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Original Research Article

Distribution of okra root rot (*Fusarium solani*) in district of Peshawar and Nowshera and characterization of different isolates

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Abstract

The root rot of okra (*Fusarium solani*) was distributed up to 32.09 to 91.14 % in ten different okra growing regions of District Peshawar and Nowshera. The highest disease severity with maximum (91.14 %) percent mortality was reported by isolate NI_2 from Garhi Momin and lowest (32.09 %) by isolate NI_4 from Balu. *In vitro,* all the isolates were observed to be significantly different by their characterization at different pH levels (5.0, 5.6 and 9.0). All the isolates grew well at pH level of 5.0 with maximum colony diameter of 76.73 mm, 1.97 g of fresh biomass and 2.29×10^6 ml^{-1} of spore production and at pH 5.6 (77.14 mm, 1.89 g and 2.16×10^6 ml^{-1}). The maximum growth among the isolates was attained by isolate NI_2 with 96.67 mm of colony diameter, 2.52 g of biomass and 3.23×10^6 $ml^{-1}$of sporulation and lowest by NI_4 (57.89 mm, 0.87 g and 0.76×10^6). *In vitro*, Dithane M-45 was found as the most effective fungicide in controlling *Fusarium solani* (6.87 mm, 0.24 g, 0.09×10^6 ml^{-1}) than Aliette (75.20 mm, 1.54 g, 1.54×10^6 ml^{-1}) and Copper oxychloride (69.10 mm, 1.55 g, 1.45×10^6 ml^{-1}) as compared to control (84.67 mm, 2.18 g, 2.14×10^6 ml^{-1}). The isolate NI_2 found as the most aggressive isolate by exhibiting the maximum growth in Aliette and Copper oxychloride amended culture media as observed in control (non poisoned).

Keywords: Okra, Root rot, *Fusarium solani*, isolates, *in vitro* and characterization.

INTRODUCTION

Okra (*Abelmoschus esculentus* L) locally known as "bhindi" is one of the most important summer vegetables in Pakistan. It is grown in all parts of the tropics and during summer in the warmer parts of the temperate region (Baloch, 1994). It is the most widely known and utilized species of the family Malvaceae (Bayer and Kubitzki, 2003; Naveed *et al*., 2009). In Pakistan, okra occupied an area of 14855 ha with a total production of 112983 tones, while in KP, it occupied an area of 1957 ha with a total production of 15630 tones (MINFAL, 2014).

Okra plants are attacked by a number of soil borne diseases caused by different fungi such as, *Fusarium solani, Macrophomina phaseolina, Rhizoctonia bataticola, R. solani, Pythium butleri, Phytophthora palmivora, Cercospora abelmoschii* and *Erysiphe cichoracearum* (Mithal, 2006). Among them, *Fusarium solani* (Mart) Sacc., the causal organism of okra root rot (Rahim *et al.* 1992; Haq *et al*., 1996) has been considered as one of the most destructive pathogens (Mithal, 2006). It has a wide host range and known to decrease both the quantity and quality of major crops including tomato (Parveen *et al*., 1993), soybean (Mousa, 1994) and other vegetables (Ghaffar, 1995). Its incidence range from 10-80% and maximum 55-80% in the crop grown on small scale as kitchen/home gardening and minimum from 10-45% in

the crop sown on large scale under field conditions (Mithal, 2006).

The plants infected with *Fusarium solani* show dark brown to black discoloration from the base of stem. Severely infected plants become dead, their roots turn dark brown in color and are badly damaged. The fungus perpetuates in the soil or gets attached to infected plants. Root rot may affect individual plant or a group of plants in patches spreading rapidly under optimum conditions (Mithal, 2006). *Fusarium* root rot is favored by higher soil temperatures (optimum 25°C to 30°C) and moderate soil moisture (Malvick, 2002). The disease severity depends upon cultural and climatic factors such as plant spacing, soil moisture, depth of planting and stress from low or high temperatures. The effect of root rot becomes most apparent during seedling stage (Aviles *et al*., 2003).

The disease can be managed through cultural practices such as time of sowing, appropriate row and plant spacing, crop rotation, seed rate, water and other nutritional requirements (Jhonson *et al*., 1997). Among them, time of sowing plays the most important role (Naz *et al*, 2009). The use of fungicides such as Benomyl and Captan is also effective against the disease (Ghaffar, 1993). The antagonistic fungi and bacteria (*Trichoderma harzianum*, *Trichoderma viride* and *Bacillus subtillis*) used as biocontrol agents have also been reported to reduce the percentage of infected plants and severity of the disease (Mithal, 2006). *Fusarium* species are highly variable because of their genetic make up and changes in the environment in which they grow (Nelson *et al*, 1983). Due to its high adoptability to environmental conditions, the variation exists among isolates of the pathogen. As far as better management is concerned, the exact identification and characterization of the isolates is very important to formulate effective control strategies accordingly.

Keeping in view the importance of the disease and losses it causes, the present study was initiated with the objectives, (1) to find out the distribution of okra root rot in the okra growing regions of district Peshawar and Nowshera and (2) to study the *in vitro* effect of different pH levels and fungicides on the mycelia growth, spore concentration and biomass on various isolates of the pathogen.

MATERIALS AND METHODS

Seedling mortality (%) due to okra root rot in District Peshawar and Nowshera

A survey was conducted in the okra growing regions of District Peshawar (Acheni Payan, Palosi, Chamkani, Jogian and Budhni) and Nowshera (Akbarpura, Garhi Momin, Zakhi Charbagh, Balu and Taru Jabba) during the 2012 growing season of the crop. At each location, five fields were randomly selected for measuring the disease severity. In each field, seedling mortality (%) was calculated at five places. Seedling mortality (%) was calculated on one meter square patch in each spot by using the following formula.

Seedlings Mortality (%) =

$$\frac{\text{Number of root rotted seedlings}}{\text{Total Number of seedlings per m}^2} \times 100$$

Infected seedlings were collected from each location and were brought to the laboratory of Plant Pathology, The University of Agriculture Peshawar for further studies.

Isolation and identification of the pathogen from diseased okra seedlings

Collected okra seedlings from ten different areas of District Peshawar and Nowshera were cut into small pieces, surface sterilized with 0.1% solution of Mercuric chloride ($HgCl_2$) for 15-30 seconds, rinsed three times with sterilized distilled water to remove the extra disinfectants and blotted dry. The treated pieces were then placed on potato dextrose agar (PDA) medium in Petri dishes under aseptic conditions, incubated at 25°C and observed regularly for the mycelia growth. The potato dextrose agar medium was prepared by using the standard procedure (for one liter of PDA, 250 g peeled potato, 20 g agar and 20 g dextrose) and was sterilized in autoclave for 20 minutes at 121°C. Streptomycin sulphate was added into the medium before pouring into the Petri plates to inhibit the bacterial growth. The pathogen was identified by using the pictorial guide of Tousson and Nelson (1976). The isolated fungus was sub cultured for further studies.

In vitro study

Effect of different pH levels on the mycelia growth, spore concentration and biomass of different isolates of the pathogen

An *in vitro* experiment was conducted to determine the effect of three different pH levels (5.0, 5.6 and 9.0) on the mycelia growth, spore concentration and biomass of the ten different isolates of the pathogen collected from various regions of district Peshawar and Nowshera. The potato dextrose agar medium was adjusted to different pH levels with the help of pH meter. After sterilization, the media was poured into Petri plates and the inoculum plug of equal diameter (5 mm) of each isolate was inoculated at the centre of plate. The data was recorded on radial mycelia growth after 5 and 10 days of the incubation at 25°C. The micro-conidial sporulation per ml was recorded in 10 days old culture of isolates with the help of haemocytometer. The fresh biomass (g) was obtained by adding the equal diameter of inoculum of each isolate in

Table 1. Disease distribution of ten isolates of okra root rot collected from ten different locations of district Peshawar and Nowshera.

Districts	Isolates	Field 1	Field 2	Field 3	Field 4	Field 5	Mean
Peshawar	PI_1	75.87 GHI	64.78 MNO	74.34 G-J	65.35 MN	72.05 IJK	70.48 E
	PI_2	74.34 J	75.87 GHI	75.98 GHI	77.72 FG	78.54 EFG	76.48 D
	PI_3	52.76 Q	58.62 P	57.14 PQ	53.75 Q	58.85 P	56.22 G
	PI_4	83.33 D	82.75 DE	82.71 DE	77.26 GH	72.56 H-K	79.71 C
	PI_5	43.43 R	37.84 ST	41.87 RS	36.00 TU	41.67 RS	40.16 H
Nowshera	sNI_1	86.42 BCD	86.03 BCD	84.74 CD	80.54 D	82.55 DEF	84.65 B
	NI_2	93.57 A	89.70 AB	89.37 ABC	92.73 A	90.31 AB	91.14 A
	NI_3	61.12 NOP	70.34 JKL	74.69 G-J	74.59 G-J	70.34 JKL	69.89 E
	NI_4	34.83 TU	31.39 UV	35.86 TU	28.55 V	29.80 V	32.09 I
	NI_5	59.96 OP	57.02 PQ	65.89 LMN	65.84 LMN	66.48 LM	63.04 F
	Mean	66.57 B	65.27 B	68.26 A	66.04 B	65.79 B	66.39
LSD for Locations (L)		2.16					
LSD for Fields (F)		1.53					
LSD for L×F		4.83					
CV (%)		5.83					

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

flasks containing potato dextrose broth adjusted to different pH levels. The flasks were incubated at 25°C and the data were recorded after 15 days. For this, a Completely Randomized two factor factorial design was framed with three replications.

In vitro efficacy of fungicides on different isolates of the pathogen

A Completely Randomized two factor factorial experiment having three replications was designed to determine the *in vitro* efficacy of three different fungicides (Aliette, Copper oxychloride and Dithan M-45) @ 1000 ppm (Nisa *et al*, 2011) against ten different isolates of the pathogen collected from District Peshawar and Nowshera. Fungicides were incorporated into potato dextrose agar medium after sterilization and then poured into petri dishes. The inoculum plug of equal diameter for each isolate was placed at the centre of Petri plate. One treatment was kept as untreated check for each isolate. The plates were then incubated at 25°C and the data on colony diameter was taken after 5 and 10 days of incubation. The data on micro-conidial concentration per ml was recorded in 10 days old culture of isolates by using haemocytometer. For fresh biomass (g), the equal inoculum of the isolates was added in potato dextrose broth flasks which were treated with different fungicides. The data were recorded after 15 days of incubation at 25°C.

RESULTS

Disease distribution (Okra root rot) in District Peshawar and Nowshera

Significant differences ($P \leq 0.05$) were observed among the ten isolates of *Fusarium solani* of district Peshawar and Nowshera (Table 1). In District Nowshera, the disease was recorded most severe with highest (91.14 %) seedling mortality in Garhi Momin (NI_2) followed by Akbarpura (NI_1) as 84.65 % while lowest as 32.09 % in Balu (NI_4). In district Peshawar, the maximum disease severity with highest (79.71 %) seedling mortality was recorded in Jogian (PI_4) followed by Palosi (PI_2) as 76.48 % and lowest (40.16 %) in Budhni (PI_5).

Effect of different pH levels on colony diameter after five days of incubation

Significant differences ($P < 0.05$) in the colony diameter of isolates of *Fusarium solani* were observed at different pH levels. The maximum (80.56 mm) colony diameter was recorded by NI_2 followed by NI_1 (65.00 mm) and NI_5 (58.11 mm), whereas the lowest colony diameter was observed in case of NI_4 (31.67 mm). All the isolates grew well at pH level 5.0 (57.03 mm) and 5.6 (56.73 mm), whereas the lowest colony diameter (45.63 mm) was recorded at pH 9.0. In case of interaction combinations between different pH levels (5.0, 5.6 and 9.0) and five isolates of district Peshawar, the maximum (57.00 mm) radial colony diameter was measured in case of isolate

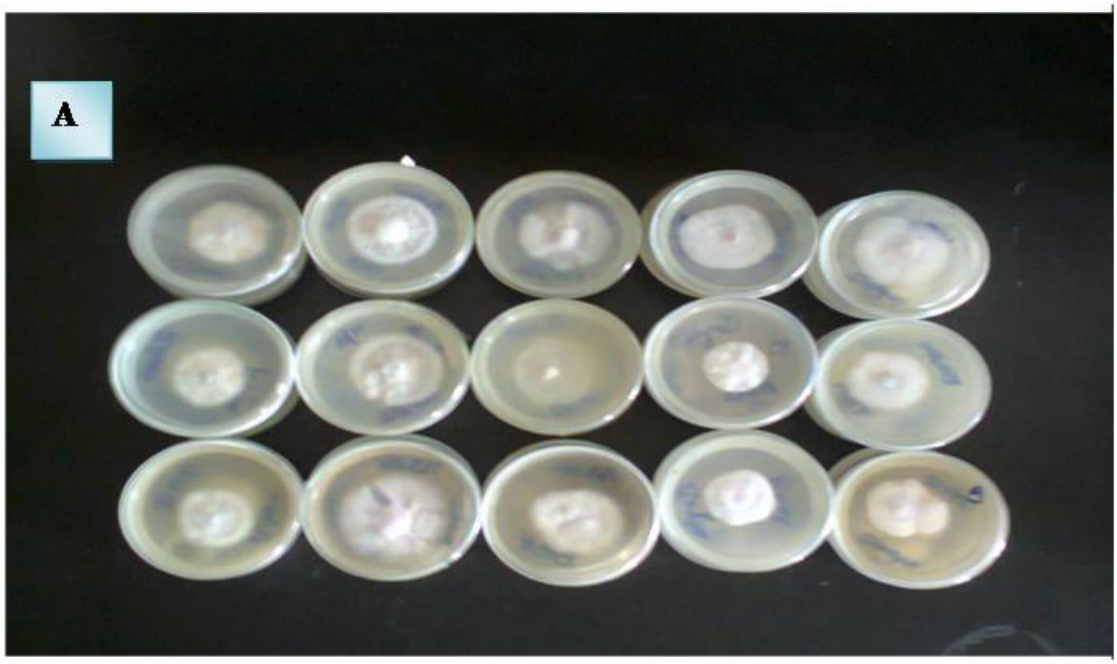

Figure 1. (A) Figure showing effect of different pH levels (5.0, 5.6 and 9.0) on the colony diameter (mm) of five isolates of Fusarium solani collected from different locations of District Peshawar after five (5) days of incubation at 25^0C.

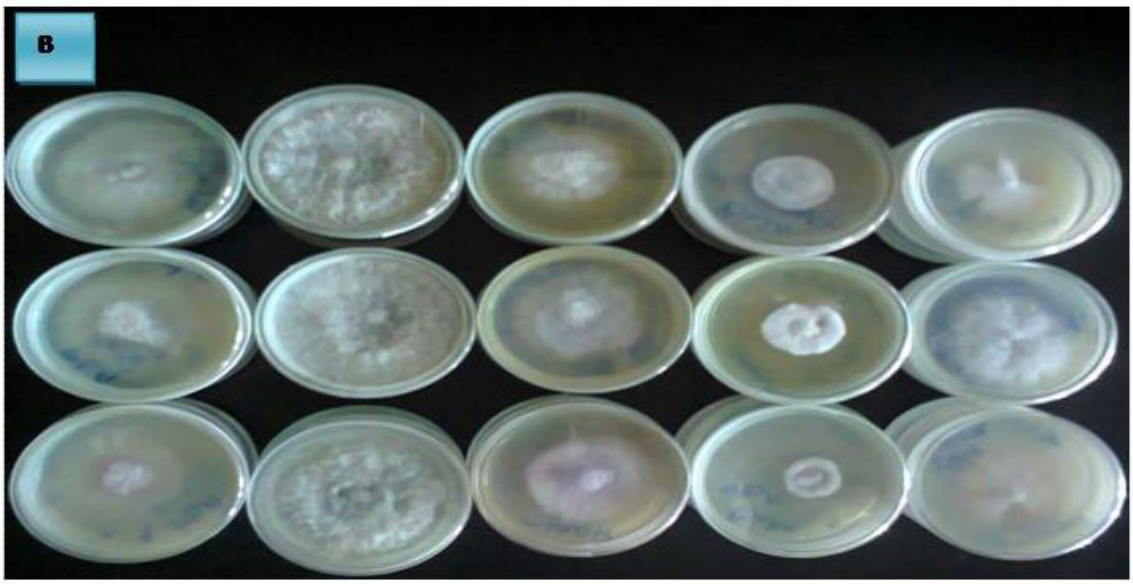

Figure 2 (B) Figure showing effect of different pH levels (5.0, 5.6 and 9.0) on the colony diameter (mm) of five isolates of *Fusarium solani* collected from different locations of district Nowshera after five (5) days of incubation at 25^0C.

PI_4 and PI_5 at 5.6 level of pH followed by isolate PI_2 and PI_3 (54.00 mm) at pH 5.0, whereas the lowest colony diameter was recorded (35.00 mm) by PI_4 at 9.0 pH level. The isolate PI_1 was found efficient in utilizing all the pH levels by exhibiting radial colony growth as 45.00 mm, 51.00 mm and 43.00 mm at 5.0, 5.6 and 9.0 levels of pH (Figure 1, A).

Contrary to the above results, the different isolates of district Nowshera exhibited maximum radial colony growth almost at every pH level. In case of interaction combinations between different pH levels and isolates of different locations, the isolate NI_2 exhibited maximum (85.00 mm, 82.33 mm and 74.30 mm) radial colony growth at 5.0, 5.6 and 9.0 pH levels (Figure 2, B). The

Table 2. Effect of different pH levels on colony diameter (mm) of ten different isolates of *Fusarium solani* after five days of incubation.

Districts	Isolates	pH levels			Mean
		5.0	5.6	9.0	
Peshawar	PI_1	45.00 L	51.00 JK	43.00 M	46.33 G
	PI_2	54.00 H	53.00 HI	*50.00 K	52.33 D
	PI_3	54.00 H	52.00 IJ	*45.00 L	50.33 F
	PI_4	40.00 N	57.00 G	35.00 O	44.00 H
	PI_5	53.00 HI	57.00 G	44.00 M	51.33 E
Nowshera	NI_1	75.00 C	70.00 B	50.00 K	65.00 B
	NI_2	85. 00 A	82.33 B	74..30 C	80.56 A
	NI_3	60.00 F	50.00 K	45.00 L	51.67 DE
	NI_4	40.00 N	35.00 O	20.00 P	31.67 I
	NI_5	64.33 E	60.00 F	50.00 K	58.11 C
	Mean	57.03A	56.73 A	45.63 B	53.13

LSD for Fields (F) 0.86
LSD for Isolates (T) 0.47
LSD for I×T 1.49
CV (%) 1.72
*Patches were formed in radial colony growth.
Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

Figure 3 (C) Petri plates showing effect of different pH levels (5.6, 5.0 and 9.0) on colony diameter (mm) of five isolates of *F. solani* on PDA after ten (10) days of incubation at 25 ^{0}C,collected from different locations of District Peshawar**.**

isolate NI_1 showed radial colony growth (75.00 mm and 70.00 mm) at 5.0 and 5.6 pH levels, whereas the isolate NI_4 showed minimum (20.00 mm) radial colony growth at 9.0 pH. In interaction combinations, the isolates accomplished maximum radial colony growth at 5.0, 5.6 and 9.0 pH levels (Table 2).

Effect of different pH levels on colony diameter of *F. solani* after 10 days of Incubation at 25^{0}C

The isolates showed significant differences ($P<0.05$) at different pH levels. The maximum colony diameter (96.67 mm) was exhibited by NI_2 followed by NI_3 (80.78 mm)

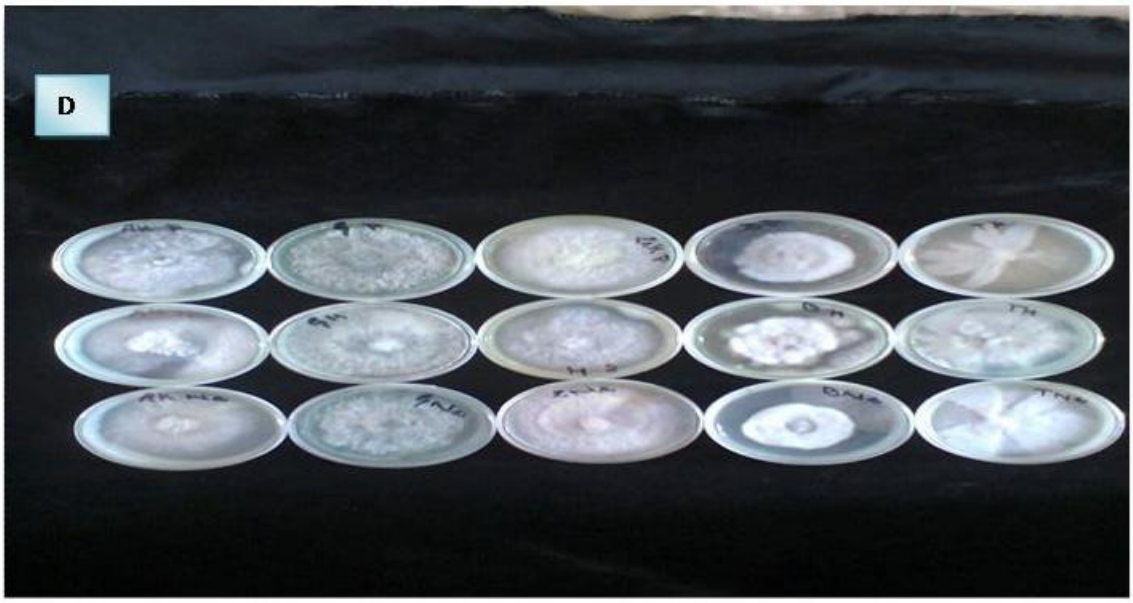

Figure 4. (D) Petri plates showing effect of different pH levels (5.6, 5.0 and 9.0) on colony diameter (mm) of five isolates of *F. solani* on PDA after ten (10) days of incubation at 25 ^{0}C,collected from different locations of District Nowshera**.**

and NI_1 (78.26 mm), while the lowest colony diameter was accomplished by NI_4 (57.89 mm). The isolates grew best at pH 5.0 and 5.6 than 9.0 with 76.73 mm, 77.14 mm and 68.13 mm. In case of interactions combinations between different pH levels and isolates of District Peshawar, the maximum (80.00 mm) radial colony growth of isolate PI_1 was recorded at 5.6 pH level followed by PI_4 (78.00 mm) and PI_5 (75.16 mm) at the same pH level (5.6). On the other hand, the isolate PI_5 exhibited minimum (61.37 mm) radial colony growth at pH level of 9.0 (Figure 3).

The isolates of district Nowshera exhibited maximum radial colony growth at every pH level (Figure 4). In case of interaction combinations between different pH levels and isolates, the maximum (100.00 mm and 90.00 mm) radial colony growth was accomplished by the isolate NI_2 at 5.0, 5.6 and 9.0 level of pH whereas the isolate NI_3,

NI_1 and NI_5 exhibited radial colony growth of 85.00 mm, 83.78 mm and 80.00 mm at pH 5.0. The isolate NI_1 and NI_3 gives maximum (81.00 mm and 80.00 mm) radial colony growth at pH 5.6. On the other hand, the isolate NI_4 exhibited minimum (51.67 mm) radial colony growth at 9.0 level of pH. In interaction combinations, the isolates accomplished minimum radial colony growth at 9.0 pH level as compared to 5.6 and 5.0 pH level (Table 2).

Effect of different pH levels on fresh biomass (g) after 15 days of incubation

The isolates were found significantly different (P<0.05) by the effect of different pH levels. The highest (2.52 g) biomass was recorded by isolate NI_2 followed by NI_3 (2.23 g) and NI_1 (1.90 g), whereas lowest showed by NI_4 (0.87 g). Among the different pH levels, the isolates showed highest (1.97 g) biomass at 5.0 and 5.6 pH level (1.89 g), whereas the minimum (1.31 g) was observed at pH 9.0. In case of interaction combinations between different pH levels and isolates of district Peshawar, the maximum biomass (2.20 g) was recorded in case of isolate PI_2 at 5.0 level of pH followed by PI_1 and PI_5 (2.0 g) at pH level 5.6, whereas the lowest was observed in case of PI_4 (0.93 g) at 9.0 pH. The isolate $PI_1$was found to be efficient in utilizing all the pH levels (5.0, 5.6 and 9.0) by exhibiting biomass of 1.80 g, 2.0 g and 1.57 g at 5.0, 5.6 and 9.0 levels of pH (Table 4).

In case of interaction combinations between isolates of district Nowshera and different pH levels, all the isolates showed maximum biomass at every pH levels. The isolate NI_2 showed highest (2.97 g, 2.50 g and 2.10 g) biomass at 5.0, 5.6 and 9.0 pH level, while the isolate NI_3 showed 2.53 g and 2.37 g of biomass at 5.0 and 5.6 pH. The isolate NI_4 showed lowest (0.50 g) biomass at a pH level of 9.0. In interaction combinations, the isolate showed maximum biomass at 5.0 and 5.6 as compared to 9.0 level of pH.

Effect of different pH levels on spore concentration

Significant (P<0.05) effect of different pH levels was observed on spore concentration per ml. Among the different isolates, the highest (3.23×10^6) spore

Table 3. Effect of different pH levels on colony diameter (mm) of ten different isolates of *Fusarium solani* after ten (10) days of incubation.

Districts	Isolates	pH levels			Mean
		5.0	5.6	9.0	
Peshawar	PI_1	70.00 H	80.00 D	68.00 I	72.67 E
	PI_2	75.00 F	70.22 H	*65.00 J	70.07 F
	PI_3	74.44 F	70.47 GH	*63.00 K	69.30 FG
	PI_4	64.75 J	78.00 E	63.00 K	68.58 G
	PI_5	72.00 G	75.16 F	61.37 L	69.51 FG
Nowshera	NI_1	83.78 C	81.00 D	70.00 H	78.26 C
	NI_2	100.00 A	100.00 A	90.00 B	96.67 A
	NI_3	85.00 C	80.00 D	77.28 E	80.76 B
	NI_4	62.33 KL	59.67 M	51.67 N	57.89 H
	NI_5	80.00 D	76.89 E	72.00 G	76.30 D
	Mean	76.73 A	77.14 A	68.13 B	74.00

LSD for Isolates (I)	0.93
LSD for Treatments (T)	0.51
LSD for I×T	1.62
CV (%)	1.34

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

Table 4. Effect of different pH levels on biomass (g) of ten different isolates of *Fusarium solani* after 15 days of incubation.

Districts	Isolates	pH levels			Mean
		5.0	5.6	9.0	
Peshawar	PI_1	1.80 GH	2.0 EF	1.57 IJ	1.79 DE
	PI_2	2.20 CD	1.90 FG	1.17 K	1.76 E
	PI_3	1.90 FG	1.70 HI	1.0 KL	1.53 F
	PI_4	1.13 K	1.50 J	0.93 L	1.19 G
	PI_5	1.61 J	2.0 EF	1.0 KL	1.53 F
Nowshera	NI_1	2.20 CD	2.10 DE	1.43 J	1.90 C
	NI_2	2.97 A	2.50 B	2.10 DE	2.52 A
	NI_3	2.53 B	2.37 BC	1.80 GH	2.23 B
	NI_4	1.17 K	0.93 L	0.50 M	0.87 H
	NI_5	2.20 CD	1.90 FG	1.57 IJ	1.89 CD
	Mean	1.97 A	1.89 B	1.31 C	1.72

LSD for Isolates (I)	0.11
LSD for Treatments (T)	0.06
LSD for I×T	0.19
CV (%)	6.84

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

concentration (ml^{-1}) was recorded in case of isolate NI_2 followed by NI_3 (3.01×10^6) and NI_1 (2.51×10^6), whereas the lowest spore concentration (0.76×10^6) was recorded in case of isolate NI_4. The isolates showed maximum (2.29×10^6 and 2.16×10^6) spore production at pH level 5.0 and at 5.6, while the lowest spore concentration (1.62×10^6) was observed at pH 9.0. In case of interaction combinations between different pH levels and isolates of district Peshawar, the highest (2.46×10^6) spore production was recorded in case of isolate PI_1 at pH level of 5.6 followed by PI_2 (2.32×10^6) and PI_1 (2.22×10^6) at pH 5.0. The lowest (0.75×10^6) spore production was recorded in case of isolate PI_4 at pH 9.0.

In case of interaction combinations between different pH levels and isolates of district Nowshera, the isolates showed maximum spore production almost at every pH

Table 5. Effect of different pH levels on spore concentration/ml of ten different isolates of *Fusarium solani*.

Districts	Isolates	pH levels Spore concentarion (10^6)			Mean
		5.0	5.6	9.0	
Peshawar	PI_1	2.22 G	2.46 EF	1.91 H	2.20 D
	PI_2	2.32 FG	1.96 H	1.41 I	1.90 E
	PI_3	1.53 I	1.41 I	1.10 J	1.35 G
	PI_4	1.45 I	1.64 I	0.75 KL	1.28 G
	PI_5	1.64 I	1.91 H	0.98 JK	1.51 F
Nowshera	NI_1	2.96 CD	2.60 E	1.98 H	2.51 C
	NI_2	3.58 A	3.26 B	2.85 D	3.23 A
	NI_3	3.26 B	3.18 BC	2.60 E	3.01 B
	NI_4	1.10 J	0.74 L	0.43 M	0.76 H
	NI_5	2.83 D	2.46 EF	2.22 G	2.50 C
	Mean	2.29 A	2.16 B	1.62 C	2.02

LSD for Isolates (I) 0.13×10^6
LSD for Treatments (T) 0.07×10^6
LSD for I×T 0.23×10^6
CV (%) 6.89

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

Table 6. Effect of fungicides on colony diameter (mm) of ten different isolates of *Fusarium solani* after five (5) days of incubation.

Districts	Isolates	Treatments				Mean
		Control	Aliette	Copper oxychloride	Dithane M-45	
Peshawar	PI_1	60.00 E	56.67 FG	46.67 JK	5.00 S	42.08 C
	PI_2	57.33 F	50.00 I	54.00 H	5.00 S	41.58 C
	PI_3	54.00 H	50.00 I	37.33 O	0.00 T	35.33 E
	PI_4	64.33 D	55.00 GH	48.00 J	8.00 R	43.83 E
	PI_5	50.00 I	32.00 P	26.00 Q	0.00 T	27.00 G
Nowshera	NI_1	75.00 B	60.00 E	42.00 M	0.00 T	44.25 D
	NI_2	90.00 A	*90.00 A	*90.00 A	5.00 S	68.75 A
	NI_3	70.00 C	50.00 I	45.00 KL	0.00 T	41.25 C
	NI_4	42.00 M	25.00 Q	40.00 N	5.00 S	28.00 F
	NI_5	60.00 E	43.33 LM	40.00 N	5.00 S	37.08 C
	Mean	62.27 A	51.20 B	46.90 C	3.30 D	40.92

LSD for Isolates (I) 0.93
LSD for Treatments (T) 0.59
LSD for I×T 1.85
CV (%) 2.79
*Resistant to fungicide.

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

level (5.0, 5.6 and 9.0). The isolate NI_2 exhibited highest (3.58×10^6, 3.26×10^6 and 2.85×10^6) spore production at 5.0, 5.6 and 9.0 level of pH, while the isolate NI_3 showed 3.26×10^6 and 3.18×10^6 spores at 5.0 and 5.6 level of pH. The least (0.43×10^6) spore production was recorded by NI_4 at pH level 9.0. In interaction combinations, the isolates showed highest spore production at 5.0 and 5.6 as compared to 9.0 level of pH (Table 5).

Effect of fungicides on colony diameter after five (5) days of incubation

The isolates showed significant differences ($P<0.05$) in

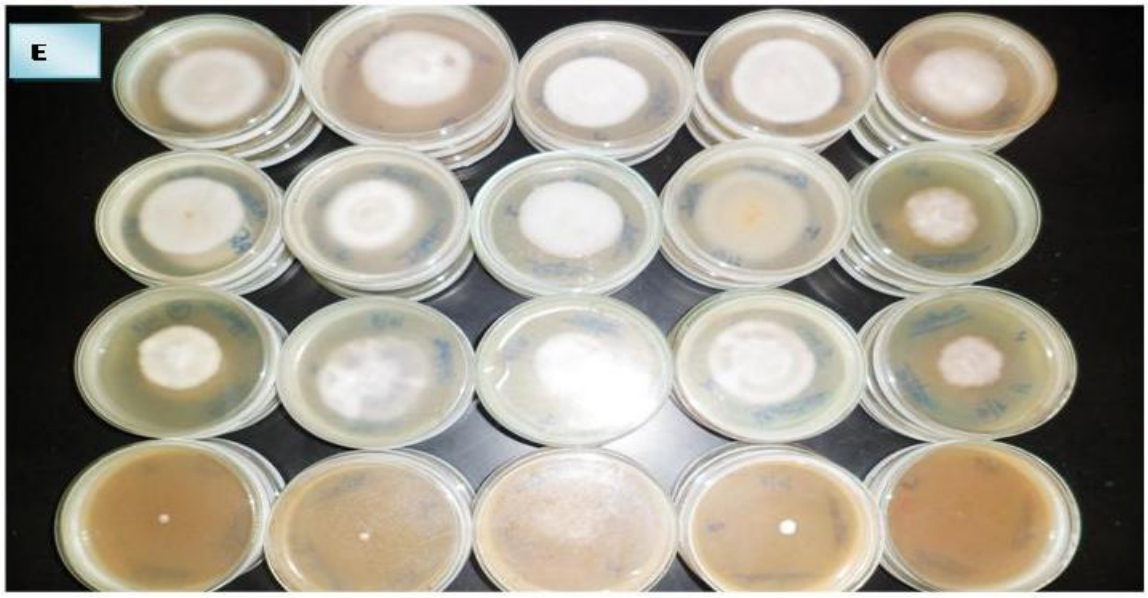

Figure 5 (E) Figure showing effect of three fungicides (control, Aliette, Copper oxychloride and Dithane M-45) on colony diameter (mm) of five isolates of F. solani on PDA after five (5) days of incubation at 25 0C collected from different locations of District Peshawar.

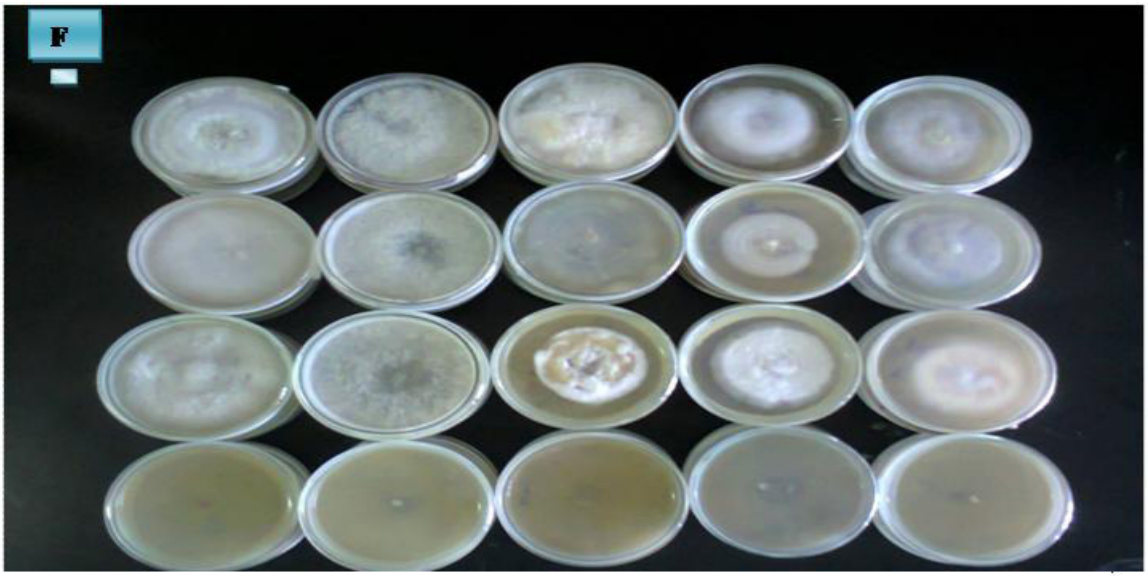

Figure 6 (F) Figure showing effect of three fungicides (control, Aliette, Copper oxychloride and Dithane M-45) on colony diameter (mm) of five isolates of *F. solani* on PDA after five (5) days of incubation at 25 ^{0}C collected from different locations of District Nowshera.

mycelia growth at 1000 ppm of fungicides. The maximum (68.75 mm) colony diameter was recorded in isolate NI_2, followed by NI_1 (44.25 mm) and PI_4 (43.83 mm), while the lowest (27.00 mm) was accomplished by PI_5 (Table 6). All the isolates were found susceptible to T_3 by exhibiting minimum (3.30 mm) radial colony diameter than T_2 (46.90 mm) and T_1 (51.20 mm) as compared to T_0 (62.27 mm). In case of interactions between different fungicides and isolates of district Peshawar, the minimum (56.67 mm and 55.00 mm) inhibition in radial colony growth as compared to T_0 (60.00 mm and 64.33 mm) was observed by the isolate PI_1 and PI_4 in T_1 followed by isolate PI_2 (54.00 mm) in T_2 as compared to T_0 (57.33 mm). The complete inhibition in radial colony growth was exhibited by isolate PI_3 and PI_5 by observing zero growth (Fig. 4.5).

In case of interaction combinations between different fungicides and isolates of district Nowshera, the isolate NI_2 was found resistant to T_1 and T_2 by observing same

Table 7. Effect of fungicides on colony diameter (mm) of ten different isolates of *Fusarium solani* after ten (10) days of incubation.

Districts	Isolates	Treatments				Mean
		Control	Aliette	Copper oxychloride	Dithane M-45	
Peshawar	PI_1	90.00 B	85.00 E	65.00 J	5.00 P	61.25 D
	PI_2	87.33 CD	75.00 H	79.00 G	5.00 P	61.58 D
	PI_3	86.33 CDE	80.00 FG	47.33 N	0.00 Q	53.42 F
	PI_4	86.00 CDE	80.00 FG	73.67 H	43.67 O	70.83 B
	PI_5	88.00 BC	54.67 L	59.00 K	0.00 Q	50.42 G
Nowshera	NI_1	90.00 B	86.00 CDE	82.00 F	0.00 Q	64.50 C
	NI_2	100.00 A	*100.00 A	*100.00 A	5.00 P	76.25 A
	NI_3	85.67 DE	81.33 F	60.00 K	0.00 Q	56.75 E
	NI_4	65.00 J	50.00 M	60.00 K	5.00 P	45.00 H
	NI_5	68.33 I	60.00 K	65.00 J	5.00 P	49.58 G
	Mean	84.67 A	75.20 B	69.10 C	6.87 D	58.96

LSD for Isolates (I) 1.13
LSD for Treatments (T) 0.71
LSD for I×T 2.25
CV (%) 2.35

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

radial colony growth as in T_0 (90.00 mm). The least (60.00 mm and 50.00 mm) inhibition in radial colony growth was observed by $NI_1$and NI_3 in T_1 as compared to T_0 (75.00 mm and 70.00 mm) while the complete inhibition in radial colony growth was measured by NI_1 and NI_3 by observing no growth in T_3. In interaction combinations, the highest inhibition in radial colony growth was measured in T_3 as compared to T_1 and T_2 (Figure 6).

Effect of fungicides on colony diameter after ten (10) days of incubation

Significant differences ($P<0.05$) in colony diameter of *F. Solani* isolates (Table 7) were observed in response to different fungicides. The maximum (76.25 mm) colony diameter was recorded by NI_2, followed by PI_4 (70.83 mm) and NI_1 (64.50 mm), whereas the lowest was observed by NI_4 (45.00 mm). The isolates were found susceptible to T_3 with minimum (6.87 mm) colony diameter than T_2 (69.10 mm) and T_1 (75.20 mm) as compared to T_0 (84.67 mm). In case of interaction combinations between isolates of district Peshawar and different fungicides application, the minimum (85.00 mm) inhibition in radial colony growth was measured by the isolate PI_1, followed by PI_3 and PI_4 (80.00 mm) as compared to T_0 (90.00mm, 86.33 mm and 86.00 mm) in treatment T_1. The minimum (79.00 mm) inhibition in radial colony growth was measured by PI_2 as compared to T_0 (87.33 mm) in T_2 whereas complete inhibition was measured by PI_3 and PI_5 by observing zero growth in T_3 (Figure 7).

In case of interactions between isolates of district Nowshera and different fungicides application, the isolate NI_2 was found resistant to T_1 and T_2 by observing radial colony growth same as in T_0 (100.00 mm) and lowest (86.00 mm and 81.33 mm) inhibition in radial colony growth was measured by isolate NI_1 and NI_3 as compared to T_0 (90.00 mm and 85.67 mm) in T_1. The minimum (82.00 mm) inhibition in radial colony growth was measured by isolate NI_1 as compared to T_0 (90.00 mm). Complete inhibition was measured by isolate NI_1 and NI_3 by observing no growth in T_3. In interaction combinations, the maximum inhibition in radial colony growth was measured in T_3 as compared to T_1 and T_2 (Figure 8).

Effect of fungicides on biomass (g) after 15 days of incubation

Significant differences in fresh fungal biomass were observed as compared to untreated check (control). The highest (2.08 g) biomass was recorded in case of NI_2, followed by NI_1 (1.86 g) and PI_4 (1.81 g) while the lowest was recorded in case of NI_4 (0.68 g). The isolates were found susceptible to T_3 with the lowest biomass of 0.24 g than T_1 (1.54 g) and T_2 (1.55 g) as compared to T_0 (2.18 g). In case of interaction combinations between fungicides and isolates of district Peshawar, the minimum inhibition (2.10 g and 1.80 g) in biomass was recorded in

Figure 7 (G) Figure showing effect of three fungicides (Control, Aliette, Copper oxychloride and Dithane M-45) on colony diameter (mm) of five isolates of F. solani on PDA after ten (10) days of incubation at 250C collected from different locations of District Peshawar.

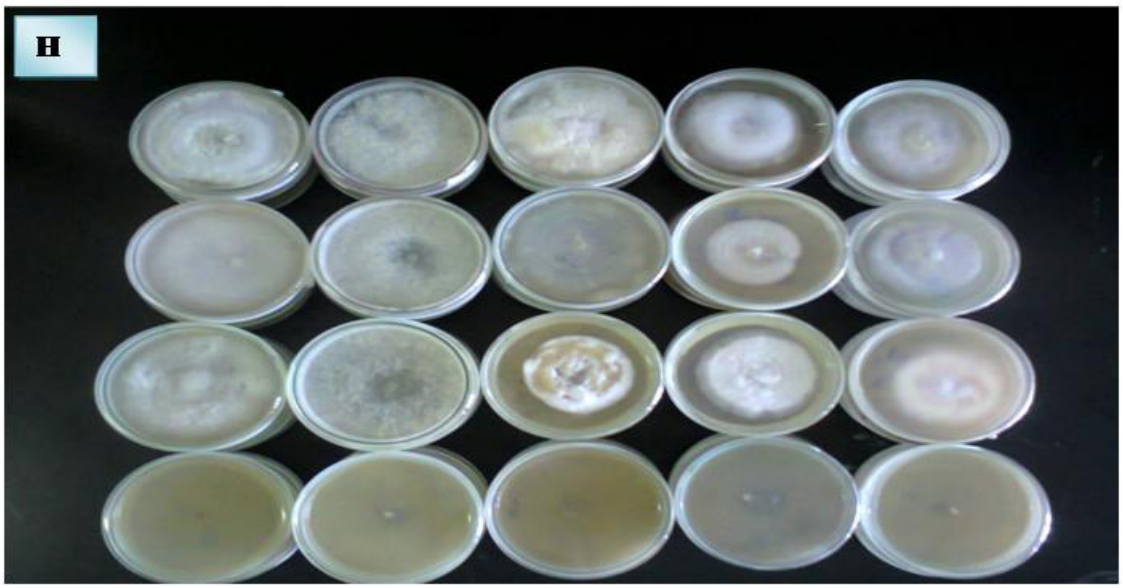

Figure 8 (H) Figure showing effect of three fungicides (Control, Aliette, Copper oxychloride and Dithane M-45) on colony diameter (mm) of five isolates of *F. solani* on PDA after ten (10) days of incubation at 25^0C collected from different locations of District Nowshera.

isolate PI_4 and PI_1 in T_1 as compared to T_0 (2.43 g and 2.20 g) followed by PI_2 by exhibiting 1.80 g of biomass as compared to T_0 (2.30 g). The complete inhibition of in mycelia growth was recorded in isolate PI_3 and PI_5 in T_3.

In interaction combinations between fungicides and isolates of district Nowshera, the isolate NI_2 gave minimum (2.30 g and 2.54 g) inhibition in mycelia growth as compared to T_0 (3.07) in T_1 and T_2. The isolate NI_1 exhibited 2.43 g and 2.20 g of biomass in T_1 and T_2 as compared to T_0 (2.81 g), whereas complete inhibition was recorded by isolate N_I and NI_3 in T_3 by observing no growth. In interaction combinations, the maximum inhibition by the isolates was recorded in T_3 as compared to T_1 and T_2 (Table 8).

Table 8. Effect of fungicides on biomass (g)) of ten different isolates of *Fusarium solani* after 15 days of incubation.

Districts	Isolates	Treatments				Mean
		Control	Aliette	Copper oxychloride	Dithane M-45	
Peshawar	PI_1	2.20 DEF	1.80 K	1.60 JK	0.20 PQ	1.38 C
	PI_2	2.30 CDE	1.60 JK	1.80 HIJ	0.30 P	1.50 C
	PI_3	2.00 FGH	1.50 K	1.00 LM	0.00 Q	1.13 D
	PI_4	2.43 CD	2.10 EFG	1.70 IJK	1.00 LM	1.81 B
	PI_5	1.84 G-J	0.80 MN	1.20 L	0.00 Q	0.96 E
Nowshera	NI_1	2.81 AB	2.43 CD	2.20 DEF	0.00 Q	1.86 B
	NI_2	3.07 A	*2.30 CDE	*2.54 BC	0.43 OP	2.08 A
	NI_3	2.10 EFG	1.50 K	1.20 L	0.00 Q	1.20 D
	NI_4	1.15 L	0.60 NO	0.80 MN	0.20 PQ	0.68 F
	NI_5	1.90 GHI	1.10 L	1.50 K	0.30 P	1.20 D
	Mean	2.18 A	1.54 B	1.55 B	0.24 C	1.38

LSD for Isolates (I) 1.15
LSD for Treatments (T) 0.09
LSD for I×T 0.29
CV (%) 13.00

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

Table 9. Effect of fungicides on spore concentration/ml of ten different isolates of *Fusarium solani*.

Districts	Isolates	Treatments				Mean
		Spore concentration (10^6)				
		Control	Aliette	Copper oxychloride	Dithane M-45	
Peshawar	PI_1	2.25 DE	1.76 F	1.55 H	0.04 O	1.40 DE
	PI_2	2.35 D	1.46 HI	1.78 F	0.05 O	1.41 D
	PI_3	1.07 K	1.30 IJ	1.07 K	0.00 O	1.06 F
	PI_4	2.17 E	1.84 F	1.34 I	0.69 LMN	1.51 C
	PI_5	1.58 GH	0.73 LM	0.86 L	0.00 O	0.79
Nowshera	NI_1	2.66 C	2.16 E	1.85 F	0.00 O	1.67 B
	NI_2	3.41 A	*2.61 C	*2.88 B	0.09 O	2.25 A
	NI_3	2.16 E	1.75 FG	1.34 I	0.00 O	1.31 E
	NI_4	1.12 K	0.65 MN	0.52 N	0.02 O	0.58 H
	NI_5	1.83 F	1.13 JK	1.34 I	0.03 O	1.08 F
	Mean	2.14 A	1.54 B	1.45 C	0.09	1.31

LSD for Isolates (I) 0.09×10^6
LSD for Treatments (T) 0.05×10^6
LSD for I×T 0.18×10^6
CV (%) 8.24

Isolates code;
Acheni Payan (PI_1), Palosi (PI_2), Chamkani (PI_3), Jogian (PI_4), Budhni (PI_5), Akberpura (NI_1), Garhi Momin (NI_2), Zakhi Charbagh (NI_3), Balu (NI_4) , Tarru Jabba (NI_5).
Values followed by same letter (s) are not significantly different from one another at 5% level of significance.

Effect of fungicides on spore concentration per ml

Significant effects of fungicides were observed on per ml of spore concentration. Among different isolates, the highest (2.25×10^6) spore production was recorded in isolate NI_2, followed by NI_1 (1.67×10^6) and PI_4 (1.51×10^6), whereas the lowest ml^{-1} spore concentration (0.58×10^6) was recorded in case of NI_4. The highest inhibition in spore production (0.09×106) was recorded in T_3 than T_2 (1.45×10^6) and T_1 (1.54×10^6) as compared to T_0 (2.14×10^6). In case of interaction combinations between fungicides and isolates of district Peshawar, the minimum (1.76×10^6 and 1.78×10^6) inhibition in spore production was recorded by isolate PI_1 and PI_3 in T_1 and T_2 as

compared to T_0 (2.25×10^6 and 2.35×10^6). The complete inhibition in spore production was recorded by PI_3 and PI_5 in T_3.

In interaction combinations between fungicides and isolates of district Nowshera, the minimum (2.88×10^6 and 2.61×10^6) inhibition in spore production was recorded in NI_2 in T_2 and T_1 as compared to T_0 (3.41×10^6), while the complete inhibition was recorded in NI_1 and NI_3 by observing zero growth in T_3. In interaction combinations, the maximum inhibition in spore production was recorded in T_3 as compared to T_1 and T_2 (Table 9).

DISCUSSION

Okra plants are attacked by a number of soil borne fungi and among them *Fusarium solani*, the causal organism of root rot is considered as the most destructive pathogen, as the disease appears at seedling stage. Since okra is a summer vegetable crop and the fungus also favours higher soil temperatures, the disease causes severe losses. The fungus is highly adoptable to different environmental conditions due to which high variations exist among the isolates. As far as better management is concerned, the characterization of the isolates is necessary to help manage this menace in a better way. In the present study, a survey was conducted to find out the disease distribution of ten different okra growing regions of district Peshawar and Nowshera, and also the various isolates of *Fusarium solani* were assessed by evaluating the *in vitro* effect of different pH levels and the efficacy of fungicides.

The disease was distributed in different areas of district Peshawar and Nowshera and was found most severe in areas of district Nowshera with the maximum seedling mortality. The disease may be severe due to poor cultural practices and climatic factors such as plant spacing and higher soil temperatures. It is evident from the previous studies that cultural and climatic factors favors the disease (Aviles *et al*., 2003). This study was further supported by Malvick (2002) that the disease is favoured by higher soil temperature and moderate soil moisture.

The isolates of *F. solani* were found significantly different by their characterization at different pH levels *in vitro*. The isolates grew best at 5.0 and 5.6 level of pH as compared to 9.0. These results were supported by Gupta *et al*., (2010) that isolates of *F. solani* isolated from guava were observed highly variable by their assessment at different pH levels. It was also reported that all the isolates showed maximum colony growth at 5.0 and 5.5 than 8.0 level of pH. These results are also in line with the results of Khilari and Ahmed *et al*., (2004) and Gandhara *et al*., (2004). There were some isolates that were found very efficient in utilizing the 5.0. 5.6 and 9.0 level of pH. Similar results were reported by Gandhara *et al*., (2004) that among various isolates of *F. oxysporum,* an isolate showed maximum growth in 5.0, 6.0 and 9.0 level of pH. This was further supported by Caracuel *et al*., (1993) that isolates of *F. oxysporum* showed maximum growth on basic medium due to the expression of ena1 gene which activated by the higher concentrations of Na^+ and was responsible to regulate the internal environment of the fungus by regulating constant condition of pH. These results are in harmony with the findings of Jamaria (1972), that strains of *Fusarium solani* grow well on a wide range of pH ranging from 3.2 to 8.3.

The isolates of *F. solani* showed variable responses to the effect of different fungicides. The isolates were found most susceptible to Dithane M-45 and was proved to be the best fungicide in controlling the pathogen. Similarly Ahmed *et al*., (2012) reported that the complete inhibition in mycelial growth was observed by Dithane M-45. These results were further supported by Nisa *et al*, (2011) and Mamza *et al*., (2008) and Rajput *et al*., (2012). The isolates were found less sensitive to Aliette and Copper oxychloride. However, some isolates were found resistant to Aliette and Copper oxychloride. This may be due to the selection pressure of particular fungicides which lead to the development of resistant genes. Similar results were reported by Rajput *et al.,* (2012) that copper oxychloride was found less effective fungicide in controlling *F. solani*. Amanda and Bruce, (1996) suggested Aliette as the less effective fungicide in controlling the *F. Oxysporum,* and the fungicide may be effected at higher concentrations. However some isolates were found very less sensitive to Aliette.

The present results are in close proximity with the findings of Maitlo *et al*., 2013 who reported seven fungicides which were applied at three different concentrations. All the fungicides inhibited the colony growth of *Fusarium solani*. But the contact fungicide (Dithane M-45) proved to be most efficient, showed complete inhibition of *Fusarium solani* followed by Alliette and Copper oxychloride at 100,150 and 50 ppm concentration.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The disease was distributed in different locations and were found most severe with highest percent seedling mortality in district Nowshera.
2. The isolates were found significantly different by their characterization at different pH levels. The isolates showed maximum radial colony growth at 5.0 and 5.6 level of pH as compared to 9.0. However, some isolates were found very efficient in utilizing all the pH levels.
3. *In vitro*, all the isolates were found most susceptible to Dithane M-45 whereas less susceptable to Aliette and Copper oxychloride.

Recommendations

1. A detail study is required for the confirmation of variations that exists among the various isolates of *F. solani* by using other factors like growth in different media, temperature.
2. The alkaline soils are recommended to grow the crop but not highly alkaline which locks up nutrients in the soil.
3. Dithane M-45 is recommended an effective fungicide for the *in vitro* management of *F. solani*.

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