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Fungal and bacterial bio-control agents in controlling citrus nematode *Tylenchulus semipenetrans* Cobb in greenhouse and field

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Abstract:

The activities of fungal bio-control agents Trichoderma hamatum, Trichoderma viride, Paecilomyces lilacinus and bacterial bio-control agents Pseudomonas fluorescens were examined against Tylenchulus semipenetrans in laboratory, greenhouse and field conditions. In laboratory the effect of cultural filtrates of bioagents was evaluated at concentrations of S (standard), S/2 and S/4. All bioagents filtrates were showed nematicidal effect against J2 of citrus nematode and the effect of them was proportional with the concentration. Highest mortality percentage was recorded by Pseudomonas fluorescens filtrate which was 94.7% at 'S' concentration followed by Trichoderma viride (86.3%) after 48h of exposure. In greenhouse test all bioagents were able to reduce the population of nematode in the soil and roots and the highest percentages of reduction were recorded in the case of Trichoderma hamatum which were 86.68% and 61% in J2 and females respectively. The bioagents also improved the growth parameters of citrus seedlings such as length and fresh weight of shoots. Finely field experiment revealed that the tested bioagents suppressed the population of J2 in soil and females in roots and the greatest percentages of reduction were recorded in the treatment of Trichoderma viride

which were 64.9% and 44.8% followed by the treatment of Trichoderma hamatum 62.2% and 44.3% in J2 and females respectively.

Key words: *Paecilomyces lilacinus, Trichoderma hamatum, Trichoderma viride, Pseudomonas fluorescens, Tylenchulus semipenetrans,* Biocontrol.

INTRODUCTION

Plant parasitic nematodes cause great economic losses to agricultural crops worldwide . Numerous nematode species are associated with the citrus rhizosphere (Cohn, 1972). However, relatively few have shown to be of economic importance, with the notable exception of *Tylenchulus semipenetrans* Cobb. 80 species and varieties of citrus are susceptible to this nematode (Muhammad et al., 2004). Yield reduction by citrus nematode depending on the citrus nematode infection and rated from 10 to 30% (Verdego and McKenry, 2004).

Tylenchulus semipenetrans was first detected on citrus roots in California in 1912 and named and described during the next 2 years (Cobb, 1913, 1914). Since its discovery, *T. semipenetrans* has been found in every citrus growing region of the world (Duncan 2005). *T. semipenetrans* , commonly known as 'citrus nematode' causes 'slow decline' disease in citrus. The pest is cosmopolitan in distribution and has been reported to cause injury to 50 species or hybrids of *Citrus* in the family Rutaceae. Higher populations are commonly found in citrus orchards established in finely-textured soils or in sandy soils with high organic matter content. (Timmer et al., 2003).

Management of the citrus nematode remains difficult as no single tactic provides adequate control of the nematode (Verdejo and McKenry, 2004). Eco-friendly control of citrus nematode could be achieved by soil microorganisms. The use of fungi and bacteria as a biocontrol agents is gaining importance

in the management of citrus nematode because they inhabit the same environment and may be affect the nematode by producing nematicidal metabolites (Anke and Sterner, 1997; Hewlett et al. , 2009) or by the direct effect on eggs and larvae (Walode et al. , 2008).

Hammam et al. (2016) reported that the fungus *Paecilomyces lilacinus* and the bacteria *Pseudomonas* are potential biocontrol agents against citrus nematode *Tylenchulus semipenetrans* and suppressed the populations of nematode and increased fruit yield of Citrus. Combination between the fungus *T. harzianum* and neem cakes reduced the population of citrus nematode, *Tylenchulus semipenetrans* (Parvatha et al. 1996).

Because of the importance of citrus nematode, the efficacy of mentioned bioagents and no adequate studies on controlling this nematode specially in the field , the present study was conducted to determine the effect of *Paecilomyces lilacinus* (Thom) Samson, *Trichoderma hamatum* (Bonord.) Bainier, *Trichoderma viride* Pers. and *Pseudomonas fluorescens* (Trevisan) Migula on the population density of *Tylenchulus semipenetrans* Cobb in the greenhouse and field.

MATERIALS AND METHODS

Isolation of fungi and bacteria

Bio-control agents were isolated from citrus rhizosphere by using dilution-plate method. From each sample, one gram soil was mixed with 100 ml of sterilized distilled water. Serial dilutions (up to $1x10^5$) were made. One ml of each suspension of fungi was poured on Potato dextrose agar (PDA) and incubate at 25 ± 2 ºC for five days and then purified and stored at 4 ºC for experimental use.

Tested bacteria were cultured on solidified nutrient agar medium with sterilized pipette and incubated at 30 ± 2 ºC. Bacterial colony purified by using streak plate method. The

purified bacteria, was then stored also at 4 ºC for experimental use. Isolated fungi and bacteria were then identified in Ministry of Science and Technology- Directorate of Agriculture Research – Department of Biotechnology.

Extraction of nematode from soil and roots

Rhizosphere soil and roots from Citrus nematode infested orchard were used to second-stage juveniles (J2) and females for this study. 250 g of citrus rhizosphere soil was placed in a beaker contain enough tap water and mixed gently to remove soil aggregation. Each sample was passed through a series of sieves (150 , 125 , 75 µm) with a supply of tap water to remove debris, and then passed through a sieves (45 , 25 µm). Nematodes were collected by washing the sieve inversely with a fairly gentle stream of water into a 250 ml beaker. Nematode suspensions were prepared for using. 1ml from each suspension of nematode was placed in counting slide by pipette for counting using light microscope.

Infected roots were washed by water to remove soil. 5g of roots were cut into 1 cm sections. They were then blender macerated in a 0.5% NaOCl solution in a food blender, at approximately 1000 rpm for two successive 15 s intervals (McSorley *et al*., 1984). The suspension was passed through a sieve as above to remove root debris and collect the female suspension. Then the number of females was counted under the light microscope by using counting slide.

Laboratory Experiment

Preparation of the culture filtrates

For production of culture filtrates of the bio- control agents, bacteria was grown in conical flasks (250 ml) containing 100 of King' s B broth medium (King et al. 1954) at 28 °C for 48 h. Fungal bioagents were grown in conical flasks (250 ml) containing 100 ml of Potato dextrose broth medium at 25±2 °C

for one week. The cultural filtrates of each bio-control agent were harvested as described by Tahikalange *et al.*, 2005. Three concentrations { S (Standard) , S/2 and S/4 } of each biocontrol agent cultural filtrate were tested. Distilled water was used as a control**.**

The effect of culture filtrates on larval mortality

To perform this test, suspension of citrus nematode J2 was adjusted to the concentration 100 J2 per ml. one ml of J2 suspension was mixed with 10 ml of culture filtrate in a petri plate (5cm)**.** One ml of J2 suspension also was mixed with 10 ml of distilled water as a control treatment**.** Each treatment was replicated three times in a completely randomized design. The numbers of live and dead nematodes were counted by light microscope after 24 and 48 h of treatment at 25 °C (Osei *et al.*, 2011). Nematodes were considered dead if they had adopted a straight shape and were immobile. The mortality percentages were recorded for each treatment by the following equation: Mortality (%) = {(C_1– C_2)/C_1} x 100 , where, C_1 is the number of live nematodes juveniles in control treatments and C_2 is the number of live nematodes juvenile counted in other treatments [Li et al. 2005].

Greenhouse Experiment

Evaluation the efficacy of bioagents against *T. semipenetrans*

This test was conducted to evaluate the efficacy of bio-control agents against *T. semipenetrans* on citrus in pots. Three months old seedlings were transplanting in pots (20 x 15cm) containing sterilize sandy loam soil. The pots were inoculated by 50ml of J2 suspension (100J2/ml) after two weeks of transplanting. After one week of inoculation 25 ml of bacterial suspension (3 x 10^8 colony forming unit (CFU) /ml) and fungal suspension (3 x 10^8 spore /ml) were added separately to each

pot around the citrus seedlings. 25 ml of bacterial and fungal suspension were added separately to Pots without nematodes. Pots with nematode only used as a positive control (control^{+}). Pots without any treatment used as a negative control (control $^{-}$).

Each treatment was replicated three times. The experiment was conducted in a completely randomized design. All pots watered regularly. After three months of nematode inoculation, the seedlings of citrus were removed carefully from pots and the roots of seedlings were washed by tap water. Nematode parameters which include number of J2 per 250 grams of soil and females per one gram of roots were recorded. Reduction of nematodes population density in soil sample and roots was calculated according to Abbott's formula: Reduction (%) = 1- (N in T/ N in C) x 100, in which N means Population of nematode, T means Treatment and C means Control(Abbott, 1925).

Plant growth parameters which include shoot length, root length, fresh weight of shoots and fresh weight of roots also recorded.

Field Experiment

This investigation was conducted in naturally infested sweet orange (*Citrus sinensis* L.) orchard to evaluate the efficacy of applying biocontrol agents against *T. semipenetrans* on citrus. Fungal bio-control agents were grown in 500 mL capacity flasks contain 250g of watery wheat grains (Mukhtar et al. 2013). Bacterium also grown in 500 mL capacity flasks contain 250ml of King' s B broth medium(King et al. 1954). Trees from orchard infested with slow decline were selected and used to achieve this experiment.

The trees were inoculated with 25g/tree of fungal pure culture and 25ml/tree of bacterial pure culture at a depth of 15-25 cm around the trunk. Each treatment was replicated three times and each replicate include one tree. Three untreated

replicates left as a control. The experiment was conducted in a completely randomized block design. Nematode population prior to the application of biocontrol agents was assessed. Nematode population in roots and soil were recorded after the application of biocontrol agents after three months. Reduction of nematodes population density in soil sample and roots was calculated according to Abbott's formula (Abbott, 1925).

Statistical analysis

Data were statistically analyzed by one -way and two- way analysis of variance (ANOVA) . Means were separated using Duncan's multiple range test (DMRT) at the 0.05 level of significance. (SPSS version 20).

RESULTS

Laboratory Experiment

The effect of culture filtrates on morality of larva

The results of this test that presented in table (1) indicated that all tested bioagent filtrates were effective against the 2nd stage juveniles of *Tylenchulus semipenetrans* and the percentage of larval mortality has been increased with the increasing of filtrate concentration and exposure duration. The results also revealed significant difference between the tested bioagents and the mortality ranged from 48.4% to 91.2% after 24h and from 53.6% to 94.7% after 48h as compared with the control 3.3% and 6.6% after 24h and 48h respectively .The bacterial bioagent *Pseudomonas fluorescens* gave the maximum mortality in J2 which was 94.7% after 48h at the " S" concentration followed by the fungal bioagent *Trichoderma viride* (86.3%). Minimum mortality was recorded in the case of *Paecilomyces lilacinus* which was 77.5% at the same concentration and the same exposure period. There is no significant difference between *T. hamatum* and *T. viride* at all tested concentrations.

Table (1) Effect of culture filtrates of Bioagents on larval mortality of citrus nematode (*Tylenchulus semipenetrans*)

Treatments	Concentrations	Mortality (%) after 24h	Mortality (%) after 48h
Pseudomonas fluorescens	S	91.2a	94.7a
	S/2	82.4b	87.6b
	S/4	59.6d	65.4e
Trichoderma hamatum	S	81.6b	84.7bc
	S/2	76.3c	80.7c
	S/4	50.3ef	57.8f
Trichoderma viride	S	82.2b	86.3bc
	S/2	77.2c	81.9c
	S/4	53.6e	58.2f
Paecilomyces lilacinus	S	74.4c	77.5d
	S/2	72.6c	75.9d
	S/4	48.4f	53.6f
Distilled water (control)		3.3g	6.6g

Each value is a mean of three replicates. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) .

Greenhouse Experiments

The effect of bioagents on population density of *Tylenchulus semipenetrans*

The results of this test that presented in table (2) revealed that all bioagents treatments gave a significant decreasing in the number of *Tylenchulus semipenetrans* 2nd stage juveniles (J2) in soil and the number of female in roots as compared with control treatment. The number of J2 in soil ranged from 3812 to 6814 per 250g of soil and the females in roots ranged from 388 to 592 per gram of roots as compared with the control in which the number of J2 in soil and females in roots were 28616, 994 respectively. Lowest number of J2 and female had been recorded in the case of the treatment *Trichoderma hamatum* which were 3812 (J2 / 250 g of soil) and 388 (female/g of roots) with reduction percentages 86.68% and 61% respectively. The result also showed significant difference between the fungal bioagents and *Pseudomonas fluorescens* in reducing the population of females in roots but there is no like that in reducing the population of J2 in soil.

Table (2) Effect of bioagents on citrus nematode (*Tylenchulus semipenetrans*) in green house

Treatments	Nematode Parameters			
	J2 / 250 g of soil x 10^3	Reduction %	Female /g of root x100	Reduction %
Tylenchulus semipenetrans (Control)	28.616a	-	9.94a	-
Pseudomonas fluorescens + Ts	6.814b	76.18	5.92b	40.5
Trichoderma hamatum + Ts	3.812b	86.68	3.88c	61.0
Trichoderma viride + Ts	4.122b	85.60	4.42c	55.5
Paecilomyces lilacinus + Ts	4.166b	85.45	4.12c	58.6

Each value is a mean of three replicates, Ts = *T. semipenetrans*, Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$).

The effect of bioagents on growth parameters of citrus plant

The results of greenhouse test that presented in table (3) revealed that all treatments significantly increase shoot length of citrus seedlings when compared with the positive control (treated with nematode only) and negative control (without treatment) and also significantly increase shoot fresh weight of seedlings as compared with the positive control (Control $^+$). Citrus seedlings that treated with *Trichoderma hamatum* alone or with nematode recorded highest shoot lengths which were 99.3cm and 97.1cm respectively, followed by the treatments of *Trichoderma viride* alone or with nematode which were 94.4cm and 91.6cm respectively.

Shoot fresh weights were also greater in seedling treated with *Trichoderma viride alone and Trichoderma hamatum* alone which were 76.6g and 74.5g respectively and without significant difference between these two bioagents.

Data in table (3) also revealed decreasing in fresh weights of roots in bioagents treatments when compared with treated control (Control $^+$) and untreated control (Control $^-$). Highest fresh weight of roots was recorded in treated control which was 104.4g followed by untreated control (99.8g). Lowest fresh weight of roots was recorded in seedling treated with *Trichoderma viride* alone which was 87.4g.

Table (3) Effect of bioagents on growth parameters of *Citrus sinensis* in greenhouse

Treatments	Plant growth parameters			
	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)
Tylenchulus semipenetrans only (Control $^{+}$)	61.6g	54.9abc	47.8d	104.4a
Pseudomonas fluorescens + Ts	88.0de	57.4abc	63.3c	97.6abc
Trichoderma hamatum + Ts	97.1ab	47.5cd	71.6ab	95.7abc
Trichoderma viride + Ts	91.6cd	40.1e	73.1ab	89.5c
Paecilomyces lilacinus + Ts	83.6e	53.2bs	62.4c	91.6bc
Pseudomonas fluorescens only	91.1cd	59.6ab	66.4bc	92.2c
Trichoderma hamatum only	99.3a	49.2bcd	74.5a	89.4c
Trichoderma viride only	94.4bc	43.1de	76.6a	87.4c
Paecilomyces lilacinus only	85.8e	56.6abc	65.7bc	89.6c
Control $^{-}$	76.5f	62.5a	63.0c	99.8ab

Each value is a mean of three replicates, Ts = *T. semipenetrans*, Control^{+} = Treatment with nematode only, Control $^{-}$ = without any treatment. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$).

Field Experiment

The effect of bioagents on the population density of *Tylenchulus semipenetrans* in the field

The observations of this test Table (4) revealed that all the bioagents are effective to reduce the population of citrus nematode in soil and showed a significant reduction in the number of J2 per 250 gram of soil when compared with control treatment . Highest efficacy was observed in the treatment of *Trichoderma viride* in which the number of J2 was 2.6 x10^3 J2 per 250 gram of soil compared with the control treatment 7.2 x10^3 J2 per 250 gram of soil. All tested fungal bioagents are significantly more effective than bacterial bioagent *Pseudomonas fluorescens* in reducing J2 in soil and among the fungal bioagents *Trichoderma viride* was the more effective than the other. Similarly highest reduction percentage was observed in the treatment of *Trichoderma viride* which was 64.9% and the other percentages ranged between 48.2% to 62.2% compared with the control(18.1%).

The population of females in roots also reduced after the application of bioagents to soil and the more effective bioagent was the fungal bioagent *Paecilomyces lilacinus* in which the

percentage of reduction was 50% but without significant difference with the other fungal bioagents. Lowest reduction percentage of females in roots was observed in the case of *Pseudomonas fluorescens* treatment (33.4%) compared with the control (9.4%).

Table (4) Effect of Bioagents on population of citrus nematode (*Tylenchulus semipenetrans*) in the field naturally infested with nematode

Treatments	J2 / 250 g soil before treatment x10^3	J2 /250 g soil after treatment x 10^3	Reduction (%)	Female /g of root before treatment x 100	Female /g of root after treatment X 100	Reduction (%)
Pseudomonas fluorescens	8.5	4.4b	48.2b	6.6	4.4b	33.4b
Trichoderma hamatum	8.2	3.1bc	62.2a	6.1	3.4c	44.3a
Trichoderma viride	7.4	2.6c	64.9a	5.8	3.2cd	44.8a
Paecilomyces lilacinus	6.7	3.2bc	52.2b	5.2	2.6d	50.0a
Control	8.8	7.2a	18.1c	14.8	13.4a	9.4c

Each value is a mean of three replicates. Means followed by the same letter in the same column are not significantly different. ($P \leq 0.05$).

DISCUSSION

The results of this study indicate that the tested bioagents possess high antagonistic activities against citrus nematode *T. semipenetrans*. This antagonism occurs over different mechanisms which consist two types of antagonism: direct antagonism which include hyperparasitism, Antibiotic-mediated suppression, Lytic enzymes and other byproducts of bioagents, and indirect antagonism which include competition and induction of host defenses.

The effect of filtrates is one of the direct mechanisms that affect the nematode so the mortality of J2 by culture filtrate of these bioagents may be due to the presences of antibiotics, extracellular enzymes or other toxic compounds in these filtrates.

Acetic acid was identified as the nematicidal principle in culture filtrates of *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* (Djian et al. 1991). Similar result by Park et.

al. (2004) suggest that the antibiotic leucinostatins in filtrate of *P. lilacinus* are indicators of nematicidal activity, whereas chitinase activity might be related to parasitism. Another enzymes, such as collagenase, chitinase and subtilisin-like serine protease corresponding to the main chemical constituents of nematode cuticle and eggshell, have been reported to be involved as a virulence factors for nematode infection. (Huang et al. 2004).

Antibiotics like 2, 4-diacetylphloroglucinol(DAPG), pyrrolnitrin, pyoluteorin and phenazines that produce by *P. fluorescens* were reported as a compounds with nematicidal effect (Hu, et. al., 2005; Timper et al., 2009 ; Nandi, et al., 2015).

Culture filtrates of *Trichoderma* spp also contain several antibiotics such as trichodermin with high nematicidal activity (Zhong-Shan, et. al. 2010). Moreover the chitinolytic system of *Trichoderma* comprises many enzymes and the list of its components is rapidly being updated as new enzymes and genes are reported. Chitinases are divided into 1,4-acetylglucosaminidases, endochitinases and exochitinases. Many 1,4-acetylglucosaminidases and their genes —*exc*$_1$*, exc*$_2$*, tvnag*$_1$*,* and *tvnag*$_2$ from *T. harzianum* T25-1*, T. atroviride* P1 and *T. virens* Tv29-8 —have been described (Kim et. al., 2002; Harman et.al., 2004)

The results from greenhouse test also revealed that the fungus *Trichoderma hamatum* was the more effective bioagent in reducing the population density of nematode in roots and soil, and increasing the growth parameters of citrus seedling such length and fresh weight of shoot and this suggest that *T. hamatum* can induce such defense enzymes and probably other defense compounds leading to systemic resistance in plants or may be the level of nutrient uptake increase because of the colonization of roots by the fungus or may be the fungus affects the expression of several genes related to the growth. Number of studies have revealed that root colonization by *Trichoderma*

spp. increased level of defense related plant enzymes as well as various peroxidase, chitinase, b-1, 3-glucanases, lipoxigenase and phenylalanine ammonia lyase (Howeel et.al., 2000; Brotman et.al. 2013). Similar result about the biocontrol efficacy of *T. harzianum* against *T. semipenetrans* in *C. jambhiri* seedlings was also reported by Walode *et al.* (2008).

This result was in accordance with the results of Shanmugaiah et al. (2009).They were reported that the plants treated with *T. viride* alone or with pathogens showed more increasing in shoot length than *Pseudomonas fluorescens* and other treatments.

Khaledi and Taheri (2016) reported that soil treatment with *T. harzianum* was more effective than seed treatment in reducing growth of *M. phaseolina* and resulted in higher plant growth parameters of soybean plant.

Results of this study also revealed that the bioagents decrease the fresh weight of roots and this reduction in root weight may be considered as an improvement of root health, because roots infection by nematode makes an increase in root weight and this results were in accordance with the results of Siddiqui and Shaukat (2004) about the effect of *Pseudomonas fluorescens* and *Trichoderma harzianum* on weight of roots inoculated with the root-knot nematode.

The present findings were showed also significant suppression of both j2 in soil and females in roots under field conditions and this antagonistic effect may due to the myco-parasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites that affect the population of nematode.

Similar results were obtained from the study of Prasad et al. (2014) about the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on the population density of root-knot nematode in soil under field condition and they reported that the fungus *Trichoderma harzianum* was more effective than the

bacteria *Pseudomonas fluorescens* in reducing population density of nematode in soil.

Another recent study revealed that the using of *T.viride* and *Pseudomonas fluorescens* in combination was more effective than the using of each one alone in controlling *Meloidogyne javanica*and these bioagents reduced the population density of nematode in soil in most tested cultivars of tomato (Saeedizadeh, 2016).

This study was confirmed the efficacy of *T. hamatum* (The first in Iraq), *P. lilacinus*, *T. viride*, and *P. fluorescens* in controlling citrus nematode *T. semipenetrans* in greenhouse and field . These bioagents could be used to develop alternative, low cost and environment friendly techniques for controlling this nematode.

REFERENCES

1. Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
2. Anke, H. and Sterner, O. (1997). Nematicidal metabolites from higher fungi. Curr. Org. Chem. 1, 361-374.
3. Howell, C.R.; Hanson, L.E.; Stipanovic, R.D. and Puckhaber, R.D. (2000). Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathol.,; 90: 248-252.
4. Brotman, Y.; Landau, U.; Cuadros-Inostroza, A.; Tohge, T.; Fernie, A.R.; Chet, I.; Viterbo, A. and Willmitzer, L. (2013). *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLoS Pathog 3:1325-1332.

5. Cobb, N.A. (1913). Notes on *Mononchus* and *Tylenchulus*. Journal of the Washington Academy of Science 3, 287–288.
6. Cobb, N.A. (1914). Citrus-root nematode. Journal of Agricultural Research 2, 217–230.
7. Cohn, E. (1972). Nematode diseases of citrus. In: Economic Nematology (J.M. Webster, Ed.). Academic, New York, 215-244 pp.
8. Djian, C.; Pijarowski, L.; Ponchet, M.; Arpin, N. and Favre-Bonvin, J. (1991). Acetic acid: a selective nematicidal metabolite from culture filtrates of *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma longibrachiatum* Rifai. Nematologica 37:101–12.
9. Duncan, L.W. (2005). Nematode parasites of citrus. pp. 437-466. In plant parasitic nematodes in subtropical and tropical agriculture (Luc M, Sikora RA, Bridge J, eds). CAB International.
10. Hammam, M. M. A.; El-Nagdi, W.M. and Abd-Elgawad, M. M. M. (2016) . Biological and chemical control of the citrus nematode, *Tylenchulus semipenetrans* (Cobb, 1913) on Mandarin in Egypt. Egyptian Journal of Biological Pest Control, 26 (2) : 345-349.
11. Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I. and Lorito, M. (2004) *Trichoderma* species opportunistic, avirulent plant symbionts. Nature Reviews 2:43-56.
12. Hewlett, T. E.; Stetina, S. R.; Schmidt, L.M.; Waters, J.P., Simmons, L.J. and Rich, J.R. (2009). Identification of *Pasteuria* spp. that parasitize *Rotylenchulus reniformis*. Journal of Nematology 41:338.
13. Hu, H. B.; Xu, Y. Q.; Cheng, F.; Zhang, X.H. and Hur, B. (2005). Isolation and characterization of a new *Pseudomonas* strain produced both phenazine 1-carboxylic acid and pyoluteorin. J Microbiol Biotechnol, 15 : 86–90.

14. Huang, X.; Zhao, N. and Zhang, K. (2004) Extracellular enzymes serving as virulence factors in nematophagous fungi involved in infection of the host. Res Microbiol 155: 811-816.
15. Khaledi, N. and Taheri, P. (2016). Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. Journal of Plant Protection Research, 56(1) : 21 – 31.
16. Kim, D.J.; Baek, J.M.; Uribe, P.; Kenerley, C.M. and Cook, D.R. (2002) Cloning and characterization of multiple glycosyl hydrolase genes from *Trichoderma virens*. Curr Genet 40:374-384
17. Li , B.; Xie, G.L.; Soad, A. and Coosemans, J. (2005) Suppression of *Meloidogyne javanica* by antagonistic and plant growth-promoting rhizobacteria. J Zhejiang Univ Sci B, 6: 496-501.
18. Mcsorley, R.; Parrado, J. L. and Dankers, W. H. (1984) . A quantitative comparison of some methods for the extraction of nematodes from roots. Nematropica 14, 72-84.
19. Muhammad, S. A., Tariq, M. and Riaz, A. (2004). Some studies on the control of citrus nematode (*Tylenchulus semipenetrans*) by leaf Extracts of three plants and their effects on plant growth variables. Asian J. Plant Sci., 3(5): 544-548.
20. Mukhtar, T.; Hussain, M.A. and Kayani, M.Z. (2013). Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathologia Mediterranea, 52 (1) : 66 - 76.
21. Nandi, M.; Selin, C.; Brassinga, A. K. C.; Belmonte, M. F.; Fernando, W. G. D.; Loewen, P. C. and de Kievit, T. R. (2015). Pyrrolnitrin and hydrogen cyanide production by *Pseudomonas chlororaphis* strain PA23 exhibits nematicidal and repellent activity against

Caenorhabditis elegans. PLoS ONE, *10*(4), e0123184. http://doi.org/10.1371/journal.pone.0123184

22. Osei, K.; Addico, R.; Nafeo, A.; Edu-Kwarteng, A.; A. Agyemang, A.; Danso, Y. and Sackey-Asante, J. (2011). Effect of some organic waste extracts on hatching of *Meloidogyne incognita* eggs. African Journal of Agricultural Research, 6(10): 2255-2259.
23. Park, J. O.; Hargreaves, J. R.; McConville E. J.; Stirling, G. R.; Ghisalberti E. L. and Sivasithamparam, K. (2004). Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. Lett Appl Microbiol. 38(4) : 271-276.
24. Parvatha, R. P.; Rao, M. S. and Nagesh, M. (1996). Management of citrus nematode, *Tylenchulus semipenetrans* by integration of *Trichoderma harzianum* with oil cakes. Nematol. Medit. 24: 265-267.
25. Prasad, G.R.G.; Ravichandra, N.G.; Narasimhamurthy, T.N.; Kumar, C.H.P. and Yadahalli, P.(2014). Management of *Meloidogyne incognita* infecting carrot by using bioagents. Journal of Biopesticides , 7 (2) : 144-150.
26. Saeedizadeh, A. (2016). *Trichoderma viride* and *Pseudomonas fluorescens* CHA0 against *Meloidogyne javanica* in the rhizosphere of tomato plants. Hellenic Plant Protection Journal. 9 (1) : 28–34.
27. Shanmugaiah, V.; Balasubramanian, N.; Gomathinayagam, S.; Manoharan, P.T. and Rajendran, A. 2009. Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plants. African Journal of Agricultural Research 4 (11) : 1220-1225.
28. Siddiqui, I. A and Shaukat, S. S. 2004. *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of

Meloidogyne javanica by *Pseudomonas fluorescens* in tomato. Lett. Appl. Microbiol., 38(2) : 169–175.

29. Sivan, A.; Elad, Y. and Chet, I. (1984). Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology, 74, 498–501.
30. Tahikalange, T. E.; Meyer, J. J. M. and Hussein, A. A. (2005). Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. J. Ethnopharmacol., 96: 515- 519.
31. Timmer, L.W.; Garnsey, S.M. and Broadbent, P. (2003). Diseases of citrus. pp. 163-196. In diseases of tropical fruit crops. CAB International, Wallingford, UK.
32. Timper, P.; Koné, D.; Yin, J.; Ji, P. and Gardene, B.B.M. (2009). Evaluation of an antibiotic-producing strain of *Pseudomonas fluorescens* for suppression of plant-parasitic nematodes. J. Nematol. 41(3): 234–240.
33. Verdejo-Lucas, S. and Mckenry, M.V. (2004). Management of the citrus nematode *Tylenchulus semipenetrans* in citrus. J. Nematol 36, 424-432.
34. Walode, N. B.; Sinha, A.K. and Neog, P.P. (2008). Biological control of citrus nematode, *Tylenchulus semipenetrans* on *Citrus jambhiri*. Indian Journal of Nematology, 38 (2) : 244 – 246.
35. Zhong-Shan Y.; Guo-Hong L.; Pei-Ji, Z.; Xi , Z.; Shao-Liu, L.; Lei, L.; Xue-Mei, N. and Ke-Qin, Z. (2010). Nematicidal activity of *Trichoderma* spp. and isolation of an active compound.World Journal of Microbiology and Biotechnology 26 (12): (2297–2302).