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ALLELOPATHIC EFFECT OF *TAGETES ERECTA* ON SOIL MICROBES AND NEMATODES OF PAPAYA

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ABSTRACT

Allelopathy refers to the process involving 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 Papaya plants spaced with 5 feet. For the first, second, third and fourth experimental plots, the Papaya plants intercropped with 2 rows, 3 rows, 4 rows and 5 rows of marigold seedlings respectively, at one foot/30cm distance within the row and between the rows. The fifth plot was considered as the control without marigold. The study ended 65 days after intercropping of *Tagetes erecta*. Soil samples were collected from all plots for microbial and nematode analyses before and after intercropping of *Tagetes erecta* in the experimental plots. T4 sample with 5 rows of *T.erecta* intercropped plants alone exhibited single gall. *M. incognita* population of J2 individuals got reduced to 87.5% in T1, 58% in T2, 47% in T3 and 33% in T4. Gall index did not decimate immediately. The population density of papaya parasitic nematode gradually decimated and was gradually increasing of beneficial bacteria while increasing the number of intercropping of *T.erecta*. Though T4 which had 5pairs of papaya plants intercropped with 5 rows of *T.erecta* showed higher reduction in nematode population it is not economical. Hence T2 which had 5pairs of papaya plants intercropped with 3 rows of *T.erecta* is advised to practice for the best and economical one.

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INTRODUCTION

Allelopathy plays a significant role in monocultures, crop mixtures or intercropping and crop rotations. Continuous monocultures increase the buildup of phytotoxins or harmful microbes or both in the growing medium and produce soil sickness and autotoxicity which in turn decrease the crop growth and yields. It also shows an important role in intercropping systems owing to inhibitory or stimulatory effects of one plant on another and increased crop quality and/ or yield. Intercropping is defined as the agricultural practice of cultivating two or more crops in the same space at the same time.[1] The purpose of this practice is to increase the yield per unit area, prevent the total crop failure, increase the yield, weed control and maintenance of soil and productivity. [2] Multiple applications of pesticides particularly nematicides are expensive and not feasible for perennial crops such as papaya when these are cultivated by small farmers as stable or little earnings crops. Alternatively, there have been a number of efforts towards the hunt for cheaper and safer substitute to chemical nematicides using antagonistic plants with nematicidal properties. 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 conscious 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 [3-5].

Tagetes is a nematode allelopathic crop and act as a non-host or a poor host and a trap crop[6] produce allelopathic compounds α ternithyl, that are fatal to Plant Parasitic Nematodes (PPNs) development and creates favorable micro environment for nematode antagonistic flora or fauna[7].Marigolds secrete thiophene, that proves antiviral, antibacterial, antifungal, nematicidal and insecticidal properties[8].Intercropping white Cabbage with *Tagetes Patula* and *Calendula officinalis L.* effectively control Pieris *brassicae L, Mamestra brassicae L. Pieris rapae L,* and pupae and larva of *Plutell xylostella* [9]. Topp *et al.*, [10] reported that the marigold rhizosphere released nematicidal compounds which are benign to microorganisms. Plant root directly influence the rhizospheric soil surrounding plant roots chemically, physically, and biologically, and creating a favourable habitat for microorganisms which in turn greatly influence the development of host plants. Certain exudates specifically engage beneficial microbes.

Carica papaya L, is one of the major fruit bearing trees growing in tropic and subtropical region. According to survey of Government of India 2014[11], 5382 million tonnes (Mt) of papaya fruits were produced from about 132 Ha. *Meloidogyne* spp., *Rotylenchulus reniformis*, *Quinisulcius acutus*, *Criconemella* spp and *Helicotylenchus dihysteria*, have been reported as a plant parasitic nematodes of papaya plants. Yet only *Rotylenchulus reniformis* and *Meloidogyne* spp. cause economically significant reduction in papaya production[12]. Up to 20% yield losses have been reported due to the infection of these two nematodes in Hawaii[13]. Rajendra Singh and wmesh kumar[14] reported that 68.42% of *Meloidogyne incognita* 43.98 % of *Rotylenchulus reniformis* were the most abundant Plant Parasitic Nematodes(PPNs) detected among 412 soil sample and roots collected from vegetable cultivating region in India. Plant competition should be reduced by choosing compatible crops as an intercropping plant which is able to exploit soil nutrients. [15-16] Growth 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 R.R. Hooks *et al* [7] 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, we focused on allelopathic effect of marigold on soil microbes and nematodes of papaya.

Objectives:

- To analyse the effect of *T.erecta* intercropped with papaya on beneficial soil bacteria.
- To find out the appropriate treatment. This could be beneficial for the effective control of the *Meloidogyne incognita* and *R.reniformis*.
- To find out the appropriate treatment this could be beneficial for the enhancement of beneficial soil bacteria

MATERIAL AND METHODS

Collection of Tagetes seeds and rising of seedlings

Seeds of *T.erecta*, were collected from local market. Care should be 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 Papaya * *Tagetes erecta* system

Five experimental plots were used and each individual plot was 10×10m and consisted of 5 pairs of Papaya plants spaced with 5 feet. For the first, second, third and fourth experimental plots the Papaya 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 *Tagetes 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 ended 65 days after intercropping of *Tagetes erecta.* Soil samples were collected from all plots for microbial and nematode analyses before and after intercropping of *Tagetes erecta* in the experimental plots.

Effect of treatments on host plants - Cobb's wet sieving method[17] Improved Bearmann Funnel Method[18]

Initial and final population densities of *M.incognita* and *Rotylenchus reniformis* from Papaya* *Tagetes 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 papaya 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[19]

The soil was collected at random from 5 sites. The soil sample was taken from rhizosphere (0-15 cm depth). Soil samples 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 [20, 21]

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 10 sec to resuspend the soil. The an aliquot of 1ml was transferred using a sterile pipette to 9 ml sterile distilled water in a test tube. This suspension was agitated manually for 10 sec and subsequently serial diluted (10-1 to 10-7). 0.2 ml 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 characteristic.

Isolation, identification and population procedure of fungi [20, 21]

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 was 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 4oC for further studies. Different colonies were selected and transferred to be identified.

Isolation, identification, and population procedure of *Actinomycetes* [22]

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 papaya 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 1 gm of the soil sample was serially diluted and 10-4 dilution was spread on nutrient agar plates or Voges-Proskaeur spread plates. The colonies that grew actively were isolated and replica plated on their respective plates. 10^8colonies were screened for plant growth promoting properties. This was followed biochemical characterization of isolates based on the morphological, physiological and biochemical features.

Gram staining for the Bacteria

A drop of sterilized distilled water was taken on the middle of the clear slide. Then a loopful bacterial suspension (young culture) was transferred to the sterilized drop of water and a very thin film was prepared on the slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three times. The slide was flooded with crystal violet solution and allowed to stand for 30 sec and then washed thoroughly with gentle stream of tap water. The slide was then immersed in iodine solution for 1 minute and washed thoroughly with 95% alcohol for 10 sec. Alcohol was drained off and washed thoroughly with gentle stream of tap water. The slide was then covered with safranin for I minute. After washing with tap water and blotted dry it and examined under microscope.

Spore staining

One drop of sterile saline water was taken on a clean glass slide for spore staining. A loopful bacterial old slant culture was taken in the drop and smeared on the slide. The film was dried over flame gentle heating. The slide was then placed over a beaker and 5% malachite green was added drop wise on the slide. Boiling of the malachite green was avoided by adding more malachite green. The slide was taken out of the stream and washed gently with tap water. The preparation was needed with safranin solution for 1 min. and washed with gentle stream of tap water, and placed under immersion lens with immersion oil.

Biochemical Test

Tryptone broth was used as a basal medium for fermentation test. A 0.01 % or phenol red was used as an indicator. Fermentation tubes with 1.0 ml of basal medium provided with indicator were made and pH of the medium was adjusted at 7.5 with NaOH, the medium was sterilized at 121°C for 15 minutes. 1.0 ml of filter sterilized glucose, arabinose, xylose and manitol was taken in each tube. The tubes were then inoculated in duplicate with fresh culture of the bacterial isolates and allowed to incubate at 37° C for 72 hrs. The change of color of the indicator to yellow indicated the production of acid.

Catalase Test

Catalase Test carried out of one drop of 30% hydrogen peroxide was placed on a slide. One loopful of the fresh bacterial culture was taken by a sterile needle and placed on the drop of hydrogen peroxide. Bubble production indicated positive result.

Hydrolysis of Starch

Hydrolysis of Starch was carried out of 10 gm soluble starch in 100 ml distilled water was heated in water bath until dissolved. 20 ml of this solution was mixed with 100 ml of melted nutrient agar and poured in the petridish after sterilization. A loopful of fresh bacterial culture was picked up by the sterile needle and stabbed on to the agar plate; After 24 hrs of incubation at 37° C, the plate was flooded with dilute iodine solution. Hydrolysis of starch was indicated by a clear zone around the growth and unchanged starch gave a blue color.

Methyl Red Test

Methyl Red Test detects acid production to a sufficient degree (below 4.5) from glucose. One ml of fresh bacterial culture grown in glucose phosphate medium was taken in a test tube. Five drops of methyl red reagent was added and read immediately. Positive tests are light red and negative are yellow.

Indole Production Test

For the Indole, one loopful fresh bacterial culture (24 hrs old) was inoculated in peptone broth and incubated at 37° C for 1-3 days, after incubation, Kovac's solution was added and shaken vigorously for one minute. A red color in the reagent layer indicated positive reaction.

Nitrate Reduction Test

Nitrate reduction test was carried out in nitrate broth. The freshly prepared cultures were inoculated in sterile nitrate broth containing tubes and incubated at 37° C for 24 hrs. At the end of incubation 0.1 ml of solutions A was added followed by solution B in equal volume. The appearance of pink deep color showed that bacterial isolates reduced nitrate to nitrite.

Voges Proskauer Test

Voges Proskauer Test carried out of one ml of fresh bacterial culture was grown in phosphate peptone medium. After addition of 0.2 ml of 40% KOH, 0.6 ml of 5% alpha napthol in absolute ethanol was added. After 10-15 minutes with vigorous shaking bright orange red color developed if acetyl methyl carbinol was present.

Citrate Utilization Test

For the Citrate Utilization Test, slope culture with a 1 inch butt of Simmon's citrate agar was inoculated by streaking over surface with a wire needle and incubated at 37° C for up to 3 days. The color of the medium changed from green to bright blue due to the utilization of citrate and when citrate is not utilized, the color of the medium remains unchanged.

RESULT

Table.1 Population density of *R. reniformis* of Papaya plants intercropped with *T.erecta*.

Treatments	Initial Population (Pi)	Final population (Pf)
T1	136±3.0	143±3.7
T2	128±4.3	94±3.5
T3	75±3.6	63±3.2
T4	89±2.7	43±2.8
Control	112±5.4	156±7.9

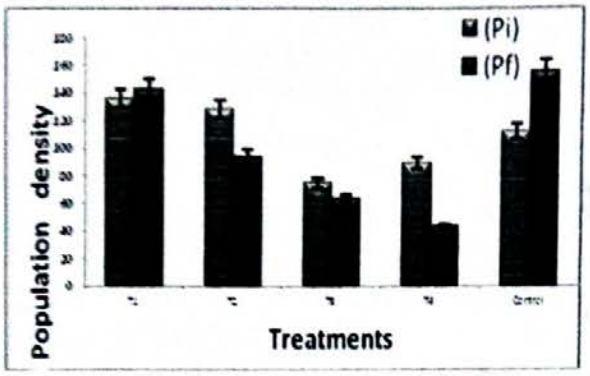

Fig.1. Change in the population density of *R. reniformis* of papaya plants intercropped with *T.erecta*.

In papaya *R.reniformis* showed an increase in control and in T1, 39% and 5%. 26% decrease was observed in the final population in T2, 16% in T3 and 52% decline in T4.Though T4 which had 5pairs of papaya plants intercropped with 5 rows of *T.erecta* showed higher reduction it is not economical. Hence T2 is advised to practice for the best and economic one. (Table 1 and figure 1).

Table.2 Population density of *M. incognita* of Papaya plants intercropped with *T.erecta*.

Treatments	Initial Population (Pi)	Final Population (Pf)	Gall index (GI)
T1	112±3.8	98±3.7	3
T2	142±2.5	82±3.5	3
T3	138±3.0	65±3.2	2
T4	128±1.4	42±2.8	1
Control	198±6.3	224±7.9	5

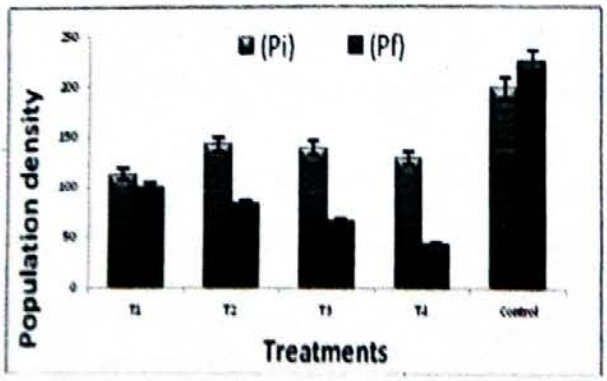

Fig.2. Change in the population density of *M. incognita* of papaya plants intercropped with *T.erecta*.

T1 = 2 rows of *T.erecta* intercropped with papaya; T2 = 3 rows of *T.erecta* intercropped with papaya;
T3 = 4 rows of *T.erecta* intercropped with papaya; T4 = 5 rows of *T.erecta* intercropped with papaya

In papaya T4 sample with 5 rows of *T.erecta* intercropped plants alone exhibited single gall. *M. incognita* population of J2 individuals got reduced to 87.5% in T1, 58% in T2, 47% in T3 and 33% in T4. Gall index did not decimate immediately. (Table 2 and figure 2).

Table.3. Diversity of microbial population in papaya rhizosphere soil.

Organism	Initial Count1 x10^6(CFU)					Final Count1 x10^6(CFU)				
	T1	T2	T3	T4	C	T1	T2	T3	T4	C
Bacteria										
Bacillus sp.	0.4	0.6	0.4	0.4	0.4	1.4	1.3	1.6	1.2	1.4
Pseudomonas sp.	0.3	0.2	0.4	0.3	0.4	1.4	1.2	1.4	1.1	1.1
Serratia sp.	0.1	0.2	0.1	0.3	0.1	0.5	0.6	0.4	0.3	0.2
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.	0.4	0.5	0.5	0.4	0.6	0.9	0.8	1.1	0.8	1.1
Actinomyces										
Streptomyces sp.	0.01	0.01	0.01	0.01	0.01	0.1	0.1	0.1	0.1	0.1

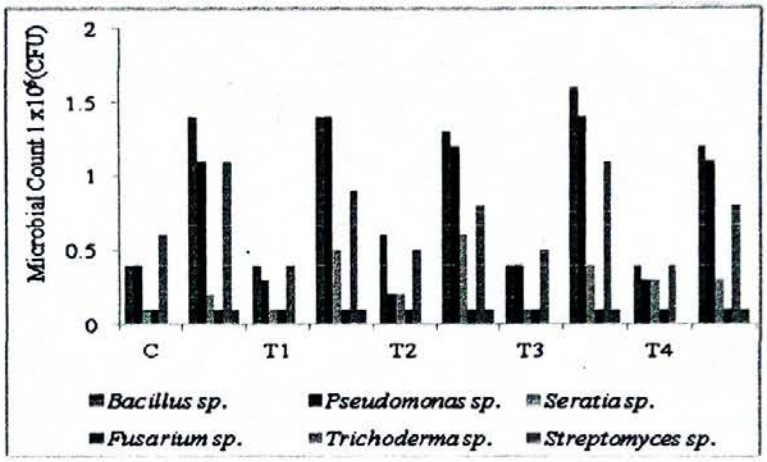

Fig. 3. Diversity of microbial population in papaya rhizosphere soil.

T1= 2 rows of *T.erecta* intercropped with papaya; T2 = 3 rows of *T.erecta* intercropped with papaya; T3 = 4 rows of *T.erecta* intercropped with papaya; T4 = 5 rows of *T.erecta* intercropped with papaya; C = Control.

Bacillus bacteria were more in T2 and T4 samples. *Serratia* showed a steady increase compared to the initial population in control there was 3.50 times increase in the final population, whereas T3 showed a 4 times increase. In the same T3 sample *Trichoderma* sp. exhibited 2.75 times increase in T1 and T4 where as in T3 it was 3.5 times. *Pseudomonas* species exhibited a six fold increase in T2 while in T1 and T4 it was twofold. Final population of *Fusarium* was either steady or there was little growth. *Streptomyces* growth was not attended by any treatment (Table3; Fig.3).

Table 4. Biochemical characteristics of the isolates.

Parameter	Strain									
	1	2	3	4	5	6	7	8	9	10
Gram's staining	-	-	-	-	-	-	-	-	+	+
Shape										
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	-	-	-	-	-	-	-	-	-	-
H2S 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	-	-	-	-	-	-	-	-	-	-

PGPR strains were isolated from soil samples collected from different selected locations of papaya 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.4). 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 H2S formation, citrate utilization, methyl red and urea hydrolysis. The biochemical characteristics of the isolates are listed in Table 4. 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

Allelopathy refers to the process involving secondary metabolites of plants, microorganisms, viruses and fungi enhance the development and growth of biological and agricultural systems. Donor plant gives helpful or harmful effect to the recipient by chemical pathway. [23] The allelopathic compounds from *T. erecta* suppress more efficiently if planted close to a nematode infected plant or nematode colonized plant host. [24] 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.

Akhtar and Mehmood, [25]used different parts of one hundred and twenty plants against root knot nematodes in lab, pot and field trials and root exudates of certain plants were reported effective. In the present study papaya T4 sample with 5 rows of *T.erecta* intercropped plants alone exhibited single gall, M. incognita population of J2 individuals got reduced to 87.5% in T1, 58% in T2, 47% in T3 and 33% in T4. Gall index did not decimate immediately. (Table 2 and figure 2). Tsay *et al*, [26] reported that intercropping water spinach (*Ipomea reptans) with Asteraceae* plants species, *T. erecta* did not produce galls and significantly reduced the root galling on spinach in green house condition. The same result was obtained by El-Hamawi *et al.*[27] who intercropped soybean with *T. erecta or T. patula.*

In papaya *R.reniformis* showed an increase in control and in T1, 39% and 5%. 26% decrease was observed in the final population in T2, 16% in T3 and 52% decline in T4.(Table 1and Figure 1) Though T4 which had 5pairs of papaya plants intercropped with 5 rows of *T.erecta* showed higher reduction it is not economical. Hence T2 is advised to practice for the best and economic one. (Table 1, 2 and fig 1,2). In another study of Govindaiah *et al.*[28] proved that the reduction in nematode root galling by the toxic nature of *Tagetes* root exudates. The present finding supported by Alam et al., [29] 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.*[30] 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. Ploeg [31] reported that *L. esculentum* fruit production was constantly higher when *Tagetes* sp was 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 plants incorporated into the soil before growing taro, *Colocastia esculenta* [32] or intercropped with soybean, *Glychines max.*[27]

T.erecta release allelochemicals into the soil which helps in extending mycorrhizal network in which the nematodes get entangled. Diversity of microbial population in *Tagetes* rhizoshere soils showed a steady status of fungal *Fusarium* and *Actinomyces Streptomyces* sp. Toxic metabolites were produced by many microorganisms, which prevent other microorganisms from competing for nutrients. This had been highlighted by Dong *et al* [33] Bacteria like *Pseudomonas* and *Bacillus* sp. which showed an increase in the final population of microbes in marigold intercropped with papaya plants rhizosphere. Bacteria like *Pseudomonas* and *Bacillus* sp. which showed an increase in the final population of microbes in marigold intercropped with papaya plants rhizosphere. *Bacillus* bacteria were more in T2 and T4 samples. *Serratia* showed a steady increase compared to the initial population in control there was 3.5 times increase in the final population, whereas T3 showed a 4 times increase. In the same T3 sample *Trichoderma sp.* exhibited 2.75 times increase in T1 and T4 where as in T3 it was 3.5 times. *Pseudomonas* species exhibited a six fold increase in T2 while in T1 and T4 it was twofold. Final population of *Fusarium* was either steady or there was little growth. *Streptomyces* growth was not attended by any treatment (Table 3; Fig.3).

Sturz and Kimpinski [34] 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.*[35] found that nematicidal compounds exuded from the *Tagetes* rhizosphere are benign to microorganisms. Sturz and Kimpinski [34] 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

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

Future Research

Tagetes erecta could be used to control the plant parasitic nematodes by promoting the growth of PGPR *Pseudomonad* strains and *Bacilli* strains. To establish the species level identity it would be subjected to 16S r RNA typing. Formulate a commercial nematicide using the α- ternithyle components from the Tagetes *erecta* root exudates and quantify the chemical components.

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Authors' statements

Competing Interest

The authors declare no conflict of interest

Abbreviations

PPNs = Plant Parasitic Nematodes
PGPR=Plant Growth Promoting Rhizosphere
RBM=Rose Bengal Medium
NA =Nutrient Agar
KA = Kusters Agar

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