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Compatibility studies among different Microbial pesticides commonly used for Soil application

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Abstract
The present investigation was carried out on a compatibility between different microbial pesticides through Dual culture technique and Pot culture assays. In dual culture, per cent inhibition of radial growth of *T. viride* in treatments *T. viride* + *P. fluorescens* and *T. viride* + *M. anisopliae*, revealed that *T. viride* covered the full plate and it showed an antagonistic effect on *P. fluorescens* and *M. anisopliae*. Thus, it was found that they were not compatible with each other. While, *P. fluorescens* inhibited *M. anisopliae* upto 19.01 per cent. In pot culture assay at 45 days after soil treatment with different combinations of microbial pesticides *viz*., *T. viride, M. anisopliae, P. fluorescens, T. viride* + *M. anisopliae, T. viride* + *P. fluorescens M. anisopliae* + *P fluorescens, T. viride* + *M. anisopliae*+*P. fluorescens* and control, at 10^{-2}, 10^{-3}, 10^{-4} concentrations revealed that *T. viride* was found to be most dominant and *M. anisopliae* growth was nil. While, *P. fluorescens* was observed at higher concentrations of 10^{-5}, 10^{-6}, 10^{-7}.

Keywords: Compatibility, microbial pesticides, soil application

Introduction
Crop losses due to pests and diseases are a major threat to the incomes of rural families and to food security worldwide (Savary and Willocquet, 2014) [8]. With the advent of chemical pesticides, this crisis was resolved to a great extent. The over dependence on chemical pesticides and eventual uninhibited use of them has necessitated for alternatives mainly for environmental concerns. Degraded soils and groundwater pollution has resulted in nutritionally imbalanced and unproductive lands. Violative pesticide residues also sometimes raise food safety concerns among domestic consumers and pose trade impediments for export crops. Therefore, an ecofriendly alternative is the need of the hour. Biopesticides or biological pesticides based on pathogenic microorganisms specific to a target pest offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health (Suman and Dikshith, 2010) [11].

Among the available methods of biological control, the pest management through microbial biopesticides started gaining momentum. Microbial pesticides are also known as Biological Control Agents. In this category, the active ingredient is a microorganism that either occurs naturally or is genetically engineered. The pesticidal action may be from the organism itself or from a substance it produces. They offer the advantages of higher selectivity and less or no toxicity in comparison to conventional chemical pesticides (MacGregor, 2006) [7].

As the microbial biocontrol agents have a complex mode of action, it is very difficult for a pest to develop resistance against biopesticides. The present group of biopesticides include viruses, bacteria, fungi and nematodes and they are used throughout the world as an alternative to chemical pesticides. Among the biocontrol agents, microbial pesticides are the most important due to easy delivery, improved formulations, a good number of pathogenic strains known and over-expression of endogenous proteins or exogenous toxins (St Leger and Wang, 2009) [10].

Broad host range, promising pathogenicity and ability to control sap sucking pests such as aphids, jassids, whiteflies (Butt, 2002) [1]; (Fan *et al*., 2007) [3] as well as pests with chewing mouthparts (Hajek and St Ledger, 1994) [6], a new era has begun in using entomopathogenic fungal formulations as the main inputs for pest management in many major crops of the country.

Due to the popularization and increased usage of microbial pesticides across the crops for pest and disease management the issues pertaining to their compatibility with each other gains

importance. The generation of information in this regard is the need of the hour. This type of situation makes it imperative to workout compatibility among the commonly recommended and used biopesticides.

Materials and Methods

The present investigations on "Compatibility and Virulence studies of Microbial pesticides commonly used in Telangana" were carried out at AICRP on Biological Control, Agricultural Research Institute, Rajendranagar, and Hyderabad during 2017-18. The materials used and the methods employed in these investigations are furnished here under. Completely Randomized Design (CRD) was followed for analyzing the data in different experiments. The data was subjected to angular transformation as per the requirement to improve homogeneity of error variances (Gomez, 1984) [5].

The microbial biopesticides commonly used for soil application *viz*., *Trichoderma viride, Metarhizium anisopliae, Pseudomonas fluorescens* were taken up for the studies for compatibility among them. Compatibility were studied under laboratory conditions through dual culture assays. Colonization ability of the same were worked out under net house conditions through pot culture studies.

Dual culture Assay

The sole treatments are entomopathogenic fungi *viz*., *Trichoderma viride and Metarhizium anisopliae* were tested individually and also with antagonistic organisms to find out any compatibility issues among them as per treatments:

T_1 = *Trichoderma viride + Metarhizium anisopliae*
T_2 = *Trichoderma viride + Pseudomonas fluorescens*
T_3 = *Trichoderma viride* (Control)
T_4 = *Metarhizium anisopliae + Pseudomonas fluorescens*
T_5 = *Metarhizium anisopliae* (Control)

The test cultures were taken from AICRP on Biological Control of Crop Pests and isolated, maintained in respective media.

Required amount of PDA media were weighed and dissolved in 100 ml of distilled water by thoroughly mixing using vertex mixer. After cotton plugging, wrapped with the paper, this media was kept in autoclave at 121 degrees with 15 lbs pressure for 15 to 20 minutes. The media was allowed to cool to a tolerable temperature for handling after sterilization. The media was poured into Petriplates and allowed it for solidification. After solidification, test culture disc of 5 mm were placed on the peripheral side of 9 cm petriplate with the PDA medium. Another test culture was placed on the opposite side of the first test culture by using sterile cork borer. In case of bacteria, a streak opposite side was done with a sterile inoculation loop. Control plate was also maintained for comparison purposes. The plates were kept for incubation at 30˚C temperature for 3 to 5 days.

After the incubation period, the radial growth of each test organism was measured using a measuring scale at 5, 7, 9 days after inoculation and per cent of inhibition were also worked out (Siddiqui and Shaukat, 2003) [9].

The compatibility was calculated by using the following formula.

$$\% \text{ of Inhibition} = \frac{\text{Control - Treatment}}{\text{Control}} \text{ x } 100$$

Pot Culture Assay

Plastic pots (1' dia) were filled with approximately 2.5 Kg sterile soil. Soil in each pot were treated with equal load of microbial pesticides (@ 60 g) as per the treatments mentioned below:

T_1 = *Trichoderma viride*
T_2 = *Metarhizium anisopliae*
T_3 = *Pseudomonas fluorescens*
T_4 = *Trichoderma viride + Metarhizium anisopliae*
T_5 = *Trichoderma viride + Pseudomonas fluorescens*
T_6 = *Metarhizium anisopliae + Pseudomonas fluorescens*
T_7 = *Trichoderma viride+ Metarhizium anisopliae +Pseudomonas fluorescens*
T_8 = Control

Test microbial pesticides @ 60 g was mixed in soil in each pot. After amending, each pot were sown with cotton seeds and three replicates were maintained for each treatment. All the agronomic practices were followed. At 45 DAS, approximately 30 g of soil were collected from each pot and estimated for the presence and growth of test biopesticides.

Preparation of Culture Media

A medium is an environment which supplies all nutrients for the growth of an organism. There were different media for the growth of fungi and bacteria. From that the following media was used for the study. PDA (potato dextrose agar), SDAY (sabourauds dextrose agar yeast), King's B medium.

The composition of SDAY media is given below.

Dextrose	-	40 g
Peptone	-	10 g
Agar agar	-	15 g
Yeast extract	-	5 g
Distilled water	-	1000 ml

Components of PDA medium

Potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml

Components of King's B medium

Proteose peptone	-	20g
K_2HPO_4	-	1.5g
$MgSO_4.7H_2O$	-	1.5g
Glycerol	-	10ml
Agar	-	15g

The study was taken up to evaluate the presence and growth of microbial pesticides. Different types of media used for different treatments.

Required amount of respective media were individually weighed and dissolved in 100 ml of distilled water by thoroughly mixing using vertex mixer. After cotton plugging, wrapped with the paper, this media was kept in autoclave at 121 degrees with 15 lbs pressure for 15 to 20 minutes. The media was allowed to cool to a tolerable temperature for handling after sterilization. The media was poured into Petriplates and allowed it for solidification.

Preparation of Stock solution and Dilutions

Ten grams of collected soil sample was weighed and mixed in 100 ml sterile distilled water by thoroughly mixing using vertex mixer, it is considered as stock solution. From the stock solution, one ml was taken and transferred to a 9 ml

dilution blank, using aseptic techniques. This was done for all the soil samples that are collected from different treatments of test bio pesticides. The solution was mixed well to obtain even distribution of organisms. With a sterile pipette, 0.1 ml of the dilution was poured into a sterile media plated in petridish and 1.0 ml was transferred to a 9.0 ml dilution blank using the same pipette. This process was repeated upto certain concentrations and the plates were rotated to ensure spreading the inoculums on media. The concentrations of 10^{-2}, 10^{-3}, 10^{-4} used for plating of *T. viride* and for *M. anisopliae*, the concentrations of 10^{-4}, 10^{-5}, 10^{-6} whereas, for *P. fluorescens* concentrations of 10^{-5}, 10^{-6}, 10^{-7} were used for plating of test microbial organisms. After that, the petriplates were placed in incubator at 25˚C to 28˚C for better incubation. The culture plates pertaining to different treatments were observed for the presence and growth of the test microbial organisms.

Results and Discussion

Dual culture Assay

Results presented in the table 1, Plate 1, revealed that the radial growth of *T. viride* (Figure 1) on PDA media with dual culture technique in treatment *T. viride* + *P. fluorescens* revealed that the culture plate recorded radial growth of 6.24 cm with 12.83 per cent inhibition at 5 DAT as against 7.16 cm in control. By the seventh day, *T. viride* covered the full plate and no clear inhibition zone was observed between the bacterial and the fungal colonies. *T. viride* showed antagonist effect on *P. fluorescens* and not compatible with each other.

The radial growth of *T. viride* (Figure 2) on PDA media with dual culture technique in treatment *T. viride* + *M. anisopliae* revealed that the culture plate recorded radial growth of 6.32 cm with 11.64 per cent inhibition at 5 days after inoculation as against 7.16 cm in control. By the seventh day after inoculation, *T. viride* covered the full plate and no clear inhibition zone was observed between the *T. viride* and *M. anisopliae* colonies. *T. viride* showed antagonist effect on *M. anisopliae* and were not compatible with each other.

The radial growth of *M. anisopliae* (Figure 3) on PDA media with dual culture technique in treatment *M. anisopliae* + *P. fluorescens* revealed that the culture plate recorded radial growth of 2.21 cm with 31.15 per cent inhibition at 5 days after inoculation as against 3.80 cm in control, 3.52 cm with 21.64 per cent inhibition at 7 days after inoculation as against 4.50 cm in control, 4.15 cm with 19.01 per cent inhibition at 9 days after inoculation as against 5.13 cm in control.

In accordance with results obtained, *in vitro* compatibility test between *P. fluorescens*-Bak150 and *T. viride*- ES1 by using dual culture plate method showed no clear inhibition zone between the bacterial (*P. fluorescens*) and the fungal (*T. viride*) colonies (Ephrem *et al*., 2011) [2].

Gokil Prasad (2013) [4] reported similar findings that compatibility of *T. harzianum* was tested against *B. bassiana*, *M. anisopliae*, *P. lecanii* and found that *T. harzianum* did not exhibit compatibility and significantly reduced average radial growth of all three fungi. Maximum inhibition (61.4%) of average radial growth of *P. lecanii* was exhibited after 12 days of inoculation. Mean inhibition of radial growth of *B. bassiana* (44.5%) and *M. anisopliae* (44.1%) after 12 days of inoculation.

Table 1: Variations in radial growth and per cent inhibition in different test bio pesticides and their combinations (Soil application) through dual culture technique

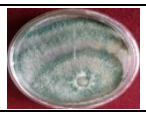
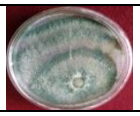
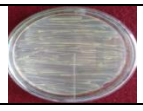
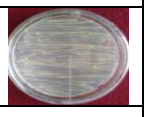
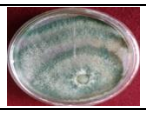
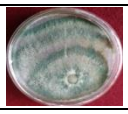
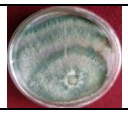
Treatments	Days after inoculation					
	5		7		9	
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
**T. viride + M. anisopliae*	6.24^{bc}	12.83^{b} (20.97)	8.00^{a}	0.00^{b} (0.00)	8.00^{a}	0.00^{b} (0.00)
**T. viride + P. fluorescens*	6.32^{b}	11.64^{c} (19.93)	8.00^{a}	0.00^{b} (0.00)	8.00^{a}	0.00^{b} (0.00)
T. viride	7.16^{a}	0.00^{d} (0.00)	8.00^{a}	0.00^{b} (0.00)	8.00^{a}	0.00^{b} (0.00)
#M. anisopliae +P. fluorescens	2.61^{e}	31.15^{a} (33.88)	3.52^{c}	21.64^{a} (27.66)	4.15^{c}	19.01^{a} (25.60)
M. anisopliae	3.80^{d}	0.00^{d} (0.00)	4.50^{b}	0.00^{b} (0.00)	5.13^{b}	0.00^{b} (0.00)
SE(m)±	0.03	0.48	0.03	0.46	0.06	0.93
CD(0.05%)	0.09	1.42	0.09	1.38	0.19	2.78

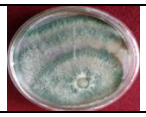
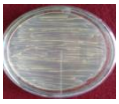
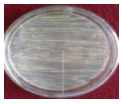
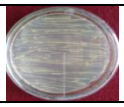
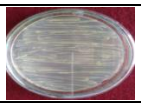
Values given in parentheses are angular transformed values

Figures indicated by same letter are not significantly different from one another as per DMRT

Note: The values indicated in the table are **Trichoderma viride* and *#Metarhizium anisopliae*

Table 2: Presence and growth of the test microbial organisms in culture plates at different concentrations

Treatments	*Trichoderma viride*			*Metarhizium anisopliae*			*Pseudomonas fluorescens*		
	10^{-2}	10^{-3}	10^{-4}	10^{-4}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-7}
T. viride									
M. anisopliae				-	-	-			
P. fluorescens							-		
T. viride + *M. anisopliae*				-	-	-			

T. viride + *P. fluorescens*			-						-
M. anisopliae + *P. fluorescens*				-	-	-			-
T. viride + *M. anisopliae* + *P. fluorescens*	-			-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-

T. viride + *P. fluorescens*

Control (T. viride)

T. viride + *M. anisopliae*

Control (T. viride)

M. anisopliae + *P. fluorescens*

Control (*M. anisopliae*)

Plate 1: Extent of antagonism/compatibility among different test bio pesticides (Soil application) through dual culture test

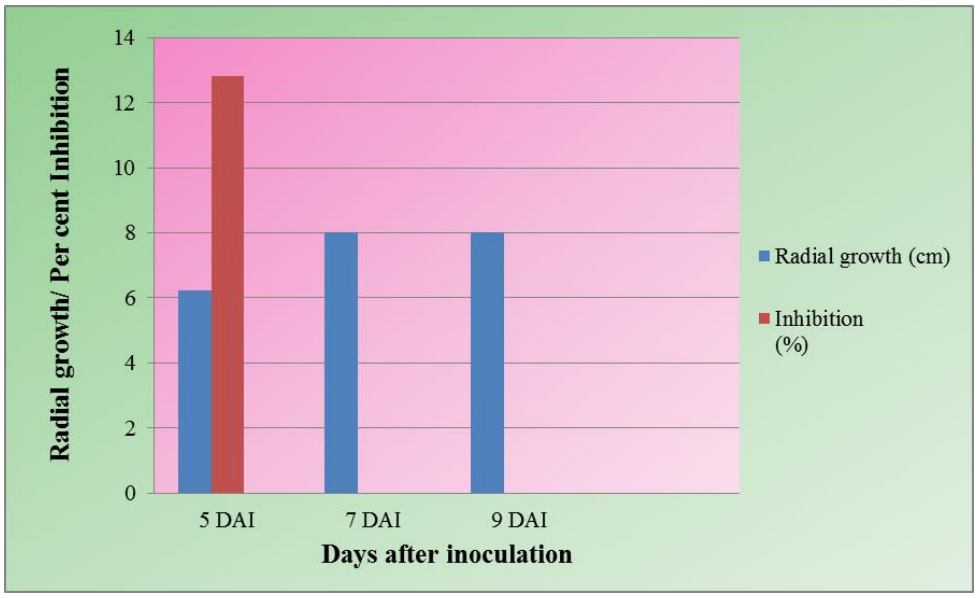


Fig 1: Radial growth and per cent inhibition of *T. viride* with *M. anisopliae*

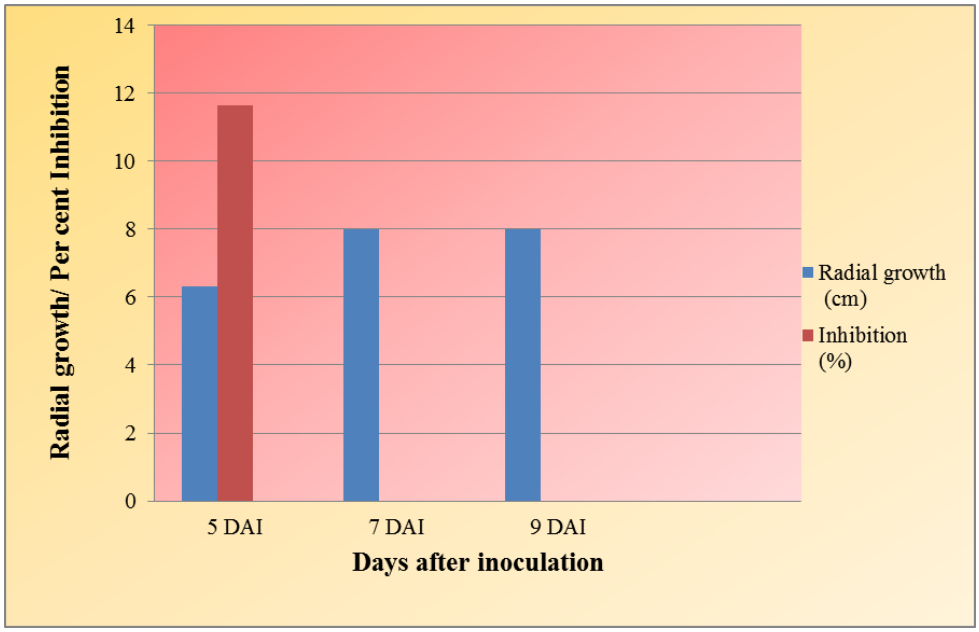


Fig 2: Radial growth and per cent inhibition of *T. viride* with *P. fluorescens*

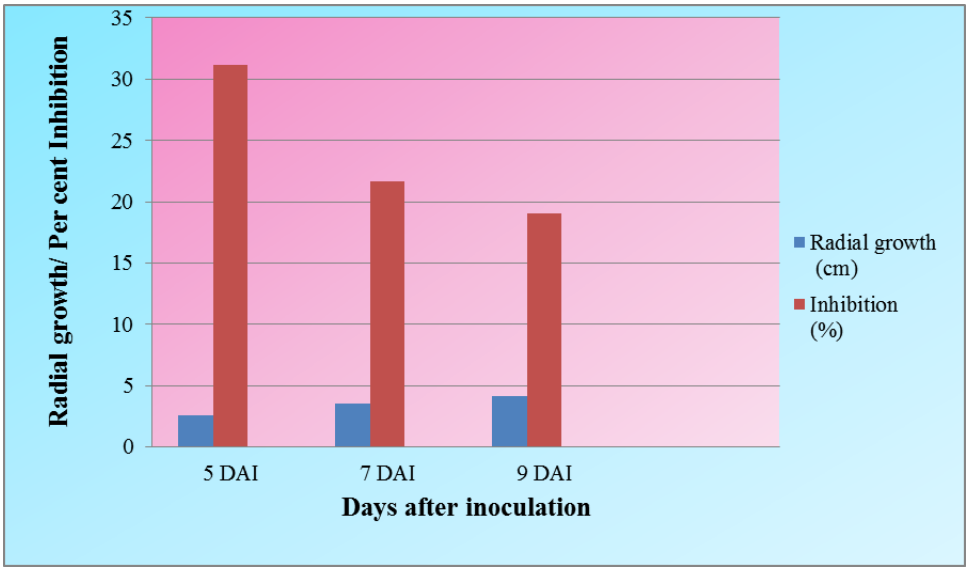


Fig 3: Radial growth and per cent inhibition of *M. anisopliae* with *P. fluorescens*

Pot Culture Assay

The present investigation was carried out by amending microbial pesticides in soil in each pot and were sown with cotton seeds and soil collected from each pot after 45 days of treatment and evaluated for the presence of test microbial pesticides by serial dilution.

The results presented in the table 2, revealed that soil treated with *T. viride*, showed the growth of *T. viride* in plates at 10^{-2}, 10^{-3} and 10^{-4} concentrations whereas, in soil treated with *M. anisopliae*, no growth was observed. In case of soil treated with *P. fluorescens*, it was observed at 10^{-6} and 10^{-7} concentrations whereas in *T. viride*+ *M. anisopliae* treated soil, *T. viride* was only observed at 10^{-2}, 10^{-3} and 10^{-4} concentrations and there was no growth of *M. anisopliae*. Soil treated with *T. viride* + *P. fluorescens*, *T. viride* was observed at 10^{-2}, 10^{-3} concentrations and *P. fluorescens* also observed in plates at 10^{-5} and 10^{-6} concentrations.

In case of *M. anisopliae* + *P. fluorescens*, *P. fluorescens* was only found at 10^{-5} and 10^{-6} concentrations whereas in *T. viride* + *M. anisopliae* + *P. fluorescens* treated soil, *T. viride* was only observed at 10^{-3} and 10^{-4} concentrations and no other microbial growth was observed in the plates. There is no growth of microbial organisms in control plates. Pertaining to the above information provided, *T. viride* is most dominant in all the treatments and showed antagonistic effect on other microbial pesticides.

References

1. Butt TM. Use of entomogenous fungi for the control of insect pests. In: Esser K., and Bennett J.W. (eds.), Mycota, Springer, Berlin, 2002, 111-134.
2. Ephrem DZ, Amutha S, Dereje G, Mesfin T, Bekele K. Biocontrol activity of *Trichoderma viride* and *Pseudomonas florescens* against *Phytophthora infestans* under greenhouse conditions. Journal of Agricultural Technology. 2011; 7(6):1589-1602.
3. Fan Y, Fang W, Guo S, Pei X, Zhang Y, Xiao Y *et al.* Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. Applied and Environmental Microbiology. 2007; 73:295-302.
4. Gokil Prasad G. Compatibility of *Beauveria bassiana* (Balsamo) Villemin with chemicals and bio-pesticides. Annals of Plant Protection Science. 2013; 21(2):360-363.
5. Gomez KA, Gomez AA. Statistical procedure for Agricultural Research. Pub. By John Wiley and sons, New York. 1984, 643-644.
6. Hajek AE, St-Ledger RJ. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology. 1994; 39:293-322.
7. MacGregor JT. Genetic toxicity assessment of microbial pesticides: needs and recommended approaches. International Association of Environmental Mutagen Societies. 2006, 1-17.
8. Savary S, Willocquet L. Simulation Modeling in Botanical Epidemiology and Crop Loss Analysis. The Plant Health Instructor. 2014, 173.
9. Siddiqui IA and Shaukat SS. Combination of *Pseudomonas aeruginosa* and *Pochonia chlamydosporia* for control of root-infecting fungi in tomato. Journal of Phytopathology. 2003; 151:215-222.
10. St Leger RJ, Wang C. Entomopathogenic fungi and the genomic era, In: Stock SP, Vandenberg J, Glazer I, and Boemare N. (eds.), Insect Pathogens: Molecular Approaches and Techniques. CABI, Wallingford, UK. 2009; 366-400.
11. Suman G, Dikshit AK. An ecofriendly approach for pest control. Journal of Biopesticides. 2010; 3(1):186-188.