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EFFECT OF FUNGICIDES, MICROBIAL ANTAGONISTS AND OILCAKES IN THE CONTROL OF *FUSARIUM SOLANI*, THE CAUSE OF SEED ROT, SEEDLING AND ROOT INFECTION OF BOTTLE GOURD, BITTER GOURD AND CUCUMBER

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Abstract

The effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani* the cause of seed rot, seedling and root infection on bottle gourd, bitter gourd and cucumber was studied *In vitro* and *In vivo.* Complete inhibition of colony growth of *F. solani* was observed where fungicides viz., Aliette, Benlate and Carbendazim @ 100 ppm were used. Carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum reduction in cucumber and bottle gourd. Root infection was completely checked by Benlate and Carbendazim in bitter gourd and was best controlled by Aliette, Topsin-M and Carbendazim in bottle gourd and cucumber. *F. solani* infested seeds of bottle gourd, cucumber and bitter gourd reduced seedling mortality and root infection when sown in mustard and neem cake amended soil. Mustard cake was found most effective at all ratios followed by neem and castor cake.

Introduction

Fusarium spp., are among the most important plant pathogens in the world and are highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes (Nelson, 1983). Species of *Fusarium* are responsible for vascular wilt eg., *F. solani* f. sp. *cucurbitae*, cause crown, foot rot and fruit rot of squash and pumpkin (Zitter, 1996), can be seed borne both internal and external and survive more than 1-2 years in seed (Watt, 2006). *F. solani* occur on seeds of bottle gourd and sponge gourd and caused significant reduction in seed germination (Shakir & Mirza, 1992; Shakir *et al*., 1995). Squash plants are highly vulnerable to attack by *F. solani* during the pre- and post-emergence stages (Nawar, 2007). Root-rot caused by *Fusarium solani* has been considered among the most deleterious diseases, which cause great losses in many parts of the world (Celar, 2000; Fantino *et al*., 1989; Madkour *et al*., 1983; Abdel-el-Rehim *et al*., 1987). 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). *Trichoderma* spp., is well documented as effective biological control agents of plant diseases caused by soil borne fungi (Sivan & Chet, 1994; Basim *et al*., 1999). A number of species within the genus *Trichoderma* are well known for their biological control capabilities against a wide range of commercially important plant pathogens (Whipps & Lumsden, 2001; McLean *et al*., 2004). They are known to produce a number of antibiotics, such as trichodermin, trichodermol A and harzianolide (Elad *et al*., 1980; Claydon *et al*., 1991). The present report gives an account of the effect of fungicides, oil cakes and microbial antagonists in the control of *Fusarium solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter 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 *F. solani*. 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 naturally infected with *F. solani* were treated with fungicides and microbial antagonist. Fungicides viz., Aliette, Benlate, Carbendazim, Ridomil, Topsin-M and Mancozeb were used @ 1, 2 and 3 g/kg of seeds. Microbial antagonist viz., *Trichoderma harzianum* @ 8×10^9 conidia ml^{-1}, *T. viride* @ 6.5×10^7 conidia/ml^{-1}, *Gliocladium virens* @ 1.7×10^8 conidia/ml^{-1}, *Bacillus subtilis* @ 1×10^9 cell/ml^{-1} and *Stachybotrys atra* @ 5.8×10^9 conidia/ml^{-1} were adjusted by means of haemocytometer. Naturally infected seeds were treated with 1% gum arabic solution prior to seed treatment with fungicides and microbial antagonists and sown in 20 cms diam., pots containing sterilized soil @ 10 seeds per pot for each treatment.

Sterilized and unsterilized 3 kg field soils in earthen pots were amended with 50,100 and 150 gm of oilcakes viz., mustard, neem and castor cake. Non-amended soil served as control. Seeds were artificially inoculated by rolling the seed on 7 days old well-sporulated cultures of *F. solani.* After 20 days of soil amendment, 10 seeds of bottle gourd, bitter 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. 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 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: Significant effect on colony growth of *F. solani* was observed in all concentration compared to control. Complete inhibition of colony growth of *F. solani* 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 at 1000 ppm (Table 1). Benlate has been reported to be most effective for checking the mycelial growth of *F. solani* (Borboru, 1984; Ahmad *et al*., 1996). Benlate completely inhibited the growth of *F. solani* at 50.0 ppm (Baird *et al*., 1994; Mathre & Johston, 1995; Nawar, 2007). Carbendazim and Carbendazim + Mancozeb gave 100 % inhibition of mycelial growth of *F. solani* at 0.2 and 0.3% concentrations (Chavan *et al*., 2009).

Artificially infested seeds of cucumber, bitter gourd and bottle gourd when treated with fungicides significantly increased seed germination and reduced seed infection by *F. solani* at all concentrations except Vitavax and Ridomil when used @1g a.i./kg seeds reduced seed germination as compared to control in cucumber and bitter gourd respectively. Maximum seed germination was recorded in cucumber by Aliette and Benlate, in bottle gourd by Benlate and Carbendazim and in bitter gourd by Benlate. Carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum reduction in cucumber and bottle gourd. (Figs. 1-3). It is reported that wilt of patchouli has been effectively managed by drenching the soil with 0.1% carbendazim, carbendazim + mancozeb (Chavan *et al*., 2009).

b. *In vivo* effect of fungicides: All the fungicides significantly increased seed germination and plant size and reduced seedling mortality and root infection by *F. solani* in bottle gourd, bitter gourd and cucumber. Best germination was obtained where infested seed of bottle gourd were treated with Aliette (92%) followed by Benlate (90%), Carbendazim, Ridomil, Mancozeb and Vitavax significantly increased germination by 84-88% as compared to control (78%). Effects of fungicides on plant size were significant but varied. Maximum reduction in seedling mortality was obtained where seeds were treated with Topsin-M (4%) followed by Ridomil Aliette, Benlate, Carbendazim , Mancozeb and Vitavax. Similarly root infection was significantly controlled by fungicidal treatments but with varied effect. Carbendazim and Topsin-M controlled maximum root infection by 6 and 8% respectively (Fig. 4). In cucumber maximum germination was observed in treatment of Topsin-M (92%) followed by Mancozeb (90%). Carbendazim and Topsin-M completely checked seedling mortality in cucumber followed by Benlate (2%) and Mancozeb (8%). Aliette, Ridomil and Vitavax reduced seedling mortality by 10-14% compared with control and were not different significantly. Minimum root infection (4%) was produced by Topsin-M and Aliette followed by Carbendazim, Mancozeb, Benlate, Vitavax and Ridomil. Plant achieved maximum size in treatments of Aliette and Topsin-M (Fig. 5). Carbendazim and Ridomil improved germination up to 88% in bitter gourd. Plants achieved maximum size where Benlate and Topsin-M was used. Root infection was completely checked by Benlate and Carbendazim. Maximum reduction in seedling mortality was produced by Benlate and Topsin-M (Fig. 6). Root infection was completely checked by Benlate and Carbendazim in bitter gourd and was best controlled by Aliette, Topsin-M and Carbendazim in bottle gourd and cucumber. Such similar observations has been made by Javed *et al*., (1997) who achieved best control of *F. solani* infection in onion by Derosal (Carbendazim) and Vitvax *In vitro* and *In vivo*. Similarly Benlate, Ridomil, Carbendazim and Vitavax when used as seed treatment controlled potato wilt pathogens viz., *F. oxysporum* and *F. solani* (Ahmad *et al*., 1996) and suppressed root colonization of mungbean by *Fusarium* spp.,(Shahzad, 1994). Bavistin showed maximum inhibition of mycelium of *Fusarium solani* under *In vitro* conditions while under field conditions; the combination of Bavistin and Dithane M-45 gave the lowest root rot incidence of *Acacia catachu* Willd. (Tomar, 2004). The pretreatment of ginger rhizomes before storing with mancozeb, carbendazim, metalaxyl or thiophanate methyl @ 0.2% reduced the decay of rhizomes by *F. solani* and increased recovery of healthy rhizomes during storage (Ram & Thakore 2009).

Table 1. Mean percent inhibition of colony growth of *Fusarium solani* 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	38.7a	41.8a	40.3a	36.4a	40.3a	36.4a	32.4a
20	64.6b	74.3b	68.2b	67.5b	59.1b	68.7b	55.1b
50	85.4c	96.4c	87.2c	84.4c	87.8c	89.4c	71.7c
100	100.0d	100.0d	100.0d	93.3d	94.2d	94.4d	94.7d
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.

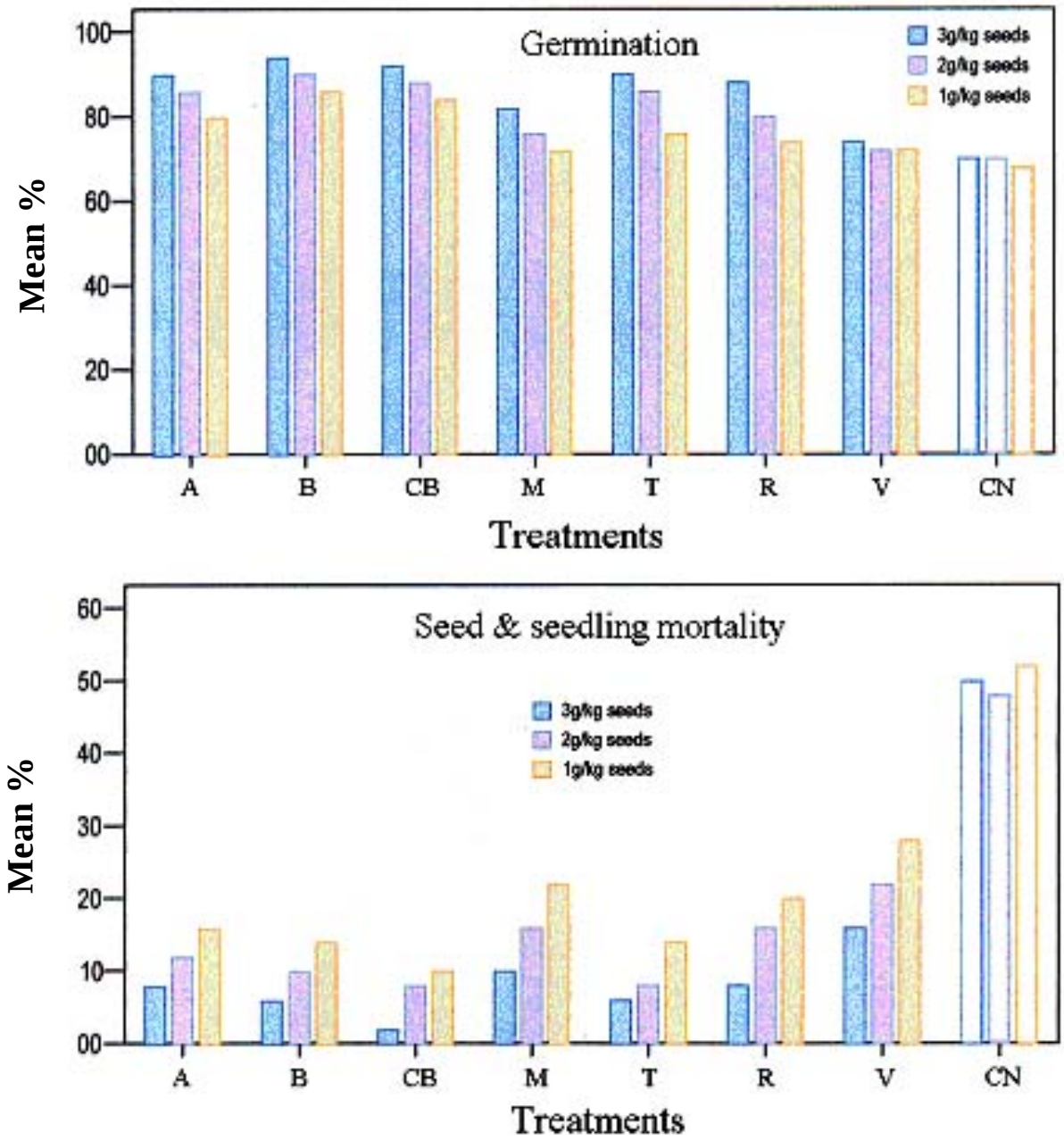

Fig. 1. Effect of seed treatment with fungicides on germination infection by *Fusarium solani* on bottle gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

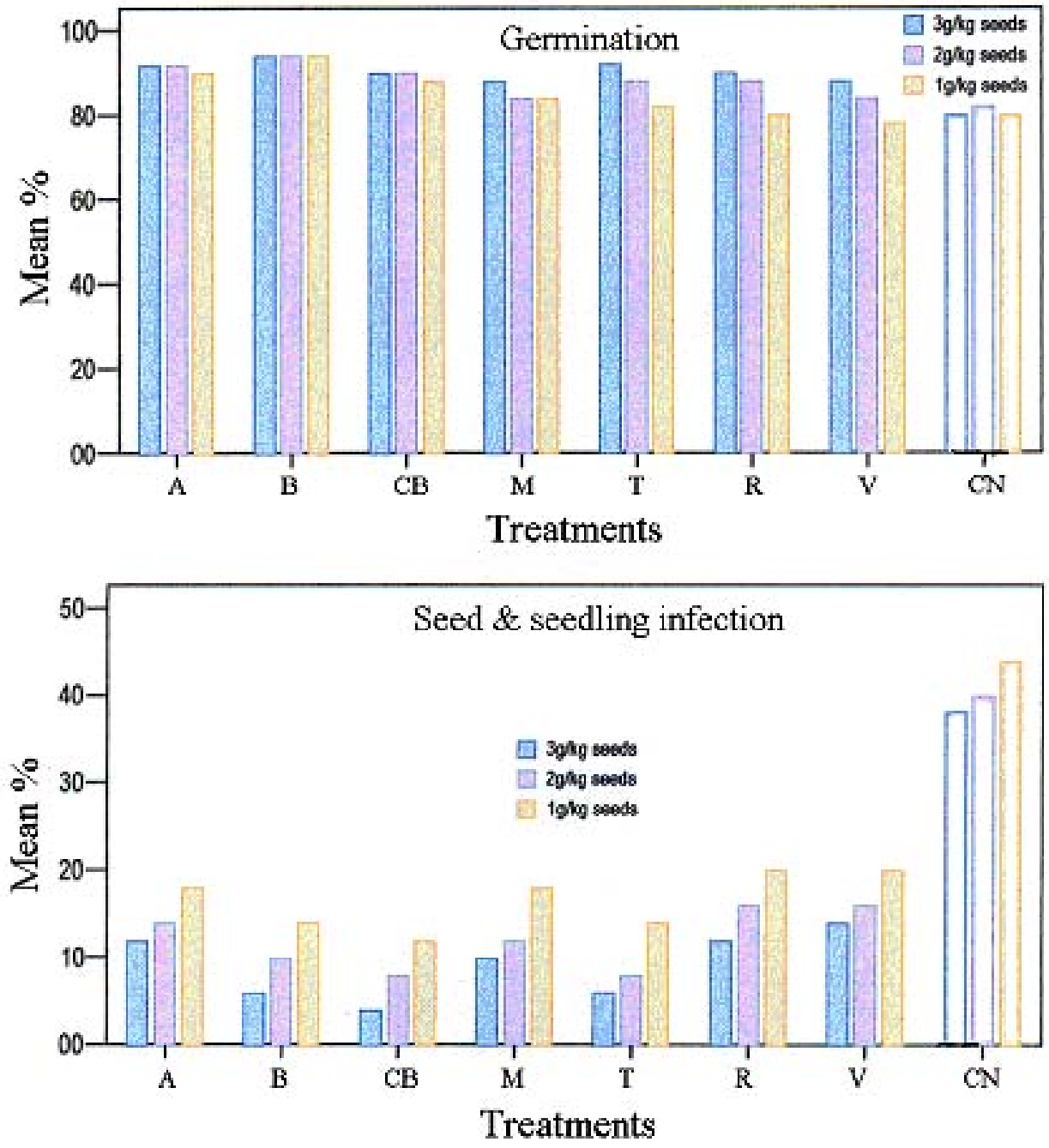

Fig. 2. Effect of seed treatment with fungicides on germination and infection by *Fusarium solani* on cucumber.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

c. *In vitro* effect of microbial antagonists: All microbial antagonists significantly reduced seed infection caused by *F. solani* in bottle gourd, bitter gourd and cucumber with no significant effect on seed germination. Maximum reduction in seed infection by *T. harzianum* was recorded in cucumber (Fig. 7a) by *T. harzianum* and *T. viride* in bitter gourd (Fig. 8a) and by *T. harzianum* and *G. virens* in bottle gourd (Fig. 9a). Ram & Thakore (2009) reported that *T. harzianum* and *B. subtilis* completely suppress the growth of *F. solani* and effectively minimized storage rot by dipping rhizomes of ginger in a combined suspension of *Pseudomonas fluorescens* and *T. harzianum* @ 0.5% for 30 min before storage.

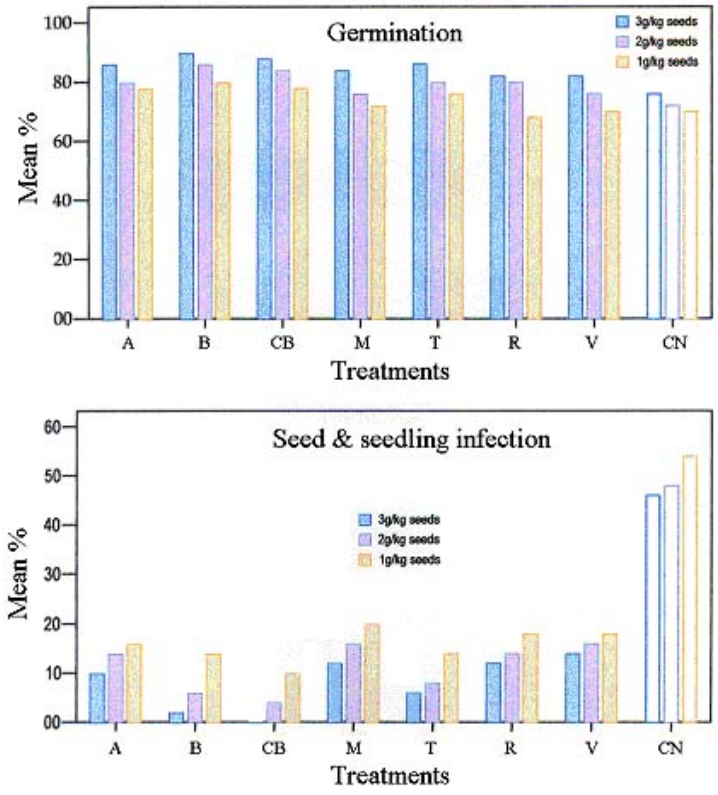

Fig. 3. Effect of seed treatment with fungicides on germination and infection by *Fusarium solani* on bitter gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

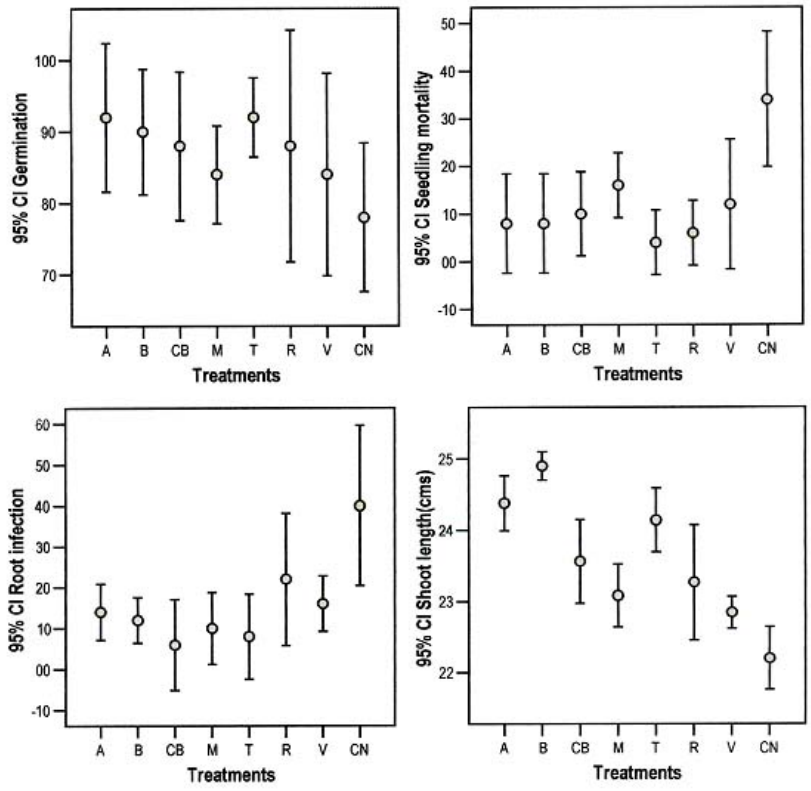

Fig. 4. Effect of seed treatment with fungicides on germination, shoot length, seedling mortality and root infection by *Fusarium solani* on bitter gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

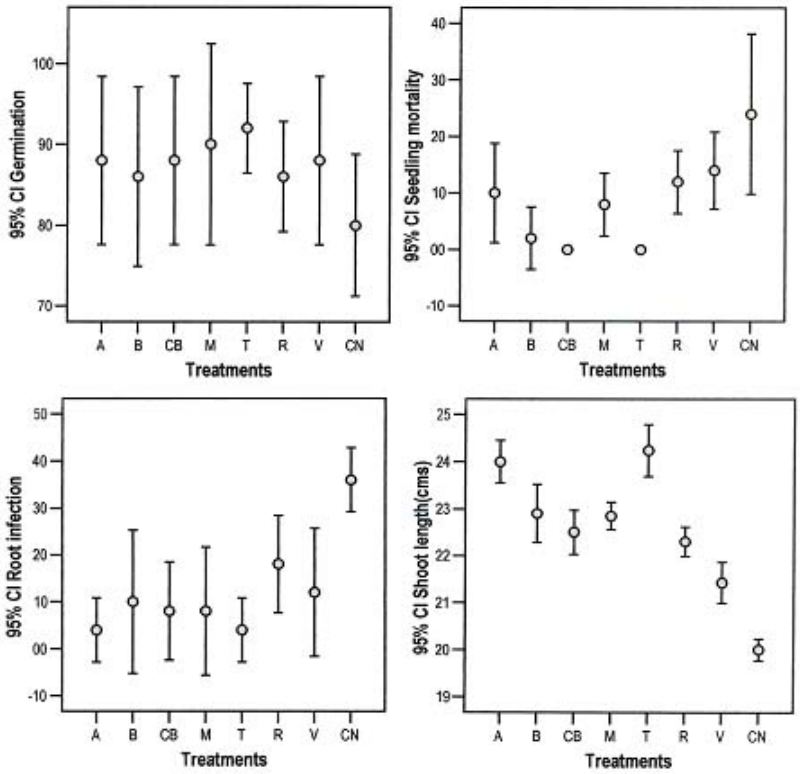

Fig. 5. Effect of seed treatment with fungicides on germination, shoot length, seedling mortality and root infection by *Fusarium solani* on bitter gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

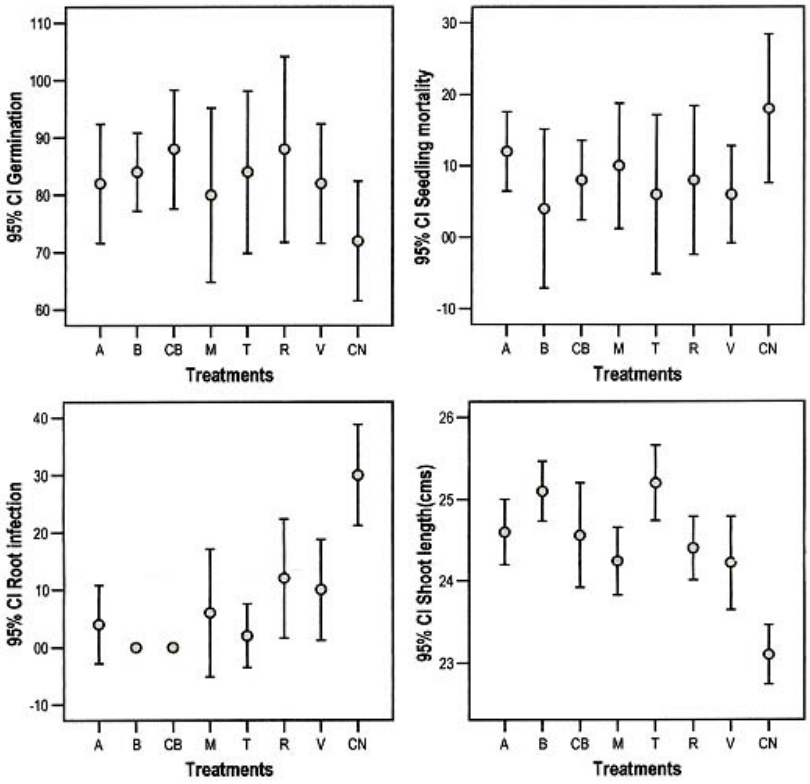

Fig. 6. Effect of seed treatment with fungicides on germination, shoot length, seedling mortality and root infection by *Fusarium solani* on bitter gourd.
A= Aliette, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin-M, R= Ridomil, V= Vitavax, CN= Control

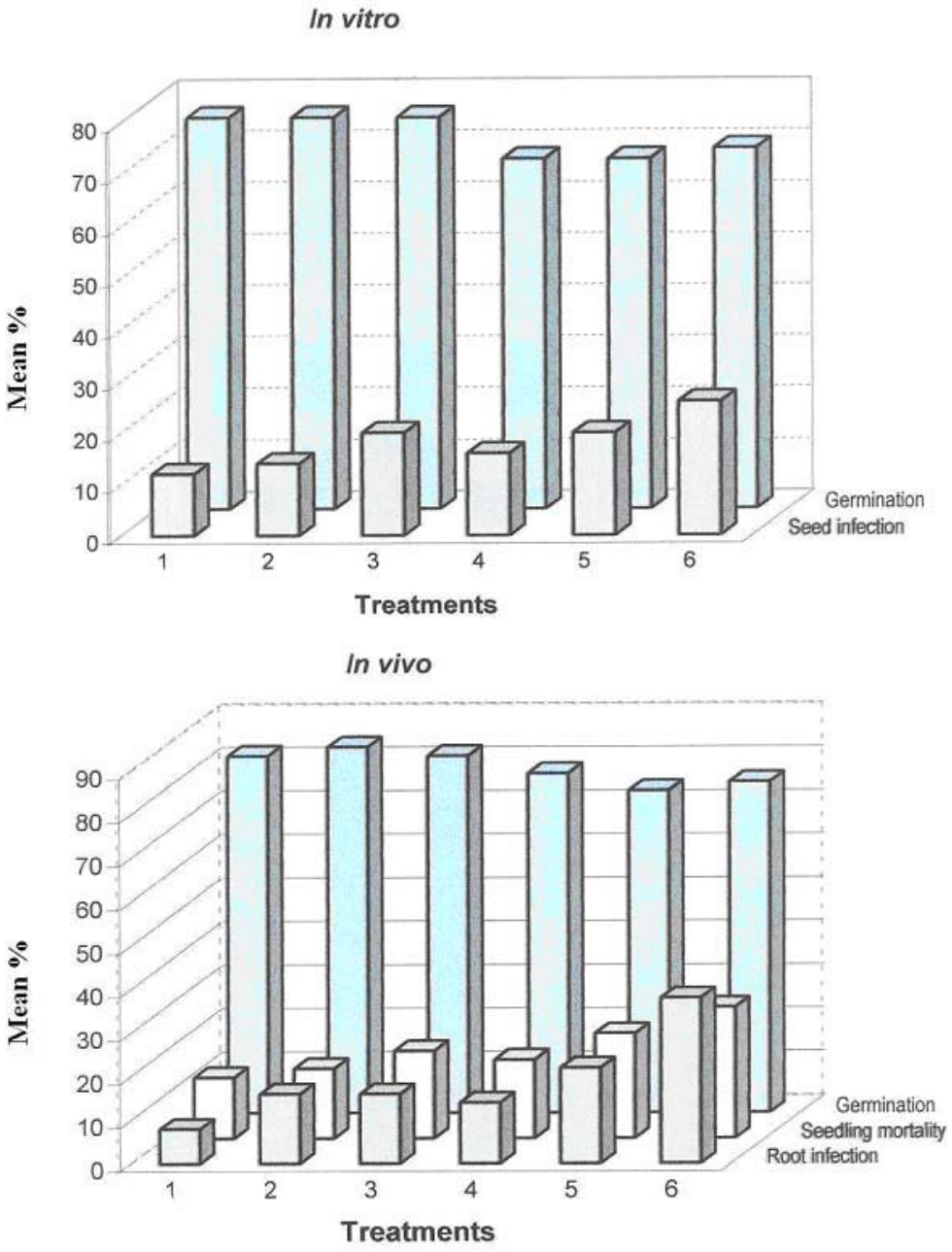

Fig. 7. Effect of microbial antagonists on germination and infection of seed, seedling and root by *Fusarium solani* on cucumber.
1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5= *Sachybotrys atra*, 6= Control.

d. *In vivo* effect of microbial antagonists: All antagonists significantly reduced seedling mortality and root infection in bottle gourd, bitter gourd and cucumber caused by *F. solani*, *T. harzianum*. *T. viride*, *B. subtilis* and *G. virens* enhanced germination. The higher reduction in seedling mortality (14%) and root infection (8%) were produced by *T. harzianum, T. viride, B. subtilis* and *G. virens* followed by *S. atra* in cucumber (Fig.7b). In bitter gourd seedling mortality was significantly reduced in treatments where *T. harzianum* and *B. subtilis* were used followed by *T. viride, G. virens* and *S. atra*. Root infection was significantly reduced but no significant differences among antagonists were seen (Fig. 8b). The higher reduction in seedling mortality and root infection of bottle gourd were produced by *T. harzianum, T. viride* and *B. subtilis* whereas *G. virens* and *S. atra* were found least effective (Fig. 9b).

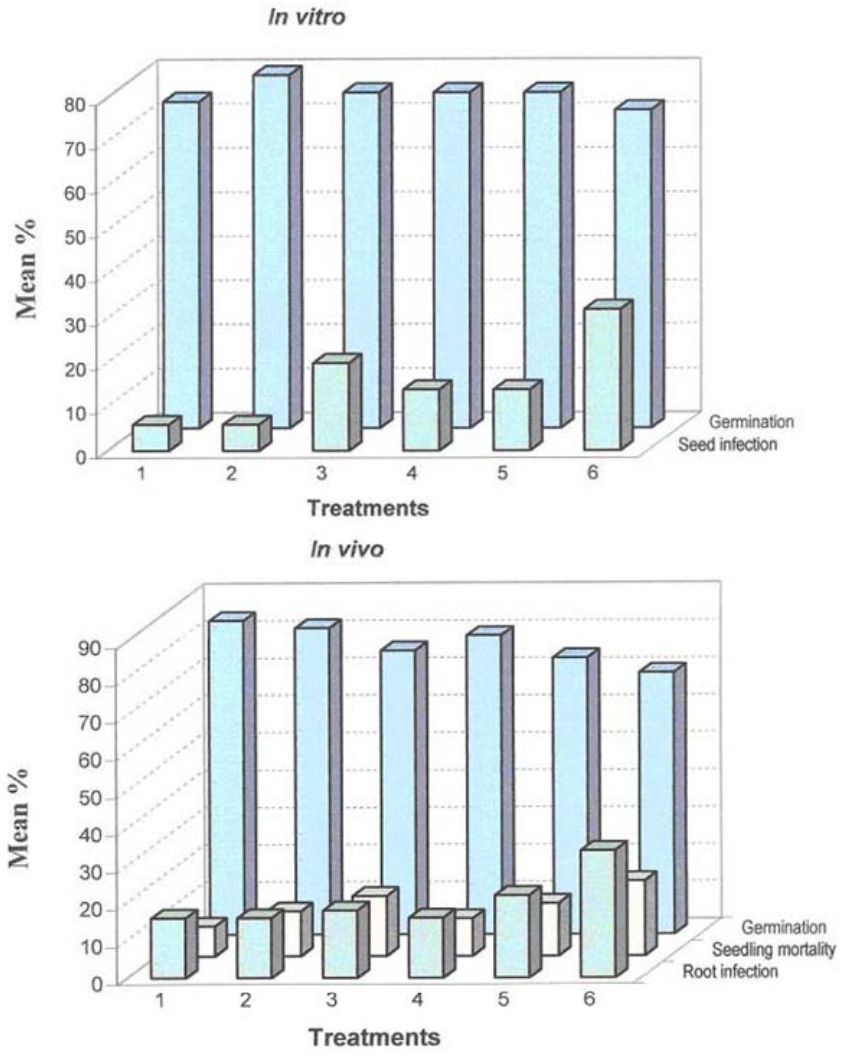

Fig. 8. Effect of microbial antagonists on germination and infection of seed, seedling and root by *Fusarium solani* on cucumber.
1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5= *Sachybotrys atra*, 6= Control.

Microbial antagonist have been used to minimize the hazardous effect of pesticides for the control of root infecting *Fusarium* spp., (Tu, 1980; Elad *et al*., 1983; Ehteshamul-Haque *et al*., 1990; Perveen & Ghaffar, 1991). Qureshi & Ghaffar (1966) found that most of the microorganisms used by them were unable to inhibit the growth of *F. solani*. In contrary to them *Trichoderma* spp., gave maximum mycelial inhibition of *Fusarium solani* (Tomar, 2004; Gupta & Misra, 2009). *Bacillus* spp., significantly reduced radial mycelial growth of *F. solani* f. sp., *tuberosi* on PDA and reduced tuber dry rot *In vivo* (Daami-Remadi *et al*., 2006). *B. subtilis* applied as a seed treatment significantly reduced seed colonization and root rot on broad bean (Sarhan, 1989) and its metabolites inhibited *F. solani* associated with wheat (Lazzaretti *et al.*, 1994). Coating of seed with *T. harzianum, T. viride* @ 1% showed improved germination, root rot reduction and better cotton seed yield (Monga & Dad Raj, 2000). Infection of *F. solani* was greatly reduced in presence of *T. harzianum, T. viride* and *G. virens* (Perveen *et al.*, 1994). 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). *Trichoderma* spp., completely suppressed wilt and showed plant growth promoting effect on guava plants (Gupta & Misra, 2009).

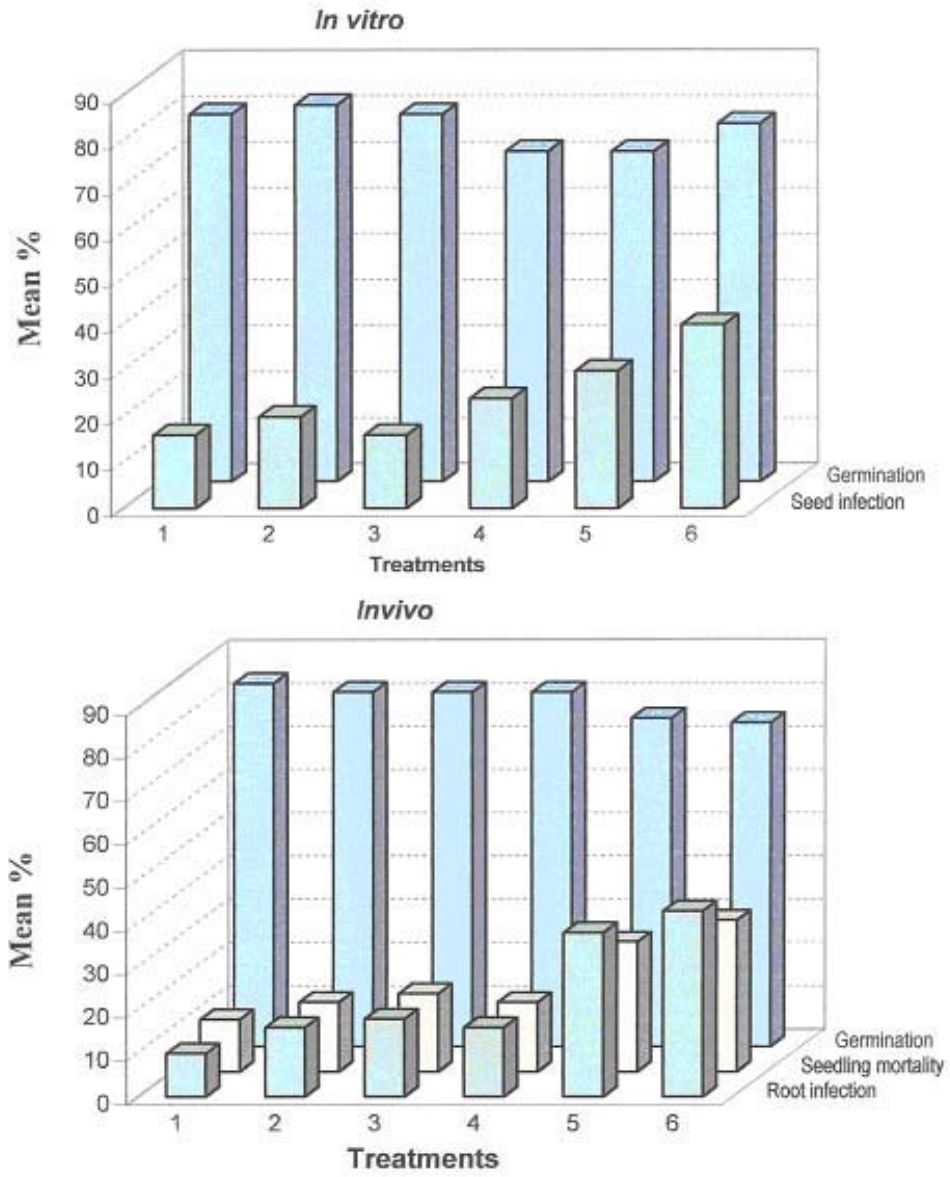

Fig. 9. Effect of microbial antagonists on germination and infection of seed, seedling and root by *Fusarium solani* on cucumber.
1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5= *Sachybotrys atra*, 6= Control.

e. Effect of oilcakes on pathogenesis: Oilcakes treatments had significant effect on seed germination, seedling mortality and plant size of bottle gourd, bitter gourd and cucumber. Mustard cake was found most effective at all ratios followed by neem and castor cake. Maximum germination (93%) and minimum seedling mortality (8.6%) in bottle gourd were recorded in treatments where neem cake was amended at the ratios of 1:20 in non sterilized soil. Plant attained maximum size (26.6cms) where sterilized soil was amended with mustard cake at the ratio of 1:30 (Table 2). Maximum seed germination (94-96%) and minimum seedling mortality (4.3-6%) of cucumber were recorded in treatments where mustard cake was amended at the ratio of 1: 20 in non sterilized and sterilized soil. Plant size was increased up to 22.3 cms in treatment where mustard and neem cake was amended at the ratio of 1: 20 in non sterilized soil (Table 3). Maximum germination (93%) and plant size (26.6 cms) and minimum seedling mortality (2.3%) in bitter gourd were observed where mustard cake was used at the ratio of 1:20 in non sterilized soil (Table 4).

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

Ratios	Mean percent				Shoot length (cm)	
	Seed germination		Seedling mortality			
	St	Nst	St	Nst	St	Nst
	Mustard cake					
1:20	90b	92b	16.3a	9.6a	24.3ab	24.5b
1:30	89b	93b	20.7a	18.6b	26.6b	25.1b
1:60	80ab	92b	33.3b	29.0c	23.0ab	20.3a
	Neem cake					
1:20	92b	93b	18.0a	8.6a	24.3ab	23.3ab
1:30	82ab	92b	20.3a	17.3b	23.6ab	25.6b
1:60	83ab	91b	34.3b	22.6bc	23.6ab	23.6ab
	Castor cake					
1:20	88b	89b	20.6a	15.6b	21.6ab	21.7ab
1:30	86b	89b	27.0ab	22.6bc	20.2a	21.6ab
1:60	80ab	87b	28.0ab	30.3c	20.0a	19.6a
Control	78a	83a	38.80c	37.3d	19.0a	21.6ab

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 solani* on cucumber.

Ratios	Mean percent				Shoot length (cm)	
	Seed germination		Seedling mortality			
	St	Nst	St	Nst	St	Nst
	Mustard cake					
1:20	96c	94c	6.0a	4.3a	21.3c	21.3c
1:30	93c	92c	8.0b	6.3a	19.5b	19.5b
1:60	87b	89bc	12.6c	13.0b	18.7ab	19.3b
	Neem cake					
1:20	90c	90c	7.3a	8.0a	20.0bc	22.3c
1:30	89c	90c	11.6bc	11.0ab	18.7ab	20.1bc
1:60	81b	84b	17.3c	17.7c	16.9a	19.5b
	Castor cake					
1:20	85b	84b	9.6a	8.6a	19.2b	19.8b
1:30	78a	80a	15.0c	10.3ab	18.6ab	18.6ab
1:60	77a	78a	19.3c	19.3c	17.5a	18.2a
Control	76a	78a	23.8d	21.8d	16.7a	17.1a

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 4. Effect of oilcakes amendment in soil on seed germination, plant size and disease caused by *Fusarium solani* on bitter gourd.

Ratios	Mean percent				Shoot length (cm)	
	Seed germination		Seedling mortality			
	St	Nst	St	Nst	St	Nst
	Mustard cake					
1:20	88c	93b	3.6a	2.3a	24.0b	26.5b
1:30	80b	83ab	7.6b	3.3a	23.1b	20.8a
1:60	79b	80ab	14.3c	9.3b	21.5a	21.8a
	Neem cake					
1:20	80b	83ab	5.0ab	3.3a	23.2b	25.3b
1:30	80b	83ab	6.6ab	4.3a	22.9ab	23.3ab
1:60	77ab	73a	16.4c	13.36c	21.9a	22.0a
	Castor cake					
1:20	79b	83ab	8.5b	4.0a	22.6ab	23.0a
1:30	70a	76a	8.3b	4.0a	22.8ab	22.4ab
1:60	75ab	76a	15.3c	16.3d	21.6a	22.0a
Control	72a	75a	18.3d	15.5d	21.1a	21.8a

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.

F. solani infested seeds of bottle gourd, cucumber and bitter gourd reduced seedling mortality and root infection when sown in mustard and neem cake amended soil. Mustard cake was found most effective in reducing seedling and root infection in cucumber. However none of the oil cakes completely controlled seedling and root infection cause by *F. solani* in cucumber, bottle gourd and bitter gourd. Contrary to our results, neem cake has been found to completely control *F. solani* infection in 40 days old plant of soybean (Ali, 1997). Such similar report has been made by Khalis & Manoharachary (1985) who found that in amended soil *F. solani* disappeared completely. The suppression of pathogenic fungi by organic soil amendment have been reported (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 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). Integrated effect of microbial antagonists, fungicides and organic amendment could kill pathogenic fungi, bacteria and nematodes and resulting in increased in yield.

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