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Tagetes Erecta With Native Isolates Of Paecilomyceslilacinus and Trichodermahamatum In Controlling Root Knot Nematode Meloidogynejavanica On Tomato

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ABSTRACT

This experiment was conducted to test the efficacy of Tageteserectaextract with bio-control agents, Trichidermahamatum and Paecilomyceslilacinus as individual treatments and in combinations on the growth of tomato plants and the development of root-knot nematode M. javanica. Inoculation of tomato plants with M. javanica(T_1)resulted in significant suppression of the length and fresh weight of shoots and roots, when compared to uninoculated plants (T_0). The means of mortality percent in J2 by fungal filtrates of P.lilacinus ,T. hamatum and T.erecta extract were 22.8 , 13.08 and 19.8% respectively.

Application of the 2 biocontrol fungi and T. erecta (individually or in combination) significantly increased all tested growth parameters of tomato. Among individual treatments, the highest value of growth parameters, length of shoot, length of root, fresh weight of shoot and fresh weight of root were recorded in P.lilacinus treatment (T_3) which were 18.07cm , 14.23cm, 21.56gm and 6.68gm respectively. In combination treatments, the maximum length of shoot (22.66 cm) and root (16.66cm) were recorded in T_8(T. hamatum + P. lilacinus + plant extract). From this study, it may be suggest that the improvement of plant growth is obtained in combination treatments than in individual treatments.

Keywords:Marigold ,Paecilomyceslilacinus, Trichodermahamatum, Tomato, Biocontrol

1.INTRODUCTION

Nematode problems are considered one of the greatest challenges facing the expansion of plantations. Root-knot nematodes, *Meloidogyne* spp., are considered one of the most economically important and complex groups of plant parasitic nematodes causing damage and high yield losses on most cultivated plants throughout the world especially in the developing countries [1].

Tomato (*Lycopersiconesculentum*, Mill.), an important vegetable crop is heavily attacked by root knot nematode, *Meloidogyne* spp. For the management of root knot nematodes although chemical nematicides were used till 1982, but due to their high cost, toxic effect on beneficial soil borne microorganism and carcinogenic effect on human an alternative approaches are practiced mainly through eco-friendly means like biological control agents, organic amendments, etc. [2]. The present investigations were thus undertaken to attempt an eco-friendly management of root knot nematode infecting tomato through integrating potential and compatible management components viz. fungal bioagents[3].

Several fungi have been developed into commercial formulations against nematodes. Among these fungi that have shown antagonism against root knot nematodes, *Pochoniachlamydosporia*, *Paecilomyceslilacinu*s , and *Trichodermaharzianum*have been found to be highly suppressive to plant nematodes, especially under greenhouse conditions[4] ,[5],[6].

Certain plants are able to kill or repel pests, disrupt their lifecycle, or discourage them from feeding. Some of these, marigolds, sesame, castorbean, and various brassicas have been used as nematode-suppressive cover crops. So the extracts of these plants or essential oils can be applied as nematicides. For hundreds of years, Indian farmers have used the neem tree (*Azadirachtaindica*) for its pesticidal, antifungal, and antifeedant properties. In research trials, potting soil amended with plant parts from the neem tree and Chinaberry tree (Meliaazadirach) inhibited root-knot nematode development on tomatoes [7].

El-Gengaihi et al.reported that, the roots of *Tageteserecta, T. patula and T. minuta* , extracted by petroleum ether and chloroform were highly potent against the reniform nematode, *Rotylenchulusreniformis* [8].

Khan and Anwer found that the incorporation of chopped green leaves of *Crotolaria*spp., *Sesbenia*spp., and *Tagetes*spp. facilitated the colonization of *Trichoderma*spp., *Paecilomyceslilacinus*, and others[9] . The significantly

greater increase in the population of BCA applied with neem leaves was apparently due to an increase in the soil's organic matter, which is required by the BCAs for colonization and multiplication[10].
So, in this study, an attempt was made to use the *Tageteserecta*extract ,*Paecilomyceslilacinus*(Thom) Samson, and *Trichodermahamatum*(Bonord.) Bainier , singly and in combination to control root-knot disease of tomato caused by *M. javanica*(Treub) Chitwood.

2.MATERIAL AND METHODS

2.1 HOST PLANT

Seeds of tomato was obtained from local market and surfaced sterilized with 1% Nacl for two minutes and sown in the plastic tray in greenhouse of College of Agriculture - University of Wasit . Then three weeks old seedlings were used in greenhouse experiment. Seedlings were transplanted in pots (15 cm diameter) containing sterilized clay loam soil.

2.2 ROOTKNOT NEMATODE INOCULUM

Eggs of root-knot nematode, *M. javanica*(Treub) Chitwoodwas collected from the infected roots of tomato (collected from a heavily infected plastic house in Kut, Iraq). The eggs had been extracted by sieving method which described by Hussey and Barker [11].It summarized by cutting the infected roots into 2-3 cm length pieces and 5gm of roots were blended in electric blender with 1% NaOCl for 1 min. Then the mixture passed through series of sieves and the eggs collected from the last sieve (25 µm) in sterile beaker .Water eggs suspension adjusted to 1000eggs/ml by using counting slide (Peters' chamber) and served as initial inoculum level for greenhouse experiment. 6 ml of this suspension were applied around each plant immediately after transplanting.

2.3 FUNGAL INOCULUM

T. hamatum and *P. lilacinus* were isolated from rhizosphere soil of greenhouse inSheikh Saad, Wasitprovince by dilution technique. Pure culture for each one prepared on PDA medium, identification conducted(in the Agricultural research office, Ministry of Science and Technology, Iraq) according to the morphological characters [12], [13], [14],[15].One week old cultures of bioagents ,maintained on PDA slants at 25 ± 2 °C were used for this study.

2.4 CULTURE FILTRATES OF FUNGI:

Biocontrol fungi were grown in conical flasks (500 ml) containing 200 ml of potato dextrose broth (PDB), autoclaved at 121°C for 20 minutes. Each flask was inoculated with 3 mm diameter discs from 7 days old pure cultures of bioagents. Cultures were filtered after 15 days of incubation at 25 ± 2 ^{o}Cthrough Whatman No.1 filter paper and served as standard (100%).Another concentrations 50% and 25% were also be made.

2.5 TGETES ERECTA PLANT EXTRACT

Marigold (*Tageteserecta*) Plants had been obtained from Agriculture college – University of Wasit . Aqueous extract of leaves of *Tageteserecta* were obtained by comminuting 25g of dried materials in a blender containing 250ml of sterilized distilled water and then filtered through muslin cloth and served as standard (S). 50% and 25% concentrations were also be made[16].

2.6 EFFECT OF T. ERECTA EXTRACTS ON FUNGAL PARASITISM

This test had been conducted by mixing 5ml of each extract concentrations with 45 ml of sterilized water agar in 250 ml flasks. Control flasks received 5 ml of distilled water. The medium was poured in a 9 cm petri plates and then inoculated after solidification with 3mm diameter disc of 7 days old culture of targeted fungi. One ml of sterile distilled water containing 1000 eggs was then spread on to each WA Petri plates [17]. Percentages of eggs parasitism by fungi were evaluated under the stereo-microscope at x 100 magnification [18].

2.7 NEMATICIDAL ACTIVITY OF FUNGAL FILTRATES AND T. ERECTA EXTRACT ON J2 MORTALITY

To investigate the effects of plant extract and culture filtrates of fungion second stage juveniles mortality, 5ml from each concentration (100 , 50 , 25%) of extract and filtrates were poured into 5 cm diameter Petri plate. At the same time fifty freshly hatched juveniles of *M .javanicain* 1ml distilled water were added to each Petri plate. Each treatment was replicated three times and kept in incubator at 280C. Fresh hatching juveniles placed in 5 ml distilled water served as control .Percentages of juvenile mortality were recorded after 24, 48 h. To confirm the death of juveniles, ten to fifteen juveniles were transferred to distilled water. If they did not move they were considered dead [19].

2.8 NEMATICIDAL ACTIVITY OF FUNGAL FILTRATES AND T. ERECTA EXTRACT ON EGG HATCHING

5ml from each concentration (100 , 50 , 25%) of extract and filtrate were poured into 5 cm diameter Petri plate. 1ml distilled water contain 100eggs were added to each Petri plate. Each treatment was replicated three times and kept in incubator at 280C. Three petri plates received 5 ml of distilled water served as control. Egg hatching inhibition (%) wasrecorded after 3 days of incubation.

2.9 EFFECT OF T. ERECTA EXTRACTS WITH T. HAMATUM AND P. LILACINUS ON ROOT KNOT NEMATODE M. JAVANIA AND GROWTH PARAMETERS OF TOMATO PLANTS IN GREENHOUSE CONDITIONS

This experiment was conducted to test the efficacy of *T. erectaextract* ,*T. hamatum and P. lilacinus* as individual treatments and in combinations on the growth of tomato plants and on the development of *M. javanica*. Three weeks old seedlings were transplanted in pots (1 plant/pot). Each pot received 5ml of extract (50%) and 5ml conidial suspension (5x106 spore/ml) which were injected 2 cm deep surrounding the bases of the plants . 6 ml of egg suspension (1000eggs/ml) were added to each pot after one week of transplanting. The experimental design was a complete randomized block with 3 replications. Pots without treatments served as control and the treatments were :

T0-Control (Negative¯) Without any treatment
T1-Control(Positive+)treated with nematode only
T2 -Nematode +*T. hamatum*
T3 -Nematode +*P. lilacinus*
T4 -Nematode + Plant extract
T5-Nematode + Plant extract + *T. hamatum*
T6-Nematode + Plant extract + *P. lilacinus*
T7 -Nematode +*T. hamatum*+ *P. lilacinus*
T8 -Nematode +*T. hamatum*+ *P. lilacinus* + plant extract.

All pots Watered regularly and data about number of galls and eggs per gram of root and plant parameters (length of shoot, length of root, dry and fresh weight of shoot and root) were evaluated after 60 days of transplanting. Reproductive factor was calculated by the formula: (Rf = Pf/Pi), where Pi = initial inoculum level and Pf = newly produced eggs).

3. STATISTICAL ANALYSIS

Statistical analyses had been conducted in Agriculture college– University of Wasitbyusing statistics system (Genstat). Least significant differences (L.S.D) were calculated at p=0.05 level to test for significant differences.

4.RESULT AND DISCUSSION

4.1. LABORATORY EXPERIMENTS

4.1.1. EFFECT OF T. ERECTA EXTRACTS ON FUNGAL PARASITISM

Extracts of T. erectashowed a significant (P = 0.05) inhibitory effect on fungal parasitism of M. javanicaeggs on water agar (Table 1).Parasitism of eggs decreased with an increase in extract concentration . A significantly greater proportion of eggs were infected in control plates (without extract). The lower rate of parasitism of eggs treated with T. erecta extract demonstrates the inhibitory effect of this extract on egg parasitism. This extracts may be affect the formation of infection organs or production of chitinolytic enzymes and toxins which are associated with egg infection [20] . In general, eggs parasitismby thefungus T. hamatum was greater significantly than the fungus P. lilacinus at all concentrations and the means of parasitism were 52.32 , 46.17% respectively .This suggests differential tolerance of T. hamatum to active ingredients in marigold leaves, or inherent variation in their parasitic capabilities against M. javanica eggs. Chaparro1, et. al.reported the tolerance of Trichodermaspp to the fungicide captan and they refer that the chemical tolerance to the fungicide was verified by means of changes at the DNA level (UP-PCR)[21] . Now it is well know that the genome of Trichodermaincludes ABC transporters (ATP binding cassette (ABC) transporters), which are members of a protein superfamily that effluxes drugs from cells of target organisms. Thus transporters may provide a mechanism of protection against cytotoxic drugs and xenobiotic agents [22].

Table (1) Effect of *T. erecta* extracts on fungal parasitism (%) of *M.javanica* eggs

Treatments (T)	Eggs Parasitism (%) Concentrations(C) of *T. erecta* extracts (%)				Means
	100	50	25	0(Control)	
T. hamatum	21.2	44.4	65.3	78.4%	52.32
P. lilacinus	19.9	39.1	58.6	67.1%	46.17
Means	20.55	41.75	61.95	72.75	
LSD (P≥ 0.05)	T= 1.31 , C= 1.85 , T × C= 1.61				

***Data are mean of three replications.**

Goswami and Archana reported that the T. viride showed more toxicity to Meloidogyneincognita thanP. lilacinus and the inhibition of eggs hatching was also greater in T. viride[23]. Owino obtained inhibition of fungal parasitism of M. javanica eggs with leaf extract of marigold at high concentrations, but at the lowest concentration investigated, significantly higher fungal parasitism of M. javanica eggs was observed[24]. However, Owino and Sikora obtained

significantly more egg parasitism by Fusarium spp. in marigold leaf-extract-amended soil than in non-amended soil. Tagetesminuta was observed to have significant inhibition on fungal parasitism of eggs of M. incognita and M. javanica in vitro[25].

4.1.2. NEMATICIDAL ACTIVITY OF FUNGAL FILTRATES AND T. ERECTA EXTRACT ON J2 MORTALITY

Mortality of juveniles of M. javanicawas affected significantly by the concentrations and exposure time in all treatments (Table 2).

Table (2) Effect of *T.erecta* extract and fungal filtrate on J2 mortality

Treatment	Concen.(%)	Mortality(%) after (hours)		Means	
		24	48		
Te-extract	100	26.2	33.2	29.7	19.8
	50	17.4	22.6	20	
	25	8.4	11.0	9.7	
Th-filtrate	100	13.4	22.8	18.1	13.08
	50	8.4	17.6	13	
	25	5.3	11	8.15	
Pl-filtrate	100	26.4	34.8	30.6	22.8
	50	19.2	25.3	22.5	
	25	12	18.6	15.3	
Control		0.66	1.6	1.13	
Means		14.23	19.85		
LSD (P≥ 0.05)	T=1.26 , C =0.45 , M=0.54 , T × C=1.26 , T × M=1.27 , M × C= 0.77, T × C × M= 1.59				

***Data are mean of three replications.**
Th= *T. hamatum*, Pl = *P.lilacinus*,Te = *T.erecta*, T= treatment, C= concentration, M= time

Percent juvenile mortality in the culture filtrates of the fungi and plant extract were found to be directly proportional to the concentration of the culture filtrates, plant extract and the duration of exposure.

Maximum mortality (34.8%) was observed in 100% concentration of the fungal filtrate of P. lilacinus after 48 h of exposure whereas it was minimum(5.3%) in 25% concentration of the fungal filtrate of T. hamatum after 24 h of exposure. The means percent mortality in fungal filtrates of P.lilacinus ,T.hamatum and T.erecta extract were 22.8 , 13.08 and 19.8% respectively.

These results are in general agree with the result of Omer et al. [26] who reported thatthe effect of root extract from T. lucida on development to hatching of Meloidogyneincognita eggs was proportional to the concentrations of plant extract and the duration of exposure.

Siddiqui et al. reported that the root extracts of Tageteserecta and Tagetespatula were more effective in the inhibition of nematode compared to the corresponding shoot extracts. Shoot extract of T. erecta caused greatest mortality of M. javanicajuvenilesand the longer exposure time could improve the activity of the extracts.Shoot extract of T. erecta caused greatest mortality of M. javanica juveniles[27].Chattopadhyay and Mukhopadhyaya reported reduced hatching after 24, 48 and 71 hours of exposure[28].

4.1.3. NEMATICIDAL ACTIVITY OF T. ERECTA EXTRACT AND FUNGAL FILTRATES ON EGG HATCHING OF M. JAVANICA

The results of the effect of T.erecta extract and fungal filtrate on egg hatching werepresented in (Table 3). All treatments at all concentrations significantly inhibited the nematode eggs hatching as compared to the control. Percent of inhibition was found to be directly proportional to the concentrations. Filtrate of P. lilacinus was the more effective on eggs hatching than the filtrate of T. hamatum and plant extract. Maximum percent of unhatchedeggs (70.3%) was observed in 100% concentration of the fungal filtrate of P. lilacinus while it was 67.9% , 54.4% in the treatments of T. erecta extract and T. hamatum filtrate respectively at the same concentration.

Table (3) Effect of T.*erecta* extract and fungal filtrate on egg hatching (%)

Treatments	% of unhatched eggs				
	Concentration(%)				
	100	50	25	Control	Means
Te- extract	67.9	38.2	11.30	3.3	30.17
Th- filtrate	54.4	32.6	8.33	0.2	23.88
Pl- filtrate	70.3	41.22	16.66	2.1	32.57
Means	64.2	37.34	12.09	1.86	
LSD (P≥ 0.05)	C= 1.06 , T= 1.23 , T x C= 2.13				

Data are mean of three replications.Th= *T. hamatum* , Pl = *P.lilacinus* , Te = *T.erecta

Nematicidal effect of fugal filtrate and plant extract may be attributed to the production of certain enzymes and toxins in the culture medium which inhibited egg hatching . Fungi are known to have proteolytic and chitinolytic activities which cause alteration in eggs' cuticular structure, changes in egg shell permeability or cause perforations in the cuticle[29],[30].

These results came in agreement with the resultsof Mokbel[31] who reported that T. hamatum culture filtrate caused 47.6% inhibition in egg-hatching of M. arenaria. The results also came in agreement with Saifullah et al. [32] who reported that the culture filtrates of T. harzianums showed strongnematicidal activity against Meloidogynesp and the percent of inhibition of egg hatching was directly proportional to the concentration of the filtrate. The role of chitinase in infecting nematode eggs were reported in Paecilomyceslilacinusand Pochoniaspp. isolated from infected nematode eggs[33] .

Aqueous root extracts singly or in combination with the leaf extracts of Tageteserecta, Cinerariamaritima and Rutagraveolens have been shown to exhibit nematicidal activity against M. arenaria, M. haplaand M. javanica[34](Sasanelli and D'Addabo, 1990).Result indicate that T.erecta was effective in management of hatching of eggs and then reduction in population of nematodes M. Javanica.

4.2 GREENHOUSE EXPERIMENT

4.2.1. EFFECT OF T. ERECTA EXTRACTS WITH T. HAMATUM AND P. LILACINUS ON ROOT KNOT NEMATODE M. JAVANIA AND GROWTH PARAMETERS OF TOMATO PLANTS IN GREENHOUSE CONDITIONS:

4.2.1.1.EFFECT ON ROOT KNOT NEMATODE M. JAVANICA

The results of the effect of various treatments individually and in combination on nematode development of M. javanica were presented in (Tables 4).All treatments individually or in combination recorded significant reduction in root galling and number of eggs as compared with the control+. However, the combination treatments were better than individual treatments in suppressing the nematode development. The lowest number of galls(5.3/gm root) and egg (3886/gm root) were recorded on the plants treated with T. erecta in combination with P. lilacinusand T. hamatum. Among individual treatments, the lowest number of galls(6.6/gm root) and eggs (5140/gm root) were recorded in P.lilacinus treatment.

Table (4) Effects of *T. erecta* extracts with *T.hamatum* and *P.lilacinus* on root galling

Treatments	Galls/gm root	Reduction(%) of galling	Eggs/gm rootx10^3	RF
N.only(control+) T_1	12.6	-	9.080	6.97
N + TeT_2	7.6	39.68	5.644	5.74
N + Pl T_3	6.6	47.61	5.140	5.72
N + Th T_4	8.3	34.12	6.122	5.94
N + Te + Pl T_5	6	52.38	4.224	4.91
N + Te + ThT_6	7.3	42.06	5.475	6.20
N + Pl + ThT_7	6.2	50.79	4.580	5.12
N + Te + Pl + ThT_8	5.3	57.93	3.886	4.66
LSD (P≥ 0.05)	2.01	3.11	2.694	1.1

*Each number is a mean of three replicates. N =Nematode ,Th=*T. hamatum*,Pl=*P.lilacinus*, Te =*T.erecta*

These results are in agree with the result of Hafeez et al. [35](2000) who reported that the treatment of tomato plants with PaecilomyceslilacinusandTrichodermaharzianumamended with organic substrate resulted in the minimum number of

galls per plant. Spiegel and Chet (1998) also reported that Trichodermaharzianumimproved growth and higher yield of M. javanica infected plants and decreased the root galling index and the number of eggs per g of root. This result has demonstrated that the combining of T. erectawithT. hamatumand P. lilacinus can provide satisfactory control of root-knot nematode M. javanica in Tomato[36].

4.2.1.2. EFFECT ON TOMATO PLANT GROWTH

The result of this study showed that the inoculation of tomato plants with M. javanica(T1)resulted in the significant suppression of the length and fresh weight of shoots and roots, when compared to uninoculated plants (T0)Table 5. Application of the 2 biocontrol fungi withT. Erecta (individually or in combination) significantly increased all growth parameters of tomato as compared with the inoculated control (T1).Among individual treatments, the highest value of growth parameters, length of shoot,length of root, fresh weight of shoot and fresh weight of root were recorded in P.lilacinus treatment (T3) which were18.07cm,14.23cm, 21.56gm and 6.68gm respectively.

Table (5) Effects of *T. erecta* extracts with *T. hamatum* and *P. lilacinus* on growth parameters of tomato plants in greenhouse conditions.

Treatments	Growth parameters			
	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)
Control $^-$ T_0	24.93	16.75	28.68	7.98
N. only(control $^+$) T_1	8.85	7.01	13.33	4.61
N + TeT_2	16.90	12.13	20.36	6.11
N + Pl T_3	18.07	14.23	21.56	6.68
N + Th T_4	15.20	10.33	20.01	5.83
N + Te + Pl T_5	18.86	14.66	23.96	6.88
N + Te + ThT_6	18.15	13.96	21.98	6.24
N + Pl + ThT_7	18.33	15.22	22.66	6.76
N + Te + Pl + ThT_8	22.66	16.66	26.95	7.20
LSD (P≥ 0.05)	2.31	2.74	1.54	0.80

Each number is a mean of three replicates. N =Nematode,Th=*T. hamatum*,Pl = *P.lilacinus*,Te =*T.erecta

In combination treatments, the maximum length of shoot, length of root (22.66 cm, 16.66cm respectively) were recorded in T8 , whereas minimum (18.15cm, 13.96cm respectively) were recorded in T6.However, this results suggest that the combination treatments were better than individual treatments in suppressing the nematode development and enhancement the growth of tomato.

The result are in accordance with the observations made by Goswami et al. [37] who reported that the length and weight of root significantly increased when tomato plants were treated with P. lilacinusand T. viride in combination with mustard cake. Leaf and stem extracts of marigold showed the best effect on most plant growth characters by suppressing the galling incidence[38].Raveendra, et al. conducted an experiment to test the efficacy of organic amendment (neem), Trichidermaviride, Tagetespatula and chemicals as individual treatments and in combinations, and they concluded that the improved plant growth is obtained in combination treatments than in individual treatments, which might be due to the additive and interactive effect of the treatment on tobacco plants[39].T. hamatum and T. harzianum resulted in the greatest reduction in nematode populations in soil and root and subsequent root knot disease severity in okra and mungbean. Similarly,longer roots in were produced in plant treated with T. hamatumwhile T. harzianum significantly increased plant height and fresh weight of shoot [40].

Moradi, et al. reported that the application of plant debris with the fungus T. harzianumimprove the biocontrol activity of the fungus in controlling the root knot nematode Meloidogynejavanica.There is a need to another research about the combination between marigold, fungal biocotrolagents, agrochemicals and naturally occurring organic matter in order to develop cost effective and environmentally safe nematode control methods which can be used in integrated pest management programs[41].

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