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Biocontrol of Root-Knot Nematode *Meloidogyne incognita* by Chitinolytic *Trichoderma* spp.

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ABSTRACT

The inhibitory potential of chitinolytic *Trichoderma* spp. was assayed against *Meloidogyne incognita in vitro* and *in vivo*. Sixteen *Trichoderma* isolates were assayed for chitinase activity using basal solid medium supplemented with colloidal chitin. Isolates recorded high chitinolytic activity were identified morphologically as *Trichoderma asperelloides*, *T. hamatum*, *T. harzianum* and *T. viride*. The four species were quantitatively assayed for total and specific chitinase activity. *T. asperelloides* showed maximum chitinase activity (1.736 U/mL). *In vitro* studies revealed that egg hatching and second juveniles vitality of root-knot nematodes were significantly inhibited by chitinase-containing growth culture of the four selected chitinolytic *Trichoderma* species. However, the reduction effect was directly correlated with the concentration of fungal growth suspension. Under greenhouse conditions, three sequentially treatments of soil with homogenized culture suspension were studied. *Trichoderma* species significantly reduced the final nematode population by 97.81% and root galling by 92.81%. Moreover, all tested *Trichoderma* species obviously promoted tomato growth compared to untreated control. Significant positive correlation between the biocontrol efficacy of *Trichoderma* species against root-knot nematodes and their chitinolytic activity was confirmed. The most potential chitinolytic species, *T. asperelloides*, also recorded the highest biocontrol activity against *M. incognita*.

Keywords: Root-knot nematode, Tomato, *Trichoderma*, chitinase.

INTRODUCTION

Nematodes are a major constraint to successful vegetable production all over the world, causing severe damage that leads to yield losses. Plant-parasitic nematodes are estimated to cause global annual losses of more than US$ 100 billion (Chitwood, 2003; Nicol et al. 2011; Degenkolb and Vilcinskas, 2016). Nematode damage in crops is non-specific and causes a range of symptoms from mild to severe, such as wilting, stunting, reduced vigor, nutrient deficiency, root lesions, reduced flowering, fruit loss, poor yield, and even death. Mild symptoms may be overlooked, and even the severe symptoms can be misdiagnosed (Nicol et al. 2011). Root-knot nematodes (*Meloidogyne* spp.) are an important group of plant parasitic nematodes that have

worldwide distributions, wide host range, short generation times, high reproductive rates and endoparasitic nature that make the control difficult (Trudgill and Blok, 2001; Manzanilla-Lopez et al. 2004; Sikora and Fernandez, 2005; Karssen and Moens, 2006). The four *Meloidogyne* spp. (*M. incognita, M. javanica*, *M. arenaria* and *M. hapla*) are the major obligate parasites on many crops worldwide (Eisenback and Triantaphyllou, 1991). Crops infected by nematodes especially vegetables such as tomato recorded yield losses of up to 80 % on heavily infested soils. All the four major species of *Meloidogyne* and their known races readily attack tomato crops in outdoor as well as in indoor cultivations (Taylor and Sasser, 1978; Sasser, 1979; Kaskavalci, 2007).

Egypt is the fifth country in the world in terms of production of tomatoes. The cultivated area reached 475.514 thousand feddan produced approximately 7.94 million tons (FAO 2016). Root-knot nematodes (*Meloidogyne* spp.), reniform nematodes (*Rotylenchulus reniformis*), cyst nematode (*Globodera rostochiensis*) and several ecto-parasitic nematodes are known to attack tomato in many different parts of the world (Esfahani, 2009). Tomato is regarded as the most favorable host for root-knot nematodes (Taylor, 1967; Dropkin, 1980).

Chitin, β 1,4-linked polymer of N-acetylglucosamine, is the second most abundant polymer in nature after cellulose and it is considered a major component of the outer shell of nematode's eggs (Haggag and Amin, 2001; Yong and Kil, 2015) as well as fungal cell wall (Haran et al. 1996). As a producer of variety of chitinases, *Trichoderma* has become an important means of biological control agent against plant diseases (Agrawal and Kotasthane, 2012).

Genus *Trichoderma* is endophytic, soil borne, green-spored ascomycetes that can be found in all soils and root ecosystems. Many species in this genus can be characterized as opportunistic a virulent plant symbionts (Harman et al. 2004; Schuster and Schmoll, 2010). *Trichoderma* sp. is capable of producing intracellular lytic enzymes including β-1, 3-glucanase, chitinase, protease and lipase which are responsible for antagonism (Elad and Henis, 1982; Haran et al. 1996; Sandhyaet al. 2004). *Trichoderma* sp. has been reported to have a biocontrol effect against plant-parasitic nematodes (Windham et al. 1989; Reddy et al. 1996; Rao et al. 1998). Low temperature scanning electron microscopic (LTSEM) studies showed that hyphae of *Trichoderma harzianum* infected potato cysts nematode eggs by penetration of cyst wall with the help of some hydrolytic enzymes or mechanically with appressorium or both (Saifullah and Khan, 2014). Biocontrol efficacy of *T. asperellum* and *T. atroviride* has been reported against *M. javanica* in soil and their ability to parasitize nematode eggs and juveniles has been observed (Sharon et al., 2001). The parasitism ability of *T.harzianum* and *T.viride* on eggs of *M. incognita* was proved. Moreover, the percentage of infected eggs of *M. incognita* increase with exposure time (Sharf and Hisamuddin, 2016). Hatching of *Meloidogyne* spp. eggs were inhibited by the culture filtrates of *Trichoderma isolates* and this inhibition was directly proportional to the culture filtrate concentration and the time of exposure (Naserinasab et al., 2011; Elgorban et al., 2014; Rompalli et al., 2016).

Therefore, the present study was conducted to estimate the efficacy of homogenized growth culture of different chitinolytic *Trichoderma* species on egg hatching and juvenile vitality of root-knot nematode *in vitro*. Moreover, the

comparative efficacy of different chitinolytic *Trichoderma* species in control of root-knot nematodes, were investigated under greenhouse conditions.

MATERIALS AND METHODS

Microbial isolates

Sixteen *Trichoderma* isolates were provided by Central Lab. of Organic Agriculture, Agriculture Research Center, Egypt. Fungal isolates were maintained on Potato Dextrose Agar (PDA).

Media Used

Gliotoxin Fermentation Medium (GFM) (Brian and Hemming, 1945) (g/L): Ammonium tartarate, 2; KH_2PO_4, 2; $MgSO_4$. $7H_2O$, 1; $FeSO_4.7H_2O$, 0.01; and distilled water up to 1000 ml. This medium is modified by supplementation with colloidal chitin 0.45% as a sole carbon source in substitution of glucose for induction of chitinase. The final pH was adjusted to 5.8.

Potato Dextrose Agar (PDA) medium (g/L): potato extract, 4; dextrose, 20; agar, 20 and distilled water up to 1000 ml. the final pH was adjusted to 5.6. It was used for preservation of the fungal culture.

Chitinase assay medium (Agrawal and Kotasthane, 2009) (g/L): MgSO4. $7H_2O$, 0.3; $(NH_4)_2SO_4$, 3; KH_2PO_4, 2; Citric acid monohydrate, 1; Colloidal chitin, 4.5; Tween-80, 200 µland distilled water up to 1000 ml.

Assay of chitinase activity

Preparation of colloidal chitin

Colloidal chitin was prepared from crab shells chitin according to Roberts and Selitrennikoff(1988).

Qualitative assay of chitinolytic activity in *Trichoderma* isolates

Qualitative chitinase production by *Trichoderma* isolates was primary screened by inoculating a fresh culture disc of *Trichoderma* isolate on chitinase assay solid medium supplemented with bromocresol purple (Agrawal and Kotasthane, 2009). Chitinase activity was detected by measuring the diameter of the purple zone after 3 days of incubation at 25±2°C. *Trichoderma* spp. were classified according to the diameter of purple zone into four categories (No chitinase activity; low activity; moderate activity and high activity) (Agrawal and Kotasthane, 2012). The most efficient isolates that gave rapid and highest chitinase activity were selected for the *in vitro* and the *in vivo* studies after the microscopic identification to the species level.

Quantative assay of chitinase activity

Total chitinolytic activity was assayed by measuring the released reducing sugars from colloidal chitin. The reducing sugars were measured using the method modified by Neish (1952). Culture plugs of young actively growing mycelium of *Trichoderma* species were inoculated in colloidal chitin supplemented broth (without bromocresol purple) and incubated at 28°C for 7 days on rotary incubator at 120 rpm. Cultural filtrates obtained by centrifugation at 8,000 rpm for 5 min at 4°C. 1 mL of supernatant was incubated with 1 mL of 1% (w/v) colloidal chitin suspension in 0.2M-phosphate buffer pH 6.5, the reaction mixture was incubated on shaking water bath at 40°C and

70 rpm for 1h. Then 1 mL of the mixture was withdrawn and added to 2 mL of copper reagent, boiled for 20 min, cooled by running tap water. Arsenomolybdate (2 mL) reagent was added and finally made up to 25 mL with distilled water. The released reducing sugars were colorimetrically measured. One chitinase activity unit was defined as 1 μmol of N-acetyl glucosamine released per one hour reaction at specified conditions.Total protein was measured using the method described by Lowry et al. (1951). Specific chitinase activity is calculated by dividing the total chitinase activity in U/mL by the protein concentration in mg/ml.

Nematode culture

A single egg mass of the root-knot nematodes, *Meloidogyne* spp. picked by fine forceps from infected eggplant as root was surface sterilized in 1:500 (*V*/*V*) sodium hypochlorite for 5 minutes. It was then transferred to a small coarse sieve lined with tissue paper, placed in a Petri plate containing sufficient amount of water. The Petri plates were incubated at room temperature for 5 days (den Ouden, 1958). Seedlings of tomato plant raised in autoclaved soil were inoculated with the progeny of the single egg mass in order to get regular supply of pure inoculum for the experiments. The root knot nematode was identified by studying the typical female perenial pattern of isolated mature females, as defined by Taylor and Netscher (1974) and its typical taxonomical characteristics mentioned by Mai and Lyon (1975).

***In vitro* experimental study**

Egg hatching bioassay

Eggs were extracted from galled tomato roots infested with *Meloidogyne incognita* using 1.5 % sodium hypochlorite solution by the method of Hussey and Barker (1973) and modified by Sikora and Greco (1990). Eggs were collected on 500 Mesh sieve and transferred to a beaker containing tap water. The selected chitinolytic *Trichoderma* species were grown separately on Gliotoxin Fermentation Medium (GFM) at 25ºC±2 and 120 rpm for 7 days under complete darkness condition. Different species were prepared separately as homogenized fungal growth culture that adjusted to contain 10^6cfu / 1 ml. Dilutions of 1, 10 and 50% were prepared from each homogenized culture suspension of *Trichoderma* species. The effect of different *Trichoderma* species on the egg hatching of *M.incognita in vitro* was investigated according to Westcott and Kluepfel (1993). One ml from each concentration of the four *Trichoderma* species growth culture was added separately into test tubes containing one ml of egg suspension (100±5 eggs per ml) of *M.incognita*. The tubes were incubated at 27 ºC in the dark for 7 days with shaking every day to ensure aeration. The total number of hatched eggs was counted. Eggs in distilled water were used as control. Morphological changes in the eggs were also observed. The experiment was laid out in completely randomized design with three replicates for each treatment. The inhibition of the egg hatching rate was calculated using the formula:

Red. (%) = (C-T)/C × 100 where,

Red.: Reduction of the egg hatching, T; Number of hatched eggs in treatment;
C: Number of hatched eggs in suspension in the control

Juveniles (J2) mortality bioassay:

The four *Trichoderma* species were screened for their antagonistic activity against second stage juveniles of *M. incognita*. Hatching of second stage juveniles was

stimulated by aerating the egg suspension with oxygen in the dark for 7 to 10 days as described by Naserinasab et al. (2011). Freshly hatched second-stage juveniles were separated from the un-hatched eggs using a modified Baermann technique (Townshend, 1963). One ml from each concentration of homogenized growth culture suspension of the four *Trichoderma* species was added separately into test tubes containing one ml of water suspension containing 100 ±5 of *M.incognita* juveniles and incubated at 28-30ºC for 24 hours. The same number of juveniles received distilled water only was served as control. Control and each treatment were replicated thrice. After 24 hours, the immobile juveniles were counted in each test tube. Juveniles were considered dead if they could not move when probed with fine needle and body become straight (Siddiqui and Shaukat, 2004). J2 mortality was calculated according to the formula:

JM (%) = (T/C) × 100 where,

JM: The mortality of J2, T: Number of dead J2 in treatment; C: The total number of J2 used in test.

In vivo experiment (Greenhouse experiment)

Experimental Site

A greenhouse experiment was carried out at Central Lab. of Organic Agriculture, Agriculture Research Center (ARC), Egypt. The experiment was conducted from August to October, 2017. The minimum and maximum temperature was about 28°C and 35°C, respectively.

Experimental design

Tomato seedlings (*Solanum lycopersicon* L. cv. Castlerock II PVP) (30 days old) were transplanted to 25 cm diameter plastic pots filled with autoclaved sand and peat moss (1:1, V: V) each pot contained one tomato seedling. Inoculation was done by making 3 holes in soil of each pot, nearby to the root at the same distance in the manner so that root not damaged. Then 3 ml of eggs suspension having approximately 3000 eggs, obtained from pure culture of *M. incognita* was injected into the holes and covered them with the soil. The homogenized growth culture at 10% concentration from each *Trichoderma* species was added separately at three intervals. The first treatment was applied one week before inoculation with eggs of *M. incognita* the second treatment was applied at the same time of inoculation and the third treatment was applied one week after inoculation (Abd El-Monem et al. 2016). Three plastic pots with tomato seedlings inoculated with 3000 eggs of *M. incognita* without *Trichoderma* treatment were served as control. Each treatment was replicated three times and all treatments were arranged in a complete randomized block design. The experiment was conducted from August to October, 2017, with minimum and maximum temperature of about 28°C and 35°C, respectively. All pots were irrigated, fertilized periodically using the same amount of water and fertilizers per each pot. Pots were kept in the greenhouse at 30°C ±5. Environmental conditions in the green house during the experiment were adjusted to be suitable for both tomato plant and nematodes.

Sixty days after nematode inoculation, tomato plants were carefully uprooted for further examination. Plant growth criteria in terms of fresh shoot and root lengths and weights as well as dry shoot weight were measured. Nematodes juveniles were extracted from soil according to the method of Goodey (1957).Number of galls and

egg masses as well as developmental stages per root system were counted. Root gall (RGI): and egg masses (EI) indices were determined according to the scale given by Taylor and Sasser (1978) as follows: 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.

Statistical analysis

Data were subjected to be analysis of variance (ANOVA). Means were compared by Duncan s multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

A wide range of *Trichoderma* species have high potentiality to produce chitinases when chitin is present as a substrate in the growth medium (Ulhoa and Peberdy, 1991; Sandhya et al., 2004; González et al., 2012 and Sharaf et al., 2012).

Qualitative assay of chitinolytic activity in *Trichoderma* isolates

The present study revealed that all tested isolates of *Trichoderma* showed chitinolytic activity at different rates when screened on solid medium supplemented with colloidal chitin 0.45 % as sole carbon source (Fig.1). Weak activity was achieved by isolates 5 and 16 while low activity was recorded in isolate 15 (Table 1).

Table 1. Qualitative assay of chitinolytic activity in *Trichoderma* isolates
High activity (H): 76-100, Moderate activity (M): 51-75, Low activity (L): 25-50, Weak activity (W):

Trichoderma Isolates	Diameter of colored zone (mm)	Activity rate
Trichoderma isolates No. (1)	90^{a}	H
Trichoderma isolates No. (2)	75^{b}	M
Trichoderma isolates No. (3)	72^{c}	M
Trichoderma isolates No. (4)	70^{cd}	M
Trichoderma isolates No. (5)	17^{j}	W
Trichoderma isolates No. (6)	64^{e}	M
Trichoderma isolates No. (7)	60^{f}	M
Trichoderma isolates No. (8)	90^{a}	H
Trichoderma isolates No. (9)	70^{cd}	M
Trichoderma isolates No. (10)	55^{g}	M
Trichoderma isolates No. (11)	90^{a}	H
Trichoderma isolates No. (12)	60^{f}	M
Trichoderma isolates No. (13)	90^{a}	H
Trichoderma isolates No. (14)	68^{d}	M
Trichoderma isolates No. (15)	33^{h}	L
Trichoderma isolates No. (16)	21^{i}	W
LSD at 1%	2.235	-

1-25 and No activity: Zero. Means in each column followed by the same letter(s) did not differ at $P \leq 0.01$ according to Duncan`s multiple range test.

Moderate activity was exhibited by isolates 2, 3, 4, 6, 7, 9, 10, 12 and 14. However, isolates No. 1, 8, 11 and 13 showed rapid response and high chitinolytic activity

consequently were selected for further studies. Agrawal and Kotasthane (2012) demonstrated that colloidal chitin media containing Bromo cresol purple pH towards alkalinity and change pH indicator dye (BCP) from yellow to purple zone surrounding the inoculated fresh culture plugs.

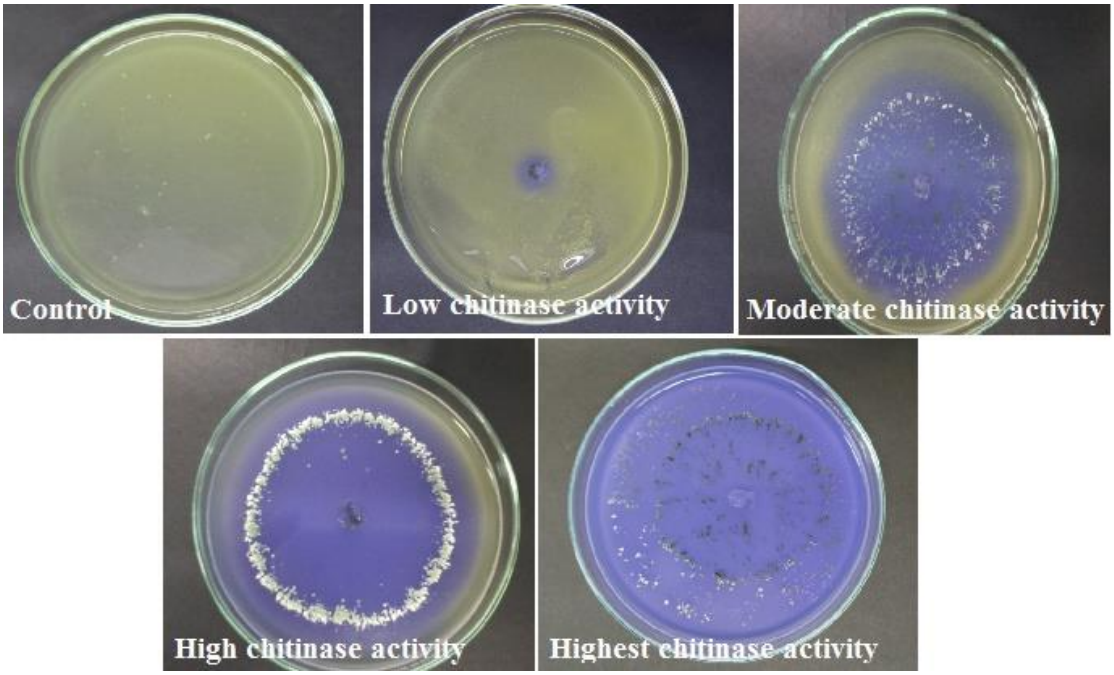

Fig. 1. Qualitative assay of chitinolytic activity of *Trichoderma* isolates on solid medium.

Microscopic identification of the four selected chitinolytic *Trichoderma* spp.:

The microscopic identification revealed that the high chitinolytic species were *Trichoderma asperelloides*, *T. hamatum*, *T. harzianum* and *T. viride.*

Quantitative assay of chitinolytic activity in *Trichoderma* isolates

Results in Table (2) indicate that *T. asperelloides* showed the highest chitinase activity (1.736 U/mL). *T. hamatum* also marked high chitinase activity (0.930 U/mL) followed by *T. viride* (0.589 U/mL). The least chitinolytic activity was achieved by *T. harzianum* (0.560 U/mL). Furthermore, *T. asperelloides* showed the maximum total protein (9.861 mg) and chitinase specific activity (0.176U/mg). Elad and Henis (1982); Haran et al. (1996) demonstrated that the biological control of *Trichoderma* genus against plant pests and diseases is based, to large extent, on their secretion of hydrolytic enzymes including β-1, 3-glucanase, chitinase, protease and lipase which are responsible for antagonism. Furthermore, the gelatinous matrix of egg masses of root-knot nematodes has been found to trigger production of proteolytic and chitinolytic enzyme by the fungus (Sharon et al., 2007; Golzari et al., 2011). Rajinikanth et al. (2016) stated that chitinolytic enzyme chi18-5 of *T. viride* played a vital role in the induction of mycosis in the nematode eggs.

Table 2. Quantitative assay of chitinolytic activity of *Trichoderma* species.

Trichoderma ssp.	Final pH	Chitinase activity (U/mL)	Total protein (mg)	Specific activity (U/mg)
T.asperelloides	7.45	1.736	9.861	0.176
T.hamatum	7.36	0.930	9.824	0.094
T.harzianum	7.5	0.560	8.362	0.066
T.viride	7.39	0.589	9.068	0.064
Blank	7.00	0.000	-	-

Efficacy of different concentrations of homogenized growth culture of chitinolytic *Trichoderma* spp. on egg hatching of *Meloidogyne incognita.*

Significant inhibition of egg hatching was showed in *Trichoderma* treated eggs compared to control (Table 3).

Table 3. Efficacy of different concentrations of homogenized growth culture of chitinolytic *Trichoderma* spp. on egg hatching of *Meloidogyne incognita*

Treatments	Treatment concentration (%)					
	1		10		50	
	No. of hatched eggs	Red. (%)	No. of hatched eggs	Red. (%)	No. of hatched eggs	Red. (%)
Control (Nematode alone)	87.66[a]	-	87.66[a]	-	87.66	-
T. asperelloides	17.00[c]	80.61	0.66[c]	99.245	0	100
T. hamatum	33.66[b]	61.60	3.00[c]	96.578	0	100
T. harzianum	42.66[b]	51.33	12.00[b]	86.31	0	100
T. viride	34.66[b]	60.46	5.66[c]	93.54	0	100
L.S.D at 0.01	12.64	-	5.42	-	-	-

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.01$ according to Duncan`s multiple range test.

Reduction percentage =Red. (%) = (C-T)/C × 100 where, T: Number of hatched eggs in treatment & C: Number of eggs hatched in suspension in the control.

At 50% concentration, the four *Trichoderma* species completely inhibited egg hatching. At 10% concentration, significant reduction in egg hatching (99.245%) was achieved by *T.asperelloides* followed by *T. hamatum* (96.578%) then *T. viride* (93.54%) and finally *T. harzianum* (86.31%). At 1% concentration, *T.asperelloides* showed (80.60%) egg hatching reduction followed by *T. hamatum* (61.60%), *T. viride* (60.46) then *T. harzianum* (51.33%).The results indicated a positive correlation between the concentration of chitinolytic activity in culture and the egg hatching inhibition efficacy. Egg infection by *Trichoderma* spp. was investigated with compound microscope. The hyphae bearing conidia and spores of different *Trichoderma* species were found around the nematode eggs causing morphological distortion in eggs which in turn failed to hatch (Fig. 2). *Trichoderma* species were able to grow on the egg surface and penetrated the egg shell as a result of their chitinolytic activity. The major inhibition mechanism utilized by *T. harzianum* against nematodes is the direct parasitism of *Meloidogyne* eggs and juveniles by using proteolytic or chitinolytic activities to induce damage in both phases as a sign of infection ability (Naserinasab et al., 2011).

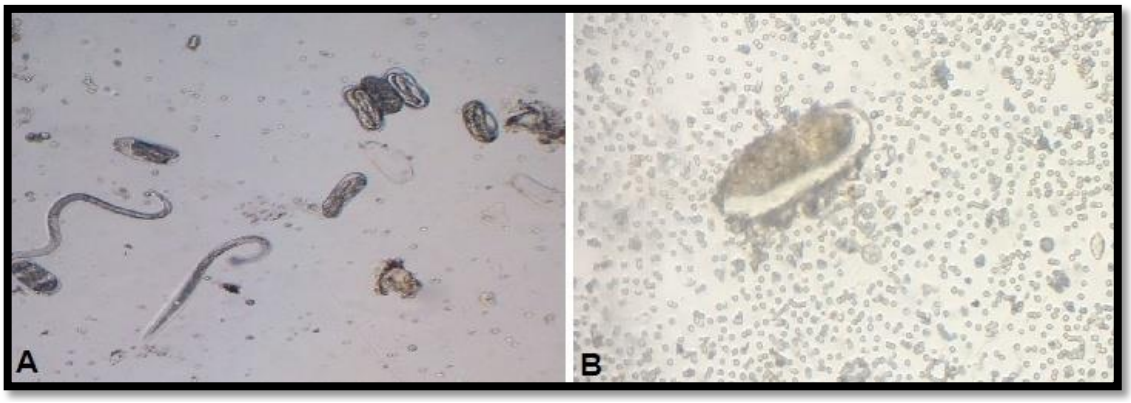

Fig. 2. Distortion caused by *Trichoderma* sp. on eggs of *Meloidogyne incognita*.
A: Untreated eggs (Normal egg hatching) **B:** *Trichoderma* treated egg (Failed to hatch)

The microscopic studies found by many researches indicated that *Trichoderma* hyphae and conidia were adhered to the surface of eggs and egg masses. Moreover, the hyphae had the ability to form trapping-rings around emerging juveniles but had no contact with adult females (Sidhu et al., 2014; Sharf and Hisamuddin, 2016). Mascarin et al. (2012) noticed that 64% of conidial attachment on eggs and second-stage juvenile were immobilized when treated with *T. harzianum*. Bokhari (2009) and Naserinasab et al. (2011) recorded that hatching of *M. javanica* eggs were inhibited by the culture filtrates of *Trichoderma* sp. and this inhibition was positively correlated with increase in the concentration of culture filtrates**.**

Efficacy of different concentrations of homogenized growth culture of chitinolytic *Trichoderma* spp. on larval mortality

The data in Table (4) indicated that the homogenized growth culture of different chitinolytic *Trichoderma* species exerted nematicidal dependent manner. However, *T. asperelloides* showed significant mortality effect (65.00 %) even at the minimum concentration (1%). The present results are in harmony with Elgorban et al. (2014) who demonstrated that treatment of *M. javanica* second-stage juveniles with culture filtrates of *T. harzianum* caused 64.5% mortality of juveniles after 72 h of exposure.

The culture filtrate of *Trichoderma* was found to be effective in suppressing the egg hatching of *M. incognita* and causing second juveniles mortality which increased with increase in time of exposure of eggs to cell free culture filtrate as well with increase in concentration (Rompalli et al.,2016).

Table 4. Efficacy of different concentrations of homogenized growth culture of chitinolytic *Trichoderma* spp. on juveniles mortality of *Meloidogyne incognita*

Treatment	Juveniles Mortality					
	Concentration (%)					
	1		10		50	
	No. of immobile juveniles	Mortality (%)	No. of immobile juveniles	Mortality (%)	No. of immobile juveniles	Mortality (%)
Control (Nematode alone)	0.00[d]	-	0.00[c]	-	0.00[b]	-
T.asperelloides	65.00[a]	65.00	79.33[a]	79.00	100.00[a]	100
T. hamatum	52.00[b]	52.00	73.66[a]	73.66	100.00[a]	100
T. harzianum	34.00[c]	34.00	51.00[b]	51.00	99.66[a]	99.66
T. viride	51.66[b]	51.66	65.66[a]	65.66	100.00[a]	100
L.S.D at 0.01	8.37	-	13.64	-	0.66	-

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.01$ according to Duncan`s multiple range test.

In vivo evaluation of chitinolytic activity of *Trichoderma* spp. against root-knot nematode (*Meloidogyne incognita*)

Effect on plant parameters

Growth parameters of *M. incognita* infected tomato (shoot length, root length, shoot fresh weight, root fresh weight and shoot dry weight) were significantly promoted with three sequential applications of homogenized growth culture of any of the four chitinolytic *Trichoderma* spp. at 10% concentration (Table 5). Fig. (3). showed the impact of homogenized growth culture of different chitinolytic *Trichoderma* spp. on tomato root fitness However, *T. asperelloides* treated plants induced the highest enhancement in plant growth parameters. Saba et al. (2012) stated that some strains of *Trichoderma* spp. establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They release a variety of compounds that stimulate systemic resistance and this explains their lack of pathogenicity to plants.

Table 5. Impact of different chitinolytic *Trichoderma* species on *Meloidogyne incognita* nematode infected tomato growth parameters under greenhouse conditions.

Treatments	Growth parameters				
	Length (cm)		Plant fresh wt. (g)		Shoot dry wt. (g)
	Shoot	Root	Shoot	Root	
Control (Nematode alone)	34.99[b]	18.32[d]	15.60[d]	4.46[b]	3.20[b]
T. asperelloides	58.30[a]	46.30[a]	43.40[a]	13.50[a]	8.50[a]
T. hamatum	55.10[a]	38.30[b]	37.10[b]	12.10[a]	7.00[a]
T. harzianum	52.20[a]	31.06[c]	33.20[c]	11.00[a]	5.86[a]
T. viride	55.10[a]	31.00[c]	36.00[bc]	10.10[a]	6.70[a]
L.S.D at 0.05	4.13	4.59	2.81	2.69	2.13

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan`s multiple range test.

Fig. 3. Impact of different chitinolytic *Trichoderma* species on root fitness of tomato infected with root-knot nematode (*Meloidogyne incognita*).

(A): Root-knot nematode infected root + *T.asperelloides*. (B): Root-knot nematode infected root + *T. hamatum*. (C): Root-knot nematode infected root + *T.harzianum.* (D): Root-knot nematode infected root + *T. viride*. (E): Root-knot nematode infected root (Control).

In the same line Shoresh et al. (2010) reported that some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and induction of plant defenses against biotic and abiotic damage

Kumar et al. (2017) demonstrated that many *Trichoderma* species were able to produce the auxin phytohormone indole-3-acetic acid (IAA), and its production has been suggested to promote root growth

Effect on nematode population and root galling

All *Trichoderma* species effectively reduced the final nematode population of *M. incognita* compared to untreated control (Table 6). However, *T.asperelloides* showed maximum inhibition (97.81%) followed by *T.hamatum* (96.31%). Additionally *T.harzianum* and *T.viride* significantly reduce the final population to 88.16% and 90.34%, respectively. On the other hand, all treatments caused significant decrease in root galling, egg masses production, which is a measure of nematode development over time, and number of eggs/egg mass, which is an indicator for female fertility (Table 7). Maximum reduction of root galling (RGI = 3.0) as well as root-knot nematodes reproduction (EI = 2.0) were obviously observed in plants treated with *T.asperelloides* and *T.hamatum*.

Table 6. Impact of homogenized growth culture of different chitinolytic *Trichoderma* spp. on population density of *Meloidogyne incognita* under greenhouse conditions.

Treatments	No. of juveniles /250 g soil	No. of females/5g of root	No. of developmental stages/ 5 g of root	Final population	Red. %
Control (Nematode alone)	338.33^{a}	82.66^{a}	21.00^{a}	441.99	-
T. asperelloides	6.00^{e}	2.33^{d}	1.33^{c}	9.66	97.81
T.hamatum	10.33^{d}	6.00^{c}	0.00^{c}	16.33	96.31
T.harzianum	40.00^{b}	4.33^{cd}	8.00^{b}	52.33	88.16
T.viride	30.00^{c}	11.33^{b}	1.33^{c}	42.66	90.34
L.S.D at 0.05	1.62	2.69	1.62	-	-

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan`s multiple range test.

Similar results were obtained by Naserinasab et al. (2011) who reported that at transplanting stage, treatment of the soil with the antagonistic *Trichoderma* caused significant suppression in the number of galls, egg masses, eggs and diameter of galls. When the tomato plants were inoculated with *T. harzianum*, root and shoot weight clearly improved as compared to both the absolute and nematode controls. Javeed and Al-hazmi (2015) stated that time of the fungus introduction into the soil played an important role in the biocontrol efficacy of the treatment. They found significant reduction in the number of root galls at all contact times of *T. harzianum*. However, when the fungal treatment was introduced two or one weeks prior to nematode inoculation, such inhibition effect of the treatment increased significantly. *T. viride*

showed a great suppression effect on the nematode reproduction and root galling of root knot-nematode on cucumber plants under greenhouse conditions (Yankova et al. 2014). Abd-Elgawad and Kabeil (2012) confirmed the ability of *T. harzianum* and a commercial suspension of *Serratia marcescens* to reduce significantly the number of egg masses and eggs per root system as compared to untreated plants. Such reduction is positively correlated with an acceleration of plant fitness. Furthermore, treatments with both biocontrol agents inhibited female fertility (eggs/egg masses) of nematodes.

Table 7. Impact of homogenized growth culture of different chitinolytic *Trichoderma* spp. on reproduction of *Meloidogyne incognita* under greenhouse conditions.

Treatments	No. of galls / 5 g of root	RGI	Red. %	No. of egg masses/ 5 g of root	EI	Red. %	No. of eggs/ egg mass	Red. %
Control (Nematode alone)	167.00[a]	5	-	70.00[a]	4	-	418.00[a]	-
T. asperelloides	12.00[d]	3	92.81	3.00[d]	2	95.71	51.33[e]	87.72
T.hamatum	13.00[d]	3	92.21	10.00[c]	2	85.71	68.33[d]	83.65
T.harzianum	33.66[b]	4	79.84	23.33[b]	3	66.67	100.00[c]	76.07
T.viride	30.33[c]	4	81.83	23.00[b]	3	67.14	105.00[b]	74.88
L.S.D at 0.05	3.08	-	-	2.85	-	-	3.48	-

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan`s multiple range test. (RGI): Root gall index and (EI): egg masses index were determined according to the scale given by Taylor and Sasser, 1978 as follows: 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.

The data confirmed that, the plant fitness in terms of plant growth parameters significantly increased by reducing the severity of nematodes infection. Furthermore, the nematicidal activity of *Trichoderma* species is directly proportional to their chitinase enzymatic activity.

Conclusion, both *in vitro* and *in vivo* results of the present study confirmed the significant role of microbial chitinase in inhibition of root-knot nematode and suggested the positive correlation between the chitinolytic activity of different *Trichoderma* species and their nematicidal activity.

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الملخص العربى

المكافحة الحيوية لنيماتودا تعقد الجذور(ميليدوجينانكوجنيتا) باستخدام بعض أنواع التريكودرما المحللة للكيتين

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فى هذه الدراسة تم تقييم القدرة التثبيطية لبعض أنواع التريكودرما المحللة للكيتين على نيماتودا تعقد الجذور(ميليدوجين انكوجنيتا) تحت ظروف كلا من المعمل والصوبة. تم اختبار قدرة ستة عشر عزلة من التريكودرما على افراز انزيم الكيتينيز وذلك باستخدام بيئة صلبة مزودة بالكيتين كمصدر أساسى للكربوهيدرات. ظاهريا (كيفيا) تم تعريف اربع عزلات فى ذات القدرة العالية على تحليل الكيتينوهى تريكودرما أسبرلويدس، تريكودرما هاماتم، تريكودرما هارزيانم و تريكودرما فيردى. تباعا تم التقييم الكمى لانزيم الكيتيناز. أظهرت النتائج أن تريكودرما أسبرلويدس كانت صاحبة أقوى نشاط انزيمى كلى (1.736 U/ml). تحت ظروف المعمل تم تثبيط حيوية يرقات نيماتودا تعقد الجذور ونسبة فقس البيض بشكل ملحوظ باستخدام المعلق المتجانس من مزراع الأنواع المختارة للتريكودرما. وجدت علاقة طردية بين زيادة التركيز وتأثير المعاملة عند التقييم. كما توافقت النتائج المتحصل عليها من تجارب المعمل مع النتائج المتحصل عليها من الصوبة حيث أظهرت معاملات التريكودرما انخفاضا ملحوظا فى أعداد النيماتودا فى الجذور والتربة وصلت الى 97.81% وذلك بالتوازى مع زيادة ملحوظة فى طول ووزن المجموع الخضرى والجذرى لنبات الطماطم المعامل بالتريكودرما مقارنة بالنبات غير المعامل. أكدت النتائج العلاقة الطردية بين القدرة التثبيطية لأنواع التريكودرما ونشاطها الانزيمى.