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CHEMICAL AND BIOLOGICAL CONTROL OF *FUSARIUM* ROOT ROT OF OKRA

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Abstract

The effect of *Trichoderma harzianum* and *T. viride* and three fungicides i.e. Benlate, Ridomil and Dithane M-45, was investigated on the management of Fusarium root rot in okra under screen house conditions. The disease incidence and percent mortality were significantly reduced ($p \leq 0.05$) by all the fungicides and antagonists when compared with untreated check plants. *T. harzianum* and Ridomil increased the yield by 83.6 and 80.2 %, respectively. Under *In vitro* study, Dithane M-45 proved to be more effective than Ridomil and Benlate used alone or integrated with any of the antagonists. Maximum colony diameter of the pathogen (6.9 cm) was recorded in control treatment. *T. viride* was less effective when used alone or with any fungicide, while *T. harzianum* reduced the colony diameter by 43.5 % under *In vitro*.

Introduction

Okra (*Abelmoschus esculentus* L.Moench) locally known as Bhindi, is one of the most important vegetable crops of Pakistan. Okra is apt to the attack of fungi such as *Macrophomina phaseolina*, *Rhizoctonia bataticola, R. solani, Fursarium solani*, *Pythium butleri*, *Phytophthora palmivora*, *Cercospora abelmoschii* and *Erysiphe cichoracearum* (Mithal, 2006). Fusarium root rot is known to decrease both quantity and quality of major crops including tomato (Parveen *et al*., 1993) and other vegetables (Ghaffar, 1995), and soybean (Mousa, 1994). The disease is caused by *Fusarium solani* (Rahim *et al*., 1992). Its incidence has been reported 10-80%, with a maximum (55-80%) in plants grown as kitchen / home gardening and minimum (10-45%) in the crop sown under field conditions. The infected plants are scattered or found in groups when the crop is grown on ridges in field or cultivated on flat beds as kitchen / home gardening. Severely infected plants are found dead, their roots turn dark brown and badly damaged. The fungus perpetuates in the soil or attached to infected plants. Naturally infected seeds of okra appear brown to black and show die-back and root rot symptoms (Mithal, 2006).

Trichoderma harzianum and *T. viride* have been reported to inhibit the mycelial growth of all root rot fungi. Soil infestation with each of the bio-control agents tested reduced the percentage of infected plants and severity of the disease (Mithal, 2006; Faheem *et al*., 2010). Ghaffar (1993) used different chemicals to control root rot caused by fungi. Seed dressing with Agrosan and Furadan resulted in 38% reduction of *Fusarium* infecting okra. Combination of Benomyl and Captan was effective against all root rot fungi. Of the various bio-agents, *Trichoderma* species have been known to suppress many soil borne fungi under greenhouse and field conditions. *T. harzianum* has been found to antagonize fungal plant pathogen and parasitic nematodes (Saifullah, 1996; Saifullah & Thomas, 1997; Siddiqui *et al*., 1999).

Trichoderma spp., are present in substantial number in nearly all agricultural soils and decaying wood (Khuls *et al*., 1999). *Trichoderma* has multiple mechanisms for control of pathogens. It may grow towards hyphae of other fungi and compete with them for food, space and other resources, coil around them and degrade cell walls of the target fungi, hence limiting the growth and activity and/or by direct consumption of the contents of the target pathogens. Individual strains may produce antibiotics which are harmful for the integrity of the target pathogen (Benitez *et al*., 2004).

This paper reports the efficacy of different chemical and biological control agents for the management of Fusarium root rot in Okra.

Materials and Methods

Isolation and identification of *Fusarium solani* from dead okra roots: Infected okra seedlings were collected from Taru Jaba, Peshawar. Small pieces of the roots were surface sterilized with 0.1% solution of mercuric chloride for 15-30 seconds, rinsed with sterile distilled water to remove the disinfectant and blotted dry. The treated pieces were placed on Potato Dextrose Agar (PDA) medium in Petri dishes under aseptic conditions and incubated at 25°C for 1 week. Mycelial growth that developed after 7 days was examined. *Fusarium solani* was isolated and subcultured for further studies.

***In vitro* study:** Two species of *Trichoderma,* viz., *T. viride* and *T. harzianum*, obtained from the Plant Pathology Department, Khyber Pakhtunkhwa Agricultural University, Peshawar and three different fungicides viz., Benlate, Ridomil and Dithane M-45 (with different dosages i.e., 100, 200, 300 mg/L) obtained from Agricultural Research Institute Tarnab, Peshawar were tested against *F. solani* alone and in combination with *Trichoderma* spp. The fungicides were incorporated in the medium before pouring into plates. *F. solani* was inoculated at the centre of Petri dish having PDA medium as 1 cm^2 inoculum plug cut out from 2-week old culture with a sterile scalpel. Inoculum plugs of the same size from the 2-week old culture of the *Trichoderma* spp. were applied at four sites around *F. solani*. The dishes were sealed with parafilm and incubated at 25°C for 15 days. In the control treatment, *F. solani* was allowed to grow alone.

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The treatments were;

- T_1= Control (only *F. solani*)
- T_2= *T. harzianum* + *F. solani*
- T_3 = *T. viride* + *F. solani*
- T_4= Dithane M 45 (@100 mg/L) + *F. solani*
- T_5= Dithane M 45 (@200 mg/L) + *F. solani*
- T_6= Dithane M 45 (@300 mg/L) + *F. solani*
- T_7= Benlate (@100 mg/L) + *F. solani*
- T_8= Benlate (@200 mg/L) + *F. solani*
- T_9= Benlate (@300 mg/L) + *F. solani*
- T_{10} = Ridomil (@100 mg/L) + *F. solani*
- T_{11} = Ridomil (@200 mg/L) + *F. solani*
- T_{12}= Ridomil (@300 mg/L) + *F. solani*
- T_{13} = *T. harzianum* + *F. solani* + Dithane M 45 (@100 mg/L)
- T_{14} = *T. harzianum* + *F. solani* + Dithane M 45 (@200 mg/L)
- T_{15}= *T. harzianum* + *F. solani* + Dithane M 45 (@300 mg/L)
- T_{16}= *T. harzianum* + *F. solani* + Benlate (@100 mg/L)
- T_{17}= *T. harzianum* + *F. solani* + Benlate (@200 mg/L)
- T_{18}= *T. harzianum* + *F. solani* + Benlate (@300 mg/L)
- T_{19}= *T. harzianum* + *F. solani* + Ridomil (@100 mg/L)
- T_{20}= *T. harzianum* + *F. solani* + Ridomil (@200 mg/L)
- T_{21}= *T. harzianum* + *F. solani* + Ridomil (@300 mg/L)
- T_{22}= *T. viride* + *F. solani* + Dithane M 45 (@100 mg/L)
- T_{23}= *T. viride* + *F. solani* + Dithane M 45 (@200 mg/L)
- T_{24}= *T. viride* + *F. solani* + Dithane M 45 (@300 mg/L)
- T_{25}= *T. viride* + *F. solani* + Benlate (@100 mg/L)
- T_{26}= *T. viride* + *F. solani* + Benlate (@200 mg/L)
- T_{27}= *T. viride* + *F. solani* + Benlate (@300 mg/L)
- T_{28}= *T. viride* + *F. solani* + Ridomil (@100 mg/L)
- T_{29}= *T. viride* + *F. solani* + Ridomil (@200 mg/L)
- T_{30}= *T. viride* + *F. solani* + Ridomil (@300 mg/L)

Each treatment was replicated five times in a Completely Randomized Design (CRD). The data were analyzed by Analysis of Variance (ANOVA) and means were separated by Least Significance Difference (LSD) test. Colony diameter of *F. solani* was measured twice at 7 days interval.

Mortality (%): The percent mortality was recorded in each treatment at the seedling stage by using the following formula;

$$\text{Mortality (\%)} = \frac{\text{Total number of dead plants}}{\text{Total number of plants}} \text{ X } 100$$

Growth Parameters and yield plant^{-1} (g): The plant height, number of fruits and yield plant^{-1} were recorded on four randomly selected plants. Then averages of that were calculated.

Re-isolation of *F. solani* from affected roots: The affected roots of okra plants were brought to the laboratory and were surface sterilized with 0.1% solution of mercuric chloride for 15-30 seconds, rinsed with sterile distilled water to remove the disinfectant and blotted dry. The treated pieces were then cultured on the PDA medium. Plates were regularly checked for the fungal growth. *Fusarium solani* was confirmed with a mycologist in the department of Plant Pathology.

Pot experiment: Anmol, a local variety of okra was obtained from the market. Six seeds/pot were sown in 15 cm diameter pots. These seeds of okra were treated either with three selected fungicides by dusting method or with two bioagents (*T. harzianum* and *T. viride*). Two days after emergence, each seedling was treated with 10 ml conidial suspension of *Trichoderma* spp. @ $5x10^5$spores/ml. Inoculum of *F. solani* (one plate per pot) was added at the time of sowing. After two weeks of germination, thinning was done in pots; leaving two plants/pot.

Six treatments were used, each replicated five times in Randomized Complete Block Design (RCBD). The data were statistically analyzed by analysis of variance (ANOVA) and means were separated by Least Significance difference (LSD) test.

The treatments were;

- T_1 = Treated with *F. solani* (Contorl)
- T_2 = Ridomil (@ 2 g/kg) + *F. solani*
- T_3 = Dithane M 45 (@ 2 g/kg) + *F. solani*
- T_4 = Benlate (@ 2 g/kg) + *F. solani*
- T_5 = *T. viride* + *F. solani*
- T_6 = *T. harzianum* + *F. solani*

Data were recorded on:

Germination (%): Germination percentage of okra was calculated by using the following formula;

$$\text{Germination (\%)} = \frac{\text{Number of germinated seed}}{\text{Total number of seed sown}} \text{ X } 100$$

Disease incidence (%): The disease incidence (%) was recorded by using the following formula (Ajmal *et al*., 2001);

$$\text{Disease Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \text{ X } 100$$

Results and Discussion

***In vitro* study of fungicides and *Trichoderma* spp., against *Fusarium solani*:** Different doses of the three fungicides (Dithane M 45, Benlate and Ridomil) at the selected concentrations and two bioagents (*T. harzianum* and *T. viridae*) alone as well as in combination reduced significantly the growth of *F. solani* (Table 1). Among the antagonists, *T. harzianum* significantly reduced (43.2%) the growth of *F. solani* than *T. viride* (20%).

All the fungicides also significantly affected the growth of *F. solani*. It was 57.9-100% by Dithane M 45, 17.4-100% by Benlate and 55-100% by Ridomil when applied (@100, 200 and 300 mg/L). With increase in concentration, the efficacy of all the fungicides was increased. However, Dithane M 45 showed better performance than others having colony diameter of 2.9 (unit) when applied @ 100 mg/L while Benlate and Ridomil have 5.7 and 3.1 (unit) respectfully, at the same concentration. Shah *et al*., (2006) worked on the effectiveness of different fungicides and reported that Dithane M 45 was best among the tested fungicides in controlling *Fusarium* sp., *In vitro*.

Table 1. Mean colony diameter (cm) of *F. solani* after 7 and 14 days of incubation at 25^c C as affected by fungicides and *Trichoderma* spp.

Treatment	Colony diameter (cm)	Decrease than control (%)
T_1= Control (only *F. solani*)	6.9 A	--
T_2= *T. harzianum* + *F. Solani*	3.9 C	43.2
T_3 = *T. viride* + *F. Solani*	5.5 B	20.0
T_4= Dithane M 45 (@100 mg/L) + *F. solani*	2.9 DE	57.9
T_5= Dithane M 45 (@200 mg/L) + *F. solani*	1.9 HI	71.6
T_6= Dithane M 45 (@300 mg/L) + *F. solani*	0.0 K	100.0
T_7= Benlate (@100 mg/L) + *F. Solani*	5.7 B	17.4
T_8= Benlate (@200 mg/L) + *F. Solani*	3.9 C	43.5
T_9= Benlate (@300 mg/L) + *F. Solani*	0.0 K	100.0
T_{10} = Ridomil (@100 mg/L) + *F. Solani*	3.1 D	55.2
T_{11} = Ridomil (@200 mg/L) + *F. Solani*	2.4 FGH	65.5
T_{12}= Ridomil (@300 mg/L) + *F. Solani*	0.0 K	100.0
T_{13}=*T.harzianum* + *F solan i*+ Dithane M 45 (@100mg/L)	1.7 IJ	76.1
T_{14}=*T. Harzianum* + *F solani* + Dithane M 45 (@200mg/L)	1.4 J	80.0
T_{15}= *T. harzianum* + *F. solani* + Dithane M 45 (@300 mg/L)	0.0 K	100.0
T_{16}= *T. harzianum* + *F. solani* + Benlate (@100 mg/L)	3.3 D	52.9
T_{17}= *T. harzianum* + *F.* + Benlate (@200 mg/L)	2.8 DEF	59.2
T_{18}= *T. harzianum* + *F. solani* + Benlate (@300 mg/L)	0.0 K	100.0
T_{19}= *T. harzianum* + *F. solani* + Ridomil (@100 mg/L)	2.4 FGH	65.5
T_{20}= *T. harzianum* + *F. solani* + Ridomil (@200 mg/L)	2.1 GHI	69.1
T_{21}= *T. harzianum* + *F. solani* + Ridomil (@300 mg/L)	0.0 K	100.0
T_{22}= *T. viride* + *F. solani* + Dithane M 45 (@100 mg/L)	2.9 DE	57.9
T_{23}= *T. viride* + *F. solani* + Dithane M 45 (@200 mg/L)	1.8 IJ	74.5
T_{24}= *T. viride* + *F. solani* + Dithane M 45 (@300 mg/L)	0.0 K	100.0
T_{25}= *T. viride* + *F. solani* + Benlate (@100 mg/L)	5.4 B	21.3
T_{26}= *T. viride* + *F. solani* + Benlate (@200 mg/L)	3.8 C	44.9
T_{27}= *T. viride* + *F. solani* + Benlate (@300 mg/L)	0.0 K	100.0
T_{28}= *T. viride* + *F. solani* + Ridomil (@100 mg/L)	3.3 D	52.9
T_{29}= *T. viride* + *F. solani* + Ridomil (@200 mg/L)	2.5 EFG	63.8
T_{30}= *T. viride* + *F. solani* + Ridomil (@300 mg/L)	0.0 K	100.0
LSD (0.05)	0.5	
C.V. (%)	32.7	

Means followed by different letters are significantly different from each other at 5 % level of significance

The combinations of antagonists and fungicides showed different performance (Table 1). *T. harzianum* with Dithane M 45 showed better performance and reduced the *R. solani* growth by 76.1-100% (@100, 200 and 300 mg/L) than check. This reduction was 52.9-100 and 65.5-100% when *T. harzianum* + Benlate and *T. harzianum* + Ridomil were applied. *T. viride* also showed poor performance in combination with fungicides. Dithane M 45 (@ 100, 200 and 300 mg/L) and *T. viride* reduced the growth of *F. solani* by 57-100%, while *T. viride* + Benlate and *T. viride* + Ridomil by 21.3-100 and 52.9-100. The combined effect of antagonists and chemicals was more than that of applied alone. Shaban and El-Bramawy (2011) found that the dual application of *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions was more effective. This could be due to the better antagonistic characteristics of *T. harzianum* i-e mycoparasitism, antibiosis, competition and production of cell wall degrading enzymes (Chet, 1987, Ghisalberti & Sivasithamparama 1991, Schirmbock *et al*., 1994). An also of better performance of Dithane M 45 which was also reported by Shah *et al*., in 2006.

Disease incidence and mortality (%): Keeping in view the results of *In vitro* study the best treatments were selected and tested for *In vivo* study. Table 2 indicated that response of *Fusarium solani* to fungicides and bio-agents. Among the fungicides, Dithane M 45 was found to be highly effective in reducing the incidence and mortality percentage of *Fusarium* root rot of Okra. The least disease incidence and plant mortality (35.6 and 17.6%) were recorded in treatment where seeds were treated with Dithane M 45 @ 2 g/Kg. This was 41.3 and 69.4% lower than untreated (check). Reduction of incidence of soil borne and seed borne diseases in a number of crops by seed treatment with Vitavax 200 have been reported by Shah *et al*., (1992). *Trichoderma harzianum* reduced the disease incidence and plant mortality by 41.9 and 27.4% respectively, more efficiently than *T. viride*. It was 30.7 and 52.3% lower than the untreated (check). These results coincide with the results of Rahim & Surrieh (1989), who reported that *Trichodeerma harzianum* was more effective in controlling the plant mortality caused by *F. solani* in Okra, while *T. viride* was less effective. Several species of *Trichoderma* have been reported (Siddiqui *et al*., 1999) to suppress soil borne disease causing fungi including *Fusarium* sp. The highest incidence and mortality (60.5 & 57.5%) were observed in the diseased (check) plants.

Table 2. Effect of seed treatment with different fungicides and bio-agents on disease incidence and plant mortality on Fusarium root rot of okra in pot experiment.

Treatment	Disease incidence (%)	Decrease than check (%)	Plant mortality (%)	Decrease than check (%)
$T_{1.}$ Untreated (Check)	60.5 A	--	57.5 A	--
$T_{2.}$ Ridomil @ 2g/kg seed	41.7 B	31.1	37.5 B	34.8
$T_{3.}$ Dithane M 45 @ 2g/kg seed	35.6 B	41.1	17.6 D	69.4
$T_{4.}$ Benlate @ 2g/kg seed	38.3 B	36.7	37.5 B	34.8
$T_{5.}$ *T. viridei*	44.6 B	26.3	32.6 BC	43.4
$T_{6.}$ *T. harzianumi*	41.9 B	30.6	27.4 C	52.3
LSD (0.05)	9.1		6.7	
C.V. (%)	17.3		15.8	

Means followed by different letters are significantly different from one another at 5 % level of probability

With increase in disease incidence, the plant mortality (%) also increased. However, the lowest disease incidence was found in plants treated with Dithane M 45 followed by Benlate. Moreover, the differences between the two were non-significant. These fungicide(s) inhibit growth and multiplication of the *F. solani* (Agrios, 1997). Bioagents also played similar role in inhibiting the *F. solani*. *Trichoderma harzianum* was found more efficient than *T. viride*.

Germination (%): Results presented in Table 3 revealed that the germination percentage was significantly affected by different fungicides and antagonists. Seeds treated with Dithane M 45 showed high percentage (93.3%) of germination than others. This was 21.6% higher than untreated (check). These results are in line with the findings of Dash & Narain (1996). They reported that pretreatment of seed with fungicide improve seed germination. Seed treatment with bio-agents i.e., *T. harzianum* and *T. viride* also showed their performance in improving germination. Both the bio-agents were non-significant between themselves in term of germination percentage.

However , *T. harzianum* and *T. viride* increased germination percentage by 13.4 and 15.1% than untreated (check). *Trichoderma* species are capable of hyperparasitising the pathogenic fungi and found to involve in protecting number of crop plants (Durrell, 1968; Barnett & Binder, 1973). With increase in disease incidence, germination percentage is decreased.

Table 3. Effect of different fungicides and bio-agents on germination of okra seeds as affected Fusarium root rot in pot experiment.

Treatment	Germination (%)	Increase over check (%)
$T_{1.}$ Untreated (Check)	76.7 B	--
$T_{2.}$ Ridomil @ 2g/kg seed	90.0 A	17.4
$T_{3.}$ Dithane M 45 @ 2g/kg seed	93.3 A	21.7
$T_{4.}$ Benlate @ 2g/kg seed	91.7 A	19.6
$T_{5.}$ *T. Viridei*	88.3 A	15.2
$T_{6.}$ *T. Harzianumi*	87.0 A	13.4
LSD (0.05)	10.1	
C.V. (%)	9.6	

Means followed by different letters are significantly different from one another at 5 % level of probability

Growth parameters and yield plant^{-1}: Data presented in Table 4 indicated the effect of different fungicides and bio-agents on the growth and yield of okra. Maximum plant height (77.7 cm), number of fruits (30.6) and yield (340.8 g) were recorded in plants raised in pots treated with *T. harzianum*. This was 45.8, 102.6 and 83.6% higher than untreated (check). The shortest plant (53.3 cm), lower number of fruits/plant (15.1) and low yield/plant^{-1} (185.6 g) were recorded in treatment having no fungicide and bio-agents. Among fungicides Dithane M 45 showed better performance. Treated plants were 24.6% taller, having 73.2% higher number of fruits/plant and produced 80.2% greater yield/plant than untreated (check). However, the bio-agent (*T. harzianum*) showed dominancy over fungicides under screen house conditions. Siddiqui *et al*., (2000) reported that *T. harzianum* elevated plant height in okra and such results were also obtained against *F. solani* in sunflower by Waffa & Amin (2001) that *Trichoderma* species controlled *F. solani* and increased the plant growth parameters.

Bio-agents have remarkable capacity of multiplication, thus, when applied; they multiply in exponential ratio and even can overcome stress condition by forming thick walled spores. Hence, bioagents are the solution for safer environmental issues and needs proper attention for seed treatment (Bharath *et al*., 2005).

Table 4. Effect of seed treatment with different fungicides and bio-agents on growth parameters and yield plant^{-1} of okra in pot experiment.

Treatment	Growth parameters		
	Plant height (cm)	No. of fruits plant^{-1}	Yield plant^{-1} (g)
$T_{1.}$ Untreated (Check)	53.3 C (--)	15.1 D (--)	185.6 C (--)
$T_{2.}$ Ridomil @ 2g/kg seed	61.9 BC (16.1)*	26.8 B (77.5)*	231.6 B (24.8)*
$T_{3.}$ Dithane M 45 @ 2g/kg seed	66.4 B (24.6)	26.3 B (73.2)	334.4 A (80.2)
$T_{4.}$ Benlate @ 2g/kg seed	63.5 BC (19.1)	20.1 C (33.1)	271.2 B (46.1)
$T_{5.}$ *T. Viridei*	63.1 BC (18.4)	24.3 B (60.9)	272.1 B (46.6)
$T_{6.}$ *T. Harzianumi*	77.7 A (45.8)	30.6 A (102.6)	340.8 A (83.6)
LSD (0.05)	10.4	3.6	42.6
C.V. (%)	13.5	12.7	12.9

Means followed by different letters are significantly different from one another at 5 % level of probability
*Percent increase over untreated (check)

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