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Research article

Biological control of root-knot nematode *Meloidogyne incognita* infesting Eggplant by the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg parasitic fungus *Verticilium chlamydosporium* under field conditions

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Key words: Microbial control, *Dactylaria brochopaga, Verticilium chlamydosporium, Meloidogyne incognita.* Yeast, Molasses, Vermiculite, Eggplant.

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Abstract

Microbial control of root-knot nematode which infested Eggplant (*Solanum melongena*) by using the nematode-trapping fungus *Dactylaria brochopaga* & *Verticilium chlamydosporium* as a combined with yeast, molasses and vermiculate (F1+F2AVYM) is reported under field condition study. The results revealed that the highest percentage reduction in number of nematode larvae per 1kg soil was achieved when applying the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. Also the highest percentage reduction in number of root-galls per plant (93.1 %) was achieved by using the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. The data revealed that the weight of fruits per plant were significantly ($P \leq 0.05$ and/or $P \leq 0.01$) increased in all nematode-trapping fungus *D. brochopaga* treatments compared to the untreated check treatment.

Introduction

Most research on microbial agents that attack nematodes in soil has concerned fungi, especially those that form traps to ensnare their prey. The nematode-trapping fungi; the first nematode- trapping fungus, the ubiquitous *Arthrobotrys oligospora*, was described by Fersenius, [1] Zopf [2] observed that the fungus could capture motile nematodes and parasitize them. Mai and Chen [3] used the nematode trapping fungi (N.T.F.) *Arthrobotrys superba, A. dactyloides, A. arthrobotryoides,* and *Dactylella doedycoides* to reduce the penetration of alfa-alfa roots by *Pratylenchus penetrans in* sterilized soil. Niblack and Hussey [4] evaluated (N.T.F.), *A. amerospora,* combined in 3 commercial preparations with *Rhizobium japanicum* inoculums for controlling of *Heterodera glycines* on soybean (Glycine max) in the field and greenhouse. In a greenhouse experiment conducted to evaluate the effect of pigeon droppings and *A. oligospora* as a soil amendment on the root- knot nematodes *Meloidogyne incognita* on Muskmelon, Ali [5] indicated that there was a trend towards nematode population diminution with greater efficiency when the fungus has been added soil 2 weeks before to nematode inoculation and planting. Inoculums density of *A. oligospora* was positively correlated with number of juveniles and galls per gram of root before treatment. The fungus, *Verticillium chlamydosporium* parasitizes eggs of root-knot and cyst nematodes [6]. Also, it infects nematode eggs and sedentary females of cyst nematodes by hyphae produced on actively growing mycelium [7]. *V. chlamydosporium* colonizes the rhizosphere, which facilitates the infection of egg masses protruding from female root-knot nematodes on infected roots [8]. The fungi *A. dactyloides, A. oligosora, Macrosporium ellipsosporium,* and *M. cionopagum*, parasites on most of the *Pratylenchus penetrans* adults and juveniles which added to the fungus cultures [9]. Anter *et al.* [10] in a greenhouse experiment, *A. conoides* and *A. oligospora* showed the highest effect on reducing *M. incognita* numbers during the first 4 weeks. *A. conoides* and *A. oligospora* showed highly influential on the activity J_2 of *M. incognita* compared to the control treatment [11]. *Arthrobotrys oligospora, A. conoides, Arthrobotrys sp., Dactylaria shelensis, and Dactylaria sp.* and *Monacrosporium* were tested by Duponnois *et al.*, [12] for their trapping ability against *Meloidogyne mayaguensis.* One *Dactylaria* strain and most of the *Arthrobotrys* strains reduced the population of nematodes. An Egyptian population of *D. brochopaga* the effect on nematodes has a great impact through production of traps which capture the larvae and dissolve nematode outer cuticle and digest the inner content of the victim [13]. Noweer and Mona, E.M. Alshalaby [14] showed that the

effect of *V. chlamydosporium* combined with some organic manure on *Meloidogyne incognita* and other soil micro-organisms on tomato under field condition decreased the counts of *Meloidogyne incognita* juveniles in soil, as well as, gall formation on roots. Noweer and Aboul-Eid [15] obtained that the biological control of root-knot nematode *Meloidogyne incognita* infesting cucumber *Cucumis sativus* L. cvs. *Alfa* by (N.T.F.) *D. brochopaga* under field conditions. They showed that the *D. brochopaga* alone or in combination with yeast, molasses and vermiculite reduced the juvenile-*M. incognita*-population density per 1 kg soil and number of root-galls per 1 gm roots. Aboul-Eid *et al.* [16] studied the impact of the nematode-trapping fungus, *Dactylaria brochopaga* as a biocontrol agent against *Meloidogyne incognita* infesting Superior grapevine. They found that all treatments significantly reduced *M. incognita* J_2 in soil and number of root galls compared with the untreated control. Significant yield increases have been observed with all treatments compared with the untreated control. Spores suspension twice applications gave the highest yield production. Noweer and Mona E.M. Al-Shalaby [17] 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 [18] found that 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 showed that the *D. brochopaga* affected the development and reproduction of *M. incognita* on sugar beet and white bean under field conditions. Noweer., [19] investigated the 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. He showed that the mixed compound treatment greatly affected the citrus nematode (*Tylenchulus semipnetrans*) 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. In the present study, the effect of the nematode-trapping fungus *D. brochopaga* & *Verticilium chlamydosporium* as a combined with yeast, molasses and vermiculate on the development and reproduction of *M. incognita* infested Eggplant plants is reported under field condition.

Materials and methods

The experiment was carried out through the period from 20 January 2018 to 27 May 2018 on an area almost ten karats' cultivated to Eggplant, *Solanum melongena*, variety Balady in Abdel-samad village, Imbaba, Giza. The soil of the experimental site is sand with a high level of natural infestation of root-knot nematodes *Meloidogyne incognita.* The seedlings of Eggplant were planted in January 20, 2018. Pre-treatment soil samples were taken, to determine the level of root-knot nematode infestation and contained from three simple samples (65 gm/sample) from each three rows per treatment as one composite soil sample (250 gm). A modified technique of Christie and Perry [20] methods had been chosen for nematode extraction because it illustrates a simpler technique requiring only minimum equipment for sandy soil processing. The sieves used in nematode processing were 2 mm hole diameter sieve and two 325 mesh sieves. Trapped Nematode suspensions washed from the last two sieves were transferred directly into glass beakers without using a centrifugal flotation technique step. Examination and counting of nematodes were made under a research microscope and root- knot nematode juveniles were identified by referring to the identification of ten root-knot females attached to the naturally- infested plant roots. Root – knot nematode identification was achieved according to Jepson Susan [21].

The fungus (Every one gm contain 5000 spores from the nematode-trapping fungus *Dactylaria brochopaga* collecting from the surface of Petri dishes contains the fungus grows on water agar medium, this fungus which has constricting rings responsible for nematode capturing through trapping mechanism with 5000 spores from the nematode egg parasitic fungus *Verticilium chlamydosporium* collecting from the slants contains the fungus grows on PDA medium this fungus which feeding on the nematode egg-masses was introduced into soil by different ways see table 1. The use of the two fungi together for different feeding method on the nematodes, whether nematode egg-masses or larvae that hatches from the nematode egg-masses. Compared with the nematicide Furidan treatment and an untreated treatment as control (Table 1). Percentage reduction in Root-knot second stage juvenile population in soil were determined according to the formula of Handerson & Tilton (Puntener) [22] where % Re= % reduction (% efficiency).

$$\text{Re \%} = 1 - \frac{J_2 \text{population in the treated plots after application} \times J_2 \text{ population in the check plots before application}}{J_2 \text{ population the in treated plots before application} \times J_2 \text{ population in the check plots after application}} \times 100$$

Data on population densities of *M. incognita* in both soil and root samples taken from the three replicates per treatment on 20/1, and 25/3 and 27/5/2018. The yield of Eggplant was taken throw the period 25/3 to 27/5/2018 at the end of experiment (table 4).

Table 1. Type and concentration of applications of the nematode-trapping fungus *Dactylaria brochopaga*, nematode egg parasitic fungus *Verticilium chlamydosporium* and other non-chemical materials in Eggplant plants.

Treatments	Type of application	Concentration/spot
(F1AVYM)	Fungus *Dactylaria brochopaga* carried on agar and vermiculite with yeast and molasses substrate	2gm (Fungus) + 3gm (Vermiculite) + 1gm (Yeast) + 1ml (Molasses)*.
(F2AVYM)	Fungus *Verticilium chlamydosporium* carried on agar and vermiculite with yeast and molasses substrate	2gm (Fungus) + 3gm (Vermiculite) + 1gm (Yeast) + 1ml (Molasses)*
(F1+F2AVYM)	Fungi carried on agar and vermiculite with yeast and molasses substrate	2gm (Fungi) + 3gm (Vermiculite) + 1gm (Yeast) + 1ml (Molasses)*.
(VY)	Vermiculite with yeast	3gm (Vermiculite) + 1gm (Yeast)*
(VM)	Vermiculite with molasses	3gm (Vermiculite) +1ml (Molasses)*
(V)	Vermiculite alone	3gm (Vermiculite) *
(FUR)	Furidan G.	1gm*
(Control)	Untreated control	Without addition

*With planting F1= *Dactylaria brochopaga*, F2= *Verticilium chlamydosporium*
A=Agar, V= Vermiculite, Y= Yeast, M= Molasses, FUR= Furidan

Results

Effects of the nematode-trapping fungus *Dactylaria brochopaga* & *Verticilium chlamydosporium* as a combined with yeast, molasses and vermiculate (F1+F2AVYM) on reproduction of root-knot nematode *Meloidogyne incognita* infesting Eggplant plants variety Balady are shown in tables (2, 3). Data in table 2 reveals that the highest decrease in the number of larvae per one kg soil was shown with treatment by (F1+F2AVYM). On March 25, 2018: The number of larvae per kg soil was decreased in almost all treatments especially it was 263 larvae per kg for the (F1+F2AVYM) compared to the untreated control 995. On May 27, 2018: The number of larvae per kg soil was decreased in almost all treatments especially it was 235 larvae per kg for the (F1+F2AVYM) compared to the untreated control 1739. The percentage reduction of the number of larvae per one kg soil was 84.7% for (F1+F2AVYM) on March 25, 2018, while it was 88.6% for (F1+F2AVYM) on May 27, 2018. Data in table (3) reveals that the highest decrease in the number of galls per 5gm roots was noted with treatment by the (F1+F2AVYM). On March 25, 2018: The number of galls per 5gm roots was decreased in almost all treatments especially it was 10 galls for (F1+F2AVYM) compared to the untreated control 36. On May 27, 2018: The number of galls per 5gm roots was decreased in almost all treatments especially it was 11 galls for (F1+F2AVYM) compared to the untreated control 94.

The percentage reduction of the number of galls per 5 gm roots were 92.9% for (F1+F2AVYM) on March 25, 2018, while it was 93.1% for (F1+F2AVYM) on May 27, 2018.

Effects on mean production of eggplant variety balady plants

The highest mean production of Eggplant variety Balady plants per one plant in the different treatments was 18 kg/plant for the (F1+F2AVYM), and it was 2160 kg per one plot (kirate). While the mean production of Eggplant variety Balady plants per one plant in the untreated control treatment was 5 k g/plant, and it was 600 kg per one plot (kirate). The length period of collection of the eggplant fruits explained that the use of difference in the quantity of production to show the efficiency of different treatments.

Discussion

The present results indicate that the fungus *Dactylaria brochopaga* or the fungus *Verticilium chlamydosporium* was affected on the development and reproduction of *Meloidogyne incognita* on Eggplant plants variety Balady under field conditions especially for the (F1+F2AVYM). This was indicated by the lower numbers of juveniles in soil, lower numbers of galls per 5gm roots, the % reduction in population density of soil larvae, in treatment of the nematophagous *A. dactyloides*. Aboul-Eid *et al.*, [13] reported that *Dactylaria brochopaga* has constricting rings responsible for nematode capturing through trapping mechanism.

Table 2. Effects of the nematode-trapping fungus *Dactylaria brochopaga* & *Verticilium chlamydosporium* alone or mixed with non-chemical materials on population density of root-knot nematode *M. incognita* larvae infested Eggplant plants.

Treatment	Population density of larvae/one kgm soil					
	20/1/2018	25/3/2018		27/5/2018		
	Initial No.	No. *	R% **	No. *	R% **	XR% ***
(F1AVYM)	445	329d	74.2	315d	90.1	80.4
(F2AVVYM)	393	305d	79.8	293d	92.4	85
(F1+F2AVYM)	411	263d	84.7	235d	86.6	88.6
(VY)	385	615c	59.9	863c	63.6	61.8
(VM)	415	629bc	62.7	1019bc	54.2	58.5
(V)	403	811ab	28.9	1325b	30	29.5
(FUR)	491	389d	82.2	1185bc	47.5	45.4
(Control)	421	995a	----	1739a	----	----

* Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.
** % Red. = % reduction (% efficiency) according to Anderson & Tilton formula.
***XR=Mean reduction due to treatments. F1= *Dactylaria brochopaga*, F2= *Verticilium chlamydosporium.*
A=Agar, V= Vermiculite, Y= Yeast, M= Molasses, FUR= Furidan.

Table 3. Effects of the nematode-trapping fungus *Dactylaria brochopaga* & *Verticilium chlamydosporium* alone or mixed with non-chemical materials on galls number of root-knot nematode *M. incognita* larvae infesting Eggplant plants.

Treatment	Number of galls/5gm roots				
	25/3/2018		27/5/2018		
	No. *	R% **	No. *	R% **	XR%***
(F1AVYM)	16c	71.4	20d	86	78.7
(F2AVYM)	12c	85.7	17d	89.5	87.6
(F1+F2AVYM)	10d	92.9	11d	95.3	93.1
(VY)	30b	21.4	56b	44.2	32.8
(VM)	32ab	14.3	70b	27.9	21.1
(V)	40a	14.3	96a	2.3	8.3
(FUR)	27b	32.1	32c	72.1	52.1
(Control)	36a	----	94a	----	----

*Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.
** % Red. = % reduction (% efficiency) according to Anderson & Tilton formula.
***XR=Mean reduction due to treatments.
F1= *Dactylaria brochopaga*, F2= *Verticilium chlamydosporium.*
A=Agar, V= Vermiculite, Y= Yeast, M= Molasses, FUR= Furidan.

Table 4. Mean production of Eggplant variety Balady plants per one plant and one plot (kirate).

Treatment	Mean of Production	
	Per one plant (Kg)*	Per one kirate (Kg)*
(F1AVYM)	12e	1440d
(F2AVYM)	11e	1320e
(F1+F2AVYM)	18e	2160e
(VY)	9c	1080c
(VM)	8bc	960bc
(V)	6ab	720ab
(FUR)	10d	1300c
(Control)	5a	600a

*Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.
F1= *Dactylaria brochopaga*, F2= *Verticilium chlamydosporium.*
A=Agar, V= Vermiculite, Y= Yeast, M= Molasses, FUR= Furidan.

The two fungi proved to be more effective nematode-antagonist and may have been affecting nematode population larvae through production of traps which capture the larvae and dissolve the nematode outer cuticle digest the inner content of the victim and parasitic on nematode eggs [23]. This information explains the results of this work. Moreover, Mankau [24] stated that the nematode- destroying fungi play a major role in recycling the carbon, nitrogen, and other important elements from the rather substantial of nematodes which browse on microbial primary decomposers. Certain fungal agents gave similar results in controlling citrus nematode in citrus groves and orchards [25]. The results of work with similar formulations of the nematode-trapping fungus *Arthrobotrys dactyloides* also has constricting rings responsible for nematode capturing through trapping mechanism has shown potential as a biological control agent against *Meloidogyne javanica* in soil microcosms [26].

Finally the (F1+F2AVYM) could be of great impact on the future of biotic and/or organic farming approach especially for the exported crops and other important foodstuff agricultural commodities especially for the two Fungi *Dactylaria brochopaga* & *Verticilium chlamydosporium* spores and broadcasted mycelia carried on granules of vermiculite, yeast, and molass.

Conclusions

Microbial control of root-knot nematode which infested Eggplant (*Solanum melongena*) by using the nematode-trapping fungus *Dactylaria brochopaga* & *Verticilium chlamydosporium* as a combined with yeast, molasses and vermiculate (F1+F2AVYM) is reported under field condition study. The results revealed that the highest percentage reduction in number of nematode larvae per 1kg soil was achieved when applying the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. The highest percentage reduction in number of root-galls per plant (93.1 %) was achieved by using the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. The weight of fruits per plant were significantly ($P \leq 0.05$ and/or $P \leq 0.01$) increased in all nematode-trapping fungus *D. brochopaga* treatments compared to the untreated check treatment. The use of the two Fungi *Dactylaria brochopaga* & *Verticilium chlamydosporium* spores and broadcasted mycelia carried on granules of vermiculite, yeast, and molass could be of great impact on the future of biotic and/or organic farming approach especially for the exported crops and other important foodstuff agricultural commodities.

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