A FIELD TRIAL TO USE THE NEMATODE-TRAPPING FUNGI *DACTYLARIA BROCHOPAGA* AND *ARTHROBOTRYS CONOIDES* TO CONTROL ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* INFESTING BANANA *(MUSA SAPIENTUM)*

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Abstract

A field trial to control the root-knot nematode *Meloidogyne incognita* infesting Banana *Musa sapientum* by using the nematode-trapping fungus *Dactylaria brochopaga* alone or mixed with nematode-trapping fungus *Arthrobotrys conoides* loaded on crushed vermiculite, molasses and yeast is reported under an open field experiment. The data revealed that the nematode-trapping fungus *D. brochopaga* in combination with nematode-trapping fungus *A. conoides*, yeast, molasses and vermiculite reduced the juvenile - *Meloidogyne incognita*-population density per one kg soil and number of root-galls per five gm roots. It was followed by the nematode-trapping fungus *D. brochopaga* in combination with yeast, molasses and vermiculite. Next ranked the nematode-trapping fungus *A. conoides* in combination with yeast, molasses and vermiculite. The weight of banana fruits per plant was significantly (P≤0.05) increased in each fungus treatment compared to the untreated diseased check.

Key words: nematode-trapping fungi, yeast, molasses, vermiculite, banana.

INTRODUCTION

Most horticultural and agricultural crops are attacked by one or more species of plant parasitic nematodes. These infections often cause severe yield loss and farmers are usually dependent on nematicides to get a substantial harvest. Several chemical nematicides cause health and environmental problems. Fungi that parasitize on nematodes have been suggested as alternative non-polluting methods of controlling plant-parasitic nematodes (Stirling, 1991; Kaya & Stock 1997). Nematode-trapping fungi are subsequently metabolized, in adhesive nets formed from inter-woven hyphae. To obtain successful plant protection, it is essential that the fungi establish, survive and proliferate through soil as mycelia capable of forming these traps. These fungi are common in a wide range of soil habitats throughout the world (Gray, 1987). Jaffee et al. (1996) found 12 species of nematode trapping fungi; these fungi are of special interest because of their possible involvement in food chain interactions. The history of attempts to use predaceous fungi to control plant-parasitic nematodes has been the subject of several reviews. Rao and Malek (1973) found that the fungi *Arthrobotrys dactyloides* (Drechsler), *A. arthrobotryoides* and *Dactylaria thaumasia* slowed the population increase of *Pratylenchus penetrans* on alfalfa in the laboratory and greenhouse. Of the three tested fungi, *A. dactyloides* was the most effective antagonist. Hertz and Dackman (1992) reported that conidia of *A. oligospora* germinated directly into adhesive traps when applied close to cow faeces on water agar plates, the conidial trap is considered a survival structure enabling the fungus to overcome fungi stasis. Traps adhere to the surface of passing nematodes, thus facilitating the spread of the fungus, before penetration of the nematode cuticle and immobilization of the nematode take place. *A. oligospora* and *A. conoides* showed high effect on the activity of the control treatment (El-Sawy, 1994). Aboul-Eid et al. (1997) found that *Dactylaria brochophaga* and *D. thaumasia var. Longa* are two common hyphomyceted nematode-trapping fungi which have prevalence in the Egyptian environmental conditions. The results of the development of kaolin-based formulations of *A. dactyloides*, a nematode-trapping fungus, have shown potential as a biological control agent against *Meloidogyne javanica* (Treub) Chitwood in soil microcosms (Stirling & Mani 1995; Jaffee, 1999). *A. oligospora, A. conoides, Arthrobotrys sp., Dactylaria shelensis, Dactylaria sp.*, and *Monacrosporium bembicodes* were tested by Duponnois et al. (1997) for their trapping ability against *Meloidogyne mayagyensis*. Most of the *Arthrobotrys* strains and one

Dactylaria strain decreased the development of the nematodes. An Egyptian population of *D. brochopaga* proved to be more effective nematode-antagonist and affecting nematode population larvae through production of traps which capture the larvae and dissolve nematode outer cuticle and digest the inner content of the victim (Aboul-Eid, 1963, and Aboul-Eid et al., 2002). Noweer, (2005) studied the efficacy of the nematode-trapping fungus *D. brochopaga* and biofertilizers, on controlling the root-knot nematode *Meloidogyne incognita* infecting Tomato. Noweer and Aboul-Eid (2013) studied the Biological control of root-knot nematode *M. incognita* infesting cucumber *Cucumis sativus* L. cvs. *Alfa* by the nematode-trapping fungus *D. brochopaga* under field conditions.Noweer and Mona (2014) studied the evaluation of Nematophagous fungi *D. brochopaga* and *A. dactyloides* against *M. incognita* infesting peanut plants under field conditions. 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.

Therefore, the objective of this study was to evaluate the effects of nematode-trapping fungi, *Dactylaria brochopaga* and *Arthrobotrys conoides,* loaded on crushed vermiculite, molasses and yeast against the root-knot nematode, *Meloidogyne incognita* infesting banana.

MATERIALS AND METHODS

Isolation and Identification of Fungi

Twenty grams of agar were added to one liter of distilled water and dissolved if needed on a water bath. The medium was then poured in 10 ml aliquots into a series of glass tubes and sterilization was made by autoclaving for 20 minutes at 15 lbs pressure. The idea of using such a poor medium is to cut down the growth of other moulds such as Mucorales and the more vigorously growing Hyphomyceted in order to give chance to the more delicate growing nematophagous fungi. Addition of steriled nematodes to culture plates stimulates trap formation in nematophagous fungi (Comandon and De Fonbrune, 1938). *D. brochopaga* and *A. conoides* were isolated from Egyptian soil by using water agar medium (Snyder and Hansen, 1947) and identified by referring to the detailed descriptions and keys offered by (Dollfuss 1946; Alexapoulos & Mism 1979; Aboul-Eid et al. 1997). Propagulus suspensions of fungus were prepared by adding sterilized distilled water to the fungus culture surface and shaking the Petri-dishes to get the propagulus suspensions.

Field Experiment

The work was carried out in a banana orchard infested with *M. incognita* through the period from May 2013 to April 2014 in a banana plantations farm located in El- Entelak village, Southern Tahrir Region, El- Behera governorate Egypt. The farm soil is sandy and the farm is planted to two years old banana *Musa cavendishii* L. cv. Grandnan. Nile water and chemical fertilizers are pumped through drip irrigation network and all other agricultural practices were applied according to the technical recommendations of banana growing. Six treatments were applied experimentally; each was replicated in ten banana spots making a total of 60 experimental spots with 2-3 banana plantations in each spot. Pretreatment soil and root samples were taken on May 6, 2013 from each spot at the rate of one stratified sample (250 g) composed of five simple samples (50 g) per spot. Soil and root samples were collected from the rhizosphere of plants, composed of almost 250 gm soil in weight- unless otherwise mentioned in descriptions of field experiments- and was taken generally from 10-30 cm depth in soil into polyethylene – plastic bags. Supporting data such as the exact locality of the sample, date of sampling, banana data, soil data and other data of importance were recorded on a fixed card to the sample bag. Bags were then sealed, carried to the laboratory and stored in a refrigerator on 5-7^{o}C until processing. Nematode suspension of M. incognita larvae extracted from infested soil, cultivated with banana by a modified method (Christie & Perry 1951) followed by a centrifugal-flotation technique (Jenkins, 1964). A modified technique of Cobb (1918) 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 (1987) and Oteifa (1963) using perennial pattern. The fungi were introduced into soil by spores and broadcasted mycelia carried on agar and vermiculite substrate with yeast and molass were incorporated into soil to a depth of three cm's. Six separate treatments were made as described in Table (1).

Table 1. Type and dose of the nematode-trapping fungi *Dactylaria brochopaga*, *Arthrobotrys conoides* and the other treatments in banana open-field experiment

Treatments	Type of application	Concentration/spot
1	Fungi *D. brochopaga* and *A. conoides* carried on agar and vermiculite with yeast and molasses substrate	*D. brochopaga* 125x100 spors and *A. conoides* 125x100 spors + 1 Kgm (Vermiculite) + 10 gm (Yeast) + 20 ml (Molasses).
2	Fungus *D. brochopaga* carried on agar and vermiculite with yeast and molasses substrate	*D. brochopaga*25x1000spors + 1 Kgm (Vermiculite) + 10 gm (Yeast) + 20ml (Molasses).
3	Fungus *A. conoides* carried on agar and vermiculite with yeast and molasses substrate	*A. conoides* 25x1000 spors + 1 Kgm (Vermiculite) + 10 gm (Yeast) + 20 ml (Molasses).
4	vermiculite with yeast and molasses substrate	1 Kgm (Vermiculite) + 10 gm (Yeast) + 20 ml (Molasses).
5	Furidan G.	20 gm
6	Untreated control	Without addition

The weight of banana fruits production of the all treatments was recorded. *M. incognita* analysis: Soil and root samples were taken before treatments (May 6, 2013) and after treatments (4/9/2013, 6/1/2014 and 28/4/2014 to estimate the populations and reduction % of nematode in soil (calculated according to Handerson & Tilton formula.

$$\textbf{Reduction \%} = 1 - \frac{\text{No. in treatment after application} *}{\text{No. in control before application}} \times 100$$

$$\frac{\text{No. in treatment before application} *}{\text{No. in control after application}}$$

(Puntener, 1981). Numbers of root galls per 5g roots were also determined.

Statistical analysis

Data were analyzed following standard procedures for analysis of variance. Differences between means were evaluated for significance according to Duncan's a new multiple range test at the 5% level of probability (Duncan, 1955).

RESULTS AND DISCUSSION

Effects of the fungus *D. brochopaga* and *A. conoides* compound with non-chemical materials on reproduction of root-knot nematode *M. incognita* - infested Banana *Musa sapientum* L. cvs. Grandnan plants are shown in tables (2, 3).

Data in table (2) reveals that the highest decreased in the number of larvae per one kgm soil were shown with treatment by fungi (*D. brochopaga* and *A. conoides*) carried on agar and vermiculite with yeast and molasses substrate.

On September 4, 2013: The number of larvae per kgm soil was decreased in almost all treatments as it was 805, 724 and 975 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (2160) for vermiculite with yeast and molasses substrate, and (1245) for Furidan G, compared to the untreated control (2705).

On January 6, 2014: The number of larvae per kgm soil was decreased in almost all treatments as it was 212, 484 and 716 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (1800) for vermiculite with yeast and molasses substrate, and (1106) for Furidan G, compared to the untreated control (1976).

On April 28, 2014: The number of larvae per kgm soil was decreased in almost all treatments as it was 192, 318 and 685 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (2106) for vermiculite with yeast and molasses substrate, and (1045) for Furidan G, compared to the untreated control (2318).

Data in table (3) reveals that the highest decreased in the number of root-galls per 5gm roots were shown with treatment by fungi (*D. brochopaga* and *A. conoides*) carried on vermiculite with yeast and molasses substrate.

On September 4, 2013: The number of root-galls per 5gm roots was decreased in almost all treatments as it was 9, 8 and 12 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (19) for vermiculite with yeast and molasses substrate, and (18) for Furidan G, compared to the untreated control (21).

On January 6, 2014: The number of root-galls per 5gm roots was decreased in almost all treatments as it was 5, 8 and 10 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried

Table 2. Effects of the Fungi *D. brochopaga* and *A. conoides* mixed with non-chemical materials on population density of root-knot nematode *M. incognita* larvae infested banana plants

Treatments	No. of Juveniles/1 Kg soil						
	Initial 6/5/2013 No. of nematodes	4/9/2013 No. of nematodes	%Red. %	6/1/2014 No. of nematodes	%Red %	28/4/2014 No. of nematodes	%Red. %
1	2650 a	805d	73%	212d	90%	192d	92%
2	2700 a	724d	76%	484cd	78%	318d	87%
3	2475 a	975cd	65%	716c	65%	685c	70%
4	2645 a	2160b	27%	1800a	18%	2106a	12%
5	2890 a	1245c	61%	1106b	54%	1045b	60%
6	2455 a	2705a	-	1976a	-	2318a	-

Data with the same letters within a column are not significantly different according to Duncan's a new multiple range test.

Table 3. Effects of the Fungi *D. brochopaga* and *A. conoides* mixed with non-chemical materials on No. of root galls of root-knot nematodes *M. incognita* in banana (in 5 gm of roots)

Treatment	No. of galls/ 5 gm roots						
	Initial 6/5/2013 No. of nematodes	4/9/2013 No. of nematodes	%Red. %	6/1/2014 No. of nematodes	%Red. %	28/4/2014 No. of nematodes	%Red. %
1	23 a	9b	65%	5d	78%	4d	86%
2	22 a	8b	67%	8c	64%	6cd	77%
3	19 a	12b	43%	10c	47%	9bc	61%
4	21a	19a	19%	18a	14%	20a	21%
5	22a	18a	26%	14b	36%	12b	55%
6	19 a	21a	-	19a	-	23a	-

Data with the same letters within a column are not significantly different according to Duncan's a new multiple range test.

on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (18) for vermiculite with yeast and molasses substrate, and (14) for Furidan G, compared to the untreated control (19).

On April 28, 2014: The number of root-galls per 5gm roots was decreased in almost all treatments as it was 4, 6 and 9 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (20) for vermiculite with yeast and molasses substrate, and (12) for Furidan G, compared to the untreated control (23).

Data in table (4) reveals that the highest decreased in the number of hatched juveniles of root-knot nematodes *M. incognita* per 5gm banana roots were shown with treatment by fungi (*D. brochopaga* and *A. conoides*) carried on vermiculite with yeast and molasses substrate.

On September 4, 2013: The number of hatched juveniles of root- knot nematodes per 5gm roots was decreased in almost all treatments as it was 72, 64 and 76 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (125) for vermiculite with yeast and molasses substrate, and (86) for Furidan G, compared to the untreated control (245).

On January 6, 2014: The number of hatched juveniles of root- knot nematodes per 5gm roots was decreased in almost all treatments as it was 28, 42 and 66 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (164) for vermiculite with yeast and molasses substrate, and (78) for Furidan G, compared to the untreated control (214).

On April 28, 2014: The number of hatched juveniles of root- knot nematodes per 5gm roots was decreased in almost all treatments as it was 16, 30 and 65 larvae per kgm for Fungi *D. brochopaga* and *A. conoides* carried on vermiculite with yeast and molasses substrate, Fungus *D. brochopaga* carried on vermiculite with yeast and molasses substrate, and Fungus *A. conoides* carried on vermiculite with yeast and molasses substrate respectively. While it was (195) for vermiculite with yeast and molasses substrate, and (62) for Furidan G, compared to the untreated control (265).

Effects on banana yield: The highest yield in the different treatments was 71 kg /spot for The fungi *D. brochopaga* and *A. conoides*, followed by 70 Kg for The fungi *D. brochopaga*. The yield for vermiculite with yeast and molasses substrate treatment was 56 kg/spot compared to 62 kg/spot for Furidan nematicide compared to 48 kg/spot for untreated control (table 5).

Table 4. Effects of the Fungi *D. brochopaga* and *A. conoides* mixed with non-chemical materials on No. of hatched juveniles of root-knot nematodes *M. incognita* in banana orchard

Treatments	No of hatched juveniles/5 gm roots						
	Initial 6/5/2013 No. of nematodes	4/9/2013 No. of nematodes	%Red. %	6/1/2014 No. of nematodes	%Red. %	28/4/2014 No. of nematodes	%Red. %
1	250 a	72c	75%	28d	89%	16d	95%
2	242 a	64c	77%	42cd	83%	30cd	90%
3	225 a	76c	71%	66c	71%	65c	77%
4	209a	125b	48%	164b	22%	195b	25%
5	214 a	86c	65%	78c	64%	62c	77%
6	212 a	245a	-	214a	-	265a	-

Data with the same letters within a column are not significantly different according to Duncan's a new multiple range test.

Table 5. Effects of the Fungi *D. brochopaga* and *A. conoides* mixed with non-chemical materials combined on banana fruits production in banana orchard infested with root-knot nematode

Treatments	Mean of banana spot production (Kg)
1	71a
2	70a
3	66ab
4	56c
5	62b
6	48c

Data with the same letters within a column are not significantly different according to Duncan's a new multiple range test.

DISCUSSION

The present results indicate that the fungus *D. brochopaga* and *A. conoides* in combination with the non-chemical materials affected the development and reproduction of *M. incognita* on banana under field conditions. This was indicated by the lower numbers of juveniles in soil, lower numbers of root-galls per 5gm roots, the % reduction in population density of soil larvae, in treatment of the nematophagous fungi *D. brochopaga* and *A. conoides* with the non-chemical materials. Aboul-Eid (1963) reported that *D. brochopaga* has constricting rings and *A. conoides* has adhesive-network responsible for nematode capturing through trapping mechanism. The 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 and digest the inner content of the victim (Aboul-Eid et al., 2002). This information's explain the results of this work. Moreover, (Mankau, 1980) 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 (Walker & Morey, 1999). The results of work with similar formulations of the nematode-trapping fungus *Arthrobotrys dactyloides* has shown potential as a biological control agent against *Meloidogyne javanica* in soil microcosms (Stirling and Mani, 1995).

Finally the fungi (*D. brochopaga* and *A. conoides*) 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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