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Research paper

Effect of the Nematode-Trapping Fungus Dactylaria Brochopaga and the Nematode Egg Parasitic Fungus Verticilium Chlamydosporium in Controlling Citrus Nematode Infesting Mandarin, And Interrelationship with the Co Inhabitant Fungi

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Abstract

In a field experiment, the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* , was evaluated against the citrus nematode, *Tylenchulus semipenetrans* infesting Mandarin, *Citrus reticulata*, , the mixed compound was applied in October 2016 at the rate ½ kg/tree, and root and soil samples were collected monthly until the next October, 2017. Successive treatment of the same mixed compound was added in the end of April 2017. For the comparison, Vydate 24% L. was applied as well as non treated check trees were left. Data revealed that the mixed compound treatment greatly affected the citrus nematode numbers both in soil and roots, in comparing with those of Vydate or induced by mixed compound was 97% and 70%; respectively in soil and roots. Rates of reproduction increase of the citrus nematode also reached 3% and 30% in both soil and roots; respectively. Vydate treatment resulted in a relatively lesser percentages. Growth of the concomitant fungus, *Trichoderma* sp. was increased specially in the last samples of October 2017, however those of fungi; *Aspergillus flavus, Fusarium* sp. And *Rhizopus* sp. was reduced, due to mixed compound treatment. *Aspergillus niger* and *Penicillium* sp. were not affected by the presence of the mixed compound. Vydate did not affect the co inhabitant fungi to a great extent.

Keywords*: T. Semipenetrans, biocontrol, Dactylaria brochopaga, Verticilium chlamydosporium, Mandarin.*

1. Introduction

Citrus plants are considered of economic importance in order to manage the citrus nematode, *T. semipenetrans* all over the world especially in tropical and subtropical countries. The fungal biocontrol of citrus nematode has been utilized in citrus groves and fields by many workers. In Florida, Trajan (1961) described the fungus *Dactylella doechsleri* as a new fungal species obtained from soil and roots of citrus plants. In a survey comprised 27 citrus groves in Florida, Walter and Kaplan (1990) recorded the infection of *T. semipenetrans* egg-masses by *Verticillium chlamydosporium, P. lilacinus, P. marquandii, Streptomyces* sp., *Arthrobotrys oligospora* and *Dactylella ellipospora.* Galuilina (1951) showed that the majority of predatory fungi found in Russia belong to Hyphomycetes. They were mainly distributed among the genera *Trichothecium, Arthrobotrys, Dactylaria* and *Dactylella.* In Egypt, Aboul-Eid (1963) reported that *A. conoides, A. oligospora, Dactylaria brochopaga* and *Dactylaria thaumasia* var. *Longa* were found in organic monured soil. Aboul-Eid *et al.*, (1997) found that *D. brochophaga* and *D. thaumasia* var. *Longa* are two of common Hyphomycetes nematode -attrapping fungi which have prevalence in the Egyptian environmental condition. In pot experiment studies, Mangat and Bhatti (1988) revealed that the maximum growth reduction of *Citrus jambdiri* seedling was obtained when *Fusarium solani* was used either simultaneously or 6 weeks after inoculation of *T. semipenetrans,* depends on type of the fungi isolate. Whereas, the nematode populations both in soil and roots were significantly reduced when the fungal inoculation preceded nematode inculcation by 6 weeks as compared with those of the nematode treated alone. Mani *et al.,* (1989) found the number of *T. semipenetrans* decreased markedly with increasing inoculum's levels of *P. lilacinus.* The population density of *T. semipenetrans* was greatly reduced and the corresponding plants growth criteria of acid lime, *Citrus aurantifolia* were effectively increased due to the biocide application by each of *P. lilacinus* (Parvatha-Reddy *et al.*, 1996a), *Glomus fasciculatum* (Reddy *et al.*, 1995), *T. harzianum* (Reddy *et al.*, 1996b) and by *Verticillium lecanii* (Reddy *et al.*, 1996c). Under field conditions, Mankau and Bartnicki (1987) found that populations of *T. semipenetarns* in established southern California citrus groves were generally associated with an abundant and highly varied flora of nematode trapping fungi, which included at least six *Arthrobotrys* spp. And a few *Monacrosporium* and *Dactylella* pp. Fernandez Diaz-Silveria and Ortega-Herra (1998) reported that loss of citrus yield due to *T. semipenetrans* averaged 9-1% in older orchards infested with high population densities. The use of *P. lilacinus* in citrus orchards decreased nematode numbers to some extent. Warrior *et al.,* (1999)

successfully utilized the fungus *Myrothecium* sp. In controlling citrus nematode in citrus fields in addition to several fruit orchards in North America. Noweer and Aboul-Eid (2013) studied the Biological control of root-knot nematode *Meloidogyne incognita* infesting cucumber *Cucumis sativus* L. cvs. *Alfa* by the nematode-trapping fungus *Dactylaria brochopaga* under field conditions. They found that the nematode-trapping fungus *D. brochopaga* alone or in combination with yeast, molasses and vermiculite reduced the juvenile-*Meloidogyne incognita*-population density per one kg soil and number of root-galls per one gm roots. Noweer and Mona E.M. Al-Shalaby (2014) evaluated nematophagous fungi *Dactylaria brochopaga* and *Arthrobotrys dactyloides* against *Meloidogyne incognita* infesting peanut plants under field conditions. They found that population densities of *M. incognita* in soil were significantly reduced in all treatments compared with control, as well as gall formation on peanut roots. Noweer (2014) studied the effects of some nematode-trapping fungi on the root-knot nematode *Meloidogyne* sp. infesting white bean *Phaseolus vulgaris* and sugar beet *Beta vulgaris* sp.*vulgaris* under field conditions. He found that the fungus *Dactylaria brochopaga* affected the development and reproduction of *Meloidogyne incognita* on white bean and sugar beet under field conditions. In the recent study, the mixed compound the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, as a new fungal nematicide was evaluated against *T. semipenetrans* infecting Mandarin and the interrelationship of the co inhabitant fungal communities.

2. Materials and Methods

In the locality of Abd-elsamad, Mansouria, Giza, Egypt, a highly infested farm of Mandarin, *Citrus reticulata* (Burm) with the citrus nematode, *T. semipenetrans* had been selected in which the experimental achievements were taken place. The form land was sandy in which Mandarin trees have been cultivated since 15 years ago. The above-ground symptoms consisted of yellowing of leaves, malnutrition and defoliated branch ends. The symptoms were particularly noticeable in the uppermost portions of the trees. In October 2016, trees were treated by the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, at the rate ½ kgm /tree every gm consist of 2000 conidia from the nematode-trapping fungus *Dactylaria brochopaga* and 1000 chlamydospores from the nematode egg parasitic fungus *Verticilium chlamydosporium* . The granular material was added to soil in circular tunnel around the stock of each tree, in 20 cm depth, then covered and irrigation was taken place. Another the mixed compound treatment was applied in the end of April 2017. For the comparison, another treatment using the chemical nematicide, Vydate 24% Liquid. At the rate 3 Liters per 600 Liters water/Acre., was applied to other trees as well. Each treatment was replicated four times involving those of non-treated trees to serve as a check. Pretreatment composite samples of both soil and roots were taken from the rhizosphere, and continued monthly until end of the trial in October 2017. Soil and root samples were collected in plastic bags and transferred directly to the lab, in which they were kept in a refrigerator under 4^{o}C., until nematode extraction. Nematodes were extracted from soil samples, of about 250cc. each, according to Cobb (1918) and Christie & Perry (1951). Data were statistically analyzed according to L.S.D. test and presented in tables 1& 2. The co inhabitant fungi in the soil rhizosphere of Mandarin trees were isolated using potato dextrose agar (PDA) media, according to Harwig *et al.*, (1979). The relative estimation of the abundant fungi was assessed four times through the year; October 2016, Dec 2016, May 2017 and October 2017. It was the categorized to five rates; (-) No fungi growth, (+) only one fungal colony, (++) from one colony to quarter the Petri-dish growth, (+++) half the Petri-dish growth, (++++) three quarter the Petri-dish growth and (+++++) all the Petri-dish growth. Growth of the assessed fungi in soil rhizosphere was estimated according to the same previous nematode replication, and means were calculated and presented in Table 3.

3. Results

Data in Table 1 indicate that adding the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* in October, 2016 directly decreased numbers of citrus nematode, *T. semipenetrans* in soil after treatment. The effectiveness of control lessened then for one month, and increased gradually but in April, 2017 resulted in excessive decreased to nematode reproduction, where the population density recorded was 95 larvae/kg. Soil, in samples of June 2017. The percent reduction of the nematode population was continued in which the population density was 65 larvae/1 kg. Soil in samples of October 2017, comparing with those of Vydate treatment and non-treated check (928 and 1006 larvae/kg.; respectively). In soil, the % reduction of the nematode numbers at the end of the trial, induced by the treatment was 97% comparing with that of the check (40%). At the end of the trial also, the % reproductive increase of *T. semipenetrans* were 3%, 71% and 60% in samples of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, Vydate and none treated check treatments; respectively. The chemical treatment with Vydate affected numbers of the citrus nematode directly after application to a great extent, and then the efficacy decreased gradually until it had vanished. Data in Table 2 revealed that numbers of the citrus nematode recovered from the incubated roots were also greatly affected by treatment of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*. Number of the citrus nematode recovered in October, 2016 was 82 larvae/5 g roots; and it reached 19 larvae by the end of trial in which the % reduction was 70%. Numbers of *T. semipenetrans* larvae proliferated from lemon roots in either Vydate treatment or non-treated check trees were not little as compared with those from biocide one. The % increase of the citrus nematode larvae reproduced on roots were 30%, 153% and 159% due to the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, Vydate and non-treated check treatments respectively. The abundant fungi in the soil rhizosphere of Mandarin trees were fluctuated variably due to the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* application (Table 3). Colonies of the associated fungi; *A. flavus*, *Fusarium* sp. and *Rhizopus* sp. were obviously decrease as a result of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* treatment, however those of *Trichoderma* sp. were increasingly propagated. Data also revealed that either *A. niger* or *Penicillium* sp. were not affected by the presence of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, all over the year of trial. *Penicillium* sp. occurred dominantly the most in which a heavy or moderate growth was proliferated from samples of Mandarin rhizosphere. The fungus persisted dominantly, in soil samples taken from the treated the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* trees. However, its number decreased progressively with the successive periodical samples until the end of the trial. It was evidently also that the presence of *A. flavus*, *A.niger*, *Fusarium* sp. and *Rhizopus* sp. were not affected in samples of Vydate and non-treated check, through the trial year to a great extent.

4. Discussion

Biocontrol management of nematodes had been intensively taken place among nematode control research in the recent decade. The present data clarify that utilization of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium*, significantly minimized numbers of *T. semipenetrans* infecting Mandarin trees both in soil and roots (Table 1 & 2). The population density, % reduction and % effectiveness control were consequently affected greatly in comparing with those of Vydate chemical treatment and non-treated check. Many fungal agents gave similar results in controlling citrus nematode in citrus groves and orchards (Mankau & Bartnicki, 1987; Walter & Kaplan, 1990; Fernandez-Diaz-Silveria & Ortega-Herro 1998; Walker & Morey, 1999 and Warrior *et al*., 1999). The highest effectiveness % of the citrus nematode control due to the mixed compound fungi was noticed directly, one month after treatment, in October 2016. The effectiveness % decreased greatly in December, and was reactivated another time in the next months, to less encouraging percentages to some extent. So, a successive treatment of the mixed compound fungi was applied at the same concentration in the end of April 2017. Consequently, the population density, % reduction and % effectiveness were acceptably reactivated nextly until the end of the trial. Similar findings were reported by Snathi *et al.*, (1998). Biocontrol agents retarded multiplication of *T. semipenetrans* on Mandarin and orange, and that was accompanied by healthy improvement of trees. No further improvement of determination in the trees health from the conditions observed four months after treatment. The reproduction increased (R.I. %) of *T. semipenetrans* was noticeably retarded either in soil or in roots of Mandarin trees (3% and 30% respectively) compared with those of Vydate treatment (71% & 153% resp.) and non-treated check also (60% & 159% resp.). Partly similar findings were reported by Reddy *et al*., (1996c) and Snathi *et al.*, (1998). On the contrary, Walker and Morey (1999) reported that under pot and field experiments in naturally infested soil with citrus nematode, microbial antagonists performed poorly against *T. semipenetrans* on oranges and Mandarin. They also noticed that dry formulation of spores of *Dactylella* sp. and *Beauveria* sp. did not reduce nematode levels. Mode of action of the utilized fungus, *D. brochophaga* had been offered by Shephered (1955). The fungus was more effective antagonist. This may have been due to affecting the nematode larvae through production of traps which capture the larvae and dissolve the outer skeleton and digest the inner content of the victim. Aboul-Eid (1963) also reported that *D. brochopaga* has constricting rings responsible for nematode capturing or trapping mechanism. Data in Table (3) revealed that treatment of the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* affected greatly growth of the abundant fungi; *A. flavus*, *Fusarium* sp. and *Rhizopus* sp., which may be less competitive. It showed also that *A.niger* and *Penicillium* sp. were not affected by the mixed compound treatment, and the latter one was the most prevalent coin habitant through the trial year. The efficacy of *D. brochopaga* and *V. chlamydosporium* on the abundant fungi may be indirectly affected *T. semipenetrans* infecting Mandarin trees. Consequently, the adversely affect on citrus nematode may be attributed to the fungal biocide itself, and the indirect action of the affected abundant antagonistic fungi. Results evidently proved also that the mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* persisted dominantly in soil, but in slightly-declined trend, until the end of trial. So that, the fungal application may better be squinty revaluated.

5. Conclusions:-

1- The mixed compound from nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* was greatly affected on the citrus nematode numbers both in soil and roots.

2- The population density, % reduction and % effectiveness control were consequently affected greatly in comparing with those of Vydate chemical treatment and non-treated check.

3- The mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* affected greatly growth of the abundant fungi.

4- The mixed compound from the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* persisted dominantly in soil, but in slightly-declined trend, until the end of trial. So that, the fungal application may better be squinty revaluated.

5- Vydate did not affect the co inhabitant fungi to a great extent.

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Table (1): Population density and reproduction of Tylenchulus semipenetrans in soil of Mandarin trees, as affected by treatment of the nematode-trapping fungus Dactylaria brochopaga and the nematode egg parasitic fungus Verticilium chlamydosporium.

*No. of Larvae/1 kg., Soil																																						
Treatment	Oct. 2016	November 2016			December 2016			January 2017			February 2017			March 2017			April 2017			May 2017			June 2017			July 2017			August 2017			September 2017			October 2017			
	No.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	No.	%R.	%EF.	%R.I.
the mixed compound	1806	782	54	85	529	78	60	496	79	88	468	80	117	393	85	131	268	91	132	246	92	124	187	95	141	177	96	156	144	98	113	81	97	136	65	97	174	3
Vydate	1107	974	17	--	715	53	--	780	48	--	841	43	--	832	44	--	780	48	--	902	38	--	910	38	--	949	35	--	900	38	--	885	34	--	928	29	--	71
Control (without treatment)	1571	1016	33	0	850	55	0	966	48	0	1067	42	2	1072	45	0	1013	45	7	984	47	0	1017	48	0	1056	43	0	895	52	0	914	47	0	1006	40	0	60
L.S.D.(0.05)	--	168			112			109			208			214			178			224			278			262			304			320			279			
(0.01)	--	--			146			142			270			279			249			292			360			342			395			416			362			

Reduction % (%R.) = $\frac{\text{Reduction in treatment}}{\text{Number before treatment}} \times 100$

Effectiveness % (%Ef.) = $\frac{\text{Reduction in treatment - reduction in control}}{\text{Reduction in control}} \times 100$

Reproduction increase % (R.I. %) = $\frac{\text{Final number}}{\text{Initial number}} \times 100$

*** Mean of four replicates.**

Table (2): Number of Tylenchulus semipenetrans larvae, on roots of Mandarin trees as affected by the nematode-trapping fungus Dactylaria brochopaga and the nematode egg parasitic fungus Verticilium chlamydosporium.

*No. of Larvae/ 5 gm., Roots.																																						
Treatment	Oct. 2016	November 2016			December 2016			January 2017			February 2017			March 2017			April 2017			May 2017			June 2017			July 2017			August 2017			September 2017			October 2017			
	No.	No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		No.	%R.		%R.I.
the mixed compound	82	41	58		34	68		26	76		19	79		16	76		11	86		9	86		8	90		13	83		10	85		8	90		19	70		30
Vydate	66	100	0		44	40		66	0		50	16		47	13		32	44		57	0		59	0		68	0		70	0		73	0		82	0		153
Control (without treatment)	99	98	0		66	38		54	49		64	32		46	17		43	52		33	61		78	13		117	0		120	0		121	0		138	0		159
L.S.D.																																						
(0.05)		51			8			9			12			8			6			11			12			18			22			16			21			
(0.01)		21			12			13			16			13			9			16			17			25			23			22			30			

Reduction % (%R.) = $\frac{\text{Reduction in treatment}}{\text{Number before treatment}} \times 100$

Reproduction Increase % (R.I. %) = $\frac{\text{Final number}}{\text{Initial number}} \times 100$

*** Mean of four replicates.**

Table (3): Persistence of Soil fungi co inhabiting rhizosphere of Mandarin trees, as affected by treatment of the nematode-trapping fungus Dactylaria brochopaga and the nematode egg parasitic fungus Verticilium chlamydosporium.

	Soil Fungi																											
	Aspergillus flavus				***A. niger***				***Fusarium* sp.**				***Penicillium* sp.**				***Rhizopus* sp.**				***Trichoderma* sp.**				**Dactylaria brochophaga**			
Treat-ments	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**	**Oct. 2016**	**Dec. 2016**	**May 2017**	**Oct. 2017**
the mixed com-pound	++	+	+	-	+	+	+	+	+	+	-	-	+++	++	+	+	+	+	-	-	-	-	+	++	-	**1.5 x10^3**	**5.5 x10^3**	**6.5 x10^3**
Vydate	+	++	++	++	+	++	++	++	++	++	++	++	+++	+++	++	+++	+	+	++	++	-	+	-	-	-	-	-	-
Control (without treat-ment)	+	+	++	++	+	+	+	++	+	++	++	+	++	++	++	+++	+	+	++	++	-	+	+	+	-	-	-	-

*** Spore/g. soil.**