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EFFECT OF COMBINED APPLICATION OF ANTAGONISTIC ORGANISMS AND ORGANIC AMENDMENTS ON THE WILT INCIDENCE OF COTTON INCITED BY *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*

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Abstract

Generally all the organic amendments suppondnrted the survival of *T. viride*and*P. Fluorescens* and the population of the antagonist increased gradually in all the organic amendment treatments up to the maximum period tested. However, the final population of *P. fluorescens* and *T. viride* was the maximum in neem cake amended soils followed by FYM amended soils (32.63; 64.00 and 30.60;62.00 × 10^{-5}cfu g^{-1} respectively). Basal application of neem cake@ 250 kg ha^{-1} + soil application (2.5 lit ha^{-1}) + seed treatment (10 ml kg^{-1} of seed) of *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) combination treatment recorded the minimum incidence of wilt (11.30%) and enhancing the growth parameters of cotton. The untreated control recorded the maximum disease (67.85%) incidence and minimum growth parameters. The same treatment recorded maximum reduction in the population of *F. oxysporum* f.sp. *vasinfectum* (8.75 × 10^{-6}) and rhizosphere population of 27.35 × 10^{-3}cfu g^{-1} soil and 32.30 ×10^{-6}cfu g^{-1} soil of *T. viride* (Tv_3) and *P. fluorescens* (Pf_7) respectively. Similarly, combined application of *P. fluorescens*, *T. viride* and neem cake increased the yield parameters like numbers of bolls $plant^{-1}$ and seed cotton yield $plant^{-1}$.

***Key words*:** Cotton, *Fusarium* wilt, *P. fluorescens*, *T. viride,* Neem cake

Introduction

Wilt of cotton caused by *F. oxysporum* f. sp. *vasinfectum* remains to be a serious threat to the cotton production worldwide. Various disease management methods including cultural, physical, chemical and biological methods have been tried in the past to manage the disease and no single management strategy could effectively manage the disease. All these methods are effective only when employed well in advance as precautionary measure (Kata, 2000).

Host resistance could be the most effective approach for managing *Fusarium* wilt of cotton. However, commercial cultivars resistant to race 4 infecting upland cultivars are limited. The strategies like employing dry heat treatments, hot water treatments, soil fumigation and solarization protocols may decrease inoculum, but their employment on a routine basis are often impractical to implement on a large scale, costly and may fail to prevent the accumulation of inoculums in fields routinely planted with cotton as the fungus can persist in fields for many years and sporulate on the roots of even the resistant cultivars.

Besides, ill effects of fungicides *viz*., environmental pollution, health hazards, phytotoxicity, development of resistance by the pathogen and also exorbitant cost necessitates the search for safe alternative management strategies. At present the idea of controlling soil-borne plant pathogens, including *Fusarium*, with biological control can have an important role in sustainable agriculture (Pandey *et al.,* 2010). As a result, disease containment through an eco-friendly biocontrol approach, using antagonistic microflora, is becoming an inevitable component in the integrated management strategy of plant diseases. The rhizosphere is the first line of defense for roots, against attack by pathogenic fungi. Therefore, there is an excellent opportunity to find rhizosphere competent microorganisms that can act as potential biopesticides. Though remarkable success has been achieved in this direction through the use of antagonistic microorganisms as biocontrol agents, the information generated on the performance of the introduced antagonists in the ecosystem under varying field conditions still remains inadequate constituting a major obstacle in the large scale adoption of this technology especially against soil borne plant pathogens. Many of the introduced antagonists failed to survive in the soil due to lack of favourable conditions like food base, moisture conditions etc. Therefore, the search for native antagonists has become imminent for exploiting these organisms to the advantage. Generally the recent investigations have focused Fov on biological control, organic amendments, naturally occurring nematicides and induced resistance (Dias-Arieira *et al.*, 2012) in combination for the management of complex pathogens. With this background in mind, the present study was planned and conducted to develop integrated

management strategy involving native biocontrol agents along with neem cake for the effective management of cotton wilt disease.

Materials and Methods

Isolation of *Fusarium oxysporum* f. sp. *vasinfectum*

The pathogen *F. oxysporum* f. sp. *vasinfectum* was isolated from the diseased roots of cotton plants showing the typical wilt symptoms by tissue segment method (Rangaswami, 1972). Infected roots and stems were washed in tap water and cut into small pieces. The pieces were surface sterilized in 1 per cent sodium hypochlorite ($NaOCl_2$) solution for 30 sec. and washed serially in sterile distilled water to remove the traces of sodium hypochlorite and then transferred to sterilized Petri plate containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (28 ± 2°C) for 5-7 days. Hyphal tips growing from infected bits were transferred to PDA slants and the fungus was purified by using hyphal tip technique (Rangaswami, 1972) and were preserved in a refrigerator at 4°C and used for further studies. The pathogen *F. oxysporum*f. sp. *Vasinfectum* was identified with the help of the descriptions by Booth (1971) and Singh (1987). The pathogenicity of the isolates was proved by Koch's postulates.

Mass multiplication of *F. oxysporum* f. sp. *vasinfectum* inoculum for soil application

The isolates of the pathogen were multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in 500 ml conical flask and autoclaved at 20 psi for two h. Four actively growing mycelial discs (9 mm) of the pathogen isolates were inoculated into each flask under aseptic condition and the flasks were incubated at room temp. (28 + 2°C) for 15 days the inoculum thus obtained was used for the experiments.

Isolation of native antagonists from rhizosphere soil

Trichoderma spp.

Cotton rhizosphere soil samples collected from different locations were used for the isolation of *Trichoderma* isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These strains of *Trichoderma* spp. were, purified following single hyphal tip method and maintained in TSM slants at 4°C in refrigerator with periodical sub-culturing. *Trichoderma* spp., thus isolated was subjected for identification based on the key to species suggested by Domsch *et al.* (1980).

Isolation of native antagonistic bacteria

Antagonistic bacteria were isolated from the rhizosphere soil collected during the survey. The soil along with root bits was mixed thoroughly and one g of rhizosphere soil was processed following serial dilution. One ml of 10^{-5} dilution was plated on King's B (KB) agar medium and incubated at room temperature (28 ± 2°C) for 48 hours (Aneja, 2003) to isolate *Pseudomonas*. The colonies fluorescencing under UV light were picked up, purified and maintained in KB slants. The efficient Pseudomonas strains identified from the in vitro dual culture assay were examined for the colony morphology, growth, pigmentation, cell shape and gram reaction as per the standard procedure given by Barthalomew and Mittewer (1950).

Preparation of liquid formulation of biocontrol agents

For the preparation of liquid formulations the method suggested by Manikandan *et al.* (2010) was followed. The most effective isolate of *P. fluorescens* and *T. viride* identified in the present study was multiplied on Nutrient, King's B and PDA broth respectively. The mother culture of *T. viride* and log phase culture of *P. fluorescens* was inoculated individually into respective broth and incubated at room temperature (28 ± 2°C). Further, the respective broths were added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

Effect of organic amendments on the survivability of bio control agents

Two hundred g. of garden land soil was filled in earthen pots (15 cm dia.). The organic amendments *viz.,* farm yard manure, press mud, poultry manure, neem cake and coir pith were incorporated in soil at 1% level (w/w) (Ayyappan, 2005). The conidial suspensions of the antagonists were prepared with adequate CFU and added to soil @ two ml/100g of soil and mixed thoroughly. The pots were maintained inside the glasshouse with judicious, uniform and regular watering. Samples were drawn periodically at 0, 30, 60 and 90 days after incubation and the population of the antagonist was assessed using serial dilution technique. For assessing the population of *T. viride* and *P. fluorescens, Trichoderma* selective medium (TSM) and King's B medium were used respectively.

Efficacy of antagonists and organic amendment (Neem cake) on plant growth and the incidence of wilt of cotton

A separate pot culture experiment was conducted by incorporating neem cake @ 2 per cent level and antagonists as per the treatment schedule to the pathogen inoculated (5% level) sick soil to assess their efficacy on the management of wilt pathogen of cotton. The following are the treatments.

Treatment details

T_1 – Neem cake soil application @ 250 kg ha^{-1} as basal application

T_2 – *T. viride* (Tv_3) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (2.5 litha^{-1})

T_3 – *P. fluorescens* (Pf_7) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (2.5 lit ha^{-1})

T_4 – *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1})

T_5 – T_2 + Neem cake soil application @ 250 kg ha^{-1}

T_6 – T_3 + Neem cake soil application @ 250 kg ha^{-1}

T_7 – T_4 + Neem cake soil application @ 250 kg ha^{-1}

T_8 – Carbendazim 50 %WP @ 4.0 g kg^{-1} as ST + Soil drench @ 0.1%

T_9 – Control

The experiment was conducted in a randomized block design with three replications where in five pots per replication and one plant per pot were maintained. The incidence of wilt (%), shoot and root length (cm), biomass of the plant (g plant^{-1}), number of bolls per plant, and seed cotton yield (g plant^{-1}) were recorded. The biomass of the plant was recorded after drying the plants in the hot air oven at 60°C until attaining a constant weight. Also, the population of the antagonists and pathogen was assessed using dilution plate technique with suitable selective media.

Results and Discussion

Effect of different organic amendments on the population of *T. viride* and *P. fluorescens*

The survival of native *T. viride* and *P. fluorescens* isolates as influenced by the organic amendments was assessed through periodical sampling and the results are presented in table 1. Generally all the organic amendments supported the survival of *T. viride* and *P. fluorescens* and the population of the antagonist increased gradually in all the organic amendment treatments up to the maximum period tested except the control. However, the final population of *P. fluorescens* and *T. viride* was the maximum in neem cake amended soils followed by FYM amended soils (32.63; 64.00 and 30.60; 62.00×10^{-5}cfu g^{-1} respectively). Other amendments *viz*., presumed, poultry manure and coirpith recorded a final *P. fluorescens* population of 28.50, 29.93 and 27.10×10^{-5} cfu $g^{-1}$of soil and *T. viride* population of 51.25, 50.33 and 49.00×10^{-5} cfu g^{-1} of soil respectively. The increase in the rhizosphere population of the antagonists might be attributed to the reason that, organic amendments produced volatile and nonvolatile substances during their decomposition and also stimulated resident and introduced antagonists (Lumsden *et al.,* 1995). Also, the organic amendments might have served as an ideal food base for the growth and multiplication of antagonists as reported by Hoitink and Boehm (1999). *T. viride* and *P. fluorescens* in neem cake formulation was found to be more promising than talc formulation and affected 60.9% control over check (Sumana *et al*., 2012). According to Ramji *et al.* (2015)neem cake was found to be best substrate for supporting the population dynamics and longevity of *T. harzianum in vitro*. Neem cake maintained with 25% moisture was able to support the longevity of *T. harzianum* for more than 105 days with a considerable level of population.

Effect of combined application of antagonists and neem cake on *Fusarium* wilt and biometrics of cotton (pot culture)

The combined application of antagonists and neem cakes are furnished in table 2. Among the treatments, basal application of neem cake@ 250 kg ha^{-1} + soil application (2.5 lit ha^{-1}) + seed treatment (10 ml kg^{-1} of seed) of *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) combination treatment (T_7) recorded the minimum incidence of wilt (11.30%). This was followed by the treatment (T_6) with *P. fluorescens* (Pf_7) as seed and soil treatment plus soil application of neem cake which recorded at par results with that of combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_7) as seed and soil treatment (T_4) in reducing the wilt incidence and enhancing the growth parameters of cotton. The untreated control recorded the maximum disease (67.85%) incidence and minimum growth parameters. Raj and Singh (1996) observed that neem, mustard and mahuva oil cakes were found most effective in reducing *Fusarium* sp. and neem cake was found most effective in controlling wilt incidence. Plant growth promoting rhizobacteria in combination with organic amendment reduced root-rot disease incidence and population of root pathogenic fungi and increase the yield in soyabean (Inam-ul-Haq *et al*., 2012).application of compatible mixture of fungal and bacterial biocontrol agents possessing various mechanism of pathogen suppression is suggested as a reliable and potential means of disease suppression (Mishra *et al.,* 2013).

Effect of combined application of antagonists and neem cake on the rhizosphere population of antagonists and *F. oxysporum* f. sp. *vasinfectum* (pot culture)

Seed treatment plus soil application with combination *T. viride* (Tv_3) and *P. fluorescens* (Pf_7) plus neem cake (T_7) resulted in the maximum reduction in the population of *F. oxysporum* f.sp. *vasinfectum* (8.75×10^{-6}) and the same treatment recorded a rhizosphere population of 27.35×10^{-3}cfu g^{-1} soil and 32.30×10^{-6}cfu g^{-1} soil of *T. viride* (Tv_3) and *P. fluorescens* (Pf_7) respectively. The treatment (T_6) with *P. fluorescens* (Pf_7) (as ST+SA) plus neem cake (as SA) reduced the pathogen population to 9.85×10^{-6}cfu g^{-1} soil and recorded a rhizosphere population of 42.30×10^{-6}cfu g^{-1} soil. Similarly, *T. viride* (Tv_3) (as ST+SA) plus neem cake (as SA) reduced the pathogen population to 10.10×10^{-3}cfu g^{-1} soil and recorded a rhizosphere population of38.45×10^{-3}cfu g^{-1} soil. Carbendazim as seed treatment (4 g kg^{-1}) and soil drenching (0.1%) caused the maximum reduction in the rhizosphere population of *F. oxysporum* f. sp. *vasinfectum* with 6.19×10^{-3}cfu g^{-1} as against 25.45×10^{-3}cfu g^{-1} soil in control (Table 3). The increase in the rhizosphere population of the antagonists might be attributed to the reason that, the organic amendments might have served as an ideal food base for the growth and multiplication of antagonists as reported by Hoitink and Boehm (1999). Besides, organic amendments increased the rhizosphere population of the antagonists (Ashwani *et al*., 2004).

Effect of combined application of antagonists and neem cake on yield parameters of cotton (pot culture)

Generally, the antagonistic treatments with integration of neem cake, showed enhanced yield attributes when compared to other treatments and control. However, among the treatments the treatment T_7 (basal application of neem cake @ 250 kg ha^{-1} +soil application (2.5 lit ha^{-1}) + seed treatment (10 ml $kg^{-1}$of seed) of *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) combination) recorded, 15.15 numbers of bolls $plant^{-1}$, 69.23 g of seed cotton yield $plant^{-1}$. This was followed by the treatment T_6 (*P. fluorescens* (Pf_7)+ neem cake), which recorded 13.40 numbers of bolls $plant^{-1}$, 58.29 g $plant^{-1}$ of seed cotton yield. The treatments T_4 and T_5 came next in the order of merit in enhancing the biometrics of cotton Table 4. *P. fluorescens* strains were found to increase plant growth and yield in various crops (Vivekananthan *et al*., 2004; Sarvanakumar and Samiyappan, 2007). Sowmya and Rao (2011) who reported that treatment of gladiolus corms with *P. fluorescens*, *P. chlamydosporia* and neem cake proved significant increase in the yield of Gladiolus. Sivakumar *et al.* (2008), reported that treatment with a combination of antagonists *viz.*, *T. viride*, *P. flourescens* and *P. lilacinus* along with neem cake significantly reduced the wilt incidence and enhanced the growth parameters and yield of tomato. The maximum root length and shoot length were recorded in rice when seeds were treated with *T. hazianum* and *T. viride* isolates (Joshi *et al.,* 2010).

Table 1 : Effect of different organic amendments on the population of *T. viride* and *P. fluorescens*

T. No.	Organic amendments	Population of *P. fluorescens* (10^5cfu g^{-1})				Population of *T. viride* (10^5cfu g^{-1})			
		0	30	60	90	0	30	60	90
T_1	**FYM**	25.50^a	27.33^c	28.67^b	30.60^b	55.75^a	58.70^a	60.93^a	62.00^b
T_2	Pressmud	25.25^a	26.60^d	27.33^c	28.50^c	55.25^c	48.67^c	50.60^b	51.25^c
T_3	Poultry manure	23.33^b	28.67^b	29.00^b	29.93^b	54.00^b	45.25^b	48.66^b	50.33^c
T_4	Neem cake	24.00^a	29.50^a	30.00^a	32.63^a	55.33^a	60.25^a	63.67^a	64.00^a
T_5	Coir pith	24.33^a	25.67^e	26.00^c	27.10^c	54.00^a	46.33^b	48.75^b	49.00^d
T_6	Control	24.00^a	24.33^f	23.80^d	21.00^d	53.33^b	40.00^d	41.33^c	40.50^e

Table 2 : Effect of combined application of antagonists and neem cake on *Fusarium* wilt and biometrics of cotton (pot culture)

T. No.	Treatments	Shoot length (cm)	Root length (cm)	Bio mass (g $plant^{-1}$)	Per cent wilt incidence	Per cent decrease over control
T_1	Neem cake @ 250 kg ha^{-1}	60.25^d	22.70^e	90.34^f	36.20^g	67.32
T_2	*T. viride* ST @10 ml kg^{-1} and SA @ 2.5 lit ha^{-1}	64.80^c	24.85^d	95.46^e	21.90^f	67.70
T_3	*P. fluorescens* ST @10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	66.36	26.30	98.97	18.80	72.29
T_4	*T. viride*+ *P. fluorescens* ST @ 10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	68.99	29.10	101.34	15.60	77.00
T_5	$T_1 + T_2$	69.41	27.21	100.67	14.00	79.36
T_6	$T_1 + T_3$	70.26	28.04	103.81	13.21	80.53
T_7	$T_1 + T_4$	71.30	30.15	106.64	11.30	83.34
T_8	Carbendazim 50% WP ST @ 4.0 g kg^{-1} and SA @ 0.1%	58.33	24.85	96.22	13.60	79.95
T_9	Control	45.60	20.45	60.46	67.85	–

Table 3 : Effect of combined application of antagonists and neem cake on the rhizosphere population of antagonists and *F. oxysporum* f. sp. *vasinfectum* (pot culture)

T. No.	Treatments	Rhizosphere population (g^{-1} of oven dry soil)		
		T. viride **(10^3cfu)**	***P. fluorescens*** **(10^6cfu)**	**Fov (10^3cfu)**
T_1	Neem cake @ 250 kg ha^{-1}	-	-	18.60
T_2	*T. viride* ST @10 ml kg^{-1} and SA @ 2.5 lit ha^{-1}	36.88	-	12.85
T_3	*P. fluorescens* ST @10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	-	41.01	11.20
T_4	*T. viride*+ *P. fluorescens* ST @ 10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	26.45	31.10	10.00
T_5	$T_1 + T_2$	38.45	-	10.10
T_6	$T_1 + T_3$	-	42.30	09.85
T_7	$T_1 + T_4$	27.35	32.30	08.75
T_8	Carbendazim 50% WP ST @ 4.0 g kg^{-1} and SA @ 0.1%	0.00	0.00	06.19
T_9	Control	-	-	25.45

Table 4 : Effect of combined application of antagonists and neem cake on yield parameters of cotton (pot culture)

T. No.	Treatments	No. of bolls $plant^{-1}$	Seed cotton yield g $plant^{-1}$
T_1	Neem cake @ 250 kg ha^{-1}	7.00^{f}	29.4^{g}
T_2	*T. viride* ST @10 ml kg^{-1} and SA @ 2.5 lit ha^{-1}	08.38^{e}	34.78^{f}
T_3	*P. fluorescens* ST @10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	10.54^{d}	44.90^{e}
T_4	*T. viride*+ *P. fluorescens* ST @ 10 ml $kg^{-1}$and SA @ 2.5 lit ha^{-1}	12.67^{c}	54.22^{c}
T_5	$T_1 + T_2$	11.80^{d}	50.26^{d}
T_6	$T_1 + T_3$	13.40^{b}	58.29^{b}
T_7	$T_1 + T_4$	15.15^{a}	69.23^{a}
T_8	Carbendazim 50% WP ST @ 4.0 g kg^{-1} and SA @ 0.1%	12.40^{c}	53.32^{c}
T_9	Control	06.02^{g}	23.11^{h}

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