an important source of foreign exchange for Pakistan from a long time. Unfortunately, its production and quality become victim of several biotic and abiotic diseases among which citrus canker induced by *Xanthomonas citri* pv. *citri* is most dangerous. The current study was planned to check the potential of nutrients and different phenolic antioxidants in the management of citrus canker. For this purpose, mixture of nutrients NPK (Compound) and B, Zn, Fe, Mg, Mn and Ca (Nutriotop) and six phenolic antioxidants (Salicylic Acid, Benzoic Acid, $K_2HPO_4$, Citric Acid, $KH_2PO_4$ and Isonicotinic acid) were evaluated under greenhouse and field conditions. Amng six phenolic antioxidants, Salicylic acid (13.18 %) disease incidence expressed followed by the citric acid (16.18), $K_2HPO_4$ (17.68), benzoic acid (19.18), $KH_2PO_4$ (20.64) and Isonicotinic acid (23.18) % as compared to the control. Nutriotop in combination with Compound expressed very low disease incidence (17.28%) as compared to Compound (26.73%), Nutriotop (32.47%) as well as control. Among nutrient mixture, combination of both Compound and Nutritop and salicylic acid in phenolic antioxidants expressed minimum disease incidence.

# VARIATIONS IN DIFFERENT ISOLATES OF *XANTHOMONAS CITRI* PV. *CITRI* CAUSING CANKER ON THE BASIS OF SYMPTOMS EXPRESSION

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Citrus is an important ancient fruit cultivated across the globe and it is an imperative source of income for many countries. Nonetheless, despite the richness of citrus production across the world, the fruit yield is constantly threatened by many diseases that can cause serious social and economic effects of the citrus growers and consumers. Citrus canker caused by *Xanthomonas citri* pv. *citri* (*Xcc*) cause huge economic and yield losses in Pakistan and all other major citrus growing countries. Disease severity and aggressiveness of *Xcc* was checked in the four tehsils of district Faisalabad and in University of Agriculture Faisalabad (UAF). Variations in the aggressiveness of *Xcc* in different isolates collected from different citrus growing localities of district Faisalabad were also checked on the basis of lesion size in green house. Disease severity of *Xcc* was high in the tehsil Sumundri 22.47% followed by 20.33% in Tandlianwala, 14.2 % Chak Jhumra, 10.13 % Jaranwala and 8.8 % in the University of Agriculture Faisalabad. Maximum lesion size (4.33) mm developed by the isolates of tehsil sumudri followed by Chak Jhumra (3.03), Tandliyanwala (2.53), UAF (2.10) and Jaranwala (1.20). In conclusion, disease severity and aggressiveness of *Xac* was found high in the orchards of tehsil sumundri.

# BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF HUANGLONGBING (HLB) IN LOCAL CITRUS CULTIVARS OF SARGODHA, PAKISTAN

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Huanglongbing (HLB), a century old widely distributed destructive bacterial disease in the citrus growing areas around the globe. The early detection of HLB is one of the key steps to manage the disease in time. Therefore, a study was carried out in citrus growing areas of Sargodha during 2018-2019 for routine, economical, quick detection and molecular characterization of HLB in infected citrus samples collected based on symptomology. The highest incidence of HLB (26%) was in Kot Momin, whereas, the least incidence (4.6%) was in Sahiwal. Similarly, local cultivars viz., mosambi **(*C. sinensis*)** and Kinnow **(*C. reticulata*)** showed the highest incidence of 26% in Kot Momin and 23% in Bhalwal, respectively. Iodo-starch test followed by biological indexing was performed to detect the HLB in citrus plants. The selected citrus samples were subjected to polymerase chain reaction followed by sequencing. Dark grey edges of the infected leaves indicated the accumulation of starch. Inoculated healthy citrus plants showed the characteristic blotchy mottling symptoms after 6-8 months of inoculation. Among the five varieties, the highest disease transmission was in Mosambi, whereas, the least transmission was in lime (16.66%). DNA was isolated from the HLB infected symptomatic leaves samples amplified the β operon (703bp) region. Sequence analysis showed that local bacterial strains had genetic variation from the strains in rest of the world. The present study revealed that iodo-starch test and vegetative propagation provided reliable, economical and quick indexing of HLB in citrus. Moreover, molecular detection aided the detection with genetic variability in bacterial strains.

# MANAGEMENT OF MANGO BACTERIAL BLACK SPOT (MBBS) THROUGH NANOPARTICLES

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Bacterial black spot disease of mango is becoming a potential threat for the mango industry of Pakistan. It causes upto 7-80% yield loss depending on disease triangle. Various chemical, antibiotic, phytoextracts and biological management strategies have been adopted to overcome this malady. These control measures are either having adverse effects on environment or in slow action. Nanotechnology is one of the rapidly advancing and most fascinating science in the field of Agriculture. Use of nanoparticles (NPs) is an environment friendly and effective approach to control bacterial diseases of plants. Silver and copper nanoparticles have excellent antibacterial properties and used as an alternative to pesticides in management of bacterial plant diseases. NPs directly affect the bacterial pathogen and activate defense system of host plant through altering its nutritional status. Silver and copper can be directly toxic to bacterial pathogens while manganese, boron, silicon and zinc act as fertilizer in host defense. As demand for food production is increases globally under changing climate, nanoparticles will play a key role to mitigate new challenges in plant disease management by reducing chemical pesticides and early detection of bacterial pathogens.

# EVALUATION OF THE CHEMICAL CONTROL OF BACTERIAL LEAF BLIGHT (*XANTHOMONAS ORYZAE PV. ORYZAE*) OF RICE IN TRANSPLANTED RICE (*ORYZA SATIVA* L.) CROP

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Bacterial leaf blight of rice caused by Xanthomonas oryzae pv. Oryzae is considered as a major threat to rice production worldwide including Pakistan. Thus field trials were conducted to evaluate different commercially available antibacterial chemicals against this destructive disease on the rice cultivar Pk-386. For this purpose, comparative efficacy of bleaching powder, Kasumin 2SL (kasugamycin), Triseen 5SL (validamycin), Profile 50WP (kasugamycine+copperoxychloride), Coopix 50WP (copper oxychloride) Thrill 20WP (bismerthiazole) was studied as foliar spray applications against BLB at their recommended doses. The experiment was laid out in randomized complete block design with three replications. Among the tested chemicals the disease incidence (60.44%), Disease severity index (16.79%) were lowest for Coopix 50WP with highest yield (6.58 tonnes ha-1) followed by Profile 50WP with 78.56%, 21.96%, and 6.44 tonnes ha-1 respectively. The performance of control plot as compared to other treatments remained poor with highest (98.08%) disease incidence, 58.01% (disease severity index) and lowest yield 50.67 tonnes ha-1. The benefit to cost ratio in cases of Coopix 50WP and bleaching powder were same (1:13) but the incremental increase in income over the control with Coopix 50WP was highest (Rs. 30330) as compared to that of (Rs.16352) with the use of bleaching powder. Therefore, on the basis of results copper oxychloride and kasugamycine+copper oxychloride coukld be recommended for the economic and efficient control of bacterial leaf blight of rice crop.

# EVALUATION OF DISINFECTANTS AND INDUCERS ON SOLANACEOUS VEGETABLES AGAINST SOFT ROT BACTERIA

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Soft rot bacteria *Pectobacterium carotovorum* (*Pcc*) is a major constraint and looming threat to solanaceous vegetable production throughout the world. Management of *Pcc* is paradox as chemicals use is not recommended due to direct consumption of vegetables. Therefore, present study was planned to see impact of different disinfectants and inducers against soft rot bacterium isolated and characterized in early part of the study. Disease was heavily present in solanaceous vegetables viz tomato, bell pepper and potato in different markets of Rawalpindi such as Pirwadae, Raja bazar sabzi mandi and Shamsabad vegetable market. Disease incidence was noted in tomato (23%), bell pepper (30%) and potato (35%) respectively. However, no disease was found in eggplant during study. isolates were found positive for loop tests, catalase test, pectinase test and anaerobic test confirmed the isolates of soft rot bacterium. *In vitro* study data showed that all disinfectants and inducers formed zone of inhibition compared to control. However, zone of inhibition varied and the maximum zone of inhibition was made by streptomycin (3.4cm), boric acid (2.9cm) and chitosan (2.7cm). *In vivo* results were obtained when disinfectants and inducers were screened in vivo against *Pcc* on the solaneceous vegetables. Boric acid and chitosan show complete effectiveness against Pcc followed by sodium hypochlorite and streptomycin. In conclusion, chitosan and boric acid emerged as most effective treatment against soft rot bacterium. It is pertinent to mention that chitosan and boric acid are safe to both for environment and human.

# MOLECULAR CHARACTERIZATION OF BACTERIAL LEAF BLIGHT PATHOGEN FROM PUNJAB, PAKISTAN

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Survey was conducted in the major rice growing areas (Kaller Belt) during the cropping season 2015, to monitor the incidence of some new disease caused by the novel pathogen. The symptoms of this disease are quite similar to that of the Bacterial Leaf Blight (BLB). The climatic changes taking place globally gives us the evidence of the presence of the novel pathogen. Results indicated that in addition to *Xanthomonas oryzae* pv. *oryzae* causing BLB there is a new pathogen *Xanthomonas oryzea pv. oryzicola* causing bacterial leaf streak of rice. Plant samples were collected from the five major districts of Punjab *i-e,* Kala Shah Kaku, Sialkot, Faisalabad, Sheikhupura and Hafizabad during 2015. The isolates obtained were subjected to biochemical identification and were found to be Gram negative, 3% KOH positive and 5% salt tolerance positive. The pathogenicity test was performed to confirm the pathogen and the isolate obtained from Kala Shah Kaku was found to be the most aggressive strain among all the five isolates. It was found that this disease inflicts almost 20% yield losses in major rice growing areas of Punjab. Molecular characterization was done by performing Polymerase Chain Reaction (PCR) followed by DNA Extraction. PCR product was used for sequencing. Phylogenetic analysis was done using that sequence and it was found that this pathogen is having a novel clade as it falls distantly from *Xanthomonas oryzae* pv. *oryzicola*.

# EVALUATION OF COTTON GERM PLASM AGAINST THE BACTERIAL BLIGHT DISEASE CAUSED BY *XANTHOMONAS AXONOPODIS PV MALVECERUM* UNDER FIELD CONDITION

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Cotton is the major crop of Pakistan. Pakistan is 5th largest cotton producing country in the world and also 3rd largest exporter of raw cotton in the world. The experiment was conducted to evaluate the performance of cotton germplasm under field condition against the bacterial blight of cotton caused by *Xanthomonas axonopodis pv malvecerum* which is gram negative bacteria. For the purpose of performance evaluation of germ plasm, nine Bt varieties such as Bt Ali Akbar 802, Bt-FH 177, Bt-FH 182, Bt-NIBGE 2, Bt-CM 615, Bt-CRS 2007, Bt-CM 616, Bt-FH 169 and Bt-IUB 222 and also three non Bt varieties like Sindh1, CRIS134 and CIM 573 were used. The screening trial was conducted for two years under field condition. According to the results six varieties Bt-CM 615, Bt-CRS 2007, Bt-CM 616, Bt-IUB 222, Sindh 1 and Bt-FH 177 were moderately resistant to the disease, three varieties Bt-Ali Akbar 802, CIM 573 and Bt NIBGE 2 were moderately susceptible to the disease and two varieties Bt-FH 182 and Bt-FH 169 were susceptible to the disease while Bt-CRIS 134 was highly susceptible to the disease. The result denoted that the 50% varieties were moderately resistant, 41.6% were moderately susceptible and 9.4% were highly susceptible and even single completely resistant variety was not exist among these popular cotton varieties.

# EFFECTIVENESS OF DIFFERENT FUNGICIDES AND PLANTS EXTRACT AGAINST THE CITRUS CANKER DISEASE CAUSED BY *XANTHOMONAS AXONOPODIS PV. CITRI*

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Citrus canker is a bacterial disease which is caused by *Xanthomonas axonopodis pv. citri.* It badly destroys citrus orchards. Citrus canker is highly susceptible in varieties like Grapefruit, lemon and limes but this disease is destruct citrus on a very large scale in citrus growing areas of Pakistan. Many practices are done to control that disease i.e. eradication, sanitation, and windbreaks these are considered well but the chemical control is not avoided to get rid of this disease. Proper spray at proper timing play a better role but many of the farmers unaware from this practice. For such situation creates badly need for ascertain the efficacy of both plant based extract and fungicide as control measure for citrus canker. Conduct a study on tree bearing citrus plant to evaluate the effectiveness of different fungicides and plant extracts against citrus canker. The five treatments i.e. Neem oil (T1), copper oxychloride (T2), *Allium cepa* extract (T3), difenoconazole (T4) and *Datura alba* extract (T5). Spray of fungicides i.e. copper oxychloride @ 3g/ liter of water and difenoconazole @ 1ml/ liter of water. Plants extracts i.e. *Alliumcepa* @ 10ml/liter of water, Neem oil@ 10ml/liter of water and *Datura alba* extract @ 10ml/liter of water on tree bearing citrus plants were evaluated. Twenty-Eight plants were used for testing which passes through five treatments including control with four replications. Spray was done on tree bearing citrus plant. Fungicidal spray i.e. Copper oxychloride controlled the disease up to 77% and difenoconazole 59% while the plants extract i.e. *Allium cepa* controlled the disease 80%, *Datura alba* extract 66% and Neem oil 62%. Timely control of citrus canker hence, copper oxychloride and *Allium cepa* extract is recommended best to the citrus growers. Compared to other treatments both these treatments resulted in higher suppression of citrus canker disease.

# EVALUATION OF DIFFERENT ANTIBIOTICS FOR THE MANAGEMENT OF BACTERIAL BLIGHT OF *EUCALYPTUS CAMALDULENSIS*

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*Eucalyptus camaldulensis* is an important tree species of the tropical, subtropical and temperate regions of the world. It belongs to the family Myrtaceae and native to Australia. There are many diseases affecting *Eucalyptus* production but bacterial blight is one of the most serious disease that causes severe damage at nursery stage. Environmental factors and disease incidence have strong linkage. After collection of diseased samples from field different experiments were conducted in the research area of the Department of Forestry and Range Management, University of Agriculture, Faisalabad (UAF). The main objective of this study was to evaluate different concentration of antibiotics to manage bacterial blight disease with antibiotic treatments. After isolation, identification and pathogenicity tests, Antibiotics such as Amikacin Sulphate, Cefuroxime, Ceftizoxime Sodium, Co-amoxiclav, Ceftriaxone, Cephradine, Ampicillin Sodium, Ampicillin/Cloxacillin and Ceftazidime with one control treatment were evaluated at 300ppm, 500ppm and 700ppm concentrations under in-vitro, greenhouse and in-vivo conditions. Ceftriaxone was found best as it formed maximum inhibition zone (12.657mm) at 300ppm, 500ppm and 700ppm concentrations in the in-vivo experiment as compared to above mentioned treatments. While the minimum disease incidence (%) was recorded by the combination of Cephradine + Ceftriaxone in the greenhouse (15.185%) and in-vivo (18.519%) experiments at 300ppm, 500ppm and 700ppm concentrations. Data was analyzed by using Statistics software.

# ANTIBACTERIAL EFFICACY OF SYNTHETIC CHEMICALS TOWARDS BACTERIAL CANKER OF TOMATO CAUSED BY *CLAVIBACTER MICHIGANESIS SUBSP. MICHIGANESIS*

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Tomato (*Solanum lycopersicum*) is an important crop belongs to the family Solanaceae. It is the major source of antioxidants and vitamins such as vitamin C, vitamin K, potassium and folate etc. A coplex of diseases is known to attack on tomato, among them bacterial canker which is caused by (*Clavibacter michiganesis subsp. michiganesis*) is the most destructive disease which causes significant losses mostly in the humid environment. Bacteria enters systemically in the vascular tissues of plant that is the main cause of destruction. Estimated yield losses due to bacterial canker ranges from 10% to 84% for individual growers. Different management strategies have been used to manage this devastating disease. Chemicals show maximum inhibitory action towards disease. In current study different chemicals alone and in combination including Bronopol, Oxolinic acid, Oxytetracycline, Copper sulphate, Antibak RZ, coper, Ziram, Mancozeb and Param were used to treat bacterial canker. Three concentrations of each chemical were used. Results showed that mixture of Coper and Ziram showed best results followed by Mancozeb, Bronopol, Oxolinic, Copper Sulphate, Oxytetracycline and Antibak RZ. It was concluded that Coper and Ziram can be used in future for the management of bacterial canker of tomato.

# INVESTIGATION OF *PSEUDOMONAS SYRINGAE* PATHOVARS ASSOCIATED WITH BACTERIAL CANKER ON PEACH IN AZAD JAMMU AND KASHMIR

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Bacterial diseases of peach caused by *Pseudomonas syringae* is an increasing problem in Azad Jamun & Kashmir (AJK). Current study was first time carried out in AJK for the determination of disease incidence and prevalence of peach fruits growing areas of Poonch division (Haveli, Bagh and Sudhanoti). Almost 75% disease prevalence was calculated in district Bagh followed by 50 % in district Poonch while there was 25% disease prevalence found in Haveli and Sudhanoti. Similarly, Maximum disease incidence was 16%, in district Bagh followed by district Poonch (15%), Sudhanoti (11%) and Haveli (8%). Twenty-nine gram negative isolates having florescent colonies were recovered on King's B media showed positive levan and tobacco hypersensitive response while negative oxidase test, pectolytic activity on potato tubers and arginine dihydrolase test confirmed *Pseudomonas syringae* isolates while positive gelatine, aesculin hydrolysis and lactate utilization test but negative tyrosinase and tartrate tests confirmed that 16 isolates were pathovar *syringae*. To refine results molecular characterization was done using two primer sets (16s rRNA and *gyrB* gene). After phylogenetic analysis it was confirmed that from 29 isolates 16 isolates were *P. syringae* pv. *syringe* while other 13 isolates were *P. syringe* pv. *morsprunorum Race 1.* The results highlighted the alarming situation of new threat in AJK, which must be further studied for its epidemiology for better management of bacterial canker of peach in local environmental conditions.

# PATHOGENIC VARIATION AND BIOCHEMICAL REACTION AGAINST *XANTHOMONAS ORYZAE* PV. *ORYZAE* CAUSED BACTERIAL LEAF BLIGHT OF RICE

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Bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* emerged as a big disease in Asia continent including Pakistan. In previous study we identified 14 isolates from different area of Sindh province. Additionally, we subjected these isolates to pH, Salt, Antibiotic and Pathogenicity. The result of pathogenicity showed that basmati 2000 variety was used. 1 isolates (5%) was resistance, 8 isolates (40%) were moderately resistance and 5 isolates (25%) were moderately susceptible and all isolates showed an obvious growth at various salt (NaCl) concentrations and pH levels. Regarding antibiotic all isolates showed different level of reaction against Ampicillin and Gentamycin.

# DEVELOPMENT OF DISEASE FORECASTING MODEL FOR BACTERIAL BLIGHT OF COTTON BASED ON WEATHER PARAMETERS WITH RESPECT TO DIFFERENT SOWING DATES

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Cotton is an important fiber cash crop extensively sown in tropical and sub-tropical areas around the globe. Bacterial blight of cotton is a re-emerging threat on cotton crop in Pakistan under climate change scenario. The present study was conducted to develop a disease forecasting model based on weather parameters according to different sowing dates. Seeds of fifteen cotton varieties/lines were sown at experimental are of Plant Pathology Research Institute, Faisalabad. Three different sowings were done at 15 days intervals. Maximum and minimum temperature, relative humidity, rainfall, and wind speed data were recorded from a mini weather station. Disease severity data were recorded using Brinkerhoff's disease rating scale at 15 days intervals after the appearance of the disease. The relative impact of different sowing dates was evaluated using analysis of variance (ANOVA). Pearson's Correlation was used to find the correlation between disease severity and weather parameters. Stepwise forward regression was used, and the model was selected based on high $R^2$, low Mallow's CP and low Mean Square Error (MSE). A significant difference in disease severity was recorded among the three sowing dates. Maximum and minimum air temperature, rainfall and relative humidity played a significant role in disease severity in all three sowings. A strong positive correlation was observed between disease severity and maximum air temperature (0.81), relative humidity (0.83), rainfall (0.71) was observed. A negative weak correlation (-0.48) was recorded between minimum air temperature and disease severity. The present study is beneficial to adopt the prophylactic measures to manage the disease according to different sowing dates.

# BIOHERBICIDAL ACTIVITY OF *PSEUDOMONAS* SPP. TO SUPPRESS THE ALLELOPATHIC STRESS OF *TRIANTHEMA PORTULACASTRUM* LEAF ON VIGNA MUNGO

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*Vigna mungo L.* (black gram) is a summer pulse crop endemic to Central Asia. It belongs to the *fabaceae* family. It is one of Pakistan's top five premium food beans and a highly cost-effective crop. Allelopathy is the term used to describe a plant's direct or indirect chemical effects on another plant. Allelochemicals in *Trianthema portulacastrum* leaf extract (0.4%, 0.8%, 1.2%, 1.6%, 2%) effect and inhibit black gram germination rate, seedling length, fresh and dry biomass of seedling from low to high concentrations. The research revealed that leaf extract of black pigweed decreases the mash bean leaf germination (33 to -6%), seedling length (10 to 3%), fresh biomass of seedling (-40 to -256%), dry biomass of seedling (-35 to 55%). There are the number of techniques to suppress the allelopathic activity of weeds like cultural tecniques and herbicides but they are non-economical and pollution causing techniques, so we utilize biological tool *pseudomonas* PF 097 as herbicides and as PGPR to decrease the *Trianthema portulacastrum* allelopathic stress and to enhance the growth of black gram. The addition of *Pseudomonas* PGPR PF097 increased mash bean leaf germination (11-38%), seedling length (43-54%), and fresh biomass of seedling (8-18%) and dry biomass of seedling (10-16%).

# CITRUS GREENING: A SERIOUS THREAT TO CITRUS INDUSTRY

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Citrus is regarded as the most vital fruit crop throughout the world belonging to family Rutaceae and its origin is South East Asia It is cultivated on commercial scale in more than 50 countries of the world.It has great importance in our daily life due to its high dietary values and is a good source of mineral and vitamins.The production of citrus is affected by a number of biotic (citrus canker, brown leaf spot, citrus greening, citrus decline and citrus melanose) and abiotic (extreme or low temperature, rainfall, wind speed and humidity) factors. Citrus greening (caused by *Candidatus liberibacter*) is one of bacterial diseases and is becoming an emerging threat in citrus production. The pathogen may affect different parts of the citrus and cause severe infection in young fruits. The disease losses reach to 74% in different citrus growing regions of the world and psyllid vector (*Diaphorina citri*) is responsible in its spreading. Citrus greening is managed by using various control strategies including sanitation practices, discarding of infected parts and portion of plants which are source of infection and by the application of different biological and chemical approaches.

# BACTERIAL LEAF SPOT: AN EMERGING THREAT TO SUCCESSFUL PRODUCTION OF CHILLI CROP

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Chili (*Capsicum annum*) is an important condiment belongs to family *solanaceae*, which is used in both fresh and preserved form throughout the world. It has many economical, medicinal and nutritional benefits. Its production is hampering due to various abiotic (drought, heat, cold, and salinity) and biotic (root, stem, leaf, and fruit rots; leaf spots, viral and powdery mildew diseases) factors. Among these biotic factors *Xanthomonas compestris* pv. *vesicatoria* is an emerging threat. So proper management has become a great concern to decrease these losses. Different management practices (cultural, biological, chemical, induce resistance, and resistant cultivars) are used to control this disease. Use of resistant variety is an operative and cost-effective strategy to control the diseases and maximize the yield in limited time. Due to changing climatic conditions and the emergence of disease epidemics, judicious application of bactericides is an alternative significant strategy for disease management and chili production improvement. Microorganism-based products can be used to boost plant development and alleviate disease-related problems. In the current climate scenario, the best control of this disease is the employment of integrated management approaches to deal with the rising threat of this disease for future food security.

# ASSESSMENT OF BACTERIAL LEAF BLIGHT OF RICE IN AGRO-CLIMATE OF SINDH PROVINCE AND CHARACTERIZATION OF ITS ASSOCIATED PATHOGEN

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Bacterial Leaf Blight (BLB) of rice has created an alarming situation in Sindh province and is causing considerable losses to this crop. There is dire need to manage this problem. For this purpose, present studies were designed to assess latest position of BLB in Sindh, to study patho-diversity of *Xanthomonas oryzae* pv. *oryzae (Xoo*) and to explore resistant germplasm. So, comprehensive survey was conducted, 147 locations were visited and 200 samples were collected from surveyed areas. Disease incidence in lower Sindh was found to be 34.1% with disease severity of 10.11%. Whereas, the disease incidence in upper Sindh was 21.65 % with 4.73% disease severity. Isolation from disease samples resulted in 120 purified colonies. Bacterial isolates of *Xanthomonas oryzae pv. oryzae* were identified on molecular basis using XOR primers. Out of 120 isolates, 34 were confirmed as *Xanthomonas oryzae pv oryzae*. All 34 strains produced BLB symptoms and were confirmed as *Xanthomonas oryzae pv oryzae* in pathoginecity studies also. These preliminary studies will be helpful in managing BLB for protection of rice crop in Sindh province.

# EFFICACY OF DIFFERENT ANTIBIOTICS AND CHEMICALS AGAINST BACTERIAL LEAF BLIGHT DISEASE OF RICE CAUSED BY *XANTHOMONAS ORYZAE PV. ORYZAE*

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Rice (*Oryzae sativa L.*), a member of Poaceae, is extensively cultivated in subtropical and tropical areas around the world. Rice is the second most widely grown cereal crop of the world and it plays a significant role in Pakistan's economy. Unfortunately, such a valuable crop is susceptible to a variety of diseases, BLB which is caused by bacterial pathogen named, *Xanthomonas oryzae pv. oryzae*. BLB (Bacterial leaf blight) of rice is among the most devastating diseases that affects the rice worldwide. Bacterial leaf blight control was accomplished by the utilization of chlorine. Anti-toxin drug specifically Agromycin 100, teramycine and streptomycin sulphate showed moderate or lower degree management of bacterial leaf blight. Use of sodium hypochlorite powder to the stale water at the pace of 20 ppm chlorine could altogether limit the infection in the field conditions. The control of bacterial blight by antibiotics is one of the most effective anti-disease countermeasures. Three antibiotics including Flare 72 WP (streptomycin sulphate), Cordate 4 WP (kasugamycin) and Castle 50 WP (kasugamycin + copperoxychloride) with combination of four fungicides named Copper oxychloride 50 WP (copper oxychloride), Nativo 75 WDG (tebuconazole+trifloxystrobin), Gem Star Super 325 SC (azoxystrobin+difenconazole) and Bordeaux mixture as foliar spray were applied against bacterial blight. The results showed that Flare 72 WP performed as the best with 92.23% disease control and increase the rice yield upto 3.55%. It is concluded that Flare 72 WP has been proved the better option for controlling BLB of rice.

# ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGEN ASSOCIATED WITH LEAF SPOT DISEASE OF CAULIFLOWER

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Cauliflower is one the important vegetables in the species *Brassica oleracea* in the genus *Brassica*, is the main member of *Brassicaeae* family. It is an annual plant which is being grown all over Punjab Province. It is propagated through seed. Pakistan included in main ten cauliflower producing countries in the world, having 11420 hectares area with an average 209010 tons of production in 2013. The important diseases of cauliflower are leaf spot, root rot, bacterial soft Rot, Blackleg, rot, Damping Off, powdery mildew and Downy Mildew. The most common disease is leaf spot caused by bacterial pathogen having complex with *Alternaria brassicicola.* These two pathogens have a major impact on the yield of this important vegetable. In the current work, a survey was in the district Faisalabad to collect infected plant samples. Then samples were treated with 70% ethanol to remove surface bacterial and fungal spores. Then samples were treated with autoclaved double distilled water and were left to dry. Samples were placed on Nutrient Agar media and then for placed for incubation at 28$^{o}$C for 24h. Next day bacteria ooze was observed around samples. With fine autoclaved spatula, the bacteria were streaked on the NA plates. Next day fine colonies were observed. From a single colony, liquid culture was prepared. 80% glycerol was used for cryo-preservation of pure bacteria. In future, infection test corn plant will conducted and pathogen will be characterized through molecular techniques.

# BIOPESTICIDES: A SAFE WAY TOWARDS PEST MANAGEMENT

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Chemical control is used to protect the plants against pathogens, but it has some disadvantages as these are not environment friendly. They are harmful for human health and even kill beneficial pests. Another disadvantage is that they effective only for a short period of time as pathogen develop resistance against chemicals. Approximately 10 lac people die every year due to pesticide's poisoning. Biopesticides are new, safe and environment friendly as compared to chemical pesticides. Use of biopesticides is specific and complex as they contain microorganisms. They are divided into three types i.e., bio fungicides, bioinsecticides and bioherbicides. Bio fungicides are made of specific microbes and natural metabolites that release antibiotics and toxin which control the growth of fungus and improve the immunity of plants. Bio insecticides contain such microbes and natural compounds that are effective against pathogens. They do not harm beneficial pests non-targeted pathogens. Bio herbicides contain microbes and natural compounds obtained from plants that are effective against different weeds. Bioherbicides target the specific functions taking place in herbs that is effective against such herbs. The mode of action differs depending on the target pathogens in plants, an endotoxin damages the digestive system, which is frequently specific to the insect pest. The bacterial biopesticide colonizes on the plant and crowds out pathogenic bacteria or fungus when applied to control them.

# EXPLORING THE INVOLVEMENT OF FUNGI IN THE PURPLE BLOTCH DISEASE OF ONION AND ITS BIOCONTROL

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Onion (*Allium cepa* L.) belongs to family Amaryllidaceae. Onion is believed to be the oldest crop known to man and it has many uses ranging from condiment to daily used vegetable. Onion is cultivated on a large area in Pakistan and the rest of the world. In Pakistan, Onion is cultivated on an area of 138 thousand with an annual yield of almost 1.5 million tons. Pakistan is the sixth largest producer of the onion in the world. Due to high water content, many fungal and bacterial pathogens attack onion crop. Almost 66 pathogens attack this crop globally including 38 fungal, 10 bacterial, three viral, one phytoplasmal, one parasitic and seven other diseases. While in Pakistan several soil borne fungal pathogens invade onion crop. Purple Blotch is caused by *Alternaria porri* (Ellis) *Cif*. It affects the onion crop adversely under field conditions. In the current work, a comprehensive survey was made in the different locations of district Faisalabad for the collection of diseased samples. During this survey samples of onion were taken and were surface sterilized with 70% ethanol for 15 sec. Later the samples were washed with autoclaved double distilled water to minimize the effect of alcohol. The samples were placed on PDA media plates (100 µg/ml Ampicillin) and were incubated at 28±2$^{o}$C for 24 h. After appearance of fungal growth around the placed samples, hyphal tip method was followed to purify the fungal pathogen involved in purple blotch disease. Four genotypes are also planted in the field conditions to check the pathogenicity of the fungus and BCA will be screened in the lab conditions to use in the field for managing this important disease. Different physiological determinants will be measured to check the possible effects of fungus on the physiology and yield of the plant. Data will be analyzed through SPSS 19 statistical software.

# DETECTION OF CITRUS CANKER IN CITRUS PLANTS WITH AN EFFICIENT IMAGE PROCESSING TECHNIQUE AND SUPPORT VECTOR MACHINE CLASSIFIER

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Leaf and fruit diseases significantly reduce the yield and quality of agricultural produce. Mainly citrus plants are affected by Citrus Canker i.e., a bacterial disease caused by *Xanthomonas axonopodis pv. citri*. Citrus plants suffering from citrus canker have white spongy spots surrounded by yellowish margins (lesions) and these spots turns greyish or brownish with passage of time. Lesions and pustules of citrus canker on leaves and fruits of citrus plants can easily be identified by appearance. Key objective of this study is the detection and classification of citrus canker through image processing method. Proposed methodology includes Pre-processing of acquired images through FDCHL, Segmentation by K-Mean algorithm, classifier, Feature Extraction by Fuzzy Feature Selection and Classification by SVM. Experimental results show that citrus canker was accurately and precisely detected by the proposed methodology. In future, efforts can be made to design, develop and commercialize a sustainable robotic system for pruning of leaves and fruits suffering from citrus canker.

# EFFICACY OF DIFFERENT CHEMICALS AGAINST THE INCIDENCE OF BACTERIAL LEAF BLIGHT DISEASE OF RICE UNDER BAHAWALNAGAR CONDITIONS

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Rice is one of the most important staple food crops after wheat in Pakistan. Rice is important food crop for more than three billion peoples worldwide. Bacterial leaf blight disease is the most devastating and dangerous disease in important rice growing areas. Different chemicals like Copper oxychloride, Copper sulphate, Streptomycin, Bismerthiazole and Kasugamycine were applied against the incidence of Bacterial leaf blight disease. Streptomycin was most effective chemicals for reducing the Bacterial leaf blight disease 88% followed by Kasugamycine, Copper oxychloride 81% and 80% respectively. Highest yield 4.2t/ha were recorded in Streptomycin treatment followed by Kasugamycine, Copper oxychloride 4t/ha and 3.89t/ha respectively. Copper sulphate least effective treatment for reducing the disease.

# CHARACTERIZATION OF EPIDEMIOLOGICAL CONDITIONS CONDUCIVE TO BLACK LEG OF POTATO

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Epidemiological conditions conducive for the development of black leg disease of potato were characterized on five potato varieties including Harmony, Lady Rosetta, Desiree, Accent and Faisalabad White. All epidemiological factors including Max and Min Temperature, Rain fall, Relative humidity and wind speed showed significant relationship with the black leg disease development. All five varieties showed maximum disease incidence when the maximum temperature was 18-26˚C. Strong correlation was seen between disease incidence and minimum temperature exhibited by high values of r i.e., 0.94, 0.94, 0.95, 0.94, 0.95 on five potato varieties Harmony, Lady Rosetta, Accent, Faisalabad White and Desiree respectively. All five varieties showed maximum disease incidence when the minimum temperature was 05-11˚C. Black leg disease was increased significantly with the increase in Relative Humidity while maximum disease incidence was seen when the RH was more than 80 percent. Rainfall also showed positive relationship with the disease development. Maximum disease incidence was observed at 4-5 km/h, higher values of r showed that there exists strong correlation between Wind Speed and disease incidence on all five varieties. Regression analysis indicated that relative humidity exhibited statistically non-significant response. So it was removed from the model The multiple regression model based on two year data: Y=24.382+0.3592X1+.0970X2-0.2551X3+1.982X4 (X1= Max.T, X2 = Mini.T, X3 = Rainfall, X5= Wind speed) explained 97% variability. The normal probability plot showed that mostly points were present on the reference line. In the residual verses fitted value, all the points were distributed within range -1 to +1. Only four points were present along the reference line.

# MANAGEMENT OF BLACK LEG OF POTATO THROUGH ANTIBIOTICS PLANT EXTRACTS AND NUTRIENTS

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For management of blackleg disease of potato nine antibiotics were evaluated under lab conditions and the two most effective were assessed under greenhouse and field conditions Among all treatments, maximum inhibition zone was expressed by ciprofloxacin (14.17) mm followed by levofloxacin (12.93), ampicillin (6.79), kanamycin (6.54), clarithromycin (5.91), azithromycin (5.14), streptomycin (4.88), amoxicillin (4.69), erythromycin (4.19) mm as compared to control. Ciprofloxacin and Levofloxacin alone and in commination expressed minimum disease under greenhouse and field conditions. Similarly, nine plant extracts were evaluated against *Pectobacterium atrosepticum* to manage blackleg disease. Maximum inhibition zone and minimum disease was expressed by *S. cumini*. Macro and micro nutrients alone and in combination were also evaluated against blackleg disease. Minimum disease was recorded when macro and micronutrients were evaluated in combination against blackleg disease.

# ANTIBACTERIAL ACTIVITY OF VARIOUS BACTERICIDES AGAINST BACTERIAL BLIGHT AND THEIR EFFECTS ON VARIOUS PHYSIO CHEMICAL ATTRIBUTES OF POMEGRANATE

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Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is a widespread disease affecting pomegranate production and quality of fruit globally. The notorious disease spread in epidemic form and affecting all available commercial cultivars of pomegranate. A field experiment was conducted for its management under south Punjab conditions during 2018 and 2019. Six times foliar application of eight treatments (seven bactericides and water as control) was done at 15 days of interval, starting from first week of May to the end of July on three pomegranate cvs. Sindhuri, Kalehar and Sava. The observations during current study indicate that among eight treatments only T7 treatment (copper oxychloride @ 0.3% + streptocycline @ 500 ppm) proved significantly effective against bacterial blight in pomegranate by reducing disease incidence and disease severity on leaves, fruits, stems and plant. The same treatment produced significantly maximum disease reduction percentage, marketable fruit percentage, fruit weight, hundred arils weight, total arils weight, peel weight, juice contents, TSS, acidity, phenolics, vitamin C and total antioxidants as compare to other treatments. The combination of antibiotics (streptocycline @ 500 ppm) and copper-based fungicides (copper oxychloride @ 0.3%) if sprayed properly with 15 days of interval the chances of bacterial blight disease attack on pomegranate would be reduced significantly with improved fruit quality attributes.

# ANTIBACTERIAL EFFECT OF *BACCHARIS SALICIFOLIA* ON ORAL AND DENTAL BACTERIAL ISOLATES IN A PAKISTANI POPULATION

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Antimicrobial drugs play very important role for the treatment and prevention of infectious diseases. Various types of emerging and re-emerging diseases are a big challenge in medical science. Chewing sticks are regularly used in Pakistan, many other Asian countries, Middle East and Africa for oral hygiene, religious and social purposes. Recently, WHO has endorsed and prompted the use of these sticks for oral hygiene. The aim of the present study was to check the antimicrobial activity of aqueous extract of *Baccharis salicifolia* twigs against oral and dental bacterial isolates. The isolated bacterial strains were identified with the help of standard biochemical methods and sequencing of 16S ribosomal DNA. It was observed that the aqueous extract of *Baccharis salicifolia* had good antibacterial effect on the growth of *Acinetobacter baumanni, Listeria monocytogenes* and *Streptococcus mutans* with maximum zone of inhibitions of 22 mm, 19 mm and 18 mm respectively. Antimicrobial susceptibility of isolated bacterial strains were also determined against commonly prescribed antibiotics included Penicillin G, Amoxicillin, Vancomycin, Azithromycin, Gentamycin, Rifampicin and Chloramphenicol by disc diffusion method. The results indicated that most of bacterial strains were resistant against selected antibiotics. *B. salicifolia* showed good antibacterial activity against orodental bacteria and the usage of chewing sticks twice a day on a normal basis might also limit the incidence of gingivitis and dental caries. Twigs of *B. salicifolia* can be used as an effective, inexpensive and commonly available herbal toothbrush for teeth cleaning and oral hygiene.

# IN VITRO AND IN VIVO MANAGEMENT MEDICINAL PHYTOEXTRACTS AGAINST *XANTHOMONAS CITRI* PV. *CITRI* CAUSING CITRUS CANKER

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Citrus is one of the world's most essential fruit crop, used as fresh fruit and juices with antioxdental properties and rich source of vitamin C. It is cultivated throughout the world but the production of citrus is affected due to various abiotic and biotic factors like extreme temperature, high humidity, rainfall and different types of microbes such as virus, bacteria, fungi and nematodes. Citrus canker caused by *xanthomonas citri* pv. *citri* is one of the bacterial infection. Various management techniques were used, such as the use of chemicals, plant extracts and antibiotics. In the present research, different plant extracts (Korr- tumma, cinnamon, black pepper, kalwanji, cloves, Taiz patta, ginger, safaida and maithy leaves) and nanoparticles (silver and copper) were used to manage the disease. Maximum inhibition zone was produced by *C. colocynthis* and *N. sativa* followed by *C. tamala, Z. officinale, P. nigrum, C. verum, S. aromaticum, E. globu, T. Graecum* mm respectively as compared to control. In greenhouse and field conditions, the most effective phytoextracts were evaluated alone and in combination and observed that minimum diseases incidence were observed *C. colocynthis* and *N. sativa.*

# MANAGEMENT OF *XANTHOMONAS CITRI* PV. *CITRI* CAUSING CITRUS CANKER THROUGH AgNPs AND CuNPs

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Citrus (*Citrus latifolia*) is an important fruit crop of the world. It is a member of the *Rutaceae* family which is originated from the subtropical and temperate regions of Southeast Asia. Citrus has purple or white flowers with good fragrance and odor and carries juicy fruit cavities known as hesperidium. Among other methods the most frequently used approach is the green synthesis of NPs using plant extract. These have some distinctive advantages like less biohazardous, being easily assessable and serve as a source of active constituents. In this experiment the pathogen were treated with nanoparticles (AgNPs and CuNpS) and the maximum inhibition zone was produced by (silver+ copper) nanoparticles follow by silver nanoparticles and copper nanoparticles mm respectively as compared to control. In greenhouse and field conditions Maximum disease incidence was noticed by copper nanoparticles and minimum disease incidence was observed when (silver nanoparticles + copper nanoparticles) were in combinations compared to the control.

# INTEGRATED MANAGEMENT OF PLANT PATHOGENIC *Streptomyces* SPECIES CAUSING POTATO SCAB IN OKARA REGION

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Potato scab (PS) also known as common scab (CS) caused by *Streptomyces scabies* is an important disease of potato all over the world. In one of the latest survey, a disease incidence of 44% was determined in district Okara for PS. PS has been identified in Pakistan as one of the dangerous diseases as far as the potato export industry is concerned. So far no potato cultivar has been found resistant to this devasting pathogen. Moreover, different group of pesticides have been tried but no adequate control measures are established and by now, there are no registered chemicals for PS management in Pakistan. Therefore, there is a dire need to find out some effective integrated management strategies for controlling this growing disease. A survey was conducted to collect potato scab affected disease samples from different areas of Okara region. Isolation, identification and purification of the pathogen (*Streptomyces* species) causing common scab of potato was done using different growth mediums. Isolates of *Streptomyces isolates* were grown on YME agar plates at 28°C and preserved at -80°C in eppendrof tubes. Afterwards, beneficial bacteria were isolated from the rhizosphere potato samples using serial dilution method. These test bacteria were then evaluated *In-vitro* for their bio-control efficacy against isolated pathogenic *Streptomyces* species. In the same way, plant extracts and chemicals were also evaluated using *In-vitro* plate assays with different concentrations. DNA extraction, PCR and 16s rRNA sequencing was done for the identification of pathogenic and bio-control isolates. Moreover, all the selected bio-control bacteria, plant extracts and chemicals were then evaluated *In-vivo* under greenhouse conditions using seed treatments. Conclusively, an integrated management strategy was developed that can be used to control *Streptomyces* species causing Potato Scab disease in potatoes.

# SECTION 3: BIOINFORMATICS IN PLANT PATHOLOGY

# HOW GENOMICS IS CHANGING OUR UNDERSTANDING ABOUT THE COMPLEX PLANT-PATHOGEN INTERACTIONS?

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Plants differ from animals regarding adaptive immunity mechanisms and need a specialized, multi-layered defense response system. Understanding plant-pathogen interaction pathway is still underway as we see advancements in the omics tools such as transcriptomics, proteomics, and metabolomics lead by next-generation sequencing technologies. These new technologies provide us an opportunity to understand and describe these interactions from a different perspective. The information gathered from the available genomics, transcriptomics, proteomics, and metabolomics tools is required for the metabolic network modeling of plants and pathosystems. Through this talk, we will look at different omics datasets such as infection of *Fusarium solani* and *Fusarium oxysproum* in common bean (*Phaseolus vulgaris*) and *Plasmodiophora brassicae* infection in Chinese cabbage (*Brassica rapa* L. ssp. pekinensis). As a result of this talk, we shall conclude on the 1) how the omics-tools help us understand the plant-pathogen interaction from different perspectives and 2) what types of differential signals are produced in plants when a plant is infected by a pathogen.

# *INSILICO* BINDING ANALYSES OF BACTERIAL TAL EFFECTORS TO THEIR NATURALLY EVOLVED SPECIFIC EFFECTOR BINDING ELEMENTS IN RICE

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Bacterial blight (BB) disease of rice is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). The disease is the outcome of the host susceptibility (S) genes induced by the bacterial Transcription Activator-like Effectors (TALEs) binding to the effector binding elements (EBEs) in the promoter of the S genes. The binding is driven by the 12th and 13th residues of each TALE repeat consisting of 34 amino acids, via specific TALEs-EBE DNAs code. An *insilico* approach was applied in the current study to determine H-bonds based binding strength and fitness/affinity in terms of buried accessible surface area (BASA) values of various TALEs to their natural and computationally predicted best matches (henceforward control) EBE DNAs. Out of the total nine TALEs studied, the representative TALE, the Tal5 had 12 and 6 H-bonds with natural and control EBE DNAs, respectively. Likewise, Tal5 had the BASA values of 169.94 and 104.112 Å$^2$ with natural and control EBE DNAs, respectively. Other eight TALEs, AvrXa7, PthXo3, TalC, PthXo1, PthXo2, PthXo6, PthXo7 and AvrXa27 also had more H-bonds and BASA values with natural EBE DNAs than the respective control ones. There, however existed, some degeneracy/exceptions to this notion. The results indicate that in general, the natural EBE DNAs seem to be evolutionarily the best selected cis-acting elements for the cognate TALEs in question.

# *IN SILICO* STUDY REVEALS VARIOUS INSIGHTS INTO EVOLUTIONARY AND FUNCTIONAL BIOLOGY OF PLANT CELL WALL DEGRADING PECTATE LYASES (PELS) FROM PLANT PARASITIC NEMATODES OF THE ORDER TYLENCHIDA

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Plant parasitic nematodes (PPNs) develop sophisticated feeding sites in plant roots, called nematode feeding sites (NFSs). The development of NFSs depends on the degradative role of certain cell wall degrading enzymes, namely, cellulases, xylananses, arabinases, and pectate lyases (Pels). The Pels are secreted by esophageal glands of PPNs into the cytoplasm of the host cell to invade and macerate the host root tissues. Considerable research has been conducted on exploring the Pels of pathogenic bacteria and fungi. However, a detailed Pel characterization has not yet been done for PPNs. This study was primarily intended to characterize and investigate the evolutionary and functional dynamics of Pels in PPNs of the order Tylenchida. Consequently, Tylenchida Pels were found to be evolutionary derived into two major clades. Clade-1 lineage meets with the Pels of sub-family_3, whereas the lineage of Clade-2 meets with subfamily_2 of polysaccharide lyases family 3. Structurally, Clade-1 Pels exhibit eight turns of β-strands, whereas Clade-2 comprises seven turns of β-strands. Molecular docking of Pels with the substrate molecule α-D-galacturonic acid revealed a mechanistically appealing role of Turn 1, β-strand, and Turn 3 in catalytic activity, ultimately enhancing the degradation mechanism of pectin through Pels.

# GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF THE PLANT DEFENSIN (*PDF*) GENE FAMILY IN SELECTED LEGUMINOUS CROPS AND THEIR EXPRESSION PROFILES IN RESPONSE TO BIOTIC AND ABIOTIC STRESSES

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Plant defensins (*Pdf)* are a smaller group of antimicrobial peptides, that are small basic peptides and have a distinctive three-dimensional folding pattern. Plant defensin involves the innate immunity of plants, structurally and functionally related to insect and mammalian defensins, and inhibits the growth of a widespread range of fungi. This study was performed a genome-wide search of *Pdf* genes and identified a *Pdf* gene in eight selected legume crops. As a result, 13 *Pdf* genes in *P. sativum*, 6 in *P. vulgaris*,16 in *M. trunculata*,4 in *C. cajan*,6 in *G. max*,5 in A. *hypogea*,5 in C. *arietum,* and 4 in *V. angularis* were identified. Phylogenetic analysis divided identified genes into two groups, *Pdf* 1 (subgroup A1, A2, A3, A4, A5) and *Pdf* 2 (B1 and B2) based on the presence of proline-rich domain at N-terminal and linker DNA domain at C-terminal respectively. Resultant grouping and subgrouping were also supported by motif analysis, and gene structure analysis. The gene structure analysis depicted the occurrence of up to eight introns in all *Pdf*. Similarly, promotor analysis exhibited that many cis-regulatory elements are present upstream of *Pdf* genes which respond to a wide range of biotic and abiotic stresses responses. Expression analysis evaluates the resistance response in legumes during biotic and abiotic stress conditions. These results suggested that *Pdf* is involved in legumes

defense response against pathogen and will provide basic insights for future research involving *Pdf.*

# GENOME-WIDE ANALYSIS OF LEGUME CROP SPECIES FOR *IN SILICO* STUDIES OF PROGRAMMED CELL DEATH (PCD) INDUCING METACASPASES GENES

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Metacaspases (MCs) are subclass of caspases and functionally characterized as cysteine proteases. The MCs are known to involve in various physiological functions of plant such as development of seed to falling of leaves, various stress responses and unfavorable environmental conditions. Most importantly, upon pathogen infection, MCs contribute to establishment of induced programmed cell death (PCD) and limit pathogen further invasion. This study was primarily designed to report and characterize the MCs in eight legume crops. As result, 15 MC genes in *A. hypogea*, 9 in *C. cajan*, 9 in *C. arietinum*, 13 in *G. max*, 13 in *G. soja*, 9 in *P. vulgaris*, 10 in *M. truncutula* and 9 in *V. radiata* were identified. Phylogenetic analysis divided identified genes into two clades, MC1 (subgroup 1A, 1B and 1C) and MC2 (2A and 2B) on the basis of presence of proline rich domain at N-terminal and linker DNA domain at C-terminal respectively. Resultant grouping and subgrouping were also supported by conserved domain analysis, motif analysis and gene structure analysis. The gene structure analysis depicted the occurrence of up to eight introns in all MCs. The gene duplication analysis revealed that most of genes might evolve by segmental duplication under the influence of purifying selection in legume plant species. Similarly, promotor analysis exhibited that large number of cis-regulatory elements are present at upstream of MC genes which response to wide range of biotic and abiotic stresses responses.

# IN-SILICO CHARACTERIZATION OF CHLOROPLASTIC, MITOCHONDRIAL, PEROXISOMAL AND CYTOSOLIC ISOFORMS OF ISOCITRATE DEHYDROGENASE (ICDH) FROM *ARABIDOPSIS THALIANA*

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Plants are subjected to various abiotic stresses which affect their development rate. In response to these environmental stresses plants adapt different defence mechanisms including the expression of stress-responsive genes. The proteins translated from these stress-responsive genes may directly cope with these stresses or may be involved in the signalling pathways. The system controlling the signalling pathways of these stress-responsive proteins should be investigated thoroughly, which include the protein characterization and their role in the regulation of glutathionylation and nitrosylation. Isocitrate dehydrogenase (ICDH) belongs to a multi-enzymatic family and it is available in different NAD and NADPH dependent isoforms. ICDH is important for a number of pathways within cell mainly for the regulation of antioxidant defence mechanisms. In current research work, accession numbers of different isoforms of ICDH were searched from literature. The online database TAIR was used to retrieve the coding sequences and protein sequences of all ICDH isoforms. 3D structures of isoforms were predicted using SWISS MODEL and Protein Data Bank was used to retrieve the structure of thioredoxin. The structures of ligands NADP/NAD, H2O2, and substrate DL-isocitrate were retrieved from PubChem. Domains and motifs of all isoforms were searched using Conserved Domain Database and MEME Suite respectively. The interaction of all isoforms with other molecules was found out through molecular docking. The results showed that the affinities of all isoforms were almost similar. So, we can say that these isoforms complement each other's activity. The results will help to better understand the mechanism of regulation involving ICDH and its different isoforms. This knowledge will be helpful to make plants adaptable in changing environment.

# SECTION 4: MOLECULAR PLANT PATHOLOGY AND BIOTECHNOLOGY

# IMPACT OF PHOTOPERIOD ON BIOTECHNOLOGICAL *RNAi* LINES OF POTATO (*SOLANUM TUBEROSUM* L.) OBTAINED ON THE BASIS OF THE *PHYTOCHROME B* GENE

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Phytochromes are a family of light-sensitive proteins that respond to red and far-red lights, which are important for plant development. Phytochromes control various molecular and cellular processes in the plant life cycle and control the expression of genes that respond to vital ambient light. The short pulses generated by the red lights prevent the loss of green pigments in the dark and the beginning of seed germination (Wang P.Hu., 1982). Since the discovery of the *Phytochromes* genes, the physiological processes from seed growth to flowering in many plants have been shown to be controlled by these genes. The activity of these genes as well involves the elongation of the hypocotyls and the synthesis of flavonoids and carotenoids. To date, many studies have begun to elucidate the molecular and biochemical mechanisms of the *Phytochromes*. They are still being studied and described with great interest as genes for photoreceptors in plants. Light (photoperiod, light intensity, wavelength) is one of the most studied environmental factors in i*n vitro* potato tuber formation studies. The effects of light are the *Phytochrome* responses, which can affect on morphogenesis through the photoperiod, light intensity, or spectral wavelength. *In vitro* studies concerning the effect of photoperiod on tuber formation began after it was hypothesized during potato cultivation experiments that tuber formation depends on photoperiod. Light also affects the morphology of the tubers *in vitro*. Microtubers induced in complete dark conditions have a long dormancy period than microtubers grown in short daylight. The main goal of our research was to study the effect of photoperiod on our new obtained biotechnological potato *RNAi* lines (*RNAi* 75-line; *RNAi* line 85; *RNAi* line 86; *RNAi* line 149; *RNAi* line 150; *RNAi* line 151). To do this, we conducted two different photoperiod conditions at 16: 8 and 0:24. The photoperiod for the formation of microtubers was 0:24 short period (22 and 30 days), which was under dark condition, and 16:8 long period (33 and 7 days) was under light condition grown explants. The number of tubers in the explants was 1.87 pieces in light conditions, and their average weight was 118 mg, while the number of tubers in dark conditions was 3.75 pieces and their average weight was 320 mg as a higher indicator. Most of the microtubures cultured under light conditions were green. This may be due to the synthesis of the alkaloid solanine. It should also be noted that the green microtubures began to grow to form buds. Similar phenomena have been studied in detail by Wang P. Hu (1982). Incubation in the dark, the cultured microtubures turned brown, but no buds were formed. The results of the experiment showed that the dark conditions were favorable for the tuberization process.

# MOLECULAR CHARACTERISATION OF *SPIROPLASMA CITRI* AGENT OF STUBBORN OF CITRUS FRUITS IN ALGERIA

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Algeria is a country known for a varietal variety of citrus fruits, which are susceptible to serious diseases such as the stubborn, causing significant economic losses. Samples of fresh leaves and orange-tree fruit (*Citrus sinensis* L.) with stubborn symptoms were detected in the Baghlia region (Boumerdes). DNA extracted from symptomatic samples were used as a template for amplification of products of 675 bp using primer pair Spiralin (SC1, SC1') by PCR. Furthermore, the spiralin gene was cloned and sequenced. Molecular characterization by sequence analysis made it possible to identify two isolates with 80% homologies (Isolate of Algeria I (MK789650) genomic sequence of 658bp and Isolate of Algeria II (MK789651) genomic sequence 644bp). Isolate of Algeria I (MK789650) has similarities of the order of 100% with several isolates and strains such as the isolate Qualubia (AM157771), GII3-3X (AM285305), strain SPF1from Iran (KT834818), Zarghan isolate (KP148814), Firuzabad I isolate (KP148817), alcanar 254 (U13994), isolate from France (U13996), strain Khafr I (JN974243), and Fewa isolate from Egypt (AM157770). Very close relationships were recorded with an isolate of Firuzabad II (KP148818), Corse isolate (U13995) and R8A2 strain (CP013197). While the isolate from Algeria II (MK789651) showed weak relations with spiralin *Spiroplasma citri* (CAJ43425) and *Spiroplasma* sp. Chis (WP_105299266).

# CREATION OF NEW BIOTECHNOLOGICAL HIGH-YIELDING WHEAT LINES (*TRITICUM AESTIVUM* L.) USING RNA INTERFERENCE (*RNAi*) TECHNOLOGY WITH THE *PHYA1* GENE

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The efficiency of modern agricultural production does not allow satisfying the constantly growing needs of humankind. Therefore, to solve the food problem, it is necessary, firstly, to increase the yield of the main food crops, including wheat. Through the efforts of traditional breeding, wheat varieties are obtained with relatively high resistance to certain unfavourable factors, however, in general, the problem of increasing wheat productivity and its resistance is still actual. Some problems are impossible or very difficult to solve with the help of classical selection. Therefore, special hopes are pinned on genetic engineering, which, continues the direction of traditional breeding to improve the genotypes of useful plants but achieve the same goals in a more efficient and faster way. RNA interference (*RNAi*) technology allows a wide range of resistance-enhancing genes, isolated from different plant species and microorganisms, to be used to create transgenic plants that acquire pre-engineered stress resistance properties. Currently, *RNAi* is actively used to regulate the expression of genes of plants, animals, and microorganisms and as a method for studying the functional genomics of organisms. The mechanism of RNA interference in cells can be triggered using vector constructs encoding small interfering siRNAs. The purpose of our research is to carry out in planta transformation of winter soft wheat of the Bardosh variety using vector constructs pHG-8_PHYA1, using RNA interference technology, in order to obtain new biotechnological early ripening and high-yielding wheat lines. The agrobacterial in planta transformation of the pHG-8_PHYA1 vector constructs into mature wheat materials of "Bardosh" was carried out. During the growing season, the T1 generation of wheat plants was obtained. After that, PCR analysis was carried out to determine the transgenic wheat plants. As a result, transformed new wheat genotypes were obtained.

# SELECTION AND STERILIZATION OF LOCAL POMEGRANATE (*PUNICA GRANATUM L.) IN VITRO* EXPLANTS

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With the help of microcloning technology in a short period of time it is possible to reproduce the required amount of high-quality plant materials, which will be free from viral and bacterial infections, and to obtain plantlets with high purity of the variety. Apical meristems are used in plant propagation *in vitro* because viruses penetrate apical meristems more slowly than other parts of plants. Therefore, parts of the apical and axillary buds (growth points) of the plant are used. Apical meristems are used in plant propagation in vitro because viruses penetrate apical meristems more slowly than other parts of plants. Therefore, parts of the apical and axillary (growth points) of the plants are used. The process of clonal micropropagation consists of several stages, the first of which is the selection of a donor plant, the separation of explants, sterilization, and obtaining a sterile culture that grows well. The selected materials were brought from the existing local pomegranate varieties in the country which are "Tuyatish", "Kazakh", "Javdari" and "Achchik dona" and planted in our experimental fields. In our study, non-woody plantlets (apical and axillary buds) were isolated from existing varieties in the experimental field in the first half of July and brought to the laboratory in special containers. In the laboratory, the explants were washed with tap water with the addition of detergent (soapy water) for 10-15 minutes and the excess part and leaves were removed. Cutting of isolated explants was performed under sterile conditions (in laminar boxes) using sterilized tools. Gradually sterilized explants were then transplanted into an artificial nutrient medium MC (Murasiga Skug). As a result of sterilization of explants collected from existing varieties in the experimental field by different methods, their effectiveness was proved to be at different rates. Survival rates of the explants varied over 15 days from the date of the experiment. Thus, in the first and second variants of the experiment *in vitro*, the yield of sterile implants was 10–30%. In the third variant of the experiment, the rentability of sterile explants of these varieties was 92% and was found to be acceptable. In particular, the use of sterilization components in combination with fungicides and antibiotics has shown good results in our pomegranate *in vitro* culture studies.

# STUDY ON THE PRODUCTION OF VARYING PERCENTAGES OF ORGANIC ACIDS FOR PREPARATION OF ANTIMICROBIAL AGENT/ PRESERVATIVES USING BLACK WHEAT GLUCOSE AS THE SUBSTRATE

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Antimicrobial agents and preservatives acquire a significant market around the globe. The different organic acids like acetic acid, propionic acid and lactic acid reserve an important position in the class of natural agents /metabolites that can be utilized as preservatives. Moreover, if the source of production is organic and the process of production is also natural i.e via fermentation process utilizing no chemical agents and preservatives with specific pH (6.0-6.3) and temperature conditions (30-33$^0$C). Different organic acids propionic acid (PA) and its derivatives can be mentioned as important chemical intermediates, which are mostly used in a variety of industrial applications as antimicrobial agents for a broad spectrum of microorganisms, an anti-inflammatory substance, exhibiting analgesic and antipyretic properties, herbicides, controlling both monocotyledonous and dicotyledonous plants, preservatives in bakery and cheese products, artificial flavours and fragrances, pharmaceuticals, precursors of cellulose acetate propionate (CAP) etc. The black wheat is the source of different active molecules that can also participate in the preparation of fortified propionic acid which can be utilized significantly for the preparation of antimicrobial agents and preservatives. The present study was performed to screen different nutrient media components utilizing black wheat-based glucose as the substrate for the production of the varying percentage of organic acids. The results showed that fermentation conditions and varying percentages of nutrients are the significant criteria for producing varying per cent of organic acid levels and this can be utilized as the basis for targeting specific organic acid in the fermentation batches.

# AN ARABIDOPSIS *RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN B* (*RBOHB*) ENCODED BY *AT1G09090* IS IMPORTANT FOR RESISTANCE AGAINST NEMATODES

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Reactive oxygen species are a byproduct of aerobic metabolic processes but are also produced by plants in defense against pathogens. In addition, they can function as signaling molecules that control various aspects of plant life, ranging from developmental processes to responses to abiotic and biotic stimuli. In plants, reactive oxygen species can be produced by respiratory burst oxidase homologues. Arabidopsis contains 10 genes for respiratory burst oxidase homologues that are involved in different aspects of plant life. Plant pathogenic cyst nematodes such as *Heterodera schachtii* induce a syncytium in the roots of host plants that becomes a feeding site which supplies nutrients throughout the life of the nematode. In line with this function, the transcriptome of the syncytium shows drastic changes. One of the genes that is most strongly downregulated in syncytia codes for respiratory burst oxidase homologue B. This gene is root specific and we confirm here the downregulation in nematode feeding sites with a promoter::GUS(β-glucuronidase) line. Overexpression of this gene resulted in enhanced resistance against nematodes but also against leaf-infecting pathogens. Thus, respiratory burst oxidase homologue B has a role in resistance. The function of this gene is in contrast to respiratory burst oxidase homologues D and F, which have been found to be needed for full susceptibility of Arabidopsis to *H. schachtii*. However, our bioinformatic analysis did not find differences between these proteins that could account for the opposed function in the interaction with nematodes.

# EXPRESSION AND FUNCTIONAL ANALYSIS OF AN ANTIMICROBIAL ARABIDOPSIS THIONIN PEPTIDE *THI2*.3 WITH ACTIVITY AGAINST DIVERSE PLANT PATHOGENS

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Thionins are produced as complex structure in the form of propreporteins in plants. This structure later, give rise to mature Thionin peptide having antibacterial and antifungal activity in most of the cases. In mature peptide, the c-terminal part, which is actually acidic, is enzymatically cleaved. Arabidopsis genome comprise of 4 thionin genes. These genes are expressed in the various tissues of the plant. In the current work, Thi2.3 gene was studied in detail for understanding the antimicrobial role of this unique thionin in the Arabidopsis genome. Over-expression lines with and without acidic domain were established in pPZP3425 plant expression vector. Only acidic domain of this important thionin was also overexpressed in the plant parts through constitutive promotor. The overexpression lines were made homozygous by using drug resistant marker. All of the established over-expression lines were tested for the expression of this important gene at transcription level by using real time PCR. Later, lines with highest expression of Thi2.3 genes were selected for infectivity assays. All of these three types of over-expression lines were subjected to testing against *Pesudomonas syrangae* pv. *tomato* DC3000 and *Botrytis cincerea*. Additionally, natural expression of Thi2.3 gene was studied through promotor: GUS fusion. Maximum expression was seen in seeds.

# GENOME-WIDE ASSOCIATION IDENTIFIES GENOMIC REGIONS IN THE BIOCONTROL FUNGUS *CLONOSTACHYS ROSEA* THAT INFLUENCE ANTAGONISM AND BIOCONTROL OF PLANT-PARASITIC NEMATODES

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Biological control is a promising approach to reduce plant diseases caused by nematodes to ensure high productivity in future agricultural production. As large-scale genomic sequencing becomes economically feasible, the impact of single nucleotide polymorphisms (SNPs) on biocontrol-associated phenotypes can be easily studied across entire genomes of fungal populations. In this study, we made use of 53 whole-genome re-sequenced *Clonostachys rosea* strains to perform a genome-wide association (GWA) study for *in vitro* antagonism against plant-parasitic nematodes. Potato dextrose broth culture filtrates from *C. rosea* was inoculated with the root-lesion nematode *Pratylenchus penetrans* and nematode mortality was determined after 24 h of incubation. *In vitro* antagonism assay against *P. penetrans* displayed a significant variation among *C. rosea* strains and suggests that GWA of the trait is possible. An empirical Bayesian multiple hypothesis testing approach identified a total of 279 SNP markers significantly (local false sign rate ≤ $10^{-10}$) associated with the trait. Two non-ribosomal peptide synthetase genes (*nps4* and *nps5*) were present in the genomic regions associated with the nematicidal activity. Gene deletion strains of *nps4* and *nps5* genes were generated and showed increased growth and conidiation rates compared to the wild type. Culture filtrates from *C. rosea* Δ*nps4* and Δ*nps5* strains exhibited reduced nematicidal activity and immobilised nematodes to a significantly ($P \leq 0.05$) lower number compared to the wild type after 24 h of incubation. Furthermore, Δ*nps4* and Δ*nps5* strains showed reduced biocontrol efficacy in a naturally nematode infested soil in a pot experiment and failed to reduce the populations of nematodes in soil or in roots of wheat as efficiently as the wild type strain. Taken together, we show that NPS4 and NPS5 are biocontrol factors in *C. rosea*, presumably by producing a hitherto unknown non-ribosomal peptide compound with nematicidal properties.

# TRANSLATION INITIATION FACTOR EIF4E DIRECTLY INFLUENCES THE VIRULENCE OF RICE BLAST FUNGUS

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Translation initiation factor eIF4E is required by almost all cellular mRNA for their translation into proteins. It regulates the recognition of 5′ cap structure of mRNA while directing ribosomes to capped mRNA. eIF4E regulates stress response in Schizo *Saccharomyces pombe* but the direct or indirect influence in the development of filamentous fungi is not yet well documented. We investigated the contribution of the eIF4E factor in the physiology and pathogenicity of the horrendous fungus known as rice blast fungus posing a serious threat to global rice and wheat production. We deployed functional genetics, histopathology, biochemical, and microscopy techniques to functionally evaluate the contributions of eIF4E in rice blast fungus. Our *Magnaporthe oryzae* eIF4E (MoeIF4E3) deletion strains showed defective growth and asexual reproduction, impaired virulence. The stress response was also altered in our deletion strains. However, a comprehensive evaluation of the translational regulatory machinery is still required to study the in-depth mechanism of this fungal-plant interaction.

# DOWN-REGULATION OF MASTER FLAGELLAR REGULATOR *FLHDC* BY A NOVEL LYSR-TYPE TRANSCRIPTIONAL REGULATOR CRGA TO LIMIT CELL MOTILITY AND VIRULENCE IN *RALSTONIA SOLANACEARUM* GMI1000

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For the invasion and colonization, bacteria need flagella for their motility to modulate bacterial virulence. There are certain regulators in *Ralstonia solanacearum* that exert both positive and negative regulation on diverse cellular functions by interacting with the promoter region. In this article, we describe that CrgA, a LysR-type transcriptional regulator possesses N-terminal helix-turn-helix motif and C-terminal LysR substrate-binding domain that suppresses the activity of *flhDC* which is a master regulator of flagellum biogenesis. Mutant deficient of *crgA* (Δ*crgA*) leads to enhanced transcription of *flhDC*. Besides this, deletion of *crgA* promotes higher expression of flagellin gene *fliC* by possessing more polar flagella than wild type GMI1000. Yeast-one-hybrid and electrophoretic mobility shift assays unveiled that CrgA interacts directly with *flhDC* promoter. Soil-soaking inoculation with the Δ*crgA* caused wilting on tomato plants before the plants were inoculated with wild type GMI1000. We conclude that *R. solanacearum* regulator CrgA negatively regulates *flhDC* and consequently affects the expression of flagellin gene *fliC* to modulate cell motility, thus affecting virulence in the host plant.

# FIRST MOLECULAR EVIDENCE OF *COLLETOTRICHUM SPP.* ASSOCIATED WITH BANANA AT UTHAL, BALOCHISTAN, PAKISTAN

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Banana (*Musa* spp.) is widely cultivated in Uthal and small light-brown to reddish-brown spots were recorded on banana fruits which turned into sunken lesion at later stage of infection. The disease incidence was recorded as 31%. The pathogen (*Colletotrichum* spp.) was isolated on PDA with white colonies which turned into dark gray. The aseptate, hyaline and cylindrical conidia were round from one end while another end was round or narrowly acute with 10.13 to 15.93 (avg. 12.91) × 3.35 to 4.16 (avg. 3.72) µm ($n$ = 50), L/W ratio = 3.10. Light brown to dark black single conidial appressoria were subglobose to slightly ellipsoidal with 4.92 to 8.52 × 3.36 to 6.71 µm, avg. 6.1 × 4.3 µm ($n$ = 25), L/W ratio = 1.29. Through PCR, calmodulin (CAL), β-tubulin (TUB2) and internal transcribed spacer (ITS) regions were amplified from a representative isolate LUAWMS with CL1/CL2, Bt1/B2t2 and ITS1/ITS4 primers, respectively. The sequences of isolate LUAWMS were exhibiting 100% genetic similarity with CAL, TUB2 and ITS sequences. Three replications of three banana fruit were sterilized with 70% (v/v) ethanol, washed thrice with sterilized water and wounded with sterilized needle. A drop of 15µL conidial suspension (1 × $10^6$ conidia/mL) of isolate LUAWMS was pipetted on each wounded fruit and sterile distilled water as used as negative control. The inoculated fruits were incubated at 28°C with 12/12 hours dark/light cycle. After 4 days, symptoms were recorded on artificially inoculated fruit and same pathogen was isolated, but no symptoms and pathogen were recorded on control, fulfilling Koch's postulates. The morpho-molecular identification confirmed first molecular evidence of *Colletotrichum* spp. associated with banana at Uthal, Balochistan, Pakistan.

# *AGERATUM ENATION* VIRUS AND ASSOCIATED DNA-SATELLITES COMPLEX INFECTING WOODY PLANT, FIRST TIME REPORTED FROM PAKISTAN

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Begomoviruses are transmitted by vector whiteflies in the family Geminiviridae which infect dicotyledonous hosts. These plant infecting viruses usually infect herbaceous plants, a recent study investigated that these are also infecting woody plants. Foliage plant parts of mulberry were collected from Lahore-Pakistan exhibiting leaf yellowing and curling symptoms. Old world begomovirus associated with DNA-satellites complex were amplified by using RCA product as PCR, cloned and sequenced. Two full-length begomoviruses clones; *Ageratum enation virus* (AEV) associated with geminivirus associated alpha satellite in the genus *Colecusatellite*; *Ageratum enation alphasatellite*, sub-family Geminialphasatellitinae and Papaya leaf curl beta satellite have been first time reported from mulberry. *Ageratum enation* virus and associated *Ageratum enation* alpha satellite were showing recombination, usually infecting crops and weeds. Weed infecting monopartite begomovirus AEV and associated DNA-satellites complex is the first time reported in a woody plant (*Morus alba*) and also the first report from Pakistan.

# DYNAMICS OF ENDOSPHERE AND RHIZOSPHERE MICROBIOMES FOR SUSTAINABLE PLANT DISEASE MANAGEMENT

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Many microbes reside in the endosphere of plants or inhabit the rhizospheric vicinity of the roots. Most of these microbial communities are beneficial for the plants and assist them to carry out various physiological processes. In addition to growth promotion, they help the plants to cope with biotic stresses and harsh environmental conditions. The microbes from the endosphere and rhizosphere employ different strategies to empower the plant to fight any invading pathogens. They, directly and indirectly, facilitate the mechanisms involved in disease resistance, i.e., they assist plants via the induction of systemic resistance against pathogens using various stimuli. Similarly, the synthesis of siderophores, secondary metabolites, and antimicrobial compounds are very important tools used by endophytes to suppress the invasion and establishment of pathogens on plants. The rhizosphere microbiome contains many microbial species that are important mediators of plant defense against pathogens. In this review, we investigate the dynamics of endosphere and rhizosphere microbiomes for the enhancement of disease resistance in plants. In addition, considering the microbiome a useful source and its future implementation for sustainable disease management, microbiome-engineering approaches have also been discussed.

# PRE-STORAGE *ALOE VERA* GEL APPLICATION REDUCES DISEASE INCIDENCE AND DECAY OF SWEET CHERRY FRUIT

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Due to high nutritional properties, sweet cherry (*Prunus avium*) exhibits high consumer demand. However, after harvest, the occurrence of decay and disease incidence cause rapid deterioration of cherry fruit quality. Postharvest shelf-life extension through the use of the chemical has raised various health-related issues with increasing demand for some safe plant-based alternatives. Therefore, the present study was conducted to check the pre-storage application of Aloe vera (ALV) gel coating on the quality of cherry fruit. Fruits of three commercial sweet cherry varieties including 'Babazai', 'Thor' and 'Spin' treated with ALV gel (0, 2, 4 and 6%). Following storage fruit were stored at 2±1°C with 90% RH for 12 days. Results indicated that fruit treated with ALV showed a reduced incidence of postharvest disease and decay as compared to untreated control fruit which started decaying within 6 days of cold storage. Fruit treated with ALV also exhibited better fruit quality viz. higher fruit firmness, reduced fruit weight loss, higher sensory quality attributes, reduced decrease in sugar contents, titratable acidity, and sugars to acid ratio. It can be concluded that pre-storage ALV application can be used effectively to reduce disease incidence and decay in cherry fruit with better eating quality.

# CRISPR/CAS BASED GENE EDITING APPROACHES FOR GLOBAL FOOD SECURITY

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An explosive increase in the world population presents a daunting challenge for the future of global food security. This ominous challenge for increased demand for food gets further complex with the rising threats from global warming and climate change. A major proportion of the rural and suburban population in developing parts of the world already face severe food shortages and malnutrition challenges among all age groups of society. So, increasing yield outputs as well as the quality of food crops is a necessity for future generations. Modern technologies for plant genetic engineering especially the CRISPR/CAS system presents a great opportunity in this regard as it provides a way for; 1) enhancement in disease resistance of crop plants 2) improvement of nutrient traits for high consuming crops 3) inducing tolerance towards abiotic stress for meeting climatic challenges 4) possibility for nascent domestication of wild plant species. CRISPR/CAS based gene editing approaches also provide an escape from many GMO restrictions enabling societal and scientific approval paving a bright passage ahead for meeting global food demands of the future.

# PATHOGENICITY AND *IN VITRO* BIO-CONTROL OF DIFFERENT ISOLATES OF *PYTHIUM APHANIDERMATUM*

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Pythium especially known as water moulds can be caused serious diseases on different crops resulting in significant crop losses. *Pythium aphanidermatum* cause severe damping-off losses in the greenhouse and fields of vegetables. A survey was carried out of seventy villages of district Hyderabad for isolation of *P. aphanidermatum* from different vegetables. The four virulent isolates of *P. aphanidermatum viz., Pa*11, *Pa*12, *Pa*14 and *Pa*16 were confirmed their pathogenic nature on Spinach. All four isolates have adverse effects on all plant developmental stages as compared to the control plant. Four fungal biocontrol agents *viz., Trichoderma harzianum, Trichoderma polysporum, Paecilomyces lilacinus* and *Paecilomyces variotii* were checked against *Pa*11, *Pa*12, *Pa*14 and *Pa*16 in dual assay test. Both *Trichoderma* sp. performed well antagonist against all four isolates of *P. aphanidermatum.* The highest inhibition percentage was recorded at 32.7% in the dual assay test of *T. harzianum* + *Pa*16. Among all four tested antagonistic fungi, three fungi *viz., T. harzianum, T. polysporum* and *P. variotii* produced negative effects on the radial growth of all four isolates of *P. aphanidermatum.*

# MOLECULAR CHARACTERIZATION OF BEGOMOVIRUS INFECTING ORNAMENTAL, SADABAHAR

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Rose periwinkle (*Catharanthus roseus*) commonly known as Sadabahar, is an ornamental and medicinal plant of the family Apocynaceae. Rose periwinkle was famous for its anti-cancerous properties due to the alkaloids that are present in it. During a survey in 2019, it is observed that rose periwinkle is associated with begomovirus symptoms of yellowing and leaf curling. Molecular detection on rose periwinkle was accomplished by DNA extraction, PCR amplification as well as sequence analysis. Results of PCR showed the required size amplicon of the 2.8 kb and 1.4 kb with Begomo 01/02 and Beta 01/02 primer respectively. Although, the amplicon size of the 480 bp and 579 bp was obtained with CLCuMuBF11/R33 and Av/Ac core primers respectively. The begomovirus sequence showed 100% identity with Cotton leaf curl Multan virus and Cotton leaf curl Multan beta satellite. It is the first time that the Cotton leaf curl Multan virus infected the rose periwinkle in Pakistan (Multan).

# PLANT HORMONES AND THEIR ROLE IN PLANT GROWTH

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Plant hormones are organic endogenous signal molecules produced within the plants in extremely low concentrations that can regulate diverse biological functions at cellular as well as at molecular levels. They control all aspects of normal plant growth and development, from embryogenesis to the regulation of organ size, reproductive development, pathogen defense, stress tolerance and even plant death. Moreover, the metabolism of hormones provides the energy and building blocks for plant life as it regulates the growth rate of the individual plant parts and integrates them to produce the shape that we recognize as a plant. Unlike animals, in which hormone production is restricted to specialized glands, each plant cell is capable of producing hormones. Furthermore, hormones as signal molecules regulate cellular processes in targeted cells locally as well as systemically when moved to other parts of the plant. Six major hormones identified are auxins, cytokinins, gibberellin, abscisic acid, ethylene and brassinosteroids. Modern studies also include jasmonates and strigolactones. A single hormone can regulate many cellular and developmental processes, while at the same time multiple hormones often influence a single process. In addition to this, a large number of related synthetic chemical compounds are used to regulate the growth of cultivated plants, weeds and in vitro grown plants and plant cells. These human-made compounds are known as plant growth regulators (PGRs). Taken together, hormones are vital to normal plant growth and development and lacking them turn plants into mostly a mass of undifferentiated cells.

# ISOLATION AND ITS-RDNA BASED MOLECULAR CHARACTERIZATION OF PLANT PATHOGENIC FUNGAL SPECIES INPOSTHARVEST CITRUS FRUITS

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The quality and quantity of citrus fruit is depreciated due to various fungal diseases encumbering the copacetic standards, thus rendering economic losses. The current study is based on the morphological, pathological and molecular identification of the fungi associated with citrus fruit diseases. The isolated pathogens from infected fruit samples, cultured on potato dextrose agar followed by morphological characterization, belonged to *Lasiodiplodia* sp., *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sp. The imperative species identification was conducted by molecular sequencing. Pathogenicity assays on three different citrus varieties were performed to detect the most viable variety to postharvest fungal diseases. All isolates displayed significant results in cv. Kinnow except for *Fusarium* sp., which displayed a high lesion area on cv. Malta and cv. Musambi. Genetic identification exhibited 95–100 % similarity with *Lasiodiplodia theobromae*, *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Penicillium digitatum*, *Fusarium solani* and *Fusarium oxysporum*. Results could be beneficial for developing the management policies to reduce the diseases in citrus orchards.

# ROLE OF SALICYLIC ACID IN CONTROLLING PLANT DISEASES

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Salicylic acid (SA) is a natural plant hormone that mitigates biotic and abiotic stresses by improving plant resistance and plays a crucial role in thermogenesis and disease resistance. It is involved in signalling and activation of plant's effector-triggered immunity system (ETI), pattern-triggered immunity (PTI) and systematic acquired resistance (SAR), which prevents the colonization and spread of pathogen on the plant surface. Activation of SAR results in acquiring resistance in distant uninfected parts of the plant against bacteria, fungi and viral attack. It acts as a stimulant or transmitter of the cell during stress conditions such as drought, cold, heat and salt stress. Its accumulation within the plant body is important for the quick response or activation of multiple modes of plant disease resistance. SA is linked by an intricate network, a protein interaction network in the plant, with many essential components of plant defense mechanism. Non-expressor plant proteins (NPR1) are regulated by SA during the pathogen attack. Production of SA increases during pathogen attack that converts inactive NPR1 into active monomers that interacts with the cis-element binding protein, TGA, present within the nucleus, which results in the expression of various SA-dependent pathogenesis-related genes. SA is involved in protecting the plant from fungal attacks and nematodes by activating the plant defense mechanism quickly as compared to the non-salicylic acid-mediated defense signalling pathway. It is also helpful in nutrient uptake by the plant during stress conditions. It increases the N, P, K, Mn and Mg accumulation within the plant body during stress. It declines the Na and Cl accumulation within the plant body when grown in saline conditions.

# A COMBINATIONAL APPROACH TO COMBAT INSECTS THROUGH NEXT-GENERATION *BT* AND PLANT LECTINS

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The efficacy of crystal protein has been assumed to be compromised because of its extensive utilization in crop plant cotton. The second-generation *Bt* vegetative insecticidal protein could be best suited as an alternative to combat such problem of resistance build-up due to its broad range of affinity with a receptor in midgut region of insects. Plants lectin provide the first direct response against the attack of insects by acting as entomotoxic protein. The combination approach of using second-generation *Bt* (VIP3A) gene with the plant lectin gene can be the best-suited approach to combat insect resistance and to minimize the 50 to 60% losses caused by chewing and sucking insects. In this study, transgenic plants of cotton expressing insecticidal toxin and plant lectin gene were developed to control the major infestation against chewing and sucking insects. The Vegetative Insecticidal Protein 3A and *Allium sativum* leaf agglutinin gene already cloned into pCAMBIA-1301 vector was taken from CEMB Plant Biotechnology Lab and was processed to be transformed into locally developed GS1 non-*Bt* cotton variety. The recombinant pCAMBIA-1301 vector containing gene cassette was transformed into the *Agrobacterium* competent cells through electroporation and confirmed through restriction digestion and polymerase chain reaction. The transformation of foreign gene (pCAMBIA_VIP3Aa + ASAL) cassette into the cotton genome was done through *Agrobacterium*-mediated shoot apex technique. Gene-specific primers of Vip3Aa and ASAL gene were used to confirm the amplification of 682 bp fragments of Vip3Aa gene and 587 bp fragments of ASAL gene in transgenic cotton plants. The 70 acclimatized transgenic cotton plants were shifted to a tunnel in which 45 plants were amplified through PCR. The results obtained through a combinational approach to combat both chewing and sucking insects determined the potential of the material to be utilized in the breeding programme for variety development.

# ANTIFUNGAL EFFECT PHYTOEXTRACTS ON MANAGEMENT OF *PHYTOPHTHORA CAPSICI* CAUSING PHYTOPHTHORA BLIGHT IN CHILLI (*CAPSICUM ANNUM* L)

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Chilli is most important commercial and domestic vegetable which is used as sauce, pickle, spice, condiment in all over the world. But now a days, great loses occur due man fungal diseases such as wilting, rots, blight, anthracnose etc. Among all Phytophthora blight is most important disease caused by *Phytophthora capsici*. Fruits may exhibit dull green, sunken, water soaked and discoloured lesions which later turn brown to black and as a result wilting, girdling and ultimately death of plant occur. Disease samples were collected from different chilli growing areas. Pathogen was isolated from infected samples and purified in diagnostic lab. To manage this phytopathogenic fungi five phytoextracts (*Allium sativum, Moringa oleifera, Zingiber officinale, Azadirachta indica and Mentha*) were used under lab and field condition. Among these phytoextracts, *Allium sativum* exhibited minimum colony growth followed by *Moringa oleifera*, *Zingiber officinale*, *Azadirachta indica* and *Mentha* as compared to control. *A. sativum* has maximum inhibitory effect against *P. capsici* at 75%.

# A SYSTEM BIOLOGY APPROACH TO EXPLORE HOST-PATHOGEN INTERACTION UNDER PHYTOCHEMICAL CROSS-LINKAGES

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Phyto-signalling molecules are minute but tangible that has rigorous roles in any plant-pathogen interaction. Certainly, most of the pathogen alters their biosynthesis, transport, degradation and cellular signalling responses to pave their virulence. Therefore, the gene expressions of such molecules with their correlated defence mechanisms were analysed in *Arabidopsis thaliana* against *Erysiphe orontii* (a potential biotroph), *Botrytis cinerea* (a potential necrotroph), *Pseudomonas syringae* (a bacterial hemibiotroph), and *Phytophthora infestans* (a fungal hemibiotroph) using molecular biology/ system biology techniques. The findings strongly suggested that each pathogen has its unique infection strategy based on up-regulation and down-regulation of host phyto-signalling genes. Our studies also explored four basic pathogenic infection maps based on cross-linking phyto-signalling molecules.

# BIOSTABILIZATION OF ARSENIC IN RHIZOSPHERE BY ENHANCED SECRETION OF EXOPOLYSACCHARIDES SECRETION AND STRENGTHENED ANTIOXIDANTS SYSTEM

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Arsenic, an analog of phosphorous can actively be absorbed by the plant and able easily incorporate into the food chain and pose serious health risks to a human. Several contemporary techniques including bioremediation, are used for stabilizing such bioactive contaminants and ensuring food safety. In this context, *Pantoea conspicua*, an arsenic tolerant plant growth-promoting rhizobacteria (PGPR) was used to alleviate the arsenate stress while improving host growth attributes. A novel strategy of biotransformation coupled adsorption was used by the strain to stabilize metal on their cell wall and avoid its entry across the membrane of their own and host cells. Host plants under arsenate induced stress show a severe reduction in their growth characteristic in terms of net assimilation rate (NAR) and relative growth rate (RGR ($p<0.05$)) whereas accumulation leads to ROS accumulation resulting in secondary oxidative damage. Due to oxidative stress, membrane disruption and electrolyte leakage occur making them prone to abiotic stressors. Inoculation of *P. conspicua*, stimulate the host to release higher quantities of indole acetic acid and stress-related primary and secondary metabolites aiding the host to sustain their normal growth. Apart from this, *P. conspicua* inoculation triggers the host to the secret bulk amount of exopolysaccharide (EPS) that help to lockdown the arsenic in the rhizosphere thus, avoiding uptake and accumulation hence, ensuring food safety and human exposure. In addition, the selected strain tends to improve the antioxidant system, which actively scavenges the ROS produced as a result, lower ROS accumulation, lower malonaldehyde production and ensuring higher membrane integrity of the host. Apart from this, higher phytohormones and metabolite production makes the strain effective phyto-stimulant and can potentially be used as a biofertilizer and bio-remediator.

# AGRICULTURAL BIOTECHNOLOGY: A WAY FORWARD FOR PROTECTING CROP PLANTS AGAINST BIOTIC STRESSES

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Plant diseases, pests and herbs significantly decreased agricultural production, putting a threat to the world's growing population's food supply. The use of biotechnology tools is reliable, quick, and accurately effective in plant protection and production policies. In this perspective, different biotechnological techniques are being studied as potential long-term control against phytopathogens. As a result, this review article provides a comprehensive overview of the many molecular techniques such as genome editing for stress tolerance, RNA interference, CRISPR/Cas9 technologies used to plant protection, pests, and insect resistance. *Bacillus thuringiensis* (Bt) *has* 300 different cry proteins that are active against a wide range of insect species. Bt toxin mainly affects the digestive system of the insects in different crops cotton, rice and maize. Some of the important plant defense proteins against insect pests are protease inhibitors responsible for protecting plants from pests and possible infections. Alpha-amylase inhibitors, trypsin inhibitors, cystatins have been transformed in the crop plants against different pests. The studies have also recognized that chemical substances like aminobutyric acid (ABA) or benzothiadiazole (BTH) can influence biotic stress tolerance in plants. RNAi has been largely used for host induced gene silencing for mainly the biotrophic and obligate plant pathogens like nematodes and viruses. Recombinant DNA technology can be successfully utilized in plants to help them fight infections. CRISPR Cas system may improve the immunity to protect themselves against viruses. S1M1o1 gene in the tomato genome confers resistance to powdery mildew fungus (Oidium neolycopersici). As a result, researchers used double sgRNA and a transgene-free powdery mildew-resistant tomato to target two key sites in the S1M1o1 gene. To summarise, rising diseases and pests, as well as ongoing scientific discoveries have made biotechnology tools are important to use in plant protection.

# APPLICATION OF FRAGMENTED AND UN-FRAGMENTED DNA FROM FUNGAL PLANT PATHOGENS AS CROP PROTECTION STRATEGY TO COPE WITH ROOT ROT IN CHILI

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Oomycetes organisms caused very devastative crop losses in vegetables and other field crops by infecting the plant both at pre and post-emergence growth stages. Chemicals and other commonly used management practices are very less effective against these organisms. The use of elicitors in crop protection is considered a sustainable alternate for inducing resistance in plants against pathogens. An investigation was made to confirm this hypothesis, for this purpose DNA of *Phytophthora capsici and Rhizoctonia solani was isolated and* fragmented in laboratory conditions. The pure fragmented and un-fragmented DNA of both pathogens was mixed and three different concentrations (20, 40, and 60 **m**g $mL^{-1}$) were sprayed on chilli plants against root rot disease. Change in resistance/susceptibility status of treated plants by inhibiting the growth of pathogens and activating the defense mechanisms in plants was studied. Interestingly, it was observed that the treated plant with fragmented and un-fragmented DNA mixtures at a concentration of 60 **m**g $mL^{-1}$ showed 25 % decrease in mortality of infected (*P. capsici and R. solani)* chilli plants *at 35 days after inoculation as compared with the control treatment not sprayed with the mixture of pathogen's self-origin* DNA fragments only inoculated with pathogen's spore suspension. This investigation shows a new perspective in crop protection especially to deal with oomycetes causing root rot in *Capsicum.*

# MOLECULAR CHARACTERIZATION AND MANAGEMENT OF *ALTERNARIA SOLANI* INCITING EARLY BLIGHT OF TOMATO THROUGH BACTERIAL STRAINS

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Tomato is grown widely in almost 140 countries with an annual production of 151 million tons and mostly grown in South America. Pure culture of *Alternaria solani* isolated from twig and leaf samples were prepared. The fungal colonies were cultured in Broth of MEA and PDA for 5-7 days at 25°C. Cetyl-trimethyl-ammonium-bromide (CTAB) extraction technique was applied for DNA extraction of *A. solani* with little improvement. Molecular identification with ITS primers of isolated fungi was undertaken and the 594 bp size of the band was obtained. The DNA sequence was submitted to Gen Bank having Gene Bank no. MN200940. The sequenced data was 100% similar to the *Alternaria* isolate Alt-C81 of Gen Bank accession code MN044802. Biological control agents were supplied and data were collected at an interval of 3, 5, 7 and 10 days. Maximum inhibition percentage after 3 days (84.23%) was recorded by *Azospirillum lipoferum* (RB-38) while the lowest inhibition (44.84%) was recorded by *Bacillus subtilis* (RB-59). After 7 days of incubation, the highest inhibition (89.13%) was observed by using *A. lipoferum* (RB-38) and the lowest (60.57%) by *Bacillus spp*. (RB-43), respectively. After 10 days of incubation, highest inhibition percentage (95.91%) was recorded by *B. subtilis* (RB-59) and lowest by *Bacillus thuringiensis* (RB-16) with a PI of and 13.71% respectively. These results suggest that the bacterial isolates studied have a good potential to be used as biocontrol agents of *A. solani* in tomatoes for sustainable production of tomatoes without using synthetic fungicides. It not gives a good perspective for sustainability but also reduces the environmental pollution.

# *IN VIVO* AND *IN VITRO* EVALUATION OF FUNGICIDES AGAINST PHYTOPHTHORA BLIGHT DISEASE OF CHILLI CAUSED BY *PHYTOPHTHORA CAPSICI*

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Different species of Phytophthora are most destructive plant pathogens of various plant species worldwide. Among all these *Phytophthora capsici* is most important oomycete plant pathogen which causes Phytophthora blight in chillies. Water soaked and discoloured lesions are formed which later turn brown to black. Samples were collected from different chilli growing region of Pakistan. *In vitro* and *in vivo* experiments were conducted in diagnostic lab and plant pathology research area of UAF, Faisalabad by using five fungicides (Nanok, Nativo, Novice, Misheal and Ellectus). To evaluate the efficacy of fungicides, three treatments as well as three concentrations were prepared by using stock solution. Among five fungicides, Nanok showed maximum inhibitory effect followed by Nativo, Novice, Misheal and Ellectus as compared to control. Nanok has maximum inhibitory effect of mycelial growth of *P. capsici* at 250ppm while100% control at 500ppm.

# FUNGICIDAL ACTIVITY OF SHOOT EXTRACT OF *SISYMBRIUM IRIO* AGAINST *FUSARIUM OXYSPORUM* F. SP. CEPAE

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To check the antifungal potential of *Sisymbrium irio* shoot extract against *Fusarium oxysporum* f. sp. cepae (FOC), the present study was carried out. In preliminary bioassays, different concentrations (1 to 5%) of leaf, stem and fruit extracts were evaluated against FOC. All the extracts were effective against the pathogen. Leaf extract was found the most effective causing 25-41% decrease in FOC biomass. The fractionation of methanolic leaf extract was done by two organic solvents namely *n*-hexane and chloroform. Different concentrations (1.56 mg $mL^{-1}$ to 200 mg $mL^{-1}$) of these fractions were tested against FOC. The *n*-hexane and chloroform fractions showed inhibitory activity against the pathogen and resulted in 77-93% and 80-96% reduction in biomass of FOC. GC-MS analysis showed the presence of 24 compounds in *n*-hexane, and 4 compounds in chloroform fraction. In *n*-hexane fraction, β-sitosterol (18.64%) was the most abundant compound followed by orotic acid, bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester (12.18%), 10-octadecenoic acid, methyl ester (7.90%) and 1,2-benzenedicarboxylic acid, diisooctyl ester (6.05%). This study concludes that *n*-hexane and chloroform fractions of methanolic leaf extract of *S. irio* contain potent antifungal constituents for the management of FOC.

# EFFECT OF DIFFERENT APPLICATIONS OF ENDOPHYTIC MICROBES ON MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF CHILLI GENOTYPES

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Chilli (*Capsicum annum* L.) belongs to the genus *Capsicum* and the family Solanaceae, which is one of the most important spice crops in tropical and subtropical countries, ranking as the fourth most farmed crop on the planet. The global population is growing at an exponential rate; by 2050, it could reach 9.1 billion people. Its output, however, is insufficient to fulfil the demands of an ever-increasing population. To increase its productivity, many chemical fertilizers have been used in the past. Chemicals are generally a restricted option due to their high toxicity and probable negative impact on the environment. The focus now is on developing a safe and environmentally friendly growth enhancement method that includes the use of endophytes. In the present study, an experiment was conducted to decipher the bio-control potential of fungal endophytes (*Trichderma sp., Piriformospora indica*) and bacterial endophytes (*Bacillus sp*., *Arthrobacter* sp., *Burkholderia phytofirmans* PsJN) on various plant Morpho-physiological parameters. In this experiment, the four varieties HP-609, ASHA-F1, RED SKY, and HP-8011 were used with four replications in a completely randomized design. Endophytes were applied through the root dipping method. Data of all the morpho-physiological parameters were recorded and all the data were analysed through the proper statistical tool. As a result, it was found that the highest dry root weight and petiole length were observed in plants treated with *Trichoderma* of cultivar ASHA-F1 and HP-609 respectively. Furthermore, maximum numbers of leaves, fresh shoot weight, fresh root weight, plant height and dry shoot weight were observed in plants treated with Arthrobacter of cultivar ASHA-F1. *Piriformospora indica* treated plants of cultivar HP-609 showed the highest petiole length. So it can be said that maximum growth was shown by plants treated with *Arthrobacter* and then *Trichoderma.* However, *Bacillus, Piriformospora indica* and PsJN also showed significant results.

# INCIDENCE AND MOLECULAR IDENTIFICATION OF ROOT-KNOT NEMATODES INFECTING TOMATO IN GILGIT BALTISTAN

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Root-knot nematode (*Meloidogyne* spp.) a parasite of almost all crops, is considered to be one of the most devastating plant parasites having a global distribution with significant economic impact worldwide. Proper detection and quantification of disease in terms of its incidence facilitates the management process. It is the first step in the selection of a suitable management strategy and cannot be ignored. Field surveys were carried out in eight localities of district Astore and Gilgit to investigate the incidence and prevalence of root-knot nematodes (RKNs), *Meloidogyne* spp., associated with tomato plantation in spring, 2019. *Meloidogyne incognita* (Kofoid and White) Chitwood was found associated with rhizosphere soils of tomato (*Solanum lycopersicum* L.). Species identification of *Meloidogyne incognita* was confirmed using perineal pattern morphology and SCAR primers. Soil pH (6.85-7.90), soil EC (0.06-0.24 d S/cm) and soil textures (Sandy loam to silt loam) were determined. Overall disease prevalence and disease incidence in the studied area was 71.87% and 36.7%, respectively. Disease galling index (2.18) and many juveniles (876) in 100 $cm^3$ soils were maximum sandy loam textures soils of Jaglote at district Gilgit. Perineal pattern morphology revealed characteristics squarish arch of *Meloidogyne incognita*. DNA amplification with SCAR primers (Finc/Rinc) produced characteristics 1200 bp size product for *M. incognita*. This is the first report of root-knot nematodes, *M. incognita* infecting tomatoes in Gilgit Baltistan.

# CRISPR-CAS9 BASED GENOME EDITING APPROACH FOR ENHANCING DISEASE RESISTANCE IN PLANTS

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A rapid increase in population is exerting serious pressure on food systems jeopardizing global food security. In addition to this, various forms of plant-pathogen cause severe losses in yield under field and storage conditions equally. In order to coup up with these challenges, genetic improvement of crop plants is essential to meet up with rising food demands around the world and to ensure food for everyone. Conventional approaches for genetic improvement take decades to produce a fruitful outcome, whereas the transgene approach has continuously met with scientific and ethical dilemmas. Therefore, a modern gene-editing technology the CRISPR-CAS9 has proven to be a revolutionizing approach to enhance disease resistance in plants. The technology has successfully been utilized in model plants as well as major crops plants; including wheat, rice, maize, potato and citrus for enhanced resistance towards various forms of phytopathogens. CRISPR-CAS9 provide a transgene-free approach for genetic manipulation of crop plants and is providing important support on the path of development of disease-free plant varieties, ultimately ensuring sustainable food productions for the future.

# MICROCLONAL PROPAGATION OF THE MEDICINAL PLANT GINKGO BILOBA IN *IN VITRO* CONDITIONS

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*Ginkgo biloba* L. is the only species of the Ginkgo family that has survived to the present day. The plant is unique with its medicinal properties. Regarding the healing properties of ginkgo biloba, scientists began to study the unique healing properties of the tree in the sixties of the twentieth century. They began to gather a lot of data about its healing power, as well as conducted research on how this plant works in the whole human body and found that it has the following healing properties: it is in the veins, capillaries improve blood circulation, as a result of which the oxygen supply to all organs and tissues of the living organism is improved, the activity of the brain and memory is restored; protects platelets from sticking and bleeding; helps relieve bronchial asthma attacks; works effectively in the treatment of insomnia and depression; an indispensable tool in the treatment of diabetes; can stop the growth of metastases in cancer. Our study aimed to optimize the *in vitro* culturing processes of Ginkgo biloba. *Ginkgo biloba* L. is of great importance as an ornamental tree and as a source of many chemicals with pharmacological properties called ginkgolides. Due to the difficulties of propagating this plant under natural conditions, low germination of seeds, it is necessary to develop methods of vegetative propagation of seedlings in vitro. There is a lot of information about in vitro propagation protocols for multi-purpose medicinal trees, but little is known about the cultivation of Ginkgo biloba in the literature. In the Laboratory of Transgenomics and Tissue Culture of the Center for Genomics and Bioinformatics, research is being conducted to grow *Ginkgo biloba* L. from apical buds in nutrient media enriched with various growth regulators and other substances in Murashige and Skoog environments.

# NANOTECHNOLOGY: A FANGLED APPROACH TOWARDS PLANT DISEASE MANAGEMENT

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Plant diseases are the major contributors to yield reduction (15-20%) in the agricultural sector. Up till now, various management strategies including cultural control, chemicals, plant extracts and antagonistic microorganisms have been used to combat plant diseases. The application of synthetic chemicals is not a healthy approach towards plant diseases as it causes environmental pollution and has health hazard effects on human beings. While plant extracts and antagonistic microorganisms are slow in action against plant diseases. Nanotechnology offers an efficient, eco-friendly alternative source for the proper management of plant diseases in agriculture. Plant-based nanotechnology is an advanced method, which certainly opens a new era in the field of agriculture to overcome such problems. Many studies have been performed to explore the mode of action of nanoparticles (NPs) against different plant pathogens. Nanoparticles (AgNPs, CuNPs) improve plant health and affective against plant pathogens in several ways as they have antimicrobial activity, genotoxicity, ROS-induced cytotoxicity and plant growth promotion. The antimicrobial activity of nanoparticles (NPs) is significantly governed by their size, concentration, shape and dose. The current study is mainly focused on the limitations in traditional plant disease management and the potential of nanoparticles (NPs) for the management of plant pathogens.

# *HY5* MANIPULATES PLANT DEFENSE AGAINST DOWNY MILDEW THAT IS LATER SUPPRESSED BY A *HYALOPERONOSPORA ARABIDOPSIDIS* (HPA) EFFECTOR

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Plant pathogens release effectors into the host cell to manipulate plant immunity, but the mechanisms of these effectors that suppress plant immunity remain unclear. ELONGATED HYPOCOTYL 5 (HY5), an important light signalling component, plays an important role in growth, development and abiotic stress. However, there is no direct evidence if HY5 participates in responding to biotic stress. Genetic and transcriptomic analysis was performed to test that HY5 positively regulates plant immunity and HaRxL5, an RXLR effector from *Hyaloperonospora arabidopsidis* (Hpa), suppresses plant immunity. HaRxL5 target proteins were screened using Immunoprecipitation-mass spectrometry and HY5 was selected to be the candidate. In vitro and in vivo assays confirmed the interaction between HaRxL5 and HY5. HY5 acts as a positive regulator in plant immunity through increasing plant defense-related genes expression. The effector HaRxL5, enhanced plant susceptibility to Hpa, interacts with host HY5. The interaction between HaRxL5 and HY5 compromises the DNA-binding activity of HY5, resulting in a decrease of defense-related genes expression, reactive oxygen species accumulation. Our study demonstrated that HY5, a critical regulator in photomorphogenesis, positively regulates plant defense against Hpa, and the effector HaRxL5 enhances enhanced plant susceptibility through interacting with HY5 to attenuate HY5 DNA binding activity.

# USING BIOCHAR IN COMBINATION WITH LOW PH PRODUCT AND BACTERIAL ANTAGONIST FOR GROWTH PROMOTION AND MANAGEMENT OF ROOT-KNOT NEMATODE IN CHILI

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The chilli (*Capsicum annuum* L.) is a popular vegetable from the Solanaceae family. Various abiotic and biotic stresses have a global impact on chilli productivity. Besides viral, fungal, bacterial, and nematode disorders, the remarkable production limitations affecting yield and quality are plant-parasitic nematodes around the globe. The root-knot nematode, *Meloidogyne incognita* form a special relationship with their host by developing knots, galls in the roots of the plant. Root-knot nematodes antagonistic microorganisms are a good option for plant management in terms of food safety and human health. Biochar can increase the availability of nutrients for the plants. Antagonistic bacteria (*Janthinobacterium* sp.) have been shown the potential of biocontrol which suppress fungal diseases in plants and can be used to protect the plants against nematode infection. To overcome this particular issue, a study was conducted in the research area of the plant pathology department at the University of Agriculture, Faisalabad. Nursery of desi chilli was taken from Ayub Agriculture research institute (AARI), seedlings were grown in suitable size pots. Grown chilli plants have been studied in response to the effect of root-knot nematode and the growth promotion in various soil amendments and the effect of bacterial antagonists have been was studied against root-knot nematode. CRD design was used for laying out the experiment and subsequent statistical analysis. This experiment was conducted by using biochar (10%, 1.5% and 2%0g), low pH product (0.5%, 10% and 1.5%g) and Janthinobacterium for growth promotion and management of root-knot nematode in chilli. Results showed that the treatment with 2.0% Biochar biochar along with Janthinobacterium in presence of nematode (T7) show maximum value for fruit length and fresh shot weight. Whereas treatment with 2.0% Biochar along with Janthinobacterium in presence of nematode (T7) show maximum value for fresh shot weight. . Similarly, the Treatment with 0.5% Low low pH product along with Janthinobacterium in presence of nematode (T2) show maximum value for fruit weight. The treatment with 1.5% low pH product along with Janthinobacterium in presence of nematode (T4) revealed a minimum value for fresh shoot weight. The treatment with 1.0% low pH product along with Janthinobacterium sp. in presence of nematode (T3) revealed a minimum value for dry shoot weight. Overall low pH product in combination with Janthinobacterium sp. gave the best results for plant growth traits. Best control of Meloidogyne incognita was observed in a consortium of treatment with 2.0% Biochar along with Janthinobacterium sp. in presence of nematode (T7) followed by the treatment with 1.5% low pH product along with Janthinobacterium in presence of nematode (T4).

# DISEASE IDENTIFICATION THROUGH VOLATILE ORGANIC COMPOUND (VOCS) EMISSIONS PROFILING IN FRUITING PLANTS

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Analysis of volatile organic compounds emissions is a natural and environmentally friendly way to improve crop defense by identifying diseases in plants. VOCs can be emitted at any moment from the leaves of plant species in response to biotic or abiotic stresses. The findings show that abiotic challenges induce the emission of isoprenoids, the most prevalent type of VOCs, which improves plant resistance either immediately through quenching reactive oxygen species (ROS) or passively by stabilizing cell membranes. Several VOCs released by leaves have been demonstrated to prevent germination and growth of plant pathogens in the experimental study, although the mechanisms of action are unknown. VOCs can affect the plant defensive system by provoking the formulation of defensive proteins and metabolites (e.g., phytoalexins) which hinder microbial populations. The release of specific VOCs 's appears doubtful because it is well known that most of the similar VOCs are emitted in response to a variety of viral and noninfectious conditions. Majority of the VOCs are associated with fungal disease in tomato plants, were also associated with ozone therapy of tomato plants. When different plant species were challenged with the same virus, the same VOCs were released.

# APPLICATION OF DIFFERENT CARRIER MATERIALS TO INTRODUCE ENDOPHYTIC BACTERIA FOR GROWTH PROMOTION IN BREAD WHEAT

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Wheat is a cereal crop from the Poaceae family. In many countries of the world especially in Pakistan *Triticum aestivum* (wheat) is main source of human diet. It contain important nutrients like carbohydrate and protein so, it is source of energy for population of entire world. For food security, economic development and human nutrition it is very important. Experiment was conducted to check the response of two endophytes **(***Burkholderia phytofiramans*, PsJN and Bacillus sp. MN54) in combination with carrier materials (talc. Powder, wheat biochar, sugarcane biochar and farmyard manure) on varieties of wheat (Ujala-16, Zincol-16 and Fathejang-16) in *in vitro* conditions. The morpho-physiological attributes of seedling (fresh root weight, shoot weight, root length, shoot length, leaf area, dry root and shoot weight) and plant mature (flag leaf area, peduncle length, internodal length, plant height, spike length, number of tillers per plant, yield/plant, spike weight, 1000 grain weight, number of grains/spike, and number of spike lets/spike) was checked. Results showed that the interaction of negative control and Zincol-16 represent maximum internodal length (18.900). Plant height was noticed in talc.powder and MN54 with Fathejang-16 interaction. Number of tillers/plant was observed in talc. Powder and MN54 with Fathejang-16 (81.300) interaction. Th interaction of talc. powder and PsJN treatment with Zincol-16 (41.650) showed maximum 1000 grains weight. The yield/plant (6.7300) was noticed maximum due to interaction of sugarcane biochar and MN54 with Fathejang-16. Among varieties, Fathejang-16 showed maximum results for plant height, peduncle length, spike length, flag leaf area, number of grains/spike, yield/plant. 1000 grain weight and spike weight was maximum in Zincol-16 cultivars. It is concluded that endophytic bacteria could be used for the growth promotion in bread wheat.

# SECTION 5: PLANT NEMATOLOGY

# INFLUENCE OF WESTERN CORN ROOTWORM SEED COATING AND GRANULAR INSECTICIDES ON SOIL NEMATODE COMMUNITIES

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Our study was conducted in a maize field to determine the impact of the season and chemical treatment on nematode communities. Field trial included the variants with granular application with tefluthrin (199.5 g a.i./ha), granular application with clothianidin (110 g a.i./ha), seed treatment with clothianidin (1.25 mg a.i/seed) and control variant without application of insecticides. The nematode community was investigated for species composition, trophic structure and biodiversity. A total of 37 nematode species and 33 genera were identified during the investigated period. *Acrobeloides nanus*, *Cephalobus persegnis*, *Eucephalobus striatus* and *Basiria gracilis* were the dominant species, accounting for 48 % of the number of nematodes. The mean abundance and species diversity index were significantly lower for the maize variant with granular application of clothianidin. Bacterial feeders were the dominant trophic group for all four variants. The population numbers of the different trophic groups were significantly different between variant with granular application of clothianidin and the control. The Sum Maturity index, Maturity index and Plant parasitic index did not show significant differences among the variants. The highest values of the Enrichment and Structure indices were observed in the first month of the investigation in all four variants. According to cluster analysis, nematode species population densities were strongly affected by the date of soil sample collection and by the variants used in the experiment.

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# PHYTOPARASITIC NEMATODES THREAT IN SUSTAINABLE AGRICULTURE: AN OVERVIEW

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Phytoparasitic nematodes are tremendous threat to agriculture production. The extent and nature of symptoms initiated and yield damage caused during root feeding depends on the nematode-crop combination. *Meloidogyne* spp. including *M. incognita* and *M. javanica* is destructive pests of field, vegetable, and fruit crops. Majority of the phytoparasitic nematodes feed on roots, ecto-endo parasitically. Nematode-root-feeding induce root galling by *Meloidogyne* spp. the obligate sedentary parasites of vascular tissues; root lesions by epidermal feeders, cortical deformation by cortical feeders. Nematode-damaged-roots exhibit reduced root system with fewer feeder roots ultimately reduced plant foliage vigor. Nematode-root-damage reduces plant ability to extract water and nutrients, the result being lack of vigor and yield loss. We observed ca. 22.4% yield losses from 19 commercially grown vegetable crops. Damage ranged from 2% for cabbage to 45% for squash, which was 35%, 80%, and 46% higher than that from developed countries, USA, and India, respectively. The main reason for more losses in Pakistan appears related to unawareness of growers about the presence of nematodes and damage they cause. Another reason might be non-availability of resistant crop cultivars and nematicides. Recommended measures against nematode diseases include use of clean nematode free planting material, resistant varieties, and crop rotations to suppress nematode infestation. Integrated nematode management can be promoted through farmers-nematologists interactions, local production of bio-control agents and strict chemical pesticide regulations. Farmer's/grower's awareness and skills are equally important in minimizing nematode infestation and yield losses, to sustain the agriculture production.

# BIOCONTROL POTENTIAL OF ENTOMOPATHOGENIC NEMATODE ISOLATES RECOVERED FROM POTATO-GROWING AREAS OF TURKEY AGAINST THE LARVAE OF *AGRIOTES RUFIPALPIS* (BRULLE, 1832) AND *AGRIOTES SPUTATOR* (LINNAEUS, 1758) (COLEOPTERA: ELATERIDAE)

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Wireworms, *Agriotes* spp. (Coleoptera: Elateridae), are one of the destructive soil-dwelling insect pests. Recently, damaging activities of wireworm larvae, which are difficult to control, have been increasingly reported in potato-growing areas of Turkey. In the current study, the potential of different entomopathogenic nematode (EPN) isolates (*Steinernema feltiae* Sf_BL22, *S. feltiae* Sf_BL24, *S. feltiae* Sf_KAY4, *Heterorhabditis bacteriophora* Hb_N3, *H. bacteriophora* Hb_KAY10, and *H. bacteriophora* Hb_AF12) obtained from domestic potato cultivation areas were tested against larvae of two most common wireworm species, *A. rufipalpis* (Brulle, 1832) and *A. sputator* (Linnaeus, 1758) (Coleoptera: Elateridae), at different temperatures (25 and 30°C) and concentrations (12.5, 25, 50, 100, and 150 IJs/cm$^2$) in both plastic cup and pot experiments. The increment in the mortality rates of the wireworm larvae were observed with the increasing temperatures and concentrations of IJs. In the plastic cup experiment, the EPN isolates were more effective against the larvae *A. sputator* 3 days after treatment (DAT), and the highest mortality (50%) was obtained by applications of *S. feltiae* Sf_BL22 and *H. bacteriophora* Hb_KAY10 isolates at 30°C. The mortality rates of *A. rufipalpis* and *A. sputator* larvae were similar 7 DAT and ranged between 72.5% and 87.5%. In the pot experiment, the mortality rates over 60% were generally achieved at the highest concentration (150 IJs/cm$^2$) 7 DAT by all isolates except for *H. bacteriophora* Hb_N3. The results indicated that native EPN isolates were highly effective against the larvae of *A. rufipalpis* and *A. sputator* and had great potential to be used in the biological control of wireworms.

# PHYTOTHERAPEUTIC IMPACT OF BOTANICALS AQUEOUS EXTRACTS ON TOMATO (*LYCOPERSICON ESCULENTUM* L.) CROP INFESTED BY ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

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Root-knot nematodes (RKNs) are important soil-borne pathogens cause heavy yield losses to tomato crop. Neem (*Azadirachta indica* L.) and hemp (*Cannabis sativa*) extracts had reputed with efficient, cost-effective and environment-friendly plant disease management tools. Aqueous leaf extracts of both plant species at three concentration levels (25, 50 and 75% w/v) and one combination (50% w/v) of each were applied on tomato cv Roma root zone to evaluate their efficiency to control and safe the crop against RKN in pot experiment under glasshouse conditions. Pots were filled with autoclaved soil, organic matter, and sand in equal amounts and aqueous extracts were mixed thoroughly at the final stage of soil preparation before transplantations. RKN 2000 J2 were inoculated 15 days after transplanting when plants successfully established their root system. The experiment was replicated thrice and terminated 70 days after nematode inoculation. Botanical aqueous extracts effectively reduced J2 penetration 70 days after inoculation and reduced the reproduction factor. The most effective concentration which reduced Galling Index (GI) was Neem (75%), followed by combined application (50+50% w/v) of hemp and neem. Interaction of both extracts positively influenced plant health and reduced nematode infestation either due to increased nutrient supply and improvement in soil health. Extracts werefound effective in the low-temperature area and can be used as alternatives to each other where other is not available.

# EFFICACY OF BIOCHAR APPLICATION FROM DIFFERENT FEED STOCKS ON PLANT GROWTH AND RESISTANCE AGAINST ROOT-KNOT NEMATODES IN TOMATO

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Tomato (*Lycopersicum esculentum Mill.*) is one of the most important vegetable crops cultivated worldwide including Pakistan. In Pakistan, the tomato is vulnerable to attack by various biotic and abiotic stresses. Among all pathogens, plant parasitic nematodes cause significant losses to vegetable crops including tomato. Root knot nematode (*Meloidogyne incognita*) is a serious threat to tomato production among all *Meloidogyne spp*. and it is a major pest causing serious crop losses globally. In the present study, the experiment was conducted to evaluate resistance response of tomato cultivar towards *M. incognita and t*o assess growth and yield response of tomato cultivar *by using biochars from different feed stocks (sugarcane, green waste, wheat straw, maize stalk) at different concentrations like 0.5, 1.0 and 1.5% (w/w) with three replications under completely randomized design* and nematode application, biochar feedstocks, biochar concentrations and all of their interaction were studied by analyzing all the data of root weight (gm), Shoot weight(gm), growth, chlorophyll contents, plant height(cm), number of galls, egg masses and no. of females. It was cleared from the results that different temperatures used for pyrolysis performed considerably well in managing the PPNs and promoting different plant physiological parameters. Green waste, maize stalk and wheat straw perform significantly better in enhancing the yield of tomato plant. Sugarcane baggase somehow contributed in enhancing overall plant growth. In 2nd experiment impact of biochar in controlling of *M. incognita* was confirmed by substantial reduction in nematode attack in biochar amended soil. Whereas, the study revealed that the appropriate biochar is 1.5% sugarcane baggase pyrolysed at 300°C in reducing overall nematode infection.

# COMPARING THE PRESENCE OF SOIL AND PLANT PARASITIC NEMATODES IN ORNAMENTAL AND FLOWERING PLANTS

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In the tropical and subtropical regions of the world, plant parasitic nematodes and soil nematodes are major pests. Nematodes are recognized as the cause of serious damages and losses to a wide range of plants including ornamental plants and flowering plants. To compare the population of plant parasitic nematodes and soil nematodes in ornamental plants and flowering plants, a survey was conducted in two different location of University of Karachi, Karachi. Soil samples were collected from three different ornamental plants and three flowering plants viz., Screw pine (*Pandanus tectorius Parkinson*), Weeping fig (*Ficus benjamina*), Devil's backbone (*Euphorbia tithymaloides*), Common zinnia (*Zinnia elegans*), Sada bahar (*Catharanthus roseus*), Minnieroot (*Ruellia tuberosa*). Soil sample was taken of each host to identify different genera of nematodes. Different quantitative and qualitative assays were applied for this. Eight different genera of plant parasitic nematodes *Helicotylenchus, Pratylenchus, Rotylenchulus, Tylenchorynchus, Hemicriconemoides, Xiphinema, Aphelenchus, Tylenchulus* were identified on the basis of their morphological characters. Six different genera of soil nematodes *Acrobeles, Acrobeloides, Cephalobus, Eucephalobus, Chiloplacus, Oscheius* were identified on the basis of their morphological characters.

# PLANT PARASITIC AND SOIL NEMATODES FROM LAWAT DISTRICT NEELUM, AZAD JAMU KASHMIR

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Lawat is situated in Neelum Valley District Neelum. Neelum Valley is 144 km long bow-shaped deeply forested region in Azad Kashmir, Pakistan. The present survey was conducted at 16 localities of district Neelum from Lawat Kashmir Pakistan. A total of 60 samples of soil were collected at the depth of 10-15cm. Plant parasitic nematodes recovered with 70% of total nematodes, whereas the occurrence percentage of free-living soil nematodes was 30.7%. The systematic studies carried out so far on plant parasitic, and soil nematodes resulted in a total of 60 samples, out of these the following nematodes are found during the current survey. *Aphelenchus*, *Aphelenchoides*, *Criconemoides*, *Filenchus*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Meloidogyne javanica*, *Paratylenchus, Pratylenchus, Psilenchus*, *Quinisulcius, Rotylenchus, Tylenchus*, *Tylenchorhynhus*, *Xiphenema,* soil nematodes *Alaimus, Acrobeloides, Acrobeles, Cephalobus, Ironus, Mesorhabditis, Mylonchulus* and *Plectus* are found from above mentioned areas. The main purpose of the survey was to observe the prevalence and incidence of disease those caused by nematodes.

# *PASTEURIA PENETRANS* ENDOSPORE ENCUMBRANCE LEVELS AND SOIL TEXTURE REGIME INFLUENCE THE POPULATION DYNAMICS OF *MELOIDOGYNE INCOGNITA* AND DEVELOPMENT OF *PASTEURIA*

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*Pasteuria penetrans* have long been recognized as promising biological control agents for *Meloidogyne incognita* and have been shown to reduce their populations below economic threshold levels and improved crop yields. The purpose of this study was to assess the increasing *Pasteuria* endospore encumbrance levels on *M. incognita* reproduction and impact of different soil textures on the development of *P. penetrans* on *M. incognita* in tomato. Five levels of encumbered J2 suspension viz., 1-2 spores/J2, 3-5 spores/J2, 7-10 spores/J2, >15spores/J2 and control were prepared and inoculated @ 500 J2 to 21 days old tomato plant from each of the treatment levels in one set of experiment. After seven days, plants were uprooted, washed and 0.1 % acid fuchsin solution and % invasion by J2 was measured. In another set of experiment, same treatments levels @ 1500 encumbered J2 were inoculated. Nematode population dynamics was assessed by determining the galling index, egg masses/plant, females/plant, infected females and % infected females at 30 days after inoculations. Root penetration by J2 was inversely related (r = 91) to increasing endospores/J2 while nematode reproduction parameters including galling index, egg masses/plant and females/plant were directly related to increasing endospores/J2. However, infection in adult females increased by increasing *Pasteuria* endospore/J2. Development of *Pasteuria* was evaluated in different textured soil viz., sandy soil, silt, clay soil, loam soil, sandy loam, loamy sand, sandy clay and clay loam. For this, 21 days old tomato seedlings growing in 10 cm dia. clay pots were inoculated with 1500 encumbered J2. After 30 days of inoculations development of *Pasteuria* was recorded in terms of infected females and No. of spores/mg root powder. The results revealed that infected females and No. of spores/mg root powder was maximum in loamy sand and sandy loam minimum in clay, sandy and clay loam soil. This study provided useful information that soil suppressiveness induced by *P. penetrans* is dependent on endospore density and development of *Pasteuria* is maximum in those soil textures having suitable ratio of sand.

# EXPLORING BIOCONTROL POTENTIAL AND MECHANISM OF CULTURAL FILTRATES FROM *VERTICILLIUM LEPTOBACTRUM* AGAINST ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, IN TOMATO

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Root-knot nematode (RKNs; *Meloidogyne* spp.), infecting the roots of almost all cultivated plants, are becoming a limiting factor in the successful cultivation of agricultural crops. Use of chemicals has being withdrawn because of their toxicity, loss of efficiency after a prolonged use and hazardous environmental concerns. A potential environmental and consumer friendly management strategy is the use of biocontrol agents. In this study cultural filtrates of *Verticillium leptobactrum* were evaluated for the management of RKN *in vitro* and *in planta* in tomato. The cultural filtrates of *V. leptobactrum* gave 96% J2s mortality and inhibited 94% egg hatching in *in vitro* experiment. According to repellence test, majority of J2s were repelled by *V. leptobactrum* filtrates. Results from *in planta* experiment also showed strong nematicidal potential of *V. leptobactrum*. Application of *V. leptobactrum* caused reduction in number of galls, galling index, and egg masses on tomato plant and enhanced plant growth parameters by lowering the reproduction factor and soil nematode population under green house conditions. *In planta* assay showed that plants treated with *V. leptobactrum* filtrates exhibited manifold increase in the accumulation of total chlorophyll and enzymes, viz. peroxidase and phenylalanine ammonia lyase (PAL) which is known to confer systemic resistance in tomato against *M. incognita*. Results revealed that along with direct nematicidal effect *V. leptobactrum* filtrates also modulated phenylpropanoid pathways led to enhanced accumulation of defense related mediator molecules and enzymes in tomato resulted in disease suppression to a significant extents. Findings of this study clearly suggest that fungal cultural filtrates of *V. leptobactrum* could be used as an effective, non-chemical and environment friendly management tool against RKN.

# OCCURRENCE AND DISTRIBUTION OF PLANT PARASITIC NEMATODES IN DIFFERENT FRUITS GROWING AREAS OF SINDH

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Agriculture is the most important sector of Pakistan. Effective improvement in the agricultural productivity depends on appropriate crop protection from pest and diseases. Among these the nematode problem in agricultural productivity is of significant importance. Plant parasitic nematodes are one of the limiting factors in agricultural production. The economic importance of nematodes is well recognized all over the world as most of the agricultural crops are damage by their continuous feeding on roots, buds stems, crowns, leaves and even seeds, resulting in low yield and poor quality fruits. A number of extensive nematodes surveys of agricultural important fruit growing areas of Sindh (300 samples from 10 localities and Baluchistan (200 samples from 10 localities) were conducted during 2017-18. About 500 soil and root samples were collected from fruits *viz*., banana, citrus, chickoo, guava, and mango, of different localities of Sindh. The results of these samples have shown prevalence of 66 nematode species belonging to 32 genera, 21 families and 7 orders *viz*., Aphelenchida, Dorylaimida, Diplogasterida, Enoplida, Mononchida, Rhabditida and Tylenchida.

# INDUCTION OF RESISTANCE IN TOMATO AGAINST ROOT-KNOT NEMATODES USING RHIZOBACTERIA

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Tomato (*Lycopersicon esculentum* Mill.) belongs to the *Solanaceae* family, is one of the important vegetable crop globally as well as in Pakistan. Owing to ever-rising consumer demand, its area under cultivation is also increasing. Nutritionally, it is rich in minerals, vitamins, essential amino acids, nutraceuticals and dietary fibers. Although tomato is high yielding crop but there are many biotic and abiotic factors creating problems for farmers. Tomato plants are susceptible to several types of fungi, bacteria and viruses. However,the infestation caused by Root-Knot Nematodes (RKN) is becoming more challenging day by day. RKN attacks tomato plants during the whole growth span. Due to its characteristic nature, symptoms like belowground obvious galls or knot-like root swellings, often develop over the period and may go unnoticed. The knots block the movement of water and nutrients to the rest of the plant resulting in stunted plant growth. Additionally, tomato plants affected by root-knot nematodes are more susceptible to soil-borne diseases caused by *Ralstonia solanacearum* (bacterial wilt), *Sclerotium rolfsii* (southern blight) *Fusarium, Pythium*, or *Rhizoctonia*. This secondary infection may lead to extensive discoloration of internal stem, root tissue and ultimately necrosis occurred. Relatively high reproduction rate and polyphagous nature make it quite hard to manage root-knot nematodes of tomato. Moreover, the application of existing nematicides have its own environmental concerns due to high toxicity and beyond the limit residual effects, resultantly, killing of beneficial soil microbiota and ground water contamination takes place. Therefore, it is direly needed to introduce environment-friendly alternative measures for the management of RKN. Moreover, resistance against RKN can be induced by application of synthetic and biotic agents, rhizobacteria are one of biotic agents which elicit host plant resistance. Rhizobacteria offer an excellent alternative in providing a natural, effective, safe, persistent and durable protection against root-knot nematodes in tomato.

# PREVALENCE AND INCIDENCE OF *MELOIDOGYNE GRAMINICOLA* (GOLDEN AND BIRCHFIELD) IN WHEAT-RICE CROPPING ZONE AND RESISTANCE EVALUATION OF INDIGENOUS RICE AND WHEAT GERMPLASM

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Rice and wheat are the most valuable cereal food cropping systems threatening by root-knot nematode Meloidogyne graminicola, one of the most important pests that caused significant reduction in yield. Among several management strategies used, resistance against *M. graminicola* could be the most helpful and reduce this issue in rice and wheat fields. The present research survey was conducted to determine the prevalence and identification of rice root-knot nematode *M. graminicola* (Golden & Birchfield) in rice zone II, Pakistan. Further, advanced lines/varieties of rice and wheat for resistance against *M. graminicola* were tested. The goal of this work was to screening wheat and rice cultivars in diverse conditions in Pakistan for resistance resources that could be utilized in farming and breeding for resistance against *M. graminicola*. The most rice cultivated field surveyed during September 2017 from zone II (Faisalabad, Hafiz Abad, Sheikhupura and Lahore districts) in Punjab Province of Pakistan. The survey results concluded that all districts have root-knot nematode infection which was 29% in Faisalabad, 35.71% in Hafizabad, 30.76% from Sheikhupura and 13.33% from Lahore district. 12 rice cultivars showed susceptible response with maximum number of galls and number of juveniles respectively while Basmati 385 showed highly susceptible against *M. graminicola*. Lowest grain weight was recorded from susceptible varieties (PK-386, 1.90g, Basmati 385, 2.36g) and maximum from tolerant varieties (Basmati-2000, 6.73g, NIAB IRRI-9, 7.20g, Basmati 198, 7.10g, Basmati-515, 6.80g). Most of the wheat varieties were found susceptible to partially susceptible. Dharrabi 2011, T-D-1, AS-2002, AAS-2011, Millat-2011, ARRI- 2011, C-217, Barani-83 showed susceptible response. There were only three varieties Shafaq-2006, Faisalabad-2008, Galaxy 2013 displayed a partially tolerant response to *M. graminicola*. None of the varieties under study showed resistance response. Appropriate extension programs must be established to explain and advise crop growers on proper nematode management strategies.

# ACTIVITY OF RHIZOSPHERE MICROBIOTA AGAINST ROOT KNOT NEMATODE OF TOMATO

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Root-knot nematodes (*Meloidogyne* spp.) are most widely distributed among all plant parasitic nematodes and are the major contributors in annual yield loss. *Meloidogyne* genus include different species that infest a vast host range of about 5500 plant species. Vegetables from *Solanaceae* family holds prime importance in our dietary routine are the major victims of such tiny creatures. Agrochemicals (nematicides) are used for the management of these nematodes. The residual effects in our diet and toxic impact of such chemicals on human as well as on the plant health can never be ignored. Furthermore, these chemicals have no sustainable nematode control potential. Consequently, development of alternate approaches is significantly important, a generous count of supportive evidence are available that confirm the efficiency of the fungal and bacterial biocontrol agents (BCA). The in-vitro and in-vivo assays were conducted for the evaluation of microbial isolates individually and in combinations on tomato plants under greenhouse conditions. The results revealed that *Pochonia chlamydosporia, Bacillus subtilis* and *Psudomonas putida* inhibited the population of J2 by 65%, 58% and 79% at 48hrs respectively. Furthermore, bacterial filtrates exhibited 41-81% nematode mortality & volatile compounds caused 21-85% J2 mortality at 48hr. *Bacillus subtilis* individually and in combinations significantly reduced the population of RKN juveniles & number of knots/g root in pot assay under green-house conditions. Additionally, improved plant growth parameters including root/shoot length, root/shoot weight, leaf area, and number of leaves were improved significantly by microbial treatments. The study concluded that beneficial microbes in plant rhizosphere can be utilized to improve plant health.

# HISTOPATHOLOGICAL VARIATIONS IN OKRA GERMPLASM DUE TO ROOT KNOT NEMATODE INFECTION AND ITS MANAGEMENT

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The present study was planned to assess the efficacy of plant growth promoting rhizobacteria (PGPR) and bio-pesticides (Match, Nova Star and Proclaim) against *M. incognita* infection on okra and to screen out the resistant germplasm of okra against root knot nematode and to check the histopathology of the infected roots of okra. For management purpose okra seeds were sown into 20cm diameter earthen pots having sterilized soil. After 10 days of germination of seeds, 20 ml of 5% sugar solution containing $10^7$ CFU/ml of each PGPR (*Bacillus subtilis, Azotobacter sp., Pseudomonas fluorescence*, *Azospirillum sp.* and *Rhizobium sp.*) and 20 ml of each bio-pesticide (Match, Nova star, Proclaim) solutions were applied in root zone. After 1 week of application of PGPR and bio-pesticides, the 5 egg masses (2000 juveniles approximately) were applied in the root zone of the plants by making holes. For histopathology, infected roots were cut into sections and stained with safranin and then slides were prepared and were observed under microscope to know the histopathological changes brought in the roots of the okra by the *Meloidogyne incognita*. From the screening experiment, 9 moderately resistant, 7 susceptible, 9 moderately susceptible and 10 highly susceptible varieties were found. Among the Plant Growth Promoting Rhizobacteria (*Bacillus subtilis*) and among the bio-pesticides (Nova star) were found to be most effective because they have promoted the plant growth upto maximum level and showed minimum number of nematode reproduction parameters. Histopathological changes observed during the experiment were breakage of tissues and cells of the cortex region, formation of giant cells, alterations in the tissues and cavities in the vascular regions were observed due to the disturbance in the tissues and presence of females.

# NOVEL SOIL AMENDMENTS TO ENHANCE RESISTANCE AGAINST *MELOIDOGYNE INCOGNITA* IN OKRA

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Crop is greatly affected by bacteria, fungi and nematodes which have great impact on yield. Among all damaging species of nematodes, *Meloidogyne* is a major constraint towards okra production, globally. Present study on soil amendments was conducted to check out the efficacy of poultry manure, farmyard manure, biochar and rock phosphate for the management of *Meloidogyne incognita*. Soil was treated with two concentrations of each treatment i.e FYM (5% ,10%), Biochar (0.5%,1%), Rock phosphate (2.5%,5%) and poultry manure (5%,10%). Results revealed that farmyard manure with concentration 5% showed a significant effect on root weight, shoot weight, and shoot dry weight. Farmyard manure helps in mitigating multiple nutrient deficiencies. Similarly, poultry manure with concentration 5% showed a maximum effect on root length. Farmyard 10% showed maximum plant height, farmyard 5% showed a maximum number of leaves and chlorophyll content. From overall results, it is concluded that farmyard manure performed best than other treatments (poultry manure, biochar, rock phosphate) against *Meloidogyne incognita* in Okra.

# SCREENING OF OIL SEED CAKES AND THEIR AMELIORATIVE POTENTIAL FOR ROOT KNOT NEMATODE MANAGEMENT IN *CYAMOPSIS TETRAGONOLOBA* L.

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Root-knot nematodes (Meloidogyne species) one of the substantial constraints of the Guar (Cyamopsis tetragonoloba) worldwide may affect more than 3000 crops worldwide, and oil seed cakes are known to exhibit the pesticidal activities. The aim of the present study was to check the nemato-toxic potential of Almond, Cotton, Jatropha, Jojoba and Neem seed cake through in vitro studies viz; juvenile mortality test and egg hatching test and in vivo studies viz; pot experiment. The findings of data suggested that tested oil seed cake extracts showed the lethal effects on 2nd stage juvenile and egg hatching, mortality was increased with the higher concentration of aqueous extracts, 100% mortality was observed at 50% and 100% aqueous extracts of all tested oil seed cakes while the ethanolic extracts found lethal at all concentrations showed 100% mortality as compare to control. Complete inhibition of egg hatching was observed with increase in concentration of aqueous extract while dynamic hatching activity was observed at different concentrations of ethanolic extracts. The amendment of oil seed cakes in soil showed significant results, successfully enhance the growth and physiological parameters in guar against Meloidogyne incognita. The most effective was Almond followed by Jatropha > Neem> Cotton>Jojoba. The highest reduction in no. of knots was found in T10 as compare to control. The approach of the study was environment friendly, cheap and easily available to farmers.

# SCREENING OF MASHBEAN GERMPLASM AGAINST ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) AND ITS MANAGEMENT THROUGH RESISTANCE INDUCERS

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Mash bean/black gram (*Vigna mungo*) is considered to be the most important pulse crop and a rich source of protein. Mash bean is attacked by several fungi, bacteria and viruses but there is another pathogen infecting mash bean known as the root-knot nematode (*Meloidogyne incognita*). So, this study was proposed to screening of mash bean germplasm to find the sources of resistance against RKN, *Meloidogyne incognita* and to evaluate the effects of different resistance inducers including salicylic acid (SA), mono-potassium Phosphate ($KH_2PO_4$), and Di-potassium Phosphate ($K_2HPO_4$) in various concentrations. Even the efficacy of three bio pesticides viz Match, Proclaim and Nova Star were tested against disease caused by *Meloidogyne incognita* in mash bean. An experiment was conducted to screen the fifty mash bean varieties in such a way that each variety was replicated 8 times. A complete randomized design (CRD) was arranged for this experiment. At the time of germination (after 30 days), the inoculation was done with RKN (*Meloidogyne incognita*) on mash bean plants. Different concentrations of resistance inducers and bio pesticides were used. At harvest different mash bean growth and *Meloidogyne incognita* reproduction parameters were assessed after 60 days of inoculation. The results of the present investigation were helpful in using resistance inducers and bio pesticides at an accurate dose and application method for the management of nematodes. From the screening experiment, 14 moderately resistant, 32 susceptible and 4 highly susceptible varieties were found. The uninoculated untreated (Healthy); inoculated and the plants treated with resistance inducers and bio pesticides were assessed for different plant growth and nematode reproduction parameters after 60 days. By increasing the levels of inoculum, plant growth characteristics found decreased and by increasing the different resistance inducers and bio pesticides concentrations, plant growth characteristics found increased whereas by increasing the levels of inoculum, the root weight and number of galls found increased and by increasing different resistance inducers and bio pesticides concentrations, root weight and number of galls found decreased.

# CONSORTIUM APPLICATION OF BACTERIAL STRAINS FOR GROWTH PROMOTION AND MANAGEMENT OF ROOT KNOT NEMATODE *MELOIDOGYNE INCOGNITA* IN OKRA (*ABELMOSCHUS ESCULENTUS*)

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Plant-parasitic nematodes (PPNs) are serious threat to global agricultural production. Among them, root-knot nematode (*Meloidogyne incognita*) is a major problem in okra production. This study was planned to assess the growth attributes and nematicidal potential of different Bacillus sp. MN54, Enterobacter sp. MN17 and B. phytofirmans PsJN and their consortium against *M. incognita* infecting okra variety. Results revealed that application of Enterobacter sp. MN17 significantly enhanced the maximum root length (19.0) of okra plants, followed by root weight (8.7) and shoot dry weight (19.6) respectively. Likewise, the nematode suppressive effect of the bacterial isolates was increased in consortium (MN 54, MN 17, PsJN) due to their synergistic effect. The treatment of Bacillus sp. MN54 + Enterobacter sp. MN17 + B. phytofirmans PsJN showed the highest reduction in number of galls (10.5), number of females (23.2), egg masses (19.5), egg mass index (4.1), and galling index (2.1) as compared to only nematode and control. The outcomes suggest that these bacillus species could be used for promoting growth attributes and suppress the *M. incognita* infection. It is concluded that the biocontrol agents managed in the consortium were more successful than those used only once to control nematode attributes, and enhanced the growing parameters compared to negative and positive control. But more research is needed to confirm these findings and to investigate the many pathways that the researched bacterial isolates cause in field situations.

# INTERACTION BETWEEN MAJOR MOSAIC VIRUSES AND ROOT KNOT NEMATODE (*MELIDOGYNE INCOGNITA*) ON CUCUMBER

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Cucumber (*Cucumis sativus* L.) is an important vegetable and member of the family Cucurbitaceae. Cucumber production technology is signified as consumed and industrialized product across globe. In Pakistan, cucumber is cultivated on 350862 ha with production 148.6 tons per annum. Cucumber is threatened by root-knot nematode and viruses. Genetic and physiological make up of cucumber is not well established, to number of viruses and nematode which causes loss up to 100%. The present study was planned to understand the infection of major mosaic viruses (*CMV, ZYMV*) and root-knot nematode with their reciprocal impact on fifteen cucumber genotypes. Seeds of cucumber genotypes were sterilized and sown in autoclaved soil to validate the impact of *CMV, ZYMV*, and *RKN* in greenhouse. Symptoms of the mosaic viruses on infected plant leaves, nematode reproduction parameters including: no. of galls, egg masses, no. of females and plant growth parameters including root length, root and shoot weight were studied. All inoculum densities caused significant reduction in growth and yield of cucumber, positively correlated with the respective symptoms. Population densities of incognita on plant growth parameters and nematode reproduction had negative correlation. Number of galls were found more on the roots of cucumber infected with nematode than with both nematode an viruses. Max pack and Beti-alpha were highly susceptible while Alpha prime and Patio showed moderate resistant response against *ZYMV, CMV* and RKN. Cucumber mosaic viruses showed synergistic effect with each other while this interaction resulted in antagonistic effect when these mosaic viruses were co-inoculated with RKN. Secondary metabolites secreted by the plants in response to pathogen play significant role in plant defense mechanism. Study on secondary metabolites in imparting resistant/susceptibility was also stepped in for conclusive findings. ELISA tests and alkaloids, flavonoids, phenols, peroxides, diphenol *picrylhydrazine* (DPPH), and catalase superoxide dismutase quantification for the infected viruses was done, compared with control. Ascorbic acid, cyclohexanol, Dibenzo ate, Benzamide, *pentadecanoic* acid, *Benzoic acid, 1,1- Benzene dicarboxylic acid, Phenol (1,1- Dimethyl ethyl),* Benzimidazole, Quinoline and Phthalic acid were found at different retention time in GC-MS data, Magnified the phytochemicals differences.

# EVALUATION OF NEMATICIDAL POTENTIAL OF DIFFERENT PHYTO-EXTRACTS AND NEMATICIDES AGAINST ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON SPINACH

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Spinach (*Spinacia oleracea* L.) is an important leafy vegetable crop that is a rich source of vitamins, proteins, minerals, and chemicals. Several pathogens attack and cause various seed-borne and leafy diseases in spinach. The root-knot nematode, *Meloidogyne incognita* is a sedentary endo-parasite and is among the most damaging agricultural pests, attacking a wide range of crops. Keeping in view the above fact, the present investigation was planned to assess the effectiveness of different Phyto-extracts (Azadirachtin, Eucalyptus, and garlic) and nematicides (Confidor, Praxis, Pirate, Match, Nova star and Proclaim) on the reproduction of root-knot nematode (RKN) (*Meloidogyne incognita*) on spinach. Effect of all treatments was checked on percent egg hatching and percent juvenile mortality under in *vitro* conditions. Results of *in vitro* experiment exploited that among all nematicides confidor and Nova star and from Phyto-extracts Azadirachtin and garlic increased the mortality and decreased the egg hatchability rate. In the second experiment was performed under in *vivo* conditions in the field area of the Department of Plant Pathology, University of Agriculture, Faisalabad. A desi variety of spinach was evaluated and the plant growth parameters (root length, root weight, shoot length and shoot weight) and reproduction parameters (number of galls, number of females, number of egg masses, and number of nematodes) of root-knot nematode (RKN) (*Meloidogyne incognita*) were assessed. The soil drench method was used for the application of treatments. After 12 days of germination, 20 ml of each treatment (nematicides and Phyto-extracts) were applied in the root zone in such a way that by creating holes around the plants' soil with the help of sharp wooden sticks. After one week of application of the above-mentioned treatments, the plants were inoculated with one thousand freshly hatched juveniles. In this experiment treatments were applied first and then inoculation was done because treatments were used as a protective measure against RKN's. Eleven treatments were used under CRD design and replicated five times. At harvest different spinach growth and *M. incognita* reproduction parameters were assessed after 60 days of inoculation. Among the nematicides (Confidor and Nova star) and the Phyto-extracts, Azadirachtin was found to be the most effective. Results of the present investigation would help use Phytoextracts and nematicides at accurate doses for the management of nematodes.

# MANAGEMENT OF ROOT-KNOT NEMATODES INFECTING ALFALFA BY PGPR, BIO PESTICIDES AND CHEMICALS

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Alfalfa (*Medicago sativa L.*) belongs to the *Fabaceae* family and has been cultivated since a long time as a food of livestock, so it is called Queen of fodder and also known as Chilean clover, Buffalo grass and Lucerne. This Study has been planned to check the efficacy of PGPR (plant growth promoting rhizobacteria), bio-pesticides (Match and Nova Star) and chemicals (Minto and Priority) against *Meloidogyne incognita* infection on Alfalfa. Seeds of Alfalfa were sown in the earthen pots, after 45 days of germination 20 ml of 5% sugar solution having $10^7$ CFU/ml of each PGPR (*Bacillus subtilis and Pseudomonas fluorescence*), 20 ml of each bio-pesticides solutions and 20 ml of chemicals at recommended dose were applied in root zone by making holes around the plants with the help of sharpen wooden sticks. After 1 week of application of PGPR, bio-pesticides and chemicals the one thousand five hundred freshly hatched juveniles were applied to the plants around the root zone by making holes. There were twenty-one treatments, and each treatment was replicated 5 times. Pots having only nematodes were served as control. After thirty-five days of nematode inoculation, plants were harvested, and data were recorded. Plant growth parameters (root and shoot lengths, root and shoot weight, number of nodules and number of leaves) and nematode reproduction (number of females, number of egg masses, number of juveniles and number of knots/galls) were assessed. In plant growth parameters *Bacillus subtilis* and *Bacillus subtilis* + Priority increased the plant growth. Maximum reduction in nematode population was assessed by the *Bacillus subtilis* + Priority and *Bacillus subtilis* + Minto.

# MULTIPLICATION OF *STEINERNEMA KRAUSSEI* & *STEINERNEMA GLASERI* ON THE LARVAE OF DIFFERENT INSECT PEST

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Entomopathogenic nematodes related to family Steinernematidae and Heterorhabitidae are obligate parasites of diverse insect pest and those EPNs are used as natural control agents. Two main genera *Steinernema* and *Heterorhabditis* and their associated bacteria (*Xenorhabditis* and *Photorhabdus)* are the most exceptional option to accompolish insect pests. The toxin released by both nematodes and microbes kill the insect pest in 24 to 72 hours which depend on the host specific and EPN culture. The main objective of the current research was to evaluate reproduction potential of *Steinernema kraussei* and *Steinernema glaseri* on four type of insect larve (Wax moth, pink bollworm, brinjal borer and armyworm) at different levels. For the production of *Steinernema kraussei* and *Steinernema glaseri* different insect larvae were collected from different area of university of Agriculture of Faisalabad and tended them in laboratory and used distinctly in laboratory experiments as the collective host insect for *Steinernema kraussei* and *Steinernema glaseri.* Entomopathogenic nematodes suspension was stored at 9$^{o}$c to test their value against various lepidopteron insect larvae. Experiment was done to check the efficacy of *Steinernema kraussei* and *Steinernema glaseri* on four insect larvae at 25±2 under lab conditions and 300 IJs were used for this experiment. The efficacy of *Steinernema kraussei* and *Steinernema glaseri* is recorded more on wax moth and army worm as compared to pink bollworm and brinjal borer. Effect of different concentrations (100, 200 and 300) of *Steinernema kraussei* and *Steinernema glaseri* was studied on different insect larvae (wax moth, pink bollworm, brinjal borer and armyworm). At 300 IJs concentration of *Steinernema kraussei* and *Steinernema glaseri* maximum reproductive potential was recorded as compared tp 100 and 200 IJs. Effect of different storage time (2, 4, 6 weeks) old culture of *Steinernema kraussei* and *Steinernema glaseri* and different larval weights (0.25g and 0.5g) on reproductive potential and population of EPN was studied on different insect larvae. Maximum reproductive potential of *Steinernema kraussei* and *Steinernema glaseri* was recorded on 2-week old culture as compare to 4 and 6 weeks old culture of EPN. Maximum population of *Steinernema kraussei* and *Steinernema glaseri* was recorded on 0.5g as compared to 0.25g larval weight.

# INTEGRATED MANAGEMENT OF ROOT KNOT NEMATODES (*MELOIDOGYNE INCOGNITA)* ON CHILI

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Chili (*Capsicum annuum* L.) belongs to Solanaceac family is an important commodity used as a vegetable, spice, medicinal herb, industrial products and ornamental plant by billions of every day. The present study was carried out by considering the above-mentioned scenario to evaluate the nematicidal activity of (Confidor 200SL, Match 050EC, Garlic Extract, FYM, Neem Extract, Confidor 200SL+ Match 050EC, Neem Extract + Match 050EC, Neem Extract + Confidor 200SL, Garlic Extract+ Match 050EC, FYM+ Match 050EC, FYM+ Confidor 200SL, FYM + Garlic Extract, FYM Neem Extract, Garlic Extract Confidor 200SL) against root knot nematode (*Meloidogyne incognita*) reproduction in chilli. The experiment was carried out in the field area of Department of Plant Pathology, University of Agriculture, Faisalabad. A desi chilli cultivar was evaluated with three replications under randomized complete block design (RCBD). Chemicals were applied following protective treatment method with the recommended dose through soil drench application method to assess the efficiency of both of the chemical and the plant extracts. Different chilli growth and nematode reproduction parameters were assessed after 60 days of inoculation. The comparative analysis of all the chemicals based on their performance revealed that the chemical "Confidor 200SL" showed maximum reduction in reproduction parameters of nematodes followed by Match 050EC, Confidor 200SL+ Match 050EC, Neem Extract + Match 050EC, Neem Extract + Confidor 200SL, Neem Extract, Garlic Extract + Confidor 200SL, Neem Extract, Garlic Extract, Garlic Extract + Match 050EC, FYM + Confidor 200SL, FYM + Neem Extract, FYM + Garlic Extract, FYM + Match 050EC, Garlic Extract and FYM. The soil drench was proved to be more efficient in controlling nematode infection.

# EFFECT OF MARIGOLD (*TAGETES ERECTA*) ROOT EXTRACT IN CONTROLLING ROOT KNOT NEMATODES (RKNs)

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Tomato (*Solanum lycopersicum*) is the second most important crop after potato that is used either in fresh or processed form. It is highly nutritious and enriched with minerals, vitamins and antioxidants. However, this crop is highly affected by various bacterial, fungal, viral, and other root pathogens. In tomatoes, root knot nematodes (RKNs) is one of the major factor that affect crop yield and these nematodes develop massive galls on the tomato roots that reduces its biological efficiency to uptake nutrients. Marigold (*Tagetes erecta* L.) is a well-known plant that is grown worldwide for ornamental, medicinal and cosmetic purposes and has allopathic properties. It contains thiophenes and *oxygen radicals like* **a**-terthienyl in its tissues that control various insects, fungus and nematodes populations. Therefore, an experiment was conducted to evaluate the allopathic impact of marigold in controlling nematodes obtained from tomato roots. In this experiment, marigold root water was extracted and is diluted with distilled water with the respective ratios; $T_1$ (1:1), $T_2$ (2:1) and $T_3$ (3:1). The results revealed that the highest mortality rate in nematodes (82.33%) was noticed in T3. Likewise, the similar concentration (T3) was also highly effective in controlling nematodes eggs hatching.

# MANAGEMENT OF FRUIT FLY THROUGH THE USE OF **ENTOMOPATHOGENIC NEMATODES**

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Fruit flies are the major pest of different vegetables and fruits causing significant losses to fruit and vegetables, as a result international trade getting limited day by day. Use of different synthetic insecticides to control fruit flies has been replaced with microbial control agents which is eco-friendly and safe. Within the microbial groups EPNs belonging to genera, *Steinernema* and *Heterorhabditis* are the more effective against economically important soil-living insects. Keeping in view the above facts the present study was carried out to examine the virulence of *Heterorhabditis bacteriophora*, *H. indica*, and *Steinernema carpocapsae* against different stages of fruit fly species under controlled set of environmental conditions. Fruit fly infested fruits was obtained from the native fruit market, in the laboratory, rearing and identification of fruit fly species was done. Three different EPN doses (50, 75, 100 $J_2$s/$ml^{-1}$) were prepared and used against larvae and pupae of different fruit fly in an *in vitro* experiment. Data regarding $J_2$ mortality was recorded with the interval of one day. Percent adult emergence was also recorded. Results regarding *Steinernema carpocapsae* shows that the interaction of concentration levels and interval days. Results of the interactions were highly significant. The maximum numerical value of *Steinernema carpocapsae* 1285 was found in *B. Correcta* + Treatment 3 + Day 5 while minimum value of *Steinernema carpocapsae* 243.5 in *B. cucurbitae* + Treatment 1 + Day 1. The maximum numerical value of *Heterorhabditis bacteriophora* 1240 was found in *B. Correcta* + Treatment 3 + Day 5 while minimum value of *Heterorhabditis bacteriophora* 394.5 in *B. cucurbitae* + Treatment 1 + Day 1. The maximum numerical value of *H. indica* 1365.5 was found in *B. Correcta* + Treatment 3 + Day 5 while minimum value of *H. indica* 376 in *B. cucurbitae* + Treatment 1 + Day 1. The information obtained and gathered in this study suggests the potential for the use of EPNs to manage fruit fly in University of Agriculture, Faisalabad but future studies should consider impact of various fruit flies i.e., *B. cucurbitae*, *B. zonata* and *B. Correcta*, days interval on *Heterorhabditis bacteriophora*, *H. indica*, and *Steinernema carpocapsae* under various treatments. Moreover, this work suggests a need for additional research to determine whether both EPNs species like that *Steinernema glaseri* and *Heterorhabditis bacteriophora* should be released for integrated biological control of fruit fly in Pakistan.

# COMPARING THE PRESENCE OF SOIL AND PLANT PARASITIC NEMATODES IN HEALTHY AND INFECTED GRASS

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In the tropical and subtropical regions of the world, plant parasitic nematodes and soil nematodes are major pests. Nematodes are recognized as the cause of serious damages and losses to a wide range of plants including healthy grass and infected grass. To compare the population of plant parasitic nematodes and soil nematodes in healthy grass and infected grass, a survey in which the analysis of soil samples collected within the premises of university of Karachi. Four samples were chosen randomly, from which two were healthy grasses and other two were infected grasses. Following names were given to the samples: HEALTHY GRASS *Cynodon dactylon* (sample1-2), INFECTED GRASS *Cynodon dactylon* (Sample 3-4). Soil sample was taken of each host to identify different genera of nematodes. Different quantitative and qualitative assays were applied for this. Eight different genera of plant parasitic nematodes *Longidorus, Tylenchorhynchus, Helicotylenchus, Hemicriconemoides, Xiphinema, Rotylenchulus, Pratylenchus and Aphelenchus* were identified on the basis of their morphological characters. And six different genera of soil nematodes *Oscheius, Cephalobus, Chiloplachus, Acrobeloides, Eucephalobus* were identified on the basis of their morphological characters.

# MANAGEMENT OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) INFECTING BERSEEM BY INTEGRATION OF PGPR, BIOPESTICIDES AND PLANT EXTRACTS

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Berseem is an essential winter fodder for animals because it is high in water, phosphorus, calcium, and nutrients, as well as being easy to digest. Different pathogens attack on Berseem, but the root knot nematode (*Meloidogyne incognita*) is one of the most damaging pathogens. As a result, this research was done to check the effectiveness of (PGPR) plant growth promoting rhizobacteria, bio-pesticides, and plant extracts. For management purpose berseem seeds were sown into earthen pots having sterilized soil. After 10 days of germination of seeds, 20 ml of 5% sugar solution containing $10^7$ CFU/ml of each PGPR (*Azotobacter spp. and Rhizobium spp.*), 20 ml solution of each bio-pesticide and 20 ml solution of plant extracts was applied by making holes with sharpen wooden sticks around the root zone of plants. After 1 week of application of PGPR and bio-pesticides, 1000 juveniles from freshly hatched egg masses were applied in the root zone of the plants by making holes. The experiment was arranged in CRD with 22 treatments and replicated 3 times. Pots with only nematodes were kept as control for comparison. After six weeks of nematode inoculation, plants were harvested and data was recorded on plant growth parameters (length of roots, length of shoots, weight of fresh roots, weight of fresh shoot, number of nodules and number of leaves) and nematode reproduction (number of knots/galls, number of juveniles, number of egg masses and number of females). For the plant growth parameters and for nematode reproduction, from PGPRs Rhizobium, from bio-pesticides Match, from plant extracts Neem gave the best result, improve the plant growth and reduced the reproduction of nematodes.

# DISTRIBUTION OF CYST NEMATODES IN GILGIT-BALTISTAN, PAKISTAN

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Cyst nematode, the Heterodera Schmidt (1871) is one of the most widely distributed and economically important plant parasitic nematode genus. During the present investigation of plant-parasitic nematode fauna from Gilgit-Baltistan, Pakistan, four cyst nematode species viz., Heterodera avenae Wollenweber, 1924; H. mani Mathews,1971; H. schachtii Schmidt, 1871 and H. zeae Koshy, Swarup and Sethi, 1971 were detected from all four districts.

# COMPARISON OF HATCHING OF NEMATODES BETWEEN TWO DIFFERENT ENTOMOPATHOGENIC NEMATODES (*STEINERNEMA PAKISTANENSE* AND *STEINERNEMA BIFURCATUM*)

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The suitability of entomopathogenic nematodes as biological control agents of specific target insects is affected by their level of infectivity and reproductive capacity. Therefore, in the present study the productivity of two entomopathogenic nematodes (*Steinernema pakistanense & S. bifurcatum*) were compared in *Galleria mellonella* larvae. The production of infective juveniles (IJ) in *G. mellonella* was significantly affected by nematode species. Emergence of IJs from both EPNs at 3 different doses of 15, 25, 35-50 IJs were observed for 3-5 days. The observed comparisons of the emergence of IJs from both EPNs at doses of 15, 25, 35-50 IJs under maintained temperature (28˚C) were more or less same indicating that under appropriate conditions *S. bifurcatum* has the equal potential and efficacy to serve as biological control as *S. pakistanense*. Our aim is in future with the help of this specie we can control various types of insect and they can play very important role for control of insect and also it is safe method (Biological control) for our environment.

# HISTOPATHOLOGICAL VARIATIONS IN EGGPLANT DUE TO ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* INFECTION

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Root knot nematodes cause heavy yield losses in eggplant and other crops. Nematode inoculum levels decide the losses caused by nematodes. In the current experiment, the influence of four inoculum levels of *Meloidogyne incognita* [250, 500, 1000, 1500 second stage juveniles (J2s) per treatment] were tested against eggplant. Eggplant seedlings were transplanted in earthen pots (6″/8″) having sterilized soil and was irrigated. After 40 days, the plants were harvested and data was recorded on the basis of plant growth parameters (root length, root weight, shoot weight, shoot length, number of leaves) and nematode reproduction parameters (number of females, number of galls, number of egg masses, number of nematodes). Different growth parameters of eggplant were affected differently by the four levels of nematode inoculum. For histopathological studies the infected plants were taken into the lab and washed. Thin sections of the infected roots of the eggplant were cut with the help of sharp blade. These sections were stained with safranine solution and then washed with distilled water to remove extra stain. After that, slides of these sections were made and observed under microscope to study the histopathology. At the higher inoculum levels, the effect on the plant growth was more and at the lower, the effect on the plant growth was less. More number of galls were present on the plants which were treated with the higher inoculum levels and a smaller number of galls were observed with the lower number of inoculum levels. Therefore, the number of juveniles or inoculum levels directly affects the plant growth. From the histopthological studies it was observed that the cells of the infected roots were found to be enlarged due to the formation of giant cells. The vascular bundles of the infected parts were disrupted and blocked.

# POTENTIAL OF ENTOMOPATHOGENIC NEMATODES FOR THE CONTROL OF THE STAGES OF FRUIT FLY (*BACTEROCERA ZONATA*)

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*Bactrocera zonata* is generally known as "Peach fruit fly". It is considered as one of the destructive pest of fruits, belongs to family Tephritidae. Bactrocera zonata is a polyphagous insect on Peach, Guava, Apple and Mango. Peach fruit fly has wide host range, it attacks approximately on 50 different plant species including wild plants. Fig, citrus and apricot are secondary host of peach fruit fly. It is widely distributed in Asia and Africa and also reported in North America. In their lifecycle 4 stages observed which are egg, larva, pupa and adult that shows peach fruit fly attain complete metamorphosis. Currently for the chemical control of *B. zonata*, Malathion and organophosphorus (Insecticides) are used. These Insecticides contaminated the environment and has a negative impact on beneficial insects (biological control agents). These Insecticides are also toxic for the human health and cause carcinogenic effects in humans. Entomopathogenic nematodes are those nematodes which are used as biological control of many serious insect pests. The two families of nematodes, Steinernematidae and Heterorhabditidae have ability to control insect pest without any negative impact on environment and other beneficial organisms. EPN are the best substitute of toxic chemical Insecticides. EPN naturally found in soil. EPN are symbiotically associated with Enterobacteriaceae (Genus: *Xenorhabdus* and *Photorhabdus*). To kill the insect (host), IJs (Infective juveniles) enter in the host's body by the natural opening like mouth, anus and spiracles and infrequently they can directly penetrate through cuticle of host. When the IJs enter in the host body, they release bacteria in the haemocoel, after the release of intestinal bacteria the process of reproduction starts as well as production of metabolites which furthermore kill the host generally within 2-3 days and provide food source for IJs.

# NEMATICIDAL ACTIVITIES OF CANNABIS SATIVA L. AND ZANTHOXYLUM ALATUM ROXB. AGAINST MELOIDOGYNE INCOGNITA

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Because of being costly and pernicious to the environment and human health, the use of nematicides has become prohibitive in many countries and the management of plant parasitic nematodes using antagonistic plants can be a very attractive alternative. In the present studies the effectiveness of aqueous extracts of *Cannabis sativa* and *Zanthoxylum alatum* was assessed on hatching, mortality and infectivity of *Meloidogyne incognita* at different concentrations viz. S, S:1, S:5, S:10, S:25, S:50 and S:100. Both the plants had significant effects on juvenile mortality and hatching inhibition in a dose-dependent manner. Mortality and hatching inhibition caused by *C. sativa* were significantly higher than that of *Z. alatum*. Time duration also affected mortality and hatching inhibition significantly. Significant inhibition in invasion of M. incognita juveniles on cucumber cv. Royal Sluis was observed by different treatments with extracts. M. incognita juveniles exposed to 'S' extracts of *C. sativa* and *Z. alatum* for 24 and 48 h caused no infection. Exposure for 12 and 6 h caused more than 95 and 90% reductions in infectivity of *M. incognita* juveniles respectively. Similarly, soil drench and root dip treatments also caused significant reductions in infection. Reduction in infectivity was found to be significantly higher with extracts of *C. sativa* as compared to *Z. alatum* and decreased in a dose-responsive manner. The results of the studies showed that the extracts of test plants, commonly found locally, possess high potentials for the control of root-knot nematodes and could be the possible replacement for synthetic nematicides.

# INDUCED SYSTEMIC RESISTANCE BY *GLOMUS MOSSEAE* (GERD. & TRAPPE) AND NEEMEX® AGAINST *MELOIDOGYNE INCOGNITA* (KOFOID AND WHITE) CHITWOOD IN EGGPLANT

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Mycorrhizal fungus (*Glomus mosseae*) and neemex are capable to defend different crops including vegetables against a variety of soil-borne pathogens, but the bio-control modes of action remain largely unknown, especially in the case of root knot nematodes. In this split root experiment which is consisting of a two-compartment set-up, mycorrhizal fungus and neemex were applied either locally (i.e. nematodes in the same compartment as MF or neemex or both) or systemically (i.e. nematodes, MF and neemex physically separated). As a first step toward the elucidation of the modes of action, the neemex and mycorrhizal fungus-induced resistance was confirmed against *M. incognita* as the number of galls were significantly lowered in treated roots. The presence of MF and neemex alone or together, locally, resulted in a significant reduction of nematode infection. The results showed that infection by the *M. incognita* was also systemically reduced by mycorrhizal fungus and neemex either alone or in combination.

# EVALUATION OF DIFFERENT INSECTICIDES FOR THE MANAGEMENT OF RICE ROOT KONT NEMATODES (*MELOIDOGYNE GRAMINICOLA*) IN *ORYZA SATIVA*

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Root knot nematodes (*Meloidogyne graminicola*) are considered as a developing problem of rice in Pakistan as well as worldwide. The purpose of current study was to evaluate 10 chemicals against root knot nematode (*M. graminicola).* The application off root-dip treatment was done by soaking of seedlings of rice cv. PK-386 in the 20-ppm solution of Rugby (Cadusafos), Furadan 3g (Carbofuran), Match (Lufenuron), Matanza (Pyriproxyfen), CARTAP 4G (Cartap Hydrochloride), Movento 240 Sc (Aspirotetramate), Actara 25wg (Thiomethaczam), Belt 48SC (Flobindiamide) and Confidor 20 SL (Amedacluprid) for 3 h before transplanting. Thirty-five days old nursery of rice cv. PK-386 was transferred in pots. Growth parameters of rice like plant height, shoot weight, root weight, grain weight and nematode reproduction parameters including number of galls, number of females, number of eggs/ 5 galls, $J_2$s /100 $cm^3$ of soil were recorded. The insecticides evaluated for egg hatching inhibition and J2s mortality test showed significant results at different concentrations and time intervals under in vitro studies. The results showed that all treatments showed significantly different response against *M. graminicola* egg hatching. Among the ten chemicals Furadan, Rugby, Match, Cartap, Movento, Actara, Belt and Confidor cause higher percentages of hatching inhibition as compared to Polo and Matanza. While among the tested doses, S (Standard/Recommended) and 2S (Double dose) concentrations were more effective as compared to S/2 (Half dose). Results also revealed that effectiveness of chemicals increases with time of exposure with chemicals. Among the ten chemicals nine (Rugby, Match, Furadan and Matanza, Cartap, Movento, Actara, Belt and Confidor) caused (88.41, 86.29, 89.69, 81.00, 63.40, 98.08, 98.48, 98.72 and 98.88) hatching inhibition respectively while Polo cause (26.13) inhibition. It is concluded that could Furadan be more effective with 2S (Double dose) concentration against *M. graminicola* in rice.

# SECTION 6: PLANT VIROLOGY

# BIOLOGY AND MOLECULAR PATHOLOGY OF PLASMODIOPHORID-TRANSMITTED ROD-SHAPED VIRUSES IN PLANTS- AN EMERGING PROBLEMS IN PAKISTAN

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Plasmodiophorid-transmitted rod-shaped viruses (genus *Furovirus*, *Pomovirus*, *Pecluvirus*, *Benyvirus*) possess divided positive-standard RNA genome, separately encapsidated in tubular rod-shaped particles and transmitted by plasmodiophorid (protozoan) vector in soil. These viruses have a wide host range and world-wide distribution. Among 13 major viruses, *Soil-borne wheat mosaic virus* (SBWMV), *Oat golden stripe virus* (OGSV), *Sorghum chlorotic spot virus* (SrCSV), *Chinese wheat mosaic virus* (CWMV), *Soil-borne cereal mosaic virus* (SBCMV) infect cereals; *Beet necrotic yellow vein virus* (BNYVV), *Beet soil-borne mosaic virus* (BSBMV), *Beet soil-borne virus* (BSBV), *Beet virus Q* (BVQ), *Potato mop-top virus* (PMTV) infect root or tuber crops and *Broad bean necrosis virus* (BBNV), *Peanut clump virus* (PCV) and *Indian peanut clump virus* (IPCV) infect leguminous crops. The molecular based mechanism of virus transmission by plasmodiophorid vector, for some better characterized viruses and role of coat protein/read-through (CP/RT) and other genes in virus transmission process have been briefly discussed. The reports on analysis of nucleotide sequences of SBWMV, BNYVV, PMTV, PCV and BSBMV indicated heterogeneity among plasmodiophorid-transmitted tubular viruses and have at least three types of genome organizations. However, coat protein/read-through (CP/RT) protein is a common feature and is found in BNYVV, SBWMV, PMTV, BSBMV. The BNYVV RT protein is involved in particle assembly and transmission by *Polymyxa betae*. Repeated manual transmission of BNYVV, SBWMV, and PMTV results in spontaneous deletions of RT domain and lose the ability of acquisition and transmission through plasmodiophorid vector. PMTV-S, a field isolate, was efficiently acquired and transmitted by a monofungal culture of *Spongospora subterranea* whereas PMTV-T which has 543 nt deletion in the 3´ halves of the RT, could not be acquired and transmitted by the same *S. subterranea* culture. The association of lack of transmissibility of PMTV-T with-in apparent deletion of sequence in RT, relative to RT of transmissible isolate PMTV-S suggests that the RT domain, encoded by PMTV-S RNA3 contains determinants that play an important role in the acquisition and transmission of PMTV by its vector (*S. subterranea*). The mechanism, by which virus particles move into or out of the protoplasm of zoospore, is a complicated issue and need further investigation. However, the process by which aviruliferious zoospores acquire virus particles *in vivo* has been proposed. Recent progress in molecular pathological strategies, to protect crop plants against major plasmodiophorid-transmitted tubular viruses, has also been briefly discussed. This paper also reviews the information on occurrence, distribution, and characterization of plasmodiophorid-transmitted tubular viruses in sugar beet and potato crops of Northwest of Pakistan.

# CITRUS TRISTEZA VIRUS (CTV); CURRENT SCENARIO IN RELATION TO PREVIOUS REPORTS

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Citrus is an economically important and most widely cultivated fruit crop being grown in over 142 countries. Pakistan is ranked 13th worldwide and exported 0.9-million-ton citrus costs 61 billion USD in the year 2020-21. In Pakistan, a number of surveys were conducted on CTV, in this regard firstly Punjab and KPK were surveyed by Catara et al. (1988) & presence of CTV was reported on basses of ELISA and Electron microscopy. Grimaldi & Catara (1989) revealed presence of thread like structures in phloem cells of columella of citrus fruits. Catara et al. (1991) surveyed more than nurseries and orchards of Punjab and found an overall incidence of about 20%, supported by the findings of Anwar and Mirza (1992) who reported 18.8% incidence of CTV. Arif et al. (2005) reported an incidence of 27% in KPK. Similarly, Iftikhar et al. (2009) extensively surveyed KPK and Punjab for two consecutive years 2006 & 2007, serologically reported an incidence of 37% & 44% in KPK and Punjab province respectively during 2005, in the following year 2006 incidence reported was 40% in KPK and 48% in Punjab. Jalial et al. (2013) reported CTV incidence in Punjab is 58% successively while Sadia et al. (2016) reported maximum incidence of 67% in Punjab. In recent study at virology lab, crop disease research institute, NARC, major citrus producing areas of Punjab divided into three north south & central zones, thoroughly sampled and incidence was recorded 73.53%, 64% & 69.15% respectively with an average of 68.9% in Punjab based on ELISA, results were verified using minor CP gene specific primers (ATGGACGACGAAACAAAGA) and (TCAACGTGTGTTGAATTTCC) that positively amplified 0.6 kb fragment of cDNA. This continuously increasing incidence is a perilous for the citrus industry of Pakistan.

# RESPONSE OF OKRA VARIETIES TO OKRA LEAF CURL VIRAL DISEASE IN RELATION TO ENVIRONMENTAL CONDITIONS

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Okra (*Abelmoschus esculentus* L.) is an important kharif vegetable of Pakistan but recently is also widely grown during Rabi season due to high nutritive value and market demand. Okra is attacked by numerous viral diseases including okra leaf curl viral disease (OLCVD) caused by okra leaf curl virus (genus *Begomovirus* and family *Geminiviridae*) transmitted through whitefly (*Bemisia tabacci*) which is considered most serious. Therefore, this study was conducted to determine response of okra varieties to OLCVD in relation to environmental conditions of this region. Experiments were conducted at Shahpur, Peer Khathi, and Tando Adam localities under natural inoculation conditions to assess response of six different okra varieties such as Tulsi, Durgah, Sabazpari, Sarahariya, Ramaan Kirshna and Pooja against the OCLVD. Trails were conducted in rabi and kharif season of 2016 and 2017, respectively. Approximately 27 plants of each variety were grown with three replications in complete randomize design in same experimental plot to record intensity of the disease. Additionally, vector population was also scouted during each observation time. Furthermore, correlation of environmental conditions with the disease incidence and severity was determined. The disease incidence and severity in Tulsi variety at all three tails was found zero during both growing seasons. The disease was first recorded at 7th week after sowing and whitefly vector of was appeared 4th and 5th weeks after sowing during kharif and rabi growing season, respectively. Pooja, Sarahariya and Subzpari varieties showed minimum the disease severity (2 rating score) followed by Durgah and Ramaan Kirshna (3 rating score) during rabi growing season. During kharif season all varieties except Tulsi showed unique disease severity score 1. Minimum 6 whiteflies/leaf were recorded in Tulsi variety during both growing seasons. Same whitefly population in Subzpari variety was observed during this growing season. In Pooja, Duragah and Sarahariya varieties 7 whiteflies/leaf was recorded during rabi and kharif seasons. Whereas, maximum 8 whiteflies/leaf were recorded in Subzpari and Ramaan Kirshna during kharif season but 7 whiteflies/leaf in Ramaan Kirshna during rabi growing season. Average minimum temperature was 18.1±0.8 and 22.1±0.7 $^0$C during rabi and kharif growing season, respectively. Whereas average maximum temperature of 35.8±0.6 and 40.9±0.4 $^0$C was recorded during rabi and kharif growing season, respectively. The relative humidity was higher (55.2±0.8 average relative humidity %) in rabi season as compared to kharif growing season (44.3±0.7 average relative humidity %). However, minimum temperature was significantly correlated with disease incidence and severity during both growing seasons. Whereas maximum temperature and relative humidity (%) during rabi season was significantly correlated with the disease incidence and severity but non-significant relationship was determined during kharif season. These investigations are suggesting that severity score (1-2) was lower in seasonal cropping, thus seasonal cropping is recommended to avoid severe threats to the crop. Incase high market demand of fresh vegetables, resistant varieties of okra such as Tulsi, Pooja, Sarahariya and Subzpari should include in cropping calendar of rabi vegetables. Furthermore, resistance potential of Tulsi variety should assessed using different isolates of the okra leaf curl virus prior to investigating genetic makeup of the variety for breeding purpose.

# SCREENING OF COTTON GENOTYPES AGAINST CLCUD BY GRAFTING METHOD

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Cotton leaf curl virus disease is an important constraint to cotton production. The resistant source is a safe solution of this hazardous problem. In this regard fifteen (15) genotypes were tested by petiole grafting. All tested genotypes CRIS-682, CRIS-683, CRIS-692, CRIS -693, CRIS-694, CRIS-695, CRIS-696, CRIS -697, CRIS-698, CRIS-748 CRIS-749, CRIS-750, CRIS-751, CRIS-753 and CRIS-754 showed susceptible reaction and required different period of symptoms development. Minimum incidence of disease was recorded in CRIS-695 and CRIS-754.

# SCREENING OF TOMATO VARIETIES FOR RESISTANCE TO TOMATO LEAF CURL VIRAL DISEASE UNDER FIELD CONDITIONS

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Tomato (*Lycopersicum esculentum* Mill.) is important vegetable in Pakistan. Tomato leaf curl viral disease caused by *tomato leaf curl virus* (TLCV) (genus: *Begomovirus*, family: *Geminiviridae*) and transmitted by whitefly (*Bemisia tabaci*) is considered as the most devastating one. Recently, available tomato varieties are not screened against this disease in the agro-ecosystem of Sindh Province of Pakistan. Therefore, in this study 12 commercial varieties of tomato such as, TO-1057, Advanta-1225, T-1359, Yaqui, Sahil, Ayushman, Early king, Advanta-1211, Kiara, Lima, Advanta-1209 and Ashoka were screened against the disease under field conditions during Rabi and Kharif growing season 2017-18. Observations were taken on disease incidence, severity, and whiteflies population at interval of week after date of transplantation. Additionally, relationship among the disease and vector insect was determined. The minimum incidence of the disease was recorded in variety Advanta-1211 (13.85%) and Lima (18.85%) followed by T-1359 (23.30%), Early king (25.51%), TO-1057 (27.21 %), Advanta-1225 (29.41%). Minimum 1-rating score was recorded in Advanta-1211, Lima and T-1359 varieties. Significantly minimum number of whiteflies was recorded in variety Lima (2.3 whiteflies), Advanta-1209 (2.3 whiteflies), Kiara (2.4 whiteflies) followed by Advanta-1211 (2.5 whiteflies). There was non-significant relationship of vector insect with both incidences as well severity of tomato leaf curl disease, $r^2$= 0.07068, $P$= 0.8272 and $r^2$= 0.2869, $P$= 0.3660, respectively. Based on the results, it is concluded that the minimum incidence and severity of tomato leaf curl disease was recorded in variety Advanta-1211, Lima and T-1359. In rabi season the disease intensity was lower as compared to kharif season. Therefore, it is suggested that variety Advanta-1211, Lima and T-1359 should incorporated in the disease management plan.

# INTENSITY OF PAPAYA RING SPOT DISEASE IN DISTRICT HYDERABAD AND SUBSEQUENT MANAGEMENT BY USING ANTIVIRAL COMPOUNDS

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*Papaya ringspot virus* (PRSV, Genus: *Potyvirus*, Family: *Potyviridae*, Order: *Patatavirales*) causing papaya ring spot disease is affecting papaya production throughout world including Pakistan. Up to 100% losses are reported due to infection of the PRSV in papaya in some region. Therefore, in the present study prevalence of papaya ring spot disease in Hyderabad district and effectiveness of Azaguanine and Ribavirin on the development of papaya ring spot disease will be assessed. Survey for occurrence of papaya ring spot disease in Hyderabad district of Sindh was carried out to record the incidence percent and disease severity. Furthermore, in order to determine pathogenic nature of the disease-causing organism, about 30 days old papaya seedlings were inoculated with sap obtained from diseased plants and results were compared with control plants were sap was not applied. For managing the disease Azaguanine and Ribavirin at concentration of 100, 500, 1000 uM were applied after development of local lesions on inoculated plant. There were five replications of each treatment. Data on the disease incidence (%) and severity was recorded after 30 days after application of the treatment. Results reveal that There was non-significant difference (Df = 4, F = 0.49, P = 0.7435) among incidence of the disease at surveyed fields of Hyderabad district. However, severity of the papaya ring spot disease was significantly varied (Df = 4, F= 1.59, P = 0.1843) at surveyed fields. Intensity of the disease in term of incidence was 100±0 % and severity was 2.2±0.5 in the inoculated plants, respectively in plants rubbed with sap obtained for symptomatic payapa plant. There was not any expression of symptoms in the plants that were un-inoculated and rubbed with carborundum powder. Furthermore, application of Azaguanine and Ribavirin at concentration of 1000 uM significantly reduced the incidence (Df = 7, F = 9.7, P = 0.0000) and severity (Df = 7, F = 3.54, P = 0.0075) of the disease. Based on present studies it is concluded that papaya ring spot disease is infecting papaya crop in Hyderabad district. However, location to location severity of the disease is change. Pathogenicity test revealed that plants rubbed with sap obtained from diseased plants were showing necrotic and water-soaked lesions. Furthermore, application of Azaguanine and Ribavirin at concentration of 1000 uM in laboratory-controlled experiment significantly reduced incidence and severity of the disease in papaya plants. It is therefore, recommended that a comprehensive survey shall be conducted to determine the intensity of papaya ring spot disease in Sindh. Effectiveness of Azaguanine and Ribavirin shall be evaluated in field experiments.

# MOLECULAR RECORD OF *POTATO LEAFROLL VIRUS* NATURALLY INFECTING TOMATO IN PAKISTAN

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Tomato (*Solanum lycopersicum* L.) is the second most important solanaceous vegetable in Pakistan with an annual production of 610,000 tonnes in 2016-2017 (Pakistan Bureau of Statistics, 2017). A survey was conducted from February to April 2019, on open-field tomato crops in the Khanewal district of Punjab. A total of ten symptomatic samples showing rolling of upper leaves and stunting and five asymptomatic samples were collected from several fields. The tomato plants were infested with small green peach aphid *Myzus persicae* Sulzer colonies on the underside of leaves. Disease incidence was 11% in the district while the disease severity index was 60%. Collected tomato samples were tested for *Potato leafroll virus* (PRLV) based on their symptoms as well as for common viruses infecting tomato (i.e., *Cucumber mosaic virus* (CMV), *Potato virus* Y (PVY), *Tobacco Etch Virus* (TEV)). Double-antibody sandwich (DAS)-ELISA method (Agdia) was performed for PLRV, CMV, TEV and Triple-antibody sandwich (TAS)-ELISA was performed for PVY. All ten symptomatic samples were found positive to PLRV only whereas all the asymptomatic ones were negative to all the tested viruses. Reverse Transcriptase Polymerase chain reaction was done after total RNA was extracted from all these samples using the Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) from Promega (USA) was used to construct the complementary DNA (cDNA) and Polymerase Chain Reaction (PCR) was performed for detection, using specific primers (PLRVF3: ATGAGTACGGTCGTGGTTAAAGG; PLRVR4: ACCCCGTTTATCATCCGCGC) designed from alignment of several isolates reported from different countries available in NCBI database. The primers amplify a 506bp region of the coat protein gene. PLRV was detected by RT-PCR in all symptomatic samples and was not detected in the asymptomatic ones. Two selected isolates were sequenced in both directions and were submitted to NCBI and were assigned with accession numbers (MN266215 and MN266216). The BLAST analysis of newly sequenced isolates of PLRV showed that they have 99.4%, 99.2% and 99.01% identity with isolates EU073861, EU073862 and JQ346189 reported from Germany, respectively. PLRV is a phloem-limited single-stranded positive-sense RNA virus, which belongs to the genus *Polerovirus* (family Luteoviridae) and is transmitted by *M. persicae* in a circulative, non-propagative manner (Gray et al., 2018). To our knowledge, this is the first molecular report of PLRV infecting tomato crops in Pakistan.

# A SURVEY FOR EPIDEMIOLOGY AND MANAGEMENT OF CITRUS TRISTEZA VIRUS DISEASE

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Citrus is the most valuable fruit all over the world as well as Pakistan due to its taste, nutritional and medicinal qualities. Citrus Tristeza Virus (CTV) is the major disease which destroys the backbone of citrus industry. The infected tree shows decline symptom in conjunction with leaf chlorosis, twigs dieback, and stem pitting. The objective of this study was to record the incidence and severity of CTV disease based upon symptomology and serological studies, and to find out the most favorable environmental conditions for CTV disease development. Surveys were conducted in 3 of District Sargodha i.e Sargodha, Kot Momin and Bhalwal. In each Tehsil, 6 fields were marked for srvey and the samples were collected based upon symptomology. The suspected samples were subjected to ELISA for the confirmation of the CTV. After confirmation disease incidence was recorded in all the fileds. In the more affected cultivar Mosambi disease severity was also recorded in all Tehsils. The data of environmental conditions was collected from Meterlogy Department, Sargodha. The data of environmental conditions were correlated with disease incidence to find out the most favorable environmental variable in disease development. The results showed that Mosabi was the most affected cultivar in all 3 Tehsils of Sargodha followed by Feutral's early and Kinnow. The severity of CTV disease indicated that older orchards are more prone to the disease than younger ones. The warm and humid conditions are the more favorable variable for the disease development. In conclusion, this study is expected to enable farmers to identify and predict the timing and severity of CTV disease. The insect vector must be controlled by using environment friendly insecticide before the onset of warm and humid conditions to avoid from severe losses caused by CTV. There is dire need for the establishment of citrus disease-free nursery as the inappropriate combination of scion and rootstock is the most promising reason for CTV disease development.

# WHITEFLY AS A VECTOR OF DISEASES IN CUCURBITS: A REVIEW

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Cucurbits are herbaceous vine plants belonging to family Cucurbitaceae and are commercially grown worldwide for consumption as a food and used in medicines. The worldwide production of cucurbits stands at 180 million metric tons. The main cucurbit crops come from 3 main genera; Watermelon from genus *Citrullus* originated from Africa, Squashes, Pumpkin and gourd from genus *Cucurbita* is native to Mesoamerica and cucumber and melon from genus *Cucumis* have their origin in South Asia. One of the major restraints in the production of cucurbits is viruses that causes stunted growth and ultimately fall in yield. Cucurbits are affected by 59 viruses from six genera (Potyviruses, Criniviruses, Cucumoviruses, Tobamoviruses, Ipomoviruses and Begomoviruses) that are mostly transmitted by aphid or whitefly. Whitefly is a vector of Criniviruses, Ipomoviruses and Begomoviruses which is a rising threat for crops of cucurbits. Criniviruses are major disease spread by *Bemisia tabaci* while begomoviruses has risen to alarming level and is serious threat for future crops. Mainly Bemisia borne viruses include Beet pseudo-yellows (BPYV) Cucurbit yellow stunting disorder virus (CYSDV) and Cucurbit chlorotic yellow virus (CCYV) these are all criniviruses except the last one which is begomovirus. The Major Bemisia borne Ipomoviruses is Cucumber vein yellowing virus (CVYV). Virus resistant varieties are developed and use to cope with this issue but still their scope is limited in some regions. This study gives a review of all the viruses spread by *Bemisia tabaci*.

# MONITORING OF CITRUS BENT LEAF VIROID IN CITRUS GROWING AREAS OF SARGODHA, PAKISTAN

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Citrus bent leaf viroid is an emerging viroid in citrus growing areas of Pakistan that effected the citrus production and quality. This study was conducted specifically for monitoring of citrus bent leaf viroid (CBLVd) in the declined and declining citrus orchards of Sargodha district. Monitoring was carried out based on symptomology including backward leaf bent of leaves, bark cracking, Pin holing, yellowing, stunting and ultimately decline. Detection of CBLVd was performed using RT-PCR assay. Primers specific for the CBLVd were used for the detection and confirmation. The interaction of CBLVD with varieties, areas and status of the orchards were monitored for the first time in Pakistan. Maximum disease incidence and severity was recorded in tehsil Kot Momin ranged from 1.76-3.53% with severe symptoms of bark cracking, backward leaf bent and stunting. Minimum disease incidence was recorded in tehsil Bhera (0.57%). Maximum infection percentage was exhibited by declined citrus orchards (2.57%) at severity index 3 showing symptoms of backward leaf bent as compared to others. Based on varietal difference maximum infection of CBLVd was recorded in Kinnow (1.97%) at severity index 4 showing stunting as compared to bark cracking and backward leaf bent. Molecular detection through RT-PCR confirmed the CBLVd on agarose gel. It is concluded that viroids have their supporting role in declined orchards. Therefore, extensive surveys are required to be investigated for the other citrus varroids and their role in decline.

# EVALUATION OF CHILLI GERMPLASM AGAINST CHILLI LEAF CURL VIRUS DISEASE AND ITS MANAGEMENT BY VECTOR CONTROL

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Chilli leaf curl virus (ChiLCV) is a major problem in chilli development in Pakistan. Present research was done to evaluate chilli germplasm against leaf curl virus. Twelve different chilli lines/varieties were tested in open field trials for their resistance/susceptibility to ChiLCV. On weekly basis data on disease severity and incidence were collected. Out of all varieties screened, Tatapuri Chilli and CH111 were highly susceptible towards the chilli leaf curl disease while CH103, CH107, CH108, CH109, GSL111, Talhari showed susceptible response whereas CBS1292 and Anaheim showed moderately susceptible response towards the leaf curl disease of chilli. Only two cultivars Hybrid-46 and Hot Queen were found to be resistant. The screening experiment showed that most of the chilli varieties are susceptible to chilli leaf curl disease and resistance is lacking in local germ plasm. In addition, the efficacy of three insecticides Imidacloprid, Polo and Emamactin was tested for the management of disease and whitefly vector in field conditions at weekly intervals. Out of three insecticides, Polo found to be more effective as compared to Emamectin and Imidacloprid against chilli leaf curl disease and whitefly vector.

# FIRST REPORT OF *COTTON LEAF CURL MULTAN VIRUS* INFECTING HOLLYHOCK IN PAKISTAN

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Hollyhock is an ornamental plant having aesthetic beauty as well as medicinal importance. Samples of hollyhock were collected from Multan based on characteristic symptoms produced by begomovirus infection include leaf curling, vein thickening, and puckering. Samples were stored at 4˚C in polythene bags after that DNA was extracted. PCR technique was optimized for the detection of begomovirus. Betasatellite was detected from the hollyhock plant with beta 01/02 and CLCuMuBF11/R33 by obtaining 1.4 kb and 479 bp respectively and sequence analysis of Beta 01/02 showed identity with Cotton leaf curl Multan betasatellite. The coat protein region was amplified by using primers Av/Ac core and showed positive results with 579bp nucleotide. Sequence analysis by using Av/Ac core primer showed that hollyhock is associated with *Cotton leaf curl Multan virus*. Begomovirus infects the hollyhock plant for the first time from the Southern part of Punjab, Multan.

# MOLECULAR CHARACTERIZATION OF BEGOMOVIRUS ON THE TOBACCO PLANT IN MULTAN

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Tobacco (*Nicotiana tabacum*) is one of the important commercially grown crops, showing the leaf curl symptoms on the viral attack. The survey was conducted on the tobacco field in 2018 and symptoms of upward leaf curling, rigid leaves, uneven leaf surface, vein thickening, and leaf banding are observed on the tobacco plant. The begomovirus was detected on tobacco plants by using molecular techniques i.e.,DNA extraction (CTAB method), PCR amplification, Sequence demarcation tool. PCR amplification was performed with Av/Ac core, Beta 01/02, Begomo 01/02, CLCuMuBF11/R33, and required amplicon of 579 bp, 1.4 kb, 2.8 kb, 478 bp respectively. After cloning, the sequencing result displayed that tobacco is associated with *Cotton leaf curl Multan virus* and Cotton leaf curl Multan betasatellite. Hence, it's essential to employ the resistant cultivar along with management strategies for controlling whitefly.

# GENETIC CHARACTERIZATION OF DISTINCT RECOMBINANT BEGOMOVIRUS INFECTING *EUPHORBIA PULCHERRIMA* IN PAKISTAN

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Begomoviruses are single stranded DNA viruses exclusively transmitted by white fly and are major threat to food security globally. In present study we report two complete nucleotide sequences of DNA-A (isolate PK1 and PK1A) of begomovirus infecting *Euphorbia pulcherrima* in Pakistan showing symptoms of yellowing and leaf curling. The DNA-A sequences were subjected to pairwise comparison and phylogenetic analysis. The results showed isolate PK1 and PK1A shared 99.7% identity with each other and maximum identity 90.8 and 90.6 with *Euphorbia leaf curl virus* [China: Shandong Qingzhou:2012] [KC852148] respectively. Two recombination events were also recorded with *Pedilanthus leaf curl virus* [PK: RYK1: Euphorbia:04] [DQ116884] as major and *Euphorbia leaf curl* [China:Guangxi 35:2002] [AJ558121] and *Euphorbia leaf curl virus* [China:Fujian:2006] [FJ487911] as minor parents. Following the latest guidelines of ICTV regarding species demarcation threshold for begomoviruses, both isolates belong to tentative new specie for which "Euphorbia yellow leaf curl virus" (EYLCuV) name is proposed.

# MOLECULAR CHARACTERIZATION OF BEGOMOVIRUS ASSOCIATED WITH PEPPER LEAF CURL DISEASE (PELCUD) ON BELL PEPPER IN DISTRICT FAISALABAD, PUNJAB, PAKISTAN

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Bell pepper (*Capsicum annuum*) is one of the most grown vegetable crops in the world. Asia produces more than 70% of the total global production of bell peppers. They are an excellent source of B vitamins. Bell pepper faces multiple diseases, but the pepper leaf curl virus disease is very common causing considerable losses to the crop. Pepper crops are badly affected by the strains of begomovirus. To overcome yield losses, effective management is essential, and exact identification of the pathogen is required for advising management strategies. The current research was planned to identify and analyze the diversity of begomovirus on the bell pepper crop in Faisalabad region of Punjab, Pakistan. Samples of infected bell pepper leaves were collected from AARI and UAF in Faisalabad. Total genomic DNA was isolated by using the CTAB method and amplified by using Begomovirus specific primers CLCV1 and CLCV2. The amplified PCR product was sequenced commercially, online BLAST of the sequences was done on NCBI to have the similarity index of the virus with other strains of Begomovirus. Phylogenetic analysis was done by using MEGA-X software and MUSCLE alignments were made between 4 sequenced isolates of PepLCuV and previously identified reference sequences of PepLCuV. Based on phylogenetic analysis, it was concluded that the associated Begomovirus is a species of Pepper leaf curl virus, as the targeted DNA sequence was 100% similar, to the isolates of PepLCV. This preliminary research work opens new frontiers for the development of sustainable management strategies for the management of Pepper leaf curl disease.

# PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CITRUS PLANTS AFFECTED BY CITRUS TRISTEZA VIRUS

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Citrus tristeza virus (CTV) is a phloem-limited virus that only affects citrus and similar plants in nature. The virus replicates in sieve elements of the phloem parenchyma cell. It is accompanied by cell-to-cell migration into a minute group of neighboring cells in host plants. The need of study is to determine the biochemical and physiological changes CTV affected plants. For biochemical characterization, 1) total soluble phenols, 2) total soluble sugar, 3) antioxidant enzymes and heavy metals status were determined. The total soluble phenols in leaf were higher than in bark and healthy leaf & bark showed higher amount of phenol than diseased leaf & bark, the sugars were higher in fruits than leaf and bark, while among the enzymes catalase (CAT) shows higher quantity followed by superoxide dismutase (SOD) and peroxidase (POD). The enzymes were higher in diseased plants when compared with healthy plants. The status of minerals and heavy metals was that iron was the most abundant in present study in Sargodha region followed by zinc, manganese, and copper, respectively in both healthy and diseased plants. Citrus plants exhibited numerous physiological changes due to CTV (transpiration rate, chlorophyll content, respiration rate, photosynthetic rate was considerably varied in healthy and diseased plants. Leaf surface area of diseased plants was much lower than of healthy ones. It is concluded that CTV affects the biochemistry and physiology of infected citrus plants which ultimately minimize the defense response and plant become prone to more losses.

# PATHOGENICITY OF *COTTON LEAF CURL MULTAN VIRUS* IN COMBINATION WITH NON-COGNATE BETASATELLITE

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Among the crop viruses, single stranded DNA viruses play a very destructive role. The crop yields in tropic and sub-tropic parts of the world are reduced due to their infection. They are mainly transmitted by whitefly. Recently, several satellites have been reported along with their helper viruses on main crops like cotton and tomatoes. In this study we used a non-cognate beta satellite along with CLCuMV to understand the viral-satellite interaction. Briefly, the begomovirus previously isolated from cotton was used in this study. The cognate betasatellites for *cotton leaf curl Multan virus* was cotton leaf curl Multan betasatellite. But instead of cotton leaf curl Multan betasatellite, we used a betsatellite isolated from vegetables. The dimeric constructs were transformed into agrobacterium and their cultures were grown at optimum temperature. The *Agrobacterium* was activated and used for inoculation transiently. The *agrobacterium* cultures of helper virus and betasatellites were mixed for their combined inoculation on young leaves of tobacco. After inoculation, the symptoms of virus/non-cognate betasatellite were monitored and recorded. The viral replication level was checked through PCR. The extent of betasatellite replication and its impact on symptoms development were established through disease scaling systems. This study was helpful in establishing the future possible link with new combination of disease epidemic.

# APPRAISAL OF PAKISTANI WHEAT AGAINST BARLEY YELLOW DWARF VIRUS AND ITS VECTOR IN PESHAWAR, KPK

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The bird cherry oat aphid, *Ropalosiphum padi* is one of the most detrimental pests of cereals, causing harm mainly by sucking cell sap from different parts of the plant and by transmitting Barley Yellow Dwarf Virus (BYDV) disease. Aphid population and BYDV occurrence was studied on 123 wheat genotypes during wheat cropping season 2020-21 which were raised at NIFA farm. The count data were subjected to different analyses: i) ANOVA was performed to determine aphid population on different dates ii) Resistance in Pakistani wheat were estimated by aphid resistance index (ARI) iii) Correlation of aphid population with BYDV incidence and percent severity. High variability in wheat aphid population was recorded on the studied genotypes. Highest mean population of *R. padi* was recorded on 16th of February (22.7 ± 0.67) while lowest was recorded on 16th of March (2.0 ± 0.00). Moreover, highest mean population of green bug, *Schizaphis graminum* was recorded on 16th of March (14.4 ± 1.04) while lowest was recorded on 16th of February (0.6 ± 0.07). According to aphid resistance index, 55 wheat genotypes were found resistant and 68 were found susceptible to wheat aphids**.** Wheat genotypes showed significant ($p \leq 0.05$) higher mean aphid population (23.3 aphids/tiller) on Tijban-10 while minimum mean population (3.3 aphids/tiller) on Karwan. BYDV incidence (infected tillers/plot) and percent severity also varied among genotypes. Maximum incidence (11) and severity (60%) was recorded during 2020-21 cropping cycle. Incidence and severity of barley yellow dwarf virus was positively correlated with abundance of bird cherry oat aphid, *Ropalosiphum padi* but not with green bug, *Schizaphis graminum*. The data of Pakistani wheat with least density of BYDV and its vector would be helpful to make varietal selections for future breeding programs. Further, it is necessary to identify the resistant genes in these genotypes that could be incorporated in future wheat cultivars to cope with this serious problem.

# COMBINING ABILITY ESTIMATES FOR COTTON LEAF CURL VIRUS THROUGH DIALLEL ANALYSIS IN CHANGING ENVIRONMENT

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The genetic components of variance for CLCV revealed higher $\sigma^2$GCA and Additive ($\sigma^2$A) than $\sigma^2$SCA and Dominance ($\sigma^2$D), which indicated additive genes were more reliable for inheritance of resistance to CLCV. The ratio of variances $\sigma^2$gca/$\sigma^2$sca was higher than unity (one) and further proved from degree of dominance $[\sigma^2A/\sigma^2D]^{0.5}$ which was greater than one and confirmed supremacy of additive genes at both locations and generations. The heritability narrow sense ($h^2$) and broad sense ($H^2$) was found higher which suggested role of additive genes, which are fixable. Therefore selection would be effective in early segregating generation according to the symptoms of cotton leaf curl virus. The GCA effects for CLCV showed that among the parents, Mac-7 found as good general combiner with highest significant negative GCA effects at both locations in $F_1$ and $F_2$ which considered as CLCV resistant parent. The SCA effects exhibited that hybrid, Mac-7 x USD16-3058, CIM-602 x Mac-7 and NIA-Noori x Mac-7 showed significant negative SCA effect in both generations and locations. It was noted that cross combinations involved good x poor and poor x good general combiner with significant SCA effect was due to complementary gene action which produce desirable transgressive segregants, these can be further studies through bi-parental mating of diallel selective mating or any other form of recurrent selection in early generation with single plant selection to exploited both additive a non-additive gene action.

# SPATIO - TEMPORAL PATTERN OF MYZUS PERSICAE (SULZ.) AND POTATO VIRUS Y DISEASE INCIDENCE IN RELATION TO EPIDEMIOLOGICAL FACTORS AND THEIR MANAGEMENT

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Potato (*Solanum tuberosum L.*) is an important vegetable crop for the majority of the world's populations. The production of potato is hindered by the attack of a number of viral diseases including PVY and PLRY that significantly reduce the potato yield by10-25% and 58-83%, respectively. *Myzus persicae* significantly enhances the spread of PVY disease under ordinary conditions. Predisposing epidemiological factors were identified in the development of Potato virus Y disease in relation to *Myzus persicae* population. Spatio-temporal pattern of *Myzus persicae* and PVY disease incidence were investigated in relation to epidemiological factors (maximum and minimum temperature, relative humidity, rain fall and wind speed) under a randomized complete block design (RCBD). Data recorded on aphid population and PVY disease incidence was graphically plotted to visualize spatio-temporal pattern. Plant extracts i.e. *Azadirachta indica* (neem), *Allium sativum* (garlic), *Zingiber officinale* (ginger) and *Allium cepa L* (onion) were evaluated to manage PVY disease and its vector. Data recorded on vector population and disease incidence was analyzed through ANOVA. Among twenty varieties/lines, FD 51-55 showed significant correlation with the environmental factors. *Azadirachta indica* at 5% concentration was effective as compared to control and other plant extracts in reducing the aphid and PVY disease incidence under field conditions.

# IMPACT OF CHILLI LEAF CURL VIRUS (*ChiLCuV* ) ON YIELD OF CHILLI PEPPER (*CAPSICUM ANNUM* L.) AND ITS HORTICULTURAL CHARACTERISTICS

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Leaf curl disease caused by *chilli leaf culrl virus (ChiLCuV)* is a severe risk for successful chilli production. This disease decreases its fruit yield and also affects the growth parameters of it. Evaluation of germplasm on the basis of its horticultural parameters is very reliable method for selection of resistant varieties/lines by recording the data of growth parameters including fresh plant weight, fresh weight of root and shoot, dry weight of root and shoot, plant height, root length, shoot length and its total fruit yield. The present study was mapped to dig out the most resistant variety with improved horticultural characteristics by screening of available chilli germplasm against *(ChiLCuV)* and its impact on horticultural characteristics. For that purpose ten varieties/lines (Fangaio, BPVLC 14-1, Big Daddy, Desi, Mexi Pak, Hot shot, Galaxy-2, Tejal, Super sky and Big Red AB) were grown under randomized complete block design (RCBD) in the research field area of Plant Virology Section, Plant Pathology Research Institute, Faisalabad. From all those varieties/lines only one (Galaxy-2) expressed resistance response (16.46%) with disease rating 2. From all the varieties/lines (Big daddy) showed the maximum fruit yield (452.24 g/plant), highest fresh weight of plant, root and shoot (378, 14.23 and 363.7 g respectively), Maximum no. of leaves (286 per plant), highest dry weight of roots (5.4 g) and root length (18.5 cm). Maximum length of Shoot (67 cm) with plant height of (81.4 cm) was expressed by BPVLC 14-1and Galaxy-2 moderately resistant group. So the final results showed that BPVLC 14-1, Galaxy-2 and Big daddy had good horticultural characteristics with improved yield and disease resistance. These varieties/lines are recommended for further use in breeding programmes.

# ASSESSMENT OF PLANT DEFENSE ACTIVATORS AND NUTRIENTS FOR THEIR ANTIVIRAL POTENTIAL AGAINST IRIS YELLOW SPOT VIRUS OF ONION *(ALLIUM CEPA*) UNDER NATURAL FIELD CONDITION

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Onion is one of most important vegetable grown worldwide and considered as rich source of vitamin B6, vitamin C, potassium and folate and it also has antioxidant properties. Out of many challenging pathogens *Iris yellow spot virus (IYSV)* causes significant yield losses and poor bulb quality in size and grade. Five plant defense activators salicylic acid (SA), citric acid (CA), benzoic acid (BA), $K_2HPO_4$, $KH_2PO_4$, solution of macro nutrients (N,P,K) and solution of micro nutrients (B, Zn, Cu, Fe, and Mn) were tested against *IYSV* under natural field conditions after confirmation of virus through ELISA and visual symptoms. Plant defense activators and nutrients were applied as foliar sprays in randomized complete block design (RCBD) while the solutions of macro and micro nutrients were evaluated under glass house conditions by following complete randomized design (CRD). All the treatments were sprayed three times in disease progress season with their three different concentrations (0.25%, 0.50% and 0.75%) with twenty days of interval. First spray was completed before inoculation of disease and remaining two were sprayed after inoculation by viruliferous thrips. Out of five plant defense activators the maximum disease was managed by salicylic acid (SA) by showing 11% disease incidence followed by Benzoic acid (BA) 13.5%, $KH_2PO_4$ 17%, $K_2HPO_4$ 27%, and citric acid (CA) 33% while in the case of Nutrients, macro nutrients showed the significant results by exhibiting 18% disease incidence while micro nutrients showed 29% disease incidence. All the experimentation was conducted in the research area of Plant Virology Section, Ayub Agricultural Research Institute, Faisalabad.

# RIBAVIRIN TARGETS SUGAR TRANSPORTER 6 TO SUPPRESS ACQUISITION AND TRANSMISSION OF RICE STRIPE TENUIVIRUS BY ITS VECTOR *LAODELPHAX STRIATELLUS*

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Rice stripe tenuivirus is one of the most destructive pathogens of rice and other cereal crops. The virus is transmitted by the small brown planthopper (SBPH, *Laodelphax striatellus*) in a circulative-propagative manner. Thus, blocking transmission by the insect vector would provide an effective strategy to prevent epidemic outbreaks of the disease. In this study, we explored the effect of ribavirin on acquisition and transmission of the virus by specifically inhibiting the expression of sugar transporter 6 (LsSt-6), which was recently reported as a key vector component for RSV transmission. Ribavirin at the highest concentration tested (250 μM) significantly reduced RSV acquisition and transmission efficiency by SBPHs through inhibiting LsSt-6 mRNA level. Survival of the model insect cell line Sf9 was 95.0 ± 2.2 and 85.6 ± 2.1% after exposure to 250 μM ribavirin or 8-azaguanine, respectively. Further study confirmed that 250 μM ribavirin also significantly reduced LsSt-6 mRNA and protein levels in Sf9 cells. However, 8-azaguanine did not significantly inhibit viral infectivity and LsSt-6 mRNA levels in SBPH or the Sf9 cell line. This result provides evidence that ribavirin has the potential to disrupt LsSt-6 expression but not others like viral RNAs to prevent acquiring RSV, which leads to less viral accumulation in SBPH tissues and thereby lower transmission efficiency.

# SECTION 7: CLIMATE CHANGE AND ABIOTIC STRESSES

# IMPACTS OF CLIMATE CHANGE ON BANANA FARMING IN BANGLADESH: CURRENT STATUS AND FUTURE PROSPECTS

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Banana (*Musa* spp.) is one of the most important fruit crops in many tropical and subtropical countries including Bangladesh. It is cultivated in more than 130 countries mainly on small farms and is an essential food for millions of people all over the world (Frison and Sharrock, 1998). In Bangladesh, it comprises nearly 42% of the total fruit production of the country. But the banana production was significantly declined by the flood, cyclones, droughts, infestation of pests and diseases due to climate change effects. We have surveyed the 100 banana orchards the effects of climate change on banana production which found that every years and flood and cyclones destroy the banana plants particularly the south-west region (Delta region) of Bangladesh that results the decrease in production. Due to increase of temperature, the banana production is decreased 30~40 % by water scarcity in Barind tract region of Bangladesh (Northern region). Moreover, pests and diseases indirectly are significantly increased due to the climate changes in Bangladesh. The banana farmers are unscrupulously used the pesticides in much higher doses for pest management, which pose a serious diseases of the human health such as cancers, heart disease, kidney disease, gastrointestinal diseases, mental retardation etc. In my keynote lecture, I will present current surveyed data on the climate change impacts on banana farming and farmers incomes and also discuss the possible strategies to adapt the climate change.

# EXTREME TEMPERATURE EFFECT ON 19 GERMPLASM OF *VIGNA RADIATA*

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In the present study, the impact of high temperature on the mung bean plant was carried out in the greenhouse. For that 19 different varieties of mung bean, i.e., swat mung, Abbas mung, Karak mung, Ramzan, Nm-28, Nm-51, Nm-54, Nm-92, Nm-98, Azri 2006, Nm-2006, Nm-2011, Nm-13-1, Nm 2021, Nm-2016, Bm-2017, Nm 19-19, Nm-20-21, Nm-121-25 were selected for this study. Various growth parameters like root, shoot length and biomass, physiological [(total chlorophyll content (TCC), carotenoid (CART), reducing sugar (RS)] and biochemical [total protein content (TPC), peroxidase (POX) and catalase (CAT) activities] were studied after 15 days of seed germination. Moreover, the experiment was categorized into two treatments, i.e., treatment 1 (control): 40 ± 5 °C, treatment 2: 50 ± 2 °C. In the whole experiment, the parameters of treatment 2 were compared with treatment 1 (control). The results revealed that the root, shoot length and biomass of each mung bean germplasm was significantly declined up to ~ 75%when grown at 50 ± 2 °C as compared to the respective control. However, swat mung germplasm exhibited maximum growth and the tolerance index (143%) compared to the rest of the germplasm grown at high temperature. Moreover, heat stress showed harmful effects on the physiological traits of mung bean germplasms. The TCC, CART, and RS in mung bean germplasms was significantly declined up to 73% in a heat stress environment. While, the biochemical traits like TPC, POX, and CAT activities in these germplasms increased their levels at high temperature. Thus, it was concluded that the mung bean germplasm "Swat mung" could be cultivated in a harsh environment like heat stress, as that plant exhibited more tolerance and showed better growth at 50 ± 2 °C. So far, there is a need to conduct the field experiment with this germplasm at high temperature so that its response in heat stress environment could be studied in detail and might be recommended as new germplasm for the farmers so that they could get a better yield of the crop.

# THE CHALLENGE OF FOOD SECURITY UNDER CLIMATE CHANGE NEXUS: RESPONSIBILITIES OF PHYTO FIGHTERS

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World population is expected to be doubled by 2050. This raises concerns about food security. Plant diseases and climate change are a major barrier in ensuring food security. About 10 to 16 % of global harvest is lost due to plant diseases. Increasing $CO_2$ emissions, are resulting in an increase in global temperature. Climate change is resulting in drought in some areas while flooding in others. Decline in forests has been seen due to abiotic stresses brought about by climate change. Such stresses alter biological processes in plants and reduce plant vigor. High $CO_2$ concentrations in air have been reported to favour plant pathogens such as *Fusarium pseudograminearum*. $CO_2$ at high levels can also increase biomass of weeds by reducing effectiveness of glyphosate herbicide, thus causing reduction in crop yield. Increasing temperature and $CO_2$ levels may have positive or negative impact on growth of plant pathogens, but potential positive impacts on pathogen growth are of higher concern. Some researches have shown that high carbon dioxide level in microclimate can increase the rate of pathogen evolution. It is a point of serious concern, as it can break plant resistance at an accelerated rate. It has also been reported that even a slight increase in $CO_2$, along with water stress can act as a stimulant for growth of mycotoxin producing fungal species. This will result in contamination of food crops by fungal mycotoxins. Climate change can alter pathogen interactions and distribution, by completing disease triangle of diseases previously not present due to unfavourable climate. Eventually, resulting in development of epidemics in new geographical regions. Change in $SO_2$ level in atmosphere has been reported to directly favor process of pathogen infection and it also indirectly affects soil composition, thus increasing the severity of plant diseases. Increased $O_3$ level in atmosphere has been reported to decrease the photosynthetic rate in plants and thus plant biomass. Climate change also effects penetration and effectiveness of fungicides and eventually has effects on IPM strategies. The impacts of changing climate on plant diseases and food security are complex and require multiple factors to be considered. A lot of research is needed in this regard. We need to combat climate change by reducing the emissions of greenhouse gases, shifting towards eco-friendly sources of energy, and reducing use of chemical fungicides. In order to ensure food security under the prevalence of plant diseases and climate change, we also require certain measures for plant protection like crop rotation, altering sowing time, using high resistance cultivars and efficient quarantine systems etc., along with combating climate change.

# CLIMATE CHANGE AND PLANT-DISEASE INTERACTION

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Climate change has become the main stream around the globe. Most of the countries are addressing this issue because climate change is the biggest threat, and is the cause of nearly 0.4 million deaths a year worldwide. Climate change putting a great impact in our agriculture. An average 0.74 $^{o}$C increases in temperature globally in the last 100 years. Temperature and carbon dioxide concentrations associated with climate change will have a substantial impact on plant-disease interactions. The impacts of climate change can be positive, negative or neutral, since these changes can decrease, increase or have no impact on plant diseases, depending on each region or period. Change in the disease pattern will also affect the reproduction, spread and severity of many plant pathogens. Simultaneously such changes will have a drastic effect on the growth and cultivation of the different crops on the Earth. Increase in temperature and CO2 concentration are also causing higher consequence of late blight (*Phytophthora infestans*) disease of potato and important diseases of rice, namely blast (*Pyricularia oryzae)* and sheath blight (*Rhizoctonia solani*). Maize crop is also badly affected by the heat stress. Changing disease pattern due to climate change has highlighted the need for future studies on such models which can predict the severity of important pathogens of major crops in real-field conditions. Simultaneously, disease management strategies should be reoriented in changing conditions with amalgamation of new strategies for sustainable food production.

# IMPACT OF ANTHROPOGENIC ACTIVITIES ON CLIMATE CHANGE AND PLANT DISEASE MANAGEMENT

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Climate change has appeared as the most prominent global environmental issue and there is a dire need to evaluate its impacts on agriculture. Anthropogenic activities are driven by demographic, social, economic, geographical, and technological changes that have a pivotal impact on climate change. An increase in global temperature up to 5 °C above pre-industrial levels representing temperature extremes, hydrological changes, $CO_2$ gas emissions, heatwaves, droughts, and floods greatly influence crop yields. Increased emissions of $CO_2$ and other greenhouse gases such as methane and nitrous oxide are major contributors to climate warming shifts. Changing weather conditions cause serious plant disease epidemics which have threatened food security. Due to climate change, harmful UV light directly reaches on earth and causes many ailments in plants. The key elements that influence disease progression are temperature, light, and water. Pant survival, vigor, rate of multiplication, rate of spore germination, distance of dissemination of inoculums, and pathogen penetration are all affected by climate change. Climate change impacts pathogens and hosts at all stages of their lives, posing a significant challenge to many pathosystems. Climate change has both positive and negative impacts on plant disease, but the exact mechanism of disease attack is not known. Several strategies for plant disease management are involved to combat this global issue by the production of resistant cultivars, crop rotation, better use of natural resources, drip irrigation, use of fungicides and biocontrol agents, soil solarization, use of healthy seeds, by changing the planting and harvesting dates of crops and diversity in cropping, etc. Intensified research on issues related to climate change could lead to improved understanding and management of plant diseases concerning future climate extremes.

# AMELIORATING EFFECT OF EXOGENOUS PROLINE AND PHOSPHORUS IN SALT STRESSED MAIZE (*ZEA MAYS* L.)

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Salinity is an important ecological factor that creates serious problems for agricultural productivity. The role of different compatible solutes in plant tolerance to salt stress is significant. This study investigated the effects of exogenous proline and phosphorus application on the growth, phosphorus content and ionic balance in maize under salt stress. Seedlings of a local maize cultivar 30Y87 were grown in pots under different proline (foliar spray ($Pr_1$, control) and 100 mM ($Pr_2$)), and phosphorus (10 mg $kg^{-1}$ sand ($P_1$) and 100 mg $kg^{-1}$ sand ($P_2$)) levels with or without 6 dS $m^{-1}$ NaCl. Phosphorus (P) content in roots and shoots, root diameter, shoot dry weight, leaf area, photosynthetic active radiation (PAR) acquisition, and ions concentrations were assessed to allow the evaluation of the effect of phosphorus and proline application in maize under salt stress. Salinity markedly reduced the P content, growth, photosynthesis efficiency and $K^+$ content, and increased the abundance of $Na^+$ and $Cl^-$ ions. However, phosphorus and proline application significantly promoted the P content and growth and ameliorate the ionic effect under salt stress, with maximum positive effect under high supply of P and Pro ($P_2$+$Pr_2$). When exposed to salt stress, maize plants under $P_2$+$Pr_2$ treatment reported 8.22% and 25.52% more phosphorus content in root and shoots, respectively compared to P1+Pr1. Also, maize plants under $P_2$+$Pr_2$ treatment showed highest root diameter, SDW and PAR acquisition compared to $P_1$+$Pr_1$ treatment where maximum reduction was observed. Additionally, $P_2$+$Pr_2$ treatment significantly ameliorate the salt effect by reducing the abundance of $Na^+$ and $Cl^-$ ions and enhancing the $K^+$ content under salinity. In sum, a high supply of proline and phosphorus ($P_2$+$Pr_2$) showed a marked improvement in P uptake, growth parameters of maize and enhanced tolerance to salt stress by maintaining ionic balance.

# PHYSIOLOGICAL CHANGES DURING DROUGHT STRESS IN MAIZE **(ZEA MAYS L.)**

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Defning the metabolic strategies used by maize to tolerate drought events will be important for ensuring yield stability in the future, but studies addressing this critical research topic are limited. To this end, the current study quantified the physiological and agronomic responses of six maize cultivars i.e. D-3464, D-6619, D-4464, D-2468, D-3377, D-3366 and then screened out a drought tolerant and drought sensitive cultivar to periods of water deficit. Drought stress caused a decline in leaf water relations, morphological characters, and photosynthetic activity, leading to decreased yield. Plants exhibited less-pronounced yield, biomass and water relations declines in the tolerant cultivar than the sensitive cultivar. These results suggested that the plant's ability to maintain functions during drought in vegetative periods are important for determining fnal productivity in maize.

# ENVIRONMENTAL POLLUTION AND ITS SMART SOLUTION BY PLANTATION IN CHANGING CLIMATE

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Environment creates favorable conditions for existence and development of living organisms. All organisms are mainly affected directly or indirectly due to environmental pollution. It is due to rapid increases in human population, rapid industrialization and vehicular emission. Environmental pollution especially by industrial revolution in urban area is a major problem facing the world today and there is an increasing awareness of the fact that a clean environment is necessary for better health of living organisms. It is our top most priority to keep our country clean and green as cleanness is a part of our faith. Primary sources of environmental pollution are increasing by anthropogenic activities, which are continuously adding different types of pollutants into environment. Industrial projects have influence on society and the environment not only in terms of benefits but also in risks and hazards. Man has been paying the cost of destruction he has produced in his/her environment and in natural process. It is exact to say that the industrial revolution of the 19th century is mainly responsible for environmental pollution. Different types of industries discharged their toxic substances in the environment, which have polluted the soil and rendered it unfavorable for plant growth. So, this environmental pollution by different industries is causing a great threat to plants, human as well as animals. There is a need to develop green spaces within and around the industrial areas for existence of better environmental conditions. As some plants can act as pollution sink for industrial wastes and known as natural lungs of the environment. If the haphazard population growth and the construction of new industrial structures go on without realizing the importance of trees then probably there would be more destructive changes for living organisms in near future in this changing climate.

# NOVEL METHOD FOR DESALINATION OF HIGH TDS BEARING WATER FOR REUSE IN AGRICULTURE

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The present investigation aimed to desalinate the high TDS bearing water (EC =14400mSm$^{-1}$) by the use of plant growth-promoting rhizobacteria (PGPR) isolated from high TDS water. The TDS bearing water has salinity level above seawater and contain traces of oil. The isolated PGPR strains W1 and W2 were characterized by 16srRNA sequence analyses as *Ralstonia pickettii* and *Brevibacillus invocatus* respectively. The 48h old culture was obtained to soak the seeds of sunflower for 2-3h prior to sowing. The seeds were allowed to grow in the field. The 48h old bacterial culture was added to diluted TDS bearing water and when the EC and TDS were decreased to 19200 mg/L, 7d of treatment, the seedlings were irrigated with diluted TDS water supplemented with 5ml of 48h rhizobacterial culture in 100ml TDS water. The high TDS bearing polluted water showed elevated level of COD (340.1 mg/L) and TDS (92020 mg/L). Both the COD and TDS values were significantly decreased following PGPR treatment. The Na ,Cl and $HCO_3$ content were increased by 3086.5% ,76% and 100% due to *Ralstonia pickettii* as compared to control .Sodium adsorption ratio (SAR), organic matter, N, K, Mg content of rhizosphere soil were increased by 196%, 110%, 228%, 120%, 663% and 100% due to PGPR compared to control. It is inferred from the present study that PGPR play positive role in bioremediation of polluted high TDS water hence alleviate the adverse effects of high TDS water and improve plant growth. The residual effect of W1 on soil organic matter and essential nutrients Ca, Mg, K and P is noteworthy for successive plantation. The addition of bacterial inocula with culture media is recommended for better effectivity of the rhizobacteria to desalinate the high TDS bearing water in addition to the seed inoculation.

# EFFECT OF EXOGENOUS APPLICATION OF NITRIC OXIDE AND ASCORBIC ACID ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF RICE (*ORYZA SATIVA* L.) UNDER WATER DEFICIT CONDITIONS

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The experiment was conducted in the botanical garden of Government College University Faisalabad, to investigate the combined and comparative effects of nitric oxide and ascorbic acid on rice (*Oryza sativa* L.) under water deficit conditions. Seed of two rice cultivars (Kissan Basmati and Basmati-515) were treatment with different levels of nitric oxide (0 and 0.1 mM) and ascorbic acid (0 and 0.1mM) and their combined treatment. Experiment was arranged in completely randomized design, with four replicates. The data was recorded for different morphological, physiological and biochemical attributes such as shoot and root fresh weight, shoot and root dry weight, chlorophyll pigment, relative water contents (RWC), phenolic and soluble proteins, total soluble sugars, total free amino acid, activity of enzymatic antioxidants (peroxidase, catalase and superoxide dismutase), and malondialdehyde (MDA) content in rice plant. Analysis of variance for data showed water deficit conditions decreased the shoot length, root length, fresh weights of shoot and root, dry weights of root and shoot of both rice genotypes. Moreover, photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) were also decreased under water deficit conditions. The present study showed that NO, AsA, and combination of NO+AsA improved the growth, physiological and biochemical attributes under both control and water deficit conditions. However, both NO and NO+AsA (combined) treatments were more effective in improving growth, physiological and biochemical attributes of rice under water deficit conditions.

# EFFECT OF FOLIAR APPLICATION OF SALICYLIC ACID TO MITIGATE THE SODIUM CHLORIDE (NACL) STRESS IN BARLEY (*HORDEUM VULGARE* L.)

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Barley (*Hordeum vulgare* L.) contains various nutrients, used as human food, animal fodder and in malting and brewing industry which belongs to of family Gramineae. Salt stress is among most challenging threat to agricultural system that effect growth, development and productivity of cereal crops. Low precipitation, dry climatic conditions, excessive use of fertilizers (especially nitrate) and erosion of soil are major issues that cause soil salinization. Salicylic acid (SA) is important in development and defense of plant against stress. In the light of above mentioned facts the goal of this study was to study impact of SA in mitigating sodium chloride stress in barley. After four weeks of germination barley was treated with sodium chloride (0 and 140mM) and salicylic acid (0 and 0.25mM). It was observed that saline stress leads to inhibition in growth, height and weight of plants. SA increased these variables when applied as foliar spray. Plant nutrients also decrease with sodium chloride stress however, increase with salicylic acid treatment. Antioxidative enzymes activities increase under stress to mitigate its negative impact. Overall, salicylic acid mitigate the negative impacts of salinity in barley.

# EFFECT OF SALT STRESS ON MORPHO-ANATOMICAL VARIATIONS IN *BRASSICA JUNCEA* AND *BRASSICA NAPUS*

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Salinity is the major factor responsible for poor germination and plant growth, eventually resulting in limiting crop yield. This experiment was investigated to study the effect of salinity on the morphology and anatomy of *Brassica juncea* and *Brassica napa*. The experiment was carried out in the research area of Postgraduate Agriculture Research Station (PARS), UAF. Two varieties of mustard i.e. super Raya and super Canola was sown in eighteen pots filled with soil, then we will use three level of salts i.e. 0 mM NaCl as control, 50 mM and 100 mM NaCl, after 35 days of germination. The pots was placed under completely randomized design (CRD) at 5% level of probability with three replicates. Experiments showed significantly result that crop growth decreased by salt stress effect. Plants root length, shoot length and fresh, dry weight showed as treatment level increased show negative effect on these parameters. Both varieties` show significant higher growth at control and growth decreased with high salt level respectively. Yield parameter such as biological yield, pod length, No of seed and grain wt reduced significantly at higher concentration of salt stress. This study conclude that sodium chloride reduced all the morphological, physiological and yield attribute significantly at higher treatment level. Anatomical studies shows that salt stress has a negative effect on the number of stomata and area of stomata as the levels of salt stress was increased as the result the reduction was occurred in both no. of stomata and area of stomata. The highest reduction was seen under the 100mM concentration of NaCl. ANOVAs factorial CRD was applied to all the parameters and statistics showed mostly significant results.

# SECTION 8: PLANT RESPONSE TO HEAVY METALS

# DISTINCT ROLE OF *SERRATIA* SP. CP-13 IN PHYSIO-BIOCHEMICAL AND YIELD ENHANCEMENTS OF CADMIUM STRESSED MAIZE (*ZEA MAYS* L.): A SUSTAINABLE PHYTO-IMPROVEMENT PERSPECTIVE

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The current work was aimed to evaluate the effect of Cd tolerant *Serratia* sp. CP-13 inoculation on Cd tolerance in maize cultivars. The two cultivars potentially differing in Cd tolerance and metabolic growth were selected as Cd-tolerant (MMRI-Yellow) and Cd-sensitive (Sahiwal-2002). A solution culture activity was performed in growth chamber with and without *Serratia* sp. CP-13 inoculation of both aforementioned cultivars growing in Cd treatments (0, 6, 12, 18, 24, 30 µM). The growth, physio-biochemical (organic acids, chlorophylls, carotenoids, soluble sugar, proline, flavonoids, anthocyanin, total protein), stress indicators, antioxidants (POD, APX, SOD, CAT) and elemental analysis (Cd, Zn, Fe, Ca, Na, Mg, K) were executed after sampling at seedling stage. Maize exhibited significant decrease in plant growth, leaf area, biomass, nutrients content, antioxidant enzymes and photosynthetic pigments in non-inoculated cv. MMRI-Yellow and cv. Sahiwal-2002, except Cd, Na, RMP, MDA and $H_2O_2$ which showed antagonistic response. However, this decrease was recorded higher in Cd sensitive Sahiwal-2002 as compared to MMRI-Yellow. The seed inoculation of CP-13 also considerably optimised the Cd stressed maize cultivars growth, biomass, nutrients and physio-biochemical attributes, except Cd, Na, RMP, $H_2O_2$ and MDA, which were decreased antagonistically in all activities. Additionally, the release of organic acids by plant roots in solution culture were also enhanced in CP-13 inoculated maize. Outcomes revealed that inoculation of *Serratia* sp. CP-13 enhanced the metabolism of both MMRI-Yellow and Sahiwal-2002 maize cultivars however; considerable higher Cd tolerance was attained by Cd-sensitive Sahiwal-2002 cultivars than MMRI-Yellow at 30 µM Cd treatments. The CP-13 based enhanced Cd tolerance might be due to the higher release of organic acids, enhanced PGP traits and reduced accretion of Cd. *Serratia* sp. CP-13 was recommended as potential bio-inoculant for bioremediation and Cd based oxidative stress mitigation synergistically in Cd stressed environments with crop plants.

# EFFECT OF CADMIUM TOXICITY ON THE GROWTH OF TWO MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] VARIETIES NM-98 AND NM-28

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Soil cadmium affects plant growth adversely and is recognized as a contaminant in the environment. High cadmium contents in soils lead to reduction in plant growth and dry matter yields. Present study was therefore, carried out to investigate the toxic effects of exogenously applied different levels of cadmium on the growth and dry matter yield of the plants of two mungbean [*Vigna radiate* (L.) wilczek] varieties ,NM -98 (Cd tolerant) NM-28(Cd sensitive). For this purpose a pot experiment was conducted and different levels of cadmium i-e 0,3,6,9 and 12ppm were exogenously applied. Different growth parameters including fresh and dry weight of roots and shoots were observed. Exogenous application of cadmium reduced all the growth parameters. The increasing concentration of cadmium level showed more deleterious effects. The mungbean variety NM-98 showed better growth in all parameters as compared to NM-28.

# APPLICATION OF *ACTINOMYCETES* ISOLATES FOR THE BIOTRANSFORMATON OF HEAVY METALS ACCUMULATED THROUG PESTICIDES IN PEACH ORCHARDS IN SWAT

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Due to extensive usage of pesticides in agriculture sector, heavy metal accumulation in soil has become one of the most serious environmental problem all over the world including Pakistan. Bioremediation has the advantages of removal of large amounts of heavy metal efficiently at a low cost. Among the microorganisms, the bioremediation ability of A*ctinomycetes* are well-documented. The present work identified a total of 22 species of *Actinomycetes* from the peach orchards having the ability of biotransformation of heavy metals (Cupper, Cu and Zinc, Zn). The physiochemical parameters of soil samples were temperature for all samples 26°C, moisture content range 25 to 8.7 and PH 7.5 to 8.8. The isolates A17 and A17.1 showed high resistance level on plates supplemented with salts of Cu and Zn. The $OD_{600}$ of selected *Actinomycetes* inoculated in media having different concentrations of CuCl2, ZnSo4 and CuCl2 + ZnSo4, decreased as the concentration of heavy metals increased. The pre-elemental analysis of soil samples showed a range of 7.3 - 19.8 mg/kg for Copper (Cu) and that of Zinc (Zn) is 18- 86 mg/kg. The bio-sorption experiment showed that isolate A17 removal is maximum than A17.1 for both Cu and Zn. Furthermore, the results also determined that in binary metal system the bio-sorption activity of both isolates increased. The post-elemental analysis of soil samples revealed that the range of Cu was decrease to 7.11 - 19.38 mg/kg and that of Zn was 17.58 - 84.19 mg/kg in different soil samples in pots experiment. Additionally, the results also determined that in binary metal system in pots experiment the activity of both isolates decreased. It is concluded from the current study that A*ctinomycetes* have transformation ability for heavy metals (Cu and Zn) both in laboratory as well as pots experiment, and it can be used as a safe, economic and eco-friendly and alternate removal strategy for heavy metals contaminated horticultural soil and agricultural fields.

# EFFECT OF EXOGENOUS APPLICATION OF MOLYBDENUM UNDER NICKEL SULFATE ($NISO_4$) STRESS ON LINSEED (*LINUM USITATISSIMUM* L.)

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Linseed (*Linum usitatissimum* l.) belongs to the family Linaceae having forty genera more than two hundred species and cultivated in subtropical and temperate zone due to commercially used for many purposes like oil, fiber and medicine. Heavy metal pollution over large region of land is caused by industrial waste activity, which has significant impact on natural vegetation. Nickel is a heavy metal that is necessary for plant growth, but is dangerous at high quantities. Various essential and micronutrients improve the yield of crops. The plant requires micronutrients in minor concentration to improve physiological character. Molybdenum is a micro-nutrient that works as a co-factor for very important enzymes of the plants such as glutamine synthase and nitrate. To study the impact of nickel sulfate and molybdenum on linseed plant, keeping this view, at the University of Agricultural Faisalabad Old Botanical Garden, a pot experiment was undertaken. A Completely Randomized design was used for the present study. Almost 10 seeds of each variety Genpol 1 and Gen pol 2 were grown in pots filled with sand. Nickel sulfate (Niso4) 500µm was applied as stress and foliar application of molybdenum (Mo) 2ppm applied to the seedling at different stages. Sample was collected, different attributes i.e. including physiological, morphological, biochemical and enzymatic content. In this study, it was discovered that nickel sulphate inhibited plant growth and reduced the concentration of carotenoids, chlorophyll (a, b) and cations ($Na^+$, $Ca^+$ and $K^+$). The data was analyzed by (ANOVA) applying by appropriate statistical software.

# EFFECT OF EXOGENOUSLY APPLIED BORON ON LINSEED (*LINUM USITATISSIMUM* L.) UNDER CHROMIUM CHLORIDE STRESS

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Linseed (*Linum usitatissimum* L.) is an important agriculture crop of Pakistan which belongs to the family Linaceae. Linseed is an important oilseed crop and cultivated on 2.8 million ha all over the world. It is also known as a flaxseed. The yield of linseed has been decreased during the last few years due to the various heavy metal stress. The harmful effect of metal on linseed plants is indicated specially reduction in weight and root and shoot length. Boron (B) is an essential micronutrient, it's a signaling molecule that increases the growth of plant by decreasing the effect of various heavy metals stress. To check the effects of Chromium chloride linseed plants, a pot experiment was conducted in the old Botanical Garden at the University of Agriculture Faisalabad, according to Complete Randomized Design (CRD). The seeds of two verities i.e., Chandni and Roshni were grown in pots having sand as a growing media. At 3rd leaf stage 500µm of $CdCl_2$ solution will be applied with Hoagland nutrient solution. To overcome the effect of $CrCl_2$ a foliar application of 2ppm Boron will be applied. Data was statistically analysed by using CO-STAT software. Morphological, physiological and biochemical parameters of plants was recorded. The result showed that metal decreased morphological attributes and physiological parameters while Boron application increased these variables. Plant nutrients, antioxidant enzymes and reactive oxygen species showed increased under metallic stress. However, Boron decreased concentration of these under stress when applied as foliar spray.

# IMPACT OF CHROMIUM ON *TRICHODERMA HARZIANUM* RIFAI

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Industrial and sewage water ejection in river and streams on daily basis and wide use of heavy metal based fungicides, bactericides and pesticides is contaminating our surroundings with various heavy metals are frequently surpasses their threshold in the environment and affecting the balance of micro-nutrients and micro-organisms in soil. *In-vitro* study was carried out in laboratory in which impact of Cr (VI) on *Trichoderma harzianum* was studied. For that two experiments i.e., solid medium assay and liquid broth assay were conducted. In solid medium assay, the radial growth, morphological alterations in *T. harzianum* and inhibition zone under the stress of Cr (VI) at different doses i.e., 0, 1000, 2000, 3000, 4000 and 5000 ppm was studied. The results showed that the radial growth was insignificantly reduced at highest dose (5000 ppm) i.e., 3.3% as compared to control. Moreover, no inhibition zone formed at different levels of Cr (VI) and the mycelial pattern of the tested fungi was crossing the disc zones. However, at 5000 ppm of Cr(VI), about 0.2 mm of concentric zone was observed. Furthermore, morphological alterations like increased in condial (globose to sub-globose) number with reduction in size (5.4 to 2.6 µm) and phialides were flask shaped with 4.6 µm in size at 1000 ppm. Whereas, not a single phialides were observed at higher doses of Cr(VI). Furthermore, in liquid broth experiment, mycelial growth (fresh and dry weight) and biochemical attributes [Total protein content (TPC), Peroxidase (POX) and Catalase (CAT) activities] were studied after 7 days of incubation. The results revealed that the fresh and dry weight of *T. harzianum* rather decreasing in the stress of Cr (VI) it was increased up to -200% as compared to control. Furthermore, the level of total protein content, catalase and peroxidase activity was increased by -10 % to -94% with the increasing concentration of Cr(VI) i.e., 1000 to 5000 ppm. Thus the findings exibited that *T. harzianum* could be used as bioremidier against Cr (VI). So far, the experiment was conducted in laboratory, further study should be carried into field to examine the efficacy of *T. harzianum* in the presence of plant that is cultivated in polluted environment contaminated with heavy metal.

# SECTION 9: MISCELLANEOUS

# SEED REGULATORY / BIO-SECURITY SYSTEM & SEED TRADE IN PAKISTAN

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The ultimate objective of all the International organizations relating to plant and human health, is to substantiate in implementation of bio-security system that ensure availability of sufficient quantity of quality food to the peoples of all the member countries. In this regards, the available facility and infrastructure in Pakistan has been reviewed in context of their regulatory functions. Since, seed is a basic input in production of healthy crop in food chain system, therefore, it has been highlighted that potential pathogens which are the major cause of low yields and toxin production in food products, must be tested as pre emptive measures and at the port of entries in the country before sowing. Importance of seed and seed borne diseases, world seed trade, market potential in Pakistan and quantum of seed import, has given to realize the future requirements of bio-security system in the country.

# DESIGNING OF A SENSOR AND MOBILE APPLICATION FOR IDENTIFICATION OF TOMATO PLANT PHYSIOLOGICAL STATE

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**Context/problematic:** Generally, the appearance of visible symptoms such as leaf depigmentation, poor fruit quality means that the disease has already created a lot of damages in the plant. The problem is therefore, how to automatically identify a diseased plant before the visible manifestation of its first symptoms. Our goals are to design firstly a fluorescence sensor able to measure physiological state bio-indicator parameters of tomato plant. Secondly, a mobile application under android studio which will receive bio-indicator parameters emitted by the sensor, perform necessary calculations to deduce the physiological state of the plant, and to show a message concerning plant state to the user through an interface. The sensor designing was done using Isis Proteus software and the mobile application using four UML (Unified Modelling Language) diagrams. The code was developed under android studio. At the end of the designing work, there emerged a sensor consisting of two stages, a transmission stage and a reception stage. Each stage consists of an assembly of electronic components allowing them to perform their function properly. The mobile application, to function properly, required the use of eight UML classes. The code of each of those classes was written and the mobile application interface is presented. This work is of great importance because; its implementation will allow to act quickly in case of plant infection, which will obviously have the effect of increasing agricultural production and food quality.

# AN ENDEAVOR TO INTRODUCE A NON-TRADITIONAL TECHNIQUE (USE OF RADIONICS COMPUTER) TO CONTROL PARASITIC SEED PLANT (CUSCUTA SPP.) ON ORNAMENTAL PLANTS, 2020

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Radionics computer was used in the selection or identification of parasitic and host plants in Kuwait. This parasite (*Cuscuta* spp.) became a big problem, when it attacked different nursery plants. Vitex and oleander were selected and identified. Sea water and urea were selected from a list to be used as herbicides by the circular movement of the pendulum on pendulum plate and sensor needle on the components of the lists. Their potencies were searched out by putting their codes (radionics rates) provided by the company on computer manual. Then host plus parasite samples were placed one by one in the treatment cup. Switches were put on as directed. Broad casting technique of treatment was applied for 10 - 20 minutes according to the requirement. Plants were checked for the herbicides reaction. Cuscuta was killed by both materials used. Vitex plants treated with sea water survived but, oleander plant treated with urea died and could not survive.

# SOIL STERILIZATION WITH NEEM BROARDCAST THROUGH RADIONICS COMPUTER: EFFECTIVENESS OF NEEM ON SOIL BORNE PESTS / ORGANISMS / CONTAMINANTS (FUNGI, BACTERIA, NEMATODES AND WEEDS) USING A NON-POISONOUS, NON-POLLUTANT AND ENVIRONMENT FRIENDLY TECHNIQUE IN THE STATE OF KUWAIT

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Soils of the contaminated areas with fungi, bacteria, nematodes and weeds are being treated with chemical pesticides to grow a healthy crop without any disorder and economic losses. But , the pesticides pollute the area and environment and also the plant products. Another technique is soil solarization which can only be adopted during summer months (July – August). Another technique was adopted here in Kuwait for the first time on trial basis. It was application of neem with code numbers and potency diagnosed using Radioninic s Computer. Then computer was used to broadcast the treatment for 10 minutes to the target area by putting the soil sample in treatment cup treatment was applied on 27thaugust, 2021. Soil samples were collected before and after treatment and populations of soil borne organisms / contaminants and their identification was recorded with the same radionics computer and the maximum circular movement of the pendulum on the pendulum plate. Root knot nematodes (Meloidogyne spp.) and other contaminants were 100 % killed within 15 days. Samples were checked 2-3 times with the same (100 %) control results.

# SANITARY AND PHYTOSANITARY MEASURES (SPS) AND PEST RISK ANALYSIS FOR MANGOES THROUGH HOT WATER TREATMENT FOR EXPORT CONSIGNMENTS IN PAKISTAN

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Pakistan is one of the largest producer and exporter of mangoes. Mango consignments exports are expected to reach 150,000 tons by the year 2020-2021. Pakistani mangoes are famous all over the world for its delicious taste and aroma. People from all over the world wait for Pakistani mangoes all the year around. In Pakistan, the mangoes are mostly produced in Sindh and Punjab provinces of Pakistan. The different varieties of Pakistani mangoes are Sindhri, which have special export quality mangoes of Sindh province and have amazing taste, aroma and color others are Chounsa, Anwar retool, Langhgra, Sunehri etc. Chounsa variety produced in Sindh and Punjab is different from each other. Sindhi Chounsa is yellow in color and have no fibers on seed while Punjabi Chounsa has white color and fibers on its seed. Anwar retool is also famous export quality mango which is called king of the mangoes in Pakistan. In Pakistan, mango harvesting starts from the month of May and lasts up to September. Five hundred mango consignments for export were inspected during the year 2020-2021**.** Most of the consignments for exports were rejected due to different reasons/objections and the key factor for export consignments rejection was due to the pests, diseases and rodents, like fruit fly, mealy bugs and plant pathogenic organisms like Anthracnose and mango malformation. Sixteen export consignments were rejected due to mealy bug detection on the port, 22 export consignments were rejected due to fruit fly and 10 mango export consignments were rejected due to fungal diseases in mangoes during the year 2020-2021. Total of 9.6% consignments for exports were rejected due to pest and diseases. To increase our export targets, we must adhere to SPS regulations by WTO/FAO, International Plant Protection Conservation (IPPC) and NPPOs, and strict compliance must be observed. We need to improve our Pest Risk Analysis (PRA) standards by adapting latest technologies to meet world standards i.e. Irradiation, HWT, Fumigation, Vapor heat treatment (VHT) etc.

# EFFECT OF UV-C AND MICROWAVE RADIATION ON SEED GERMINATION AND PLANT GROWTH IN CORN AND PEA

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With increase in use of electromagnetic devices, it is need of time to explore their adverse effects on environment and living organisms. Two devices i.e. microwave oven and ultraviolet lights which have been added to present lifestyle ranges in cooking and sterilization processes are most frequently used which are under study. The effects of electromagnetic radiations received artificially from sources such as Microwave and Ultraviolet have influenced seed germination *in vitro.* The plant growth was also noticed in vivo. The test seeds examined under ultraviolet and microwave radiation intensities included Corn (*Zea mays* L.) and Pea (*Pisum sativum* L.). The experiment was divided in two sets. The first set was devised through examining corn and pea seeds exposed to Microwave radiation range of 2450 MHz for 0 (control), 2, 3, 7, and 9 seconds while in other set of experiment, seeds were exposed to ultraviolet radiation 253.7nm for 0 (control), 10, 30, 60 and 90 minutes. After exposure of seeds to various ranges of variable radiations, the treated seeds were sown in different pots containing 500 g soil fertilizer mixture (4:1). After 48 days of seeds sowing plants were harvested and their shoot length, were recorded. The differences in growth pattern were recorded and compared using statistic 8.1 for level of significance.

# ALLELOPATHIC EFFECT OF *(CALOTROPIS PROCERA)* ON GERMINATION, GROWTH, CHLOROPHYLL CONTENT AND MINERAL UPTAKE OF FIVE AGRONOMIC CROPS

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The present research was conducted to find out the allelopathic capability of *Calotropis procera* (C.P) on germination indices, growth and mineral uptake of five cultivated crops, namely Tomato *(Solanum lycopersicum*), Gram (*Cicer arietinum*), Mung Bean (*Vigna radiata*), Fenugreek (*Trigonella foenum-graecum*) and Lettuce (*Lactuca sativa*). The selected species were grown in petri dishes for germination indices and in pots to examine growth potential and mineral uptake under four different *Calotropis procera* aqueous extracts (CPAE) (0% Control, 10% T1, 20% T2, and 30% T3) for 30 days after germination in pots. The data revealed significant reduction ($p<0.05$) in Germination indices i.e. germination percentage (GP %), germination rate (GR), germination index (GI), coefficient of velocity of germination (CVG) under increasing extract concentration whereas mean germination time (MGT) significantly ($P<0.05$) increased with increasing extract concentration. The data obtained from pot seedling revealed significant reduction in root shoot length, fresh and dry weight of seedling in all studied species. Allelopathic stress also induced the inhibition in mineral uptake and reduction of chlorophyll content in all species with increasing plant extract.

# YIELD PRODUCTION OF *PLEUROTUS OSTREATUS* - P1 USING DIFFERENT SUBSTRATES ON APPLE POMACE AS HORTICULTURAL WASTE AND WITH DATE PALM LEAVES, BANANA LEAVES

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Apple pomace is a byproduct from apple industry and considered also horticultural waste and has the potential to support the growth of microorganisms. In this study, the effect of apple pomace on the growth rate of *Pleurotus ostreatus* was investigated. *Pleurotus Spp.* is an important mushroom group and extensively used in food handling industries and has pharmaceutical uses. Its capability to produce in a varied range of temperatures and to consume existing lingo cellulosic materials is reason to be reflected as an extremely fascinating mushroom for manufacture. Different substrates such as cereal straws, corncobs, coffee grounds and urea are used in greater part to create mushroom. To check the influence of various substrates such as apple pomace, date palm leaves and banana leaves proceeding the cultivation and production of Oyster mushroom, On the basis of growth of mycelia, primordial appearance time, yield of mushroom, colonization time, fresh weight and biological efficiency were analyzed. Different treatments were used and significant results indicated that among all the treatments used best growth of mycelia, earlier formation of pin head, better yield and days to fruiting were observed in T3( 50:50%(Date palm leaves + Banana leaves) with 5% apple pomace,T5 (25:75%(Date palm leaves +Banana leaves) with 5% apple pomace, 50:50%(Date palm leaves + Banana leaves), T2 (50:50%(Date palm leaves +Banana leaves) with 2% apple pomace, T4 25:75%(Date palm leaves +Banana leaves) with 2% apple pomace, T1 (50:50%(Date palm leaves + Banana leaves). By using standard statistical method, results were evaluated. Maximum yield (439.3) and maximum biological efficiency (89.1) was obtained from treatment T3 (50:50% (Date palm leaves + Banana leaves) with 5% apple pomace. Above findings showed that treatment T3 was the best substrate as compared to all others for the production of mushroom respectively.

# USE OF TEA WASTE (LIPTON) WITH COMBINATION OF VARIOUS MIXTURE OF AGRICULTURAL WASTE MATERIALS (WHEAT STRAW, COTTON WASTE, CORN MEAL, THATCH GRASS) FOR CULTIVATION OF *PLEUROTUS PULMONAIOUS*

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The waste of agriculture was used for mushroom cultivation was good for the elimination of ecological pollution and had better impact on enviourmental and friendly attitude. Mushroom comprised a filthy extent of carbohydrate, protein, lipids and fibers. Tea waste (Lipton) latterly along by agriculture unused substantial (straw of wheat, corn meal, cotton waste and thatch grass) were applied to make compost by means of substrate for the rearing of mushroom (*Pleurotus Pulmonarius*). Gypsum and wheat bran were applied to be pre-owned as auxiliary stuff estimated 5 % of the substrate dehydrated mass in entire conducts. Complete randomized design (CRD) was applied to the experiment having arranged five different ratios (100% tea waste, 75% tea waste+ 25% agri. waste, 50% tea waste+ 50% agri. waste, 25% tea waste+ 75% agri. waste, 0% tea waste+ 100% agri. waste). Substrates arranged by standard strategies and put into the autoclave plastic packs, can sack immunized by 10% bring forth. Bring forth run pinhead development first gathering time, absolute yield, organic effectiveness (%) and nature of mushroom was watched. The time taken for 100% produce run were run to fluctuate between 19-36 days and time for pinhead arrangement was in the scope of (54.5 to 67.4) days. in spite of the fact that were no factually huge yield contrast related with the forced medicines, the mean most noteworthy yield was found in , 50% tea waste+ 50% agri. waste (410.14 g), Trailed by medications 25% tea waste+ 75% agri. (379.98 g), 0% tea waste+ 100% agri. waste (338.88 g), 75% tea waste+ 25% agri. waste (291.22 g), 100% tea waste (249.46 g), The efficiencies of different blend were somewhere in the range of 83.8% and 52.08%.As a conclusion that the leaves of Tea waste used as substrate are appropriate and new organic material for production of Oyster mushroom (*Pleurotus pulmonarius*) when mixed with waste of agriculture material like wheat straw, cotton waste, corn meal and thatch grass. This experiment has given us very promising outcomes that the Tea waste plentifully present, can be monetarily when utilized in blends with Agri. waste or other cellulosic material for mushroom development, in this way its utilization is prescribed to farmers in this nation where huge volumes of tea waste are disposed of as agricultural waste material.

# EFFECT OF PLANTING DENSITY ON GROWTH PERFORMANCE, YIELD AND YIELD COMPONENT OF MAIZE

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Recommended planting density is considered as a bench mark for maximum crop yield, especially for maize. Plant density (PD) results in pronounced stalk lodging and kernel abortion in this species. An experiment comprises three hybrids viz., Zhengdan 958, Longping 206 and Jinqiu 119 was conducted during 2016 and 2017 to determine the effects of high PD on grain yield, cob characteristics, dry matter accumulation (DMA), leaf area, photo synthetically active radiation (PAR), radiation use efficiency (RUE) and stalk lodging. Five PDs (4.5, 6, 7.5, 9 and 15 plants $m^{-2}$) were used. Increased PD was associated with an increase in PAR, RUE, DMA and grain yield, but also increased lodging percentage. Grain yield augmented at PD6 (9.44%) and PD7.5 (2.98%), but decreased to 12.5% at PD15 because of enhanced lodging that was 60%. The increased plant density reduced cob length (-8.8%), individual kernel weight (-3.66%), kernels per ear (-5.81%) and lodging risk (+60%). These findings suggested that two hybrids i.e., Longping 206 and Jinqiu119 performed well at PD6 and PD7.5. However, cultivar Zhendan 958 can be cultivated at increased plant density up to PD9 and PD15.

# CHALLENGES AND OPPORTUNITIES OF COTTON IN PAKISTAN

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Cotton is an important cash crop of Pakistan and it plays a vital role in the economic viability of the country. Cotton and cotton products contribute 0.8% in GDP and 60 % in the foreign exchange earnings of this country. More than 40% industrial level is directly or indirectly engaged with cotton industry. There are two main provinces, Punjab and Sindh, for cotton production in Pakistan. Punjab province produces 70% of total production. Pakistan has potential of producing 30 million bales from an area of 10 million acres. But after achieving a milestone of 14.8 million bales in 2011-12, cotton production in Pakistan had been hovering around 10 million bales which is far below than that the potential. Moreover, cotton production is on declining trend since 2015 and we have witnessed the lowest figure, i.e. 5.6 million bales, during the last 35 years. The main challenges to cotton productivity in Pakistan include climate change (high temperature and erratic rainfall), old Bt Bollgard1 technology, seed quality, pest problem particularly whitefly and pink bollworm, cotton leaf curl virus disease, insecticide resistance, high infestation of weeds, shortage of labor for picking, low adaption of mechanization and low profitability of cotton. Cotton researchers in Pakistan have been striving to resolve these issues since 1992. The Pakistani Scientist has succeeded in development of highly heat tolerant varieties (MNH-1020 and FH-Super cotton) which have been commercialized for general cultivation in Punjab province. The work on varieties possessing genes for bollworms and glyphosate resistance is also under progress. There is a great deal of opportunities for improvement of cotton sector in Pakistan. Our Government is keen to uplift this industry through employing latest cotton production technologies, improving fiber quality and upgrading ginning industry. We are ready to have collaboration with other countries for promotion of cotton in the world.

# STUDY ACCENTUATE THE TRAINING NEEDS OF RICE GROWERS REGARDING VALUE CHAIN IN TEHSIL NOWSHERA VIRKAN, GUJRANWALA

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Rice (*Oryza sativa L.*) is the world's largest food source after wheat. About half of the world's population is grown with rice. More than 114 countries of world are engaged in the cultivation of rice. Along with developed countries, many developing countries are also cultivating rice for employment, food and income generation. The contribution of the Asian countries in rice production in international market is almost 90%. Pakistan has prominent eminence in international rice market due to a big sharing contribution. But since a few years, the quality of Pakistani rice is rapidly deteriorating with a prominent decline in international market. This study was conducted for the training needs of Tehsil Nowshera Virkan to improve rice production, processing, storage, marketing and export value. Tehsil Nowshera Virkan was selected because it has the area of largest rice production district of Punjab. There were 605 registered growers of rice in tehsil Nowshera Virkan. The sample of 120 respondents was calculated through www.surveysystem.com by keeping confidence interval 5 and confidence level 95%. The list was generated from the Agricultural Extension Department of tehsil Nowshera, Virkan. Data was collected from respondents using a well-structured and pre-checked interview schedule. The collected data was analyzed using statistical software, the Social Science Statistics Package (SPSS). Research shows that the problems that are causing production, processing, and exports to decline include an expensive labor for plant protection measures, lack of knowledge about protection measures, a labor shortage, a lack of irrigation water, and skill. We conclude that there is a labor shortage, an energy crisis, a mix of different rice, varieties, difficult to meet international standards, delayed payments to exporters, low prices on international markets, high prices on domestic markets. The recommendations to overcome these problems include strengthening the extension services, promoting DSR, improving R&D services, encouraging farm mechanization of standard by market committee subsiding the energy and implementation of Government policies.

# AWARENESS OF MAIZE GROWERS REGARDING PEST MANAGEMENT IN DISTRICT SAHIWAL

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Maize is the third grain crop in Pakistan. It contributes 2.1 percent to agricultural supplementation and 0.4 percent to GDP. In Pakistan maize has been planted on more than 118 million hectares and annual production amount 600 million tons. Maize production in Pakistan is low as compared with other countries worldwide. Different type of bacteria, virus, nematodes, and fungus are the pest of maize affecting crop yield. Maize is one of the oldest crops grown in the world. The grains of maize contain 10% protein, 9.5% fiber, 72% starch, 3% sugar, 1.7% ash and 4.8% fat, widely used in milk, poultry and other animal feeds. Factor responsible for low maize productivity are the poor pest management, lack of certified seed/species, lack of recommended agronomic technologies, fertilizer and pesticides. In present study farmer's awareness level in maize production regarding pest management and control has been studied. Total 120 respondents were selected by using a sample size calculator (www.surveysystem.com) with a confidence level of 95% and a confidence interval of 7. Questionnaire tool was used for data collection which included both open ended and close ended questions for getting require information. The data were analyzed through statistical package for social sciences SPSS. Results show that awareness level of recommended seed rate, mean and SD (3.46±1.41), ) different bacteria, virus, nematodes, and fungus, mean and SD (2.80±1.40) , IPM technologies, mean and SD (2.98±1.13), pest control, mean and SD (3.45±1.17), harvesting and storage technology, mean and SD (3.43±1.10), advanced agricultural extension techniques, mean and SD (3.38±1.74), Research and pre base production, mean and SD (2.81±1.24), storage, mean and SD (2.66±2.14) packing and marketing, mean and SD (2.59±2.11) certification (2.33±1.33) Balance fertilizer application (2.00±2.33) maize by-products, mean and SD (1.84±3.16). These values show an information gap in pest knowledge and its control. Present study recommends that there is dire need of farmers training regarding pest management and control through latest technology. Various stake holders should take interest to enhance maize production through pest management.

# AUTOMATION IN THE FIELD OF DIAGNOSTIC PLANT PATHOLOGY IS A KEY TO COMBAT THE PLANT PATHOGEN

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Agriculture sector in all over the world face a lot of problems from sowing to harvesting, among them plant pathogen infections is a key factor causing severe yield losses and effecting the quality of the plant products. With rapid increase of human population, the curve for food demand is highly increasing day by day. Accurate and precise diagnostic of plant diseases is a major challenge in the way of plant disease management. The swift development of novel technological approaches leads the researcher, extension experts and farmer to detect and identify the plant diseases timely. Many DSP (Decision support system) and databases have been developed for detection and identification of plant disorder. Different investigation has taken place under the title of machine learning for detection and identification of plant diseases, some machine learning approach are support vector machine (SVM), random forest, Convolutional neural networks (CNN), Artificial Neural Network (ANN), Deep learning (DL), K-means method, and fuzzy logic etc. Here, a comprehensive study on detection of plant diseases by machine learning systems is performed. During this study, different Artificial Intelligence (AI) approaches like DL, CNN and ANN were found more accurate and having ability to detects number of plant diseases in different crops like Cotton, Wheat, Rice, Maize, Chili, Potato, Tomato, Cucumber, Peas, Cassava, Mango, Grapes, Banana, Peach, Berries, Apple, Tea, etc. However, an accurate diagnostic is a key challenge for pathogen control and should drive the development of robotic and mechatronics solutions for effective management of plant diseases.

# ASSESSMENT OF RESIDUAL PESTICIDES IN SOME SEASONAL VEGETABLES AND FRUITS FROM LOCAL MARKETS OF LAHORE, PAKISTAN

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Pesticides are mostly used in crop production such as, in fruits and vegetables with an ignoring attitude about their drastic effects on environment and human health. Pesticides get deposit in the fruits and vegetables in both forms as processed or in raw. The main channel of pesticide transfer to consumer is the consumption these of fruits and vegetables. Different types of insects attack on different types of fruits and vegetables and spoil them. To avoid the vegetables and fruits from those insects, pesticides are sprayed on them. Due to spray of pesticides insects remain far from them but these chemicals penetrate in the foodstuff and become the part of these fruits and vegetables. The aim of this research work was to find out the amount of pesticides retained in the fruits and vegetables. For that purpose, different fruits and vegetables were collected from different markets and analyzed through Gas chromatography tandem with flame iodized detector. Five standards were run on the GC-FID, Chlorpyrifos, Fipronil tech, Lambda tech, Bupril tech and Delta tech. These five pesticides are mostly sprayed in Pakistan since last 20 years. Residues were extracted from samples with acetone, followed by extraction step and using of the GC-FID. Maximum numbers of pesticide residues were analyzed in Ginger which were Chlorpyrifos, Delta tech and Bupril tech. Beet, Lady finger, Brinjal, and Cabbage showed the presence of two pesticides. Chlorpyrifos was detected in almost all vegetables and fruits. In case of fruit chlorpyrifos and delta were analyzed in cherry.

# A RECENT ENDEAVOR TO CONTROL CUSCUTA SPECIE, A PHANEROGAMIC SHOOT PARASITE OF NUMBER OF PLANTS BY THE USE OF A NON-TRADITIONAL TECHNIQUE, RADIONICS COMPUTER TREATMENT

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Radionic computer was used for the identification of parasite and the host plant in Kuwait during 2020. Vitex and oleander were selected as test plants. Sea water and urea were selected from the list of radionic equipment rate sheets to be used as herbicides by putting their codes on the computer rate number matrix as directed by the company. After finding out the doses as in the manual, both the parasite and host plants were placed one by one in the treatment cup. The radionics materials as chemicals were applied through broadcast technique with the help of radionic computer for the duration of 10-20 minutes. Cuscuta was killed by the use of both radionics used. Vitex plants treated with sea water survived whereas the oleander did not survive by the use of urea.

# EFFECTIVENESS OF NEEM ON SOIL BORNE PESTS/ORGANISMS/CONTAMINANTS (FUNGI, BACTERIA, NEMATODES AND WEEDS) USING A NON-POISONOUS, NON-POLLUTANT AND ENVIRONMENT FRIENDLY TECHNIQUE IN THE STATE OF KUWAIT

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Soils of the contaminated areas with fungi, bacteria, nematodes and weeds are being treated with chemical pesticides to grow a healthy crop without any disorder and economic losses. But the pesticides pollute the area and environment and also the plant products. Through radionically with the help of specific computer designed for the purpose. The technique is so simple and efficient that it works thousands of kilometers from the lab. Another technique is soil solarization which can only be adopted during summer months (July-August). Another technique was adopted here in Kuwait for the first time on trial basis. It was application of neem with code numbers and potency diagnosed using Radionics computer. Then computer was used to broadcast the treatment for 10 minutes the target area by putting the soil sample in cup treatment was applied recently. Soil samples were collected before and after treatment and populations as soil borne organisms/contaminants and their identification was recorded with the same radionics computer and the maximum circular movement of the pendulum on the pendulum plate. Root knot nematodes (Meloidogyne spp.) and other contaminants were 100% killed within 15 days. Samples were checked 2-3 times with the same (100%) control results.

# SECTION 10: INSECTS IN RELATION TO PLANTS

# SUSCEPTIBILITY OF THE PEACH FRUIT FLY *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE) TO ENTOMOPATHOGENIC FUNGI

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Guava is one of the most popular fruit in the world by its taste and nutritional value. In Pakistan, it is the third most rapidly growing fruit. The peach fruit fly, *Bactrocera zonata* is one of the major hindrances in the yield and productivity enhancement of fruits. The entomopathogenic fungus is considered an effective managemental practice against fruit flies. In order to estimate the efficiency of two species of fungi, *Beauveria bassiana* and *Metarhizium anisopliae* were tested at concentrations of conidia, $1\times10^8$, $1\times10^7$ and $1\times10^6$ against adults of *B. zonata* in the laboratory conditions. The mortality was recorded after 7 and 15 days of treatment. Both fungal species were found significant at the concentration of $1\times10^8$. For *B. bassiana* mortality observed were 40% and 43.33% at the interval of 7 and 15 days respectively, on the concentration of $1\times10^8$ conidia/ml. While *M. anisopliea* showed 35% and 40% mortality against adults of *B. zonata*. The use of EPF in IPM plans can lead to effective management against fruit fly since there is a need for sustainable and environmentally safe practices in fruit farming.

# EFFECT OF SECONDARY METABOLITES OF ENTOMOPATHOGENIC FUNGI ON COTTON MEALYBUG *PHENACOCCUS SOLENOPSIS*

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Microbial pest control is one of the emerging alternative method to control destructive insect pests of crop plants. Fungi have the capability to produce a remarkable number of secondary metabolites, which possess insecticidal properties against insect spp. Accordingly, this study was conducted by using five different concentrations of secondary metabolites of well-known entomopathogenic fungi (EPF) namely, *Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa, Verticillium lecanii* and *Paecilomyces lilacinus* against *P. solenopsis.* Among all five tested EPF metabolites, the higher concentration of *M. anisopliae* (50%) and *B. bassania* (50%) followed by *P. lilacinus* (50%) were effective in controlling the pest population, they caused 50.5%, 49.23% and 41.33% *P. solenopsis* mortality, respectively. The 2nd most effective treatments were 40% concentration of *M. anisopliae* (36.9%) and *B. bassania* (35.6%) followed by *P. lilacinus* (31.5%). Among all EPF metabolites *V. licanii* and *I. farinose* were found not effective against *P. solenopsis*. The results of present study revealed that of *M. anisopliae, B. bassania* and *P. lilacinus* have the potential to integrate in pest management strategies.

# IDENTIFICATION OF BEHAVIORALLY REPELLENT AND ANTENNAL ACTIVE COMPOUNDS OF DIFFERENT PLANT ESSENTIAL OILS AGAINST A DENGUE VECTOR, *AEDES AEGYPTI*

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*Aedes aegypti*-borne diseases contribute significantly to death, poverty, social debility, and disease burden all over the world. Among these illnesses, yellow fever is the most harmful disease spread by *Ae. aegypti*. Mosquito repellents are known to play an important role in preventing vector-borne diseases by reducing man-vector contact. Repellents derived from plants are considered more eco-friendly, biodegradable, and non-toxic to human and domestic animals in relation to synthetic repellent. The repellency of essential oils against *Ae. aegypti* was evaluated using the human bait technique. Furthermore, essential oils were tested in electrophysiological bioassays using electroantennography (EAG) and antennal active compounds were identified using gas-chromatography-coupled mass spectrometry. The essential oils of *Origanum majorana* and *Origanum vulgare* proved the most effective repellent (100%) against *Ae. aegypti* followed by *Ocimum basilicum* (89.25%), *Curcuma longa* (75%), and *Amomum subulatum* (65.45%) respectively. This study suggests that essential oils can be used as an alternative to hazardous synthetic mosquito's repellent.

# CHEMICAL COMPOSITION, REPELLENT AND INSECTICIDAL POTENTIAL OF SIX PLANTS ESSENTIAL OILS AGAINST *AEDES AEGYPTI*

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*Aedes aegypti* L. is the primary vector of dengue virus. Keeping in mind the harmful effects of synthetic chemical-based intervention measures, bio-degradable and eco-friendly essential oils extracted from either the leaves, rhizomes, and/or bark of *Mentha longifolia*, *Salsola imbricata, Erigeron bonariensis*, *Conyza canadensis, Ailanthus altissima,* and *Zanthoxylum armatum* were investigated for their repellent and larvicidal potential against *Ae. aegypti*. Chemical components of all tested essential oils were also identified using gas chromatography-mass spectrometry (GC-MS). The essential oils of *M. longifolia*, *S. imbricata*, *E. bonariensis*, *C. canadensis*, *A. altissima,* and *Z. armatum* exhibited 97.6%, 92.8%, 40.2%, 41.7%, 29.1%, and 13.2% repellency against *Ae. aegypti* at a tested dose of 33.3 µg/cm$^2$. Furthermore, in larvicidal bioassay, the $LC_{50}$ results depicted that the larvae of *Ae. aegypti* were more susceptible to *E. bonariensis* ($LC_{50}$, 26.0 mg/L) followed by *C. canadensis* ($LC_{50}$, 35.7 mg/L), *Z. armatum* ($LC_{50}$, 35.9 mg/L), *M. longifolia* ($LC_{50}$, 39.3 mg/L), *S. imbricata* ($LC_{50}$, 124.2), and *A. altissima* ($LC_{50}$, 333.6 mg/L), respectively. The most abundant constituents in the essential oils of *E. bonariensis*, *C. canadensis*, *Z. armatum*, *M. longifolia*, *S. imbricata*, and *A. altissima* were matricaria ester 43.1%, matricaria ester 31.7%, 2-undecanone 75.2%, piperitone oxide 45.5%, carvone 39.9%, and eugenol (24.4%), respectively. Our study suggests that essential oils can be used in controlling insect pests, especially insect pests of medical importance.

# ASSESSMENT OF DIVERSITY AND RELATIVE ABUNDANCE OF INSECT FAUNA RELATED TO WHEAT (TRITICUM AESTIVUM) FROM MULTAN

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Wheat, *Triticum aestivum* (Linnaeus) is the major cereal crop of Pakistan and plays an important role in economy. Pakistan is the 8th largest wheat producing country. Biodiversity is variation of life; insects are the largest and most diverse group of organisms in the world. During present study, the research was conducted in the field of department of Agriculture Science and Technology BZU Multan and five different varieties of wheat were selected for the assessment of diversity and incidence of pest and predator population and from each variety 21 plants were selected. Data was recorded from January to April Collection of insects was carried out by using Arial net and aspirator on daily basis. A total of 30192 specimens of insect belonging to 4 different orders include Hemiptera, Coleoptera, Diptera, and Lepidoptera were recorded from five varieties of wheat. Maximum population of insect (7520) observed in dilcash-2019 variety while minimum in Fakhre Bakhar (4707) variety. During January the population of pest and predator were minimum due to foggy weather but in February to mid of March the population of aphids and their predators increase and highest population was recorded from start of February to mid of March but population of aphid decreases after mid due to increase in temperature.

# CHARACTERIZATION OF CHEMICAL COMPOSITION OF PLANT ESSENTIAL OILS AND EVALUATION OF THEIR POTENTIAL AS OVIPOSITION DETERRENTS, PUPICIDAL, AND ADULT REPELLANTS AGAINST *BACTROCERA ZONATA*

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*Bactrocera zonata* (Tephritidae: Diptera) is a polyphagous and devastating insect pest of fruits and vegetables. Due to the prevalence of resistance against synthetic insecticide, there has been a shift in interest to develop plant-derived eco-friendly chemicals compatible with integrated pest management (IPM). This study has evaluated the efficacy essential oils of *Syzgium aromaticum, Conyza sumatrensis, Azadirachata indica Partheenim hysterophorus, Ocimum basilicum, Amomum subulatum, Origanum vulgare,* and *Eucalyptus globulus* on oviposition deterrence, pupae, and adults of *B. zonata*. The chemical composition of the essential oils was evaluated through Gas-Chromatography-Mass Spectrometry (GC-MS). We treated the fruits with 0.1% and 1% concentration of essential oils and five replications for each treatment were done. In multi-choice bioassay, eight bananas, each treated with different essential oil, and were given to 24 hrs starved females (20) in one replication of *B. zonata*. Their oviposition deterrence and adult repellency was observed for five hrs from onset of experiments. Essential oil of *S. aromaticum* proved very effective against *B. zonata* which was followed by *C. sumatrensis, A. Indica, P. hysterophorus, O. basilicum, A. subulatum, O. vulgare, and E. globulus* as oviposition deterrent, pupicidal, and adult repellence. The result of the study shows that plant based essential oils can be used in controlling *B. zonata* without harming the humans and the environment.

# MOSQUITO REPELLENT POTENTIAL OF *CARPESIUM ABROTANOIDES* ESSENTIAL OIL AND ITS MAIN COMPOSITIONS AGAINST A DENGUE VECTOR, *AEDES AEGYPTI*

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Disease vectoring mosquitoes are a serious threat to humans. However, till today only few mosquito repellents have been identified. The current study was conducted to evaluate the repellent potential of *Carpesium abrotanoides* essential oil against *Aedes aegypti* females by human bait technique. Essential oil was extracted by steam distillation process while the identification of chemical constituents was carried out by gas chromatography-mass spectrometry. Time span repellent bioassays of *C. abrotanoides* essential oil in comparison to DEET were performed at three different doses (33µg/cm², 165µg/cm² and 330µg/cm²) under laboratory conditions. Highest repellency periods for essential oil and DEET were observed at the tested dose of 330µg/cm² with 315 min and 720 min respectively. Lowest repellency period of 45 min for essential oil and 105 min for DEET was recorded at the tested dose of 33µg/cm². Major constituent's caryophyllene (24.3%) and *trans*-nerolidol (12.0%) of *C. abrotanoides* essential oil were also evaluated as repellents at three different doses (330µg/cm²,165µg/cm² and 33µg/cm²) against *Ae. aegypti*. Surprisingly, *trans*-nerolidol completely inhibited *Ae. aegypti* landings for 45 min when tested at 330µg/cm². However, caryophyllene did not completely inhibit *Ae. aegypti* landing even after immediate application at the tested dose of 330µg/cm². At the tested dose of 330µg/cm², mixture (*trans*-nerolidol+caryophyllene) completely inhibited *Ae. aegypti* landing for 60 min indicating the synergistic effect of caryophyllene. Hence, *C. abrotanoides* as well as its major constituent, especially *trans*-nerolidol have a potential to formulate mosquito repellent comparable of DEET.

# EVALUATION OF ESSENTIAL OILS OF DIFFERENT PLANT SPECIES AS REPELLENTS AGAINST *AEDES AEGYPTI*

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*Aedes aegypti*, which is the main vector of dengue virus, is one of the deadliest mosquito species. Repelling or keeping the mosquitoes away from biting humans is one the protection strategies being used to protect human population. Plant essential oils are a natural source of mosquito repellents. In the current study, essential oils, extracted through steam distillation, of aerial parts of *Perovskia atriplicifolia*, *Citrus reticulata* (leaves and fruit peel), *Chenopodium ambrosioides, Eucalyptus camaldulensis* (leaves and buds), *Cymbopogon citrateus, Mentha longifolia, Salvia moocroftiana* and the bark of *Azadirachta indica* were tested for their repellence potential. Human bait technique was used to evaluate the essential oils. Chemical analyses of essential oils were done using gas chromatograph-mass spectrometer (GC-MS). The all 11 essential oils were first tested at the dose of 33µg/cm$^2$, while the five most active essential oils were further investigated by testing them in time span bioassays. Essential oil of *P. atriplicifolia* with its major constituents (camphor 19.7%, eucalyptol 12.1% and limonene 10.9%) exhibited the longest repellence up to 75 min, 90 min and 135 min, which was followed by *C. reticulata* (fruit peel) with 60 min, 90 min and 120 min, *M. longifolia* with 45 min, 60 min and 90 min, *C. reticulata* for 30 min, 45 min and 75 min, while the shortest repellence time of just up to 15 min, 30 min and 60 min time interval was exhibited by *C. ambrosioides* when tested at the doses of 33µg/cm$^2$, 165µg/cm$^2$ and 330µg/cm$^2$, respectively. Importantly, at the highest dose of 330µg/cm$^2$, *P. atriplicifolia* and *C. reticulata* (fruit peel) exhibited complete protection for 60 min and 30 min time intervals while *M. longifolia* and *C. reticulata* showed complete protection only immediate after application significantly. However, *C. ambrosioides* did not exhibit complete protection even at immediate after application. So, the present study suggests that *P. atriplicifolia* essential oil itself and its major compounds may be used for commercial production of the mosquito repellents.

# CHEMICAL COMPOSITION AND BIOACTIVITY OF SELECTED ESSENTIAL OILS AGAINST AN INSECTICIDE RESISTANT POPULATION OF *AEDES AEGYPTI*

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*Aedes aegypti* (Diptera: Culicidae) is disease-causing insect pest. Synthetic repellents of mosquitoes have harmful effects on humans and the environment. Plants based essential oils of *Syzygium aromaticum*, *Tagetes minuta*, *Lipidium pinnatifidum, Origanum vulgare*, *Mentha spicata*, *Agrimonia eupatoria* were evaluated for their repellent potential against susceptible and resistance (temephos) *Ae. aegypti* by using the human bait technique. Chemical components of essential oils were analysed by gas chromatography-mass spectrometry (GC-MS). The essential oils of *L. pinnatifidum, O. vulgare*, *A. eupatoria*, *S. aromaticum*, *T. minuta*, *M. spicata* at the dose of 33µg/cm$^2$ showed 100%, 94%, 87%, 79%, 73%, 62%, and 53% repellence respectively, against susceptible *Ae. aegypti* and 93%, 88%, 86%, 72%, 62% and 44% repellence respectively against temephos resistance *Ae. aegypti*. Furthermore, at tested higher doses (165µg/cm$^2$, 330µg/cm$^2$) all the tested essential oils showed more repellence against susceptible *Ae. aegypti* than the resistant *Ae. aegypti*. Benzyl cyanide (94%), carvacrol (20%), α-pinene (62%), Piperitenone oxide (49%), dihydrotagetone (21%), α-turmerone (40%), and eugenol (92%) were the major compounds in the essential oils of *L. pinnatifidum*, *O. vulgare*, *A. eupatoria*¸ *M. spicata*, *T. minuta*, and *S. aromaticum*, respectively. The result of study demonstrates that temephos resistance population of *Ae. aegypti* have cross resistance against essential oils. However, the essential oils can be used in controlling the insect pest population.

# APIVECTORING FOR ENHANCED FRUIT QUALITY AND YIELD: A REVIEW

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Worldwide, 35% of the crop production depends upon animal pollination and bees have been documented as the most efficient pollinators providing this ecosystem service. Insect pollination has been found to increase 40-50% market value of fruits as compared to self and wind pollination treatments. Highly pollinated flowers yield low deformed fruit, higher weight, longer shelf life and enhance postharvest quality of fruit because of intense coloration and lower level of sugar- acid ratio. However, in agricultural fields, highly disturbance of appropriate landscape, reduction in nesting and habitats sites along with abundance of pollinators leading to inadequate pollination that result in low food quality. Therefore, supplemental pollination by honey bees is necessary for obtaining good commercial production in pollinator dependent crops. Bees have been reported to carry a variety of microscopic particles i.e. pollen, virus, bacterial and fungal spores. Therefore, foraging activity of bees is being used for transferring bio-control agent to antagonize various pests and pathogens like *Botrytis cinerea*. Moreover, supplementing honey bees pollination also help in improving yield and fruit quality in this crop. The spores of bio-control agents (antagonists) are added to the dispensers that are fitted in front of honey bee hives. Bees pick up these spores between their hairs while leaving the nesting and shift these to the flowers during their visitation and help in managing plant diseases. This "Apivectoring/entomovectoring (bees for transfe technology" is based on the principle of sustainable agriculture as it offers both ecological and economic profits. Previously, entomovector technology is found effective for managing fire blight in two fruit crops i.e. pear and apple and grey mould in strawberry and raspberry. Moreover, honey bees have also been found to effectively manage grey mould in strawberry through dissemination of *Trichoderma* inoculum. Therefore, honey bees enhance fruit yield and quality through spreading bio-control agents for the management of diseases and improved pollination services in pollinator dependent fruit crops.

# COMPARATIVE STUDY OF FOUR COMMERCIALLY AVAILABLE SYNTHETIC MOSQUITOES REPELLENTS AND DEET AGAINST *AEDES AEGYPTI*

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Mosquitoes are the most dangerous creature on the earth. They act as a vector of most threatening diseases in human, e.g., dengue fever, Chikungunya fever, yellow fever, filariasis, west Nile virus and encephalitis etc. It is estimated that about 3.9 billion people in more than 128 countries are at risk due to dengue fever, moreover about 390 million people suffered from dengue fever with 2.1 million severe dengue cases and 21000 deaths. Numerous measures have been taken in response of mosquitoes borne diseases to protect humans such as biological, chemical, personal and environmental protection. The most adopted measure by the humans to protect themselves from mosquito bite is the use of synthetic repellent in the urban areas. In the current study commercially available mosquito repellents (Mospel lotion, Mospel cream, Tiger and Inseguard) were evaluated in comparison with DEET (N, N-diethyl-benzamide) against females of *Ae. aegypti*. The aim of this study is to examine the claimed protection period mentioned on the commercial product of mosquito repellents by keeping DEET as standard. According to the results, DEET provided highest protection for more than 8 hours against females of *Ae. aegypti* as compared to the all four commercial mosquito repellent products containing DEET as an active ingredient at the same tested doses. Hence proved that mentioned protection periods on commercially available products are false.

# EVALUATION OF ANTENNAL ACTIVE AND BEHAVIORALLY REPELLENT COMPOUNDS OF ESSENTIAL OILS AGAINST YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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*Aedes aegypti play a vital role in the transmission of dengue hemorrhagic fever, dengue fever, and many other infectious viral diseases including, chikungunya, Zika, and yellow fever. Synthetic insecticides, which are used for* Ae. aegypti*, have become a public health problems and contamination of environment. Essential oils are the natural alternative of synthetic insecticides, due to no harmful impacts on humans. The objective of current study was to evaluate the repellent activity of essential oils, derived from* Euclyptus pillata*,* Euclyptus globulus*, and* Euclyptus camaldulensis *through steam distillation. The repellent potential of essential oils against* Ae. aegypti *was carried out through human bait technique.* Electroantennography (EAG) was used for the electrophysiological bioassays of essential oils. Furthermore, antennal active compound was identified with the help of gas-chromatography-coupled mass spectrometry. The essential oil of *E. camaldulensis* proved very effective against *Ae. aegypti* at the tested dose of 166.5µl/cm$^2$. Our study suggests that plants-based products can be used in insect pest management without harming the environment.

# LARVICIDAL ACTIVITY OF FIVE ESSENTIAL OILS AGAINST AEDES AEGYPTI L.

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*Aedes aegypti* L. is the primary vector of various awful diseases all over the globe, especially in tropic and subtropic regions. Effective control measurements were done for the control of this mosquito. In view of the adverse effects of synthetic chemical-based intervention and resistance to mosquito, biochemically degradable, and environmentally friendly, the essential oils obtained from the flowers and leaves of, *Lipidium latifolium*, *Origanum majorana*, *Amomum sabulatum*, *Melia azedarach*, and *Nigella sativa* plants were investigated against the larvae of *Ae. aegypti*. The chemistry of all the tested essential oils was identified by gas chromatography-mass spectrometry (GC-MS). LC50 results represent the 2nd stage of *Ae. aegypti* larvae appeared, more sensitive to *L. latifolium* ($LC_{50}$, 26.03 ppm) followed by *O. majorana* ($LC_{50}$, 35.75 ppm), *A. sabulatum* ($LC_{50}$, 39.29 ppm), *M. azedarach* ($LC_{50}$, 124.2), and *Nigella sativa* ($LC_{50}$,333.6 ppm), respectively. The study suggests that essential oils can be used in controlling insect pests, with no adverse effects on the humans and environment.

# EVALUATION OF CHEMICAL COMPOSITION, ADULT REPELLENCE AND OVIPOSITION DETERRENCE POTENTIAL OF PLANT ESSENTIAL OILS AGAINST FEMALE FRUIT FLY *BACTEROCERA ZONATA* **(DIPTERA: TEPHRITIDAE)**

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Peach fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae: Diptera), is the multivoltine, polyphagous and devastating insect pest causing serious threats to fruits and vegetables. Incidence of insecticide resistance modulates the management interest to develop new chemicals which should be ecosystem friendly and compatible with the integrated pest management (IPM) strategies. Plant derived essential oils are eco-friendly and effective for managing insect pests. This study has evaluated the efficacy of *Ocimum basilicum, Eucalyptus globulas, Lipidium apetalum* and *Tagetes minuta* plant derived essential oils on repellency and oviposition deterrence of *B. zonata*. We treated the fruits with 1%, 0.1% and 0.01% concentrations of essential oils and five replications for each treatment were completed. In the multi-choice bioassay, four bananas, each treated with a different essential oil, were given to 24 hrs starved females (20) of *B. zonata* in one replication and their repellency and oviposition deterrence behavior was observed visually for five hrs from the onset of the experiment. In two-choice bioassay, one solvent treated and one essential oil treated banana fruits were given to 20 starved females. The results showed that *L. apetalum* essential oil significantly repelled *B. zonata* at all concentrations as compared to other oils. Number of oviposition were negligible on treated fruits compared to control at 1% and 0.1% concentrations while at 0.01% concentration significantly lowest oviposition was observed on *L. apetalum* treated fruits. Our results suggests that all the tested essentials oils can be used in IPM programs for the management of *B. zonata* but efficacy of *L. apetalum* was maximum among the all other tested essential oils.

# POTENTIAL OF FOUR WILD PLANTS ESSENTIAL OILS IN INHIBITING BLOOD SEEKING BEHAVIOUR OF *AEDES AEGYTPI* FEMALE MOSQUITOES

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*Aedes aegypti* (Diptera: Culicidae) mosquito is an important vector of many disease-causing pathogens. An effective way to escape from these mosquitoes borne diseases is to protect ourselves from the mosquitoes' bites. In the current study, essential oils of *Lipidium pinnatifidum, Mentha longifolia, Origanum vulgare* and *Agrimonia eupatoria* were evaluated for their repellent potential against female *Ae. aegypti*. Essential oils were extracted by using steam distillation process from freshly collected aerial parts of the plants and tested against 4-5 days old gravid females of *Ae. aegypti* through human bait technique for repellency and repellent longevity assays. The chemical composition of essential oils was investigated by gas chromatography-mass spectrometry (GC-MS). The essential oils of *L. pinnatifidum, M. longifolia*, *O. vulgare* and *A. eupatoria* at the dose of 33µg/cm$^2$ showed 100%, 94%, 87%, and 83% mosquito repellent activity respectively. Furthermore, *M. longifolia* and *O. vulgare* essential oils exhibited 100% repellency at tested dose of 165µg/cm$^2$ whereas *A. eupatoria* essential oil showed 100% repellency only at 330µg/cm$^2$. In the time-span bioassay, *M. longifolia* and *O. vulgare* essential oils showed protection against *Ae. aegypti* bites for 90 and 75 min respectively whereas both *A. eupatoria* and *L. pinnatifidum* were found active for 45 min. Benzyl cyanide (94%), piperitone oxide (34%), carvacrol (20%) and α-pinene (62%) were most abundant compounds in *L. pinnatifidum*, *M. longifolia*, *O. vulgare* and *A. eupatoria* essential oils respectively. The current study demonstrates that *M. longifolia* and *O. vulgare* essential oils possess potential to be used as an alternative to synthetic chemicals to protect humans from mosquito's bites.

# EVALUATION OF CHEMICAL COMPOSITION, PUPICIDAL, ADULTICIDAL, AND ADULT REPELLENCE EFFECTS OF DIFFERENT ESSENTIAL OILS AGAINST *MUSCA DOMESTICA* L.

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*Musca domestica* is a cosmopolitan insect pest spreading various diseases in humans and other animals. Essential oils from plants may provide environment-friendly alternatives of conventional synthetic insecticides. The effect of essential oils extracted from seven different plant species: *Tagets minuta*, *Mentha arvensis*, *Calappa romanzoffiana*, *Curcuma longa*, *Eucalyptus pillata*, *Dedonia viscosa*, and *Artemisia brevifolia* on feeding behavior as well as pupicidal and adulticidal were evaluated against *M. domestica*. The chemical composition of essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS). In the feeding bioassay, all the tested essential oils, when tested at 10% concentration, showed 100% repellence, except *E. pillata,* which showed significantly less repellence (90.8%) against *M. domestica*. At 1% concentration, *M. arvensis* showed a 100% pupicidal effect followed by *T. minuta*, *C. romanzoffiana*, *C. longa*, *E. pillata*, *D. viscosa*, and *A. brevifolia* was 85%, 62.5%, 55%, 50%, 40% and 27.5%, respectively. However, at 10% concentration, both *M. arvensis* and *C. romanzoffiana* showed 100% pupicidal effect followed by *T. minuta* (87.5%), *C. longa* (77.5%), *E. pillata* (75%), *D. viscosa* (70%), and *A. brevifolia* (62.5%). In case of adulticidal, experiment, *E. pillata* and *C. romanzoffiana* showed 100% mortality followed by *C. longa* (62.5%), *A. brevifolia* (37.5%), *T. minuta* (25%), *M. arvensis* (25%), *D. viscosa* (2.5%). Our results show that the essential oils used in the current study have a potential to be used to deter oviposition and inhibit feeding in adult female flies of *M. domestica*. Moreover, the tested essential oils or their constituents can also be used as pupicidal and adulticidal.

# EVALUATION OF PLANT ESSENTIAL OILS FOR THEIR LARVICIDAL POTENTIAL AGAINST DENGUE VECTORING MOSQUITO, *AEDES AEGYPTI* (L.)

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*Aedes aegypti* (L.) (Diptera: Culicidae) is known as the main vector of the dengue virus. Like other insects, the excessive use of synthetic chemicals to control mosquitoes including *Ae. aegypti* has become a serious problem. By keeping in view, the negative impacts of chemical insecticides, the current study was conducted by using plant-derived essential oils as a natural resource against *Ae. Aegypti* larvae. Essential oils were extracted from *Mentha spicata*, *Tagetes minuta*, *Curcuma longa*, and *Syzygium aromaticum*, by utilizing steam distillation, while chemical analysis was carried out by gas chromatography and mass spectrometry (GC-MS). Larvicidal bioassays were performed under laboratory conditions at different concentrations of essential oils ranging from 50-1600ppm. *M. spicata* proved most effective as larvicide ($LC_{50}$ 488.29 ppm) followed by *T. minuta* ($LC_{50}$ 786.41ppm) and *C. longa* ($LC_{50}$ 1597.67 ppm), and *S. aromaticum* ($LC_{50}$ 1829.55 ppm) against *Ae. aegypti*. Piperitenone oxide (49%), dihydrotagetone (21%), α-turmerone (40%) and eugenol (92%) were the major compounds in the essential oils of *M. spicata*, *T. minuta*, *C. longa*, and *S. aromaticum*, respectively. The essential oils of plants have ability to control the insect pests and have no harmful effects on humans and the environment.

# EVALUATION OF REPELLENT ACTIVITY OF PLANT ESSENTIAL OILS AGAINST *AEDES AEGYPTI*

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*Aedes aegypti* is the primary vector of various pathogenic diseases throughout the world, especially in tropical and subtropical regions of the world. By keeping in view the harmful impacts of synthetic chemicals based intervention measures, current study investigated plant essential oils as best natural alternates of synthetic repellents as they are bio-degradable and eco-friendly. Six plant essential oils (*Mentha longifolia, Erigeron bonariensis, Zanthoxylum armatum, Conyza Canadensis, Ailanthus altissia* and *Salsola imbricata*) were investigated as repellents against the females of *Ae. aegypti*. Human bait technique was used to check the repellent potential of the essential oils while the chemical analysis was carried out by gas chromatography and mass spectrometry (GC-MS). Highest repellency (97.59%) was exhibited by *M. longifolia* while the lowest repellency (13.21%) was observed for at the tested dose of 33ug/cm$^2$ against *Ae. aegypti.* Current research may be helpful as plant essential oils results indicate a strong potential in replacing synthetic repellent available in the market.


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