Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

ASSESSMENT OF ANTAGONISTIC POTENTIAL OF BACTERIA AS BIOCONTROL AGENT AGAINST *ALTERNARIA* LEAF SPOT OF TURNIP

[a]Insha Saeed*, [b]Sabir H. Khan, [c]Abdul Rasheed, [d]Muhammad M. Jahangir, [e]Abdul Jabbar, [f]Hafiz M. F. Shaheen [e]Wasi-ud-Din, [f]Komal Mazhar

[a] *Department of Plant Pathology, Islamia University Bawalpur, Bahawalpur, Pakistan.*
[b] *Plant Pathology Research Institute, Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan.*
[c] *Office of Research Innovation and Commercialization, University of Agriculture, Faisalabad, Pakistan.*
[d] *Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan.*
[e] *Department of Plant Pathology, PARS campus, University of Agriculture Faisalabad, Pakistan.*
[f] Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan.

A B S T R A C T

Alternaria leaf spot is a severe disease of turnip which produces necrotic spots on leaves. In the current study, six varieties of turnip were tested against this pathogen to check their susceptibility. Among these, purple gold variety was moderately susceptible on which three antagonistic bacteria i.e. *Bacillus subtilis, Streptomyces hydrogenans* and *Pseudomonas flourescene* were evaluated against *Alternaria brassicocola* with three different concentrations 2, 3 and 5%. Among these *B. subtilis* gave best control with 23.852% disease incidence followed by *Streptomyces hydrogenans* (22.183%), *Pseudomonas flourescene* (21.742%) as compare to control. The pure sample was further used for dual culture technique and the inhibition zone technique against target pathogen. Results from the dual culture test exhibited that all the antagonistic bacteria repressed mycelial progress of pathogenic fungus but *B. subtilis* effectively reduced the development of fungi within 7 days with difference of 3.1Cm; *Streptomyces hydrogenans* inhibited the growth within 12 days of incubation with difference of 2.9Cm while *Pseudomonas flourescene* was also shown competitive effect but inhibit the growth after 15 days of incubation with difference of 1.12Cm. In case of inhibition zone technique three antagonistic bacteria with three different concentrations of 2, 3 and 5mL were effectively used against *A. brassicocola.* Among these three antagonistic bacterial isolates *B. subtilis* significantly exhibited the strongest antagonism against *A. brassicocola* with 2mL concentration followed by *Streptomyces hydrogenans* and *Pseudomonas flourescene*. A distinct inhibition zone of each treatment was measured. However, *B. subtilis* (2.8190Cm) gave a significantly greater diameter of inhibition zone than *S. hydrogenans* (2.8095) and *P. flourescene* (2.5048) after seven days of incubation respectively. Data were examined statistically to certify usefulness of bacterial antagonists against *Alternaria brassicocola*.

Keywords: *A. brassicocola, B. subtilis, P. flourescene, S. hydrogenanas,* Biological control, Turnip.

INTRODUCTION

Turnip (*Brassica rapa*) is a herbaceous annual or biennial root crop which belongs to Brassicaceae family. It's starchy roots and leaves are edible. It is highly nutritious forage crop with a short growing season and grown in temperate climates worldwide (Barari *et al*., 2005). It is believed to have originated almost 4,000 years ago in Central and Southern Europe (Rakow, 2004). The total cultivated area of turnip in Pakistan is 15.766 thousand hectares with total seed production of 240 to 280kg/acre and 8 to 10 tonnes average yield per acre. Whereas in Khyber Pakhtunkhwa province of Pakistan the cultivated area for turnip constitutes 2.997 thousand hectares with production of 1.40 million tonnes having average yield of 0.042082million tonnes per hectare (Qasid *et al*., 2018).

Turnip is a rich source of essential nutrients like vitamins,

Submitted: June 28, 2021
Revised: July 17, 2021
Accepted for Publication: November 25, 2021
* Corresponding Author:
Email: drsabirkhan62@gmail.com

minerals, water, protein, fats, carbohydrates and ash. Above-ground foliage parts normally contain crude protein (20 to 25%), consumable waterless substance (IVDDM) (65 to 80%), nonaligned cleansing agent (NDF) (nearby 20%) and harsh detergent fiber (ADF) (about 23%). The roots contain crude protein (10 to 14%) and IVDDM (80 to 85%) (Naglaa *et al*., 2017).

Turnip is prone to a number of pathogens including viruses, bacteria, nematodes and fungi. Among fungi *Alternaria brassicicola* is one of the important necrotrophic, Deuteromycetes fungal species? which damages the plants of many families for example *Cucurbitaceae, Brassicaceae* and *Solanaceae* (Anuj *et al*., 2013). *A. brassssicocla* is the causal agent of leaf spot of turnip, persist saprophytically outside their hosts. Usually its spores are produced during night and released in the day (Meah *et al*., 2017). Optimum temperature for spore production ranges between 23.8 to 27.7°C, which favors the development of new spores within short time. Prolonged dew period and frequent rainfall are favourable conditions for disease development (Su'udi *et al*., 2013). The main sources of transfering spores from one area to another are splashing water, tools, wind, human activity and animals (Manhas and Kaur, 2016).

Most of the progressive growers around the world often depend on chemical fungicides for disease management and over past hundreds of years these chemical fungicides have played a very important role in improving crop yields. However, the extensive use of toxic fungicides has created several environmental and economic complications. In addition, it is probably the main cause of development of resistant strains of plant pathogens against traditional synthetic pesticides. In this situation, biological control is the most sustainable approach for management of plant pathogens. It is the most accepted alternative option for plant disease management (Kumar *et al*., 2012). Among the microbial biocontrol agents, bacteria are able to produce wide spectrum of antifungal agents and effectively control phytopathogens both *in-vivo* and *in-vitro* conditions (Das *et al*., 2015). The establishment of any plant disease is due to the relationship between a vulnerable host, virulent pathogen and the conducive environmental factors (Gul and Tak, 2016). To reduce the frequency of disease outbreak, it is obligatory to build strong relationships between biological control mediators and the constituents of disease development triangle (Arun, 2008).

The biocontrol mechanisms contain prompted resistance, hypo virulence and inhibition of enzymes elaborate in plant pathogenicity (Xu *et al*., 2011). In the biological control number of antagonistic bacterial microbes such as *Bacillus subtilis, pseudomonas fluorescens* and *Streptomyces hydrogenans* have been tested with dual culture technique to control *A. brassicocola* (Abdalla *et al*., 2014). These selected agents express powerful biocontrol abilities *in-vitro* than applied to field by directly introduced the suspension into plant tissues (Sakalauskas *et al*., 2014).

Research reports suggest that *B. subtilis* produce various types of antimicrobial compounds such as peptides (kim *et al*., 2010) secreted enzymes, proteins (Baysal *et al*., 2013) and unstable organic substances (Tan *et al*., 2013) which make them appropriate and effective biocontrol agents (Baffoni *et al*., 2015). *Streptomyces hydrogenans* is an actinomycetes and gram-positive bacteria which plays a vital role in controlling pathogen by manufacturing cyclic lipopeptide fengycin (Chandrakar and Gupta, 2018). Due to production of enzymes, it hinders the expansion of possible fungal pathogens (Zaynab *et al*., 2018) which destroy the fungal cell wall (Errakhi *et al*., 2007; Getha *et al*., 2005). *S. hydrogenans* survive in the soil layers near the roots of the pharmaceutical plants and some belongs to water that are not considered as strong biocontrol agents (Thangapandian *et al*.,2007). *Pseudomonas fluorescens* is nonpathogenic saprophytes that inhabit soil, water and plant surfaces. It is a communal gram negative, rod-shaped bacterium. As the name indicates, it discharges a resolvable greenish fluorescent pigment called fluorescein, mainly when iron is less available (Naglaa *et al*., 2017). It include in the essential group of bacteria known as plant Growth Promoting Rhizobacteria (PGPR) which shows a significant role in the enhancement of plant, encouraged complete resistance, biological mechanism of pathogens etc (Babu *et al*., 2000). So keeping in view the problems and constrains of synthetic fungicides the and benefits of microbial bio control agents the present study is mainly designed to explore the antifungal potential *B., S. hydrogenans* and *P. flourescene* against *A. brassicocola*.

MATERIALS AND METHODS

Establishment of disease screening trial: Seed of six varieties of turnip were taken from Oilseed Research Institute, Ayub Agriculture Research Institute (AARI), Faisalabad. The experiment was set up as Randomized Complete Block Design (RCBD) with plant to plant 25 Cm and row to row 75 Cm distance having three replications

of each variety. All the cultural practices were done uniformly as required.

Disease incidence: The disease rating scale of Li *et al.* (2007), 0-6 disease rating scale for assessment of disease incidence was followed.

$$\text{Disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total number of plants}} \times 100$$

Table 1. Disease rating scale used for screening against Alternaria leaf spot of turnip

Grade	Disease %	Reaction	Symbol
0	0	Immune	I
1	1-10%	Highly Resistant	HR
2	11-20%	Resistant	R
3	21-30%	Moderately Resistant	MR
4	31-50%	Moderately susceptible	MS
5	50-75%	Susceptible	S
6	>75%	Highly Susceptible	HS

MR= Moderately Resistance, MS= Moderately Susceptible, S= Susceptible, HS= Highly Susceptible

Isolation, purification and identification of *A. brassicacola*: Turnip leaves having visible symptoms of Alternaria leaf spot were collected from research area, Institute of Horticultural Sciences (IHS), UAF and preserved at 4˚C. *A. brassicocola* was isolated from infected leaves of turnip using a standard plating technique on PDA. The infected leaves were cut down into small pieces by using sterilized scissor along with some healthy portion. After cutting into small pieces, chopped parts were sterilized with 70% ethanol for 30 seconds. After three serials washing in sterilized distilled water these pieces were dried using blotting paper and inoculated on PDA plates. The inoculated plates were placed at 28±˚C in an incubator. Fungal growth was observed on plates which was further purified and examined under the microscope. The isolated fungus was identified on the basis of morphological characters including conidial shape and number of septa (Luna *et al*., 2002).

Preparation of inoculum and Pathogenicity test: Spores of fungus were separated from PDA culture with the help of glass separator in a container. Muslin cloth was used to stain the fungal suspension for removal of mycelial content (Zhang *et al*., 2021). The specifications of haemocytometer were divided into three dimensions; 0.0625 mm^2, 0.05 mm^2 and 0.04 mm^2. The number of spores were diluted to 1x $l0^6$ spores/mL as a standard spore mixture with 2, 3 and 5mL concentration (Garba *et al*., 2017).

For confirmation of Alternaria leaf spot of turnip on turnip plants, pathogenicity test was performed by following the Koch's postulates. Pathogen was isolated from infected portions of turnip plants and was re-inoculated on healthy turnip plants. The plants sprayed with sterile distilled water were used as control, symptoms were observed at regular intervals. Later on, pathogen was re-isolated with the culture isolated from parental infected plant.

Application of inoculum: The turnip plants were sprayed with distilled water to increase humidity, after two hours of water application the spore suspension was sprayed twice in the evening. The inoculation process was repeated thrice at 4th, 8th and 12th days of initial inoculation. To make environment conducive for infection, the field was irrigated before and after the inoculation. Plants was observed daily for symptoms development. Initial symptoms appeared after 5 days and characteristic symptoms produced after 7 days of inoculation (Garba *et al*., 2017). Percentage of disease incidence was recorded.

Collection and multiplication of antagonistic bacteria: Three antagonistics bacteria *Bacillus subtillis, Psuedomonas fluorescens* and *steptomyces hydrogenans* were collected from the Phyto-bacteriology lab of the Department of Plant Pathology, University of Agriculture, Faisalabad. After collection, each of the antagonistic bacterium was further multiplied on Nutrient Agar (NA) plates.

Antagonistic activity assay against *A. brassicacola:* Three antagonistic bacteria (*Bacillus subtilis, Pseudomonas fluorescens* and *Streptomyces hydrogenans*) were collected from the Phyto-bacteriology lab of Department of Plant Pathology, University of Agriculture, Faisalabad. After collection, each of the antagonistic bacterium was further multiplied on Nutrient Agar (NA) plates. *In-vitro* antagonistic activity of the above bacterial strains was initially tested against *A. brassicacola* on PDA plates. The bacterial inoculum of the active isolates was picked aseptically and placed in the center of Petri dish containing fungal growth. The Petri dishes were

incubated at 28±^{0}C for three days. With the help of digital Vernier caliper inhibition zones were measured after 24 hours with regular intervals of time of 7 days.

Evaluation of different antagonists against mycelium growth of *A. brassicacola* by Dual culture technique: Antagonistic bacteria were evaluated for antagonism against *A. brassicocola* on PDA by dual culture technique. 20 mL of PDA was distributed in sterilized Petri plates and allowed to solidify. A mycelial plug from an actively growing *A. brassicocola* on PDA was taken and placed at one side of Petri plate. The antagonists bacteria was inoculated at just opposite side of the same plate by leaving 3-4 Cm distance (Das *et al.,* 2015). In case of control, only test fungus was grown in the centre of the plate. Petri dishes were incubated at 25±2^{0}C. After incubation, the diameter of *A. brassicocola* was noted regularly until it covered the whole Petri plate in control treatment. The zones of inhibition were measured with digital vernier caliper in two perpendicular directions (Manoj *et al.,* 2014).

Preparation of Antagonistic bacterial suspension: Fresh culture of three selected antagonistic bacteria (*B. subtilis, S. hydrogenans* and *P. flourescene*) were taken and prepared a suspension. For this fresh colony were picked and added to the nutrient broth medium. The inoculated broth was kept for overnight shaking in a shaking incubator (Robus Technologies, SI900R) at a rate of 200 rpm. The cfu level of the broth was measured using spectrophotometer (uv/vis spectrophotometer, uv-1100) and was adjusted to a final concentration of 1 x 10^8 CFU/mL.

Evaluation of different antagonistic bacteria against *Alternaria* leaf spot of turnip under field condition: Prepared fungal spore suspension was applied to the turnip plants by spraying method to produce disease symptoms. After 15 days, symptoms were appeared, and the development of symptoms are noted with regular intervals of time.

The first spray of antagonistic bacteria was done as the first symptoms of disease were appeared and other subsequent sprays were done with 3 days interval. Each treatment was repeated three times. Disease incidence was recorded weekly and percentage of disease incidence was calculated by following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total number of plants}} \times 100$$

STATISTICAL ANALYSIS

Statistically data were analyzed by using SAS / STAT software (Filzonmer *et al*., 2018) which was work under Completely Randomized Design (CRD) for laboratory experiment and Randomized Complete Block Design (RCBD) for field experiment to test the significance of antagonistic bacteria for the management of Alternaria leaf spot disease.

RESULTS

In-vitro assessment of antagonistic bacteria against *Alternaria* leaf spot of turnip by using inhibition zone technique: Maximum diameter of inhibition zones were expressed by *Bacillus subtilis* (2.82Cm) followed by *Streptomyces hydrogenans* (2.81Cm) and *Pseudomonas flourescene* (2.5Cm) as compared to control (0). Interaction between treatment and their concentration (TxC) maximum inhibition zone (3.31Cm) are expressed by *Bacillus subtilis* at 2mL concentration followed by 3 and 5mL concentration at the same concentration remaining treatment (*Streptomyces hydogenans* and *Pseudomonas flourescene*) expressed maximum inhibition zone as compared to control. Interaction between treatments and days (TxD) maximum inhibition zone (1.54, 2.36, 2.58, 2.98, 3.24, 3.41 and 3.62Cm) expressed by *Bacillus subtilis* among *Streptomyces hydrogenans* (1.71, 2.28, 2.58, 2.97, 3.13, 3.36 and 3.64Cm) and *Pseudomonas flourescene* (1.72, 2.13, 2.39, 2.61, 2.76, 2.88 and 3.04Cm) as compared to control (0) after 24 hours respectively. Interaction between Treatment, Concentration and Days maximum inhibition zone (2.20, 2.63, 3.07, 3.53, 3.70, 3.90 and 4.17Cm) expressed by *Bacillus subtilis* after 24 hours continue with 7 days at 2mL concentration among 3 and 5mL concentration, at the same concentration remaining treatments (*Streptomyces hydrogenans* and *Pseudomonas flourescene*) expressed maximum inhibition zone as compared to control (0.00) after 24 hours continue with 7 days respectively.

In-vitro assessment of antagonistic bacteria against Alternaria leaf spot of turnip by using dual culture technique: Maximum inhibition of mycelial growth was expressed by *Bacillus subtilis* with difference of 3.0333Cm followed by *Streptomyces hydrogenans* (2.9000Cm) and *Pseudomonas flourescene* (1.1214Cm) as compared to control (5.4810). Interaction between Treatment and Days maximum growth of *Alternaria brassicocola* was inhibited by *Bacillus subtilis* with maximum difference of 3.03Cm among *Streptomyces hydrogenans* with 2.90Cm and *Pseudomonas flourescene* with 1.12Cm as compared to control 5.5Cm.

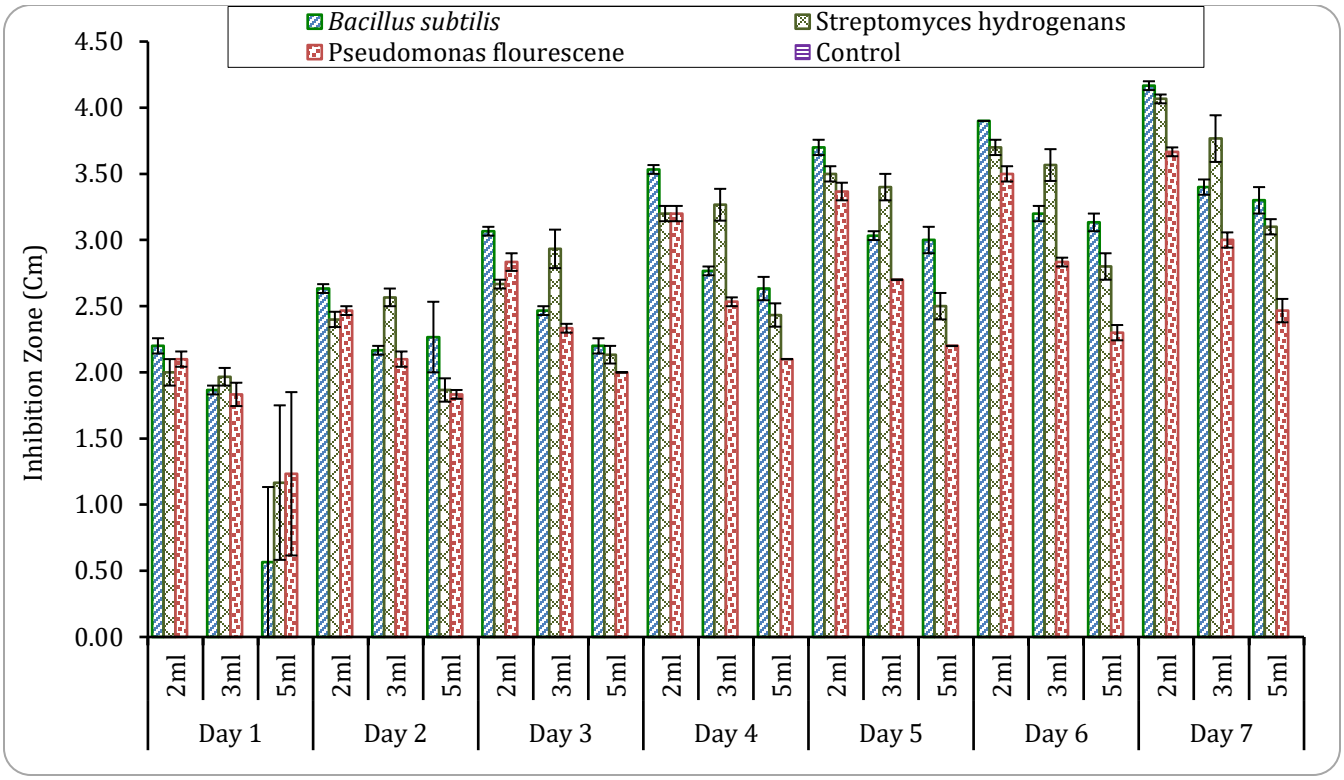

Figure 1. Evaluation of various antagonistic bacteria, concentrations and days against Alternaria leaf spot of turnip by using inhibition zone technique

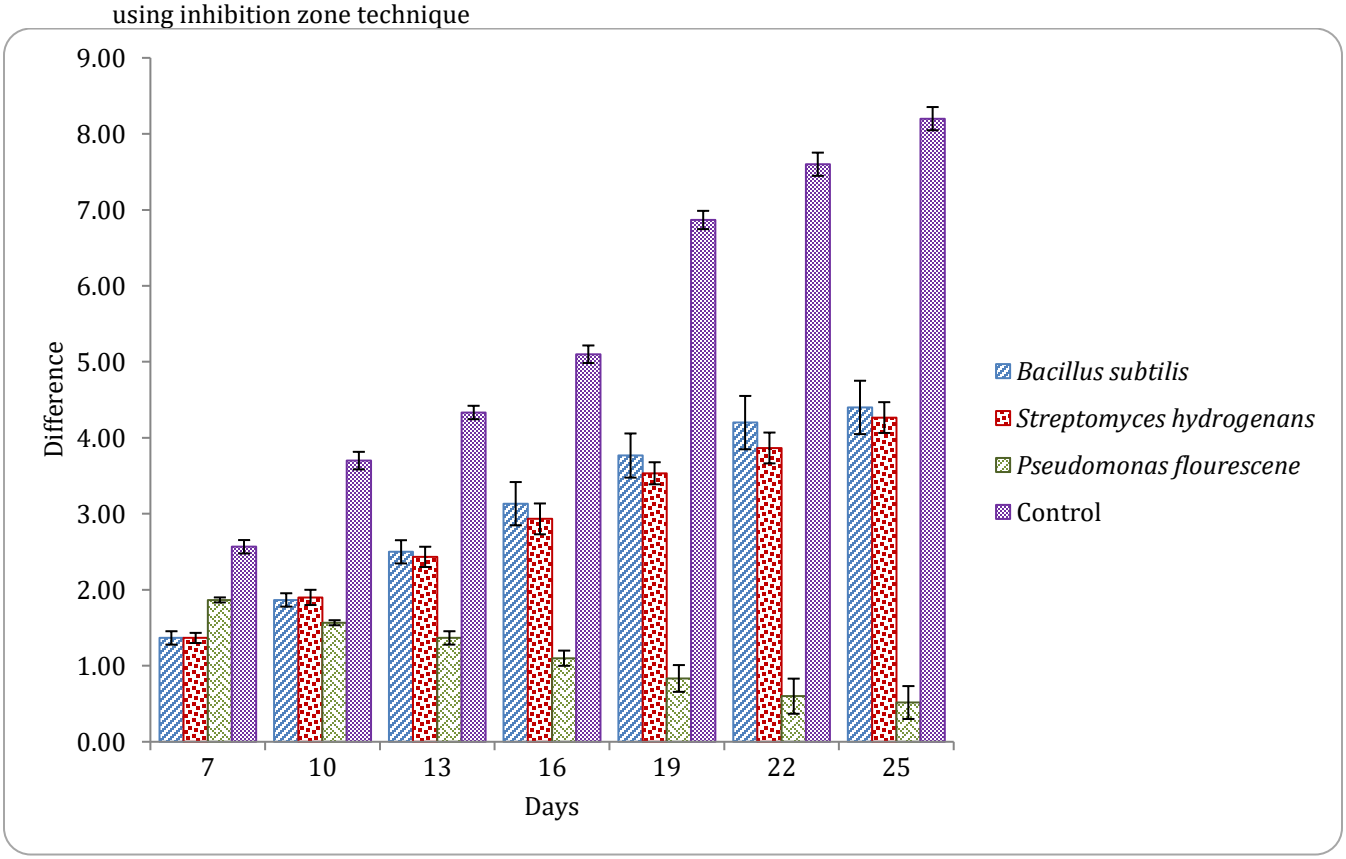

Figure 2. Evaluation of various antagonistic bacteria and days against Alternaria Leaf spot of turnip by using dual culture technique

Management of Alternaria leaf spot of turnip through antagonistic bacteria under field condition: Minimum disease incidence (21.74%) expressed in *Pseudomonas flourescene* after that *Streptomyces hydrogenans* (22.18%) and *Bacillus subtilis* (23.85%). Interaction among treatments and their concentrations (TxC) minimum diseased incidence (21.742%) expressed by *Pseudomonas flourescene* at same concentrations remaining treatments expressed less disease incidence as compared to control (Figure 1).

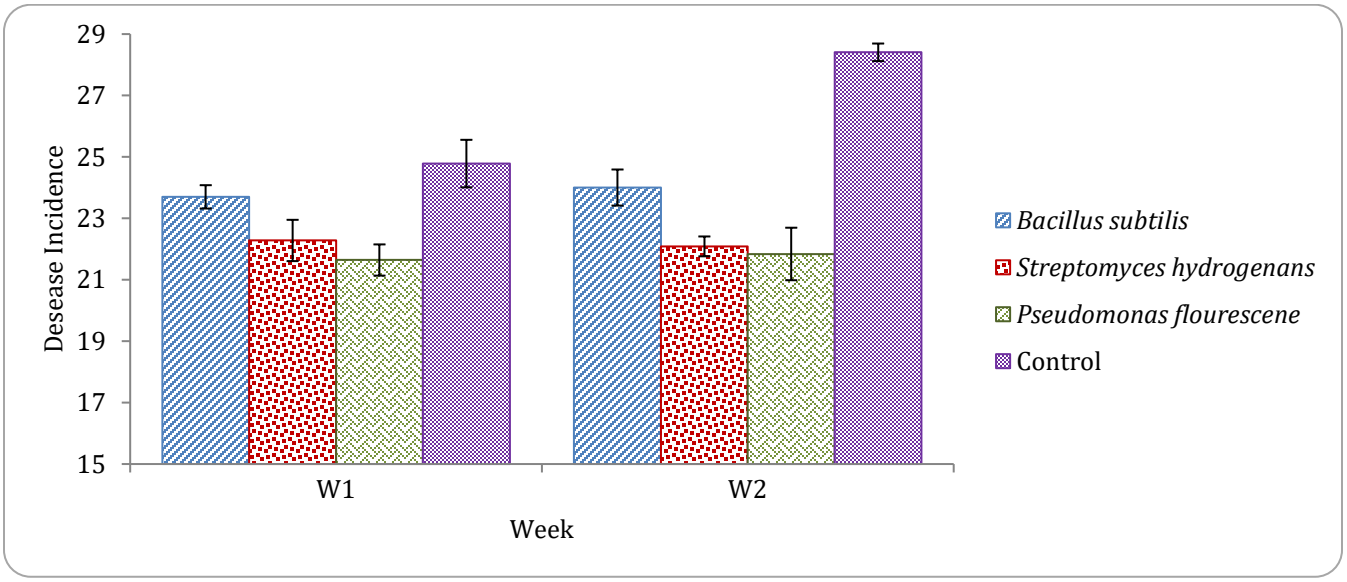

Figure 3. Effect of various antagonistic bacteria against *Alternaria* leaf spot of turnip under field conditions

DISCUSSION

Various factors are involved for producing the symptoms of a fungal disease including genetic makeup of a host, infection time, strain of fungi, environmental conditions and plant age. Alternaria leaf spot is one of the most destructive fungal disease caused by *A. brassicocola.* It potentially reduces the production of turnip by attacking on all above ground parts of plant which results in complete drying of leaves. It's estimated that leaf spot disease decrease yield up to 47% (Qasid *et al*., 2018). Development of disease in epidemic form depends on the virulence of pathogen, time of infection, favorable conditions of environment and susceptible host. To overcome these problems in future use of antagonists are effective and durable solution to save turnip crop from the infection of *A. brassicacola.* The effective solution includes the screening of available germplasm to check the relative susceptibility of plant material.

Biological control of plant pathogens becomes an integral part of pest management in light of the environmental and health issues attributed to the use of fungicides in agriculture (Kaur and Manhas. 2014). It is environment friendly and appropriate alternative method with no adverse effect of plant. In this method different biocontrol agents such as bacteria and fungi are used to suppress the population of pathogen (Sanzani *et al*., 2014).

Antagonistic organisms were collected from Bacteriology laboratory, Department of plant pathology, University of Agriculture Faisalabad. The antagonistic activity against *Alternaria citri* was tested by Dual Culture technique. For this purpose 4 antagonistics (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces hydrogenans*) were collected and applied through dual culture technique under lab conditions.

Six turnip varieties were grown and treated with *Alternaria brassicaola,* out of this most susceptible variety (purple gold) was further used for research process against Alternaria leaf spot of turnip. Three antagonistic bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces hydrogenans)* were evaluated against *Alternaria brassicacola* under both field and lab condition. Current study revealed that *B. subtilis* was proved most effective *in-vivo* condition by controlling 23.852 % disease incidence. However, under *in-vitro* condition *Bacillus subtilis* and *Pseudomonas flourescens* show maximum result.

In contemporary studies Tozlu *et al*., 2018 determined the antifungal activities of of *B. subtilis, B. megaterium, B. pumilus*, *P. polymyxa*, *P. fluorescens, Bacillus thuringiensis* subsp. kurstakii and *Bacillus sphaericus* against *A. alternate. B. pumilus* controlled 87.6-65.89% disease among all antagonists. Sundaramoorthy, (2012) revealed that both *Pseudomonas flourescens* and *B. subtilis* indicated best results against phytopathogenic fungi. According to Gul and Tak, (2016) culture filtrates from two isolates (*Bacillus subtilis* and *Bacillus amyloliquefaciens*) expressed strong efficacy against Alternaria spp under *vitro* condition. Abdalla *et al.*

(2014) isolated different species of Bacillus from the rhizosphere of tomato plants to be used as natural bio-control agents against *Alternaria alternata* under *in vitro* condition. It was found that four (Bacillus B25, B35, B41, B45) exhibited strong antagonism against Alternaria spp.

Recent study showed similar results as reported by Ankur *et a.* (2018), Naglaa *et al.* (2017) who selected different species of Bacillus such as *B. subtilis, B. megaterium, B. pumilus* and *B. cereus* and evaluated against numerous species of Alternaria. Results indicated that *B. subtilis* and *B. megaterium* expressed significant effect by inhibiting maximum growth of fungus (Deleu *et al*., 2008).

It has been identified that *B. subtilis* is capable to enhance the growth of plant (Choudhary *et al*., 2009). By using various mechanisms, *Bacillus subtilis* improves plant growth, such as perfection of plant nourishment, generation of complete resistance, injuriousness to pests and as antagonist against pathogens (Ali *et al*., 2016). *B. subtilis* produces wide range of secondary metabolites mediating antibiosis recognized for decades. Various types of antimicrobial compounds such as peptides, enzymes, proteins, and unstable organic substances which makes them appropriate and proper biocontrol agents (Baffoni *et al*., 2015). At least 4-5% of its genome is specific for antimicrobial compounds (Sumi *et al.*, 2015).

Bacillus species showed strong antimicrobial activity against genus Alternaria (Abbo *et al*., 2014). Upshots of the current study is supported by the findings of Matar *et al.* (2009), Trivedi and Pandey, (2008) and Velmurugan *et al*. (2009) who evaluated *B. subtilis, B. megaterium, B. pumilus* and *B. cereus* and reported that plates of genus Alternaria which were inoculated with *B. subtilis* and *B. megaterium* showed significantly less mycelial growth during the incubation period and indicated the ability of these bacteria to inhibit *in vitro* mycelial growth of many plant pathogenic fungi. However, no significant differences in the inhibition of genus Alternaria were observed between the species of *B. pumilus*, *B. cereus* and the control treatment. Kokalis *at el*. (2002) reported that *in vitro* inhibitory activity of these bacterial species translated well into the *in vivo* test; *B. subtilis* and *B. megaterium* showed the highest inhibition effect *in vitro* against mycelial growth of genus Alternaria. Their effects *in vivo* test on genus Alternaria disease incidence were also the best. The fourth species *B. cereus* showed low suppressive effects against disease incidence during both seasons. These results are in agreement with the laboratory findings published by (Kokalis *at el*., 2002).

Because of their wide diversity, bacteriocins display different modes of action such as protoplasm vesicularization, pore formation or cell disintegration. They are generally bactericidal and produce bacteriostatic activities due to their amphiphilic or hydrophobic properties (Gautam and Sharma, 2009). For example lantibiotic have dual mode of action, firstly they inhibit the cell wall synthesis of the pathogen through binding to lipid which is the major transporter of peptidoglycan subunits across the inner cell membrane (Chen *et al*., 2008). Secondly lipid are used as a docking molecule to insert the lantibiotic in the membrane leading to pore formation and ultimately to cell death (Chatterjee *et al*., 2005). This dual action has been reported for subtilin, a class of bacteriocin which is active against broad range of plant pathogens. It also produces Bacillomycin which helps in forming particular metabolic compounds to restrict growth of fungus. Bacillomycin interacts with the fungal cell wall and destroys leaf lamina which results in the leakage of contents eventually causes the death of cell and restricts the growth of fungus (Mardanova *et al*., 2016).

CONCLUSION

It is concluded from the recent research study that;

- The most susceptible variety of turnip was purple gold which exhibit clear symptoms of Alternaria leaf spot.
- In both *in vivo* and *in vitro* experiments, amongst three antagonistic bacteria *Bacillus subtilis* control maximum disease incidence followed by *Streptomyces hydrogenans* and *Pseudomonas flourescene*.

REFERENCES

Abbo, A. S., M. O. Idris and A. M. Hammad. 2014. The antifungal effects of four tomato rhizosphere Bacillus spp. against *Alternaria alternata*. International Journal of Science and Research, 3: 1324-1328.

Abdalla, S., S. A. A. Algam, E. A. Ibrahim and A. M. El Naim. 2014. In Vitro Screening of Bacillus Isolates for Biological Control of Early Blight Disease of Tomato in Shambat Soil. World Journal of Agricultural Research, 2: 47-50.

Ali, G. S., A. S. A. El-Sayed, J. S. Patel, K. B. Green, M. Ali, M. Brennan and D. Norman. 2015. Ex Vivo Application of Secreted Metabolites Produced by Soil-Inhabiting Bacillus spp. Efficiently Controls Foliar Diseases Caused by Alternaria spp. Applied Environmental Microbiology, 82: 478-490.

Ankur, V., Kumar, S., Shina, H.A. Jaiswal, S. 2018. Evaluate the efficacy of bio-control agents and botanicals against early blight of potato caused by *Alternaria solani*. . Journal of Pharmaceutical Innovation, 7: 28-30.

Anuj, M., R. Roychowdhury and J. Tah. 2013. Alternaria pathogenicity and its strategic controls. Research Journal of Biology, 1: 1-9.

Arun, K. 2008. Biocontrol of plant diseases: Need to tap the options. Journal of Arid Legumes, 5: 99-108.

Babu, S., K. Seetharaman, R. Nandakumar and I. Johnson. 2000. Biocontrol efficacy of *Pseudomonas fluorescens* against *Alternaria solani* and tomato leaf blight disease. Annals of Plant Protection Sciences, 8: 252-254.

Baffoni, L., F. Gaggia, N. Dalanaj, A. Prodi, P. Nipoti, A. Pisi, B. Biavati and D. Di Gioia. 2015. Microbial inoculants for the biocontrol of Fusarium spp. in durum wheat. BMC Microbiology, 15: 242-242.

Barari, H., S. M. Cook, S. J. Clark and I. H. Williams. 2005. Effect of a turnip rape (*Brassica rapa*) trap crop on stem-mining pests and their parasitoids in winter oilseed rape (*Brassica napus*). Biocontrol, 50: 69-86.

Baysal, Ö., D. Lai, H.-H. Xu, M. Siragusa, M. Çalışkan, F. Carimi, J. A. T. Da Silva and M. Tör. 2013. A proteomic approach provides new insights into the control of soil-borne plant pathogens by Bacillus species. PLoS One, 8: e53182.

Chandrakar, S. and A. K. Gupta. 2018. Studies on the Production of Broad Spectrum Antimicrobial Compound Polypeptide (Actinomycins) and Lipopeptide (Fengycin) from Streptomyces sp. K-R1 Associated with Root of *Abutilon indicum* against Multidrug Resistant Human Pathogens. International Journal of Peptide Research and Therapeutics, 25: 779-798.

Chatterjee, C., M. Paul, L. Xie and W. A. van der Donk. 2005. Biosynthesis and Mode of Action of Lantibiotics. Chemical Reviews, 105: 633-684.

Chen, H., X. Xiao, J. Wang, L. Wu, Z. Zheng and Z. Yu. 2008. Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. Biotechnology letters, 30: 919-923.

Choudhary, D. K. and B. N. Johri. 2009. Interactions of Bacillus spp. and plants – With special reference to induced systemic resistance (ISR). Microbiological Research, 164: 493-513.

Das, M. P., P. V. Devi and Y. Yasmine. 2015. A study on antagonistic potential of bacteria against phytopathogenic fungi. international Journal of Pharmaceutical Sciences Review and Research, 34: 191-193.

Deleu, M., M. Paquot and T. Nylander. 2008. Effect of fengycin, a lipopeptide produced by *Bacillus subtilis*, on model biomembranes. Biophysics Journal, 94: 2667-2679.

Errakhi, R., F. Bouteau, A. Lebrihi and M. Barakate. 2007. Evidences of biological control capacities of Streptomyces spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Beta vulgaris* L.). World Journal of Microbiology and Biotechnology, 23: 1503-1509.

Filzmoser, P., K. Hron and M. Templ. 2018. Compositional Tables. Springer Series in Statistics. Springer International Publishing, 227-243.

Garba, M., H. Makun, A. Jigam, H. Muhammad and B. Patrick. 2017. Incidence and toxigenicity of fungi contaminating sorghum from Nigeria. World Journal of Microbiology, 4: 105-114.

Gautam, N. and N. Sharma. 2009. Bacteriocin: safest approach to preserve food products. Indian Journal of Microbiology, 49: 204-211.

Getha, K., S. Vikineswary, W. H. Wong, T. Seki, A. Ward and M. Goodfellow. 2005. Evaluation of Streptomyces sp. strain g10 for suppression of Fusarium wilt and rhizosphere colonization in pot-grown banana plantlets. Journal of Industrial Microbiology & Biotechnology, 32: 24-32.

Gul, N. and H. Tak, 2016. Prevalence of Trichuris spp. in small ruminants slaughtered in Srinagar District (J&K). Journal of Parasitic Diseases, 40: 741-744.

Kaur, T. and R. K. Manhas. 2013. Antifungal, insecticidal, and plant growth promoting potential of *Streptomyces hydrogenans* DH16. Journal of Basic Microbiology, 54: 1175-1185.

Kim, P. I., J. Ryu, Y. H. Kim and Y.-T. Chi. 2010. Production of Biosurfactant Lipopeptides Iturin A, Fengycin and Surfactin A from *Bacillus subtilis* CMB32 for Control of *Colletotrichum gloeosporioides*. Journal of Microbiology and Biotechnology, 20: 138-145.

Kokalis–Burelle, N., C. S. Vavrina, E. N. Rosskopf and R. A. Shelby. 2002. Plant and Soil, 238: 257-266.

Kumar, P., R. C. Dubey and D. K. Maheshwari. 2012. Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiological Research, 167: 493-499.

Li, C. X., K. Sivasithamparam, G. Walton, P. Salisbury, W. Burton, S. S. Banga, S. Banga, C. Chattopadhyay, A. Kumar, R. Singh, D. Singh, A. Agnohotri, S. Y. Liu, Y. C. Li, T. D. Fu, Y. F. Wang and M. J. Barbetti. 2007. Expression and relationships of resistance to white rust (*Albugo candida*) at cotyledonary, seedling, and flowering stages in Brassica juncea germplasm from Australia, China, and India. Australian Journal of Agricultural Research, 58: 259.

Luna, C. L., R. L. R. Mariano and A. M. Souto-Maior. 2002. Production of a biocontrol agent for crucifers black rot disease. Brazilian Journal of Chemical Engineering, 19: 133-140.

Manhas, R. K. and T. Kaur. 2016. Biocontrol Potential of *Streptomyces hydrogenans* Strain DH16 toward *Alternaria brassicicola* to Control Damping Off and Black Leaf Spot of *Raphanus sativus*. Frontiers in Plant Sciences, 7: 1869-1869.

Manoj, K. M., Singh, R. Tomer, A. 2014. In vitro evaluation of antagonistic activity of *Pseudomonas flourescene* against fungal pathogen. Journal of Biopesticides, 7: 43-46.

Mardanova, A. M., G. Fanisovna Hadieva, M. Tafkilevich

Lutfullin, I. Valer'evna Khilyas, L. Farvazovna Minnullina, A. Gadelevna Gilyazeva, L. Mikhailovna Bogomolnaya and M. Rashidovna Sharipova. 2017. *Bacillus subtilis* Strains with Antifungal Activity against the Phytopathogenic Fungi. Agricultural Sciences, 08: 1-20.

Matar, S., S. A. El-Kazz, E. E. Wagih, A. I. El-Diwany, H. E. Moustaf, G. A. Abo-Zai, H. E. Abd-Elsala and E. E. Hafez. 2008. Antagonistic and inhibitory effect of *Bacillus subtilis* against certain plant pathogenic fungi, I. Biotechnology(Faisalabad), 8: 53-61.

Meah, M. B., F. H. Tumpa and M. A. Hossain. 2017. Effect of media composition on vegetative and reproductive growth of *Alternaria brassicicola* and *Bipolaris sorokiniana*. Current Agriculture Research Journal, 5: 266-278.

Naglaa H., S. Nakasuji, M. M. Elsharkawy, H. A. Naznin, M. Kubota, H. Ketta and M. Shimizu. 2017. Biocontrol Potential of an Endophytic Streptomyces sp. Strain MBCN152-1 against *Alternaria brassicicola* on Cabbage Plug Seedlings. Microbes Environment, 32: 133-141.

Qasid, A., S.U. Rehman, I. Jan, M. Ali, M. Faheem, F. Sattar, M. Sayam, M. and W. Sajjad. 2018. Effect of different plant spacing on the production of turnip (*Brassica rapa* L.) under agro-climatic conditions of Swabi (Pakistan). Pure and Applied Biology, 7: 243-247.

Rakow, G. 2004. Species origin and economic importance of Brassica, Brassica. Springer, 54: 3-11.

Sakalauskas, S., A. Kačergius, D. Janušauskaitė and D. Čitavičius. 2014. Bacteria with a broad-spectrum of antagonistic activity against pathogenic fungi of cereals. Zemdirbyste-Agriculture, 101: 185-192.

Sanzani, S. M., M. G. Li Destri Nicosia, R. Faedda, S. O. Cacciola and L. Schena. 2014. Use of quantitative PCR detection methods to study biocontrol agents and phytopathogenic fungi and oomycetes in environmental samples. Journal of Phytopathology, 162: 1-13.

Su'udi, M., J. M. Park, S. R. Park, D. J. Hwang, S. C. Bae, S. Kim and I. P. Ahn. 2013. Quantification of *Alternaria brassicicola* infection in the *Arabidopsis thaliana* and *Brassica rapa* subsp. *pekinensis*. Microbiology, 159: 1946-1955.

Sumi, C. D., B. W. Yang, I. C. Yeo and Y. T. Hahm. 2015. Antimicrobial peptides of the genus Bacillus: a new era for antibiotics. Canadian journal of microbiology, 61: 93-103.

Sundaramoorthy, S. 2012. Consortial Effect of Endophytic and Plant Growth Promoting Rhizobacteria for the Management of Early Blight of Tomato Incited by *Alternaria solani*. Journal of Plant Pathology & Microbiology, 60: 59-67.

Tan, Z., B. Lin and R. Zhang. 2013. A novel antifungal protein of *Bacillus subtilis* B25. Springerplus, 2: 543-543.

Thangapand, V., P. Ponmurugan and K. Ponmurugan. 2006. Actinomycetes Diversity in the Rhizosphere Soils of Different Medicinal Plants in Kolly Hills-Tamilnadu, India, for Secondary Metabolite Production. Asian Journal of Plant Sciences, 6: 66-70.

Tozlu, E., N. Tekiner, R. Kotan and S. Örtücü. 2018. Investigation on the biological control of *Alternaria alternata*. Indian Journal of Agricultural Sciences, 88: 1241-1247.

Trivedi, P. and A. Pandey. 2008. Plant growth promotion abilities and formulation of *Bacillus megaterium* strain B 388 (MTCC6521) isolated from a temperate Himalayan location. Indian Journal of Microbiology, 48: 342-347.

Velmurugan, N., M. S. Choi, S. S. Han and Y. S. Lee. 2009. Evaluation of antagonistic activities of *Bacillus subtilis* and *Bacillus licheniformis* against wood-staining fungi: In vitro and in vivo experiments. The Journal of Microbiology, 47: 385-392.

Xu, X. M., P. Jeffries, M. Pautasso and M. J. Jeger. 2011. Combined Use of Biocontrol Agents to Manage Plant Diseases in Theory and Practice. Phytopathology, 101: 1024-1031.

Zaynab, M., M. Fatima, S. Abbas, Y. Sharif, M. Umair, M. H. Zafar and K. Bahadar. 2018. Role of secondary metabolites in plant defense against pathogens. Microbial Pathogenesis, 124: 198-202.

Zhang, Y., H. Zhu, Y. Ye and C. Tang. 2021. Antifungal Activity of Chaetoviridin A from *Chaetomium globosum* CEF-082 Metabolites Against *Verticillium dahliae* in Cotton. Molecular Plant-Microbe Interactions, 34: 758-769.

Contribution of Authors:

Insha Saeed	:	Designed and conduct experiments.
Sabir H. Khan	:	Supervised and layout the experiments.
Abdul Rasheed	:	Data analysis and critically reviewed the manuscript.
Muhammad M. Jahangir	:	Help in manuscript writeup.
Abdul Jabbar	:	Data collection and compilation.
Hafiz M.F. Shaheen	:	Help in manuscript write up.
Wasi-U- Din	:	Editing the manuscript.
Komal Mazhar	:	Reference correction and reviewed the manuscript.