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EFFECT OF FUNGICIDES, MICROBIAL ANTAGONISTS AND OIL CAKES IN THE CONTROL OF *FUSARIUM OXYSPORUM,* THE CAUSE OF SEED ROT AND ROOT INFECTION OF BOTTLE GOURD AND CUCUMBER

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Abstract

Fungicides, microbial antagonists and oilcakes were used *In vitro* and *In vivo* to control *Fusarium oxysporum*, the cause of seed rot, seedling and root infection of bottle gourd and cucumber. Aliette, Benlate and Carbendazim completely inhibit the colony growth of *F. oxysoprum* @ 100 ppm whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth at 1000 ppm. Fungicidal treatment of bottle gourd and cucumber seeds artificially infested with *F. oxysporum* significantly reduced seedling mortality and root infection. Benlate, Carbendazim and Topsin-M completely checked seedling mortality in bottle gourd. Microbial antagonists viz., *Trichoderma harzianum, T. viride, Gliocladium virens, Bacillus subtilis* and *Stachybotrys atra* significantly reduced seedling mortality and root rot infection of *F. oxysporum* in bottle gourd and cucumber *In vitro* and *In vivo. T. harzianum* was found most effective in reducing seedling mortality and root infection in cucumber and bottle gourd. Mustard cake supplemented at high ratio reduced seedling mortality and markedly increased germination in bottle gourd and cucumber.

Introduction

F. oxysporum is an abundant and active saprophyte in soil and organic matter and its some specific forms are plant pathogenic (Smith *et al.,* 1988). The fungus can be spread short and long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 2005). Browning of the vascular tissue is strong evidence of *Fusarium* wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Jones *et al.,* 1982; Smith *et al.,* 1988). *Fusarium oxysporum* is a ubiquitous phytopathgen causing root rot, vascular wilt and damping off in many plant species (Saremi, 1996). This is usually involved in vascular wilt syndrome and reported as one near devastation of the commercial banana industry in the 1960s by panama wilt caused by *Fusarium oxysporum* f. sp.*cubense* (Leslie & Summerell, 2006). Many pathogenic strain designated *formae speciales* exist within *F. oxysporum* such as *F. oxysporum S*chlecht. f. sp., *cucumerinum* Owen., causing economic damage to cucumber (Booth, 1971). Some species of *Fusarium* are responsible for vascular wilt e.g., *Fusarium* wilt of melon caused by *F. oxysporum* f. sp., *melonis* (Zitter, 1996). *F. oxysporum S*chlecht. f. sp. *cucumerinum* has been isolated from the stem, peduncle, fruits and seed of cucumber plants and seed transmission occur at low but significant rate (Jenkins & Wehner, 1983). Use of environmentally friendly biological control agents can more affectively control the soil borne phytopathogens (Park, 1989; Saleem *et al*., 2000). A single application of a biological control agent may provide ample protection of the infection site as seed treatments for protection against seed decay and seedling damping-off diseases. Some of the common antagonists include *Bacillus subtilis*, *Gliocladium virens* and *Trichoderma* spp., *B. subtilis* and *G. virens* utilize antibiosis as the main mechanism of antagonism, whereas *Trichoderma* spp., use mycoparasitism as the chief mechanism of antagonism (Baker & Paulitz, 1996). From several studies, it has been confirmed that *Trichoderma* spp., have antagonistic and biological control potential against a diversity of soil borne pathogens (Grondona *et al*., 1997; Hanson & Howell, 2004; Bajwa *et al.,* 2004; Afzal *et al.,* 2013). Harman (2000) showed that *Trichoderma* sp., was used as commercial bio-fungicides to control a range of economically important soil-borne fungal plant pathogen. The mode of action of the well known antagonist *Bacillus subtilis* has been attributed to antibiosis (Nandi & Sen, 1953). Incorporation of mustard amended in soil was found to reduce population of *F. oxysporum* f. sp. *lycopersici* and wilt on tomatoes (Raj & Kapoor, 1996). A significant reduction on pathogenic effect of *F. oxysporum f.sp. lycopersici* occurred in neem kernel cake powder amended soil (Kimaru *et al.,* 2004). The present report gives an account of the effect of fungicides, oil cakes and microbial antagonists in the control of *Fusarium oxysporum* the cause of seed rot, seedling and root infection of bottle gourd and cucumber.

Materials and Methods

Seven fungicides viz., Carbendazim (Benzimidazole), Topsin-M (Thiphanate-Methyl), Aliette (Fesetyl-Al 80% up), Benlate (Triadimenol), Vitavax (Carboxin), Ridomil (Metalaxyl acylatenine) and Mancozeb (Dithane M-45) were evaluated against colony growth of *Fusarium oxysporum*. Fungicides were used @ 10, 50, 100, 500, 1000 and 10,000 ppm concentration in autoclaved PDA medium by poisoned food techniques (Borum & Sinclair, 1968). Five mm diameter agar disk of test fungi were cut from 8-10 days old culture plate by using sterile cork borer and placed in the centre of Petri plates containing different concentration of fungicides. There were four replicates of each treatment. The plates without fungicides served as control. The inoculated plates were incubated at 28ºC. The radial growth was recorded after 7-10 days of incubation when the fungus covered the plates completely in control. The percent inhibition (PI) of the fungus over control was calculated by using the following formula:

$$PI = \frac{(A-B)}{A} \times 100$$

where A is colony growth of the fungus in control plate and B is colony growth of the fungus in treated plate.

Seeds after sterilization with 2% $NaOCl_2$ were dipped in1% gum arabic solution and coated by rolling the seed on 7 days old well-sporulated cultures of fungi *Fusarium oxysporum.* Infested seeds were treated with fungicides viz., Aliette, Benlate, Carbendazim, Ridomil, Topsin-M and Mancozeb @ 1, 2 and 3 g/kg of seeds and microbial antagonist viz., *Trichoderma harzianum* @ 8 x 10^9 cfu/ml, *T. viride* @ 6.5 x 10^7 cfu/ml, *Gliocladium virens* @ 1.7 x 10^8 cfu/ml, *Bacillus subtilis* @ 1 x 10^9 cfu/ml and *Stachybotrys atra* @ 5.8 x 10^9 cfu/ml. Seeds of bottle gourd and cucumber were plated in 9 cm diameter plates, 5 seeds per plate and 50 seeds per treatment. The another set of seeds were sown in 20 cms diam., pots containing 3 kg sterilized soil @ 10 seeds per pot for each treatment.

Effect of oil cakes viz., mustard, neem and castor cake was evaluated by amending 50,100 and 150 gm in 3 kg sterilized and unsterilized field soils in earthen pots. Non-amended soil served as control. Seeds were artificially inoculated by rolling the seed on 7 days old well-sporulated cultures of *Fusarium oxysporum.* After 20 days of soil amendment 10 seeds of bottle gourd and cucumber were sown in each pot and replicated 5 times. Pots were placed in screen house benches and were regularly observed for the development of symptoms and after 40 days plants were removed and infection percentage were recorded. The roots were washed with sterilized distilled water and 1 cm long root piece after surface disinfection with 2% $NaOCl_2$ for 2 minutes were transferred on PDA plates supplemented with Benzyl Penicillin Sodium (2.5 ml/litre) and Streptomycin Sulphate (2.5 ml/litre) and incubated at 28°C for 7 days to confirm root infection and colonization by pathogen. Data were subjected to Duncan's multiple range tests at P= 0.05 depending upon the experimental design by SPSS version 12.

Results and Discussion

a. *In vitro* effect of fungicides: Complete inhibition of colony growth of *F. oxysoprum* was observed where fungicides viz., Aliette, Benlate and Carbendazim @ 100 ppm were used whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth @ 1000 ppm (Table 1). Similar reports have been made by Shahzad (1994) that Vitavax inhibited colony growth *Fusarium oxysporum* at 10,000 ppm. Benlate has been reported to be most effective for checking the mycelial growth of *F.oxysporum* at low concentrations (Hussain *et al.,* 1981; Shahzad, 1994; Arshad *et al.,* 1996). The effectiveness of Benlate, Topsin-M, Bavistin and Vitavax has already been reported against pathogenic fungi viz., *Fusarium oxysporum* (Chavan *et al.,* 1977; Ahmad *et al.*, 1996).

Cucumber and bottle gourd seeds when artificially infested and treated with fungicides significantly increased seed germination and reduced seed infection by *F. oxysporum* at all concentrations. Maximum seed germination was recorded where Benlate, Carbendazim and Topsin-M were used @ 3g a.i. / kg. Seed borne infection of *F. oxysporum* was completely controlled with maximum germination in both cucumber and bottle gourd (Figs. 1 & 2).

Table 1. Mean percent inhibition of colony growth of *Fusarium oxysporum* on potato dextrose agar by 7 fungicides using poisoned food techniques.

Concentrations ppm	% Inhibition by fungicides						
	Aliette	Benlate	Carbendazim	Mancozeb	Ridomil	Topsin-M	Vitavax
10	37.2a	44.3a	30.2a	27.4a	38.5a	33.3a	30.7a
20	58.9b	71.5b	53.4b	48.8b	55.7b	58.3b	50.5b
50	86.5c	94.1c	72.5c	71.7c	82.3c	79.0c	71.0c
100	100.0d	100.0d	100.0d	90.1d	93.9d	89.0d	92.2d
500	100.0d	100.0d	100.0d	100.0e	100.0e	100.0e	100.0e
1000	100.0d	100.0d	100.0d	100.0e	100.0e	100.0e	100.0e

Mean followed by the same letter within a column are not significantly different at (p=0.05) according to Duncan's multiple range test.

b. *In vivo* effect of fungicides: Fungicidal treatment of bottle gourd and cucumber seeds artificially infested with *F. oxysporum* significantly reduced seedling mortality and root infection. Benlate, Carbendazim and Topsin-M completely checked seedling mortality in bottle gourd. Ridomil, Aliette, Mancozeb and Vitavax reduced 14-16% seedling mortality as compared to control (48%). Similarly root infection was significantly controlled by Topsin-M (2%) followed by Benlate, Carbendazim, Aliette, Mancozeb, Ridomil and Vitavax in descending order. All the test fungicides improved seed germination and plant size (Fig. 3).

Best germination of cucumber seeds were observed where seeds were treated with Carbendazim (88%) followed by Topsin-M and Benlate (84%). Plants achieved maximum size where seeds were treated with Aliette, Benlate and Carbendazim followed by Topsin-M, Mancozeb, Ridomil and Vitavax. However plant size significantly increased in all treatments of fungicides. Benlate and Carbendazim reduced 4 and 8% seedling mortality and root infection respectively. Vitavax, Aliette, Topsin-M and Ridomil significantly reduced 8-16% seedling mortality and 10-18% root infection whereas Mancozeb was found least effective showing 28 and 20% seedling mortality and root infection respectively (Fig. 4). Seed treatment with Benlate (benomyl) and Bavistin (Carbendazim) inhibited growth of *F. oxysporum* on PDA medium and protected seedlings of onion from pre- and post-emergence damping off in green house trial (Abdelrazik *et al.*, 1990). There is another report that Benomyl+ thiram (1.5+0.45g a.i. /kg seeds) inhibited colony growth of *F. oxysporum* and seed contamination, increased the emergence of seedlings and reduced the percentage of post-emergence damping off in onion (Ozer & Koycu, 1998). Carbendazim had totally suppressed *F. oxysporum* on tomato seeds (Kumar & Lokesh, 1999) and on soybean *In vitro* (Singh, 1997). Dipping ginger seed rhizome in Carbendazim solution had reduced seed and soil borne incidence of *F. oxysporum In vivo* (Rana & Sharma, 2001).

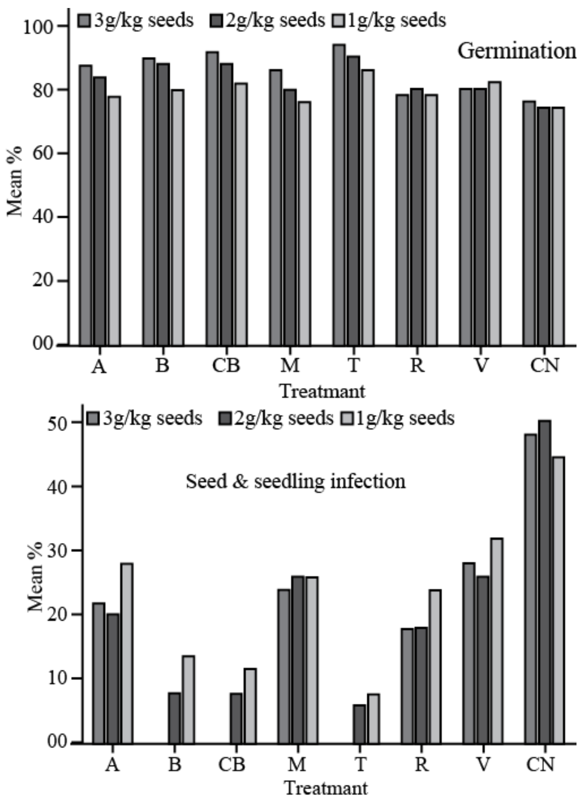

Fig. 1. Effect of seed treatment with fungicides on germination and infection by *Fusarium oxysporum* on bottle gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

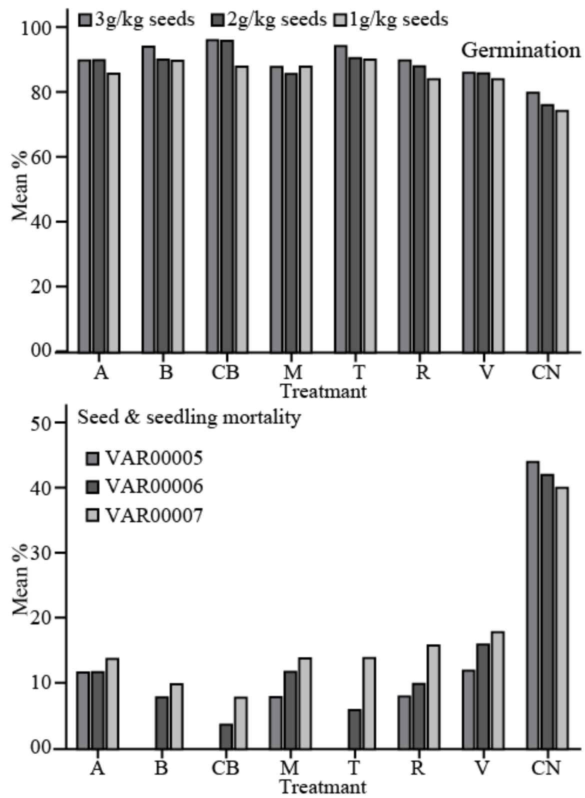

Fig. 2. Effect of seed treatment with fungicides on germination and infection by *Fusarium oxysporum* on cucumber.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

c. *In vitro* effect of microbial antagonists: On seed germination Microbial antagonists significantly reduced seed infection in bottle gourd and cucumber caused by *F. oxysporum*. Significant increase in seed germination of bottle gourd and cucumber was recorded in treatments of *T. harzianum* and *Gliocladium virens* whereas *B. subtilis* reduced seed germination in cucumber as compared to control (Fig. 5a, 6a). *Trichoderma* species are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity (Elad *et al.*, 1982).

d. *In vivo* effect of microbial antagonists: Maximum reduction in seedling mortality and root infection of bottle gourd were observed in *T. harzianum* followed by *T. viride* and *B. subtilis*. *Stachybotrys atra* was comparatively found least effective (Fig. 5b). In cucumber *T. harzianum* was found most effective in reducing seedling mortality. Similarly root infection was significantly reduced by *T. harzianum*, *T. viride* and *G. virens* (Fig. 6b). Microbial antagonist have been used to minimize the hazardous effect of pesticides for the control of root infecting *Fusarium* spp., (Elad *et al.*, 1983; Ehteshamul-Haque *et al.*, 1990; Perveen & Ghaffar, 1991). *Trichoderma* species suppressed the deleterious soil microbes by competing at the active sites, reduced disease development and subsequently stimulated the growth and yield of plants (Ahmed & Upadhyay, 2009). Non pathogenic strains of *F. oxysporum* have been reported most effective and consistent in controlling *Fusarium* wilt disease of melons and other crops (Lakrin & Fravel, 1998).

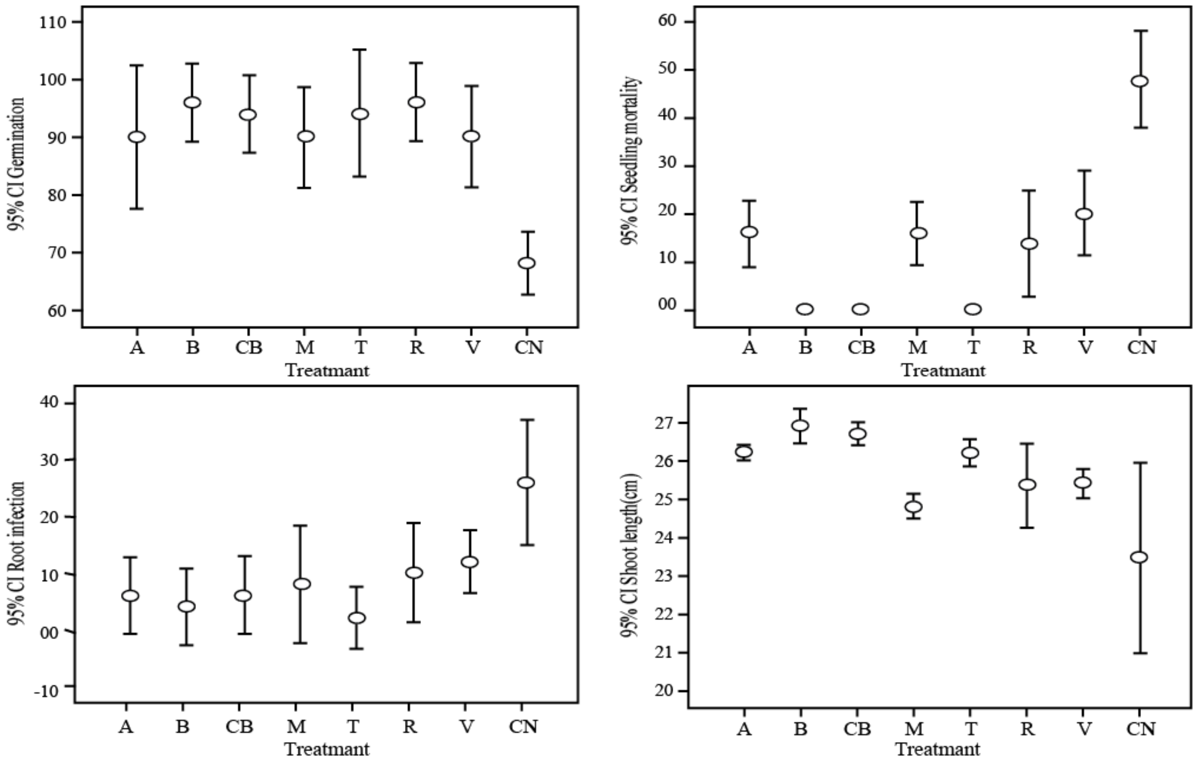

Fig. 3. Effect of seed treatment with fungicides on germination, shoot length, seedling mortality and root infection by *Fusarium oxysporum* on bottle gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

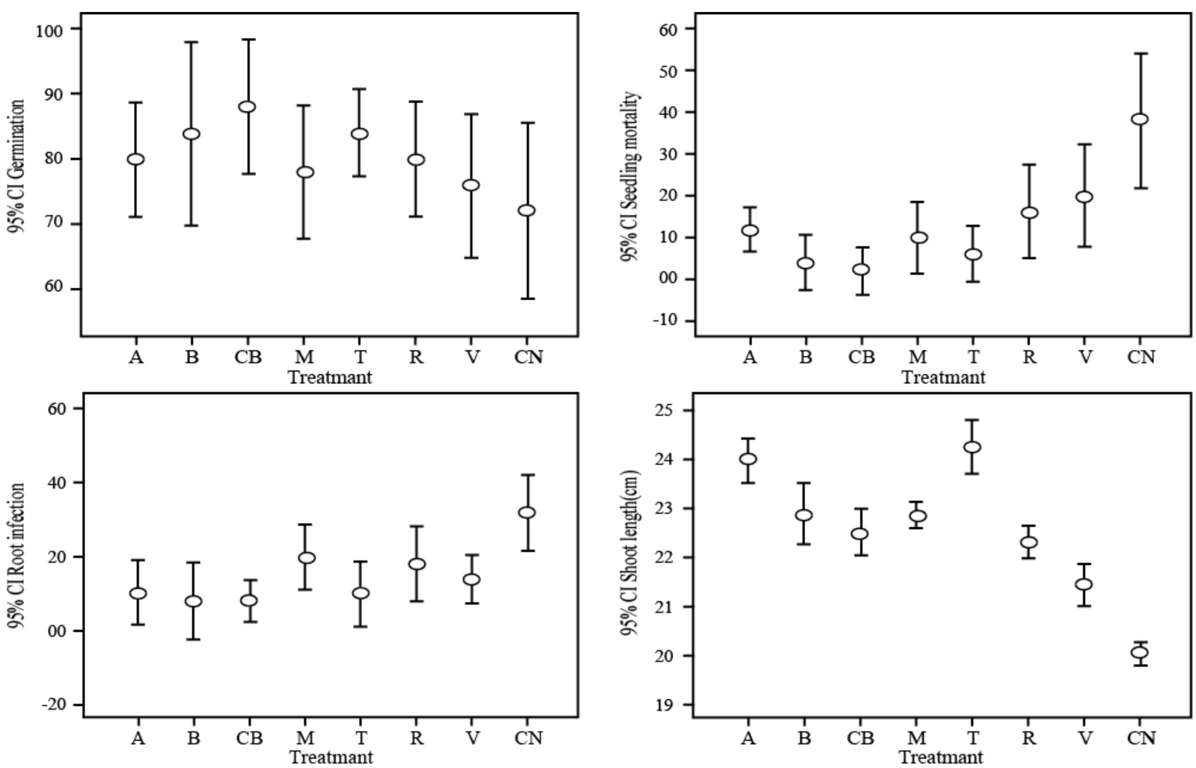

Fig. 4. Effect of seed treatment with fungicides on germination, shoot length, seedling mortality and root infection by *Fusarium oxysporum* on cucumber.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

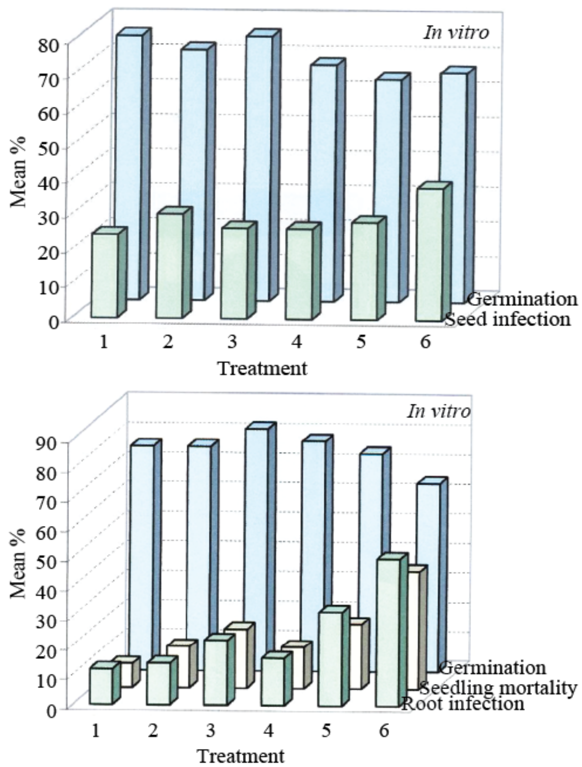

Fig. 5. Effect of microbial antagonists on germination and infection on seed, seedling and root by *Fusarium oxysporum* on bottle gourd.
1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5= *Sachybotrys atra*, 6= Control

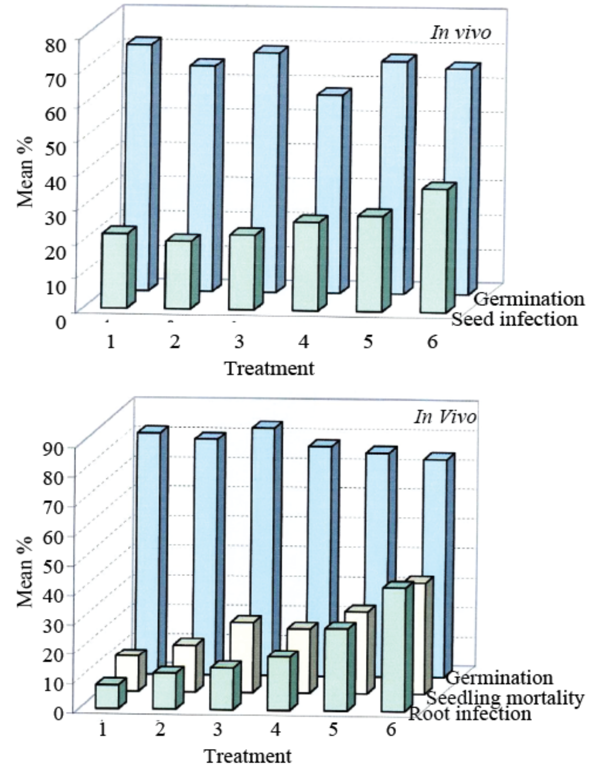

Fig. 6. Effect of microbial antagonists on germination and infection on seed, seedling and root by *Fusarium oxysporum* on cucumber.
1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5= *Sachybotrys atra*, 6= Control

e: Effect of oilcakes on pathogenesis: Significant effect on seed germination, plant size and seedling mortality was observed where infested seeds of bottle gourd and cucumber were sown in non-sterilized soil amended with mustard, neem and castor cake in all ratios and neem and castor cakes at the ratios of 1:20 and 1:30. Mustard and neem cakes were found most effective in enhancing seed germination and plant size as well as minimizing seedling mortality in bottle gourd. Mustard cake amended in non sterilized soil at the ratio of 1:20 showed maximum increase in plant size (20cm), seed germination (86%) and up to 2.2% reduction in seedling mortality (Table 2). Significant increase in seed germination and plant size of cucumber were recorded where oilcakes were used in high level in soil. Similarly seedling mortality was reduced when oilcakes were used in soil in 1:20 and 1:30 ratios (Table 3).

In the present study oil cakes amendment in soil significantly controlled the seed borne diseases produced by *Fusarium* spp., in cucumber and bottle gourd. Mustard cake supplemented at high ratio reduced seedling mortality caused by *F. oxysporum* and markedly increased germination in bottle gourd and cucumber. Mustard cake was found most effective in reducing seedling and root infection in cucumber. Soil amendment with oil cakes has been found to reduce the infection of root knot nematodes and *Fusarium* spp., on mung bean (Ehtashamul Haq *et al.*, 1993). However none of the oil cakes completely controlled seedling and root infection cause by *Fusarium* spp., in cucumber and bottle gourd. The suppression of pathogenic fungi by organic soil amendment have been reported by earlier workers (Chandra *et al.*, 1981, Singh & Singh, 1982, Khan *et al.*, 1973) The suppression of pathogenic fungi was due to fertility factor, stimulatory/inhibitory action of the degradation product because in amended soil fungal numbers were greatly increased and maximum fungal population were obtained between 20-45 days after which there was gradual fall (Khalis & Manoharachary, 1985). No significant difference on effect of organic amendment on disease expression by pathogenic seed borne fungi was observed between sterilized and non- sterilized soil. Organic amendment produced volatile and non volatile substances during their decomposition and also stimulate resident and introduced antagonists (Lumsden *et al.,* 1983). Integrated experiment using biocontrol agent i.e., *T. harzianum* along with neem cake and Bavistin gave maximum disease reduction of *Fusarium* yellows and improved the overall growth of gladiolus (Nand, 2002). In solarized soil, *Trichoderma harzianum* and *T. viride* alone and in combination gave maximum reduction of *Fusarium* wilt of carnation and also enhanced the plant growth parameters (Kumar, 2005). Plant growth promoting rhizobacteria in combination with organic amendment reduce root-rot disease incidence and population of root pathogenic fungi and increase the yield in soyabean (Inam-ul-Haq *et al.,* 2012).

Integrated effect of microbial antagonists viz., *Trichoderma harzianum, T. viride, Gliocladium virens* and *Bacillus subtilis*, fungicides viz., Benlate, Carbendazim and Topsin-M and amendment of mustard cake could play dual role in controlling the disease and reduced the hazardous effect of chemicals.

Table 2. Effect of oilcakes amendment in soil on seed germination, plant size and disease caused by *Fusarium oxysporum* on bottlegourd.

Ratios	Mean percent				Shoot length (cm)	
	Seed germination		Seedling mortality			
	St	Nst	St	Nst	St	Nst
Mustard cake						
1:20	85b	86b	27a	22a	19.6c	20.0c
1:30	82b	83b	37ab	47b	19.0c	19.4c
1:60	76a	80b	57bc	52b	18.3b	18.6bc
Neem cake						
1:20	80b	85b	37ab	37ab	18.8bc	19.0c
1:30	75a	84b	52b	50b	18.9bc	19.2c
1:60	74a	83b	62c	45b	19.0c	18.8bc
Castor cake						
1:20	79ab	82b	50b	35ab	18.5b	18.7bc
1:30	75a	81b	52b	47b	18.3b	18.3b
1:60	73a	81b	62c	57bc	18.1b	18.6bc
Control	73a	78a	68c	61c	17.1a	16.9a

Mean followed by the same letter within a column are not significantly different at (p=0.05) according to Duncan's multiple range test. St = Sterilized soil, Nst = Non-sterilized soil

Table 3. Effect of oilcakes amendment in soil on seed germination, plant size and disease caused by *Fusarium oxysporum* on cucumber.

Ratios	Mean Percent				Shoot length (cm)	
	Seed germination		Seedling mortality			
	St	Nst	St	Nst	St	Nst
Mustard cake						
1:20	80b	90c	13a	17a	19.1c	19.7c
1:30	77b	86bc	15a	20a	19.2c	19.4c
1:60	71a	79b	32b	32b	17.2a	18.4b
Neem cake						
1:20	81b	86bc	25a	27a	19.3c	19.3c
1:30	75a	81b	40bc	37b	19.1c	18.9bc
1:60	67a	78b	45bc	42bc	17.9ab	18.7bc
Castor cake						
1:20	80b	84b	15a	27a	18.4b	18.7bc
1:30	72a	83b	22a	35b	18.0b	17.7ab
1:60	70a	76ab	40bc	52c	17.2a	17.4a
Control	68a	71a	58c	58c	16.8a	17.3a

Mean followed by the same letter within a column are not significantly different at (p=0.05) according to Duncan's multiple range test. St = Sterilized soil, Nst = Non-sterilized soil

References

Abdelrazik, A.A., F.G. Fahey, A.M. Amein and A.I. El-Amein. 1990. Role of onion seeds in transmission of damping-off causal fungi and chemical control of the disease. *Assiut J. of Agri. Sci.,* 21: 173-193.

Afzal, S., S. Tariq, V. Sultana, J. Ara and S. Ehtashamul-Haque. 2013. Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. *Pak. J. Bot.,* 45(4): 1455-1460.

Agrios, G.N. 2005. *Plant Pathology*, 3rd. ed. Academic Press, Inc.: New York. 803 pp.

Ahmad, M., M.A. Khan, F. Ahmad and S.M. Khan. 1996. Effectiveness of some fungicides on the colony growth of *Fusarium oxysporium* f. sp., *tuberosi* and *Fusarium solani* associated with potato wilt. *Pak. J. Phytopathol.,* 8: 159-161.

Ahmed, M. and R.S. Upadhyay. 2009. Role of soil amendment with plant growth promoting fungi and wilt pathogen on growth and yield of tomato. *J. Mycol. Pl. Pathol.,* 39: 312-316.

Arshad, M., M. A. Dogar, A. Rashid and T. Mahmood. 1996. Fungi associated with soybean seed and their chemical control. *Pak. J. Phytopathol.,* 8: 147-151.

Bajwa R, I. Mukhtar and T. Anjum. 2004. *In vitro* biological control of *Fusarium solani* cause of wilt in *Dalbergia sissoo Roxb*. *Mycopath,* 2: 11-14.

Baker, R. and T.C. Paulitz. 1996. Theoretical basis for microbial interaction leading to biological control of soilborne plant pathogens. In: *Principlesand Practice of Managing Soilborne. Plant Pathogens.* (Ed.): R. Hall. St. Paul, MN, APS Press: pp. 50-79.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Inst, Kew, Surrey, England. 237, pp.

Borum, D.F. and J.B. Sinclair. 1968. Evidence of systemic fungicides protection against *R. solani* with Vitavax in cotton seedlings. *Phytopathol.*, 58: 976-980.

Chandra, S., M. Raizada and K.K. Khanna. 1981. Effect of organic amendment on the rhizosphere microflora of tomato. *Proc. Indian Acad. Sci. B*., 90: 189-197.

Chavan, V.N., B.B. More, B.K. Knode and P.G. Utikar. 1977. *In-Vitro* inhibitory effect of certain fungicides on the growth of *Fusarium oxysporum* f. sp. *lycopersici. Rev. Pl. Path.,* 58: 244.

Ehteshamul-Haque, S. and A. Ghaffar. 1993. Use of rhizobia in the control of root rot disease of sunflower, okra, soybean and mungbean. *J. Phytopathol.*, 138: 157-163.

Ehteshamul-Haque, S., M.J. Zaki and A. Ghaffar. 1990. Biological control of root disease of okra, sunflower, soybean and mungbean. *Pak. J. Bot.*, 22: 121-124.

Elad, Y., I. Chet and J. Katan. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Can. J. Microbiol*., 28: 719-725.

Elad's Y., I. Chet, P. Boyle and Y. Henis. 1983. Parasitism of *Ttichoderma* spp., on *Rhizoctonia solani* and *Sclerotium rolfsii*. Scanning electron microscopy and fluorescence microscopy. *Phytopath.,* 73: 85-88.

Grondona, I., R. Hermosa, M. Tejada, M.D. Gomis, P.F. Mateos, P.D. Bridge, E. Monte and I. Garcia-Acha. 1997. Physiological and biochemical characterization of *Trichoderma harzianum*, a biological control agent against soilborne fungal plant pathogens. *Appl. Environ. Microbiol.* 63: 3189-3198.

Hanson, L.E. and C.R. Howell. 2004. Elicitors of plant defense responses from biological control strains of *Trichoderma virens*. *Phytopathology*, 94: 171-176.

Harman, G.E. 2000. Myths and dogmas of biocontrol: Changes in perceptions of derive from Research on *Trichoderma harzianum* T-22. *Plant Dis.*, 84: 377-393.

Hussain, T., M. Ali and M. Yaqub. 1981. Studies on seedling disease and seed treatment of cotton. *The Pak. Cotton,* 25: 197-207.

Inam-ul-Haq, M., S. Mehmood, H. M. Rehman, Z. Ali and M. I. Tahir. 2012.Incidence of root rot diseases of soyabean in Multan Pakistan and its management by the use of Plant growth promoting rhizobacteria. *.Pak. J. Bot.,* 44(6): 2077-2080.

Jenkins, S.F. Jr. and T.C. Wehner. 1983. Occurrence of *Fusarium oxysporum* f. sp., *cucumerinum* on green house-grown *Cucumis sativus* seed stocks in North Carolina. *Plant Dis.,* 67: 1024-1025.

Jones, J.P., J.B. Jones and W. Miller. 1982. *Fusarium wilt on tomato.* Fla. Dept. Agric. & Consumer Serv., Div. of Plant Industry. *Plant Pathology Circular,* No. 237.

Khalis, N. and C. Manoharachary. 1985. Studies on the mycoflora changes in oil cake amended and unamended soils. *Indian Phytopath.,* 38: 462-466.

Khan, W.M., A.M. Khan and S.K. Saksena. 1973. Influence of certain oil cake amendment on nematodes and fungi in tomato field. *Acta. Bot. India*., 1: 49-54

Kimaru, S.K., S.W Waudo, E. Monda, A.A. Seif and J.K. Birgen. 2004. Effect of Neem Kernel Cake Powder (NKCP) on *Fusarium* Wilt of Tomato when Used as Soil Amendmen, *Journal of Agriculture and Rural Development in the Tropics and Subtropics.* 105(1): 63-70.

Kumar, R. 2005. *Management of Fusarium wilt of carnation through non-chemical methods*. *M Sc. Thesis.* Dept. Myco. *Plant Pathology*, India.

Kumar, T.S.J. and S. Lokesh. 1999. Evaluation of seed mycoflora of tomato and their management *In vitro. Seed Res.*, 27: 181-184.

Lakrin, R.P. and D.R. Fravel. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. *Plant disease*, 82: 1022- 1028.

Leslie, J.F. and B.A. Summerell. 2006. *The Fusarium Laboratory Manual*. Blackwell Publications Professional, 2121 State Avenue, Ames, Iowa 50014, USA: 388pp.

Lumsden, D.R., J.A. Lewis and G.C. Papavizas. 1983. Effect of organic amendments on soil-borne plant diseases and pathogen antagonist. In: *Environmentally Sound Agriculture* (Ed.): W. Lockeretz, Praeger, New York: pp. 51-70.

Nand, S. 2002. *Studies on biological control of Fusarium yellows of gladiolus. M Sc. Thesis.* Dept. Myco. *Plant Pathology*, India.

Nandi, P. and G.P. Sen. 1953. An antifungal substance from a strain of *B. subtilis. Nature* 172: 871-872.

Ozer, N. and N.D. Koycu. 1998. Evaluation of seed treatments for controlling *Aspergillus niger* and *Fusarium oxysporum* on onion seed. *Phytopathologia Mediterranea*, 37: 33-40.

Park, J.H. 1989. Biological control of *Phytophthora* crown rot and root rot of greenhouse pepper with *Trichoderma harzianum* and *Enterobactor agglomerans* by improved methods of application. *Korean J. Pl. and Pathology*, 5: 1-12.

Perveen, S. and A. Ghaffar. 1991. Effect of microbial antagonist in the control of root rot of tomato. *Pak. J. Bot.,* 23: 179-182.

Raj, H. and I.J. Kapoor. 1996. Effect of oil cake amendment of soil on tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*. *Indian Phytopatholgy,* 49: 355-361.

Rana, K.S. and B.K. Sharma. 2001. Role of seed and soil borne inoculums in the spread of ginger yellows and efficacy of fungicides against seed borne infection. *Himachal J. Agri. Res.,* 25: 27-30.

Saleem A., K. Hamid, A.H. Tariq and F.F. Jamil. 2000. Chemical control of root and collar rot of chillies. *Pak. J. Phytopath.*, 12: 1-5.

Saremi, H. 1996. Ecology and taxonomy *Fusarium* species. *Ph. D Thesis.* Dept. Sydney University, N. SW. Australia.

Shahzad, S. 1994. *Studies on soil borne root infecting fungi with special reference to the control of root rot and root knot disease complex*. *Ph. D Thesis*. Dept. Bot., Univ. Karachi, Pakistan. pp. 299.

Singh, D.P. 1997. Efficacy of fungicidal treatment in different graded seeds in soybean. *Legumes Research*, 20: 124-126.

Singh, N. and R. S. Singh. 1982. Effect of oil cake amended soil atmosphere on pigeon pea wilt pathogen. *Indian Phytopath.*, 35: 300-305.

Smith, I.M., J. Dunez, D.H. Phillips, R.A. Lelliott and S.A. Archer, eds. 1988. *European handbook of plant diseases*. Blackwell Scientific Publications: Oxford. 583pp.

Zitter, D.L., D.L. Hopkins and C.E. Thomas. 1996. *Compendium of Cucurbit Diseases*. APS Press, St. Paul, MN. pp. 732.

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