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Assessments of biocontrol agents and organic amendments on wilt disease of cotton

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Abstract

The pot culture experiments to examine the antagonistic effects of the individual and combined application of organic manures and antagonists (neem cake, FYM, neem cake + *Trichoderma viride*, Neem cake + *P. fluorescens*, FYM + *T. viride* and FYM + *P. fluorescens*), as well as biocontrol approach in the botanical garden, Department of Botany, Government Arts College, Musiri. The obtained results demonstrated that when different organic manures and antagonists (*T. viride* + neem cake) were mixed, the cotton plant (*Gossypium hirsutum* L.) responded with more significantly improved germination, growth, and yield parameters. The same treatment recorded maximum reduction in the population of *F. oxysporum* f. sp. *vasinfectum* (8.75×10^{-6}) and maximum rhizosphere population of 27.35×10^{-3} cfu g^{-1} soil and 32.30×10^{-6} cfu g^{-1} soil of *T. viride* and *P. fluorescens* respectively. The control group had the lowest levels of germination, growth, and yield parameters. Overall, as compared to the control, the combined application of organic manures greatly improved the cotton plant's qualitative and quantitative aspects.

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Introduction

India's 4 million farmers cultivate the cotton crop over an area of 7.4 million hectares, making it a significant commercial crop. India holds the top spot in terms of acreage, accounting for about 25% of the world's cotton crop. In India, cotton has a prominent place among all cash crops. About 65% of the raw materials used by the Indian textile industry are cotton, making it a vital raw material. With approximately 1500 mills, 4 million handlooms, 1.7 million power looms, and hundreds of garment, hosiery, and processing facilities, the Indian textile sector plays a vital role in the nation's economy and employs over 35 million people directly or indirectly (Sankaranarayanan *et al.*, 2011).

Fusarium oxysporum f. sp. *vasinfectum* (FOV) is a soil- and occasionally seed-borne fungal pathogen that causes fusarium wilt in cotton. Of the eight harmful races that have been identified globally (Hillock, 1992), in US cotton, races 1, 2, 3, 4, and 8 as well as a number of novel genotypes have been discovered (Davis *et al.*, 2006; Holmes *et al.*, 2009; Cianchetta and Davis, 2015; Cianchetta *et al.*, 2015; Halpern *et al.*, 2020). An early-season disease called Fusarium wilt, induced by FOV4, results in seedling wilt and mortality from the moment of emergence until the late square stage. Most infected plants show chlorotic, necrotic, or drooping leaves beyond the square stage, however plant fatalities are also seen (Zhang *et al.*, 2020c).

Utilising biocontrol agents is a feasible substitute to reduce yield loss. But the sustainability of a biocontrol agent needs to be thoroughly addressed, particularly in tropical nations where the soils are generally deficient in organic matter. As a result, using a biocontrol agent that is similar to the pathogen might be an option. Hostile but non-pathogenic *Fusarium* is a good substitute (Minuto *et al.*, 1995ab; Joshi *et al.*, 2013). Additionally, the negative impacts of fungicides such as health risks, phytotoxicity, disease resistance, environmental pollution, and expensive cost make the quest for safe alternative management options imperative. Currently, the concept of using biological management to eradicate soil-borne plant diseases, such as *Fusarium*, can be crucial to sustainable agriculture (Pandey *et al.*, 2010).

In general, biological control, organic amendments, naturally existing nematodes, and induced resistance in combination for the management of complex infections have been the focus of earlier research (Dias-Arieira *et al.*, 2012). The use of *Trichoderma* sp. in the crop field is reported to have a significant effect on yield and maintaining soil micro floral population (Susiana *et al.*, 2018). Also, it suppresses the activity of pathogenic microorganisms by enzymatic activities (Vinale *et al.*, 2008). Therefore the study was undertaken and conducted to develop integrated management strategy involving native biocontrol agents along with neem cake, FYM, *Trichoderma* sp. and *P. fluorescens* for the effective management of cotton wilt disease.

Materials and methods

Isolation of Fusarium oxysporum f. sp. vasinfectum

Using the tissue segment approach, the pathogen *F. oxysporum* f. sp. *vasinfectum* was identified from infected roots of cotton plants exhibiting the characteristic signs of wilt (Rangaswami, 1972). The infected stems and roots were chopped into little pieces after being cleaned with tap water. The pieces were placed on a sterilised Petri plate with potato dextrose agar (PDA) after being surface sterilised in a 1% sodium hypochlorite ($NaOCl_2$) solution for 30 seconds. They were then repeatedly cleaned in sterile distilled water to get rid of any remaining sodium hypochlorite. For five to seven days, the Petri plates were incubated at room temperature (28 ± 2°C). Hyphal points developed from infected portions were moved to PDA slants, where the hyphal tip method was used to purify the fungus (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).

Native antagonists (Trichoderma spp.) isolated from rhizosphere soil

Using the *Trichoderma* selective medium (TSM) and soil dilution plating method, soil samples from various cotton rhizosphere sites were utilised to isolate *Trichoderma* isolates (Elad and Chet, 1983). These *Trichoderma* sp. strains were cultured on TSM slants at 4°C in a refrigerator and purified using the single hyphal tip technique. They were also periodically subcultivated. After being isolated, *Trichoderma* spp. were identified using the Domsch *et al.*, 1980 proposed species key.

Native antagonistic bacterium isolation

From the rhizosphere soil that was collected for the survey, antagonistic bacteria were identified. After carefully mixing the soil with the root fragments, one gramme of rhizosphere soil was treated by serial dilution. To isolate *Pseudomonas*, one millilitre of a 10-5 dilution was plated on King's B (KB) agar medium and cultured for 48 hours at room temperature (28 ± 2°C) (Aneja, 2003). The colonies that fluoresced when exposed to UV light were collected, cleaned, and kept in KB slants, following the standard protocol provided by Barthalomew and Mittewer (1950).

Preparing the biocontrol agents' liquid formulation

For the preparation of liquid formulations the method suggested by Manikandan *et al.*, (2010) was followed. Nutrient, King's B, and PDA broth were used to multiply the isolates of *P. fluorescens, Bacillus subtilis* and *T. viride* that proved to be the most successful in the current investigation. After being separately inoculated into their respective broths, the log phase culture of *P. fluorescens*, *Bacillus subtilis* and the mother culture of *T. viride* were cultured at room temperature (28 ± 2°C). Additionally, two percent glycerol was added to each of the broths. The formulation was evaluated for sufficient CFU after the incubation time using the serial dilution plating technique. The resulting formulation was then sealed in plastic containers and utilised for additional research.

Impact of organic amendments on biocontrol agent population

Earthen pots (15 cm in diameter) were filled with 200 g of garden soil. At a 1% level (w/w), the organic amendments neem cake, farm yard manure, cow dung, vermicompost and press mud were added to the soil (Ayyappan, 2005). Conidial suspensions of the antagonists were made, applied to the soil at a ratio of two milliliters per 100 grammes, and thoroughly mixed. Watering the pots within the glasshouse required judgement, consistency, and regularity. Samples were taken 0, 30, and 40 days into the incubation period, and the antagonist population was evaluated using the serial dilution method. King's B medium was employed for *P. fluorescens, Bacillus subtilis* and *Trichoderma* selected medium (TSM) population assessments, respectively.

Plant growth the frequency of cotton wilt affected by antagonists and an organic amendment (neem cake)

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 treatment combinations were Neem cake (250 kg ha^{-1}), FYM (250 kg ha^{-1}), Neem cake (250 kg ha^{-1}) + *T. viride* (10.0 ml kg^{-1}), Neem cake (250 kg ha^{-1}) + *P. fluorescens* (10.0 ml kg^{-1}), FYM (250 kg ha^{-1}) + *T. viride* (10.0 ml kg^{-1}), FYM (250 kg ha^{-1}) + *P. fluorescens* (10.0 ml kg^{-1}) and control.

The experiment was conducted in a randomized block design with three replications where in five pots per replication and one plant per pot was maintained. The incidence of wilt (%), germination percentage (%), shoot and root length (cm), biomass, number of flowers per plant, number of bolls per plant and seed cotton yield (g plant-1) were recorded. Also, the population of the antagonists and pathogen was assessed using dilution plate technique with suitable selective media.

Results and discussion

The antagonistic applications of both *T. viride* and *P. fluorescens* soil were observed maximum population (20.91 10^{-3} cfu g^{-1} soil) of *T. viride* and (26.82×10^{-6} cfu g^{-1} soil) of *P. fluorescens* was substantially demonstrated the best rhizosphere competency.

Table 1. Effect of soil application with antagonists of *F. oxysporum* f. sp. *Vasinfectum* on the rhizosphere population

SL	Treatments	*T. viride* (10^3 cfu)	*P. fluorescens* (10^6 cfu)	*Bacillus subtilis* (10^6 cfu)	Fov (10^3 cfu)
1.	Control	0.00	0.00	0.00	34.35
2.	*T. viride*	20.13	0.00	0.00	9.91
3.	*P. fluorescens*	0.00	19.41	0.00	10.66
4.	*Bacillus subtilis*	0.00	0.00	15.40	15.62
5.	*T. viride + B. subtilis*	15.64	0.00	14.65	11.64
6.	*T. viride + P. fluorescens*	26.82	20.41	0.00	7.74

Whereas, compared to the population of 34.35 10^{-3} cfu g^{-1} soil in the control, the combined application of *T. viride* and *P. fluorescens* decreased the population of *F. oxysporum* f. sp. *vasinfectum* to a minimum of 7.74 (Table 1).

The separate antagonistic applications also recorded a maximum rhizosphere population (20.13 × 10^{-3} cfu g^{-1} soil) of *T. viride*. The growth rate of Foc was reported to have reached 0.83–0.87 cm/day by Kalman *et al.*, (2020). The synthesis of hydrolytic enzymes, like chitinase, β-1,3-glucanase, and proteases, which can lyse pathogenic fungal cells (Lopez *et al.*, 2019), competition for nutrition and colonisation of the rhizosphere niche (Rana *et al.*, 2019), and production of siderophores and antibiotics (Kumar *et al.*, 2018; Panchami *et al.*, 2020) are some of the ways that rhizosphere bacteria can inhibit the growth of pathogens.

Application of neem cake and FYM were found to be better in microbial population when compared to control. However, other amendments of Cow dung, V. compost and pressmud also found to least population were observed. In addition, the population of *T. viride* and *P. fluorescens* was the maximum in neem cake amended soil followed by FYM soil (64.67; 63.41 and 33.67; 30.70 ×10^{-6} cfu g^{-1} respectively) than control at after 30 days analysis. The same application of organic amendments resulted in the lowest population of *Bacillus subtilis* (31.55; 28.88 %) than other amended soil respectively (Fig. 1).

These results are consistent with those of Lavanya *et al.* (2016), who suggested that when compared to soil treated without jeevamrutha and panchagavya, the maximum population of general bacteria, fungi, actinomycetes, *Pseudomonas* and PSB was observed in the soil treated with jeevamrutha at 1000 l ha^{-1} and 7.5% panchagavya in field bean.

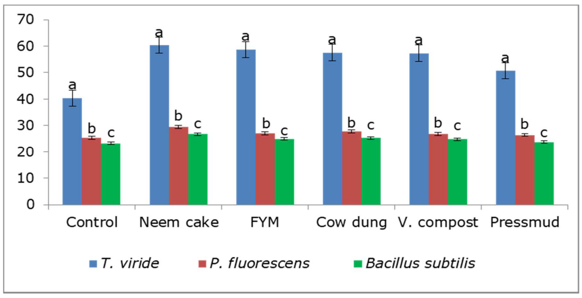

Fig. 1. Impact of various organic amendments on *Bacillus subtilis*, *P. fluorescens*, and *T. viride* populations

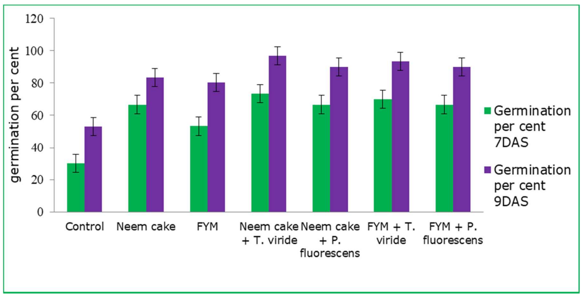

Fig. 2. Germination percentage as influenced by the application of organic manure and antagonists at 7th and 9th DAS

Fig. 2 shows the effect of all the organic amendments and antagonists showed enhanced growth promotion when compared to control. Among the organic amendments such as neem cake and FYM were recorded 83.33±2.49 and 80.33±2.40 per cent of seed germinations. The highest germination was recorded in Neem cake + *T. viride* being 73.3300±2.19 at 7th DAS and 96.66±2.89 at 9th DAS followed by 70.00±2.1 and 93.33±2.29 percentage of FYM + *T. viride*.

Table 2. Effect of combined application of antagonists and neem cake on Fusarium wilt and biometrics of cotton

Treatments	Shoot length (cm)	Root length (cm)	Biomass (g plant[-1])	Percentage of wilt incidence
Control	26.3 ±0.819	8.2 ±0.246	62.73±1.88	60±1.82
Neem cake	28.4 ±0.852	10.7 ±0.321	65.95±1.97	40±1.21
FYM	27.7 ±0.852	8.8 ±0.264	63.72±1.91	46.66±1.39
Neem cake + *T. viride*	34.1 ±1.023	13.4 ±0.393	73.20±2.19	20±0.60
Neem cake + *P. fluorescens*	29.7 ±0.891	10.8 ±0.264	67.17±2.01	33.33±0.99
FYM + *T. viride*	31.5 ±0.945	12.1 ±0.393	70.45±2.11	26.66±0.79
FYM + *P. fluorescens*	29.1±0.87	9.4±0.28	65.38±1.96	33.33±0.99

According to Lokesh *et al.* (2012), plants treated with organic manures and green leaf manures produced significantly more bolls per plant, mean boll weight, and kapas weight per plant. This could be because the plants performed better in terms of growth parameters like plant height, number of monopodial branches, leaf area index, SPAD value, and total dry matter production. The neem cake promoted stress-free plant development and reduced sucking bugs (Veena *et al.*, 2017). Conversely, vermicompost provided more nutrients and moisture (Reddy *et al.*, 2017).

Soil application of neem cake, FYM combined with antagonists are furnished in Table 2. Among the treatments, basal application of neem cake Neem cake + *T. viride* recorded the minimum incidence of wilt (20.33±0.60%). This was followed by the application of FYM + *T. viride* reducing the wilt incidence and enhancing the growth parameters of cotton. However, the untreated control recorded the maximum disease (60.84±1.82%) incidence and minimum growth parameters. Mishra *et al.*, 2013 observed that the 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. Also, in tomato, minimum wilt incidence of 14.9 per cent was recorded by seed priming with *T. viride* and soil application of neem cake (Barnwal *et al.*, 2011).

Fig. 3 clearly revealed that the two types of mono inoculations, the significantly different number of flowers and number of bolls were recorded in neem cake and FYM it was 23±0.69, 22±0.66 and 20.9±0.62 and 20.5±0.61 than control. Despite that, the combined inoculation of neem cake + *T. viride* and FYM + *T. viride* showed a highest number of flowers per plant 28±0.84, 26±0.78, 24.5±0.73 and 23.6±0.68 than other inoculations.

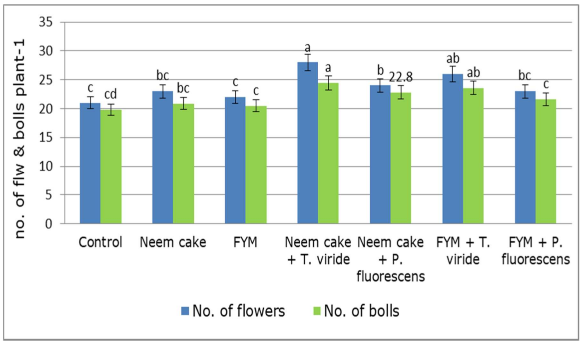

Fig. 3. Influence of different manures and antagonists on yield parameters of *Gossypium hirsutum* L.

Table 3. Influence of different manures and antagonists on yield parameters of *Gossypium hirsutum* L.

Treatments	Seed cotton yield (g) plant[-1]
Control	51.0±1.53
Neem cake	78.56±2.35
FYM	71.29±2.13
Neem cake + *T. viride*	86.21±2.58
Neem cake + *P. fluorescens*	80.18±2.40
FYM + *T. viride*	82.35±2.47
FYM + *P. fluorescens*	78.64±2.35

Furthermore, quick vegetative growth could have given buds plenty of nourishment to expand into floral buds, which would have led to early blooming and the first harvest of fruit were reported by Pariari and Khan (2013) in chilli and Jamir *et al.* (2017) in sweet pepper. Applying FYM and inorganic nitrogen to onions resulted in the highest output of marketable onions (Gererufae *et al.*, 2020). Tomato plants treated with FYM, VC, and waste decomposer showed increases in fruit yield per plant and per square metre, as well as in fruit weight and quantity per plant (Rajya *et al.*, 2015; Meena *et al.*, 2021).

Table 3 distinctly displayed that the two types of mono inoculations, the maximum seed cotton yield were recorded in neem cake and FYM it was 78.56±2.35 and 71.29±2.13 than control. Despite that, the combined inoculation of neem cake + *T. viride* and FYM + *T. viride* showed a highest seed cotton yield 86.21±2.58 and 82.35±2.47 than other inoculations. Benefits of organic manures, such as vermincompost and chicken manure, on crop productivity, radish growth, and quality aspects were also reported by Singh *et al.*, 2016. Fruit output and fresh fruit weight were increased when vermin-compost, FYM, chicken manure, and neem cake were applied to chilli peppers; poultry manure outperformed the other three (Soreng and Kerketta, 2017). In addition, Shwetha *et al.* (2009) found that the soybean yield in treatments treated with organic manures in the combination of beejamrutha + jeevamrutha + panchagavya was significantly higher than that of RDF + FYM when compared to the control.

Conclusion

Based on resistance to antagonism against *Fusarium oxysporum*, this study developed a strategy for screening biocontrol drugs against *Fusarium wilt*. To effectively manage cotton Fusarium wilt illnesses, two potential biocontrol microorganisms were obtained: *Trichoderma viride*, *Pseudomonas fluorescent* and a mixture of neem cake. Mainly, neem cake and the combination of *Trichoderma viride* produced the positive results in *Fusarium* wil reduction, cotton yield and fiber quality, performing even better than the standard other treatments. Our findings developed a novel technique for screening biocontrol agents from rhizosphere soil for cotton Fusarium wilt disease.

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