

Original Article

# Efficacy of *Purpureocillium lilacinum* AUMC 10149 as biocontrol agent against root-knot nematode *Meloidogyne incognita* infecting tomato plant

Eficácia de *Purpureocillium lilacinum* AUMC 10149 como agente de biocontrole contra o nematoide *Meloidogyne incognita* que infecta plantas de tomate

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## Abstract

Root-knot nematode *Meloidogyne incognita* is among the biotic factors which has greatly affected both the yield and the quality of the tomato crop. The egg parasitic nematode, *Purpureocillium lilacinum* (PI) is considered as one of the most promising agents in controlling and overcoming this plant pathogen. The nematicidal effect of the native isolate PI AUMC 10149 on second stage juvenile's survival and egg hatching of *M. incognita* at different times of exposure was tested *in vitro*. The obtained data showed that PI gave a maximum percentage of J2 mortality (97.6%) and egg hatching inhibition (79.8%) after 72 hours of exposure. The potentiality of PI as well as Bio-Nematon to control *M. incognita* infecting tomato was conducted using different times of application *in vivo*. Nine treatments with five replicates were used for such bioagents compared with the nematicide Oxamyl. Each seedling was inoculated with 1000 J2s of nematode/pot and 10 mL of PI ( $1 \times 10^{10}$  CFU/mL) or Bio-Nematon spore suspension ( $1 \times 10^8$  CFU/mL) 10mL/pot. The results indicated that the most effective treatments in reducing nematode population, number of galls and egg masses of *M. incognita* in plant roots was performed with treatment by PI pre-planting and post-infection with PI (Rf 1.9) giving a significant enhancement in plant length (64.9%), fresh weight (72.52%) and shoot dry weight (163.41%) without negatively impacting environment. Therefore, the present study confirmed that using *P. lilacinum* AUMC 10149 can be used as a practical supplement to environmentally friendly disease management of root-knot nematodes in Egypt.

**Keywords:** tomato, biocontrol, *Purpureocillium lilacinum*, *Meloidogyne incognita*, Bio-Nematon.

## Resumo

O nematoide-das-galhas *Meloidogyne incognita* está entre os fatores bióticos que afetaram enormemente a produção e a qualidade da cultura do tomate. O nematoide parasita de ovos, *Purpureocillium lilacinum* (PI), é considerado um dos mais promissores agentes no controle e superação desse fitopatógeno. O efeito nematicida do isolado nativo PI AUMC 10149 na sobrevivência de juvenis de segundo estágio e na eclosão dos ovos de *M. incognita* em diferentes momentos de exposição foi testado *in vitro*. Os dados obtidos mostraram que o PI deu um percentual máximo de mortalidade de J2 (97.6%) e inibição da eclosão dos ovos (79.8%) após 72 horas de exposição. A potencialidade de PI e de Bio-Nematon para controlar *M. incognita* infectando tomate foi conduzida em diferentes tempos de aplicação *in vivo*. Nove tratamentos com cinco repetições foram usados para tais bioagentes em comparação com o nematicida Oxamyl. Cada muda foi inoculada com 1.000 J2s de nematoide / vaso e 10 mL de PI ( $1 \times 10^{10}$  CFU/mL). Ou suspensão de esporos Bio-Nematon ( $1 \times 10^8$  CFU/mL) 10mL/pot. Os resultados indicaram que os tratamentos mais eficazes na redução da população de nematoides, número de galhas e desovas de *M. incognita* nas raízes das plantas foram realizados com PI pré-plantio e pós-infecção com PI (Rf 1.9), dando um aumento significativo no comprimento da planta (64.9%), massa fresca (72.52%) e massa seca da parte aérea (163.41%) sem impactar negativamente o meio ambiente. Portanto, o presente estudo confirmou que o uso de *P. lilacinum* AUMC 10149 pode ser usado como um suplemento prático para o manejo ecologicamente correto de nematoides-das-galhas no Egito.

**Palavras-chave:** tomate, biocontrole, *Purpureocillium lilacinum*, *Meloidogyne incognita*, Bio-Nematon.

## 1. Introduction

In Egypt, tomato (*Solanum lycopersicum* Mill.) is one of the most important crops in terms of production volume

and cultivated area (Akladiou et al., 2015). It is considered one of the most important vegetables for either local

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consumption or being exported to the European countries. The cultivation of tomato is usually limited by biotic and abiotic factors. Phytonematodes especially the root-knot nematode (RKN) *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, among the biotic factors which have greatly affected both yield and quality of tomato crop (Dahlin et al., 2019). Plant nematodes can complete their life cycles within a short period on susceptible crop such as tomato and accumulate to a maximum rate during its maturity while plants often die before attaining maturity (Gine and Sorribas, 2017). Although root-knot nematodes (*Meloidogyne* spp.) can be managed effectively by chemical nematicides, they have many disadvantages as many of them are expensive and pose many risks to the environment and human (Peiris et al., 2020). Biocontrol agents have been received more attention in management of these parasitic nematodes as a safe way to control agricultural pests and less expensive while reducing the danger to surrounding environment (Arthurs and Dara, 2019). Nematophagous fungi are natural antagonists with the capacity to parasitize nematodes (Campos, 2020).

*Purpureocillium lilacinum* (Thom) Samson previously known as *Paecilomyces lilacinus* is a saprophytic soil fungus and considered as one of the most promising biocontrol agents and an important egg pathogenic fungus of cyst and root-knot nematodes (Ahmed and Monjil, 2019). This fungus can infect all life stages of the root-knot nematode (Yang et al., 2015). The degree of egg mass colonization is determined by standard isolation techniques that are related to neither fungal density in soil nor the efficacy to reduce the nematode damage on tomato roots (Dahlin et al., 2019). Fungal parasitism can cause up to 90% of eggs and 75-80% of egg masses or cyst destruction (Sharf et al., 2011). The aim of the present study was to determine the efficacy of new strain *P. lilacinum* AUMC 10149 against root-knot nematode *M. incognita* *in vitro* and under greenhouse conditions compared to Bio-Nematon (commercial product of *P. lilacinum*) on tomato plants.

## 2. Materials and Methods

### 2.1. Biocontrol fungus

The tested fungus *P. lilacinum* (*P. lilacinus*) (Thom) Samson in this study was isolated from soil samples collected from Mangrove soil of Ras Mohammed protected area at Sharm El-Sheikh, Egypt. It was identified morphologically by Assiut University Mycological Centre (AUMC), Egypt and deposited in AUMC with accession number 10149. The identification of *P. lilacinum* was confirmed by molecular methods which carried out by Microbial Genetics Department, Genetic Engineering and Biotechnology Division, National Research Center,

Dokki, Giza, Egypt. Genomic DNA of the tested fungus was extracted and purified according DNeasy Blood & Tissue Kits (QIAGEN-Germany) and the non-coding fungal region. ITS was amplified and sequenced using ITS1 and ITS4 primers (Table 1). BLAST sequence analysis led to the identification of *P. lilacinum* (GenBank with accession number MW281802).

### 2.2. Preparation of *P. lilacinum* culture

Culture of the *P. lilacinum* was prepared on Czapek's liquid medium in 250-mL conical flasks. The flasks contained 50 mL of sterilized medium were inoculated with a 5 mm diameter disk of 10 days old culture of *P. lilacinum* and incubated at  $28 \pm 2$  °C for 10 days. The whole content of each flask was collected and then blended with a known volume of sterilized distilled water in an electric blender for 2 min to prepare fungal suspension for experiment. A spore suspension of *P. lilacinum* was counted at  $1 \times 10^{10}$  CFU/mL by a haemocytometer.

### 2.3. Bionematicide

Bio-Nematon® 1.15%WP, a commercial product of *P. lilacinum* contains  $1 \times 10^8$  CFU/g of fungus and was obtained from Nematode Diseases Research Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

### 2.4. Chemical nematicide

Vydate® 10%G (Oxamyl): Methyl N N-dimethyl-N [(methyl) carbamyloxy]-1-thioxamidate was used at the rate of 0.3g/pot.

### 2.5. Preparation of *M. incognita* inocula

Root galls were collected from infected tomato plants and washed well with tap water to remove soil particles. The plant roots were cut to 1-2 cm and placed in a jar containing 500 mL of 0.5% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). The jar was shaken well for two minutes to separate most of *M. incognita* eggs inside egg masses located on plant roots; then, the solution was filtered through 60 and 500 Mesh sieves. The eggs on 500 mesh were washed thoroughly with sterilized tap water to remove NaOCl residue. After that, the eggs were transferred to a conical flask and incubated at  $28 \pm 2$  °C for three days according to Coyne et al. (2007), then eggs hatched in water and active juveniles (J2) of *M. incognita* were collected.

### 2.6. *In vitro* experiment

The effect of *P. lilacinum* was tested on survival second stage of *M. incognita* juveniles (J2) and egg hatching in comparison with commercial product of *P. lilacinum*

**Table 1.** Primer codes (F and R) and their nucleotide sequence.

Primer Code	Sequence	Product Size
(ITS-1) F	5'- TCCGTAGGTGAACCTGCCG -3'	600bp
(ITS-4) R	5'- TCCTCCGCTTATTGATATGC-3'	

(Bio-Nematon) under laboratory conditions (Table 2). One hundred of 3 days old active juveniles and eggs were pipetted in vials. One mL of bio-agent suspensions of *P. lilacinum* and Bio-Nematon were separately added at the rate of 1mL/vial. Distilled water was added to the same number of eggs and juveniles and served as a control. Each treatment was replicated five times at room temperature. The percentages of egg hatching, and juvenile's mortality were recorded after 24, 48 and 72 hours under a microscope.

### 2.7. In vivo experiment

A greenhouse experiment was conducted to evaluate the antagonistic properties of *P. lilacinum* strain against root-knot nematode, *M. incognita* infecting tomato in comparison with commercial bioagent Bio-Nematon. A total of forty-five plastic pots (16 cm diameter) each one containing 1kg of sterilized loam sand soil (1:1) were planted with tomato seedlings (3-weeks old), cv. Castle Rock. Both of *P. lilacinum* ( $1 \times 10^{10}$  CFU/mL) and Bio-Nematon ( $1 \times 10^8$  CFU/ mL) were applied at (10 mL/ pot) as a soil drench. Second stage juveniles of *M. incognita* were added one day after transplanting at the rate of 1000J2s/pot. Nine treatments of pots were prepared with five replicates for each treatment including a positive control (inoculated with nematode) and a negative control (without nematode) as follows:

- 1) T1= Pots inoculated with *P. lilacinum* one week before transplanting (10mL/pot);
- 2) T2= Pots inoculated with Bio-Nematon one week before transplanting (10mL/pot);
- 3) T3= Pots inoculated with *P. lilacinum* (10mL/pot) one week after second stage of *M. incognita* juveniles (J2) inoculation;
- 4) T4= Pots inoculated with Bio-Nematon (10mL/pot) one week after second stage of *M. incognita* juveniles (J2) inoculation;
- 5) T5= Pots inoculated with *P. lilacinum* (10mL/pot) one week before transplanting and one week after second stage of *M. incognita* juveniles (J2) inoculation;
- 6) T6= Pots inoculated with Bio-Nematon (10mL/pot) one week before transplanting and one week after second stage of *M. incognita* juveniles (J2) inoculation;

- 7) T7= Pots inoculated with Oxamyl (10%) (0.3g/pot) two days after second stage of *M. incognita* juveniles (J2) inoculation;
- 8) T8= Pots inoculated with second stage of *M. incognita* juveniles (J2) (positive control);
- 9) T9= Pots contained healthy plants free *P. lilacinum* and second stage *M. incognita* juveniles (J2) (negative control).

This experiment was performed completely in a greenhouse of Nematode Diseases Research Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt under favorable environmental conditions (day length 12-14 h, temperature 28-30 °C and humidity 65%).

### 2.8. Plant and nematode parameters

Plants were collected after 60 days of nematode infection. The tomato growth criteria including plant length, plant weight and shoot dry weight were measured. Plants were uprooted carefully. The roots were washed gently by tap water to separate soil particles. Then, roots were stained with acid fuchsin in lactic acid for counting phases of nematode i.e. developmental stages, females, galls, and egg masses under binocular (Byrd et al., 1983). Juveniles of nematode were extracted from soil using modified Baermann technique then, counted using a stereoscopic microscope with count slide (Hooper et al., 2005). The nematode reproduction factor (Rf), Galls (GI) and egg masses indices (EI) were measured on scale of 0-5 according to Taylor and Sasser (1978) where, 0= no galls or egg masses; 1=1-2 galls or egg masses; 2=3-10 galls or egg masses; 3=11-30 galls or egg masses; 4=31-100 galls or egg masses and 5= more than 100 galls or egg masses per root system.

### 2.9. Determination of resistance related enzymes

Peroxidase activity (PO) was determined in fresh leaves of tomato according to Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of  $H_2O_2$  at 425 nm. using a UV Spectrophotometer.

Polyphenol oxidase (PPO) activity was determined according to Cho and Ahn (1999). The activity of (PPO) was expressed as the change in absorbency of 1.0 mL of extract per min at 420 nm. using UV spectrophotometer.

**Table 2.** Effect of *Purpureocillium lilacinum* and Bio-Nematon (1.15%WP) on percentage of egg hatching inhibition and mortality of second stage *M. incognita* juveniles (J2) at different exposure periods under *in vitro* conditions.

Treatment	Egg hatching inhibition%			J2 mortality %		
	24h	48h	72h	24h	48h	72h
<i>P. lilacinum</i>	90 <sup>a</sup>	88.4 <sup>a</sup>	79.8 <sup>a</sup>	54.4 <sup>a</sup>	76.0 <sup>a</sup>	97.6 <sup>a</sup>
Bio-Nematon	85.6 <sup>a</sup>	70.8 <sup>b</sup>	55.8 <sup>b</sup>	28.4 <sup>b</sup>	43.2 <sup>b</sup>	59.2 <sup>b</sup>
Control	63.6 <sup>b</sup>	37.6 <sup>c</sup>	12.8 <sup>c</sup>	1.2 <sup>c</sup>	3.6 <sup>c</sup>	8.4 <sup>c</sup>
LSD	13.81	9.63	7.00	6.502	8.859	5.442

Values are means of five replicates. Within a column, the marked values of the same letters are not significantly different while those have different letter(s) are significantly different using LSD at  $P \leq 0.05$ .

### 2.10. Data analysis

The data obtained were statistically analyzed with SPSS 20.0 software. One-Way analysis of variance (ANOVA) was used to determine the significance difference between the treatments. The least significance difference (LSD) was calculated at  $P < 0.05$ .

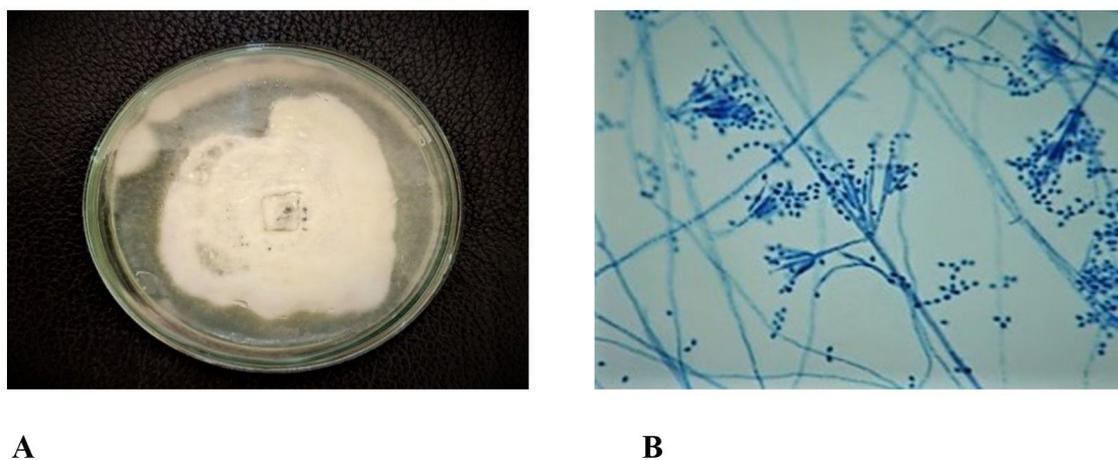
## 3. Results

To confirm the morphological identification of *P. lilacinum* AUMC 10149 (Figures 1A and 1B), the used fungus was subjected to molecular characterization by DNA-sequencing based method. PCR amplicon of ITS region was successfully obtained and then sequenced. The sequence was submitted to GenBank with accession number MW281802. *P. lilacinum* showed 99% homology with sequences of *P. lilacinum* available in GenBank.

The phylogenetic relationship showed that *P. lilacinum* was closely related and grouped with *P. lilacinum* (Figure 2).

Both tested bioagents *Pl* and Bio-Nematon (1.15%WP) gave a remarkable effect on egg hatching inhibition and juvenile's survival compared with control treatment at different exposure periods (Table 2). Generally, using *Pl* gave the highest degrees of egg hatching inhibition and J2 mortality as compared with Bio-Nematon.

Irrespective to time of application, the results showed that most of treatments caused a significant enhancement in plant growth criteria with various degrees (Table 3). The highest plant length was recorded in T5 with mean length percentage of (64.9%) followed by T3 (55.49%) then T7 (43.46%) with significant difference as compared with positive control T8. On the other hand, the highest percentages of improvement in plant weight were recorded in treatments T1, T3 and T5 with incidence percentages of 58.69, 76.26 and 72.52%, respectively.



**Figure 1.** (A) Macroscopic view of *P. lilacinum* AUMC 10149 incubated for 10 days on Czapek's medium; (B) Microscopic view (400X) of *P. lilacinum* AUMC 10149 indicating the chains of elliptical conidia with divergent branches.

**Table 3.** Effect of *Purpureocillium lilacinum* and Bio-Nematon (1.15%WP) on tomato plant growth criteria at different application times under greenhouse conditions.

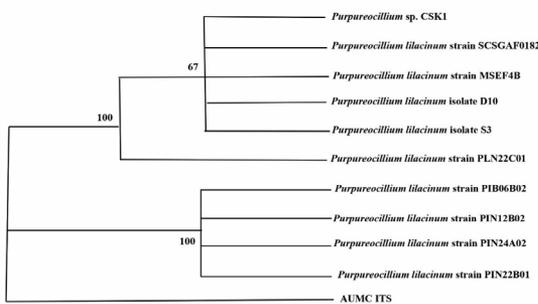
Treatments	Plant length (cm.)	Increase %	Plant weight (g.)	Increase %	Dry weight (g.)	Increase %
T1	46.6 <sup>cd</sup>	21.99	16.98 <sup>ab</sup>	58.69	2.95 <sup>ab</sup>	139.84
T2	39.6 <sup>de</sup>	3.66	13.22 <sup>de</sup>	23.55	1.98 <sup>c</sup>	60.98
T3	59.4 <sup>ab</sup>	55.49	18.86 <sup>a</sup>	76.26	2.66 <sup>b</sup>	116.26
T4	41.6 <sup>de</sup>	8.90	13.8 <sup>cd</sup>	28.97	1.97 <sup>c</sup>	60.16
T5	63 <sup>a</sup>	64.9	18.46 <sup>a</sup>	72.52	3.24 <sup>a</sup>	163.41
T6	48.8 <sup>c</sup>	27.74	14.44 <sup>bcd</sup>	34.95	2.57 <sup>b</sup>	108.94
T7	54.8 <sup>b</sup>	43.46	16.12 <sup>abc</sup>	50.65	2.78 <sup>ab</sup>	126.01
T8	38.2 <sup>e</sup>	-	10.7 <sup>e</sup>	-	1.23 <sup>d</sup>	-
T9	47 <sup>cd</sup>	23.04	13.16 <sup>de</sup>	22.99	2.02 <sup>c</sup>	64.23
LSD	5.612		2.849			

Values are means of five replicates. Within a column, the marked values of the same letters are not significantly different while those have different letter(s) are significantly different using LSD at  $P \leq 0.05$ .

Similar results were noticed with plant dry weight which was significantly improved with T5 (163.41%) and T1 (139.8%) compared with positive control T8. To our knowledge, it is better to mention that the treatment of tomato plants with the *Pl* gave higher plant growth criteria than the treatment of Bio-Nematon.

Data represented in Table 4 pointed that the total *M. incognita* nematode population was decreased by all treatments with reproduction factor (Rf) ranged from 0.9 to 3.9 as compared to nematode alone T8. Numbers of nematode populations in soil and developmental stages in roots of tomato are significantly suppressed in all treatments as compared to the positive control treatment T8. The highest reduction in total nematode population was achieved in T7 followed by T5 and T6 with percentages of 85.3, 68.4 and 65.0% respectively.

The lowest effective treatments in reducing number of J2 in soil were recorded in T1 and T2 with percentages of 45.1 and 36.8%, respectively. On the other hand, the highest reduction rate in J2 number was observed in treatments T7 and T5 with percentages of 85.2 and 68.4%



**Figure 2.** Dendrogram illustrating relatedness of genus *Purpureocillium* sp. AUMC 10149.

respectively. However, a significant reduction in number of females was noticed in all tested treatments except T2. The conventional nematocide, oxamyl T7 was superior, exceeded other treatments and significantly suppressed number of females in roots.

Nevertheless, the most effective treatment in reducing *M. incognita* in roots and soil was recorded in T5 with reproduction factor =1.9. While T2 ranked as less effective treatment with reproduction factor =3.9. Data also revealed that root galling of *M. incognita* was significantly reduced by all tested treatments with root gall index (RGI) ranged from 3.0 to 5.0. Similar result was shown with egg masses index (EI) which ranged from 2.0 to 5.0. The highest reduction was recorded with T7 followed by T5 with root galls and egg masses indices 3.0; 4.0 and 2.0; 3.0, respectively.

The present study revealed the action of some resistance enzymes including peroxidase (PO) and polyphenol oxidase (PPO) activities in fresh leaves of tomato infected with *M. incognita* (Table 5). It was noticed that all treatments induced an increase in enzymes activity compared to nematode alone (positive control). T2 (346.0%) recorded the highest significant PO activity followed by T5 (137.5%), then T4 (104.6%). The lowest percentage in PO activity was observed in T1 (5.1%). Conversely, the highest increase in PPO activity was more pronounced in T1 (93.5%) then T6 (82.6%). While T4 (4.3%) showed the lowest percentage in PPO activity.

#### 4. Discussion

In recent years, biological control agents had attained importance in modern agriculture to decrease the hazards of the intensive use of chemicals for pest and disease control of tomato as well as harmful to human health and environmental risks (Akladios et al., 2015). Accordingly, the biocontrol efficacy of new isolate nematode-pathogenic

**Table 4.** Effect of *Purpureocillium lilacinum* and Bio-Nematon (1.15%WP) on nematode indexes (*M. incognita*) at different application times under greenhouse conditions.

Treatment	No. of nematode in soil	R. %	No. of nematode in root		Total nematode (soil + root)	R. %	Rf	Root galls No.	RGI	Egg mass	EI
			Develop. Stage	Females							
T1	3210 <sup>bc</sup>	45.1	34.2 <sup>b</sup>	75.6 <sup>b</sup>	3319.8 <sup>bc</sup>	45.1	3.3	101.2 <sup>c</sup>	5	52.4 <sup>bc</sup>	4
T2	3700 <sup>b</sup>	36.8	30.6 <sup>bc</sup>	124.6 <sup>a</sup>	3855.2 <sup>b</sup>	36.2	3.9	142.4 <sup>b</sup>	5	103.6 <sup>a</sup>	5
T3	2648 <sup>c</sup>	54.8	24.8 <sup>bcd</sup>	66.8 <sup>bc</sup>	2739.6 <sup>c</sup>	54.7	2.7	85.2 <sup>c</sup>	4	49.6 <sup>c</sup>	4
T4	3144 <sup>bc</sup>	46.3	23 <sup>cd</sup>	87 <sup>b</sup>	3254 <sup>c</sup>	46.2	3.3	97.0 <sup>c</sup>	4	73.6 <sup>b</sup>	4
T5	1850 <sup>d</sup>	68.4	19.6 <sup>de</sup>	44 <sup>c</sup>	1913.6 <sup>d</sup>	68.4	1.9	55.8 <sup>d</sup>	4	30.2 <sup>cd</sup>	3
T6	2028 <sup>d</sup>	65.3	28 <sup>bcd</sup>	63.2 <sup>bc</sup>	2119.2 <sup>d</sup>	65.0	2.1	78.8 <sup>cd</sup>	4	48.4 <sup>c</sup>	4
T7	866 <sup>e</sup>	85.2	9.6 <sup>e</sup>	13.2 <sup>d</sup>	888.8 <sup>e</sup>	85.3	0.9	14.6 <sup>e</sup>	3	7.6 <sup>d</sup>	2
T8	5852 <sup>a</sup>	---	71.8 <sup>a</sup>	123 <sup>a</sup>	6046.8 <sup>a</sup>	---	6.0	186 <sup>a</sup>	5	112 <sup>a</sup>	5
LSD	582.17		10.71	25.99	594.28			24.68		23.68	

Values are means of five replicates. Within a column, the marked values of the same letters are not significantly different while those have different letter(s) are significantly different using LSD at  $P \leq 0.05$ .

**Table 5.** Activities of peroxidase (PO) and polyphenol oxidase (PPO) enzymes in fresh leaves of tomato plants infected with *M. incognita* and treated with *P. lilacinum* and Bio-Nematon (1.15%WP) at different exposure periods under greenhouse conditions.

Treatments	Peroxidase enzyme (PO)	Increase%	Polyphenol oxidase (PPO)	Increase%
T1	0.45 <sup>e</sup>	5.1	0.086 <sup>b</sup>	93.5
T2	1.942 <sup>a</sup>	346.0	0.074 <sup>b</sup>	67.4
T3	0.548 <sup>f</sup>	24.1	0.076 <sup>b</sup>	65.2
T4	0.888 <sup>c</sup>	104.6	0.048 <sup>c</sup>	4.3
T5	1.03 <sup>b</sup>	137.5	0.05 <sup>c</sup>	10.9
T6	0.804 <sup>d</sup>	88.7	0.049 <sup>c</sup>	82.6
T7	0.56 <sup>f</sup>	31.7	0.05 <sup>c</sup>	8.7
T8	0.434 <sup>g</sup>	--	0.047 <sup>c</sup>	--
T9	0.702 <sup>e</sup>	60.9	0.15 <sup>a</sup>	165.2
LSD	0.0383		0.0137	

Values are means of five replicates. Within a column, the marked values of the same letters are not significantly different while those have different letter(s) are significantly different using LSD at  $P \leq 0.05$ .

fungus *P. lilacinum* AUMC 10149 against root-knot nematode *M. incognita* infecting tomato plant was studied. The present work showed that application of *P. lilacinum* against root-knot nematode, *M. incognita* has a suppressive effect as a biocontrol agent compared with Bio-Nematon (commercial product of *P. lilacinum*). *In vitro*, the use of *P. lilacinum* gave maximum mortality percentage of 97.6% for second stage of *M. incognita* (J2) after 72 h and suppressed egg hatching with percentage of 79.8% after the same period. These results are compatible with Ahmad et al. (2019) who tested the ability of local isolates of *Purpureocillium* to reduce the nematode eggs hatching of *Meloidogyne* spp. They discovered that the isolate *P. lilacinum* has the greatest potential destruction of nematodes eggs by 67.9%. The mechanism of fungal antagonist *P. lilacinum* against root-knot nematode, *Meloidogyne* spp. has been studied by many researchers (Huang et al., 2004; Khan et al., 2006; Lopez-Llorca et al., 2008). Swarnakumari and Kalaiarasan (2017) illustrated that fungal mycelium started attaching on surface of the egg on the first day after inoculation. Penetration of fungal mycelium on eggshell was initialized by appressorium formation on the second day. Fungal growth was recorded on the egg surface at 72 h after conidial inoculation. Eggs were completely colonized by the fourth day after inoculation.

When a hypha of *Pl* faces nematode egg surface, it responds thigmotropically by forming appressoria, followed by adhesion of an adhesive on the egg for better linkage between *Pl* and the host. Then, the fungus uses various enzymatic and mechanical methods for penetrating the host (Lopez-Llorca et al., 2008). The secretion of proteases and chitinase by *Pl* led to egg penetration by breaking down of shells layers of egg nematode causing the disappearance of the lipid layer and more reduction of chitin layer after enzyme treatment (Khan et al., 2006). Moreover, the fungal hyphae will fill the eggs and then appear to the egg surface resulting in the first vegetative of growth and conidia.

For infection of J2, *Pl* needs to overcome the nematode cuticle which is considered a non-cellular layer production of the hypodermis. After penetration of the cuticle layer by *Pl* hyphae, the nematodes are paralyzed, invaded and then digested (Huang et al., 2004). Over the years, fungal metabolites in culture filtrate of *Pl* have been extracted and tested against infection process with J2 such as paecilotoxin, leucinotoxins, chitinases, proteases, and acetic acid (Yang et al., 2015). So, it can be recommended using *Pl* biological properties for controlling *Meloidogyne* spp.

The greater decrease of plant growth criteria was obtained with treatment of nematode only that may be due to the faster invasion of tomato roots by higher numbers of J2 and the eventual maturation of adult females of *M. incognita*. The developing giant cell systems and disruption of the growing xylem vessels resulted in a change in plant physiological functions such as transporting nutrients and water from the root to the whole plant, nutrient uptake, photosynthesis process and expose the plant to another pathogen infection (Saikia et al., 2013). On the other hand, the application of *P. lilacinum* before planting and after infection showed a significant improvement in plant growth criteria compared with other treatments. Similar findings of improved tomato plant criteria by *P. lilacinum* have been recorded by Ahmed and Monjil (2019).

Generally, the mechanism for improving plant growth parameters induced by the biocontrol agents as fungi is unknown. Our hypothesis may be attributed to some physiological changes induced in the plant such as changes in cell wall composition and activation of plant defense mechanism. Also, uptake of enhanced nutrient, suppression of disease, integration of mineral nutrition through solubilization of minerals or production of plant hormones as a result of the use of *Pl* (Hol and Cook, 2005).

The effectiveness of Bio-Nematon against *M. incognita* infecting various crops i.e. cowpea and eggplant was more pronounced when applied as post nematode inoculation (Metwally et al., 2019; Khairy et al., 2021). In present study, it was noticed that the applying Bio-Nematon

before transplanting and nematode inoculation showed no effect in reduction of egg masses number. This result agreed with the findings of Oclarit and Cumagun (2009) who conducted that egg mass count in plants treated with the lowest concentration of the biocontrol agent was not significantly different from the uninoculated control.

The lowest effective treatments in reducing number of J2 in soil were recorded in treatment with Bio-Nematon and *P. lilacinum* before transplanting. However, Kalele et al. (2010) documented that both pre-planting and at planting application of *Pl* 251 were found to decrease populations of nematode and root galling in both cucumber and tomato. On the other hand, the nematicidal activity by *P. lilacinum* before planting and after infection was found greater to reduce nematode population compared with control treatment. This proved the ability of using *P. lilacinum* as a biocontrol agent of *M. incognita*. Our results are in accordance with Sarven et al. (2019) who reported that treatment of soil with *P. lilacinum* PLSAU-1 at  $1 \times 10^6$  CFU/g soil before transplanting and after 3 days of brinjal transplantation decreased a maximum of 72% gall index and 84% egg masses of *M. incognita*.

The reduction in nematode parameters such as gall formation and reproduction factor is dependent on plant, fungus, and nematode species as well as prevailing environmental conditions (Campos, 2020). The presence of *P. lilacinum* in the rhizosphere of plant roots at the time of penetration of pathogen may reduce the number of juveniles as it colonizes the roots and protects its surface from attack of root-knot nematode that could enter the roots (Esfahani and Pour, 2006).

The saprophytic fungi may induce systemic resistance by activation of various defense related enzymes i.e. peroxidase (PO) and polyphenol oxidase (PPO). In different plants, increased activity of PPO or PO has been occasioned by biocontrol agent strains (El-Deriny, 2016). In this study we noticed that whether *Pl* or Bio-Nematon increased activity of PO and PPO at different time of application. This finding is in accordance with those reported by Kavino et al. (2007) who conducted that the bio-agent *Pseudomonas fluorescens* might stimulate the production of biochemical compounds associated with the host defense. Of these, early induction of PO is more important as it is the first enzyme in the phenylpropanoid pathway, which leads to production of phytoalexin and phenolic substances leading the formation of lignin (Bruce and West, 1989). Peroxidase activity is vital in the intensification of cell walls in resistant plants at the border of infection and is considered as significant components of active defense response of nematode invaded tissue (Zacheo et al., 1995). PPO-generated quinones are highly reactive and may cross-link or alkylate proteins, leading to the commonly observed brown pigments in damaged plant tissues (Constabel and Barbehenn, 2008).

## 5. Conclusion

The present study demonstrates that treatment of tomato plants with the native isolate *P. lilacinum* AUMC 10149 against root-knot nematode *M. incognita* revealed

that *Pl* has a promising bioagent potential and can be used to reduce virulence of tomato infection. Therefore, our results confirmed that *P. lilacinum* could be used as a practical bioagent for controlling of root-knot nematodes in Egypt. However, more studies must be conducted under field conditions to confirm these results and reach the optimal rates of use.

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