Egypt. J. Agronematol., Vol. 21, No.2, PP. 59-78 (2022)
DOI: 10.21608/EJAJ.2022.257669

Nematicidal Effect of Three *Trichoderma* spp. on the Suitability of Tomato Plants for *Meloidogyne incognita* Reproduction

Abdelhadi A. I. Ali; Abdelrahman Eldeeb; Mahmoud M. Ramadan and Ramadan M. El-Ashry

Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

Corresponding author email: Aaalai@agri.zu.edu.eg

Received: 22 July2022 *Revised:23 August 2022* *Accepted: 28 August 2022*

ABSTRACT

Soil-borne root-knot nematode (RKN) *Meloidogyne incognita*, has become a chronic pest that must be dealt with repeatedly every season, particularly during flowering and fruiting periods. So, this situation heightens to search for safe, effective, and extended-release alternatives for chemical nematicides to combat RKN. So, the potential nematicidal activity of *Trichoderma album, T. harzianum* and *T. viride* against RKN eggs hatching and second stage juveniles (J2) survival was determined *in vitro*, besides their interaction effect on reducing reproduction factor (Rf) of *M. incognita* in infected tomato roots in pot soils and promoting plant growth parameters under subtropical greenhouse conditions. *In vitro*, *Trichoderma* spp. showed a limited nematicidal effect against RKN with the various concentrations. *T. viride* exhibited a weak nematicidal effect against eggs and J2 of RKN. Moreover, the concentration of 1×10^8 spores/ml displayed maximum ovicidal effect on RKN eggs and *T. album* potency surpassed *T. viride.* Conversely, *T. harzianum* caused the highest significant ovicidal and larvicidal effect at the concentrations of 1×10^7 and 1×10^8 spore/ml and exceeded oxamyl potency after 6 days with eggs and at 1×10^8 spore/ml after 14 days incubation with J2s. Generally, *Trichoderma* spp. showed a significant ovicidal effect against RKN surpassing oxamyl nematicide in contrast to the larvicidal effect which was very limited compared with oxamyl. In pots, plants inoculated with *Trichoderma* spp. achieved higher plant fitness indices compared to the control, presumably related to changes in plant resistance after proving *Trichoderma* root colonization. The number of galls/root, egg masses/root, J2s/100 g soil, eggs/100 g soil and Rf in *Trichoderma* treatments showed a mediated significant reduction lower than oxamyl potency which ascertained the ability of *Trichoderma* spp. with various concentrations to keep RKN population below the economic threshold or avoid plant damage resulted from RKN infection by multiple mechanisms of action including parasitism, root colonization, plant resistance and growth promoting.

Keywords: *Meloidogyne incognita*, *Trichoderma*, ovicidal, larvicidal and root colonization.

INTRODUCTION

When the human being directly intervenes in the eco-system for self-interestedness, he becomes interested in what is directly related and excludes everything that is not directly beneficial to him. These measures caused an imbalance in the ecosystem after the shortage of biodiversity. Nematodes are an important component of the soil eco-system particularly phytonematodes that are a major worldwide limitation to economic crop production. Root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid & White) Chitwood (El-Ashry et al., 2020) is a vital species via the formation of giant cells and considered a major threat to agriculture (more than 2000 plant species), reduced plant growth parameters and productivity, or even plant death (Karssen et al., 2013) in subtropical climate causing significant losses in crop yields worldwide (Barker, 1998; McCarter, 2008). The effective control of RKN under tropical conditions is limited (dos Santos Pereira et al., 2020). Growers used

many measures to reduce RKN population including cultural practices (crop rotation, resistant cultivars) and chemical control but these control measures lack options for crop rotation which always contains resistance cultivars with low or no economic value and with no durable resistance in tropical or subtropical areas. As well, chemical control was the most effective and popular tool for growers in the urgent situation for controlling of RKN for decades (Moens et al., 2009).

Thus, the use of biological control agents (BCAs) which contain more than 200 specific organisms is known to be natural enemies of RKN (Freitas et al., 2009; Kerry, 1990) and cause no adverse damage to environment and human (Ferraz and Brown, 2002; Freitas et al., 2009). Nowadays, alternative control strategies appeared and showed effective performance against phytonematodes but the timing appears to be critical for their effectiveness (Giannakou et al., 2002).

Fungi are BCAs that can trap or hyperparasite the nematodes (Huang et al., 2004) either on nematode eggs for instance, *Paecilomyces* sp. (Khan et al., 2004) and *Pochonia* sp. (Tikhonov et al., 2002) and suppress egg hatching, consequently reducing nematode populations in plant root or pot soils. Whereas *Trichoderma* spp. fungi are widely distributed in soil and active mycoparasites with a considerable potency for foliar application (Mora and Earle, 2001) and controlling soil-borne diseases (Larralde-Corona et al., 2008) such as phytonematodes. Nearly 254 *Trichoderma* species have been formerly (Bissett et al., 2015) and 71 new species were identified along 2015 to 2018 years, and there are undoubtedly many more awaiting discovery (Qiao et al., 2018).

Various studies were conducted under greenhouse conditions to define the most effective *Trichoderma* species which *T. harzianum* considered one of the excellent control bioagents (Sharon et al., 2001) against root knot and citrus nematodes. Zhang et al. (2014) demonstrated *T. longibrachiatum* as a strong parasitic bioagent and lethal effect against *Heterodera avenae* cysts *in vitro*. Moreover, *T. longibrachiatum* and *Mortierella alpine* are used as BCAs for the sustainable management of RKN on selected crops (AL-Shammari et al., 2013). In 2008, a study conducted by Sahebani and Hadavi (2008) revealed two main suppression mechanisms by *T. harzianum* one of them via the increase in extracellular chitinase activity which is used as indicator of eggs infection capability and inducing plant defense mechanisms leading to systemic resistance. Krif et al. (2022) exhibited the beneficial use of biological nematicides as one of the most acceptable alternative methods in nematode management by the farming community. Besides, significantly improved tomato growth parameters: plant length, shoot fresh weight and, root systems in tomato crop. The beneficial effect of combined *T. viride* with composted plant residues or water-extractable fraction of vermicomposts enhanced the growth of vegetables such as tomato and their tolerance against *M. incognita* (dos Santos Pereira et al., 2020; Zhang and Zhang, 2009). Currently, BCAs such as *Trichoderma* spp. Achieved significant success in the control of various plant diseases, improving vegetable growth, decomposition process and bioremediation besides, secondary metabolites production in agroecosystem and utilization of *Trichoderma* in friendly agriculture practices (Zin and Badaluddin, 2020). Moreover, the reduction of RKN damage is not accompanied by disturbing the beneficial microorganism communities and the natural environment resulting from the application of pesticides.

Up till now, less attention has been given to BCAs in the management of plant diseases. BCAs approved to be efficient in reducing damage caused by RKN without disturbing the beneficial microorganism community and natural environment. So, the

present study was carried out to determine the comparative efficacy *of Trichoderma* spp. (*T. album, T.viride* and *T. hamatum*) as BCAs compared to the nematicide, oxamyl against *M. incognita* eggs and juveniles stages *in vitro* and clarify the ability of *Trichoderma* spp. To colonize tomato root and their influence by reducing reproduction factor of *M. incognita* in plant root and promote plant growth parameters under greenhouse conditions.

MATERIALS AND METHODS

1-Inoculum Preparation

1.1-Root-knot Nematode, *M. incognita*:

The isolate of *M. incognita* inoculum was identified using the juvenile measurements and examination of the perineal pattern system of adult females (Eisenback and Triantaphyllou, 1991; Jepson, 1987). The RKN pure culture was established on the tomato susceptible cultivar Super Strain B inoculated with a single egg mass to provide the required inoculum for the subsequent experiments.

The infected roots of tomato were cut into 2 cm long pieces and placed in a flask 600 ml containing 200 ml of 0.5% sodium hypochlorite, then the flask was capped tightly and shaken for 3 minutes to dissolve partially the gelatinous matrix to free eggs from egg masses (Hussey and Barker, 1973). The liquid suspension of eggs was poured through a 200 mesh sieve nested upon a 500 mesh sieve. Eggs collected on the 500 mesh sieve were immediately washed to remove sodium hypochlorite residue under a slow stream of tap water. To obtain newly hatched juveniles (J2), free eggs of RKN were incubated in Petri dishes at 25±1°C until hatching then collected by using a micropipette.

1.2- Inocula of Tested *Trichoderma* Species:

Trichoderma album, *T. viride* and *T. harzianum* were obtained from Plant Pathology Laboratory, Faculty of Agriculture, Zagazig University, which cultured on potato dextrose agar (PDA) in Petri dishes for 7 days at 25±2 °C. Ten Petri dishes for each fungus were used to collect sufficient conidia for the experiment. The conidia suspension of the tested *Trichoderma* species was prepared by flooding each dish with 10 ml of sterilized distilled water containing 0.01% (V/V) Tween 80, and the agar surface was scraped gently with a sterile glass rod and collected in 250 ml beakers. The suspension was filtered through sterile double-layered muslin cloth to remove mycelium fragments. The density of conidia in spore suspension was assessed using a Neubauer hemocytometer to use these concentrations as stock to prepare the subsequent suspension of different densities: 1×10^4, 1×10^5, 1×10^6, 1×10^7 and1×10^8 spore/ ml and stored at 4 °C.

2- Nematicidal Effect of Tested *Trichoderma* spp. on Egg Hatching and Juvenile Mortality of *M. incognita in vitro*:

2-1. Ovicidal activity of *Trichoderma* spp. on individual eggs hatching:

The extracted eggs of *M. incognita* from infected roots of tomato were suspended in water and counted using a counting slide under a stereomicroscope. The egg numbers were adjusted to about 1000 eggs/ml by concentrating the suspension using a centrifuge. Approximately, 200 nematode-free eggs were transferred in 0.2 ml of egg suspension to expose spore capacity 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spore/ ml with the three tested *Trichoderma* spp. (*T. album, T. viride, T. harzianum*). The Petri dishes (9 cm) were incubated at 25 ±3˚C and observed for 14 days at the latest after inoculation besides control treatment. The number of hatched J_{2s} was expressed as

a cumulative number of viable J_2s. The percentage of hatching inhibition was calculated in comparison with control according to the following equation:

$RI\ (\%) = (X - O)/X \times 100$, Where:

RI (%): Relative percentages in inhibition of egg hatching.

X: Number of J2s hatched in the sterile water control

O: Number of J2s hatched in each treatment.

2.2. Larvicidal activity of *Trichoderma* spp. on 2nd juveniles of *M. incognita*:

The previously indicated concentrations were prepared in a 5 ml final volume. Each suspension involved 0.2 ml of J2s stock suspension (1000 J2/ml) containing 200 J2 were pipetted into sterilized Petri dishes. The control plates contained 5 ml of sterile water contained 0.2 ml of J2 stock suspension. The Petri dishes were incubated at 24±2° C. The process of infection was observed periodically until 18 days after inoculation. The percentage of immotile J2 of *M. incognita* was calculated, and immotile second-stage juveniles were allowed to recover in tap water for 5 h. Those that remained inactive were considered dead (Meyer et al., 2004). Each treatment consisted of five replicates. The percentage mortality of the J2 in each concentration was corrected using Abbott's formula (Finney, 1971):

$C = (O - X)/(100 - X) \times 100$, Where:

C is the corrected mortality %; O is the observed mortality %.

X is the percentage of the control J_2 that have died at the relevant observation time.

3- Pots Experiments:

Plastic pots 25 cm diameter was filled with 2 kg/pot consisted of non-sterilized sandy soil (70.1% sand, 12.3 % clay, 8.1% silt), amended with 62.4 g composted chicken manure and 3 mg urea fertilizer per kilogram of soil to determine *Trichoderma* spp. effectiveness under temperate greenhouse conditions.

Experiments were carried out with the tomato plant (*Solanum lycopersicum* cv. 016). The seedlings at the 3-4 leaf stage (10 cm high) were separately transplanted from the seedling tray to plastic pots and irrigated. Seven days after planting, seedlings (except healthy plant control pots) were inoculated with 1000 J2s of *M. incognita* diluted in 2 ml using a micropipette. This volume was poured around each seedling into two holes 4 cm deep made using a pencil and covered with soil after inoculation.

This experiment contained 6 treatments each has 5 replicates, as follow:

- Control treatments included a negative control (plants without RKN and *Trichoderma* spp.) and positive control (plants inoculated with RKN only).
- *Trichoderma* spp. treatments (included *T. album*, *T. viride* and *T.harzianum*). Each pot received 10 ml of 1×10^6 spores/ml concentration implemented as soil drenching after inoculation with RKN.
- Oxamyl (Oxineem El-Nasr 24% SL) at 0.3 ml/plant as soil drenching after RKN inoculation.

Sixty days after inoculation, roots and surrounding soil in the pots were soaked in water for 2 hours to facilitate removing adhering soil and keep egg masses on the root surface. The measured tomato plant growth traits included fresh weights of root and shoot (g), number of leaves/plants, leaves weight /plant (g), and stem diameter (mm). While the recorded parameters related to RKN reproduction included the mean numbers of egg masses, galls (categorized galls based on volume), galls /root (root gall index), egg

masses/root (egg mass index), J2/100 g soil and eggs /100 g soil besides reproduction factor (Rf).

Nematode root densities were assessed from 2 g of root subsamples removed after chopping (1–2 cm) the whole root of each plant (Sharon et al., 2007). For enumeration of J2 in the soil, the samples of soils were extracted by the modified centrifugal-floatation method (Barker, 1985). Both root gall index (RGI) and egg-mass index (El) were calculated according to the scale given by (Taylor and Sasser, 1978). The final population densities of nematodes were determined and Rf = Final population/ initial population (Oostenbrink, 1968) was calculated.

4- Colonization of *Trichoderma* spp. Tomato Plant Roots:

To ascertain hyphae of *Trichoderma* penetration into infected epidermal and cortical tissue of the root and colonization of tomato roots by *Trichoderma* species. The roots were surface sterilized with 0.5% sodium hypochlorite for 3 minutes, washed in sterilized distilled water and repeated several times and then dried among sterilized filter papers. Small pieces of 7 mm length of roots were cut by a sterile scalpel, transferred to Petri dishes containing water agar (WA) medium and dishes were incubated in an inverted position at 28± 2°C and observed daily for 5 days. Once fungal growth occurred, the developed fungal colonies were picked up and purified using the hyphal tip techniques. Growing colonies were transferred to slopes of potato-dextrose agar (PDA) medium. Identification of the isolated fungi was possible after 7 days of incubation at 25±2 °C. Isolation, purification and identification were carried out according to the morphological characteristics of each fungus in Plant Pathology Department, Faculty of Agriculture, Zagazig University.

5- Statistical Analysis:

The replicates of each treatment were arranged in a completely randomized design. The experiments data were subjected to analysis of variance (ANOVA) based on an applied design using MSTAT version 4. Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS AND DISCUSSION

Ovicidal and Larvicidal Effect of *Trichoderma* Species on *M. incognita*:

As shown in Table (1), the application of *Trichoderma* spp. (*T. album* and *T. viride*), with several concentrations (spores /ml) at different exposure periods (days) on root knot nematode eggs exhibited a highly negative effect on the hatchability of *M. incognita* eggs (number of J2 emerged) due to the toxicity of *Trichoderma* spp. As concentrations of the tested *Trichoderma* spp. increased, numbers of the emerged juvenile decreased. The highest concentration of tested *Trichoderma* 1×10^8 spore/ml displayed the maximum effect (33.33%) of egg hatchability after one day without remarkable differences between *T. album* and *T. viride*. After three days of treatment, *T. album* (55.96%) displayed more toxicity against the hatchability of RKN eggs than *T. viride* (51.26%). With the time elapsed, the same trend was observed after 14 days and the number of emerged J2 were 125.80 and 128.40 in Petri dishes treated with 10^8 spores /ml with *T. album* and *T. viride,* respectively compared to 196.40 J2 in the control treatment (distilled water) with percentages of egg hatching reduction, 35.95 and 34.62% respectively. On the other hand, oxamyl has the highest effect compared to *Trichoderma* species at various concentrations at all different times of immersion.

Table 1: Number of emerged juveniles and non-hatched percentages of RKN, *Meloidogyne incognita* eggs, inoculated with *Trichoderma album* and *T. viride in vitro*.

Treatments	Concentrations (Spores/ml)	Incubation period (Days)				
		1	3	6	10	14
Control		2.40 [a]	55.40 [a]	124.60 [a]	181.40 [a]	196.40 [a]
T. album	10^4	2.20 [a] (8.33)	47.60 [b] (14.08)	121.60 [a] (2.41)	174.20 [b] (3.97)	179.80 [b] (8.45)
	10^5	2.00 [ab] (16.67)	41.60 [c] (24.91)	113.20 [b] (9.15)	160.00 [c] (11.80)	171.00 [c] (12.93)
	10^6	2.00 [ab] (16.67)	34.60 [d] (37.55)	104.80 [c] (15.89)	127.40 [d] (29.77)	146.00 [d] (25.66)
	10^7	1.80 [b] (25.00)	28.80 [e] (48.01)	70.00 [d] (43.82)	110.60 [e] (39.03)	136.00 [e] (30.75)
	10^8	1.60 [b] (33.33)	24.40 [f] (55.96)	53.00 [e] (57.46)	107.60 [e] (40.68)	125.80 [f] (35.95)
Oxamyl		1.00 [c] (58.33)	12.20 [g] (77.98)	31.20 [f] (74.96)	54.00 [f] (70.23)	73.80 [g] (62.42)
Control		2.40 [a]	55.40 [a]	124.60 [a]	181.40 [a]	196.40 [a]
T. viride	10^4	2.00 [ab] (16.67)	49.40 [b] (10.83)	124.60 [a] (0.00)	156.80 [a] (13.56)	187.00 [b] (4.79)
	10^5	2.00 [ab] (16.67)	43.80 [c] (20.94)	116.80 [b] (6.26)	148.20 [b] (18.30)	174.80 [c] (11.00)
	10^6	2.00 [ab] (16.67)	35.80 [d] (35.38)	108.40 [c] (13.00)	123.80 [c] (31.75)	149.60 [d] (23.83)
	10^7	1.80 [b] (25.00)	30.20 [e] (45.49)	74.20 [d] (40.45)	106.20 [d] (41.46)	141.40 [e] (28.00)
	10^8	1.60 [b] (33.33)	27.00 [f] (51.26)	64.20 [e] (48.48)	101.60 [d] (43.99)	128.40 [f] (34.62)
Oxamyl		1.00 [c] (58.33)	12.20 [g] (77.98)	31.20 [f] (74.96)	54.60 [e] (70.23)	73.80 g (62.42)

*Each replicate containing 200 eggs.

*Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; **Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test.

Under *in vitro* conditions, *T. harzianum* presented a significant effect on *M. incognita* egg hatching after 6 days of treatment. Maximum egg hatching value (17.80) was obtained from control (distilled water) on the first day compared to 7.80, 0.60, 0.00, 0.00 and 0.00 eggs with *T. harzianum* concentrations of 1×10^4, 10^5, 10^6, 10^7 and 10^8 spore/ml, respectively and the percentages of efficiency against egg hatching were 56.18, 96.63, 100, 100 and 100%, respectively. The highest egg hatching value was obtained from control and reached 198/ 200 eggs on the 6th day. Also, the parallel values with the abovementioned *T. harzianum* concentrations were 55.20, 49.20, 43.00, 32.80, 32.80 and 24.20/200 eggs, respectively. On the other hand, concentrations of 1×10^7 and 10^8 spore/ml were more effective than oxamyl treatment at all tested incubation periods under laboratory conditions (Table 2).

The effects of various *Trichoderma* spp. concentrations with different times elapsed on J2 mortality of *M.incognita* are shown in Table (3). The mortality percentages of *Trichoderma album* after 7 days post-treatment were 2.80, 2.80, 3.90, 6.50 and 2.80% with concentrations of 10^4, 10^5, 10^6, 10^7 and 10^8 spore/ml, respectively. The results suggested that the efficiency of *T. album* remarkably increased with an increase of the initial concentrations, and that time elapsed to achieve maximum effect efficiency occurred at 14 and 18 days.

Table 2. Number of emerged juveniles and non-hatched percentages of RKN, *Meloidogyne incognita*, eggs inoculated with *Trichoderma harzianum in vitro*.

Treatments	Concentrations (spores /ml)	Incubation period (Days)			
		1	**2**	**4**	**6**
Control *		17.80 [a]	55.40 [a]	148.40 [a]	198.00 [a]
T. harzianum	10^4	7.80 [b] (56.18)	12.60 [b] (77.26)	19.00 [b] (87.20)	55.20 [b] (72.12)
	10^5	0.60 [c] (96.63)	2.00 [c] (96.39)	5.40 [c] (96.36)	49.20 [c] (75.15)
	10^6	0.00 [c] (100)	1.60 [c] (97.11)	5.00 [cd] (96.63)	43.00 [d] (78.28)
	10^7	0.00 [c] (100)	0.80 [c] (98.56)	3.80 [de] (97.44)	32.80 [e] (83.43)
	10^8	0.00 [c] (100)	0.80 [c] (98.56)	2.80 [e] (98.11)	24.20 [f] (87.78)
Oxamyl		12.20 [g] (77.98)	31.20 [f] (74.96)	54.00 [f] (70.23)	73.80 [g] (62.42)

*Each replicate containing 200 eggs; Eggs in distilled water were used as control

*Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; **Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's test.

The effects of various *Trichoderma* spp. concentrations with different times elapsed on J2 mortality of *M.incognita* are shown in Table (3). The mortality percentages of *Trichoderma album* after 7 days post-treatment were 2.80, 2.80, 3.90, 6.50 and 2.80% with concentrations of 10^4, 10^5, 10^6, 10^7 and 10^8 spore/ml, respectively. The results suggested that the efficiency of *T. album* remarkably increased with an increase of the initial concentrations, and that time elapsed to achieve maximum effect efficiency occurred at 14 and 18 days. Moreover, results clearly indicated low percentages of mortalities in J2s of *M. incognita* reached 4.70, 5.20, 10.00, 13.90 and 18.40 % after 14 days post-treatment, respectively while after 18 days reached only 6.60,7.10, 12.50, 15.60 and 21.50% with the concentrations of 10^4, 10^5, 10^6, 10^7 and 10^8 spore/ml, respectively. Indeed, the mentioned results reported a tiny effect of *T. album* compared to RC of oxamyl after 7, 14 and 18 days. On the other hand, the toxicity of oxamyl against J2 of *M.incognita* reached 83.60, 98.10 and 100.00% after the latest mentioned times exposure.

The effect of *T. viride* on the mortality of *M. incognita* at the mentioned concentrations was studied to assess *in vitro* larvicidal effect on J2 (Table 3). All tested concentrations had a slight larvicidal effect on mortality of J2 *M. incognita* and the highest concentrations, 10^7 and 10^8 spore/ml were more effective in killing infective juveniles than those resulted from concentrations of 1×10^4, 10^5 and 10^6 spore/ml and produced mortalities of 6.20 and 12.60% compared to 2.80, 4.60 and 5.40% after 7 days, respectively. After 14 days, the mortality percentages were 9.90, 17.60, 5.20, 9.50 and 12.00 %, respectively. After 18 days, J2 mortalities reached only 6.20, 10.40, 13.20, 14.20 and 20.50% at concentrations of 1×10^4, 10^5, 10^6, 10^7 and 10^8 spore/ml, respectively.

T. harzianum concentrations of 1×10^4, 10^5, 10^6, 10^7, and 10^8 spore/ml showed high mortality of *M. incognita* J2 *in vitro* tests, compared with others tested *Trichoderma* spp. (*T. album* and *T. viride*). After 7 days, of *T. harzianum* application, a positive response in J2 mortality percentage was observed at concentrations of 1×10^4, 10^5, 10^6, 10^7, and 10^8 spore/ml scoring 7.30, 14.30, 18.40, 21.50 and 37.30% mortalities, respectively compared to 83.60% with the treatment of oxamyl. Whereas, after 14 days of treatment, mortality percentages in J2 of *M.incognita* increased reaching 50.55,

64.10, 78.20, 94.80 and 100.00% at 10^4, 10^5, 10^6, 10^7, and 10^8 spore/ml compared to 98.10% in the treatment of oxamyl. The concentration of 10^8 was the most effective concentration among others and surpassed oxamyl after 14 days resulting in 100% J2 mortality (Table 3).

Table 3: Numbers of dead juveniles and mortality percentages of RKN, *Meloidogyne incognita* inoculated with *Trichoderma album, T. viride* and *T. harzianum in vitro***.**

Treatment	Concentrations (Spores/ml)	Incubation period (Days)				
		3	7	10	14	18
T. album	10^4	1.80 d (0.90)	5.60 d (2.80)	6.60 e (3.30)	9.40 e (4.70)	13.20 e (6.60)
	10^5	2.00 d (1.00)	5.60 d (2.80)	6.80 e (3.40)	10.40 e (5.20)	14.20 e (7.10)
	10^6	2.20 d (1.10)	7.80 d (3.90)	11.60 d (5.80)	20.00 d (10.00)	25.00 d (12.50)
	10^7	6.60 c (3.30)	13.00 c (6.50)	17.20 c (8.60)	27.80 c (13.90)	31.20 c (15.60)
	10^8	10.20 b (5.10)	25.60 b (12.80)	28.60 b (14.30)	36.80 b (18.40)	43.00 b (21.50)
Oxamyl		45.60 a (22.80)	167.20 a (83.60)	170.80 a (85.40)	196.20 a (98.10)	200.00 a (100.00)
T. viride	10^4	1.80 d (0.90)	5.60 d (2.80)	5.60 d (2.80)	10.40 e (5.20)	12.40 f (6.20)
	10^5	2.20 d (1.10)	9.20 cd (4.60)	9.20 cd (4.60)	19.00 d (9.50)	20.80 e (10.40)
	10^6	6.60 c (3.30)	10.80 c (5.40)	10.80 c (5.40)	24.00 c (12.00)	26.40 d (13.20)
	10^7	6.60 c (3.30)	12.40 c (6.20)	12.40 c (6.20)	19.80 d (9.90)	28.40 c (14.20)
	10^8	10.20 b (5.10)	25.20 b (12.60)	25.20 b (12.60)	35.20 b (17.60)	41.00 b (20.50)
Oxamyl		45.60 a (22.80)	167.20 a (83.60)	170.80 a (85.40)	196.20 a (98.10)	200.00 a (100.00)
T. harzianum	10^4	2.50cd (1.25)	14.60e (7.30)	38.20d (19.10)	101.10cd (50.55)	135.40c (67.70)
	10^5	2.80cd (1.40)	28.60d (14.30)	89.80c (44.90)	128.20c (64.10)	172.00b (86.00)
	10^6	4.60bc (2.30)	36.80cd (18.40)	103.60bc (51.80)	156.40bc (78.20)	198.00a (99.00)
	10^7	6.00bc (3.00)	43.00c (21.50)	119.60b (59.80)	189.60b (94.80)	200.00a (100)
	10^8	9.40b (4.70)	74.60b (37.30)	127.60b (63.80)	200.00a (100.00)	200.00a (100)
Oxamyl		45.60 a (22.80)	167.20a (83.60)	170.80a (85.40)	196.20a (98.10)	200.00a (100)

Each replicate containing 200 J2; Figures in parenthesis are percentages of juvenile mortality.
* J2 in distilled water was used as control while oxamyl was used for nematicidal comparison.
**Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test.

Trichoderma is a genus of filamentous free-living soil-inhabiting fungi, the teleomorph-bearing belonging to the Hypocreales order of the Ascomycota division. *Trichoderma* is a genetically diversified genus that includes several strains with significant agricultural and industrial potential. Additionally, *Trichoderma* spp. could enhance the survival potential, competition for nutrients, growth and reproduction under different conditions, besides, their ability to tolerate different stress conditions.

Besides their abilities as antagonistic facultative parasitic fungi, they could sometimes act as parasites when conditions are favorable (Sudantha and Suwardji, 2021), and are a resistant variety of contaminants, such as pesticides, heavy metals, and polyaromatic hydrocarbons (Ali and Ramadan, 2019), therefore *Trichoderma* used for remediation pollutants from soil. Moreover, enhances the compatibility of the premix or tank mixed with other bacterial bioagents e.g., *Bacillus subtilis* and *B. licheniformis* (Silva et al., 2017).

Under laboratory conditions, *T. harzianum* displayed more virulence and infectivity than *T. viride* due to genetic variability, pathogenic capabilities, and origin of the isolate (Al-Hazmi and TariqJaveed, 2016). The obtained results are in harmony with Dababat et al. (2006); Pandey et al. (2003); Sharon et al.(2001) on the role of *T.harzianum* in the reduction of gall formations on tomato roots infected with *M. incognita* or *M. javanica* and ability of *T. viride* in reduction of galls and final population of *M. incognita* in pots of chickpea under field conditions (Trudgill, 1991).

Trichoderma spp. can parasite eggs or have opportunistic and toxic properties on RKN (Jaideep et al., 2008). Mycoparasitism involves morphological changes, such as coiling and formation of appressorium-like structures, which serve to penetrate the host and contain high concentrations of osmotic solutes. *T. harzianum* infection and parasitism of RKN eggs and juveniles were confirmed *in vitro* on dual plates (Singh et al., 2017) and eggs were colonized gradually with a long incubation period (Trifonova and Vachev, 2010). Sidhu et al. (2014) revealed that RKN egg masses, J2s and adult females collected from tomato roots grown in non-sterile manure-rich soil and scanning with electron microscopy exhibited prolific fungal growth of *Trichoderma* sp. on the egg masses. As well as the fungus colonized both pre-infectious, J2s and *Meloidogyne* eggs by attaching fungal hyphae and conidia to the surfaces of eggs and egg masses and then the hyphae made trapping rings around J2 as it emerged but had no contact with adult females. Furthermore, *Trichoderma* colonized J2 penetration holes in the root. The role of gelatinous matrix (GM) in spore attachment and the generation of fungal coils are required for parasitism. Whereas, Fucose-specific antibody and a fucose-binding lectin enhanced spore attachment to the nematode surface (Spiegel et al., 2007). Increase contact time between *M.incognita* stages (eggs and J2) and *Trichoderma* spp. in Petri dishes with favorable conditions of temperature increased the fungus and corrupting the vitelline layers of chitin and lipids, causing juvenile death (Rodriguez-Kabana et al., 1987). Furthermore, the eclosion of J2 is affected by the presence of the fungus (Mukhtar and Pervaz, 2003; Stirling and West, 1991).

Many studies explored the nematicidal effect of *Trichoderma* culture suspension and cultural filtrate against RKN showed an ovicidal and larvicidal effects raised with the increase in concentration and exposure time (Anusha and Shripad Kulkarni, 2016; Rekha Arya, 2016). The comparison showed surpassing some species like cultural filtrate of *T. viride*, most likely as a result of the greater concentration of phenols (Singh et al., 1983), besides other antinematode compounds produced in cultural filtrates directly affected the hatching of RKN eggs and juveniles mortalities but their ability to penetrate the host root was limited (Singh et al., 2015). The cultural filtrate of *Trichoderma* does not contain only secondary metabolites and antagonistic compounds but also involved extracellular degraded enzymes. The effect of the fungal metabolites is not necessarily killing but inhibiting the growth and reproduction of fungal pathogens and nematodes causing fungistasis (Benítez et al., 2004)**.**

Trichoderma produced several hydrolytic enzymes as *T. harzianum* produces amylases (de Azevedo et al., 2000), cellulases from *T. reesei* (Ahamed and Vermette, 2009), 1,3 β-glucanases from *T. harzianum*, *T. koningii* (Monteiro and Ulhoa, 2006;

Noronha and Ulhoa, 2000), chitinase from *Trichoderma* species (Sayed et al., 2019; Spiegel et al., 2007) and proteinase from *T. atroviride* (Spiegel *e*t al., 2007) able to degrade cell wall for fungi. In concerning of egg hatching and J2 vitality, chitinase-containing growth culture was associated with the concentration of fungal growth suspension as a renewable source of chitinase (Sayed et al., 2019). Chitin, an insoluble linear b-1,4-linked polymer of N-acetylglucosamine, is a common constituent of a wide range of organisms, including fungi, insects, and crustaceans, and it occurs also in nematode eggs (Flach et al., 1992). Besides chitinases enzymes, *Trichoderma* secretes proteinase Prb1 and other proteases during nematode parasitism accompanied by induction of chitinolytic activities i.e., endochitinases CHIT36 and CHIT42 and the N-acetylglucosaminidases CHIT102 and Nag1, in *T. asperellum*-203 and *T. atroviride* (Spiegel et al., 2007). The integration among different extracellular enzymes from different BCAs, *Bacillus* sp. and *Trichoderma* sp. caused a significant reduction in J2 hatching of *M. incognita* as shown with biological products based on enzyme mixtures (Frederic et al., 2020). Moreover, *Trichoderma* at species varied greatly in virulence and parasitic activity as observed between *T. harzianum* and *T. viride* on *M. incognita* (Sanjeev and Sharma, 2003) and isolates of *T. harzianum* (Th43-14) (Pinzon Espinoza et al., 2015) and *T. asperellum* (Ta1) (Abdel-Lateif and Bakr, 2018). Under *in vitro* bioassay of *Trichoderma* spp. other factors affect hatching percentages and nematode infective juveniles mortality as fungal isolate levels of aggressiveness and virulence, culture suspension and culture filtrate against RKN, concentration and exposure period (Sanjeev and Sharma, 2003; Wajahat et al., 2021) and laboratory potency may reach 100 % or less but within the accepted limits (Abdoulkader and Mansoure, 2015).

Biocontrol efficiency of the tested *Trichoderma* spp. in enhancing plant growth and suppressing RKN reproduction on tomato plant in pots

The greenhouse experiment on tomato plants was harvested at 60 days after inoculation (DAI) with RKN J2s to estimate the role of *Trichoderma* spp. in comparison with oxamyl against the reproduction and damage severity of *M. incognita* (Table 4). A comparison among *Trichoderma* spp. inoculated infected tomato plants in most cases decreased RKN reproduction and severity of damage besides improved plant growth parameters, while differences in gall numbers were not significant ($P \leq 0.05$) between *T. harzianum* and *T. viride*. Application of *Trichoderma* species reduced the number of galls and their size by more than 4 mm or <4 to 2 mm and the lower number of galls and size were obtained from pots treated with *T. harzianum.*

On the other hand, oxamyl treatment achieved the lowest *M. incognita* reproduction and damage severity as indicated by galls (14.00), egg masses (16.40), juveniles (4.00/100g soil) and eggs (18.40) (Tables 4&5). Malformation in root shape was detected by the large size of galls which increased root sternness, oxamyl treatment was more effective in reducing root sternness (9.00 galls) compared with *Trichoderma* spp., *T. harzianum* (11.40) with insignificant ($P \leq 0.05$) differences (Table 4). Generally, plant growth traits were improved in *Trichoderma* spp. and oxamyl treatments, over positive control, although *T. album*, *T. viride* and *T. harzianum* did not necessarily statically different ($P \leq 0.05$) in tomato plants growth (Table 4).

For example, fresh root weight resulted from creative treatments by *T. album*, *T. viride* and *T. harzianum* were 11.94,12.06 and 12.46 g, respectively. Moreover, the number and weight of leaves statically not different ($P \leq 0.05$) and scored 17.80 (32.27), 17.80 (30.00) and 18.40 (36.68), respectively.

Table 4: Biocontrol efficiency of *Trichoderma* spp. in comparison with oxamyl on tomato plant growth parameters and root-knot nematode, *Meloidogyne incognita* galls and egg masses.

Treatments	Plant growth parameters					Nematode parameters			
	Fresh root weight	Fresh shoot weight	No. of leaves /plant	Leaves weight /plant	Stem diameter (mm)	No. galls	No. egg masses	No. galls ≥ 4 mm	No. galls <4 to 2 mm
Healthy plants	27.55^{a}	94.43^{a}	23.00^{a}	38.66^{a}	6.60^{a}	0.00^{e}	0.00^{e}	0.00^{d}	0.00^{e}
Infected plants	10.55^{d}	49.34^{e}	13.60^{d}	24.64^{e}	4.20^{d}	70.40^{a}	87.60^{a}	16.00^{a}	33.60^{a}
T.album+M.incognita	11.94^{cd}	68.79^{d}	17.80^{c}	32.27^{c}	5.80^{bc}	55.60^{b}	69.80^{b}	13.20^{b}	20.60^{b}
T.viride+M.incognita	12.06^{cd}	63.22^{d}	17.80^{c}	30.00^{d}	5.20^{c}	41.60^{c}	68.00^{b}	12.40^{b}	15.80^{c}
T.harzianum+M.incognita.	12.46^{c}	76.69^{c}	18.40^{bc}	36.68^{b}	6.40^{ab}	36.20^{c}	49.80^{c}	9.80^{c}	11.40
Oxamyl	16.81^{b}	86.18^{b}	19.80^{b}	36.26^{b}	5.60^{c}	14.00^{d}	16.40^{d}	0.80^{d}	9.00^{d}

Each value is a mean of five replicates.

The same letter (s) in each column indicates no significant differences at P ≤0.05 according to Duncan's multiple range test.

On other hand, stem diameter in *T. harzianum* (6.40) exceeded that found in oxamyl treatment and surpassed another *Trichoderma* spp. In general, plant growth parameters and reproduction of *M.incognita* were affected by *Trichoderma* spp. with less effectiveness when compared with oxamyl.

Under greenhouse conditions, a positive correlation was recorded between root weight and *Trichoderma* concentrations (Wajahat et al., 2021). Besides *T. harzianum* proved to be a good promoter of root growth (Erazo et al., 2020) and significantly increased shoot weight, also *T. harzianum* showed slightly increases or decreases in plant parameters than *T. viride* (Mukhtar, 2018) through decreasing the effect of the nematode on plant height, stem diameter, number of leaves, and root fresh weight (Hernandez-Ochandia et al., 2015). A significant positive correlation was found between *Trichoderma* spp. as biocontrol agent against RKNs and their chitinolytic activity, where *T. asperelloides*, was the most promising chitinolytic species, demonstrating the greatest activity against *M. incognita* (Sayed et al., 2019).A direct effect of plant growth and nematode population density reduction extended to 90 days after inoculation with no significant differences between treatments (Devi and Richa Sharma, 2002).

In general, *Trichoderma* isolates increased top fresh weights and reduced galling indices. The fungus-nematode interactions included several direct and indirect mechanisms that took place in soil and plant roots (Spiegel et al., 2007). The obtained results in Table (5) revealed that all tested *Trichoderma* spp. as BCAs were relatively reduced galling and egg masses/root as compared with infected plants. Moreover, *Trichoderma* spp. significantly decreased egg numbers and J2/100 g soil subjected to treatment with 1×10^6 spores/ml. In the three *Trichoderma* spp. treatments mean numbers of *M. incognita* J2 and eggs were higher than oxamyl treatment scoring 12.40 (83.40), 16.20 (68.80), 10.60 (62.00) and 4.00 (18.40) with *T. album*, *T. viride*, *T. harzianum* and oxamyl, respectively. Also, the numbers of galls and egg masses per root system on the tested tomato cultivar 016 with *T. album*, *T. viride*, *T. harzianum* and oxamyl were 55.60(69.80), 41.60(68.00), 36.20(49.80) and 14.00(16.40), respectively. The parallel values of their RGI and EI were 4.20(4.80), 3.80(4.00), 3.00(3.20) and 2.20 (2.40) respectively. On the other hand, the total number of J2/100 g + eggs/100 g and Rfs were 95.80 (1.676), 85.00 (1.487), 72.60 (1.270) and 22.40 (0.392), respectively.

RKN infections increased the levels of reactive oxygen species (ROS; H_2O_2 and O_2) and lipid peroxidation in tomato roots. These free active radicals caused damage to plant cells. Root colonization with *T. harzianum* significantly reduced the levels of ROS, malondialdehyde, and electrolyte leakage, which were associated with increased accumulation of multiple secondary metabolites such as cellulose, and flavonoids, lignin and phenols 75 days after inoculation with *M. incognita*. Moreover, *T. harzianum* inoculation before RKN infestation significantly increased the activity of pathogenesis-related proteins such as β-1,3-glucanase, chitinase, protease, and amylase (Yan et al., 2021). *Trichoderma* spp. are a beneficial root endophyte colonized root that can reduce infections by parasitic nematodes by triggering host defense (Martínez-Medina et al., 2017).

Several *Trichoderma* spp. strains have been tested in pots to induce plant defense mechanisms against *Meloidogyne* in tomato plants (Pocurull et al., 2020). In the shoot system, accumulation of total chlorophyll and some enzymes, such as chitinase, phenylalanine ammonia-lyase (PAL), and peroxidase, which are known to confer systemic resistance, increased significantly in inoculated plants as a result of modulated phenylpropanoid pathways (Singh et al., 2017).

Table 5: Galling and reproduction of *Meloidogyne incognita* on tomato plants treated with *Trichoderma* spp. under greenhouse conditions.

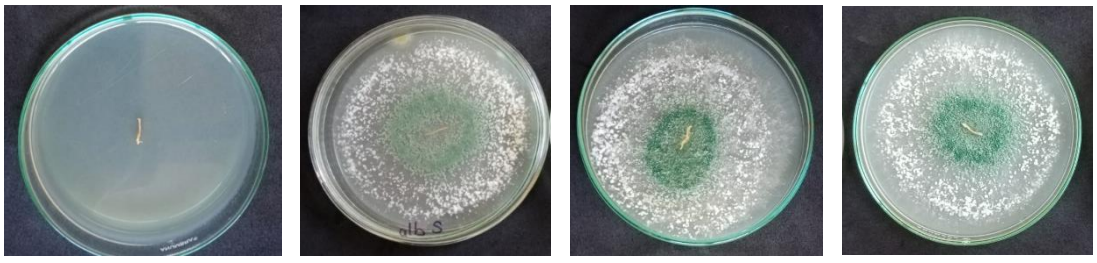
Treatments	Number of galls/roots (RGI)*	No. of egg masses/root (EI)	No. J2/100 g soil	No. eggs/ 100g soil	Reproduction factor (Rf)
Infected plants	70.40 [a] (4.20)	87.60 [a] (4.80)	22.40 [a]	110.00 [a]	2.317
T.album+M.incognita	55.60 [b] (3.80)	69.80 [b] (4.00)	12.40 [c]	83.40 [b]	1.676
T. viride+ M. incognita	41.60 [c] (3.40)	68.00 [b] (4.00)	16.20 [b]	68.80 [c]	1.487
T.harzianum+M.incognita	36.20 [c] (3.00)	49.80 [c] (3.20)	10.60 [c]	62.00 [c]	1.270
Oxamyl	14.00 [d] (2.20)	16.40 [d] (2.40)	4.00 [d]	18.40 [d]	0.392

Rf = Final population/Initial population. Final population= No. J2/ 100g soil +No. eggs/100g soil/Initial population.
*Root-gall index (RGI) or egg mass index (EI) calculated according to Taylor and Sasser (1978) as: 0 = no galls or egg masses, 1=1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100 and 5 = more than 100 galls or egg masses.

Resistance and tolerance are genetically independent and should be evaluated separately as well as, nematodes reproduction levels on plant tissues are used to measure plant resistance while tolerance used to show damage level. Application of bio-agents increased the level of Peroxidase (PO), polyphenol oxidase (PPO), Phenylalanine (PAL) and superoxide dismutases (SOD) in tomato roots during different times of observation followed by SOD, while PPO and PAL were observed in low quantity (Abd-Elgawad and Kabeil, 2012, Chandrawat et al., 2018). Induced systemic resistance in inoculated roots with *Trichoderma* is responsible for reduction in root penetration and retarding nematode development within all life stages (Spiegel et al., 2007).

Trichoderma species colonization on tomato roots:

To prove the efficiency and antagonistic potential of *Trichoderma* spp. against *M. incognita*, small parts from infected tomato roots were subjected to reisolation on nutritive media. *Trichoderma album*, *T. harzianum* and *T. viride* were found in roots of plants grown in soil previously inoculated with concentrations of 10^6 spore/ml (Fig.1). Sixty days after inoculation, the *Trichoderma* growth varied depending on the efficacy of tested species. Moreover, *T. harzianum* induced a high growth response in tomato plants compared with *T. album* and *T. viride.*



Non inoculated *T. album* *T. harzianum* *T. viride*

Figure. 1: Tomato root colonization proof by re-isolation of the tested *Trichoderma* spp. from tomato root after 60 days of *Meloidogyne incognita* inoculation.

In the present study, the bio-control activity of *Trichoderma* spp. can be explained by the ability of *Trichoderma* to inhibit J2 of *M. incognita* in the rhizosphere and reduce reproduction (galls formation and egg masses) of nematode or population density in roots and pot soils of tomato. These positive effects of *Trichoderma* may be varied with different plant species; thus the genetic base of such interactions seems not to be predominant and respond positively or negatively to different strains.

Trichoderma strains must colonize plant roots (Martínez-Medina et al., 2017) and produce host defense before stimulating plant growth and protecting against infections. Colonization implies the ability to adhere and recognize plant roots, penetrate the plant, and withstand toxic metabolites produced by the plants in response to invasion by a foreign microorganism, whether pathogen or not (Benítez et al., 2004). Rhizosphere modification means the ability of some applied BCAs to change in microhabitat conditions in favor of BCA growth and survival such as *Trichoderma* spp. (Benítez et al., 2004). All the investigated *Trichoderma* species became established in the soil and their populations grew over time besides, isolated earlier from soil and egg masses and nematode females extracted from the tomato-infected roots (Jaideep et al., 2008; Sankaranarayanan et al., 2002; Yaziji et al., 2013). In contrast, the population of nematodes decreased over time through rhizosphere competence and antagonistic potential (Mujeebur Rahman Khan et al., 2018; Rao et al., 1997). Biological control with *Trichoderma* spp. are suitable for managing wilt disease complex associated with RKN infection because of the antagonistic fungus control disease complex consisting of soilborne fungal disease such as *Fusarium oxysporum* f. sp. *lycopersici* and RKN and when used *T. viride* (Pinki Meena et al., 2020, Singh and Prasad, 2015).

CONCLUSION

Successful potential use and evaluation of *Trichoderma* spp. as BCAs in managing phytonematodes is a key to complex interactions with beneficial microbes and plants in the soil ecosystem. Effective *Trichoderma* colonization, competence, production enzymes, the absorption of nutrients from the soil and enhancements of root system architecture development promotes plant growth parameters and are probably linked to the largest long-term effects on productivity. Resistance of *Trichoderma* to several agrochemicals makes it a favorite for integrated plant disease management based on a combination of physical, chemical, and biological means. Therefore, the current study provided information on the resistance and tolerance of tomato plants to *M.incognita* under greenhouse conditions. The application of *Trichoderma* with other BCAs will be encouragement growers in developing countries for protecting valuable crops under field conditions in integrated pest management (IPM) programs and rechecked synthetic pesticides in PPNs management since BCAs are valuable and inexpensive tools against the uncritical use of pesticides.

REFERENCES

Abd-Elgawad, M.M.M. and Kabeil, S.S.A. (2012). Biological control of *Meloidogyne incognita* by *Trichoderma harzianum* and *Serratia marcescens* and their related enzymatic changes in tomato roots. Afr. J. Biotechnol. 11 (96): 16247–16252.

Abdel-Lateif, K.S. and Bakr, R.A. (2018). Internal transcribed spacers (ITS) based identification of *Trichoderma* isolates and biocontrol activity against *Macrophomina phaseolina*, *Aspergillus niger* and *Meloidogyne incognita*. Afr. J. Microbiol. Res. 12 (30): 715–722. https://doi.org/10.5897/ajmr2018.8915

Abdoulkader, M. and Mansoure, R. (2015). Evaluation of the efficiency of some antagonistic fungi to control root-knot nematode *Meloidogyne incognita* on tomato. Arab J. Plant Prot. 33 (2):201–207.

Ahamed, A. and Vermette, P. (2009). Effect of culture medium composition on *Trichoderma reesei's* morphology and cellulase production. Bioresour. Technol. 100 (23): 5979–5987.

Al-Hazmi, A.S.and TariqJaveed, M. (2016). Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. Saudi J. Biol. Sci. 23 (2): 288–292.

Ali, A.A.I. and Ramadan, M.M. (2019). *In vitro* integration of *Trichoderma harzianum* with chemical pesticides pertain to different classes. J. Plant Prot. Pathol. 10 (9):431–436. https://doi.org/10.21608/jppp.2019.57395

AL-Shammari, T.A.; Bahkali, A.H.; Elgorban, A.M.; El-Kahky, M.T. and Al-Sum, B.A. (2013). The use of *Trichoderma longibrachiatum* and *Mortierella alpina* against root-knot nematode, *Meloidogyne javanica* on tomato. J. Pure Appl. Microbiol. 7: 199–207.

Anusha, B.G. and Shripad Kulkarni (2016). Bioefficacy of some fungal biocontrol agents against *Meloidogyne incognita* infecting tomato. J. Mycol. Plant Pathol. 46 (1): 84–88.

Barker, K.R. (1998). Introduction and synopsis of advancements in nematology. Plant Nematode Interact. 36:1–20.

Benítez, T.; Rincón, A.M.; Limón, M.C. and Codon, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7 (4): 249–260.

Bissett, J.; Gams, W.; Jaklitsch, W. and Samuels, G.J. (2015). Accepted *Trichoderma* names in the year 2015. IMA Fungus 6 (2): 263–295.

Chandrawat, B.S.; Siddiqui, A.U.; Bhati, S.S. and Vinod Saharan (2018). Induction of defence enzymes using bio-agents in tomato infected with root-knot nematode, *Meloidogyne incognita*. Indian J. Nematol. 48 (2): 139–145.

Dababat, A.A.; Sikora, R.A. and Hauschild, R. (2006.) Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato. Commun. Agric. Appl. Biol. Sci. 71 (3): 953–961.

de Azevedo, A.M.; De Marco, J.L. and Felix, C.R. (2000). Characterization of an amylase produced by a *Trichoderma harzianum* isolate with antagonistic activity against *Crinipellis perniciosa*, the causal agent of witches' broom of cocoa. FEMS Microbiol. Lett. 188 (2): 171–175.

Devi, L.S.and Richa Sharma (2002). Effect of *Trichoderma* spp. against root-knot nematode *Meloidogyne incognita* on tomato. Indian J. Nematol. 32 (2): 227–228.

dos Santos Pereira, T.; Gomes Macêdo, A.; da Silva, J.; Borges Pinheiro, J.; Monteiro de Paula, A.; Biscaia, D. and Busato, J.G. (2020). Water-extractable fraction of vermicomposts enriched with *Trichoderma* enhances the growth of bell pepper and tomato as well as their tolerance against *Meloidogyne incognita*. Sci. Hortic. 272, 109536. https://doi.org/10.1016/j.scienta.2020.109536

Eisenback, J.D. and Triantaphyllou, H.H. (1991). Root-knot nematodes: *Meloidogyne* species and races. Man. Agric. Nematol. 1: 191–274.

El-Ashry, R.M.; Ali, A.A.I. and Awad, S.E. (2020). Enhancing application efficiency of *Pseudomonas* spp. and *Serratia marcescens* isolates against *Meloidogyne incognita* in tomato plants. Egypt. Acad. J. Biol. Sci. F Toxicol. Pest Control 12 (2): 127–145. https://doi.org/10.21608/eajbsf.2020.116752

Erazo S., N.S.; Echeverria Guadalupe, M.M.; Jave Nakayo, J.L.; Leon Reyes, H.A; Lindao Cordova, V.A.; Manzano Ocana, J.C. and Inca Chunata, N.M. (2020). Effect of *Pleurotus ostreatus* (Jacq.) and *Trichoderma harzianum* (Rifai) on *Meloidogyne incognita* (Kofoid & White) in tomato (*Solanum lycopersicum* Mill.).Acta Sci.-Biol.Sci.42(e47522). https://doi.org/10.4025/actascibiolsci.v42i1.47522

Ferraz, L. and Brown, D. (2002). An Introduction to Nematodes: Plant Nematology.

Finney, D.J. (1971). Probit analysis, 3rd ed. Cambridge University Press. Cambridge, UK.

Flach, J.; Pilet, P.-E.and Jolles, P. (1992). What's new in chitinase research? Experientia 48 (8): 701–716.

Frederic, J.B.; Costa, C.A. da; Rocha, F. da S. and Fernandes, M. de F.G. (2020). Application forms and mode of action of biocontroller in the management of *Meloidogyne incognita* in tomato. Hortic. Bras. 38 (3):254–260. https://doi.org/10.1590/S0102-053620200303

Freitas, L.G.; Dallemole-Giaretta, R.; Ferraz, S.; Zooca, R.J.F. and Podestá, G.S. (2009). Controle biológico de nematóides: estudo de casos. Zambolim Picanço MC Controle Biológico Pragas E Doenças Ex. Práticos Viçosa Universidade Fed. Viçosa 1, 41–82.

Giannakou, I.O.; Sidiropoulos, A. and Prophetou-Athanasiadou, D. (2002). Chemical alternatives to methyl bromide for the control of root-knot nematodes in greenhouses. Pest Manag. Sci. Former. Pestic. Sci. 58 (3): 290–296.

Hernandez-Ochandia, D.; Rodriguez, M.G.; Peteira, B.; Miranda, I.; Arias, Y. and Martinez, B. (2015). Effect of *Trichoderma asperellum* Samuels, Lieckfeldt and Nirenberg strains on the development of tomato and *Meloidogyne incognita* (Kofoid and White) Chitwood population. Ef. Cepas *Trichoderma Asperellum* Samuels Lieckfeldt Nirenberg Sobre El Desarro. Tomate *Meloidogyne incognita* Kofoid White Chitwood 30 (2): 139–147.

Huang, X.; Zhao, N. and Zhang, K. (2004). Extracellular enzymes serving as virulence factors in nematophagous fungi involved in infection of the host. Res. Microbiol. 155 (10): 811–816.

Hussey, R.S.and Barker, K.R. (1973). Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Report. 59: 1025–1028.

Jaideep G.; Pandey, R.K.; Tewari, J.P. and Goswami, B.K. (2008). Management of root knot nematode on tomato through application of fungal antagonists, *Acremonium strictum* and *Trichoderma harzianum*. J. Environ. Sci. Health B 43 (3): 237–240. https://doi.org/10.1080/03601230701771164

Jepson, S.B. (1987). Identification of root-knot nematodes (*Meloidogyne* species)., 1st ed. CAB International, Wallingford, UK.

Karssen, G.; Wesemael, W. and Moens, M. (2013). Root-knot nematodes., in: Plant Nematology. CABI, pp. 73–108.

Kerry, B.R. (1990). An assessment of progress toward microbial control of plant-parasitic nematodes. J. Nematol. 22 (4S): 621.

Khan, A.; Williams, K.L. and Nevalainen, H.K. (2004). Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol. Control 31 (3): 346–352.

Krif, G.; Lahlali, R.; El Aissami, A.; Laasli, S.-E.; Mimouni, A.; Serderidis, S.; Picaud, T.; Moens, A.; Dababat, A.A. and Fahad, K. (2022). Efficacy of authentic bio-

nematicides against the root-knot nematode, *Meloidogyne javanica* infecting tomato under greenhouse conditions. Physiol. Mol. Plant Pathol. 118,:101803.

Larralde-Corona, C.P.; Santiago-Mena, M.R.; Sifuentes-Rincon, A.M.; Rodríguez-Luna, I.C.; Rodriguez-Perez, M.A.; Shirai, K. and Narvaez-Zapata, J.A. (2008). Biocontrol potential and polyphasic characterization of novel native *Trichoderma* strains against *Macrophomina phaseolina* isolated from sorghum and common bean. Appl. Microbiol. Biotechnol. 80 (1): 167–177.

Martínez-Medina, A.; Appels, F.V. and van Wees, S.C. (2017). Impact of salicylic acid- and jasmonic acid-regulated defences on root colonization by *Trichoderma harzianum* T-78. Plant Signal. Behav. 12 (8): e1345404.

McCarter, J.P. (2008). Nematology: terra incognita no more. Nat. Biotechnol. 26 (8): 882–884. https://doi.org/10.1038/nbt0808-882

Meyer, S.L.; Huettel, R.N.; Liu, X.Z.; Humber, R.A.; Juba, J. and Nitao, J.K. (2004). Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile motility. Nematology 6 (1): 23–32.

Moens, M.; Perry, R.N. and Starr, J.L. (2009). *Meloidogyne* species–a diverse group of novel and important plant parasites. Root-Knot Nematodes 1: 483.

Monteiro, V.N. and Ulhoa, C.J. (2006). Biochemical characterization of a β-1, 3-glucanase from *Trichoderma koningii* induced by cell wall of *Rhizoctonia solani*. Curr. Microbiol. 52 (2): 92–96.

Mora, A.A. and Earle, E.D. (2001). Resistance to *Alternaria brassicicola* in transgenic broccoli expressing a *Trichoderma harzianum* endochitinase gene. Mol. Breed. 8 (1):1–9.

Mujeebur Rahman Khan; Irfan Ahmad and Faheem Ahamad (2018). Effect of pure culture and culture filtrates of *Trichoderma* species on root-knot nematode, *Meloidogyne incognita* infesting tomato. Indian Phytopathol. 71 (2): 265–274. https://doi.org/10.1007/s42360-018-0031-1

Mukhtar, T. (2018). Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. Pak. J. Zool. 50 (4): 1589–1592. https://doi.org/10.17582/journal.pjz/2018.50.4.sc15

Mukhtar, T. and Pervaz, I. (2003). *In vitro* evaluation of ovicidal and larvicidal effects of culture filtrate of *Verticillium chlamydosporium* against *Meloidogyne javanica*. Int. J. Agric. Biol. 5 (4): 576–579.

Noronha, E.F. and Ulhoa, C.J. (2000). Characterization of a 29-kDa β-1, 3-glucanase from *Trichoderma harzianum*. FEMS Microbiol. Lett. 183 (1): 119–123.

Oostenbrink, M. (1968). Major characteristics of the relations between nematodes and plants., in: International Symposium of Nematology (8th) Antibes Sept. 8-14, 1965.

Pandey, G.; Pandey, R.K. and Pant, H. (2003). Efficacy of different levels of *Trichoderma viride* against root-knot nematode in chickpea (*Cicer arietinum* L.). Ann. Plant Prot. Sci. 11 (1): 101–103.

Pinki Meena; Chandrawat, B.S. and Ahir, R.R. (2020). Bio-agents for management of wilt complex in tomato by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *Lycopersici*. Ann. Plant Prot. Sci. 28 (2): 160–162. https://doi.org/10.5958/0974-0163.2020.00042.7

Pinzon Espinoza, L.F.; Candelero de la Cruz, J.; Tun Suarez, J.M.; Reyes Oregel V.; and Cristobal Alejo, J. (2015). *Meloidogyne incognita* control in tomato (*Solanum lycopersicum* L.) to the implementation of *Trichoderma harzianum*. Control *Meloidogyne incognita* En Tomate *Solanum Lycopersicum* Con Apl. *Trichoderma Harzianum* 19 (1): 5–11.

Pocurull, M.; Fullana, A.M.; Ferro, M.; Valero, P.; Escudero, N.; Saus, E.; Gabaldon, T. and Javier Sorribas, F. (2020). Commercial formulates of *Trichoderma* induce systemic plant resistance to *Meloidogyne incognita* in tomato and the effect is additive to that of the Mi-1.2 resistance gene. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2019.03042

Qiao, M.; Du, X.; Zhang, Z.; Xu, J. and Yu, Z. (2018). Three new species of soil-inhabiting *Trichoderma* from southwest China. MycoKeys 63.

Rao, M.S.; Reddy, P.P. and Nagesh, M. (1997). Management of root-knot nematode, *Meloidogyne incognita* on tomato by integration of *Trichoderma harzianum* with neem cake. Z. Pflanzenkrankh. Pflanzenschutz 104 (4): 423–425.

Rekha Arya (2016). Combined effect of *Trichoderma harzianum* and Vermicompost in the management of root knot nematode (*Meloidogyne incognita*) in tomato cv Pusa Ruby. Indian Phytopathol. 69 (4s): 464–467.

Rodriguez-Kabana; R., Morgan-Jones, G. and Chet, I. (1987). Biological control of nematodes: Soil amendments and microbial antagonists. Plant Soil 100 (1): 237–247. https://doi.org/10.1007/BF02370944

Sahebani, N. and Hadavi, N. (2008). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Soil Biol. Biochem. 40 (8): 2016–2020.

Sanjeev S. and Sharma, G.C. (2003). Assessment of nematophagous fungi against *Meloidogyne incognita* on tomato. Ann. Agric. Res. 24 (3):583–586.

Sankaranarayanan, C.; Hussaini, S.S.; Kumar, P.S. and Rangeswaran, R. (2002). Parasitism of *Meloidogyne incognita* eggs by *Fusarium oxysporum* and other fungi. Indian J. Nematol. 32 (1): 33–36.

Sayed, M.; Abdel-Rahman, T.; Ragab, A. and Abdellatif, A. (2019). Biocontrol of root-knot nematode *Meloidogyne incognita* by chitinolytic *Trichoderma* spp. Egypt.J.Agronematol. 18 (1): 30–47. https://doi.org/10.21608/ejaj.2019.52842

Sharon, E.; Bar-Eyal, M.; Chet, I.; Herrera-Estrella, A.; Kleifeld, O. and Spiegel, Y.(2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91 (7): 687–693.

Sharon, E.; Chet, I.; Viterbo, A.; Bar-Eyal, M.; Nagan, H.; Samuels, G.J. and Spiegel, Y. (2007). Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur. J. Plant Pathol. 118 (3): 247–258.

Sidhu, R.K.; Shikha Sharma and Parshad, V.R. (2014). Scanning electron microscopy of parasitic association of soil fungus *Trichoderma* sp. with root-knot nematode *Meloidogyne incognita*. Afr. J. Microbiol. Res. 8 (45): 3770–3774.

Silva, J. de O., Santana, M.V., Freire, L.L., Ferreira, B. da S., Rocha, M.R. da (2017). Biocontrol agents in the management of *Meloidogyne incognita* in tomato. Cienc. Rural 47(10): e20161053.

Singh, A.U. and Prasad, D. (2015). Role of soil fungi on wilt incidence and *Meloidogyne incognita* on tomato. Ann. Plant Prot. Sci. 23 (2): 365–368.

Singh, K.; Srivastava, M.; Singh A.; Dwivedi K.; Srivastava Y. and Ratan V. (2015). Evaluation of culture filtrates of *Trichoderma* spp. on egg hatching and juveniles mortality of root knot nematode. Ann. Plant Prot. Sci. 23 (2):377–380.

Singh, S.P.; Veena Pant; Khan, A.M. and Saxena, S.K. (1983). Inhibitory effect of culture filtrates of some rhizosphere fungi of tomato as influenced by oilcakes on the mortality and larval hatch of *Meloidogyne incognita*. Nematol. Mediterr. 11 (2):119–123.

Singh, U.B.; Shailendra Singh; Deepti Malviya; Rajan Chaurasia; Mohd. Imran; Rai, J.P. and Sharma, A.K. (2017). Harnessing biocontrol potential of *Trichoderma*

harzianum for control of *Meloidogyne incognita* in tomato. Indian Phytopathol. 70 (3): 331–335.

Spiegel, Y.; Sharon, E.; Bar-Eyal, M.; Maghodia, A.; Vanachter, A.; Assche, A; van, Kerckhove, S. van; Viterbo, A. and Chet, I. (2007). Evaluation and mode of action of *Trichoderma* isolates as biocontrol agents against plant-parasitic nematodes. Bull. OILBSROP 30 (2): 129–133.

Stirling, G.R. and West, L.M. (1991). Fungal parasites of root-knot nematode eggs from tropical and subtropical regions of Australia. Australas. Plant Pathol. 20 149–154.

Sudantha, I.M. and Suwardji, S. (2021). Biodiversity of *Trichoderma* antagonist saprophytic fungi and its use for biocontrol of *Fusarium* wilt disease on shallots at Lombok Island, West Nusa Tenggara, Indonesia. IOP Conf. Ser. Earth Environ. Sci. 886 012123. https://doi.org/10.1088/1755-1315/886/1/012123

Taylor, A.L. and Sasser, J.N. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Dept. of Plant Pathology, North Carolina State University, USA.

Tikhonov, V.E.; Lopez-Llorca, L.V.; Salinas, J. and Jansson, H.-B. (2002). Purification and characterization of chitinases from the nematophagous fungi *Verticillium chlamydosporium* and *V. suchlasporium*. Fungal Genet. Biol. 35 (1): 67–78.

Trifonova, Z. and Vachev, T. (2010). Efficacy of *Trichoderma* species on invasion and development of root-knot nematode *Meloidogyne incognita* in tomato agroecosystem. J. Balk. Ecol. 13 (4): 379–384.

Trudgill, D.L.(1991). Resistance to and tolerance of plant parasitic nematodes in plants. Annu. Rev. Phytopathol. 29:167–192.

Wajahat A.; Tariq M. and Tooba H. (2021). Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in tomato. Pak.J.Zool.53(1):119-125. https://doi.org/10.17582/journal.pjz/20190905100940

Yan, Y.; Mao, Q.; Wang, Y.; Zhao, J.; Fu, Y.; Yang, Z.; Peng, X.; Zhang, M.; Bai, B.; Liu and A. Chen, S. (2021). *Trichoderma harzianum* induces resistance to root-knot nematodes by increasing secondary metabolite synthesis and defense-related enzyme activity in *Solanum lycopersicum* L. Biol. Control 158. https://doi.org/10.1016/j.biocontrol.2021.104609

Yaziji, M.; Allouf, N. and Kassam, R. (2013). Effectiveness of filtrates of local isolates of *Trichoderma* fungi to control different stages of root knot nematode (*Meloidogyne incognita*) under laboratory conditions. Arab J. Plant Prot. 31(3): 252–261.

Zhang, S.; Gan, Y.; Xu, B. and Xue, Y. (2014). The parasitic and lethal effects of *Trichoderma longibrachiatum* against *Heterodera avenae*. Biol. Control 72: 1–8.

Zhang, S. and Zhang, X. (2009). Effects of two composted plant pesticide residues, incorporated with *Trichoderma viride*, on root-knot nematode in balloonflower. Agric. Sci. China 8 (4): 447–454. https://doi.org/10.1016/S1671-2927(08)60231-X

Zin, N.A. and Badaluddin, N.A. (2020). Biological functions of *Trichoderma* spp. for agriculture applications. Ann. Agric. Sci. 65 (2):168–178.

الملخص العربي

التأثير الإبادي لثلاث أنواع من التريكودرما علي ملائمة نباتات الطماطم لتكاثر نيماتودا تعقد الجذور

عبدالهادى عبد الحميد إبراهيم على - عبدالرحمن الديب - محمود محمد رمضان - رمضان محمد أحمد العشري

قسم وقاية النبات - كلية الزراعة – جامعة الزقازيق - مصر.

تعد نيماتودا تعقد الجذور *Meloidogyne incognita* من أهم الأمراض المتوطنة بالتربة والتي لابد من التعامل معها كآفة مزمنة بشكل متكرر كل موسم، خاصة خلال فترات الإزهار والإثمار، لذلك يحثنا هذا الموقف للبحث عن بدائل آمنة وفعالة وممتدة المفعول لمكافحة نيماتودا تعقد الجذور عوضا عن مبيدات النيماتودا الكيماوية. لذلك تم معمليا تقييم فاعلية كل من *Trichoderma album* و*T. harzianum* و *T. viride* ضد فقس البيض وموت الطور اليرقي الثاني لنيماتودا تعقد الجذور، إلى جانب تأثيرها على تكاثر نيماتودا تعقد الجذور عن طريق تقليلها لمعامل تكاثرها في جذور الطماطم المصابة بالأصص وتعزيزها لنمو النبات تحت الظروف شبه الاستوائية.

معمليا وجد تأثير إبادي محدود لأنواع التريكودرما ضد نيماتودا تعقد الجذور عند معاملتها بتركيزات مختلفة من الفطر، حيث أظهر فطر *T. viride* تأثير إبادي ضعيف ضد البيض والأطوار اليرقية للنيماتودا. علاوة على ذلك أظهر التركيز ١×١٠^٨ جرثومة\مل أعلى تأثير إبادي ضد بيض النيماتودا، كما تفوق *T. album* علي سابقه، وفي المقابل أظهر النوع *T. harizianum* أعلي تأثير إبادي معنوي ضد البيض والأطوار اليرقية عند تركيزي ١×١٠^٧ و١×١٠^٨ جرثومة\مل ليتجاوز فاعلية مبيد الأوكساميل بعد ٦ أيام من المعاملة في حالة البيض وأيضا تفوق التركيز ١×١٠^٨ جرثومة\مل بعد ١٤ يوم في حالة اليرقات. وبشكل عام أظهرت أنواع التريكودرما المختلفة تأثير إبادي معنوي ضد البيض متجاوزًا مبيد الأوكساميل في حين كان التأثير الإبادي على اليرقات محدودًا للغاية مقارنة مع الأوكساميل.

في تجارب الأصص، حققت معاملات نبات الطماطم المصابة بنيماتودا تعقد الجذور والملقحة بأنواع التريكودرما مؤشرات نباتية أعلى عند مفاضلتها مع نباتات المقارنة. وربما ترجع التغيرات الناتجة لمقاومة النبات خاصة بعد ثبوت قدرة التريكودرما على استعمار الجذور. كما أبدت معايير الإصابة بالنيماتودا كقياسات عدد العقد الجذرية\الجذور، وعدد كتل البيض\الجذر، عدد البيض والأطوار اليرقية /١٠٠ جم من التربة، ومعامل التكاثر النيماتودا انخفاضًا معنويًا أقل من فاعلية مبيد الأوكساميل والتي توكد على مقدرة أنواع التريكودرما بدرجات متفاوتة على إبقاء تعداد نيماتودا تعقد الجذور دون الحد الاقتصادي الحرج أو تُجنب النبات الضرر الناتج عن الإصابة بالنيماتودا من خلال آليات فعل متعددة تشمل التطفل واستعمار الجذور ومقاومة النبات وتحسين النمو.