A comparative study between some biocontrol agents and the nematicide furfural on growth of tomato plants and pathogenesis of meloidogyne javanica under green-house condition

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دراسة مقارنة بين بعض العوامل الاحيائية ومبيد الفورفورال على معايير نمو نباتات الطماطة وإمراضية نيماتود تعقد الجذور *Meloidogyne javanica* في البيوت الزجاجية

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المستخلص

نفذت تجربة لدراسة تأثير ثلاث عوامل مكافحة أحيائية مع أحد فطريات المايكورايزا ومبيد الفورفورال ضد نيماتود العقد الجذرية *Meloidogyne javanica* التي تصيب نباتات الطماطة *Lycopersicon esculentum* في البيوت الزجاجية . عوامل المكافحة الأحيائية هي *Paecilmyces lilacinus* ، *Trichoderma harzianum* ، T.viride وفطر المايكورايزا *Glomus mosseae* والتي قورنت مع مبيد الفورفورال . الفورفورال كان أفضل معاملة في تحفيز نمو النبات واختزال عدد العقد الجذرية لكل نبات. الفطر *T. harzianum* كان أفضل عوامل المكافحة الأحيائية في زيادة طول المجموع الخضري والجذري والتي كانت 16.2 سم و 26.1 سم على التوالي . الفطر *Paecilomyces lilacinus* كان أفضل عوامل المكافحة الأحيائية في اختزال تعقد الجذور (57.53 سم%) . دمج عوامل المكافحة الأحيائية وفطر المايكورايزا ومبيد الفورفورال معاً كان أقل المعاملات تأثيراً في تحفيز نمو النبات واختزال تعقد الجذور حيث كانت النسبة المئوية للاختزال 31.43% .

Abstract

An experiment was conducted to study the effect of three fungal biocontrol agents along with *G.mosseae* and nematicide furfural against root knot nematode *Meloidogyne javanica* infecting tomato plants(*Lycopersicon esculentum*) under greenhouse condition. Bioagents are *Paecilomyces lilacinus*, *Trichoderma harzianum* , *T. viride* and mycorrhizal fungus *Glomus mosseae* which were compared with the nematicide furfural . The furfural compound was the best treatment in promoting plant growth and reduction the number of root galls/plant . The result showed that the *T. harzianum* was the best biocontrol agent in increasing shoot length and root length 16.2 % , 26.1% respectively .The *P. lilacinus* was the best biocontrol agent in reduction root galling (57.53%). Combination of biocotrol agents with *G. mosseae* and furfural was the least effective treatment in promoting plant growth and the reduction of root galling was 31.43% .

Key words: Biocontrol , Meloidogyne javanica, Glomus , Paecilomyces,Trichoderma , Furfural, Lycopersicon esculentum .

Introduction

Tomato (*Lycopersicon esculentum*, Mill.) , an important vegetable crop in Iraq . It is regarded as a most favorable host for root – knot nematode. All the 4 major species of *Meloidogyne* and their known races readily attack tomato crops in indoor and outdoor cultivations. Root-knot nematodes cause as high as 85% suppression in the yield of tomatoes (1) .

For the management of root knot nematode , chemical nematicides is a good strategy and were used till 1982 . However public concern about nematicides residues in food and environment and also the development of nematicides resistance by pathogens has increased the search for alternative means of controlling disease. Due to the problems caused by chemical control, mainly their deleterious effects on human health and environment, development of alternative control methods is of great importance (2).

The present investigation was thus undertaken to attempt an eco-friendly management of root knot nematode infecting tomato through integrating potential and compatible management components viz. fungal bioagents (3,4) . Recent study indicated that the combined effect of *Glomus moseae* , *Paecilomyces lilacinus* and Neem cake (*Azadiracbta indica*) facilitated the sustainable management of *Meloidogyne incognita* on eggplant under field condition reported. Another study revealed that pre-inoculation of tomato , tobacco , oat and carrot with *Glomus moseae* reduced the penetration of *M. incognita* (5,6) . The efficiency of *T. harzianum* was found to be comparable to that of carbofuran followed by *P. lilacinus*, *Arthrobotrys oligospora* and neem compound. Besides reducing nematode infestation, the biocontrol agents also enhanced the growth of the plant (7) .

Raja & Renganathan (8) reported that the integration of different methods of application of *P. lilacinus* viz., seed treatment, seedling treatments and soil application treatments under field conditions exhibited an increase in plant growth parameters (shoot length, shoot weight, root length and root weight) in the fields trial.

The nematicidal potential of eco-friendly - furfural was discovered by Rodriguez-Kabana and Walters (9), and further demonstrated against a variety of phytoparasitic nematode including *Meloidogyne arenaria*, *M. incognita* , and *Pratylenchus* (10 , 11) . El-Mougy, *et al*. (12) indicated that the furfural combined with bioagent treatment proved to have suppressive effect against nematode *M. incognita* and the numbers of nematodes in soil declined sharply in direct response to furfural application with the sharpest reductions in its population.

The direct mode of action of furfural against nematodes has been described as the destruction of the nematode cuticle (13). Residues of furfural have not been detected in plant tissue or soil after multiple applications, because the compound, which has a short half life , is degraded through oxidative and reductive analogs to carbon dioxide and acetic acid. The nematophagous fungi *Paecilomyces* and *Trichoderma* are a biocontrol fungi with a potential range of activity to control the worldwide most important plant parasitic nematodes. This biological nematicides may be safe agents in an integrated approach to control mainly sedentary nematodes. The aim of this research was to compare between the effect of biocontrol agents (*P. lilacinus*, *T. harzianum* , *T. viride* and *G. mosseae*) and furfural on: 1) Growth parameters of tomato plants , 2) Pathogenesis of root knot nematode *M. javanica* .

Materials and Methods

Nematode culture

Nematode eggs were extracted from two month old heavily galled tomato roots, using the modified extraction technique of Hussey and Barker (14) . The suspension of eggs was then adjusted to 2000 eggs / ml , 3 ml of suspension per one pot were used in this study and added at the time of transplanting .

Plant culture

Tomato cultivar (*Lycopersicon esculentum* Mill.) seedlings were transplanted in 25 cm diameter plastic pots (one/pot) containing 2 Kg aerated sterilized sandy loam soil . Ten days after transplanting they were treated with antagonistic fungi .

Fungal isolates

Isolates of fungi (*P. lilacinus*, *T. harzianum* , *T. viride* and *G. mosseae*) used in this study were obtained from Department of Biotechnology- Directorate of Agriculture Research - Ministry of Science and Technology .The fungi *P. lilacinus*, *T. harzianum* and *T. viride* were cultured in a flasks (250 ml) containing 50 g of autoclaved wheat grains and incubated at 25 + 1^{o}C for ten days .

Inoculation

An inoculums of 100 gm of *G. moseae* containing 60 chlamydospores / gm was placed as a thin layer 2 cm below the soil surface in each pot. Three grams of wheat grains infected with the fungi (*P. lilacinus, T. harzianum* and *T. viride*) per 1 Kg soil were used.

The nematicide furfural (Group Guard) was obtained from Deraawi Company for agricultural Service – Baghdad –Iraq .The rate of application of furfural was 1ml/Kg soil (9) , two days after transplanting the furfural added to the soil .

Treatments of the experiment were as follow :

T1= Control $_1$: Without any inoculation (Negative control)

T2= Control $_2$: Inoculation with nematode alone (Positive control)

T3= Inoculation with *T. harzianum* + Nematode

T4= Inoculation with *T.viride* + Nematode

T5= Inoculation with *P. lilacinus* + Nematode

T6= Inoculation with *G.mosseae* + Nematode

T7= Treatment with furfural + Nematode

T8= Recombination (T.h. +T.v. + P.l. + G.m. + fur.) + Nematode

The treatments were laid out in a completely randomized design (CRD) and each was replicated 3 times . Plants were harvested two months after nematode inoculation . Data dealing with length and weight of shoots and roots beside the gall number per plant were calculated. All obtained data were subjected to the analysis of variance and means compared according to the least significant difference (LSD) at the 5% level of probability in Wasit University, College of Agriculture.

Results

The effect of biocontrol agents *P. lilacinus*, *T. harzianum* , *T. viride* , *G. mosseae* and furfural on the growth of tomato plants and pathogenesis of *M. javanica* are presented in Tables 1, 2. The results showed significant differences in plant growth parameters in all treatments compared with the positive control (T2 nematode alone) Tables 1 . The most effective treatment in increasing growth parameters was the furfural treatment (T7) which produced maximum, shoot length (19.7 cm), Root length (26,9 cm), Wet weight of roots (0.97 gm) and dry weight of roots (0.096 gm) followed by the treatment *T. harzianum* for the same parameters (16.2 cm) ,(26.1 cm) , (0.58 gm) and (0.054 gm) respectively . The furfural treatment was the most effective one also in increasing wet weight of shoot (3.18 gm) and dry weight of shoot (0.316 gm) followed by the treatment *G. mosseae* for the wet weight of shoot (1.72 gm) and *T. viride* for the dry weight of shoot (1.55 gm) and they differ significantly . There is no significant differences between biocontrol agents (*P. lilacinus*, *T. harzianum* , *T. viride*) in shoot length.

Recombination treatment (T8) was the least effective in all growth parameters except dry weight of root. *T. Viride* treatment(T4) gave lowest value in dry weight of root (0.035 gm) .

Table (1) : Effect of biocontrol agents and furfural in growth criteria of tomato plants

Treatments	Shoot length (cm)	Root length (cm)	Wet weight of shoot (gm)	Dry weight of shoot (gm)	Wet weight of roots (gm)	Dry weight of root (gm)
Negative control T1	21.2 ^{d}	31.6 ^{e}	4.10 ^{b}	0.410 ^{b}	1.40 ^{b}	0.154 ^{b}
Positive control T2	8.50 ^{a}	9.10 ^{a}	0.62 ^{a}	0.060 ^{a}	0.20 ^{a}	0.021 ^{a}
T. harzianum + Nematode	16.2 ^{bc}	26.1 ^{d}	1.42 ^{a}	0.141 ^{a}	0.58 ^{a}	0.054 ^{a}
T.viride + Nematode	13.6 ^{b}	15.5 ^{b}	1.57 ^{a}	0.155 ^{a}	0.39 ^{a}	0.035 ^{a}
P. lilacinus + Nematode	13.9 ^{b}	20.2 ^{c}	1.35 ^{a}	0.132 ^{a}	0.45 ^{a}	0.044 ^{a}
G.mosseae + Nematode	12.3 ^{a}	16.5 ^{b}	1.72 ^{a}	0.127 ^{a}	0.41 ^{a}	0.043 ^{a}
Furfural + Nematode	19.7 ^{cd}	26.9 ^{d}	3.18 ^{b}	0.316 ^{b}	0.97 ^{b}	0.096 ^{b}
Recombination	11..3 a	13.5 ^{b}	1.35 ^{a}	0.099 ^{a}	0.31 ^{a}	0.044 ^{a}
LSD (0.05)	**3.83**	**3.62**	**1.43**	**0.14**	**0.73**	**0.062**

***Each value is an average of three replicates . Same letter(s) are not significantly different (P=0.05) by Least Significant Difference Test . Negative control : Without any inoculation . Positive control : Inoculation with nematode alone .**

The results showed also significant differences in roots galling in all treatments compared with the comparison treatment ($control_2$) Table 2 . The most effective treatment in reduction the galls number on roots of the seedlings was the furfural treatment in which the number of galls per plant was 16 compared with the comparison treatment(105), followed by the treatments *T. viride* , *G. mosseae* , *T. harzianum* and *P. lilacinus* which were 57 , 53.6 , 49.3 and 44.6 respectively . Less reduction in galls formation was observed when the antagonistic microorganisms were combined with furfural application and the number of galls per plant was 72.

Data in table 2 indicated the activity of tested treatments on galls formation by nematode and the highest percent of reduction was recorded in the treatments of furfural (84.77%) followed by the treatments *P. lilacinus* and *T. harzianum* which were 57.53% and 53.05% respectively . *T. viride* and recombination treatments showed the less performance with values of 45.72% and 31.43% reduction respectively . There is no significant differences between *P. lilacinus* and *T. harzianum* treatments and between *T. viride* and *G . mosseae* treatments in reduction galls number .

Table (2): Effect of BCAs and furfural in nematode galling on tomato plants

Treatments	No. of Gall / Plant	Reduction %
Negative control T1	0	-
Positive control T2	105 [a]	-
T. harzianum + Nematode	49.3 [de]	53.05
T.viride + Nematode	57 [c]	45.72
P. lilacinus + Nematode	44.6 [e]	57.53
G.mosseae + Nematode	53.6 [cd]	49.96
Furfural + Nematode	16 [f]	84.77
Recombination (all Treatments)	72 [b]	31.43
LSD (0.05)	5.45	-

*** Each value is an average of three replicates . Same letter(s) are not significantly different (P=0.05) by Least Significant Difference Test . Negative control : Without any inoculation . Positive control : Inoculation with nematode alone .**

Discussion

Results of the study indicate that using microbial agents and furfural suppressed the root-knot nematodes and resulted in positive changes in plant growth. Application of furfural to soil causes shifts in the composition of the soil microbial community and this is among many factors that affect the fate of agricultural chemicals in the soil. Microorganisms are either capable of utilizing a chemical as a nutrient source or cometabolizing compounds which, in turn, affect the entire soil food web and promote growth of plant (15) .

Furfural also reduced gall formation by nematode, this reduction could result from a reduction of nematode population in soil. Moreover, many scientists supervised laboratory and field trails concerning the efficacy of furfural against soil microorganisms. They recorded that furfural is a natural bactericide, fungicide, insecticide and nematicide which interacts with the cuticle of the nematode, effectively stripping the protective layers which results in the cuticle swelling and disintegrating. Movement of the nematode is impeded and it subsequently dies through dehydration or attack by parasitic organisms. (16 , 17).*T. harzianum* and then *P. lilacinus* were the best bioagents in reduction root galls and promoting growth of tomato. This effect may be attributed to the toxic, and enzymatic principles such as acetic acid and proteolytic and chitinolytic enzymes released during fungal interaction with nematode which might cause killing /inhibiting the *M. javanic* directly or indirectly promoting plant growth and ultimately enhancing yield component of tomatoes . Similar results reported that soil application with *T. harzianum* resulted in the greatest reduction in nematode populations in soil and subsequent root knot disease severity in okra and mungbean . Similarly *T. harzianum* significantly increased plant height of okra and mungbean (18). *T. harzianum* were found to be most effective than *P. lilacinus* in controlling M. incognita and enhanced all plant growth characters , and they concluded that biocontrol agents are effective agents in controlling root-knot nematode and are ecologically safe for (19) .

Trichoderma has not only been proved to parasitize nematodes and inactivate pathogen enzymes but also help in tolerance to stress conditions by enhanced root development. It participates in solubilization of inorganic nutrients. Thus, *Trichoderma* colonized roots require lesser supply of manmade nitrogen fertilizers (20). Moreover, the activity of *P. lilacinus* attributed to ability to infect eggs, juveniles and females of *M. javanica* by direct hyphal penetration (21).

G. mosseae inoculation also had a least but beneficial effect on plant growth and gall formation and this result coordinates with that of Khalil1, *et al*. (22) who indicate that *G. macrocarpum* was the least effective fungus on the formation of gall by *Meloidogyne incognita* . Arbuscular mycorrhizal fungi (AMF) are widely used in nurseries as they enhance nutrient and water availability. They have also been used to improve the growth of tomato plants (23 , 24).The suggested mechanisms of Arbuscular mycorrhizal fungi against plant parasitic nematodes are summarized as follow: i) improved plant vigor and growth to offset yield loss normally caused by nematodes, ii) physiologically alteration or reduction of root exudates that are responsible for chemotactic attraction of nematodes, iii) directly retarding nematode development or reproduction within the root tissue, and iv) enhance and encourage the endophytes and endoparasitic-nematodes to compete for the same site in the root. Also, higher chitinase activity and ß-1, 3-glucana in roots was recorded (25 , 26).From the other hand the synergistic effect of biocontrol agents and nematicide furfural (Recombination) was the least effective in most growth parameters as well as gall formation . This may be due to antagonistic effect between them or because of the qualitative as well as quantitative microbial changing that occur after the addition of treatment components . Similar results were reported by El-Mougy *et al*. (12) referred to that the combined treatments with furfural and either bacterial or fungal bioagents showed a lower effect, although they reduced the disease incidence by more than 41% . Another study revealed that combination of *G. intraradices* and *T. viride* to control root-knot nematode *Meloidogyne hapla* did not result in synergism (27).

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