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Research Article

Efficacy of bio-control agents and fungicides in management of mulberry wilt caused by *Fusarium solani*

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ABSTRACT: A study was conducted to know the efficacy of potential biocontrol agents and fungicides against mulberry wilt, *Fusarium solani*. Three antagonists *viz., Trichoderma viride., Pseudomonas fluorescens* and *Bacillus subtilis* and six fungicides *viz*., carbendazim, mancozeb, zineb, copper oxy chloride, tebuconazole and pre-mixture fungicide (carbendazim 75% + mancozeb 25%) were tested under *in vitro* and in pot culture against wilt pathogen. The results showed that *Trichoderma* and the bacterial bioagents significantly reduced the mycelial growth of the pathogen. Among the fungicides, mixture of carbendazim + mancozeb (0.1 %) completely inhibited the mycelial growth of the pathogen. In pot culture studies, the minimum (10.5 %) incidence of wilt was observed in soil drenching with carbendazim (0.1%) which was on par with soil application of consortia (Seri bed waste+Pf1+Bs4+Tv1+Neem cake) which showed 12.3 per cent incidence as compared to maximum (46.7 %) wilt incidence in control. Similarly, in field studies also recorded the minimum incidence in soil drenching with carbendazim (0.1%) followed by soil application with consortia.

KEY WORDS: Bioagents, fungicides, *Fusarium solani*, mulberry, wilt

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INTRODUCTION

Mulberry [*Morus* spp.] is a fast growing deciduous woody perennial plant. Mulberry foliage is the only food for the silkworm [*Bombyx mori* (L.)] and is grown under various climatic conditions. Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area have a direct bearing on cocoon harvest. Due to repeated harvesting of leaves, the soil nutrient get depleted and makes plant vulnerable to soil borne diseases wilt and root rot. Many soil borne diseases were very serious due to perennial nature of the crop and soil borne habitat of pathogens. Wilt of mulberry caused by *Fusarium solani* (Mart) Sacc. is a serious and economically important disease and widely prevalent throughout the country. The disease has been reported to cause severe yield losses. The incidence of disease has been reported from 50 to 80 per cent at flowering and at crop maturity stages (Siddaramaiah and Hegde, 1990). The pathogen is more alarming due to the ability to thrive well in soil and fast spread of disease once occurred besides absence of disease resistant varieties and inadequate control measures against this disease (Vineet *et al.,* 1998). The disease primarily spreads through contaminated soil, irrigation, diseases saplings and farm implements. Fungicides control of the disease is the most common method in the farming practice. Due to soil health imbalance, development of resistance strains of the pathogens, environmental pollution issues and as well as potential threat to silkworm, biological control method has been considered as a promising approach for managing of soil borne diseases. Therefore, the present study was undertaken to evaluate bio-agents *viz*., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* alone, or in combination with effective fungicides under *in vitro,* pot culture and field studies against mulberry wilt pathogen *F. solani*.

MATERIALS AND METHODS

Isolation and pathogenicity

Wilt infected plants of mulberry were collected from farmer's field of Tamil Nadu and the fungus was isolated from infected root portion on PDA (Fig. 1, 2 and 3). The pathogenicity was proved by soil infestation technique (Khalifa, 1991) and the pathogen was identified as *Fusarium solani* by fungal identification service, Agharkar Institute, Pune and also further confirmed by using molecular

method.

Fig. 1. Wilt incidence in mulberry field.

Fig. 2. *Fusarium solani* infected plant.

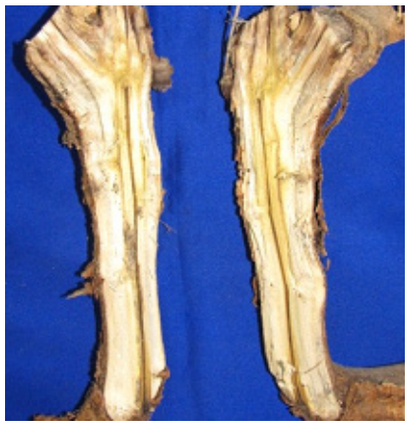

Fig. 3. *Fusarium solani* infected root.

Molecular identification of *Fusarium solani* by genus specific ITS primers

To confirm isolates of *F. solani* by using 18S rDNA specific primers ITS-F (5`-CAACTCCCAAACCCCTGTGA-3`) and ITS-R (5`-GCGACGATTACCAGTAACGA-3`) were used to get 596 bp amplicon of ITS region (Abd-Elsalam *et al.,* 2004). Amplification was conducted in a total reaction volume of 25 µl. The PCR settings used were as follows: a hold of two min at 95°C, 30 cycles of one min at 94°C, thirty sec at 55°C and one min at 72°C and a final extension of ten min at 72°C. The PCR products were resolved on 2 per cent agarose at 50V stained with ethidium bromide (0.5µg/ml) and photographed and analyzed using gel documentation system (Alpha Innotech Corporation, San Leandro, California).

Isolation and collection of biocontrol agents

Ten isolates in *Bacillus* spp., seven isolates in *Pseudomonas* and ten isolates of *Trichoderma viride* were isolated. One isolate of *Pseudomonas* (Pf1) and one isolate of *Trichoderma* (Tv1) were collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. Biocontrol agents were isolated from the rhizosphere soil samples collected from mulberry field by serial dilution technique (Pramer D, Schmidt EL. 1956. Experimental soil microbiology. Buffer Publication company, Minneapolis, USA. 107p.) using *Trichoderma* selective medium for *T. viride*, King's B (KB) medium for *Pseudomonas* and Nutrient agar (NA) for *Bacillus* spp.

BIOCHEMICAL TESTS FOR ASSESSING ANTAGONISTIC POTENTIAL

Siderophore production assay

Bacterial cultures were streaked on succinate medium amended with indicator dye. In case of *Trichoderma,* a 5 mm-mycelial disc of the fungus was placed at the centre of the plate containing the same medium. Change of blue colour of the medium surrounding the growth of the culture to fluorescent yellow indicated the production of siderophore. The reaction of each fungal and bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands, 1987).

Hydrogen cyanide (HCN) production assay

The antagonistic bacteria were streaked on KB medium amended with glycine. In case of *Trichoderma,* mycelial disc of the fungus was placed at the centre of Petri plate containing PDA amended with glycine. Sterile filter paper saturated with picric acid solution was placed in the upper lid of the petri plate. The dishes were sealed with parafilm and incubated at 28°C for 48 h. A change in colour of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction respectively (Bakker and Schippers, 1987).

In vitro evaluation of bio-agents against *Fusarium solani*

Isolated and collected efficient strains of *T. viride* and two PGPR bacterial bioagent *P. fluorescens* and *B. subtilis* were evaluated for their antagonistic activity against *F. solani* by dual culture technique (Dennis and Webster,

1971). The antagonist bacteria culture was streaked at one side of Petri plate (1 cm from the edge of the plate) plated on PDA medium and mycelial disc (5 mm diameter) of 8 days old culture of *F. solani* was placed on the opposite side perpendicular to the antagonist streak. Five days old *Trichoderma* isolates were placed on one side of the Petri dish amended with PDA medium and a mycelial disc (5 mm diameter) of 8 days old *F. solani* culture was placed on the opposite side of the Petri dish perpendicular to the disc. Four replications for each bio-agent along with control have been taken for the studies. All the plates were incubated at 28±1°C for 8 days and colony diameter of the pathogen was measured periodically and per cent growth inhibition over control was calculated.

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

Where, C- Mycelial growth of pathogen in control

T- Mycelial growth of pathogen in dual culture plate

In vitro efficacy of fungicides against F. solani

Six fungicides *viz*., carbendazim, mancozeb, zineb, copper oxy chloride, tebuconazole and pre mixture fungicide (carbendazim 75% + mancozeb 25%) at 0.1%, 0.2%, 0.3%, 0.4% concentrations were evaluated against the *F. solani* by poisoned food technique (Schmitz, 1930). Media was separately poisoned by adding desired quantity of different fungicides in 250 ml flasks containing 100 ml warm and sterilized PDA. Poisoned medium was poured in sterilized plates @ 20 ml plate^{-1}. The plates were inoculated aseptically by centrally placing one 5 mm mycelial disc of the pathogen. Three replications were made for each concentration of each fungicide along with suitable control and the plates were incubated for 8 days at 28±1 °C. Radial mycelial growth of the pathogen was measured periodically in cross way. The values were transformed to square root transformation and then analyzed for further studies.

Evaluation of bio-agents and fungicides under green house

Pot culture experiment was laid out in Completely Randomized Design under glass house condition to test the efficacy of fungicides (carbendazim, pre mixture fungicide (carbendazim + mancozeb), tebuconazole) and biocontrol agents (*Pf* 1*, Bs*4, *Tv*1) along with seri bed waste and neem cake. Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1h for 2 consecutive days and filled in pots. Sand-maize medium (9:1) was used for mass multiplication of the pathogen. Pots of 30x30cm diameter containing autoclaved soil were inoculated with sand-maize inoculum @ 50g/kg soil and left for 5 days for the establishment of inoculum. Mulberry plants were grown in pots after soil drenching with fungicides and bio-agents separately. The pots were arranged in completely randomized design with three replications for each treatment. Untreated but inoculated pots were maintained as control. Wilt incidence was recorded from initiation of wilting till 60 days of the crop age. The treatment details were as follows.

Treatments

T_1 – Soil application of consortia (Seri bed waste + Pf_1 + Bs_4 + Tv_1 + neem cake) @ 200g/ plant

T_2 – Soil application of *Pseudomonas florescence* (Pf_1) @ 10 g/ plant.

T_3 – Soil application of *Bacillus subtillis* (Bs_4) @ 10 g/ plant.

T_4 – Soil application of *Trichoderma viride* (Tv_1) @ 50 g/ plant

T_5 – Soil drenching of tebuconazole @ 0.1 %

T_6 – Soil drenching of carbendazim @ 0.1%

T_7 – Soil drenching of pre mixture fungicide (carbendazim 75% + mancozeb12%) @ 0.1%

T_8 – Control

Evaluation of bio-agents and fungicides under field condition

Field experiment was carried out at Annur region of Coimbatore district, Tamil Nadu. Six month old mulberry plant variety V1 was used to test the efficacy of biocontrol agents (Pf1, Bs4, and Tv1) and fungicides (carbandazim 0.1%, pre mixture fungicide (carbendazim + mancozeb 0.1%) and tebuconazole 0.1%) at 30 days interval as per mentioned treatments. The experimental design consisted of a randomized complete block with three replicates per treatment and 15 plants for each replicate. Disease incidence and severity were assessed on 60 days after inoculation. The plant leaf yield was recorded in all the treatments.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were compared by Duncan's Multiple

Range Test (DMRT).

RESULTS AND DISCUSSION

Isolation and identification of pathogen

The seven isolates were isolated from the infected tissues of mulberry root and identified as *F. solani* based on morphological characters, mycelial growth and characters of microconidia, macroconidia and chlamydospores. It was also confirmed by Agharkar Mycological Research Institute, Pune as *F. solani* (accession no: NFCCI-3057).

Biochemical assay test for antagonist

Siderophore production by bioagents

Production of the siderophores has been linked to the disease suppressive potential of certain florescent pseudomonads. In the present study, the two isolates of *P. fluorescens* (Pf1 and Pf7) and the two isolates of *T. viride* (Tv1 and Tv11) were shown positive for siderophore production, while the two isolates of *B. subtilis* (Bs1 and Bs9) failed to produce siderophores (Table 1; Fig. 4). The siderophore of *P. fluorescens* GL20 inhibited spore germination and hyphal growth of *F. solani in vitro* and reduced the disease incidence with enhanced plant growth (Lim *et al*., 1999). The hydroxymate type of siderophore was produced by *P. fluorescens* (Ling *et al*., 1992). Loper and Buyer (1991) reported that iron deficiency, the culture filtrate of all strains of *Trichoderma* contained coprogen, coprogen B and ferricrocin as siderophore. *T. longi* and *T. pseudokoningii* produced fuigen type of siderophore.

Table 1. Production of siderophore, HCN by bacterial and fungal bioagents

S. No	Isolates	Siderophore production	HCN Qualitative
1	Pf_1	+++	+++
2	Pf_7	+++	++
3	Bs_4	-	-
4	Bs_9	-	-
5	Tv_1	+++	+++
6	Tv_{11}	++	-
7	Control	-	-

(- negative, ++ moderate, +++ strong production)

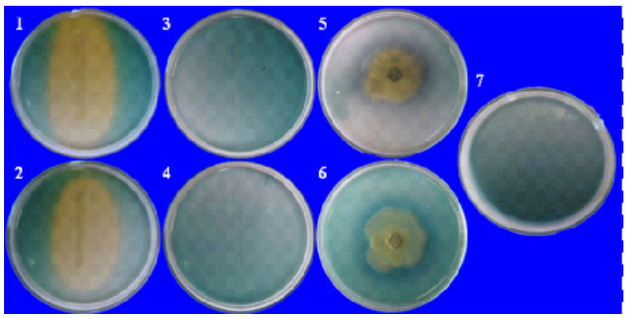

Fig. 4. Assay of Siderophore production.
1. Pf_1, 2. Pf_7, 3. Bs_4, 4. Bs_9, 5. Tv_1, 6. Tv_{11} & 7. Control

HCN production by bioagents

The production of volatile cyanide is very common among the rhizosphere pseudomonads (Bakker and Schippers, 1987; Dowling and O'Gara, 1994). The present study revealed that two isolates of *P. fluorescens* (Pf1 and Pf7) and one isolates of *T. viride* (Tv1) were positive for HCN production, while the two isolates of *B. subtilis* and one isolates of *T. viride* (Tv_{11}) failed to produce HCN (Table 1; Fig. 5). The involvement of HCN in the suppression of pathogen was reported by several workers in different hosts (Defago *et al*., 1990; Wei *et al*., 1991; Meena *et al.,* 2001).

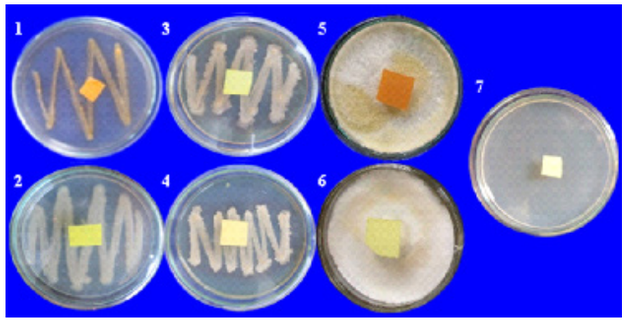

Fig. 5. Assay of HCN production.
1. Pf_1, 2. Pf_7, 3. Bs_4, 4. Bs_9, 5. Tv_1, 6. Tv_{11} & 7. Control

Molecular identification and detection of *F. solani*

Molecular tools have been used extensively as genetic markers in different fungal populations (Bentley *et al*., 1994 and Bridge *et al*., 1997). The result of molecular detection showed that the total genomic DNA from the fungus was extracted by CTAB method and the presence of DNA was checked on agarose gel-electrophoresis. Discrete bands were observed on agarose gel indicated the presence of DNA. The total genomic DNA was amplified by polymerase chain reaction (PCR) with ITS 1 (5`-CAACTCCCAAACCCCTGTGA-3`) and ITS 4 (5`-GCGACGATTACCAGTAACGA-3`) primers with the amplified amplicon size of 596bp (Fig. 6).

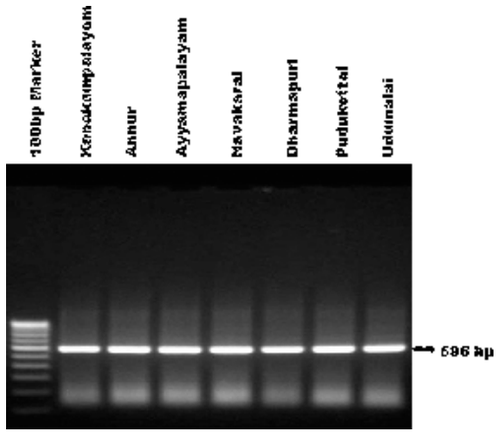

Fig. 6. Agarose gel electrophoresis of the PCR products amplified from genomic DNA of Fusarium solani with rDNA intervening sequence specific primers ITS1F and ITS4 R.

In vitro evaluation of bioagents against *Fusarium solani*

Trichoderma viride (Tv1) showed maximum inhibition of mean mycelial growth of *F. solani* (56.6 mm) in dual culture and was found significantly superior over the bacterial bioagent. *P. fluorescens* and *B. subtilis* reduced the growth of the pathogen i.e. 50.0 and 44.3 mm at maximum level, respectively as compared to 90.0 mm in control. *P. fluorescens* was found more effective than *B. subtilis* for inhibition of *F. solani* (Table 2). Due to fast growing ability and strong antagonism of *T. viride*, it covered the entire plates within 4 days of incubation, thereby inhibited the growth of *F. solani*. Maximum growth inhibition of *Fusarium* spp. by *T. viride* and *P. fluorescens* has been reported earlier by Madhukeshwara and Seshadri (2001), Malathi (2010), Rangeshwaran *et al*. (2002) and Yadav *et al*. (2007). It is reported that the antibiotics i.e. phenazine-1-carboxylic acid and oxychlororaphin have been found to contribute for the biocontrol of Fusarium wilt of crop plants (Anjaiah *et al*., 2003). *Trichoderma viride* was best in inhibiting the growth of *F. solani* in mulberry root rot by 73.6 per cent (Choudhari *et al.,* 2012).

In vitro screening of fungicides against *Fusarium solani*

In the present study, all the six fungicides were found to be effective at an extent of 5.8 - 100 per cent reduction of mycelial growth. Among them, carbendazim and pre mixture fungicide carbendazim + mancozeb at 0.1 per cent were completely inhibited the mycelial growth (100%) of *F. solani* in all the concentration followed by tebuconazole (0.1%) and recorded the reduction of 66.5 per cent (Table 3). Carbendazim (100 mg/ml) inhibiting the mycelial growwth of *F. solani in vitro* (Gupta *et al*., 1997). Carbendazim was observed to reduce the mycelial growth of *F. oxysporum.* f.sp. *ciceri* (Subhani *et al*., 2011).

Table 2. Screening of bio-agents against *Fusarium solani* under *in vitro* conditions

Trichoderma viride			*Pseudomonas fluorescens*			*Bacillus subtilis*		
Isolates code	Mycelial growth of the pathogen (mm)*	Inhibition (%) over control*	Isolates code	Mycelial growth of the pathogen (mm)*	Inhibition (%) over control*	Isolates code	Mycelial growth of the pathogen (mm)*	Percent inhibition over control*
Tv_1	42.3^{a}	56.6^{a} (48.8)	Pf_1	45.7^{a}	50.0^{a} (44.9)	Bs_1	59.2^{bcd}	34.2^{de} (35.7)
Tv_2	46.5^{abc}	48.8^{bcd} (44.3)	Pf_2	65.9^{de}	27.7^{f} (31.7)	Bs_2	56.5^{abc}	37.2^{bcd} (37.5)
Tv_3	44.1^{ab}	51.1^{bc} (45.6)	Pf_3	70.1^{ef}	22.2^{g} (28.1)	Bs 3	57.8^{bcd}	35.7^{cd} (36.7)
Tv_4	49.7^{c}	45.5^{d} (42.4)	Pf_4	60.3^{cd}	33.3^{d} (41.8)	Bs_4	50.1^{a}	44.3^{a} (41.7)
Tv_5	44.5^{ab}	51.1^{bc} (45.6)	Pf_5	74.7^{f}	17.7^{h} (24.9)	Bs_5	56.3^{abc}	37.4^{bcd} (37.7)
Tv_6	50.1^{c}	44.3^{ab} (46.9)	Pf_6	59.2^{c}	34.4^{c} (35.9)	Bs_6	61.5^{cd}	31.6^{ef} (34.2)
Tv_7	50.7^{c}	44.4^{d} (41.8)	Pf_7	53.5^{b}	41.1^{b} (39.8)	Bs_7	54.9^{abc}	39.0^{bc} (38.6)
Tv_8	43.8^{ab}	51.2^{abc} (46.2)	Pf_8	62.7^{cd}	31.1^{e} (33.8)	Bs_8	58.2^{bcd}	35.3^{d} (36.4)
Tv_9	46.9^{bc}	48.8^{bcd} (44.3)	Control	90.0^{g}	-	Bs_9	53.8^{ab}	40.2^{b} (39.3)
Tv_{10}	47.3^{bc}	47.7^{cd} (43.7)	-	-	-	Bs_{10}	61.6^{cd}	31.5^{ef} (34.1)
Tv_{11}	43.5^{ab}	51.6^{bc} (45.9)	-	-	-	Bs_{11}	63.4^{d}	29.5^{f} (32.9)
Control	90.0^{d}	-	-	-	-	Control	90.0^{e}	-

*Values are the mean of the three replications; Values in parentheses are arcsine transformed; Means followed by a same letter are not significantly different at the 5 % level by DMRT

Table 3. Screening of different fungicides against *Fusarium solani* under *in vitro* conditions

Chemicals	Diameter of mycelial growth (mm)*				Per cent inhibition over control*			
	0.1%	0.2%	0.3%	0.4%	0.1%	0.2%	0.3%	0.4%
Carbendazim	00.0^{a}	00.0^{a}	00.0^{a}	00.00^{a}	100.0^{a} (84.6)	100.0^{a} (84.6)	100.0^{a} (84.6)	100.0^{a} (84.6)
Mancozeb	68.2^{c}	60.3^{c}	56.7^{c}	54.90^{b}	24.2^{c} (29.4)	33.0^{c} (35.0)	37.0^{c} (37.4)	39.0^{b} (38.6)
Zineb	74.2^{c}	72.1^{d}	70.3^{d}	60.90^{b}	17.5^{c} (24.7)	19.8^{c} (26.4)	21.8^{c} (27.8)	32.3^{b} (34.6)
Pre mixture fungicide (Carbendazim + mancozeb)	00.0^{a}	00.0^{a}	00.0^{a}	00.00^{a}	100.0^{a} (84.6)	100.0^{a} (84.6)	100.0^{a} (84.6)	100.0^{a} (84.6)
Copper oxy chloride	90.0^{d}	88.0^{e}	84.7^{e}	82.10^{c}	00.0^{d} (0.1)	02.2^{d} (8.5)	05.8^{d} (14.0)	08.70^{c} (17.2)
Tebuconazole	30.1^{b}	24.7^{b}	10.0^{b}	00.04^{a}	66.5^{b} (54.7)	72.5^{b} (58.4)	88.8^{b} (71.0)	97.6^{a} (84.6)
Control	90.0^{d}	90.0^{e}	90.0^{f}	90.00^{d}	-	-	-	-

*Values are the mean of the three replications; Values in parentheses are arcsine transformed; Means followed by a same letter are not significantly different at the 5 % level by DMRT

Efficacy of biocontrol agents and fungicides against wilt disease

Pot culture experiment

Soil drenching of carbendazim (0.1%) recorded a disease reduction of 77.5 per cent and followed by the soil application of consortia (Seri bed waste + Pf_1 + Bs_4 + Tv_1+ neem cake) @ 200g which has recorded 73.6 per cent reduction of wilt disease at 45 days after inoculation. The number of leaves significantly increased in all treated plants ranged from 32.3 to 75.5 per plant when compared to control (17.8 per plant) (Table 4). This study was supported by several earlier studies wherein rhizobacterial strains have been found to increase the plant growth after seed inoculation and were called as Plant Growth Promoting Rhizobacteria (Kloepper *et al*., 1980). The mechanisms of growth promotion by these PGPR are complex and appear to comprise both changes in the microbial balance in the rhizosphere and alteration in the host plant physiology (Glick *et al*., 1999).

Field experiment

Field experiment showed that maximum reduction was recorded in carbendazim (0.1%) (74.2%) treated plots followed by Consortia (Seri bed waste + Pf_1 + Bs_4 + Tv_1+ Neem cake) 200 g/plant recorded (68.2%). The highest leaf yield was recorded in Consortia (Seri bed waste + Pf_1 + Bs_4 + Tv_1+ Neem cake) applied plots recorded the maximum leaf yield of 3.5 kg/plant and followed by Carbendazim applied plots recorded the leaf yield of 3.4 kg/plant respectively. However, the control showed that lesser reduction of disease (45.0 %) and lesser yield (2.2 kg/plant) (Table 5). The results are in agreement with findings of Sugha *et al*., (1995) who have reported that carbendazim was very effective in reducing the *Fusarium* wilt disease of cotton. *P. fluorescens* isolate Pf1 showed the maximum inhibition of mycelial growth of *P. aphanidermatum* and increased plant growth promotion in tomato and hot pepper (Ramamoorthy *et al*., 2002).

Table 4. Efficacy of biocontrol agents and fungicides against wilt disease under pot culture conditions

T. No	Treatment	Disease incidence (%)*	Reduction over control (%)*
T_1	Soil application of consortia (Seri bed waste+ Pf_1+ Bs_4+ Tv_1+ neem cake) @ 200 g/plant	12.3^{a} (20.5)	73.6^{ab} (59.1)
T_2	Soil application of *Pseudomonas fluorescens* (Pf_1) @ 50 g/plant	15.7^{b} (23.3)	66.3^{b} (54.5)
T_3	Soil application of *Bacillus subtilis* (Bs_4) @ 50 g/plant	23.9^{d} (29.2)	48.8^{d} (44.3)
T_4	Soil application of *Trichoderma viride* (Tv_1) @ 50 g/plant	19.8^{c} (26.4)	57.6^{c} (49.3)
T_5	Soil drenching of tebuconazole @ 0.1%	27.2^{e} (31.4)	41.7^{d} (40.2)
T_6	Soil drenching of carbendazim @ 0.1%	10.5^{a} (18.9)	77.5^{a} (61.8)
T_7	Soil drenching of pre mixture fungicide @ 0.1%	26.3^{de} (30.8)	43.6^{d} (41.3)
T_8	Inoculated control	46.7^{f} (43.1)	-

*Values are the mean of the three replications; Values in parentheses are arcsine transformed; Means followed by a same letter are not significantly different at the 5 % level by DMRT

Table 5.Efficacy of biocontrol agents and fungicides against wilt disease under field condition

T. No	Treatment	% disease incidence*	% reduction over control*	Leaf yield (Kg/ Plant)
T_1	Soil application of Consortia (Seri bed waste +Pf_1+ Bs_4+ Tv_1+ Neem cake) @ 200 g/plant	14.3^{b} (22.2)	68.2^{b} (55.7)	3.5^{a}
T_2	Soil application of *Pseudomonas fluorescens* (Pf_1) @ 50 g/plant	15.6^{c} (23.2)	65.3^{b} (53.9)	3.2^{b}
T_3	Soil application of *Bacillus subtilis* (Bs_4) @ 50 g/plant	23.5^{e} (28.9)	47.7^{d} (43.7)	2.9^{c}
T_4	Soil application of *Trichoderma viride* (Tv_1) @ 50 g/plant	20.3^{d} (26.7)	55.5^{c} (48.1)	2.9^{c}
T_5	Soil drenching of Tebuconazole @ 0.1%	29.8^{f} (33.0)	33.7^{e} (35.5)	2.8^{cd}
T_6	Soil drenching of Carbendazim @ 0.1%	11.6^{a} (19.9)	74.2^{a} (59.6)	3.4^{ab}
T_7	Soil drenching of pre mixture fungicide Carbendazim + Mancozeb @ 0.1%	34.5^{g} (35.9)	23.3^{f} (28.8)	2.7^{cd}
T_8	Control	45.0^{h} (42.1)	-	2.2^{e}

*Values are the mean of the three replications; Values in parentheses are arcsine transformed; Means followed by a same letter are not significantly different at the 5 % level by DMRT.

Results clearly indicated that bio-agents consortia (Seri bed waste+ Pf_1+ Bs_4+ Tv_1+ Neem cake) and systemic fungicide carbendazim were very effective against *F. solani* both *in vitro* and in field condition. Therefore, soil drenching with bioconsortia or carbendazim may be used for the management of the wilt of mulberry apart from increase in the leaf yield of mulberry crop by PGPR action of the microbial agents.

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