

**BIOLOGICAL CONTROL OF THE CITRUS NEMATODE,
TYLENCHULUS SEMIPENETRANS ATTACKING CITRUS SINENSIS
NAVAL ORANGE TREES**

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ABSTRACT: *This work was conducted to study the biological control of citrus nematode Tylenchulus semipenetrans infesting citrus orchards at Menoufia University.*

The experiments were applied at the citrus experimental farm of the Faculty of Agriculture, Menoufia governorate on, Citrus sinensis (naval orange) and in the Biological Control Laboratory of Faculty of Agriculture, Menoufia University.

Citrus trees naturally infested with citrus nematode Tylenchulus semipenetrans were chosen to conduct a biological control experiment by soil application of the mycorrhizal fungus; Glomus mosseae which was obtained from Cairo Mircen, Ain Shams University, Egypt, mychorrhiza inocula were introduced around the trees trunks (1×10^8 spores/ml) and was flattened with soil at the rate of 25 ml / tree. Also, the nematophagous fungus, Arthrobotrys conoides was obtained from Agricultural Botany Department, Faculty of Agriculture, Menoufia University. Spore suspension was adjusted to a concentration of 1×10^8 spores/ml distilled water at a rate of 50 ml/ tree. In addition, local bacterial strain of Azotobacter chroococcum, was obtained kindly from Pharmaceutical Department National Research Center, Giza, Egypt, the bacterial strain contained 10^7 cell/ml and applied at the rate of 50 ml/ tree. Tervigo™ is a suspension concentrate (SC) containing 20g/L abamectin with the addition of an iron chelate Fe-EDDHA 400g/L produced by Syngenta East Africa Ltd was applied at the rate of 25 ml per tree.

The above materials were applied singly or in combinations, monthly soil samples were taken to laboratory where nematodes were extracted, identified and counted until the end of the experiments.

The obtained results indicated that the nematophagous fungus, Arthrobotrys conoides treatment recorded the highest reduction percentages of citrus nematode as 66.5 % compared to other bioagents when singly applied.

Application of the three bioagents when applied together (A. conoides, G. mosseae, A. chroococcum) recorded the highest reduction percentages against citrus nematode recording 84.5 %, compared to 82.7 % at the nematicide, Tervigo.

The treatment of Mycorrhizal fungus, Glomus mosseae recorded the least reduction percentages of citrus nematode as 46.1 %.

The treatment of the three bioagents when applied together recorded the highest increase in number of fruits per branch as 200 %, in addition the treatment caused 84.6 % increase in the weight of orange fruit, also, the treatment increased the fruit yield by 108.9 % compared to control trees.

Key words: *Bioagents, citrus nematode, Vesicular-arbuscular mycorrhizal, trapping fungi, Azotobacter*

INTRODUCTION

Citrus-parasitic nematode, *Tylenchulus semipenetrans* Cobb., consider one of the most serious pest of citrus trees is capable of damaging mature trees and down grading fruit quality. It is a parasite of several woody plant species, and aptly named because it is ubiquitous in the citrus producing regions of the world (Sorribas et al., 2008). *Tylenchulus semipenetrans* nematode is well-adapted to citrus, with very high numbers required to significantly affect the growth and health of its host (Maafi and Damadzadeh, 2008).

Despite the long history of biological control in plant pathology, recent advances in biotechnology have sparked renewed interest and vigor in the pursuit of biocontrol as an emerging technology. Although chemical nematicides such as organophosphate and carbamate nematicides are highly effective in controlling nematodes, their usage has been restricted in recent years because of increasing concerns about their effects on the environment and food (Anastasiadis et al., 2008; Kiewnick and Sikora, 2006).

Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*) are obligate symbionts that increase nutrient uptake by plants, especially phosphorus and other minor elements (Gerdemann, 1968; Smith, 1987). The mycorrhizal fungi contribute to the control of plant diseases, and the mechanisms by which they do so have been well documented (Ahmed et al., 2009). This mycorrhizal working on increasing nutrient uptake, growth rates and hormonal activity in roots (Linderman, 1992).

Arthrobotrys conoides is trapping fungi which immobilize nematodes using non-adhesive knobs and constricting rings. They ensure active nematodes using one or more types of mycelial

traps. Some *Arthrobotrys* species have been formulated and applied, but they have given mixed results (Viaene et al., 2006). Antagonistic fungi are continuously attracting great attention as potential alternatives to chemical control of root-knot nematodes (Seyedeh et al., 2015). They are natural enemies of nematodes and are considered as ideal agents for controlling parasitic nematodes of plants and animals (Moosavi and Zare, 2012).

Azotobacter chroococcum; strains are implicated in the control of plant parasitic nematodes, such products may be toxic to nematodes directly or it may be indirectly suppress nematode population by modifying the rhizosphere environment (Youssef and Eissa, 2014, Mishra et al., 1987).

Azotobacter chroococcum has been reported to inhibit hatching of juveniles of *Meloidogyne incognita* and its penetration in roots (Chahal, 1988).

Therefore, the objective of the present study aimed to evaluate the combined effects of three biocontrol agents i.e. *Glomus mosseae*, *Arthrobotrys conoides* and *Azotobacter chroococcum* singly or in combination on the development of citrus nematode, *Tylenchulus semipenetrans* infecting citrus trees.

MATERIALS AND METHODS

The experiments were applied at the citrus experimental farm of the Faculty of Agriculture, Menoufia University on *Citrus sinensis* (naval orange) and in the Biological Control Laboratory of Faculty of Agriculture, Menoufia University.

The isolates of *mycorrhizal fungus*, *Glomus mosseae* was obtained from Cairo Microbiological Resources Centre (Cairo Mircen), Ain Shams University, Egypt. Mycorrhizal inocula were introduced around the trees trunks as 25 ml /tree (1×10^8 spores/ml) and was

flattened with soil. Every tree receiving endo-mychorrhiza was each inoculated with (10g) infested soil and (1.5 g) of the onion roots colonized by *G. mosseae*. (Amin and Mostafa, 2000).

Arthrobotrys conoides fungus was obtained from Agricultural Botany Department of Faculty of Agriculture, Menoufia University *A. conoides* was identified and classified according to (Cooke and Godfrey, 1964) and (Van Oorschot, 1985).

The obtained nematophagous fungus was maintained in Petri dishes contained CMA (15 g/l) media by using 5 mm fungal disc from 7-days-old culture and incubated at $25 \pm 2^\circ\text{C}$ for 7-10 days. The obtained nematophagous fungus was purified by hyphal tip and single spore isolation technique on CMA medium. Spore suspension was adjusted to a concentration of 1×10^8 spores/ml distilled water at a rate of 50 ml / tree.

Efficient bacterium local strain of *Azotobacter chroococcum*, was obtained kindly from Pharmaceutical Department National Research Center, Giza, Egypt. The aforementioned bacterial strain was prepared for application according to the method described by (Mahfouz, 2003). The prepared inocula from bacterial strain contained 10^7 cell/ml at a rate of 50 ml/ tree.

Tervigo™ is a suspension concentrate (SC) containing 20g/L abamectin with the addition of an iron chelate Fe-EDDHA 400g/L produced by Syngenta East Africa Ltd. Abamectin as an active ingredient provides effective control of nematodes, while the iron chelate is a micro fertilizer that provides crop enhancement especially in alkaline soils. Tervigo was applied as 2.5 ml per tree as soil drench around the roots.

All the treatments were applied singly or combination after taking a sample of the soil (pre-treatment) and then mixed it

with the soil around the trees. The mean average of air temperature was ranged from 22-26 C° and the relative humidity ranged between 60-70%. Nematode counts were made after 1,2,3 and 4 months of application.

Vegetative characters:

At the end of experiment, branch length (cm), number of leaves in (20cm), number of fruits in branch, fruit weigh (g) and weight of tree yield were evaluated.

Statistical analysis:

All obtained data were subjected to ANOVA test using a computer program (Costat 22, 1998) to determine Duncan's multiple range test and the LSD 5% (least significant difference). In addition Abbott's formula was used to determine the increase percentages of vegetative characters.

Reduction percentages were counted according to the formula of Henderson and Tilton (1955) and (Fleming and Retnakaran, 1985):

$$\text{Reduction \%} = \left[1 - \left(\frac{\text{Treatment after}}{\text{Treatment before}} \times \frac{\text{Control before}}{\text{Control after}} \right) \right] \times 100$$

$$\text{Increase or decrease \%} = \frac{\text{Control} - \text{treatment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

The obtained results in Tables (1 & 2) indicated that the nematophagous fungus, *Arthrobotrys conoides* treatment recorded the highest reduction percentages of citrus nematode as 66.5% compared to other bioagents when applied singly.

The treatment of the three bioagents when applied together (*Arthrobotrys conoides*, *Glomus mosseae*, *Azotobacter chroococcum*) recorded the highest reduction percentages against citrus nematode recording 84.5 %, compared to 82.7% at the nematicide Tervigo.

Table 1: Effect of bio-agents on the population density of *Tylenchulus semipenetrans* infected Naval orange trees under field conditions.

Treatments	Aver. no. of <i>T. semipenetrans</i> juveniles/ 100 g soil					
	Days after-treatments					
	Before treatment	30	60	90	120	Overall mean
<i>G. mosseae</i>	1812.2	1731.0	1592.0	1411.0	1261.0	1561.4b
<i>A. conoides</i>	1768.0	1503.0	1253.0	997.5	765.0	1257.3c
<i>A. chroococcum</i>	1837.0	1791.0	1512.0	1349.0	1115.0	1520.8b
<i>G. mosseae</i> + <i>A. conoides</i>	1693.0	1361.0	1046.0	697.0	502.0	1059.8c
<i>G. mosseae</i> + <i>A. chroococcum</i>	1781.2	1610.0	1433.0	1301.0	1097.0	1444.4 b
<i>A. conoides</i> + <i>A. chroococcum</i>	1603.0	1314.0	979.0	597.0	471.0	992.8c
<i>G. mosseae</i> + <i>A. conoides</i> + <i>A. chroococcum</i>	1798.3	1403.0	1002.0	520.0	361.0	1016.8c
Tervigo	1749.0	1296.0	1011.0	693.0	390.0	1027.8c
Control	1823.0	1897.0	1971.0	2138.0	2354.0	2036.6 a
LSD 5%						325.0

The means with the same letter at column are not significantly different at 0.05% level.

Table 2: Reduction percentage of *Tylenchulus semipenetrans* infected Naval orange plants under field conditions.

Treatments	Reduction %				
	30 Days	60 Days	90 Days	120 Days	Overall mean
<i>G. mosseae</i>	8.2	18.8	33.6	46.1	26.7
<i>A. conoides</i>	18.3	34.4	51.8	66.5	42.8
<i>A. chroococcum</i>	6.3	23.9	37.4	52.9	30.1
<i>G. mosseae</i> + <i>A. conoides</i>	22.8	42.8	64.9	77.0	51.9
<i>G. mosseae</i> + <i>A. chroococcum</i>	13.1	25.6	37.7	79.5	38.9
<i>A. conoides</i> + <i>A. chroococcum</i>	21.2	43.5	68.2	77.3	52.6
<i>G. mosseae</i> + <i>A. conoides</i> + <i>A. chroococcum</i>	25.0	48.5	75.3	84.5	58.3
Tervigo	28.8	46.5	66.2	82.7	56.1

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The treatment of mycorrhizal fungus, *Glomus mosseae* recorded the least reduction percentages of citrus nematode as 46.1%.

The obtained results in Table (3) indicated that the treatment of the three bioagents when applied together (*A. conoides*, *G.mosseae*, *A.chroococcum*) recorded the highest increase in number of fruits per branch as 200 %, in addition the treatment caused 84.6 % increase in the weight of orange fruit, also, the treatment increased the fruit yield by 108.9 % compared to control trees.

The obtained results are confirmed by those of Ismail and Fadel (2004) who studied the field efficacy of three isolates of *Bacillus thuringiensis* Berliner against the citrus nematode, *Tylenchulus semipenetrans* and registered good results.

In addition, Eissa *et al.* (2005) studied the effect of some bioagents and oxamyl in controlling some species of plant

parasitic nematodes and found that the application of some bioagents and oxamyl successfully controlled *Meloidogyne incognita*, *Helicotylenchus exallus* and *Criconemoides* spp. infesting banana cv. Williams, under field conditions in Egypt.

Ismail and El-Nagdi (2005) evaluated the effects of biocides containing *Trichoderma harzianum*, *Bacillus subtilis* and *Bacillus thuringiensis* subsp. kurstaki, as well as of latex bearing plants in controlling *Rotylenchulus reniformis* infesting chamomile (*M. chamomilla* [*Chamomilla recutita*]), and found that all applied biocides and latex-bearing plants significantly decreased the number of *R. reniformis* juveniles in soil, females and egg-masses on root, total final nematode population and consequently, the rate of nematode build-up, and improved the growth and yield of chamomile plants compared to the untreated control.

Table 3: Increase or decrease percentages of some vegetative characters on Naval orange as influenced by treatment applications.

Treatments	Branch length(cm)	Number of leaves in (20cm)	Number of fruits in branch	Fruit weigh (g)	Weight of tree yield
<i>G. mosseae</i>	+34.8	+28.6	+150.0	+57.6	+60.3
<i>A. conoides</i>	+21.2	+14.3	+50.0	+50.0	+50.0
<i>A. chroococcum</i>	+9.1	+14.3	+50.0	+40.7	+47.4
<i>G. mosseae</i> + <i>A. conoides</i>	+54.5	+57.1	+150.0	+69.2	+80.7
<i>G. mosseae</i> + <i>A. chroococcum</i>	+39.4	+42.9	+100.0	+61.5	+78.2
<i>A. conoides</i> + <i>A. chroococcum</i>	+27.3	+28.6	+100.0	+65.4	+79.5
<i>G. mosseae</i> + <i>A. conoides</i> + <i>A. chroococcum</i>	+95.5	+71.4	+200.0	+84.6	+108.9
Tervigo	+74.2	+28.6	+150.0	+65.4	+78.2

Mahdy *et al.* (2006) evaluated powder formulation of *Bacillus thuringiensis* or *Trichoderma harzianum* Rifai as seed coating or soil application for the management of root-knot and root rot disease complex, caused by *Meloidogyne javanica* and the fungus *Rhizoctonia solani* (Anamorph), on soybean plants, and found that the number of galls, root galling, egg masses and disease severity were sharply reduced on plants treated with both biological control agents, either as seed or soil application, compared with the non-treated plants.

Eissa *et al.* (2007) studied the influence of some biological control agents, Nemaless (containing a strain of *Serratia marcescens*), *Bacillus thuringiensis* B.t.NRC60 and Promot (containing *Trichoderma koningii* and *T. harzianum*) in controlling *Meloidogyne incognita* on banana cv. Williams, and recorded that the second stage juveniles (J2) significantly decreased ($P \leq 0.05$ and/or 0.01 levels) by all the biological control agents.

Kalele *et al.* (2010) reported that antagonistic fungi are continuously attracting a lot of attention as alternatives to chemical control of root-knot nematodes, specially, the egg-pathogenic fungus *Paecilomyces lilacinus*, particularly strain 251 which has shown promising potential as a biological control agent against various plant-parasitic nematodes.

Soliman *et al.* (2011) assessed the influence of *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, mixed genera of Arbuscular mycorrhizae (AM) fungi and oxamyl against *Meloidogyne incognita* on *Acacia farnesiana* (L.) Willd and *A. saligna*, and the obtained results indicated that both oxamyl and Arbuscular mycorrhizae were the most

effective treatments in decreasing the final nematode population in both soil and roots, number of galls and rate of buildup of root knot nematode, also, they recorded the maximum plant growth, nodulation parameters and chemical components in the leaves of the two species.

Castillo *et al.* (2012) investigated single and joint inoculation of olive planting stocks cvs Arbequina and Picual with the arbuscular mycorrhizal fungi (AMF) *Glomus intraradices*, *Glomus mosseae* or *Glomus viscosum*, and the root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica*, on plant performance and nematode infection, and concluded that prior inoculation of olive plants with AMF may contribute to improving the health status and vigour of cvs Arbequina and Picual planting stocks during nursery propagation.

Zakaria *et al.* (2012) evaluated the efficacy of some bioagents and soil amendments, as a single or combined treatments, in controlling root-knot nematode *Meloidogyne incognita* infecting cucumber. Each of the fungus *Verticillium chlamydosporium* and the symbiotic bacterium *Photorhabdus luminescens*, as single or joint treatments significantly reduced gall formation and other criteria on cucumber roots. Maximum reduction in gall formation, female numbers, egg-mass production, developmental stages and final population of juveniles in soil, was acquired by these treatments, *V. chlamydosporium* + *P. luminescens*, *P. luminescens* +compost (C) and *V. chlamydosporium* + *P. luminescens* + animal compost (AC), compared with the control and other treatments. Applications of all treatments significantly promoted plant growth i.e. length of shoot and root, fresh and dry

weight of shoot and root, number of leaves, flowers, fruits and weight of fruits per each plant compared to control (infested plants with nematode only and healthy plants).

Finally, it could be concluded that the nematophagous fungus, *Arthrobotrys conoides* treatment recorded the highest reduction percentages of citrus nematode as 66.5 % compared to other bioagents when singly applied , while the treatment of the three bioagents when applied together (*Arthrobotrys conoides* , *Glomus mosseae*, *Azotobacter chroococcum*) recorded the highest reduction percentages against citrus nematode recording 84.5%, compared to 82.7% at the nematicide Tervigo.

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المكافحة البيولوجية لنيماتودا الموالح التي تهاجم أشجار البرتقال أبو سره

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الملخص العربي

أجريت هذه التجارب على أشجار الموالح الموجودة بمزرعة كلية الزراعة - جامعة المنوفية وتم معاملة الأشجار بفطر الميكورهيذا وفطر الأثروروبوتوريس وكذلك ببكتريا الأروتوباكتر وتم تطبيق هذه المعاملات منفردة أو مجتمعة ومقارنة تأثيرها على نيماتودا الموالح بالمبيد الكيماوي (تيرفيجو). وفي نهاية التجربة تم دراسة تأثير هذه المعاملات على الصفات الخضرية والثمارية للأشجار محل الدراسة.

أجريت هذه الدراسة على أشجار البرتقال أبوسره وذلك باستخدام فطر الميكورهيذا (الجلومس) والتي تعيش معيشة تكافلية مع جذور الأشجار وتسهل إمتصاص الماء والأملاح والعناصر الغذائية للأشجار مما يزيد من مقاومتها لنيماتودا الموالح الضارة، كما تم استخدام فطر آخر وهو فطر الأثروروبوتوريس وهو فطر صائد للنيماتودا، وأخيرا تم استخدام بكتريا الأروتوباكتر وتم إضافة هذه المعاملات منفردة أو مجتمعة وكانت أهم النتائج المتحصل عليها هي :

أظهرت النتائج أن أفضل المعاملات المنفردة كانت لفطر الـ *Arthrotrys conoides* حيث قلل متوسط أعداد نيماتودا الموالح من (١٧٦٨,٠) قبل المعاملة بالفطر) إلى (٧٦٥,٠) خلال ١٢٠ يوم من تطبيق المعاملة وذلك بنسبة موت وصلت إلى (٦٦,٥)٪ في الشهر الرابع. كما أوضحت النتائج بان المعاملة المجتمعة بفطر (الميكورهيذا + الأثروروبوتوريس + بكتريا الأروتوباكتر) كانت أفضل معاملة حيث قللت نيماتودا الموالح من (١٧٩٨,٣) إلى (٣٦١,٠) وذلك بنسبة موت وصلت إلى (٨٤,٥)٪ في الشهر الرابع والأخير للتجربة وذلك مقارنة بالمبيد النيماتودي تيرفيجو الذي أدى إلى نسبة موت وصلت (٨٢,٧)٪ في الشهر الرابع للتجربة.

أظهرت النتائج بان المعاملة بفطر الميكورهيذا (منفردا) أعطى أقل نسبة موت حيث وصلت في الشهر الرابع والأخير من التجربة إلى (٤٦,١)٪. وانعكست هذه النتائج على الصفات الخضرية والتي تمثلت في طول الفرع وعدد الأوراق في (٢٠سم) من الفرع وأعطت المعاملة الثلاثية (الميكورهيذا + الأثروروبوتوريس + بكتريا الأروتوباكتر) أفضل النتائج حيث أدت إلى زيادة طول الفرع بنسبة (٩٥,٥)٪ ونفس المعاملة أدت إلى زيادة أعداد الأوراق إلى (٧١,٤)٪ مقارنة بالكنترول. أما بالنسبة للقياسات الثمرية فكانت المعاملة الثلاثية (الميكورهيذا + الأثروروبوتوريس + بكتريا الأروتوباكتر) أفضل المعاملات حيث أدت إلى زيادة عدد الثمار بالفرع بنسبة (٢٠,٠)٪ مقارنة بالكنترول كما أدت إلى زيادة وزن الثمرة بنسبة (٨٤,٦)٪ وزيادة إنتاج الشجرة بنسبة (١٠٨,٩)٪ مقارنة بالكنترول.

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