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Antagonistic effect of *Tagetes erecta* on soil microbes and nematodes of banana

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ABSTRACT:

Antagonism process involves secondary metabolites of plants, microorganisms, viruses and fungi enhancing the development and growth of biological and agricultural systems. Five experimental plots were used and each individual plot was 10×10m and consisted of 5 pairs of Banana plants spaced with 5 feet. For the first, second, third and fourth experimental plots the Banana plants intercropped with 2 rows, 3 rows, 4 rows and 5 rows of *Tagetes erecta* seedlings respectively, with one foot/30cm distance within the row and between the rows. The fifth plot was considered as the control without *T. erecta*. The study was ended 65 days after intercropping of *T. erecta*. Soil samples were collected from all plots for microbial and nematode analyses before and after intercropping of *T. erecta* in the experimental plots. *R. Similis* took 65 days to get reduced to 25% in T4 treatments. In T1 there was an increase of 30% as found in the control. T2 showed a 13% decline and 16% decline in T3. P. Coffee in banana plants gave an increase of 25% in control, 10% decrease in T1, 1.5% decrease in T2, 58%decline in T3 and 70% decline in T4. Tagetes intercropped banana soil Actinomycetes and fungus Fusarium were not affected by the exudates of Tagetes. Trichoderma exhibited deterioration in the T1 & T2 treatment. All the three bacteria under study thrived better in the Tagetes treated banana soil. T2 treatment had more response showing comparatively slower growth than in T3 & T4 treatments. The population density of Banana parasitic nematode gradually decimates and gradually increasing of beneficial bacteria while increasing the number of intercropping of *T. erecta*

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Introduction

Antagonism plays a significant role in monocultures, crop mixtures or intercropping and crop rotations. Intercropping is defined as the agricultural practice of cultivating two or more crops in the same space at the same time (Hugar and Palled 2008). The purpose of this practice is to increase the yield per unit area, prevent the total crop failure, increase the yield, and weed control and maintenance of soil and productivity (Ijoyah and Dzer 2012).

Plant based bio-nematicides have the relative advantages of being inexpensive and local availability over the chemical nematicides. Besides, their environmental security in an environmentally aware world also holds guarantee for their suitability and use by resource-poor farmers. A variety of medicinal and antagonistic plants have been tested for their effectiveness towards the control of root knot nematodes over the years (Ibrahim *et al.*, 2006; Abo Elyousr *et al.*, 2010; Hussain *et al.*, 2011).

Tagetes is a nematode allelopathic crop and act as a non-host or a poor host and a trap crop (Pudasaini *et al.*, 2008) produce allelopathic compounds α ternithyl, that are fatal to Plant Parasitic nematodes (PPNs) development and creates a favorable micro environment for nematode antagonistic flora or fauna (Cerruti *et al.*, 2010). Marigolds secrete thiophene, that proves antiviral, antibacterial, antifungal, nematicidal and insecticidal properties (Riga *et al.*, 2005). Intercropping white Cabbage with *Tagetes patula* and *Calendula officinalis* L. were effectively control Pieris *brassicae* L, *Mamestra brassicae* L., *Pieris rapae* L., and pupae and larva of *Plutell xylostella* (Beata Jankowska et al., 2013). *Pratylenchus coffeae* is the next most important nematode to the *R. similis* infestation in banana. In India, 44.4% yield loss caused by root-lesion nematode in banana cv. Nendran (Sundararaju and Kumar, 2003) Nendran and Kalyan Bale were highly infected by *P. coffeae* and *R. similis* respectively (Sundararaju and Cannayane, 2002). In Tamil Nadu, 30.9% reduction in yield of Poovan banana due to root-knot nematode was reported (Jonathan and Rajendran 2000). The yield of banana was significantly increased. 12 kg/plant banana was harvested when intercropped with *Tagetes* spp., whereas 9 kg/ plant was obtained with the untreated control (Sundararaju and Cannayane, 2002).

Growing competition between the marigold and cash crop can be reduced by intercropping *Tagetes* with a perennial or biannual cash crop, because the leaf canopy between *Tagetes* and cash crop will be at different levels. (Cerruti *et al.*, 2010) suggested that thorough descriptions of experimental designs, methods such as pot or plot sizes, distances between plants, accurate numbers of *Tagetes* and host plants, biomass or relative sizes of the plants involved, and environmental conditions are needed for the clear understanding of intercropping studies. Therefore, in this article we focused on these suggestions through the allelopathic effect of

marigold on soil microbes and nematodes of Banana.

Material and Methods

Collection of Tagetes seeds and rising of seedlings

Seeds of *T. erecta* were collected from local market. Care was made for seed germinating capacity, health and purity. Seeds were not treated with any sort of chemical. Plants were first grown in sterilized sandy loam soil in earthen pots. Plants were allowed to grow for 30 -days.

Intercropping design for Banana with *T. erecta* system

Five experimental plots were used and each individual plot was 10×10m and consisted of 5 pairs of Banana plants spaced with 5 feet. For the first, second, third and fourth experimental plots the Banana plants intercropped with 2 rows, 3 rows, 4 rows and 5 rows of *T. erecta* seedlings respectively, with one foot/30cm distance within the row and between the rows. The fifth plot was considered as the control without *T. erecta*. The experiment was rain fed while manual weeding was carried out to keep the plots free of weeds. Fertilizers were not applied in the experimental plots. The study was ended 65 days after intercropping of *T. erecta*. Soil samples were collected from all plots for microbial and nematode analyses before and after intercropping of *T. erecta* in the experimental plots.

Effect of treatments on host plants - Cobb's wet sieving method (Cobb., 1918) **Improved Bearmann Funnel Method** (Ijoyah and Fanen, 2012; Hooper, (1986).

Initial and final population densities of *M. incognita* and *Rotylenchus reniformis* from banana *T. erecta* system were determined. For this purpose, composite soil samples were collected from each plot from five different sites at the beginning of the planting of *T. erecta* seedling in banana field after 65 days. Five soil samples were taken from each treatment. Samples were mixed thoroughly and nematodes were extracted from a 200cc soil sample using Improved Bearmann Funnel Method. The extracted nematodes were killed by heat and fixed in 4% formaldehyde and identified by morphological characteristic and then counted using a binocular microscope and nematode counting chamber.

Isolation of antagonistic micro-organisms from the soil sample of host plants

Fungi were isolated on Rose Bengal Agar (RBA), bacteria on Nutrient Agar (NA) and *Actinomycetes* on Kusters Agar (KA) by serially dilution of soil samples. Soil dilution plates were incubated at 30±1°C for 24-48 hours for bacterial growth and 4-5 days for fungal growth. Representative colonies on the plates were selected subcultured and kept at 4°C until further use. Soil samples for microbial study.

Soil samples for microbial study (Ijoyah and Jimba, 2012; Tresner and Hayer, 1970)

The soil was collected at random from 5 sites. The soil sample was taken from rhizosphere (0-15 cm depth). Soil sample collected from different sites were kept in plastic bags until further use. The population density of microbes

in the soil was determined by serial dilution plate count method. Isolation, identification, and the count of population density of microorganism used a selective medium. Identification of microorganism was estimated by morphology, physiological and microscopicaly.

Isolation, identification and population procedure of bacteria (Cobb, 1918; Hooper, 1986; Ellis, 1968a; Ellis, 1968b)

The identification of microbe's population used fresh soil. The population of bacteria was determined by serial dilution plate count method. 10g fresh soil was suspended into 90 ml distilled water solution and agitated for 1h to provide mechanical desegregation of cells. Subsequent dilutions were prepared by manually shaking the suspension for 10sec to resuspend the soil. Then an aliquot of 1ml was transferred using a sterile pipette to 9ml sterile distilled water in a test tube. This suspension was agitated manually for 10sec and subsequently serial diluted (10-1 to 10-7). 0.2ml of soil suspension from each of the dilution was spread plated on the Nutrient agar medium. The number of bacteria colony was estimated after 2 -3 days of incubation at 28±2°C by plate count method. Selected colonies were transferred to NA medium. The isolates of bacteria were identified by using morphological, physiological and biochemical characteristics.

Isolation, identification and population procedure of fungi (Cobb, 1918; Hooper, 1986; Ellis, 1968a; Ellis, 1968b)

One g of soil was suspended into 9ml of distilled water in Erlenmeyer flask and agitated on shaker for 1h at 120 rpm. The soil extract was diluted from 10-1 to 10-7 spread 0.2ml of soil sample suspension from each serial dilution on to isolation Rose Bengal Medium. It was incubated at 28°C for 3-7 days. Alternatively, 1g soil sample of each treatment were serially diluted up to 106 dilutions with saline. Dilutions 104−106 were placed on Rose Bengal Medium by spread plate technique and incubated at 28±2°C for 4 days. The most prominent colonies were isolated and maintained on RBM slants at 4°C for further studies. Different colonies were selected and transferred to be identified.

Isolation, identification, and population Procedure of *Actinomycetes* (Tresner and Hayer, 1970; Tsao et al., 1960)

The soil samples were pretreated with calcium chloride and then dried at 450c for an hour in order to reduce the incidence of bacteria and molds. This modified procedure was found to be suitable for the isolation and identification of *Streptomyces* sps. The pretreated soil sample was serially diluted upto 10-7 in sterile saline (0.85%). Then 0.5ml of serially diluted sample was spread on Kusters Agar medium. The plates were incubated at room temperature for up to 7days after incubation well developed, powdery, leathery and aerial mycelium producing colonies were selected as *Streptomyces,* which were confirmed by microscopic observation.

Certain microbes inhabiting the rhizosphere zone of banana intercropped plants Tagetes were grown for a period of 65 days and the soil samples were collected after the fully

grown of Tagetes plants. *Tagetes* were uprooted and 1gm of the soil sample was serially dilution was spread on nutrient agar plates. The colonies that grew actively were isolated and replica plated on their respective plates.

Result

In banana plants intercropped with marigold, *R. similes* took 65 days to get reduced to 25% in T4 treatments. In T1 there was an increase of 30% as found in the control. T2 showed a 13% decline and 16% decline in (T3 Fig 1).

Fig.1. Change in the population density of *R. similes* of banana plants intercropped with marigold

T1 = 2 rows of marigold intercropped with banana; T2 = 3 rows of marigold intercropped with banana; T3 = 4 rows of marigold intercropped with banana; T4 = 5 rows of marigold intercropped with banana *P. coffeae* in banana plants gave an increase of 25% in control, 10% decrease in T1,1.5% decrease in T2, 58%decline in T3 and 70% decline in T4 (Fig 2).

T1= 2 rows of marigold intercropped with banana; T2 = 3 rows of marigold intercropped with banana; T3 = 4 rows of marigold intercropped with banana; T4 = 5 rows of marigold intercropped with banana; C = Control; CFU = Colony Forming Unit.

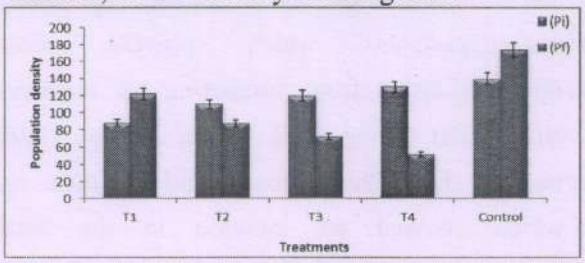
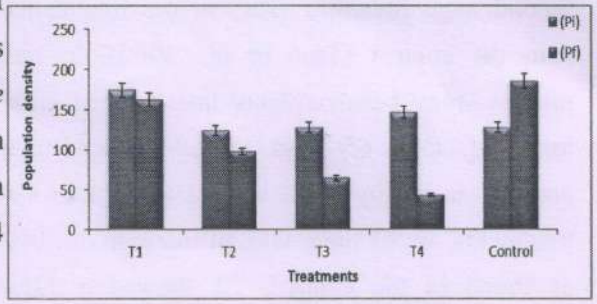


Fig.3. Change in the population density of *Helicotylenchus* sp. of banana plant intercropped with marigold

Tagetes intercropped banana soil *Streptomyces* and fungus *Fusarium* were not affected by the exudates of *Tagetes*. *Trichoderma* exhibited increases in the T1 and T2 treatment but more increase in T5. All the three bacteria under study thrived better in the *Tagetes* treated banana soil. T3 treatment had more response showing comparatively slower growth than in T2 and T4 treatments (Table 1).

PGPR strains were isolated from soil samples collected from different selected locations of banana marigold system in Tamilnadu, India. Appropriately diluted rhizosphere soil samples were spread on to the nutrient agar medium and



screened. 10 isolates were randomly picked up and chosen for biochemical analysis (Table 2). The 2 soil isolates were identified as *Bacillus* sps because they are aerobic, motile, rod shaped bacterium, endospore forming, Gram positive, positive for catalase, nitrate reduction, Voges-Proskaeur, formed acid from glucose, hydrolyzed starch and gelatin and negative for H_2S formation, citrate utilization, methyl red and urea hydrolysis. The biochemical characteristics of the isolates are listed in Table

2. Based on the morphological, physiological characteristics and biochemical tests, the remaining 8 isolates were identified as *Pseudomonas* sps. Raffinose and trehalose sugars which are toxic to microbes were absent in present analysis.

Discussion

The allelopathic compounds from *T. erecta* suppress more efficiently if planted close to a nematode infected plant or nematode colonized plant host (Ellis, 1968b; Koon-Hui Wang, 2010). It is an ideal practice for many small farmers, who can also add a subsidiary income through intercropping of *Tagetes erecta.* Root exudates of selected cultivars of *Tagetes* sp. is (*T. patula, T. erecta T. minuta,)* significantly reduced the population density of second-stage juveniles (J2s) in the treatments than the control (Tsao *et al.,* 1960). In the present study banana plants intercropped with marigold, took 65 days to get reduced the population density of R. similes to 25% in T4 treatments. In T1 there was an increase of 30% as found in the control. T2 showed a 13% decline and 16% decline in T3. *P. coffeae* in banana plants gave an increase of 25% in control, 10% decrease in T1, 1.5% decrease in T2, 58%decline in T3 and 70% decline in T4. *Helicotylenchus* sp. was also affected by marigold intercropping. There was a 6% decrease in the final population of T1, 20% of decline in T2, 50% decrease in T3 and 70% decline in T4 (Fig 1, 2, 3).

n another study of Govindaiah *et al.,* 1991; Alam *et al.,* 1979 proved that the reduction in nematode root galling by the toxic nature of *Tagetes* root exudates. The present finding supported by Alam *et al.,* 1979; El-Hamawi *et al.,* 2004 the population density of banana parasitic nematode pests such as *Radopholus similis, Hoplolaimus indicus, R. reniformis* and *Helicotylenchus multicintus,* were suppressed when *T. erecta* was intercropped with banana. In contrast, Powers *et al.* 1993 confirmed when cucurbits intercropped with *Medicago sativa* (alfalfa) or *T. patula* had no influence on populations of various nematode genera as compared to cucurbit monocrops and that *L. esculentum* fruit production was constantly higher when *Tagetes* sp were intercropped and gave yields comparable to those obtained with *L. esculentum* grown in fumigated soils. The same results were obtained when *T. erecta* was grown as a cover crop and the residues of this plant incorporated into the soil before growing taro, *Colocastia esculenta* (Sipes and Arakaki, 1997) or intercropped with soybean, *Glychines max.* Govindaiah *et al.,* (1991) *T. erecta* release allelochemicals into the soil which facilitate in extending mycorrhizal network in which the nematodes get intertwined. Diversity of microbial population in *Tagetes* rhizoshere soils showed a steady status of fungal *Fusarium* and *Actinomyces streptomyces* sp. Toxic metabolites were formed by many microorganisms, which prevent other microorganisms from competing for nutrients and habitat (Dong *et al.,* 2006; Akhtar, 1998) bacteria like *Pseudomonas* and *Bacillus* sp. which showed an increase in the final

Table 1. Diversity of microbial population in banana rhizosphere soil

Organism	Initial Count 1 x 10^6 (CFU)						Final Count 1 x 10^6 (CFU)					
	T1	T2	T3	T4	T5	C	T1	T2	T3	T4	T5	C
Bacteria												
Bacillus sp.	0.3	0.4	0.2	0.4	-	0.2	2.8	2.3	2.5	2.8	-	1.5
Pseudomonas sp.	0.3	0.4	0.2	0.1	-	0.2	2.5	2.3	2.4	2.5	-	1.3
Serratia sp.	0.2	0.1	0.2	0.2	--	0.1	0.8	0.9	1.2	1.1	--	0.4
Fungi												
Fusarium sp.	0.1	0.1	0.1	0.1	-	0.1	0.1	0.1	0.1	0.1	-	0.1
Trichoderma sp.	0.5	0.5	0.6	0.5	-	0.5	0.8	0.8	1	0.9	-	1.2
Actinomyces												
Streptomyces sp.	0.01	0.01	0.01	0.01	-	0.01	0.1	0.1	0.1	0.1	-	0.1

Table 2. Biochemical characteristics of the isolates

Parameter	Strain									
	1	2	3	4	5	6	7	8	9	10
Gram's staining	-	-	-	-	-	-	-	-	+	+
Motility	M	M	M	M	M	M	M	M	M	M
Indole test	-	-	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-	-	-
Voges Proskauer test	-	-	-	-		-	-	-	+	+
Cytochrome oxidase test	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	+	+
Citrate utilization	+	+	+	+	+	+	+	+	-	-
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+
Nitrite reduction	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
Acid from Glucose	+	+	+	+	+	+	+	+	+	+
Acid from Fructose	+	+	+	+	+	+	+	+	+	+
Acid from Inositol	-	-	-	-	-	-	-	-	-	-
Acid from Lactose	+	+	+	+	+	+	+	+	+	+
Acid from Maltose	+	+	+	+	+	+	+	+	+	+
Acid from Mannitol	+	+	+	+	+	+	+	+	+	+
Acid from Raffinose	-	-	-	-	-	-	-	-	-	-
Acid from Sorbitol	+	+	+	+	+	+	+	+	+	+
Acid from Sucrose	+	+	+	+	+	+	+	+	+	+
Acid from Xylose	+	+	+	+	+	+	+	+	+	+
Acid from Trehalose	-	-	-	-	-	-	-	-	-	-

population of microbes in marigold intercropped with Banana plants rhizosphere. *Tagetes* intercropped banana soil *Streptomyces* and fungus *Fusarium* were not affected by the exudates of *Tagetes*. *Trichoderma* exhibited increases in the T1 and T2 treatment but more increase in T5. All the three bacteria under study thrived better in the *Tagetes* treated banana soil. T3 treatment had more response showing comparatively slower growth than in T2 and T4 treatments (Table 2). Sturz and Kimpinski 2004 reported that several bacterial species isolated from *T. erecta* and *T. patula* caused the gradual decrease in *P. penetrans* nematode population densities in potato plant soil. Topp *et al* 1998 found that nematicidal compounds exuded from the *Tagetes* rhizosphere are benign to microorganisms. Sturz and Kimpinski 2004 supported this theory that some rhizobacteria living in the marigold rhizosphere are suppressive to root lesion and other nematodes. Hence intercropping could be more capable for disease pest control when antagonistic plant is used.

Conclusion

T. erecta L. has fungicidal, nematicidal properties because of the occurrence of thiophenes in its tissues. The microclimate in Banana associated with *T. erecta* could directly decimate the population of nematodes and indirectly control harmful microbes by promoting the growth of PGPR *Pseudomonads* strains and *Bacilli* strains.

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