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RESEARCH ARTICLE

Efficacy of antagonists, oil cakes and fungicides against *Fusarium* wilt disease in brinjal

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ABSTRACT

An investigation was conducted to demonstrate the efficacy of different antagonists, oil cakes and fungicides with known activity against brinjal vascular wilt, *Fusarium solani*. Three different antagonistic genera *viz*., *Bacillus* sp., *Pseudomonas* sp. and *Trichoderma* spp., seven different oil cakes, and four different commercial fungicides were tested against the pathogen under *in vitro* and field conditions. Among the *Pseudomonas* isolates tested *in vitro*, Pf2 was most effective against the pathogen. Among *Trichoderma* sp. tested *in vitro*, TV1 was most effective against *Fusarium solani.* The isolates of *Bacillus* sp. screened for antifungal activity against *Fusarium solani* Bs1 was most effective against the pathogen. Among the oil cake extracts tested *in vitro*, neem cake extract (5%) effectively reduced the growth of *F. solani.* Among the fungicides tested *in vitro,* carbendazim at 500 ppm was most effective against *F. solani.* The effective antagonists, organic amendments and carbendazim were screened *in vitro* and further confirmed in pot culture experiments. Results of the pot culture experiment indicated that soil application of *T. viride* (SA) @ 2.5 kg·ha^{-1} had the maximum disease reduction over control. The most effective treatments, and their combinations, screened in laboratory and pot culture were tested against *F. solani* under field condition. Results of the field experiment indicated that treatment with *T. viride* (SA) @ 2.5 kg·ha^{-1} had the maximum disease reduction over control and improved plant growth and yield of Brinjal (35.0 Mt.ha^{-1}).

Keywords: *Bacillus subtilis, Brinjal, Fusarium solani, Oil cakes, Pseudomonas fluorescens, Trichoderma viride*

INTRODUCTION

Brinjal (*Solanum melongena* L.) is grown as an important vegetable crop all over the world, mostly in the Indian subcontinent and southeast Asia. Among the different diseases that attack brinjal crops, wilt has become a major significant disease, causing a significant reduction in yield. The wilt disease of brinjal is characterized by yellowing of foliage, drooping of apical shoot leading to the ultimate death of the whole plant. Brinjal yield is drastically reduced due to infection by the wilt pathogen *Fusarium solani* (Sacc.), a fungus that can survive in the soil for many years (Chakraborty and Chatterjee, 2008). Ingeneral, the genus *Fusarium* is a severe destructive disease on crop plants. They cause chlorosis, necrosis, premature leaf drop, browning of the vascular system, and wilting, all of which consequently cause significant yield losses. *Fusarium* species exhibit a high level of host specificity, are classified into more than 120 formae speciales and races on the basis of plant species and cultivars they infect(Armstrong and Armstrong, 1981). The genus of wilt pathogens such as *F. oxysporum, F. solani, F. graminearum*, and *F. verticillioides* (Khan *et al*., 2017). Effective management of crop disease is generally with the use of synthetic pesticides (Kiran *et al.,* 2006). Application costs of synthetic fungicides are comparatively higher, particularly in countries where pesticides are imported, pollution to soil, water, and air by the accumulation of chemical residues and development of resistance races require that alternatives be developed for control of pathogens (Parthasarathy *et al*., 2016). Control of vascular wilt disease is challenging due to the long perseverance of the quiescent structures in the field and the broad host range of some species. The pathogen is difficult to manage once it spreads the vascular tissue and chemical fungicides seem to be ineffective (Deketelaere *et al*., 2017). Reducing the primary inoculum in the soil has been considered as an important goal and can be accomplished by several management strategies. Management of soil-borne pathogens with biocontrol agents and

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antagonistic microorganisms in the soil is an active, non-chemical control method. Fungal antagonists and botanicals have been tested under *in vitro* and *in vivo* for antimicrobial activity against *Fusarium solani* (Narayanan *et al*., 2015). Biological control has a prospective for the management of soil-borne diseases. A range of soil microorganisms has confirmed activity in the control of various soil-borne plant pathogens, including *Fusarium* wilt pathogens (Larkin and Fravel, 1998). Rhizosphere competence of antagonist is a pre-requisite for biological control of soil- borne pathogens and is associated with production of higher amounts of cellulolytic enzymes, increased saprophytic ability, and manipulation of the environment around a crop plant to favor organisms that contribute to crop health and vigor rather than with pesticides which destroy a range of microorganism including fungal pathogens. The domestic antagonist is most virulent against wilt pathogens because they have evolved under local conditions (Najar *et al.,* 2011). Organic amendments, have the capability to modify soil characteristics such as the concentration of nutrients (e.g., P, K, Fe), pH, NO_3 content, organic material, and structure, which decisively imparting the colonization, adherence, survival, reproduction and competency of beneficial microorganisms in the soil. The objective of this research was made with this background, to evaluate the individual and integrated efficacy of oilcakes, antagonists and fungicides to offer ecofriendly management of soil-borne pathogen *F. solani*.

MATERIAL AND METHODS

A survey was conducted on the occurrence of wilt disease in different brinjal growing areas of Tamil Nadu, *viz*., Madurai (Checkkanurani, Surakundu), Tirunelveli (Athuvazhi), Virudhunagar (Pudhur), Trichy (Kalapatti) and Dindugal (Nilakottai). In each field, 100 plants were selected at random and the number of infected plants by *Fusarium solani* were recorded. *Fusarium solani* was isolated from wilted brinjal plants and maintained in pure culture on potato dextrose agar (Chakraborty and Chatterjee, 2008).

Isolation of antagonists from the rhizosphere region

Antagonistic fungi and bacteria were isolated from rhizosphere soil collected from the above mentioned different brinjal growing areas of Tamil Nadu, India. Plants were gently removed from the soil with intact roots and the excess soil adhering to roots gently removed. Ten-g of rhizosphere soil was transferred to a 250 mL Erlenmeyer flask containing 100 mL of sterile distilled water. After thorough shaking, the organisms in the suspension were isolated by serial dilution. From the 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, dilutions 1 mL of each aliquot was pipetted out, and separately placed in sterilized Petri dishes containing *Trichoderma* special medium (TSM), King's B medium (King *et al*., 1954), nutrient agar medium(NA) and gently rotated clockwise and counter clockwise for uniform distribution and incubated at room temperature (28±2°C) for 24 hrs. Colonies with characteristics of *Bacillus* spp. or *Pseudomonas* spp. were isolated and purified using a streak plate method (Rangaswami, 1993) on nutrient agar and King's B media. *Trichoderma* sp. isolated from TSM medium was purified on PDA. Pure cultures were maintained on agar slants and stored at 4°C.

Talc based formulation

In India, talc based formulations of *T. viride* was developed at Tamil Nadu Agricultural University, Coimbatore for seed treatment of pulse crops and rice (Jeyarajan et al., 1994). *Trichoderma* is grown in the liquid medium is mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade. The talc formulations of Trichoderma has a shelf life of 3 to 4 months. It has become quite popular in India for the management of several soil-borne diseases of various crops through seed treatment at 4 to 5 g/kg seed. Several private industries produce large quantities of talc formulations in India for supply to the farmers. The annual requirement of Trichoderma has been estimated as 5,000 tones to cover 50 per cent area in India (Jeyarajan, 2006).

Screening fungal antagonists against F. solani in vitro

Three isolates of *T. viride*(TV1,TV2,TV3) and *T. harzianum*(TH1,TH2,TH3) were screened against *F. solani* using a dual culture technique (Dennis and Webster, 1971). *Trichoderma* sp. were placed opposite each other near the periphery of the Petri plate and incubated at room temperature (28±2°C). After four days of incubation, mycelial growth of the pathogen and the inhibition zone were measured in treated and control plates. Percent inhibition (PI) of mycelia growth was calculated using the formula of Pandey *et al.* (2000). Overgrowth of antagonists over the pathogen was measured seven days after incubation.

Screening of bacterial antagonists under in vitro

Bacterial isolates (Pf1,Pf2,Pf3) were tested for their inhibitory effect on the growth of *F. solani* using a dual culture technique (Dennis and Webster, 1971). Each bacterial isolate was streaked on one side of a Petri dish (1 cm away from the plate edge) on PDA medium and a 5-day old mycelial disc (8 mm dia) of *F. solani* was placed on the opposite side of the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature (28±2°C) for 4 days. After four days the pathogen growth and inhibition zone were measured.

Efficacy of oil cake extracts against F. solani in vitro

One-g of each oil cake(Neem, Castor, Pungam, Groundnut, Gingelly, Cottonseed, Mahua) was made into powder separately and soaked in 1.25 mL of sterile distilled water overnight. The material was ground using a pestle and mortar, filtered through a muslin cloth and the filtrate centrifuged at 10,000 rpm for 15 min. The supernatant served as the standard extract solution (100%) (Dubey and Patel, 2000). The efficacy of oil cake extract was tested against *F. solani* using the technique of Schmitz (1930). Fifty-mL of freshly prepared PDA was placed in a conical flask. Aqueous extracts of oil cake 5 mL were mixed with 45 mL of PDA medium to obtain a 5% concentration and sterilized. The sterilized PDA medium (15 mL per Petri dish) was poured into sterilized Petri dishes and allowed to solidify. A 9 mm mycelial disc of *F. solani* was taken from an actively growing culture and placed at the centre of each Petri dish which was incubated at room temperature. The PDA medium without extract of oil cake served as control. The radial growth of *F. solani* was recorded after seven days of incubation and expressed as growth inhibition percentage.

Effect of fungicides against F. solani in vitro

The efficacy of fungicides was tested against *F. solani* using the technique of Schmitz (1930). Concentrations of 100, 200 or 500 ppm(Carbendazim, Propiconazole, Tebuconazole, Fosteyl aluminum) were added with 100 mL sterilized PDA medium. The medium was poured into a sterilized Petri dish and allowed to solidify. A 9 mm mycelial disc of *F. solani* was taken from an actively growing culture, placed at the centre of each Petri dish and incubated at room temperature. The medium without fungicide served as control. The radial growth of *F. solani* was recorded after seven days of incubation and expressed as growth inhibition.

Efficacy of antagonists, organic amendments and fungicide against F. solani wilt of Brinjal in pot culture

A pot culture experiment was conducted in a greenhouse. Talc based formulations of antagonistic bacteria and fungi were delivered as soil applications at 30 and 60 days after sowing. The *F. solani* was multiplied on sand maize medium and incorporated in pots at 5% (w/w). The observations on percent disease incidence of wilt were recorded. Each treatment was replicated three times. The treatments were: T1 = *T. viride* 1 Soil Application @ 2.5 kg·ha^{-1}; T2 = *T. harzianum* 2 Soil Application @ 2.5 kg·ha^{-1}; T = *P. fluorescens* 1 Soil Application @ 2.5 kg·ha^{-1}; T4 = *P. fluorescens* 2 Soil Application @ 2.5 kg·ha^{-1}; T5 = *Bacillus subtilis* 1 Soil Application @ 600 g·ha^{-1}; T6 = *B. subtilis* 2 Soil Application @ 600 g·ha^{-1}; T7 = *Methylobacterium* Soil drench (SD) 2.5 kg·ha^{-1}; T8 = Carbendazim (0.1%) SD; T9 = Neem cake @ 150 kg·ha^{-1}; T10 = Mahua cake @ 150 kg·ha^{-1}; T11 = Gingelly cake @ 150 kg·ha^{-1}, and T12 = untreated control.

Effect of antagonists, organic amendments and fungicide against F. solani wilt incidence and yield of brinjal in field

A field experiment was conducted to develop management practices against *F. solani* wilt of Brinjal. The effective treatments tested under pot culture were evaluated in the field. The brinjal cv. Vijay (Ankur Seeds Private Limited, Nagpur, India) was used, the plot size of 5 × 4 m^2. After leveling plots, composted materials and fertilizers were applied at recommended rates as per Horticulture Crop Production Guide, 2008 and seedlings were planted in rows of 45 × 15 cm spacing and later thinned. Plants were uniformly irrigated after planting. Subsequent irrigation was applied three days after planting and thereafter plants were irrigated uniformly at weekly intervals. Observations on disease incidence and yield were taken at 60 DAS.

Statistical analysis

Pot culture and laboratory experiments were conducted with a Completely Randomized Design. The field experiment was arranged in a Randomized Complete Block Design. Data were subjected to analysis of variance (Gomez and Gomez, 1984). Duncan's Multiple Range Tests was used to separate means. Percent values were transformed by arcsine or square root.

RESULTS AND DISCUSSION

Brinjal is a commercial vegetable crop in India which attacked by several diseases of fungi and bacteria.

Table 1. Effect of *Trichoderma* spp. against mycelial growth of *Fusarium solani* (*in vitro*)

Treatments	Mycelial growth (cm)**	Per cent reduction over Control	Inhibition zone (mm)
Trichoderma viride (TV1)	2.66	69.77	1.37
Trichoderma viride (TV2)	2.76	68.63	0.76
Trichoderma viride (TV3)	3.64	58.63	0.42
Trichoderma harzianum (TH1)	2.85	67.61	1.32
Trichoderma harzianum (TH2)	2.87	67.38	1.36
Trichoderma harzianum (TH3)	2.86	67.55	1.43
Control	8.8	-	-
CD (P=0.05)	0.2		

* Mean of three replications

Among them, vascular wilt is an important restriction in brinjal production because of *Fusarium*

spp. penetrate through the roots and proliferate in vascular tissue (Boyaci *et al*., 2011) which makes it difficult to control. The results of the present study summarized the interactions between the antagonists and the pathogen under *in vitro* and *in vivo* conditions.

Table 2. Effect of different isolates of *Pseudomonas fluorescens* against *Fusarium solani* (*in vitro*)

Treatment	Mycelial growth (cm)**	Per cent reduction over control	Inhibition zone (mm)
Pseudomonas fluorescens (Pf1)	4.76	46.51	0.56
Pseudomonas fluorescens (Pf2)	4.67	47.52	0.76
Pseudomonas fluorescens (Pf3)	4.88	45.16	0.53
Control	8.90	-	-
CD (P=0.05)			0.18

* Mean of five replications

Effect of Trichoderma spp. against mycelial growth of F. solani

The antagonistic action of the selected fungal bio-agents against the *F. solani* f. sp. *melongenae* was performed by dual culture technique. Three isolates each of *T. viride* and *T. harizanum* were collected from Brinjal growing areas of Tamil Nadu and tested for antagonistic activity against *F. solani* f. sp. *melongenae* with a dual culture technique. Mycelial growth of the *F. solani* f. sp. *melongenae* ranged from 2.66 to 3.64 cm.

Table 3. Effect of *Bacillus subtilis* against the mycelial growth of Fusarium solani (*in vitro*)

Treatment	Mycelial growth (cm)**	Per cent Reduction Over Control	Inhibition Zone (mm)
Bacillus subtilis (Bs1)	3.67	59.76	0.66
Bacillus subtilis (Bs2)	3.86	57.62	0.86
Bacillus subtilis (Bs3)	3.76	58.75	0.76
Control	8.93	-	-
CD (P=0.05)	0.20		

* Mean of five replications

Among the tested isolates, *T. viride* (TV1) recorded the maximum (69.77%) inhibition on the mycelial growth (2.66 cm) with 1.37 mm of inhibition zone followed by *T. viride* (TV2) (Table 1). Inhibition of growth of the *F. solani* f. sp. *melongenae* by *T. viride* is also in confirmatory with the similar reports of Chakraborty and Chatterjee (2008) *Trichoderma harzianum* and *T. viride* resulted in decreasing population of *F. solani* in soil, there by deterring disease incidence in field condition. Bernal *et al*. (2001) observed effective antagonism between *Trichoderma* spp. and *Fusarium oxysporum* f. sp. *cubense*. Abou-Zeid *et al*. (2007) found that biological agents (*T. viride* and *T. hamatum*) significantly inhibited the radial mycelial growth of the pathogenic fungi-*Fusarium oxysporum*. Both *T. harzianum* and *T. viride* specifically reduced incidence of wilt disease up to 86 and 83%, respectively (Chakraborty *et al.,* 2008).

Table 4. Efficacy of different oil cake extracts on the mycelial growth of *Fusarium solani* (*in vitro*)

Treatment	Mycelial growth (cm)*	Per cent reduction over control
Neem cake (5%)	2.66	70.64
Castor cake (5%)	8.86	1.12
Pungam cake (5%)	6.75	24.85
Groundnut cake (5%)	6.36	29.01
Gingelly cake (5%)	6.25	30.25
Cotton seed cake (5%)	8.65	3.37
Mahua cake (5%)	3.36	62.56
Control	8.96	-
	0.21	-

* Mean of three replications

Effect of P. fluorescens isolates against F. solani

Bacteria have been explored as biocontrol agents, plant growth promoters and inducers of disease resistance (Parthasarathy *et al*., 2016). To exploit this, the present study among the three isolates of *Pseudomonas* sp. tested for their antagonistic activity against *Fusarium solani* f. sp. *melongenae* by dual culture, Pf2 significantly exerted highest (47.52) per cent reduction of mycelial growth with 0.76 mm inhibition zone followed by Pf1 (Table 2). The mode of action of *P. fluorescens* were more effective than other bacterial BCA's as production of siderophores, production of HCN (Ahl *et al*., 1986), and induced systemic resistance (Van Peer *et al.,* 1991). Strains of *P. fluorescens* showed inhibitory action against chickpea wilt pathogen *F. oxysporum* f. sp. *ciceris* (Vidhyasekaran and Muthamilan, 1995). Thangavelu *et al.* (2001) screened 11 isolates of *P. fluorescens.* Among them Pf10 was most effective in inhibiting mycelial growth of *F. oxysporum* f. sp. *cubense.*

Effect of B. subtilis against the mycelial growth of F. solani

Three isolates of *Bacillus sp.* screened for antifungal activity against *F. solani* f. sp. *melongena*, Bs1 significantly recorded maximum 59.76% reduction of mycelial growth with 0.66 mm inhibition zone followed by BS3 (Table 3). Similarly, in brinjal, Podile and Dube (1985) found that inoculation of *B. subtilis* Sy1 promoted germination and early development of Brinjal seed. In Brinjal, *B. subtilis* Sy1 not only antagonized several pathogenic fungi but also promoted seedling growth and increased stress tolerance. This might be due to production of antibiotics such as iturin, bacillomycin, zwittermycin A and surfactin (Yu *et al.,* 2002). Kilian *et al*. (2000) summarized a number of mechanisms that could contribute to increased yields and reduced attack by pathogens following application of *B. subtilis*,

such as competition by temporary colonization of the rhizosphere and rhizoplane, induced resistance by activation of defense genes in plants, promotion of plant and root growth, and formation of growth controlling substances like cytokinin and auxin.

Table 5. Effect of different fungicides against *Fusarium solani* in vitro

Treatments	Per cent growth inhibition (cm)			% growth inhibition		
	Concentration (ppm)			Concentration (ppm)		
	100	200	500	100	200	500`
Carbendazim (BAVISTIN)	2.75	2.85	1.47	69.30	68.19	83.59
Propiconazole (TILT)	2.66	2.85	1.76	70.31	68.19	80.35
Tebuconazole (FOLICUR)	4.75	3.85	3.66	46.98	57.03	59.15
Fosetyl aluminium (ALIETTE)	2.92	2.75	2.85	67.41	69.30	68.19
Control	8.96					

* Mean of four replications CD (P=0.05)

Treatment 0.09

Concentration 0.07

Treatment x Concentration 0.1

Efficacy of oil cakes on mycelial growth of F. solani

The results on the efficacy of extracts of seven oil cake extracts were tested against the growth of *F. solani*. The result showed that neem cake (5%) significantly recorded maximum (70.64%) reduction of mycelial growth over control followed by mahua cake (5%) recorded 62.56% reduction of mycelial growth over control respectively (Table 4). Addition of organic residues to soil was effective for management of soil-borne diseases.

Table 6. Effect of antagonists, organic amendments and fungicides on wilt incidence of brinjal plants in pot culture

Treatments	*Disease incidence (%)						Mean Disease incidence (%)*	Per cent reduction over control (%)
	10 DAS	25 DAS	40 DAS	55 DAS	70 DAS	85 DAS		
Trichoderma viride (Tv1) SA @ 2.5 kg/ha	0.84 (5.25)	2.67 (9.40)	6.92 (15.25)	8.46 (16.90)	7.64 (16.04)	8.25 (16.92)	5.79	88.58
Trichoderma harzianum (Tv2) SA @ 2.5 kg/ha	2.22 (8.56)	4.65 (12.45)	6.87 (15.19)	8.81 (17.26)	10.58 (18.98)	11.02 (19.38)	7.35	85.50
Pseudomonas fluorescens (Pf1) SA @ 2.5 kg/ha	2.35 (8.81)	6.56 (14.84)	9.52 (17.97)	12.33 (20.55)	14.68 (22.52)	16.45 (23.92)	10.31	79.67
Pseudomonas fluorescens (Pf2) SA @ 2.5 kg/ha	2.28 (8.68)	6.45 (14.71)	9.38 (17.83)	12.25 (20.48)	14.55 (22.42)	16.26 (22.78)	10.19	79.90
Bacillus subtilis (Bs1) SA @ 600 g/ha	3.82 (11.27)	10.64 (19.03)	17.62 (24.81)	21.62 (27.70)	27.62 (31.70)	30.64 (33.61)	18.57	63.38
Bacillus subtilis (Bs2) SA @ 600 g/ha	2.62 (9.31)	10.32 (18.73)	15.34 (23.05)	20.26 (26.75)	26.34 (30.87)	30.12 (33.28)	17.5	65.49
Methylobacterium soil drenching 0.1%	2.34 (8.79)	6.75 (15.05)	10.15 (18.57)	13.12 (21.23)	15.22 (22.96)	18.36 (25.37)	10.99	78.33
carbendazim soil drenching 0.1%	0.75 (4.96)	2.78 (9.59)	6.89 (15.21)	8.56 (17.01)	7.72 (16.13)	8.35 (16.79)	5.84	88.48
Neem cake @ 150 kg/ha	3.06 (10.07)	8.35 (16.79)	13.25 (21.34)	16.86 (24.24)	21.24 (27.44)	22.25 (28.14)	14.16	72.08
Mahuva cake @ 150 kg/ha	2.85 (9.71)	5.92 (14.08)	13.68 (21.70)	16.92 (24.28)	18.72 (25.63)	20.46 (27.24)	13.09	74.19
Gingelly cake @ 150 kg/ha	2.96 (19.90)	8.64 (17.09)	14.56 (22.43)	17.83 (24.97)	20.56 (26.96)	22.65 (28.41)	14.53	71.35
Untreated Control	9.41 (11.86)	30.97 (33.56)	52.41 (46.38)	65.32 (53.92)	72.41 (58.32)	73.85 (59.25)	50.72	-

*Mean of three replications; *Figures in the parantheses are arc sine transformed values; DAS= Days After Sowing

CD (P=0.05)

Treatments = 0.19

Days = 0.13

Treatments × Days = 1.46

Huber and Watson (1970) summarized mechanisms of organic amendments influencing soil-borne diseases by increasing biological buffering capacity of soil and reducing pathogen numbers during anaerobic decomposition of organic matter. This study confirms earlier result reported by Komathi (2002) who stated that 10% concentration of mahua cake extracts produced total inhibition of mycelial growth of *Sclerotium rolfsii* (Sacc.). Mahua cake extracts controlling mycelial growth of *S. rolfsii* (Alice *et al*., 1998).

Table 7. Effect of antagonists, organic amendments, and fungicides on wilt incidence of brinjal plants in field condition

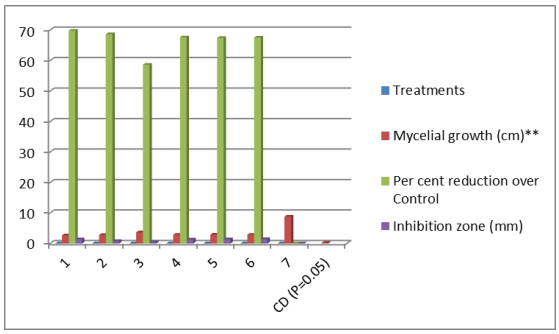
Treatments	**Disease incidence (%)						Mean Disease incidence (%)	Per cent reduction over control (%)	Plot yield (20 m²) (kg)	Yield t/ ha
	10 DAS	25 DAS	40 DAS	55 DAS	70 DAS	85 DAS				
Trichoderma viride (TV1) SA @ 2.5 kg/ha	0.86 (5.32)	2.58 (9.24)	6.75 (15.05)	8.46 (16.90)	7.35 (15.73)	7.25 (15.62)	5.54	87.96	70.00	35.0
Pseudomonas fluorescens (Pf1) SA @ 2.5 kg/ha	3.64 (10.99)	7.68 (16.08)	12.55 (20.74)	14.82 (22.64)	19.26 (26.03)	23.78 (29.18)	13.62	70.41	65.00	32.5
Bacillus subtilis (Bs1) SA @ 600 g/ha	3.40 (10.62)	8.44 (16.88)	14.85 (22.66)	17.65 (24.84)	23.54 (29.02)	29.78 (33.07)	16.27	64.66	62.00	31.0
Methylobacterium soil drenching 0.1%	3.75 (11.16)	5.86 (14.00)	9.55 (18.00)	11.96 (20.23)	14.67 (22.52)	16.95 (24.31)	10.45	77.30	60.00	30.0
T1 + T2 + T3 + T4	0.75 (4.96)	2.65 (9.36)	6.89 (15.21)	8.55 (17.00)	7.72 (16.13)	8.35 (16.79)	5.81	87.38	59.00	29.5
carbendazim soil drenching 0.1%	2.22 (8.56)	4.65 (12.45)	6.87 (15.19)	8.81 (17.26)	10.58 (18.98)	11.02 (19.38)	7.35	84.03	57.00	28.5
Neem cake @ 150 kg/ha	3.36 (10.56)	5.75 (13.87)	9.15 (17.60)	11.18 (19.53)	13.35 (21.43)	16.08 (23.64)	9.81	78.69	53.00	26.5
Mahua cake @ 150 kg/ha	3.03 (10.02)	5.78 (13.91)	9.34 (17.74)	11.95 (20.22)	14.28 (22.20)	16.55 (24.00)	10.15	77.95	52.00	26.0
Untreated Control	8.64 (17.09)	20.56 (26.96)	37.68 (37.86)	45.48 (42.40)	49.28 (44.58)	57.74 (49.55)	36.56	-	49.00	24.5

*Mean of three replications; *Figure in the parentheses are arc sine transformed values; DAS= Days After Sowing
CD (P=0.05)

Treatments	=	0.13
Days	=	0.11
Treatments × Days	=	0.33
Treatment × Days × yield	=	6.5

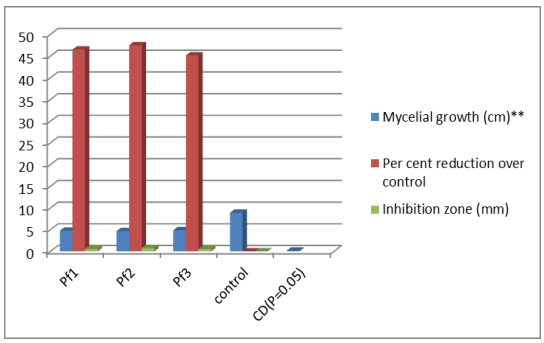
Effect of fungicides against F. solani

Among fungicides tested carbendazim (100, 200, or 500 ppm); propiconazole (100, 200, or 500 ppm), tebuconazole (100, 200, or 500 ppm), fosetyl aluminum (100, 200, or 500 ppm) were tested.



Graph 1. Effect of *Trichoderma* spp. against mycelial growth of *Fusarium solani* (*in vitro*)

Among this carbendazim @ 500 ppm had the maximum growth inhibition (83.59%) followed by propiconazole at 500 ppm (80.35%) (Table 5). Present findings are in similarity with the previous reports. Mayur *et al*. (2001) reported that carbendazim was the most effective to inhibit the radial mycelial growth of chickpea wilt pathogen *F. oxysporum* f. sp. *ciceri* at 100 ppm.

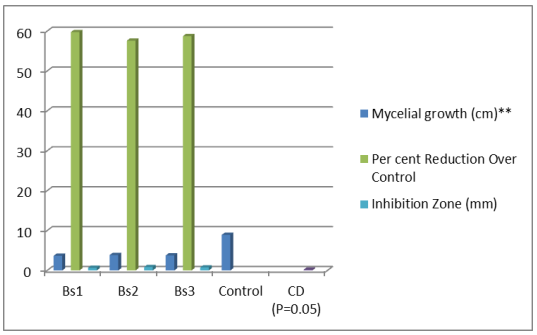


Graph 2. Effect of different isolates of *Pseudomonas fluorescens* against *Fusarium solani* (*in vitro*)

Bhaskar *et al*. (2003) reported that carbendazim was found to reduce the disease incidence of dry corm rot disease in Colocasia caused by *Fusarium solani*. Chakraborty *et al*. (2009) cited that soil solarization integrated with applications of carbendazim was found to effective to control the *Fusarium* wilt of eggplant.

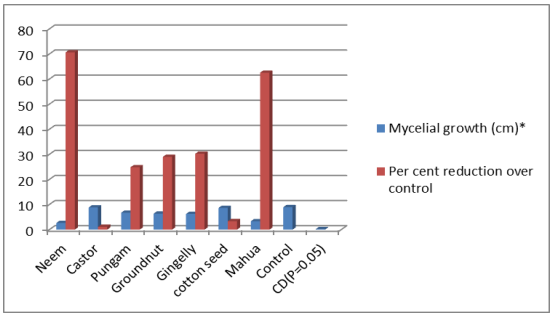
Effect of antagonist, organic amendments and fungicides on wilt incidence of Brinjal in pot culture

On Brinjal plants the *T. viride* SA @ 2.5 kg·ha^{-1} had less disease incidence (5.79%) indicating an 88.58% disease reduction followed by soil drenching with carbendazim 0.1 % providing an 88.48% reduction of the disease (Table 6).



Graph 3. Effect of *Bacillus subtilis* against the mycelial growth of *Fusarium solani* (*in vitro*)

Combined application of two, or more, biocontrol strains to enhance levels and consistency in disease control (Pierson and Weller, 1994). Similar findings were observed by Gasoni *et al*. (1998) who reported that treatment of radishseed with *B. cereus* and *P. fluorescens* effectively controlled *Rhizoctonia* damping off in greenhouse studies.

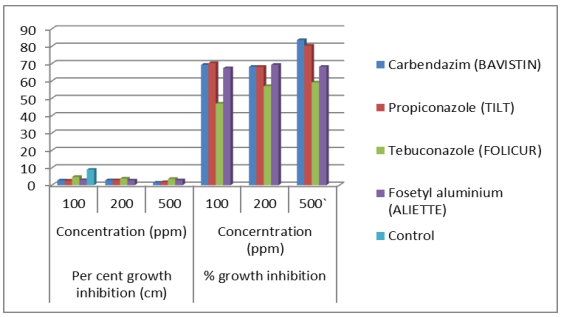


Graph 4. Efficacy of different oil cake extracts on the mycelial growth of *Fusarium solani* (*in vitro*)

Saravanakumar (2002) reported that a mixture of two PGPR strains was effective in reducing disease incidence of root rot of green gram. High antagonistic action of the *Trichoderma* spp. perceived against the test fungi might be due to fast growing nature, rapid sporulation and toxic metabolite producing capacity.

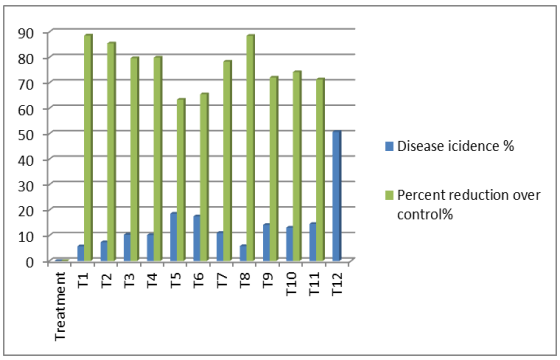
Effect of antagonist, organic amendments and fungicide combination on wilt incidence of Brinjal plants under field condition

Brinjal root incidence was reduced by soil application of *T. viride* SA @ 2.5 kg·ha^{-1}, (Trichoderma is grown in the liquid medium is mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade,talc based *T.viride* is applied as soil application @2.5 kg/ha). a minimum root wilt incidence (7.25%) on 85 DAS followed by combination of *T. viride* SA @ 2.5 kg·ha^{-1} + *P. fluorescens* SA @ 2.5 kg·ha^{-1} + *B. subtilis* SA @ 600 g·ha^{-1} + *Methylobacterium* soil drench (0.1%) with a root wilt incidence of 8.35% on 85 DAS.



Graph 5. Effect of different fungicides against *Fusarium solani in vitro*

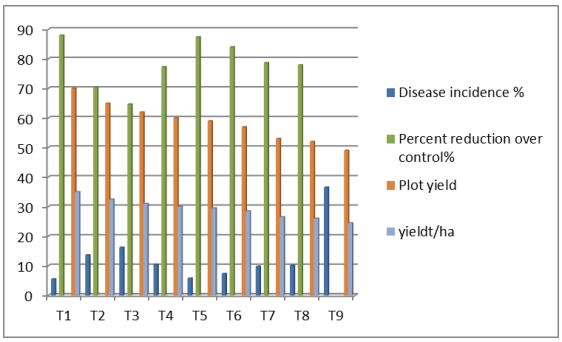
The untreated control had a 57.74% reduction on 85 DAS. Percent mean disease incidence and percent reduction over control was maximum in soil application *T. viride* (5.54, 87.96%) followed by TV + PF + BS + MB (5.81, 87.38%) (Table 7).



Graph 6. Effect of antagonists, organic amendments and fungicides on wilt incidence of brinjal plants in pot culture

The plot yield indicated that significant yield was observed in *T. viride* soil application followed by soil application of *P. fluorescens*, Soil application of *B. subtilis* (62.00) and soil drench of *Methylobacterium* (60.00). Marimuthu *et al*. (2002) reported a synergistic effect of combined application of *Azospirillum* and *P. fluorescens* Pf1 to reduce root rot incidence and enhance plant growth and yield of cotton under field conditions.

Yield increase under field conditions might be due to growth-promoting compounds gibberellin, cytokinin, or auxin from tryptophan, produced by biocontrol agents increased growth rate and yield of cotton under field conditions (Pal *et al*., 2000). Najar



Graph 7. Effect of antagonists, organic amendments, and fungicides on wilt incidence of brinjal plants in field condition

et al. 2011 evaluated native biocontrol agents isolated from rhizosphere against *F. solani* f. sp. *melongenae* causing wilt disease of Brinjal. The bacterial bioagent *P. fluorescens* followed by *T. viride* were effective in reducing disease incidence of Brinjal wilt. *Trichoderma* spp. inhibits pathogenic invasion through mycoparasitism, antibiosis and competition (Anwar *et al*., 2008), lysis and penetration of pathogenic hyphae (Dennis and Webster, 1971).

CONCLUSION

Based on the experimental results, it can be concluded that *Trichoderma* spp. was found to be very effective in controlling the *Fusarium* wilt of eggplant. It can be used either singly or through integration with other bio-control agents for disease control. Application of biocontrol methods, and integrated disease management, may be useful as an alternative over conventional chemical control methods. Also, it could be cost-effective, eco-friendly and suitable for sustainable organic farming.

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