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BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* BY *ARTHROBOTRYS OLIGOSPORA*

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ABSTRACT

Experiments were conducted to investigate the effect of *Arthrobotrys oligospora* against *Meloidogyne incognita* infecting tomato plants. Effect of *A. oligospora* on capture of *M. incognita* juveniles and the trapping rate *in–vitro* were calculated. Different density of *A. oligospora* i.e.1, 3 and 5% of soil weight used to evaluate its effect on nematodes and plant growth parameters of tomato plants under green-house conditions. Data showed that the *A. oligospora* formed adhesive, three-dimensional networks and the trapping organs have the ability to trap the second stage juveniles of *M. Incognita*. *In-vitro* test was carried out by exposing the nematode juveniles to *A. oligospora culture* at 24, 48 and 72 hours. The trapping rate of juveniles by the trapping organs increased by increasing the exposure time at (72 hours). Results of *in-vitro* test showed significant reduction in nematode criteria comparing to the untreated control. Tomato growth parameters were also significantly enhanced.Thus; the present study showed the possibility of using *A. oligospora* as a bio-control agent to root-knot nematode, *M. incognita*.

Key words: Nematode-trapping fungi, *Arthrobotrys oligospora*, Biological control, *Meloidogyne incognita*.

INTRODUCTION

Root-knot nematodes are obligate parasites and therefore obtain nourishment for their development and reproduction from living plant cell. The root damage from phytoparasitic nematode parasitism leads to stunted growth, and crop producing low yields. *M. incognita* cause severe damage, high losses and limiting the crop production. *Meloidogyne* spp. cause annual losses of about USD$ 100 billion worldwide (Brand *et al*., 2010). *Meloidogyne* spp., were found in many locations especially in the new reclaimed areas in Egypt (Ibrahim *et al*., 2010; Bakr *et al*., 2011).

Chemical methods have been worldwide used and more effective in plant parasitic nematodes management but are not eco-friendly and in the course of time may cause serious threat to the ecological balance. So that, searching for eco-friendly control methods for plant–parasitic nematodes is urgently needed to avoid the highly toxic of nematicides to human, animals and the environment (Abawi and Widmer, 2000).

A large number of fungi are known to trap or prey on nematodes. There are more than 50 species of predacious fungi which capture and kill nematodes in soil (Siddiqui and Mahmood, 1996). Nematophagous fungi have been subjected as a source of biological nematicides to reduce the significant economic damage caused by plant-parasitic nematodes because of their association with each other in the rhizosphere.*Arthrobotrys oligospora* is a nematode-trapping fungus live mainly as saprophytes. In the presence of nematodes, *A. oligospora* enters the parasitic stage by forming complex three-dimensional networks to trap nematodes (Yang *et al*., 2011). The trapping initiates a series of processes including adhesion, penetration, and immobilization of nematodes (Barron, 1977; Nordbring-Hertz, 2004). The ability to trap nematodes makes it an attractive candidate as a bio-control agent for controlling plant parasitic nematodes. Application of this fungus has given very interesting results against root-knot nematodes.

The objectives of this study were to isolate, characterize, and evaluate the bio-

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control efficacy of *A. oligospora* as a biological control strategy against root-knot nematodes in infected tomato plants.

MATERIALS AND METHODS

Isolation, Purification and Multiplication

Nematophagous fungi have been isolated and maintained on Corn Meal Agar Medium (CMA) at 25°C as recommended by Singh *et al*.,(2005). The fungus was kept for one week to observe the morphological characters of their conidia, and conidiophores. Morphological identification, was done according to Watanabe (2002) using the stereomicroscope.

DNA extraction, PCR and sequencing

DNA extraction was performed using the DNeasy Plant Mini Kit, (Qiagen, Germany) applying only the procedures mentioned in the kit and the quality and quantity of the DNA were evaluated using the spectrophotometer. Amplification was done based on the universal primers ITS3 and ITS4 primers(White et al., 1990) which giving a ~330 bp PCR product in a 50 µL volume containing 10× Dynazyme buffer, 0.2 mM each dNTP, 0.75 ng each primer, 2 U Taq DNA polymerase , and genomic DNA (10–15 ng/µL). PCR reactions were carried out in Eppendorf Master Cycler gradient Machine with the following thermal profile: 1 cycle of 5 min at 95°C; followed by 35 cycles of 95°C for 1 min, 53°C for 30 s, 72°C for 1min; with, final extension 72°C for 10 min. DNA fragments resolved by gel electrophoresis on 1.5% agarose gels as shown in Fig (2).PCR products with target fragment were purified from the agarose gel using E.Z.N.A. Gel Extraction kit (Omega Bio-Tek).Target fragment sequenced at the Haartman Institute, University of Helsinki, Finland using ITS3 (GCATCGATGAAGAACGC AGC) and ITS4 (TCCTCCGCTTATTGATATGC) primers (White et al, 1990).Alignment of this sequence was used for comparison with that of available rRNA sequences available in Gen Bank (NCBI, http://www.ncbi.nlm.nih.gov/genbank/).

Trap organs formation by *Arthrobotrys oligospora in- vitro*

Formation of trap organs was done by culturing the nematophagous fungi on Petri dishes(9 cm in diam.) containing a film layer of CMA medium for 7 days .Four disk from the newly hyphal tip were picked by cork borer, and inoculated in the middle of four new plates containing CMA medium. The plates were incubated at 25c˚ for 7 days. The fungi trapping organs was studied microscopically.

Trapping of *Meloidogyne incognita* juveniles by *Arthrobotrys oligospora In-vitro*

A. oligospora cultured on Petri dishes containing CMA medium for 7 days .Then using the cork borer transfer 4 disks from the newly hyphae to the middle of four new Petri dishes Containing CMA. The plates were incubated at 25c˚ temperature for 4 days. Then the old disc was removed and at the same place 300 µ water containing 500 J2s of *M. incognita* were added to induce the development of trapping devices. Plates were incubated in the dark at 25c˚. Trapped juveniles of *M. incognita* by *A. oligospora* were determined after 24, 48 and 72 hrs of nematode inoculation using a stereomicroscope. Trapping rate calculates according to Duponnois *et al.* (1995) as follow:

$$\text{Trapping Rate} = \frac{\text{Trapped Juveniles}}{\text{Total Juveniles}}$$

Photographs

Photographs were taken with a light microscope equipped with a camera (Leitz, Laborlux S/Leica, DFC 420 C).

Greenhouse experiment

Preparation of *M. incognita* inoculum:

Stock culture of pure root-knot nematode, *M. incognita* was cultured and maintained on tomato plants (*Lycopersicum esculentum* Mill. Cv. Beto 86) kept in pots under green-house conditions for six weeks at Faculty of Agriculture, Menoufia University,Shebin El-kom, Egypt. Three months old tomato roots infested with *M. incognita* were used to prepare nematode inoculum. Roots were removed from the pots and gently washed with tap water to remove the adherent soil

particles. Roots were cut into small pieces and then were macerated for two periods of 10 seconds each at high speed using a blender. This method released the highest number of nematodes from roots. The macerated root solution was then placed in a flask containing sodium hypochlorite (NaOCl). Water was added to adjust the final concentration of NaOCl to 0.5% as described by Hussey and Barker (1973). The solution in the flask was vigorously shaken for 3 minutes to release the eggs from the egg matrix as NaOCl removes the gelatin matrix of egg masses. The solution was then poured through different size sieves to remove the root tissue. Eggs were collected on the 20 micrometer (µm) sieve and washed several times with tap water to remove residual NaOCl. Eggs were then transferred to a flask containing tap water then counted under light microscope. The extracted eggs were transferred into Baermann trays with soft tissue paper to allow egg hatching. After 72 hours the extractions were collected and examined under stereomicroscope and counted the new hatched second stage number in each ml.

Preparation of *A. oligospora* inoculums

A. oligospora inoculum was prepared by inoculating 1000 ml flask contains 300 ml of Corn Meal Broth (CMB) autoclaved for 20 min at 121°C with equal disk from the stored culture. Flasks incubated on a rotary shaker at 100 rpm for 7 days at 25°C. Mycelium was harvested from the liquid cultures by filtration using sterilized filter paper (Whatman No.1), rinsed with sterilized distilled water, and homogenized in a blender with sterilized water. The fungal spore suspension was counted by Hemicytometer slide and adjusted to a concentration of $1x10^4$ spores / ml for inoculation. The homogenized mycelium mixed with pot soil at 1%, 3% and 5% (10, 30 and 50ml /kg soil) of soil weight.

The experiment was carried out under greenhouse conditions to evaluate the effect of *A. oligospora* on root-knot nematode management. Three weeks old tomato seedlings CV. Beto 86 were transplanted in plastic pots (15 cm in diam.) containing sterilized mixed sand / clay soil (2/1 v/v) to avoid biological factors. Fungal suspension at 10,30 and 50 ml /plant ($1x10^4$ spores / ml) were inoculated around the root zone by pipetting in 4 holes at transplanting time. At the same time of transplanting one thousand of *M. incognita* juveniles (J_2S) were inoculated by pipetting in 3-4 holes around the root. Plants received sterilized water served as control. Each treatment was replicated three times. Plants were daily watered and weekly fertilized with a nutrient solution as described by Epestein (1972). After two months from nematode inoculation plants were removed; uprooted and their roots were carefully washed under running tap water to remove soil particles. Nematode related parameters i.e.: number of galls and egg-masses/root system were recorded .Egg-masses were counted as described by Daykin and Hussey (1985) by dipping the washed root system in aqueous solution of phloxin-B (15 mg/l of water) for 20 min to emphasize egg-masses. The egg-masses take a pink-red colour, while the roots remain uncoloured or only very slightly stained. Number of J2s / 250 g soil, Females /root system and developmental stages as well as were recorded. The plant growth parameters viz., shoot and root fresh and dry weights were recorded. Data statically analysis using Duncan's Multiple Range test (P=0.05) using costat 6.3 version program.

RESULTS

Isolation

Microscopically observation of fungi clear that the Conidiophores hyaline, erect, simple or rarely branched, bearing 2–6 conidia sympodially on sterigmata in the apical parts. Conidia sympodulosporous, hyaline, ovate, 2-celled, composed of large apical cells and small basal one, apiculate and truncate at base as shown in Fig (1), which is almost describe the fungus *A. oligospora.*

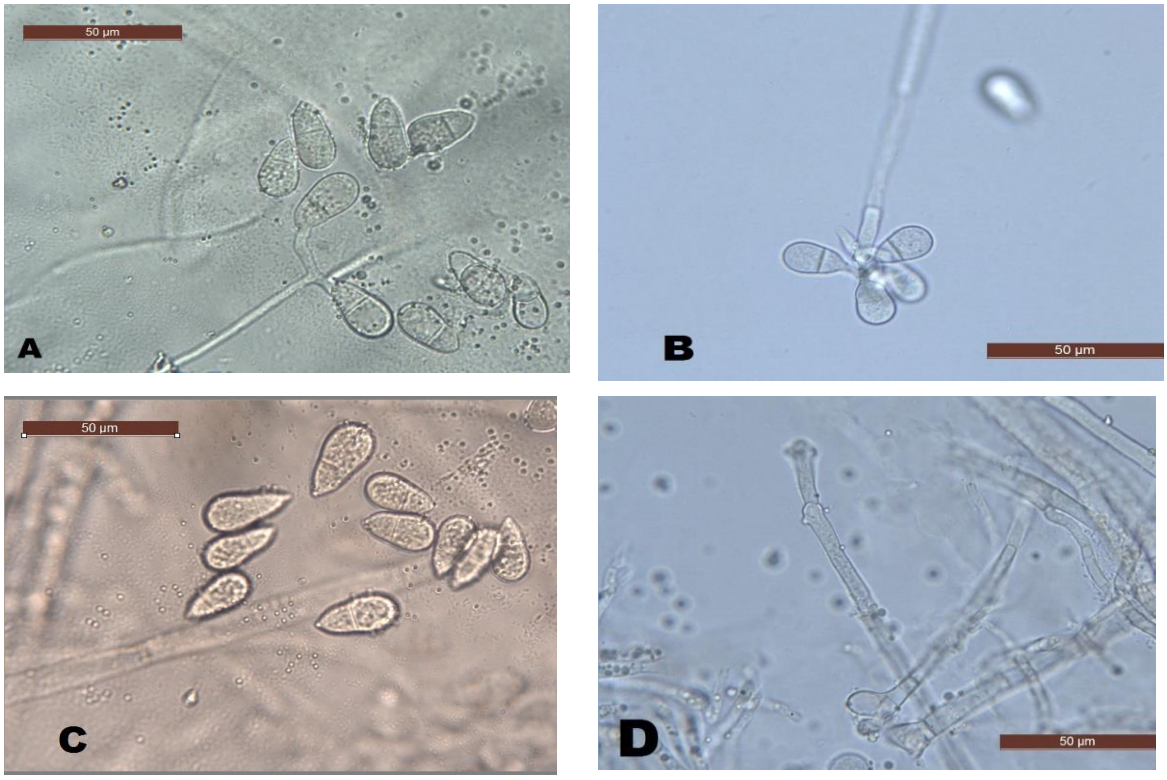

Fig (1): *A. oligospora* Conidiophores and Conidia. A: hyaline, erect, simple or rarely branched. B: bearing 2–6 conidia sympodially on sterigmata in the apical parts.C: Conidia hyaline, ovate, 2-celled, composed of large apical cells and small basal one, apiculate and truncate at base.D: sterigmata on the conidiophore apical parts.

Sequence analysis

PCR amplified and sequenced. For the species identification, we obtained the five best hits for the 18S rRNA gene sequences from GenBank using blastn . We obtained close relatives (100% similarity), which indicated that this isolate probably belonged to the *Arthrobotrys* The 18S rRNA sequence that we obtained was not specific enough to differentiate *Arthrobotrys* species; however, the best candidate was *A. oligospora*.

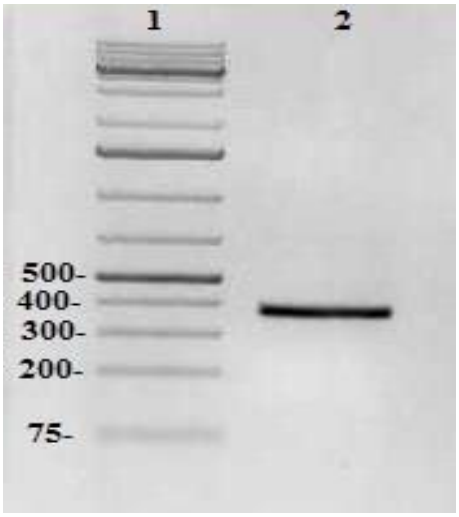

Fig. (2).Agarose gel electrophoresis showing 1: 1kb plus ladder 2:target fragment

Trap organs formation by *A. oligospora in- vitro*

Microscopically observation of *A. oligospora cultivated on* CMA plates for nematode trapping organs clear that the fungi formed adhesive three-dimensional network as shown in Fig. (3). the adhesive network trap may consist of a single ring or a fully developed three-dimensional network. This network formation can capture nematode juveniles during movement.

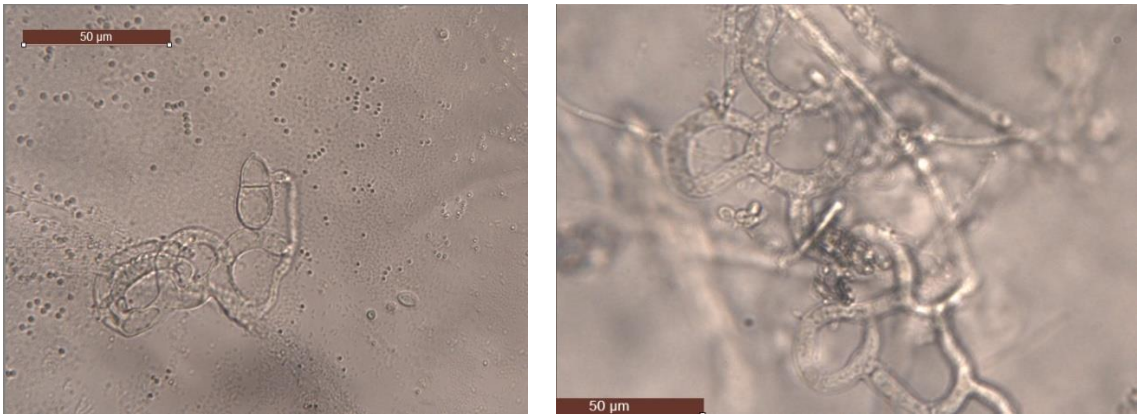

Fig (3):Three-dimensional adhesive net forming by *A. oligospora*.

Trapping of *M.incognita* by *A.oligospora*

Microscopically studies of *A. oligospora* activity against *M. incognita* juveniles revealed that the trapping structures were observed after 24 hours from *M. incognita* juvenile's addition as a response of juveniles presence. The formed adhesive three dimension network viewed captures the juveniles of root-knot nematodes by the fungus organs. The processes start by trap formation then Attraction of nematodes larvaes, Adhesion of trapping organs to juveniles, Capturing, Penetration and immobilization the nematodes by the fungus and digestion and assimilation the nematodes as shown in Fig (4).

The trapped nematodes were digested completely after 48 hours from trapping. Trapping rate of *M. incognita* by *A. oligospora was different according to the time* of observation after nematodes inoculation. As appeared in Fig.(5A) number of captured second stage of *M. incognita* by *A. oligospora* is different after 24 ,48 and 72 hours of nematodes inoculation. The highest numbers of trapping juveniles was recorded after 72 hours from nematodes inoculation. In turn, the trapping rate of *M. incognita* second stage by *A. oligospora* take the same trend as shown in Fig.(5B). The highest trapping rate was recorded after 72 hours from nematode additions to the fungus culture. Results clear that *A. oligospora* trapped 48 and 74% of *M. incognita* juveniles within 48 and 72 hours respectively in vitro.

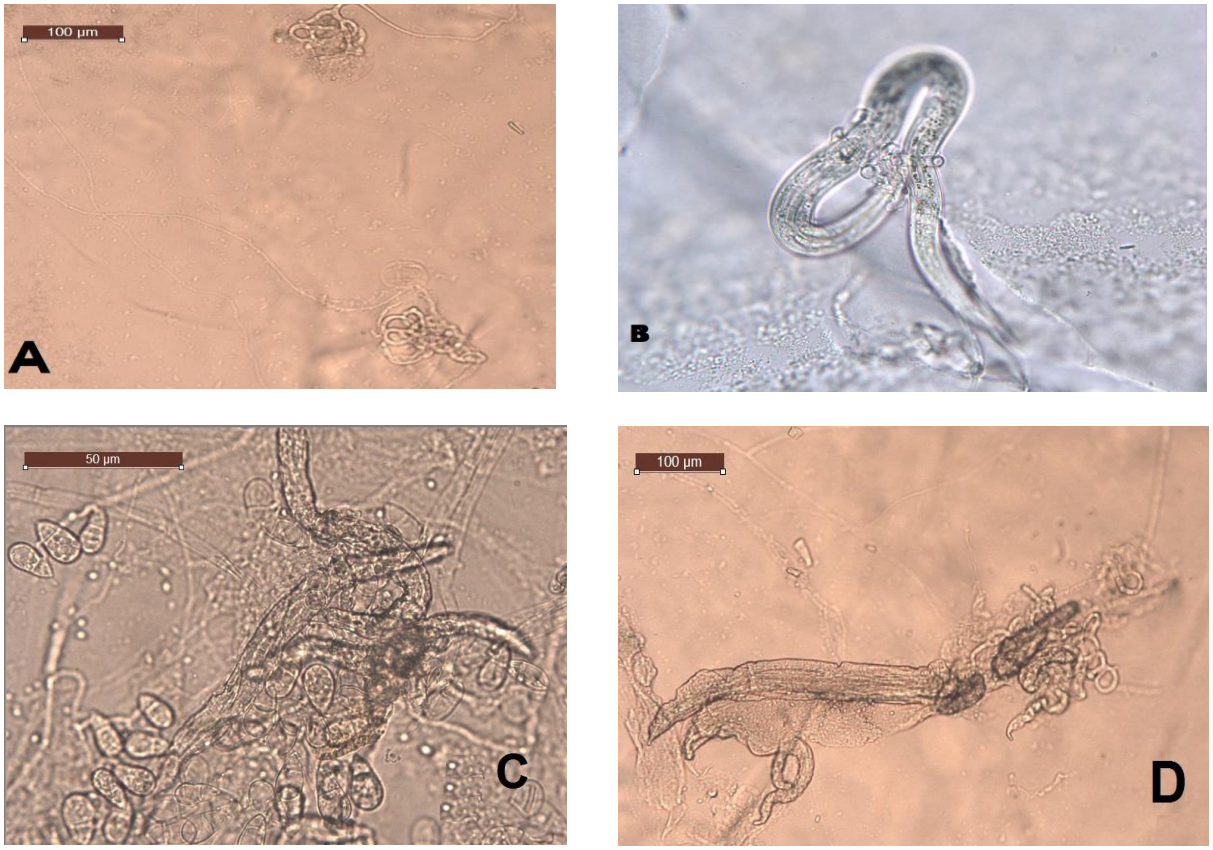

Fig. (4): The parasitic stage steps of *A. oligospora* :A) Trapp formation B) Adhesion and Capturing. C) Penetration and immobilization. D) Digestion and assimilation.

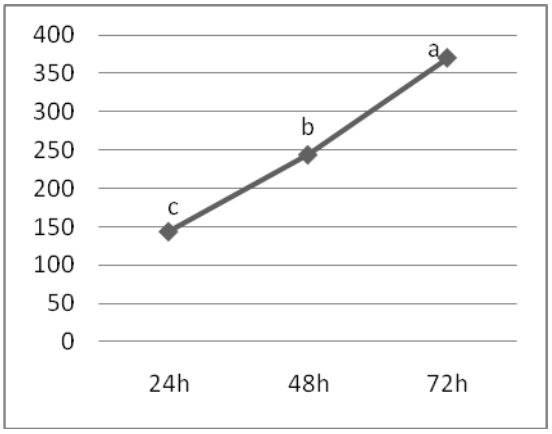

Fig. (5A):Number of second stage of *M. incognita* trapping by *A. oligospora*

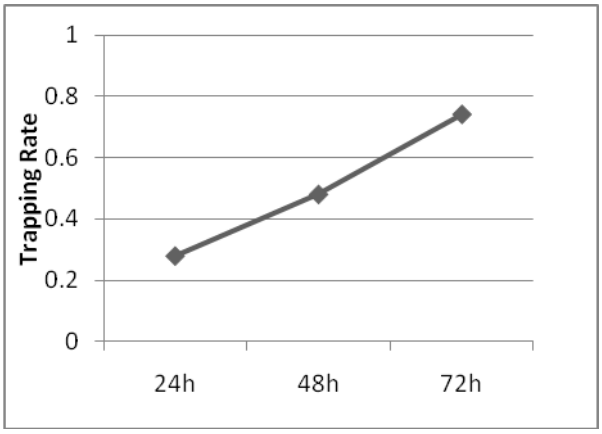

Fig. (5B):Trapping rate of *M. incognita* second stage by *A. oligospora*

Results of the present study showed that all related root-knot nematodes and plant growth parameters of tomato plants were affected by using the different density of *A. oligospora* under greenhouse conditions. The reduction in number of J2s /250 g soil varies from treated and non-treated pots. The highest reduction in number of J2s/250g soil were recorded in the treated pots with *A. oligospora* at 50 ml/plant compared to control as shown in Fig (6). Examination of plant root system revealed that the number of galls and egg masses/root system were affected markedly by all applied treatments as

shown in Fig (6). Females, eggs/egg-mass and developed stages/root system were also decreased, the lowest reduction was occurred also with the treatment of *A. oligospora* at 50ml/plant as shown in Fig (7). Recorded data clear that shoot, root fresh and dry weight of tomato plants were also affected by the fungi treatments compared with the non-treated one. Results also revealed that tomato plants grown on pots treated with *A. oligospora* showed the highest fresh shoot, root weight and dry shoot weight as presented in Fig. (8).

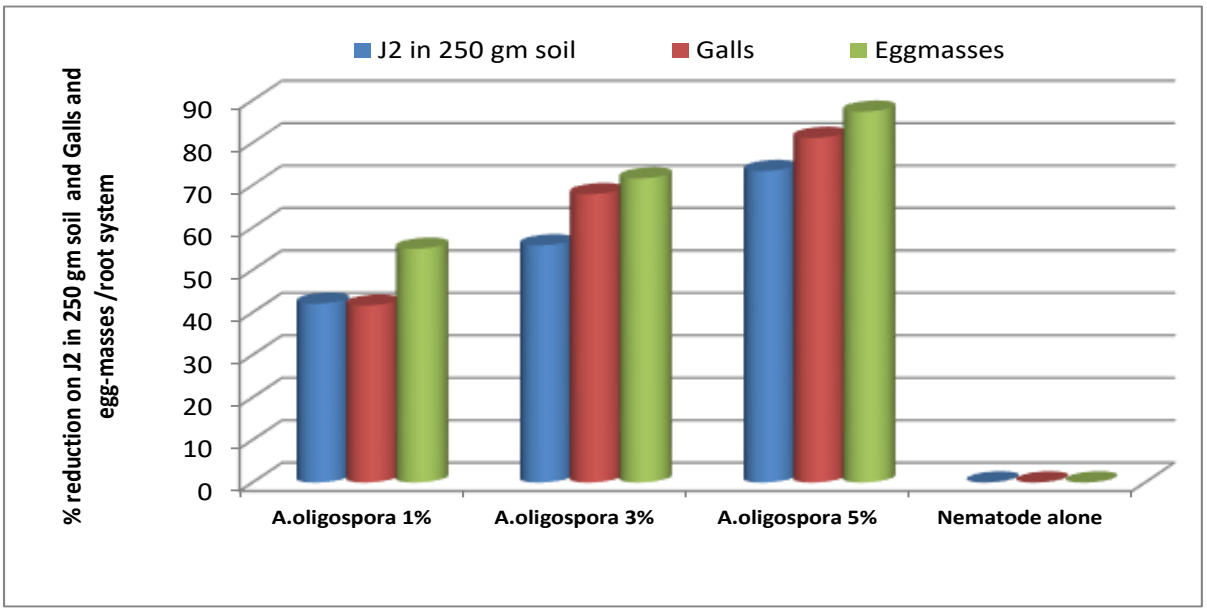

Fig. (6): Effect of *A. oligospora* in % reduction on number of second stage in pot soil, number of galls and egg-masses /root system.

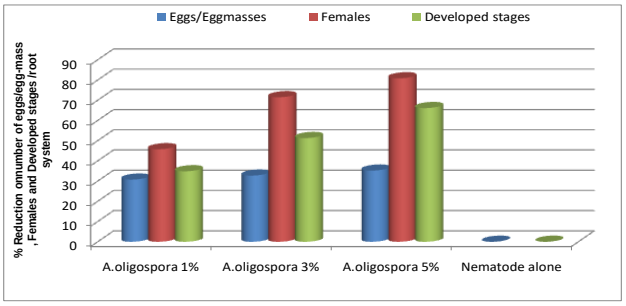

Fig. (7): Effect of A. oligospora in % reduction on number of eggs/egg-mass, females and developed stages /root system/root system.

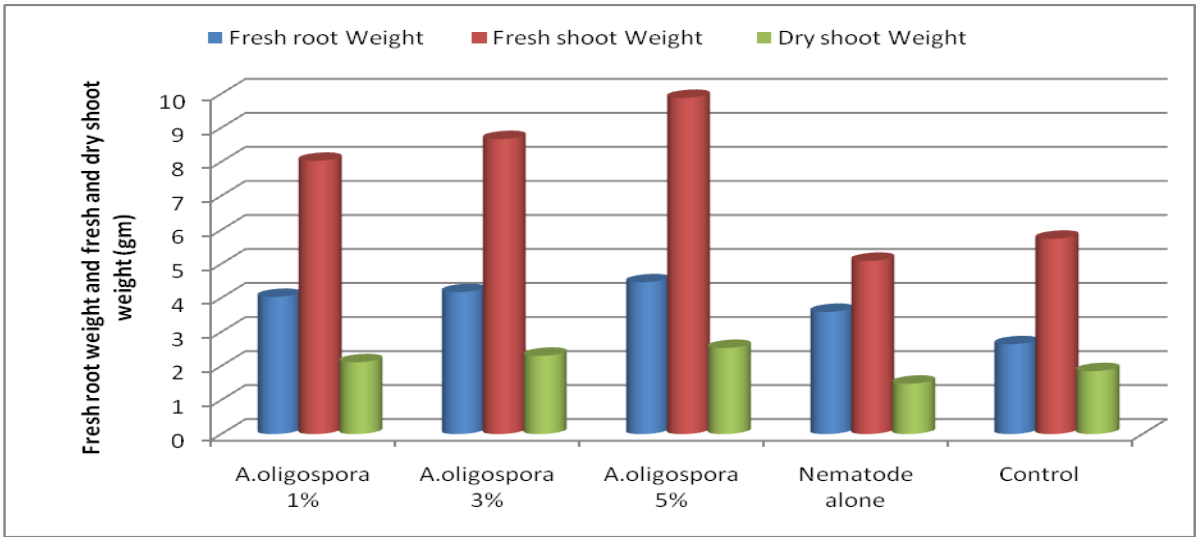

Fig. (8): Effect of *A. oligospora* on fresh root weight and fresh and dry shoot weight of tomato plants.

DISCUSSION

The nematophagous fungus *A. oligospora* was isolated in this study and observed for nematodes trapping organs under laboratory conditions. Evaluated against root-knot nematodes in vitro clear that the fungus have a trapping organs can capture *M. incognita* juveniles and totally digested after capture. Results of current study revealed that *A. oligospora* capture the highest number of root-knot nematodes *M. incognita* after 72 hours and this finding in according to those by Duponnois *et al.* (1995;1996) and Alkader, (2008). In-vitro trapping of *M. incognita* by *A. oligospora* increased with time and this may be revealed to the nematodes mobility on the plates and the fungus density and the nutrient supply. The trap formation was found to be highly influenced by several other factors like pH, temperature, nematode species, nematode population and nutritional level etc. (Balogh *et al*., 2003; Singh et al., 2011; Xu *et al*., 2011).Addition of organic matter to the soil also enhanced and increased the nematophagous fungus density (Jaffee, 2002; 2004). Nematode trapping fungi produce chemo-attractants substrates which attract nematodes near the fungal mycelia that help the trapping organs to capture nematodes. Literatures revealed that *A. oligospora* produce two pathogenicity factors- a carbohydrate binding protein (lectin) and an extracellular serine protease (Ahman *et al*., 2002; Yang *et al*., (2007). Extracellular proteases play an important role in degrading structural components of the nematodes. Proteases have been found to be involved in immobilization of nematodes captured by *A. oligospora* (Tunlid and Jansson 1991; Tunlid *et al*., (1994). The ability of nematophagous fungi to destroying nematodes is correlated to its extracellular proteases production amount, the more extracellular proteases production the better efficiency in killing nematodes (Bedelu *et al*., 1998).

Evaluation the fungus against root-knot nematodes under glasshouse conditions indicated that *A. oligospora* offers good opportunity to be used as potential bio-control agents against *M. incognita* in tomato this results in agreement with this obtained by Tsay *et al*.,(2006) and Singh *et al*., (2012). Results showed decreasing in number of juveniles in soil, galls and egg masses/root system. The lower number in galls, egg-masses and juveniles as a result to affecting the second stage juveniles by the application of the fungus which decreased the infection and penetration of J2s to the plant roots. Similar results were observed by Singh *et al*. (2007); Sharma and Pandey (2009) and Jamshidnejad *et al*.,(2013) as they reported that *A. oligospora* significantly decreased the mean numbers of galls, egg-masses and egg per egg-mass on tomato plants infected by *M. javanica*. Our results cleared

that plant growth parameters markedly influenced by the application of *A. oligospora* against the root-knot nematodes *M. incognita* Similar results were obtained by Sharma and Pandey (2009) and Singh et al*.,* (2012).The increasing in plant growth parameters may be referred to affecting the root system by the fungus application as the root system became more effective in water and nutrients uptake and transition.

For the wide application of *A. oligospora* as an environment eco-friendly bio-control agent against root-knot nematodes in crop production, there is an urgent need to develop some efficient mass production system of the fungi at a commercial scale for field application. The long-term protection of plants against root-knot nematodes will be more effectively when the bio-control agents applied combined with other management strategies as part of an integrated pest management program.

REFERENCES

Abawi, G.S. and Widmer, T.L. (2000). Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. Appl. Soil Ecol. 15:37-47.

Ahman, J.; Johansson T.; Olsson M.; Punt, P.J.; Hondel, C. and Tunlid A (2002). Improving the pathogenicity of a nematode trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. Appl. Environ. Microbiol. 68(7):3408-3415.

Alkader, M.A. (2008).In vitro Studies on Nematode Interactions with their Antagonistic Fungi in the Rhizosphere of Various Plants. PhD thesis ,Faculty of Forest and Environmental Sciences, Albert Ludwigs Universität, Freiburg im Breisgau, Germany.214 page.

Bakr, R.A.; Mahdy, M. E. and Mousa,E. M. (2011). A Survey of Root-knot and Citrus Nematodes in Some New Reclaimed lands in Egypt. Pakistan Journal of Nematology, 29(2):165-170.

Balogh, O.; Tunlid A. and Rosén S. (2003). Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora*. Fungal Genetics and Biology 39:128- 135.

Barron, G.L.(1977). The Nematode Destroying Fungi. Canadian Biological Publications Ltd, Ontario, Canada. 140 pp.

Bedelu, T. ; Gessesse, A. and Abate, D. (1998).Relation of protease production to nematode degrading ability of two Arthrobotrys spp. World Journal of Microbiology & Biotechnology 14:731-734.

Brand, D.; C. R. Soccol; A. Sabu and S. Roussos (2010).Production of fungal biological control agent through solid state fermentation: A case study on *Pacecilomyces lilacinus* against root-knot nematodes. Applied Mycology. Int., 22:31-48.

Daykin,M.E. and R.S. Hussey (1985). Staining and histopathological techniques in nematology. In: Barker, K.R.,C.C. Carter and J. N. Sasser (eds), An advanced treatise on Meloidogyne, vol.II Methodology, pp.39-48. north Carolina state University Graphics, Raleigh.

Duponnois,R.; Mateille,T. and Gueye,M. *(1995)*. Biological Characteristics and Effects of Two Strains of *Arthrobotrys oligospora* from Senegal on *Meloidogyne* Species Parasitizing Tomato Plants. Biocontrol Science and Technology ,5:*517-525.*

Duponnom, R. ; Mateille, T.; Sene, V. ; Sawadogo, A. and M. Fargette (1996). Effect of different west African species and strains of *Arthrobotrys* nematophagous fungi on *Meloidogyne* species. entomophaga ,41 (3/4) 475-483.

Epestein,E.(1972).Mineral nutrition of plants :Principles and prespection. pp.39,wiley Newyork.

Hussey, R. S.and K. R. Barker (1973).A comparison of methods collecting inocula of Meloidogyne spp. including a

new technique. Plant Disease Reporter, 57:1025-1028.

Ibrahim, I. K. A.; Mokbel, A. A. and Handoo, Z. A. (2010). Current status of phytoparasitic nematodes and their host plants in Egypt. Nematropica, 40:239-262.

Jaffee, B.A.(2002). Soil cages for studying how organic amendments affect nematode-trapping fungi. Applied Soil Ecology 21:1–9.

Jaffee, B.A. (2004). Do organic amendments enhance the nematode-trapping fungi *Dactylellina haptotyla* and *Arthrobotrys oligospora*?. Journal of Nematology 36:267–275.

Jamshidnejad, V.; Sahebani, N. and Etebarian, H. (2013). Potential biocontrol activity of *Arthrobotrys oligospora* and *Trichoderma harzianum* BI against *Meloidogyne javanica* on tomato in the greenhouse and laboratory studies. Archives of Phytopathology and Plant Protection, 46(13):1632-1640.

Nordbring-Hertz, B. (2004) Morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora*-an extensive plasticity of infection structures. Mycologist 18: 125–133.

Sharma,P. and Pandey,R.(2009). Biological control of root-knot nematode; *Meloidogyne incognita* in the medicinal plant; *Withania somnifera* and the effect of biocontrol agents on plant growth. African Journal of Agricultural Research, 4 (6):564-567.

Siddiqui, Z. A. and Mahmood, I. (1996). Biological control of plant parasitic nematodes by fungi: a review. Bioresource Technolog. 58:229-239.

Singh, K. P.; Jaiswal, R. K.; Kumar, N. and Kumar, D. (2007). Nematophagous Fungi Associated with Root Galls of Rice Caused by *Meloidogyne graminicola* and its Control by *Arthrobotrys dactyloides* and *Dactylaria brochopaga*. *Journal of Phytopathology*, 155:193–197.

Singh, R. K.; Kumar, N. and K. P. Singh (2005).Morphological Variations in Conidia of *Arthrobotrys oligospora* on Different Media. Mycobiology 33(2): 118-120.

Singh, R. S.; Bhari, R. and Kaur, H. P. (2011). Current trends of lectins from microfungi. Critical Reviews in Biotechnology,31, No. 3: 193-210 (doi: 10.3109/ 073 885 51.2010. 505911).

Singh, U.B. ;A. Sahu, N. Sahu, R.K. Singh, S. Renu, D.P. Singh, M.C. Manna, B.K. Sarma, H.B. Singh and K.P. Singh (2012). Arthrobotrys oligospora-mediated biological control of diseases of tomato (Lycopersicon esculentum Mill.) caused by Meloidogyne incognita and Rhizoctonia solani. Journal of Applied Microbiology 114, 196-208.

Tsay, T. T.; Chen, P. C. and Wu, W. S. (2006). A new method for isolating and selecting agents with high antagonistic ability against plant parasitic nematodes. Plant Pathol. Bull. 15:9-16.

Tunlid, A. and Jansson, S. (1991). Proteases and their involvement in the infection and immobilization of nematodes by the Nematophagous fungus *Arthrobotrys oligospora*. Applied and Environ. Microbiol.57(10): 2868-2872.

Tunlid, A.; Rosen,S.; Ek, B. and Rask, L.(1994). Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. Microbiology , 140: 1687- 695.

Watanabe,T. (2002). Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species. 2nd ed. 506 page. CRC PRESS, Boca Raton London New York Washington, D.C.

White, T.J.; Bruns. T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis, M.A.; Gelfand, D.H.; Sninsky, J.J.; White T.J., eds).

Academic Press, New York, USA: 315–322.

Xu, L. L.; Lai, Y. L. and Liu X. Z. (2011). Effects of abscisic acid and nitric oxide on trap formation and trapping of nematodes by the fungus *Drechslerella stenobrocha* AS6.1. Fungal Biology. 115:97-101.

Yang, J.; Wang, L.; Ji, X.; Feng, Y.; Li, X.;Zou,c.; Xu,J. ;Ren,Y.;Mi,Q. ;Wu,J. ;Liu,S.; Liu, Y. ;Huang,X. ;Wang,H. ;Niu,X. ;Li,J. ; Liang,L. ; Luo,Y. ;Ji,k. ; Zhou,W. ;Yu,Z. ; Li,G. ;Liu,Y. ;Li,L. ;Qiao,M. ; Lu Feng,l. and Zhang, K-Q. (2011) Genomic and Proteomic Analyses of the Fungus *Arthrobotrys oligospora* Provide Insights into Nematode-Trap Formation. PLoS Pathogens, .7(9): e1002179. doi:10.1371/journal.ppat.1002179.

Yang, J.; Tian, B.; Liang, L. and Zhang, K-Q (2007). Extracellular enzymes and the pathogenesis of nematophagous fungi. Appl. Microbiol. Biotechnol. 75:21–31.