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

BIOLOGICAL STUDIES ON SEED BORNE MYCOFLORA OF EXOTIC TOMATO SEED

[a]Hayat U. Tareen, [b]Ch. A. Rauf, [c]Ghulam Qadir, [a]Abdul R. Bhutta
[a] Federal Seed Certification and Registration Department, Islamabad, Pakistan.
[b] Department of Plant Pathology, PMAS- Arid Agriculture University Rawalpindi, Pakistan.
[c] Department of Agronomy, PMAS- Arid Agriculture University Rawalpindi, Pakistan.

A B S T R A C T

Sustainable vegetable farming is under stress due to the presence of biotic and abiotic factors. Most of the diseases/stress caused by fungi that survive mostly in seed. The current study is carried out to evaluate the presence of fungi on exotic seeds of tomato. This research work included the isolation and identification of seed-borne mycoflora on ten tomato cultivars. It was found that some variety was highly resistant, and some cultivars show was highly susceptible amongst the ten cultivars. Pathogenicity of four highly aggressive seed-borne fungi viz *Alternaria alternate, Rhizoctonia solani* and *Fusarium moniliforme* were assessed through lab based i*n-vitro* and *in vivo* techniques. All the germinated seeds were infected by these fungi with varying degree of variability or aggressiveness Disease index varied from 0.29 to 0.91 for all tested materials. All pathogenic fungi on each cultivar significantly reduced germination and produced more abnormal seedlings compared to control. The most common four seed-borne fungi (*Aspergillus niger, Alternaria alternata, F. moniliforme,* and *F. oxysporum*) were selected to study their transmission from seeds to seedlings. Results revealed that pathogenic fungi move from seed to seedling and other part of plant but saprophytic fungi were can not able to move in plant parts systemically. While the means value showed that the highest transmission was observed during germination stage followed by seedling stage on leaves and on stem. The outcome of this study will help to regulate the import of unhealthy vegetable seeds and also help in quarantine pathogens associated with imported seed, that prevent agricultural and natural resource from risk associated with the entry of seed borne pathogens in the country.

Keywords: Seed born fungi, mycoflora, tomato, pathoginicity, germination, exotic.

INTRODUTION

Tomato *(Lycopersicon esculentum* Mill.) belongs to family Solanaceae, its fruit have reasonable amount of soluble sugars, several organic acids and large amount of vitamins A and C as well as essential minerals and other nutrients (Rick, 1980). They dilute and purify the blood and regulate the digestive system (Holden *et al*., 2002) .It is grown throughout the year in different parts of Pakistan. Average tomato yield is 11.05 tonnes per hectare, total area under cultivation is 44.46 thousand hectares and total production is 491.4 thousand tonnes (GOP, 2013). Tomato is major vegetable however, their production per unit area is lower as compared to potential yields due to unavailability of pure and high quality seed and resistant to various diseases for obtaining maximum yields. The major problem for low yield is unavailability of disease free seed. Seed and soil-borne diseases are considered as important factors for yield loss. Mostly, diseases spread due to seed-borne mycoflora. Seed and soil borne diseases take a heavy toll of vegetable production in Pakistan.

Pathogen free quality seed is instantly needed for ideal plant propagation. Seed is a source of several diseases from biotic agents resulting in significant losses of crop yields (Islam *et al.,* 2009; Anwar and McKenry, 2012). Spread of seed-borne diseases across international borders is very common, and also complicated to identify due to rare typical symptoms on seed surfaces (Nishikawa *et al.,* 2006). Some of the seed-borne fungi cause seed rot, decrease seeds germination, and cause pre- and post-germination death (Haikal, 2008). It emphasizes for appropriate steps for managing pathogen free, healthy and viable seeds (Farrag and

* Corresponding Author:
Email: tareen_emp@yahoo.com

Moharam, 2012). The fungi that associated with seed may be present internally or externally in seed or attached as contaminant. Most of the fungal seed contaminations are either due to attack in the field internally (seed sheaths, embryo and endosperm), or as an external infectivity (Ismael, 2010). Such seeds are normally weak in vigor and longevity and will fail to germinate or emerge.

Although major portion of tomato seeds in Pakistan is being imported but still the quality is unstable and uncertain in the country. Therefore, there is a dire need to test the quality of seed by diagnosing the seed borne diseases of these exotic vegetables to passively check the introduction of exotic pathogens in the country. Keeping in view these implications and importance of healthy seed, this study was carried out to achieve the following objectives:

a. To study the nature, frequency and distribution of seed borne mycoflora of exotic tomato seed, representing important vegetables family i.e. tomato *(Lycopersicon esculentum)* solaneceae,
b. To study the pathogenicity, adaptability and mode of transmission from seed to seedling.
c. To studies the effect of fungal infection on seed and its quantitative role on germination

MATERIALS AND METHODS

This study was undertaken at central seed health laboratory of Federal Seed Certification and Registration Department, Islamabad. Seeds of 10 cultivars of tomato (solaneceae) were studied for detection of seed borne mycoflora. Seeds were obtained from the field stations of Federal Seed Certification and Registration Department located in Karachi and Lahore (both are entry ports of imported seed).

Isolation and identification of seed borne mycoflora: Seeds of selected exotic (imported) tomato were treated with 1 % sodium hypochlorite (NaClO) for 7-10 minutes depending on the type of seeds. After treatment, the seed were washed with sterilized distilled water and later dried on blotter paper. In total 10 cultivars and respective country from where they import, were used for test including Saffal-60 (India), Founto (India), Red-Stone (China), Rio-General (India), Advanta-1209 (India), FMX-1077 (USA), Rio-Fuego (India), Rio-Grande (India), Roma-VF (Holland) and Baby red (India).

Blotter paper method: This method was proper used for the detection of seed borne mycoflora for all the crops as suggested by ISTA (2007)). Four hundred seeds were plated equidistantly in Petri dishes of 9 cm diameter on three layered well soaked filter paper under sterilized condition and were incubated at 20 ± 2 °C under 12 hours of alternate cycles of near ultraviolet light (NUV) and darkness for seven days. The NUV light enhances the sporulation of many fungi. On 8th day, the plates were examined for presence of fungi under stereo-binocular microscope, and further confirmation was made under compound microscope.

Fungi identification: Identification of these fungi were based on their external morphological characters. Fungal habit characters included colony color, mycelium growth, spore shape and size, as described in the Identification Keys of Barnett (1960), Booth (1977), and Nelson *et al*. (1983).

Pathogenicity test of isolated fungi from vegetable seeds: Confirmation of the pathogen was made by pathogenicity tests. The specific pathogenicity test was performed by the following method as reported by Iftikhar *et al.* (2008), Giri *et al.* (2001).

Test tube cotton swab method: Five seeds of selected cultivars sown individually in test tubes (20 cm x 3 cm) were prepared by filling with cotton in the bottom of the tube. 20 ml of sterilized distilled water in tube was added and were covered with aluminum foil. The test tubes were autoclaved. Seeds were surface treated with 1% sodium hypochlorite solution for 10 minutes and rinsed with distilled water, then placed on the moist cotton swab in the test tube. One disk of 5 mm of fungal isolate was placed adjacent to the targeted seeds. In the last, tubes were covered again with aluminum foil and were placed artificial growth chamber at ambient temperature (Giri *et al*., 2001).

Data regarding the severity of diseases were expressed on the basis of percent infected area of leaf, stem or root according to diseases rating scale described by James (1971) as given below:

Table 1. Disease rating scale.

Scale	Range	Scale	Range	Scale	Range
0	0.0	1	1-10	2	11-25
3	26-50	4	51-75	5	76-100

During the experiment, pathogenic nature of the pathogen (pathogen variability / aggressiveness) was studied according to the Koch's Postulates.

Effects of seed borne fungi on seed germination: One hundred artificially infected seeds of tomato were placed separately on anchor brand paper of a size 24 × 48 cm, with four replications. Paper with seeds were rolled and put in polyethylene bags and incubated at 20±2 °C for eight days. To study the effect of artificially inoculated seed-borne fungi on germination, the method of Baggett and Fraizer (1973) was employed as seed coating with fungal cultures. First, seeds were surface-sterilized, and then these seeds were mixed with the desired inoculums in conical flasks. For proper mixing, flasks were corked and shaken for about one hour on mechanical shaker. After this, the seeds remained in the inoculum for 8-10 hours and then taken out and kept on sterile paper towel overnight and the following parameters were recorded (all in %):

i. Germinated seeds (normal and abnormal)
ii. Un-germinated / dead seeds
iii. Quantitative role of fungi (un-germinated and abnormal seeds)

The quantitative role of seed borne fungi on germination, abnormal seedling and un-germinated seeds were observed using blotter paper technique. Only one seedling was planted in one Petri dish (dia. 9 cm).

Transmission of fungi from seed to seedlings: The most common four seed-borne fungi (*A. niger, A. alternata, F. moniliforme* and *F. oxysporum*) were selected to study their transmission from seeds to seedlings. All these four pathogenic fungi were isolated at different growth stages, viz., germination, seedling, leaves and stem of vegetable plants at 5, 15, 30 and 45 days after sowing, respectively.

Statistical analysis: Data were statistically analyzed by using Analysis of Variances (ANOVA) and means were compared using (LSD) at 5 percent level of significance as described by Chase and Brown (2000).

RESULTS AND DISCUSSION

Isolation and identification of seed-borne mycoflora from tomato seeds: Ten cultivars of tomato (Saffal-60, Founto, Roma-VF, Rio-General, Advanta-1209, FMX-1077, Baby-Red, Rio-Grande, Red-Stone and Rio-Fuego) were tested against seed-borne mycoflora to evaluate their resistance / susceptibility. In total, ten genera and eleven species of fungi (*Alternaria alternata, Aspergillus niger, Botrytis cinerea, Cladosporium herbarium, Drechslera* sp., *Fusarium oxysporum, F. moniliforme, F. oxysporum, Pythium aphanidermatum, Rhizoctonia solani, Verticillium albo-atrum* and *Trichoderma viride*) were isolated and identified from the seeds of all tomato cultivars. Cultivar Red-Stone (89.5 % healthy and 10.5 % infected seeds) was highly resistant, and cultivar Rio-Fuego (72.5 % healthy and 27.5 % infected seeds) was highly susceptible amongst the ten cultivars. Five fungal pathogens, viz., *A. alternata, A. niger*, *Drechslera* sp., *F. oxysporum, and F. moniliforme*, remained dominant in most of the tomato varieties (Founto, Roma-VF, Rio-General, FMX-1077, Red-Stone and Rio-Fuego).

Abdel-Mallek *et al.* (1995) isolated 39 fungal species (from 16 genera) from healthy tomatoes, and *Aspergillus niger* being the most prevalent (in 84.6 % samples). Diseased tomatoes had eleven seed-borne fungal species, where the most common were *A. alternata, Aspergillus niger* and *Rhizopus stolonifer.* Bankole (1996) isolated internal seed-borne mycoflora of 2 tomato varieties reporting 18 species (in 9 genera) the most abundant were *Aspergillus flavus, A. niger, Alternaria longissima, Fusarium spp.* and *Phoma destructive*. *Phytophthora erythroseptica* causes wilting and stunting in tomatoes (Naseema *et al*., 1983). Prevalence of *Phoma* spp. and *F. moniliforme* was higher on both tomato and pepper seeds (Nutsugah, 2004).

Muhammad *et al.,* (2004) isolated 8 fungal species from rotten tomato fruits, among which only *A. flavus* and *A. niger* were the most prevalent. Bankole (1996) reported *Aspergillus, Cladosporium* and *Fusarium* genera as the most common on tomato seeds and isolate *Phoma destructiva* from two tomato varieties. Further, he described the similar seed borne fungi as in this study. Ismael (2010) obtained the similar results from tomato seeds in Sulaimania, and in Germian region. Fakir (2001) reported six seed-borne mycofloral diseases of tomato, viz., *Alternaria solani*, *Aspergillus flavus*, *Penicillium* spp., *A. fumigatus*, *Fusarium oxysporum*, and *Phytophthora infestans*. a number of seed borne pathogens parasitized Tomatoes seed including *Fusarium oxysporum* f. sp. *lycopersici*, (Ivanović and Mijatović, 2003).

Present study results are in conformation with the findings of other researchers that seeds of the tomato are potentially vulnerable to seed-borne fungi. The majority of the isolated seed-borne fungi have been considered to be pathogenic (Mathur, 1983; Elarosi, 1993). Many other researchers also isolated several fungal species from different vegetables. Wahid and Ali

(1990) isolated numerous pathogenic mycoflora from the seeds of major vegetable grown in both seasons recieved from different locations of Pakistan.

Generally, in all crop / vegetable seeds, almost same factors are accountable for the cultivar differences of resistance to fungal infection (Chandrashekar and Satyanarayana, 2006). Another factor that may contribute to fungal infection is grain hardness that may different from variety to variety. Softer grains have greater infection compared to harder grains (Glueck and Rooney, 1978). Variation in isolation frequency could be due to difference in the moisture content of seeds, varietal differences and seed susceptibility to infection besides specific environmental conditions there in. Some other factors that contribute in differences in isolation frequency may be due to variation in the moisture content of seeds and seed susceptibility to infection in addition to environmental conditions.

Pathogenicity test of isolated fungi from tomato seeds: Pathogenicity of four highly aggressive seed-borne fungi (*A. niger, A. alternata, F. oxysporum* and *F. moniliforme*) of tomato was assessed on ten cultivar / varieties. Results presented in Table 2 indicated that all the germinated seeds were infected by these fungi. The disease index for the first fungi (*Alternaria alternata* ranged from 0.27 to 0.71 with a mean of 0.48. Baby-Red seeds got the highest susceptibility (0.71), and Red-Stone was the least susceptible (0.27) against *A. alternata*. In case of *Aspergillus niger,* the disease index having a mean value of 0.46, as Rio-Grande had the highest susceptibility (0.65), and Rio-Fuego got the lowest value (0.31).

Table 2. Percentage of healthy and infected seeds of tomato cultivars, and their associated mycoflora.

Sr.	Cultivars	Healthy seeds	Infected seeds	Associated seed-borne mycoflora
1	Saffal-60	83.3 bc	16.7 cd	*Aspergillus niger*, *Botrytis cinerea, Drechslera* sp., *Fusarium oxysporum*
2	Founto	79.0 cd	21.0 bc	*Alternaria alternata, A. niger*, *Drechslera* sp., *F. moniliforme*
3	Red-Stone	76.5 de	23.5 ab	*A. alternata, A. flavus, A. niger, F. oxysporum, Drechslera* sp.
4	Rio-General	73.7 de	26.3 ab	*A. alternata, A. niger, Cladosporium herbarium, Drechslera* sp., *F. oxysporum, Pythium aphanidermatum, Rhizoctonia solani, Verticillium albo-atrum*.
5	Advanta-1209	82.5 bc	17.5 cd	*A. flavus, C. herbarium. P. aphanidermatum, V. albo-atrum*
6	FMX-1077	78.5 cd	21.5 bc	*A. alternata, B. cinerea, Drechslera* sp., *F. oxysporum, Trichoderma viride*
7	Baby red	87.0 ab	13.0 de	*B. cinerea, C. herbarum, R. solani*
8	Rio-Grande	84.5 ab	15.5 de	*C. herbarum, P. aphanidermatum, R. solani, V. albo-atrum*
9	Roma-VF	89.5 a	10.5 e	*A. alternata, B. cinerea* and *F. oxysporum*
10	Rio-Fuego	72.5 e	27.5 a	*A. alternata, A. niger, C. herbarum, Drechslera* sp., *R. solani*
CV		2.8	11.6	
LSD		5.5	5.4	

Table 3. Disease index of three seed-borne mycoflora on different tomato cultivars during Pathogenicity test.

Sr.	Cultivars	*A. alternata*	*A. niger*	*F. oxysporum*	*F. moniliforme*	Average
1	Saffal-60	0.67 b	0.40 ef	0.74 a	0.53 e	0.59
2	Founto	0.45 e	0.49 c	0.63 b	0.40 f	0.49
3	Red-Stone	0.27 g	0.37 f	0.53 c	0.77 a	0.48
4	Rio-General	0.53 d	0.45 d	0.42 e	0.63 c	0.51
5	Advanta-1209	0.34 f	0.41 def	0.49 cd	0.71 b	0.49
6	FMX-1077	0.42 e	0.55 b	0.78 a	0.59 d	0.58
7	Baby red	0.71 a	0.42 de	0.53 c	0.26 g	0.48
8	Rio-Grande	0.30 g	0.65 a	0.45 de	0.42 f	0.45
9	Roma-VF	0.63 c	0.49 c	0.42 e	0.49 e	0.50
10	Rio-Fuego	0.45e	0.31 g	0.43 e	0.58 d	0.45
	Average F	0.478	0.46	0.54	0.54	
	CV	4.25	5.50	4.37	3.71	

Data in a column bearing different letter(s) have statistically significant difference at $P \leq 0.05$.

The disease index for third fungi *Fusarium oxysporum* ranged from 0.42 to 0.78 with a mean value of 0.54. The FMX-1077 cultivar had the highest susceptibility (0.78), while Rio-General and Roma-VF were the least susceptible (0.42) however disease index for fourth fungi *F. moniliforme* ranged from 0.26 to 0.77 with an average of 0.54. Red-Stone cultivar was the most susceptible (0.77), while seeds of Baby red had the lowest susceptibility (0.26).

Shovan *et al.* (2008) performed the pathogenicity test with 33 isolates of *F. oxysporum* in pot culture. All the tested isolates were found to be pathogenic. *Fusarium* and *Alternaria sp.* inoculation on tomato seed reduced seed germination and produced wilting (Perveen, 1996). *A. niger* was moderately pathogenic on tomato fruits (Muhammad *et al.,* 2004). *F. oxysporum* is the most hazardous pathogens for plant wilting (Wagner, 2004). Tomato plants disease wilting and dying caused by *F. oxysporum* due to the colonization of underground organs and xylem (Ito *et al.,* 2005). Wagner *et al.* (2001) also reported that tomato seedlings show decrease in plant fluorescence resulting from the pathogen presence *F. oxysporum.* This pathogen caused wilting and seriously endangers tomato production (Djordjević *et al.,* 2011). *F. oxysporum* is the main source of tomato wilt and fruit rot; the symptoms appear on older plants with typical signs of leaf chlorosis (Ignjatov *et al.,* 2012). *Fusarium* species are responsible for vascular wilt, and can be seed-borne both internal and external and survive for more than 1-2 years in seed (Watt, 2006).

Generally, results proved the virulence of fungi to infect all the tested seeds with different degrees. It also attributed differences in susceptibility to infection and genetic structure of each seed. These findings are in similar with those reported by Nguyen *et al*. (2005). However, various root exudates of different varieties may protect the plants from fungal infection (McLaren *et al.,* 2004). Fungi mostly use plant sugars for growth and respiration which affects the carbohydrate content of seeds leaving them irregular and abnormal besides weak health of the growing seedlings (Wildermuth *et al.,* 1992; Al-Abdalall-Amira, 1998). Disorder of food changes in infected plants also explains the consumption of lipids and proteins by fungus in seeds. Fungi cause the maximum seed damage including reduced germination and seedling vigour among the seed-borne pathogens. Most of the frequently isolated fungal species are not pathogenic.

Effects of seed-borne fungi on seed germination and seedlings: Ten species of different seed-borne fungi (*Alternaria alternata, Aspergillus niger*, *Botrytis cinerea, Cladosporium herbarium, Drechslera* sp., *Fusarium oxysporum, F. moniliforme, Pythium aphanidermatum, Rhizoctonia solani* and *Verticillium albo-atrum*) were artificially inoculated on tomato seeds. Percentage seed germination was more than 80%, the highest seed germination percentage (93.0 %) was observed in check (control), while the lowest seed germination percentage (81.3 %) was observed in *Alternaria alternata* (Table 3).

Although, percentage germination of artificially inoculated seeds tomato was more than 80 % but the range of percentage of abnormal seedlings was higher over that of normal ones. The highest abnormal seedlings percentage of tomato was observed with two seed-borne fungi; *Fusarium oxysporum* (71.0 %) and *Cladosporium herbarium* (72.0 %), while the lowest abnormal seedlings (21.5 %) were observed in check treatment.

Percentage of un-germinated / dead seeds ranged from 7.0 to 18.7 %. The highest percentage of un-germinated/ dead seeds was observed with *A. alternata* (18.7 %) While the lowest un-germinated / dead seeds (7.0 %) were recorded in control.

Habib *et al.* (2007) observed that all the inoculated fungi reduced the seeds viability. Inoculation of tomato seeds with conidia of *A. alternata* reduced the germination rate and created wilting with larger prevalence (Perveen, 1996). The presence of pathogenic fungi that reduced germination rate due to the growth of newly emerging shoots. Use of NaOCl serves only for surface disinfection of seeds, whereas fungus penetrates the deeper layer of the cells prior to germination (Melchers, 1956). Occurrence of *Aspergillus* spp., on seeds of some vegetables in greater amount and its association with un-germinated seeds point out that species of saprophytes *Aspergillus* may cause low germination in seeds (Shakir and Mirza, 1992). Ijaz *et al.* (2001) also reported that *A. niger* is a damaging storage fungi that affected the seed quality and reduces seed germinability. Saad *et al.* (1988) observed that *Aspergillus flavus* and *Fusarium solani* were associated with damage to plumule, radical and hypocotyl of germinating seedlings. Such fungi in our study also reduced seedling development. Lower germinability of seeds is attributed to damaged embryos from deeper infection. All the fungi used in this study induced disease symptoms on

germinating seedlings. Ismael (2010) observed harmful influence on germination rate of pepper and tomato cultivars by some fungal exudates. Fungal exudates significantly decreased germination rate of all the tested solanaceous seeds.

Transmission of seed-borne mycoflora in tomato seed: Percentage transmission of seed-borne mycoflora in tomato seed was recorded during the germination stage of tomato seeds due to *A. alternata* was 84.3 %, while during the seedling stage it was 76.7 % (Table 4).

Table 4. Effect of different seed-borne mycoflora on the seed germination and seedlings condition of tomato.

Sr. #	Seed-borne mycoflora	Germination	Abnormal seedlings	Normal seedlings
			%	
1	*Alternaria alternata*	81.3 e	65.0 bc	16.3 d
2	*Aspergillus niger*	85.5 bcde	63.0 bc	22.5 bcd
3	*Botrytis cinerea*	88.7 abcd	61.3 c	25.0 bc
4	*Cladosporium herbarium*	91.0 ab	72.0 a	19.0 cd
5	*Drechslera* sp.	83.5 cde	66.7 abc	16.8 d
6	*Fusarium oxysporum*	90.0 abc	71.0 ab	19.0 cd
7	*F. moniliforme*	88.0 abcde	61.5 c	26.5 b
8	*Pythium aphanidermatum*	85.3 bcde	63.2 abc	22.0 bcd
9	*Rhizoctonia solani*	84.0 cde	64.7 abc	19.3 cd
10	*Verticillium albo-atrum*	82.5 de	64.0 abc	18.5 cd
11	Control	93.0 a	21.5 d	71.5 a
	CV	3.21	5.91	11.60
	LSD	6.79	8.84	7.11

Data in a column bearing different letter(s) have statistically significant difference at P ≤ 0.05.

Table 5. Transmission (%) of four seed-borne fungi at different growth stages in tomato.

Growth stage	Days	*Alternaria alternata*	*Aspergillus niger*	*Fusarium oxysporum*	*Fusarium moniliforme*	Average	LSD value
Germination	5	84.3 a	76.5 b	83.7 a	86.3 a	82.7	4.2
Seedling	15	76.7 ab	66.7 b	83.3 a	73.3 ab	75.0	10.9
Leaves	30	46.7 a	0.0 c	33.3 b	36.7 b	29.2	9.4
Stem	45	23.3 a	0.0 b	16.7 a	20.0 a	15.0	7.7
Average		57.8	35.8	55.1	53.3		

Means in each row with similar letter(s) are at par at $P = 0.05$

On the leaves its inoculum density was decreased to 46.7%, while on stem it was reduced to half (23.3 %) as compared with that on leaves. Transmission percentage of *A. niger* recorded at germination stage of tomato seeds was 76.5 %, which was comparatively lower than that for *A. alternata*. During seedling stage it was 66.7%; but this fungus was not transmitted in leaves and stem.

Fusarium oxysporum transmission was 83.7 percent during the germination stage of tomato seeds, However, its transmission in leaves drastically reduced to 33.3 %, and on stem it was further reduced to half 16.67 % as compared with leaves. Results on transmission of *F. moniliforme* showed the highest value (86.3 %) during the germination stage, which was comparatively much higher than for all other tested fungi. While during seedling stage it was 73.3 %, but transmission of this fungus transmitted in leaves was nearly half (36.7%) as compared with seedling stage, and in stem it was recorded only 20%

Mean transmission values of these four fungi indicated the highest transmission for *A. alternata* (57.8 %) followed by *F. oxysporum* (55.1 %), *F. moniliforme* (53.3 %) and *A. niger* (35.8 %). Mean value of transmission during different growth stages showed the highest transmission during germination (82.7 %) followed by seedlings (75.0 %), leaves (29.2%) and stem (15.0 %) during the seedling stage.

Zida *et al*. (2008) also foundd similar results. However, considering the high infection level encounter in the

seeds, further studies are required to clarify the correct role of these fungi in seeds. Seed-borne fungi are able to infect seedlings and move up the stem to the inflorescence through the vascular system. Elwakil and Ghoneem (1999) studied transmission of three seed-associated *Fusarium* species (*F. moniliforme, F. oxysporum,* and *F. solani*) in growing *Psyllium* plants. Only *F. solani* restricted to the roots and hypocotyls of the plants (12.5 % at 120 d). At the seedling stage (35 d after sowing), the recovery of *F. moniliforme* was 90 % in roots and 10 % in hypocotyl and lower stem. In the mature plant (120 d), the fungus did not move beyond the upper stem. Recovery of *F. oxysporum* from the stem showed that the fungus reached the top of the stem. Elizabeth *et al.* (2008) reported that *Alternaria* and *Fusarium* associated with seed caused the deterioration of the seed and seedling quality. It revealed that these tested species were pathogenic to different growing stages of tomato crop. These pathogens cause disease at various stages of plant growth from seed germination to crop maturity (Dumbre *et al.*, 2011).

REFERENCES

Abdel-Mallek, A.Y., S.K. Hemida and M.M.K. Bagy. 1995. Studies on fungi associated with tomato fruits and effectiveness of some commercial fungicides against three pathogens. Mycopathol. 130: 109-116.

Anwar, S.A. and M.V. McKenry. 2012. Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses. Pak. J. Zool. 44: 327-333.

Baggett, J.R. and N.A. Fraizer. 1973. Seasonal pattern of *Fusarium* root rot development in resistant vs. susceptible cultivars of *Phaseolus vulgaris* L. Hort. Sci. 8: 214-216.

Bankole, S.A. 1996. The distribution and pathogenicity of the seed mycoflora of tomato varieties cultivated in western Nigeria. Afr. Crop Sci. J. 4: 491-496.

Barnett, H.L. 1960. Illustrated genera of imperfect fungi. Burgess Pub. Co. Minn. 241 P.

Booth, C. 1977. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.

Chandrashekar, A. and K.V. Satyanarayana. 2006. Disease and pest resistance in grains of sorghum and millets. J. Cereal Sci. 44: 287-304.

Chase, W. and F. Brown. 2000. General Statistics, 4th Ed., John Wiley and Sons, NY, USA.

Djordjević, M., B., Zečević, R. Djordjević, J.I. Zdravković and M. Mijatović. 2011a. str. 69.Uticaj rase 3 fuzarioznog uvenuća na pojedine sorte i hibride paradajza. Zbornik abstrakata IV simpozijuma Sekcije za oplemenjivanje organizama Društva genetičara Srbije, Kladovo.

Dumbre, S., D. Guldekar and S.R. Potdukhe. 2011. Survey of seed borne fungi of onion (*Allium cepa* L.) from various locations of Maharashtra. J. Soils Crops 21: 221-224.

Elarosi, H. 1993. Diseases of Vegetables. New Publishing House, Alexandria. 310 p. (In Arabic).

Elizabeth, Z.P., S. Paco, L. Vibeke, S. Phillipe, S. Irenee and N. Adama. 2008. Importance of seed borne fungi of sorghum and millet in Barkina Faso and their control using the plant extracts. Pak. J. Biol. Sci. 11: 321-331.

Elwakil, M.A. and K.M. Ghoneem. 1999a. Detection and location of seed-borne fungi of blonde *Psyllium* and their transmission in seedlings. Pak. J. Biol. Sci. 2: 38-44.

Fakir, G. A. 2001. An annotated list of seed borne disease in Bangladesh. Seed Pathol. Lab., Dept. of Plant Pathol., BAU, Mymensingh, Bangladesh. 41 P.

Farrag, E.S.H. and M.H.A. Moharam. 2012. Pathogenic fungi transmitted through cucumber seeds and safely elimination by application of peppermint extract and oil. Not. Sci. Biol. 4: 83-91.

Giri, G.K., R.M. Gade and C.U. Petail. 2001. Seed borne *Bipolaris sorokiniana* in wheat and its chemical control. J. Soils Crops 11: 109-112.

Glueck, J.A. and L.W. Rooney. 1980. Chemistry and structure of grain in relation to mold resistance. In: Sorghum Diseases - A World Review. Proc. Int. Workshop on Sorghum Diseases. Dec. 11-15, 1978, Hyderabad, India. pp. 119-140.

GOP, 2013. Fruit, vegetables and condiments statistics of Pakistan 2011-12, Government of Pakistan Ministry of National Food Security & Research Islamabad.

Habib, A., S.T. Sahi, M.U. Ghazanfar and S. Ali. 2007. Location of seed borne components and impact on seed germinability. Int. J. Agri. Biol. 9: 514-516.

Haikal, N.Z. 2008. Effect of the filtrate of pathogenic fungi of soybean on seed germination and seedling parameters. J. Appl. Sci. Res. 4: 48-52.

Holden M.A.J.M., S.A. Bhagwat, K.Y. Patterson. 2002. Development of a multi-nutrient data quality evaluation system. J. Food Comp. Anal. 15:339-348.

Ignjatov, M., D. Milošević, Z. Nikolić, J. Gvozdanović-

Varga, D. Jovičić and G. Zdjelar. 2012. *Fusarium oxysporum* as causal agent of tomato wilt and fruit rot. Pestic. Phytomed. (Belgrade) 27: 25-31.

Ijaz. A., S.A. Anwar, A. Riaz and M.S.A. Khan. 2001. Seed-borne pathogens associated with wheat and their role in poor germination. Pak. J. Phytopathol. 13: 102-106.

Islam, S.M.M., M.M.I. Masum and M.G.A. Fakir. 2009. Prevalence of seed-borne fungi in sorghum of different locations of Bangladesh. Sci. Res. Essay 4: 175-179.

Ismael, J.H.S. 2010. Isolation and identification of some fungi from certain solanaceous seeds in Sulaimania and Germian regions and their exudate effects on germination rate. Agric. Biol. J. North Amer. 1: 615-619.

ISTA. 2007. International rules for seed testing. Proceedings of International Seed Testing Association (ISTA). 8303 Bassersdorf, Switzerland.

Ito, S., A. Nagata, T. Kai, H. Takahara and S. Tanaka. 2005. Symptomless infection of tomato plants by tomatinase producing *Fusarium oxysporum* formae speciales nonpathogenic on tomato plants. Physiol. Mol. Plant Pathol. 66(5): 183-191.

Ivanović, M. and M. Mijatović. 2003. Patogene gljive semena povrća. Poljoprivredni fakultet, Novi Sad.

James, C. 1971. Reprint No. 28. The 19th Int. Seed Testing Cong., Vienna, Austria. 8 P.

Mathur, S.B. 1983. Testing seeds of tropical species for seed borne diseases. Seed Sci. Technol. 11: 113-128.

McLaren, D.L., R.L. Conner, R.G. Platford, J.L. Lamb, H.A. Lamey and H.R. Kutcher. 2004. Predicting diseases caused by *Sclerotinia sclerotiorum* on canola and bean, a western Canadian perspective. Physiol. Plant. 120: 489-497.

Melchers, L.E. 1956. Sorghum kernel smut infection in relation to seed germination. Trans. Kansas Acad. Sci. 59:320-6.

Muhammad, S., K. Shehu and N.A. Amusa. 2004. Survey of the market diseases and aflatoxin contamination of tomato (Mill.) fruits in Sokoto, northwestern Nigeria. Nutr. Food Sci. 34: 72-76.

Naseema, A., S. Balakrishnan and M.C. Nair. 1983. Seed borne fungi associated with vegetables. Agri. Res. J. Kerala 21: 32-37.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. Fusarium Species: An Illustrated Manual of Identification. The State Univ. Press. 203 P.

Nguyen, T.T., P.W.J. Taylor, R.J. Redden and R. Ford. 2005. Resistance to *Ascochyta rabiei* (Pass.) Lab. in a wild *Cicer* germplasm collection. Animal Prod. Sci. 45: 1291-1296.

Nishikawa, J., T. Kobayashi, K. Shirata, T. Chibana and T. Natsuaki. 2006. Seed borne fungi detected on stored solanaceous berry seeds and their biological activities. J. Gen. Plant Pathol. 72: 305-313.

Nutsugah, S. K. 2004. Seed borne mycoflora of major food crops in Ghana. J. Sci. Technol. 24: 22-31.

Perveen, S. 1996. Studies on some seed borne fungal diseases of tomato. Ph. D. thesis. Dept. of Botany, Univ. of Karachi, Pakistan.

Rick, C.M. 1980. Tomato. *In:* Hybridization of Crop Plants. American Society of Agronomy/Crop Science Society of America, Madison, Wisconsin, USA. pp. 669-680.

Saad, S., Raghunathan, A.N. and Shetty, H.S. 1988. Seed mycoflora of cowpea (*Vigna unguiculata* (L.) Walp) and their pathogenic importance. Seed Sci. Technol. 16: 541-548.

Shakir, A.S. and J.H. Mirza. 1992. Seed-borne fungi of Bottle gourd from Faisalabad and their control. Pak. J. Phytopathol. 4: 54-57.

Shovan, L.R., M. K.A. Bhuiyan, N. Sultana, J.A. Begum and Z. Pervez. 2008. Prevalence of fungi associated with soybean seeds and pathogenicity tests of the major seed-borne pathogens. Int. J. Sustain. Crop Prod. 3: 24-33.

Wagner, A. 2004. Fungal communities colonizing stems of hot pepper (*Capsicum annuum*). Phytopathol. Pol. 33: 23-29.

Wagner, A., A. Strudzińska and W. Michałek. 2001. Pathogenicity of some soil-borne isolates of *Fusarium oxysporum* to tomato. *In:* Proc. 5th Cong. of Europ. Found. for Plant Pathology. Biodiversity in Plant Pathology. Sep. 18-22, 2000. Taormina – Giardini Naxos, Italy. pp. 339-341.

Wahid, S. A. and S. Ali. 1990. Proceeding seed pathology in Pakistan. Federal seed certification department, government of Pakistan, Islamabad. pp. 135-150.

Watt, B. A. 2006. *Fusarium* rot of cucurbits. Fact Sheet of Insect and Plant Disease Diagnostic Lab., Pest Management Office, University of Marine Cooperative Extension. UMCE Home page.

Wildermuth, G. B., R. D. Tinline and R. B. McNamara. 1992. Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. Aust. J. Agri. Res. 43: 45-58.

Zida, P. E., P. Sérémé, V. Leth, P. Sankara, I. Somda and A. Néya. 2008. Importance of seed-borne fungi of sorghum and pearl millet in Burkina Faso and their control using plant extracts. Pak. J. Biol. Sci. 11: 321-331.