Efficiency of Jordanian *Trichoderma harzianum* (Rifai) Isolates against *Meloidogyne javanica* (Treub) on Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

The efficacy of three Jordanian *Trichoderma harzianum* isolates; Th1, Th2 and Th3, against *Meloidogyne javanica* on tomato was evaluated *in vitro* and *in vivo*. The effects of their product compounds on hatching and mortality of second stage juveniles' larvae (J2) under laboratory conditions were tested. In a plastic house experiment, the three Jordanian *T. harzianum* isolates increased significantly plant height and fresh shoot weight. *T. harzianum* isolates in combination with nematode did not significantly affect shoot fresh weight and plant height compared with nematode treatment. Root galling and reproductive factor were reduced in all isolates, the resulting reduction reached up to 50%. The study of culture filtrates (autoclaved and non-autoclaved) and extracts of Trichoderma-treated soils (autoclaved) at all concentrations significantly inhibited eggs' hatching and increased J2 mortality. The inhibition and J2 mortality increased with increasing concentration of the culture filtrates and soil extract of *T. harzianum* isolates. The autoclaved culture filtrates were less effective than the non-autoclaved ones. Th1 was more effective than the other ones in J2 mortality at 50% and 100% concentrations of autoclaved soil extract. In conclusion, this study identified three Jordanian *T. harzianum* isolates that were active on soil and capable of producing compounds against *M. javanica*.

Keywords: Tomato, *Trichoderma harzianum*, *Meloidogyne javanica*, Jordanian isolates, Biological control.

INTRODUCTION

Root Knot Nematodes (RKN) belonging to the genus *Meloidogyne* are among the important pests attacking economic plants throughout the world. RKN attack nearly every crop grown and cause average annual yield losses of about 5% (Sasser, 1987; Sasser and Freckman, 1987). The estimated average annual losses of irrigated vegetable crops cultivated in the Jordan Valley due to RKN are nearly 15% (Abu-Garbieh, 1994). However, 70 species of *Meloidogyne* have been described, where *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood and *M. arenaria* (Neal) (Chitwood) are extremely polyphagous apomictic species. These three are distributed worldwide and account for the majority of crop losses due to RKNs (Nickle, 1991). In Jordan, these three species were isolated and identified from soil and plant samples collected from major irrigated areas in Jordan; where *M. javanica* being the most common isolated one (Abu-Garbieh *et al*., 2005).

Several control measures were employed to control RKN in infested areas. Chemical control (fumigants and non-fumigants) is the most common method (Minton *et al*., 1980; Walker and Watchel, 1988; Lamberti *et al*., 2000). Although fumigant and non- fumigant nematicides are highly effective against RKNs, they are harmful to environment and human as well as expensive.

Biological control with fungi and bacteria is the core of

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integrated pest management (IMP) (Davis *et al*., 1988; Holland *et al*., 1999; Sharon *et al*., 2001; Meyer *et al*., 2004; Abu-Dhaim *et al*., 2005) to control plant parasitic nematodes. Among the antagonistic fungi, species of *Trichoderma* have been used as biocontrol agents against nematodes. Soil treatment by *T.harzianum (*T-12) and *T. koningii* (T-8) resulted in a reduction in egg production of *M. arenaria* (Windham *et al*., 1989; Rao *et al*., 1998; Sharon *et al*., 2001).

Various mechanisms were suggested for the biocontrol action of *Trichoderma* spp. against phytopathogenic antibiosis, competition, mycoparasitism and enzymatic hydrolysis (Sivan and Chet, 1992; Elad, 1995; Al-Ameiri, 2001). All mechanisms can potentially be involved in the nematode biocontrol process (Elad, 1995; Sharon *et al*., 2001; Meyer *et al*., 2004).

Therefore, this study was conducted in order to evaluate the three Jordanian isolates of *T. harzianum* on tomato growth in terms of the ability of the isolates against colonization of *M. javanica* on tomato root plants under plastic house conditions, and to investigate the activity of produced compounds by these isolates on egg hatching and second stage juveniles (J2) from culture and treated soil extracts.

MATERIALS AND METHODS

Nematode Inoculum

The root-knot nematode *M. javanica* isolates were obtained from infected tomato plants (*Lycopersicon esculentum* Mill) from Ghor Al-Safy and propagated on tomato plants cv. GS12 grown in plastic pots under plastic house conditions at 24±3ºC and 15: 9 h (L: D), at the Agricultural Research Station, Mu'tah University. Eggs were separated from egg masses with sodium hypochlorite (0.5%, 1 min) and hatched in water to produce infective second stage juveniles (J2).

Trichoderma Isolates and Preparation

Wild types of Jordanian isolates (Table 1), *T. harzianum*-(Th1, Th2, Th3) mutagenesis by benomyl tolerant as described by Ahmad and Baker (1987)-were grown on potato dextrose agar (PDA) containing 10 µg a.i. benomyl/ ml at 24±2ºC in the incubator.

Table 1. *T. harzianum* isolates, site of collection and cultivated plant.

Trichoderma harzianum isolate number	Location	Cultivated plant
Th1	Rabba Station	Tomato
Th2	Karak Valley	Tomato
Th3	Ghor Al-Safy	Tomato

Seedlings

Three weeks old tomato seedlings cv. GS12 averaging 5 cm in height were grown in a plastic house at the Agricultural Research Station, Faculty of Agriculture, Mu'tah University. The experiments were conducted under controlled conditions of 22±4ºC and 16: 8 h (L: D). One seedling was planted in each pot.

The Effect of *Trichoderma harzianum* Isolates on Tomato Growth, Root Galling and Nematode Reproduction

The soil used in this study was analyzed at the Central Laboratory, Faculty of Agriculture, Mu'tah University. The soil had the following characteristics; a pH of 7.2, total N of 0.22%, P of 160 ppm, K of 350 ppm and 2.1% organic matter. The soil was taken from a deep layer, free of root debris, and was free of soil infested pathogens (sterilized by oven). For experiment with J2-infested soil and *T. harzianum*, five days before planting, the J2 were added to the pots (3000/pot) at a depth of 2.5cm into the rhizosphere using 3 holes made with a plastic rod, and half of the *T. harzianum* dishes 9 cm in diameter. Four days old cultures of fungus were added to the pots (Saydam

et al., 1973). Control treatment was carried out with tap water and potato dextrose agar (PDA). The experimental treatments were: (Mj, Mj+Th1, Mj+ Th2, Mj+Th3, Th1, Th2, TH3 and control), where (Mj= *M. javanica*, Th= *Trichoderma harzianum* and 1-3 are the isolate numbers). Each treatment was replicated four times. The plants were daily irrigated and fertilized (twice during the experimental period) with 1.5 g/L water (N: P: K 20:20:20 and TE) to insure a proper plant growth.

The layout of the experiment was a randomized complete design (RCD). Nine weeks after the seedlings were inoculated with *M. javanica*, plant shoot fresh weights and heights were measured. Root-galling was assessed following a scale (0-5) according to visible galls on the root system; where: 0 = no galling; 1 = 1-9 galls/plant; 2 = 10-19 galls/plant; 3 = 20-29 galls/plant; 4 = 30-39 galls/plant and 5 = more than 40 galls/plant (Abu-Gharbieh *et al*., 1978). Reproduction factor (RF) was calculated according to the formula RF = PF/PI; where PI represents the initial population (3000 J2), and PF the final population of eggs and J2 as recorded at the end of the experiment.

Effect of *T. harzianum* Cultural Filtrates on Egg Hatching and J2 Mortality of *Meloidogyne javanica*

To determine the efficiency of *T. harzianum* isolates against J2 and egg hatching, potato dextrose (PD) medium was autoclaved, seeded with 0.5 cm of *Trichoderma harzianum* disc and incubated for three days. After two weeks of incubation, the fungal biomass was removed by sieves and centrifuged at 10,000 g for a half of an hour and sequentially filtered to ensure that there was no fungal growth. The products were divided into two samples; one of them was autoclaved at 121ºC, 15Ib/in^2 for 15 minutes and the other non-autoclaved (two treatments). The cultured filtrate was used in four concentrations of 100%, 50%, 25% and zero, as control. The J2 and egg masses were collected from infected roots after harvesting, placed in 0.5% sodium hypochlorite for two minutes, then removed in sterilized distilled water, and after that hatched 100 J2 and one egg mass (separately) were transferred into Petri dishes containing 10 ml of the fungal filtrate at the above-mentioned concentrations with five replicates per treatment. Three days later, the percentage of mobile J2 was determined by counting mobile versus nonmobile (straight) J2, and the number of J2 hatched from the egg mass was recorded five days later.

Effect of Soil Extracts on Egg Masses and J2 Mortality of *Meloidogyne javanica*

One hundred grams of soil samples from experiment pots treated with *Trichoderma* isolates were collected after harvesting. The fungus was extracted with 100 ml of distilled water by vigorous shaking for 1 hour in a 500-ml Erlenmeyer flask, and allowed to precipitate for 30 minutes. The supernatants were collected, centrifuged at 10,000 g for 10 minutes, and the resulting supernatant was filter-sterilized with a filter paper (Whatman) three times. Then, four concentrations of 100%, 50%, 25% and zero of the prepared sterile products, autoclaved at 121ºC, 15Ib/in^2 for 15 minutes, were used to study the effect of soil extract on J2 or hatching from egg masses which were tested *in vitro* in sterile Petri dishes. A total of 100 J2 or one egg mass (separately) with five replicates per concentration (autoclaved) was placed into every Petri dish (containing 10 ml of each concentration). Plates were incubated at 25°C. After 3 days of incubation, the percentage of mobile J2 was determined by counting mobile versus nonmobile J2 (straight), and the number of J2 hatched from the egg mass was recorded five days later.

Statistical Analysis

Data analyses were performed using MSTATC program package. Treatment means (plant growth, galling index Rf, egg hatching and J2 mortality) were

compared using Least Significant Difference (LSD) at 5% probability level according to Steel and Torri (1980).

RESULTS

The Effect of *Trichoderma harzianum* Isolates on Tomato Growth, Root Galling and Nematode Reproduction

Growth of tomato plants (shoot fresh weight and plant height) inoculated with *T. harzianum* alone increased significantly more than those inoculated with nematode only (Figure 1, A and B). This increase reached more than 50% in plant height. The two isolates Th1 and Th2 with nematode treatment increased plant growth, but there was no significant influence on plant height.

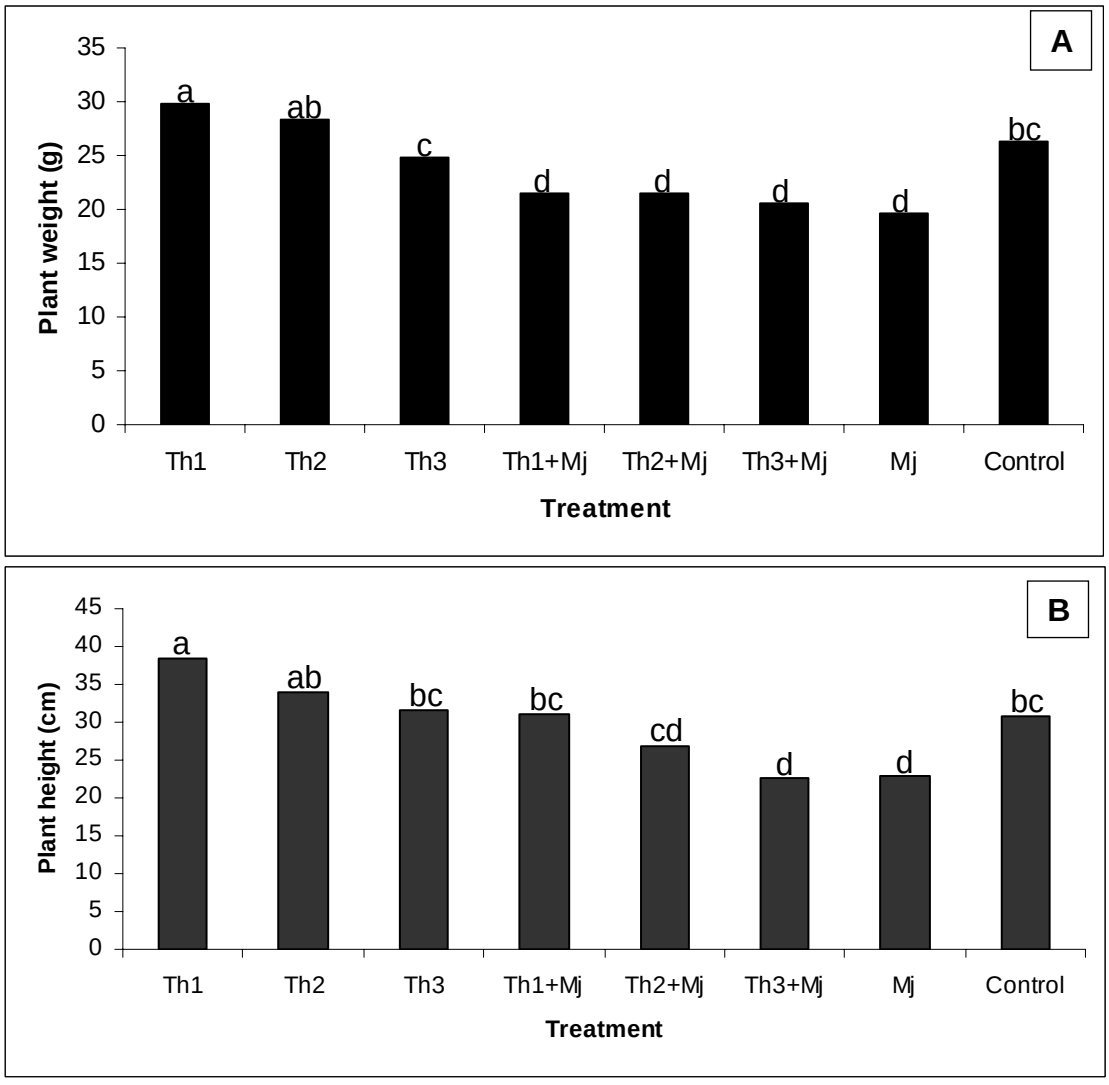

Figure 1. Effect of three Jordanian *Trichoderma harzianum* isolates and *Meloidogyne javanica* alone, or in combination, or with none of them on plant weight (A) and shoot height (B) of tomato compared to control treatment.

Treatment with *T. harzianum* significantly decreased root galling compared to nematode treatment alone (Figure 2A). This reduction reached more than 50% with Th3 the least isolate. Reproduction factor was significantly lower when nematodes were effectively treated with *T. harzianum* isolates (Figure 2B).

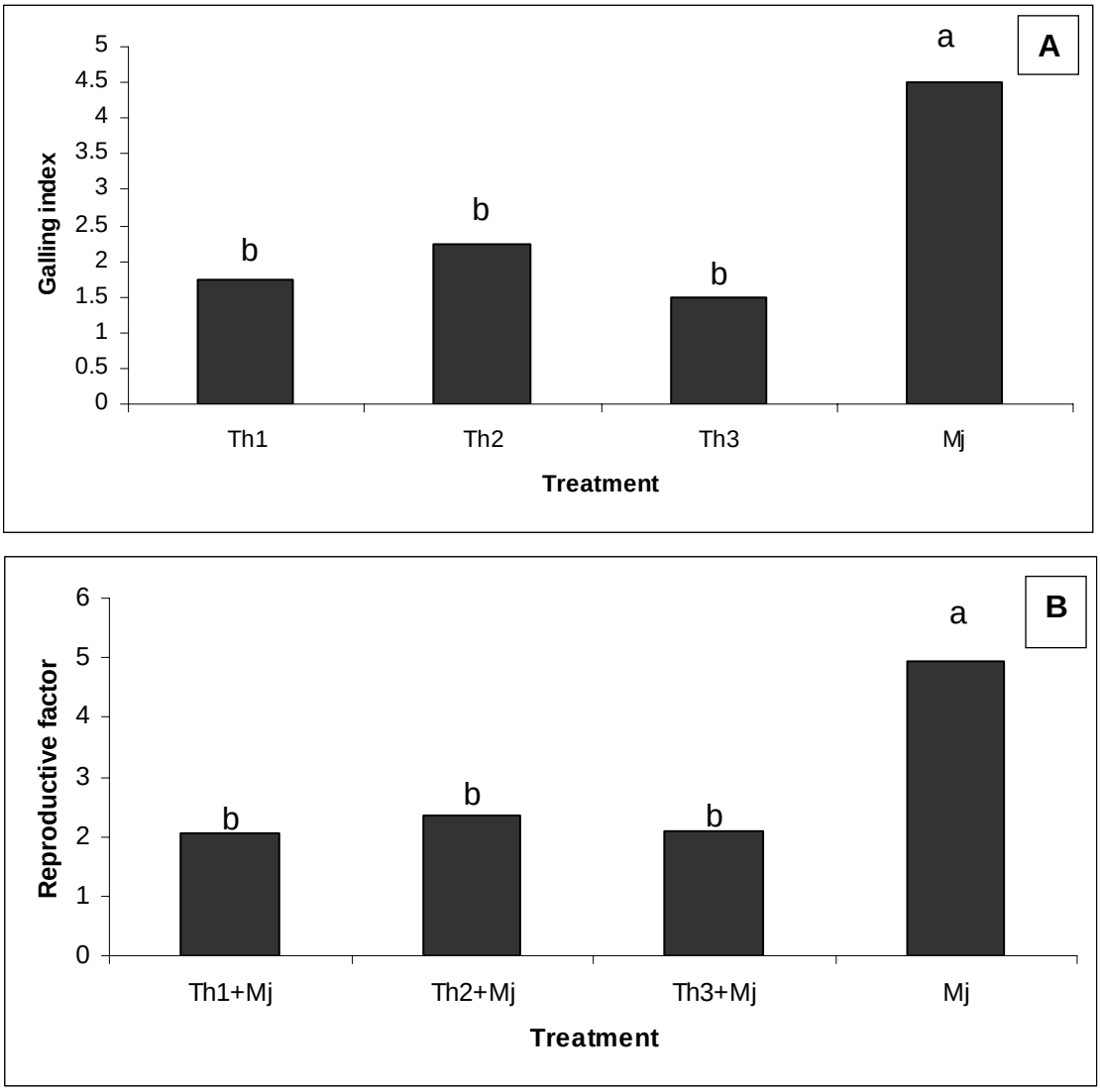

Figure 2. Effect of three *Trichoderma harzianum* isolates on galling index (A) and reproductive factor (B) of *Meloidogyne javanica* on tomato. Root-galling on a scale (0-5); where: 0 = no galling; 1 = 1-9 galls/plant; 2 = 10-19 galls/plant; 3 = 20-29 galls/plant; 4 = 30-39 galls/plant; and 5 = more than 40 galls/plant.

Effect of Culture Filtrates of *Trichoderma harzianum* on Egg Hatching and J2 of *M. javanica*

J2 hatching reduced significantly in all treatments treated with *T. harzianum* culture filtrate concentrations (non-autoclaved) than untreated eggs (Figure 3A) with an average of 12.13 and 20.4 for 100% and 25% filtrate concentrations, respectively. Among isolates under the same concentration, there were no significant differences except for Th2 and Th3 isolates at 50% concentration, in which they were 16 and 12 in J2 hatching, respectively.

Culture filtrates (non-autoclaved) of the bioagent isolates increased significantly the mortality of J2 with increasing the filtrate concentration (Figure 3B). The increase was 14 folds compared to the control. Isolate Th1 at 100% concentration significantly differs compared with the other treatments. The average of concentrations indicated significant differences among isolates of *Trichoderma*.

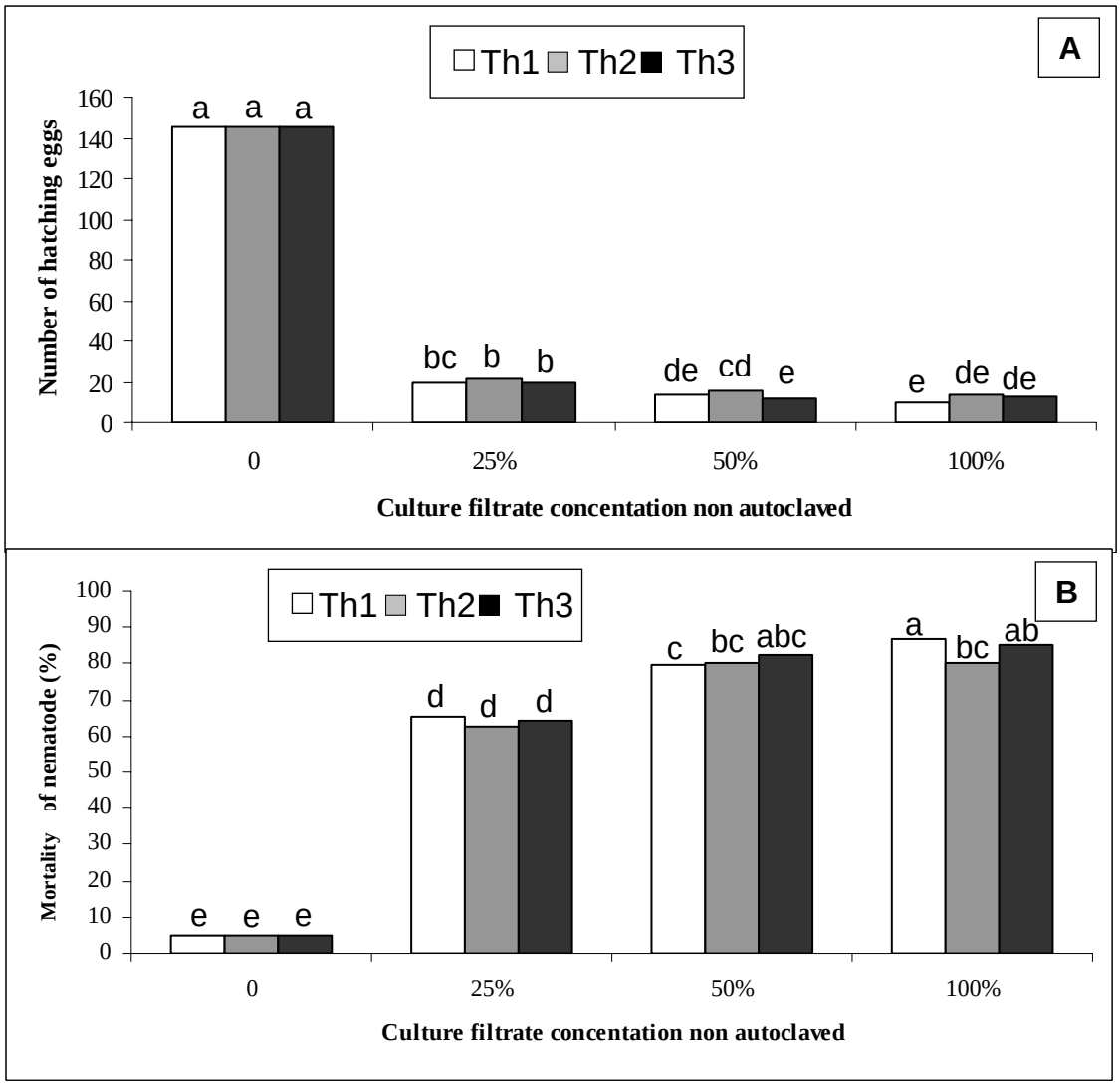

Figure 3. Effect of three Jordanian *Trichoderma harzianum* isolates from culture (non-autoclaved) on the number of hatching eggs (A) and J2 mortality percent (B) of *Meloidogyne javanica*.

The number of hatched J2 decreased significantly within the autoclaved culture filtrate concentrations, compared to the control (Figure 4A), and there were no significant differences among the isolates under the same concentration except for isolate Th 2 (31.8) and Th 3 (26.0) at 50%.

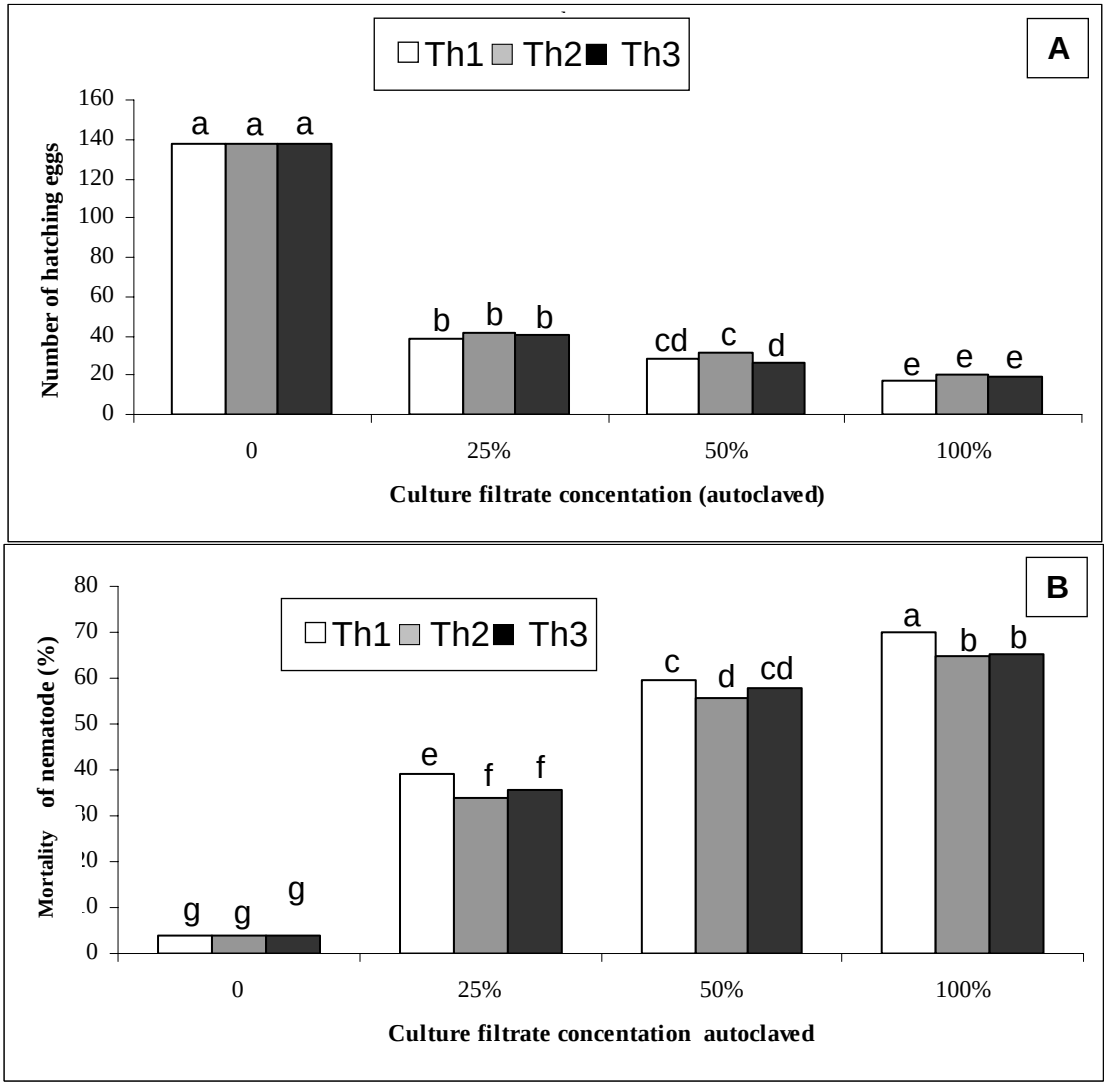

Figure 4. Effect of three Jordanian *Trichoderma harzianum* isolates product compounds from culture (autoclaved) on the number of hatching eggs (A) and J2 mortality percent (B) of *Meloidogyne javanica*.

M. javanica J2 exposed to autoclaved culture filtrates significantly increased the mortality than untreated J2 (Fig. 4B). Isolate Th1 caused a significant increase in J2 mortality than the other isolates (Th2, Th3) at all concentrations with an average mortality of 43.15%, 39.60% and 44.10%, respectively.

Effect of Soil Extracts on Egg Hatching and J2 of *M. javanica*

Exposed egg mass to extracts of *Trichoderma* isolates treated soils decreased significantly the number of egg

hatching with increasing soil extract concentration (Figure 5A), but there was no significant effect within the isolates at the same soil extract concentration. The average hatching decreased from 42.25 to 39.45 between isolates Th2 and Th3 at 25% and 100%, respectively. Mortality of J2 treated by *Trichoderma* isolates was significantly increased compared with the control treatment (Figure 5B). Significant differences among *Trichoderma* isolates appeared in J2 mortality at 50% and 100% concentrations. Th1 was more effective than the other ones.

DISCUSSION

The results of this study showed that the Jordanian *Trichoderma* isolates increased plant growth significantly when used alone (Figure1, A and B). These results agree with those obtained by other researchers using different *Trichoderma* spp. (Al-Ameiri, 2001; Sharon *et al*., 2001; Al-Ameiri, 2007). This effect might be attributed to the influence of *Trichoderma* on nutrient uptake and subsequently enhance plant growth. Plant growth in pots treated with *Trichoderma* isolates and *M. javanica* treatments increased without significant differences when compared with M. *javanica* treatment alone.

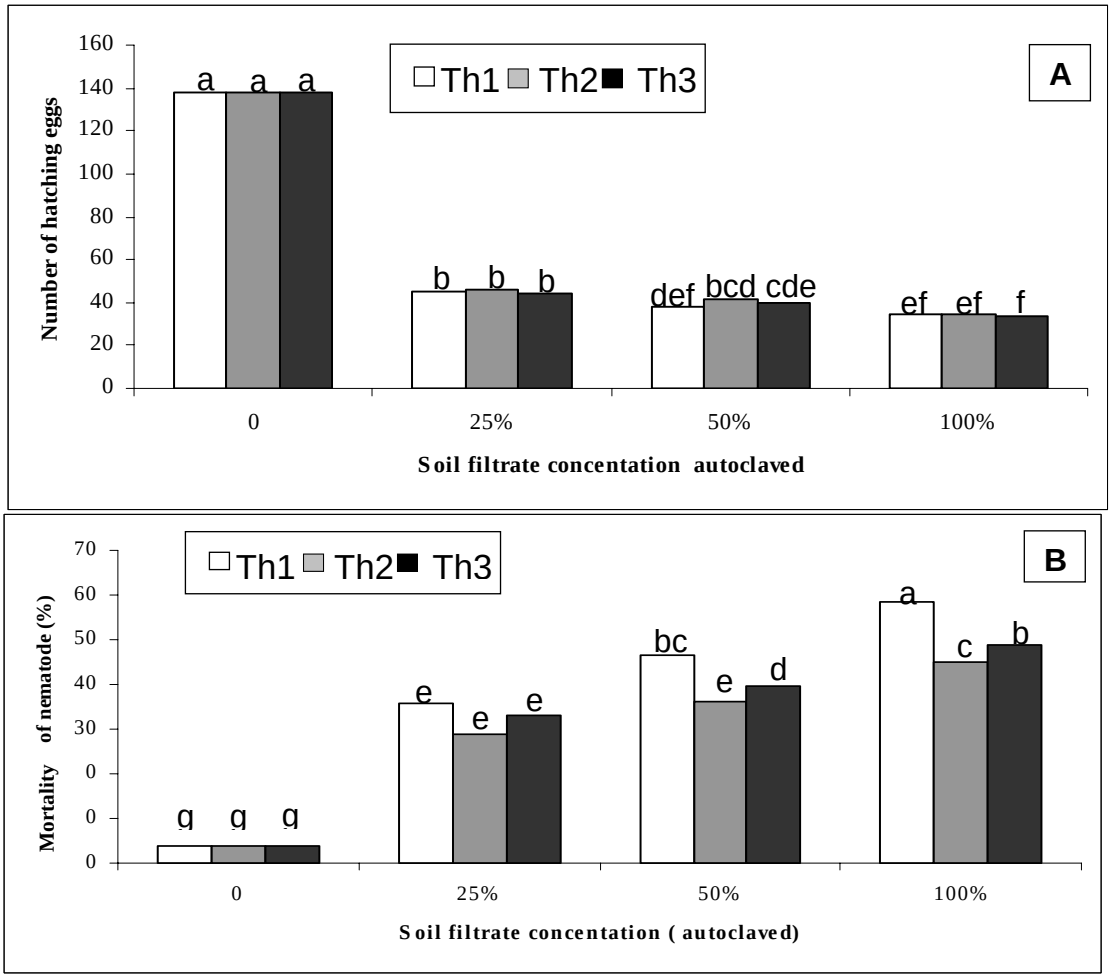

Figure 5. Effect of three Jordanian *Trichoderma harzianum* isolates product compounds soil-treated (autoclaved) on the number of hatching eggs (A) and J2 mortality percent (B) of *Meloidogyne javanica*.

This result agree with the results of Dababat and Sikora (2007), who showed that there were no significant variations between weights and heights of plants inoculated with *M. incognita* alone and with different *Trichoderma* spp. and *M. incognita*. In this study, the biocontrol isolates and nematode were added at the same time, five days before planting. This time was not enough for biocontrol adaptation and the J2 of nematode became younger (less sensitive to *Trichoderma* products) or entered the root system (protected from *Trichoderma* products and parasitism) causing infection and leading to reduce plant growth.

Trichoderma isolates significantly reduced galling index (Figure 2A). The same results were obtained by Sharon *et al*. (2001), who reported that *T. harzianum* reduced galling of root-knot nematode *M. javanica* on tomato plants. Furthermore, Dababat and Sikora (2007) used two species of *Trichoderma* (*T.viride* and *T. harzianum*) and found a significant reduction in tomato root galling infested with *M. incognita*. Our results are in agreement with Pandey *et al.* (2003) who used different treatments of *Trichoderma viride* against *M. incognita* in chickpea, in which all treatments of *T. viride* decreased galling and the final nematode population densities in both field and pot experiments. These results might be due to the reduction in the population migratory of ecto-parasitic nematodes (J2) that caused infections. *Trichoderma* isolates did not completely prevent the infection of RKN nematode galling. This could be due to the effect of the ratio between males that leave the roots and females that stay inside the roots causing root galling, and using short exposure time J2 to biocontrol agent in this study.

The effects of culture filtrates of *Trichoderma* isolates significantly reduced the number of hatching eggs and increased J2 mortality of *M. javanica* (both autoclaved and non-autoclaved) (Figures 3 and 4), and soil extracts treated with different isolates of *T. harzianum* (Figure 5). With all treatment concentrations, the lower concentration was found less effective than the higher ones. These results agree with those obtained by Dababat and Sikora (2007), who found that culture filtrates of different species of *Trichoderma* with a concentration of 50%, were less effective on egg hatching and J2 mobility than 90% concentration. Our study showed that the effect of *Trichoderma* metabolism compounds decreased egg hatching and J2 mortality with lower concentrations. This might be due to the fact that the product compounds chitinase and protease were not sufficient to be more effective.

Culture filtrates of *Trichoderma* isolates considerably affected egg hatching and J2 mobility (non-autoclaved and autoclaved) (Figures 3 and 4). This proved that the genus *Trichoderma* has the ability to produce different products that affect egg hatching and J2 mobility. These products with protolytic activity were affected by heat, and some of them were heat resistant, affecting J2 cuticle. The J2 cuticle is composed mainly of protein (Blaxster and Robertoson, 1998). Second stage juveniles of the migratory endoparasitic nematode *Raodpholus simlis* were more sensitive to fungal metabolites than older juvenile stages or adults (Amin, 1994). Results obtained from this study agree with the results of Al-Ameiri (2001), who found that the culture filtrates of *T.harzianum* (autoclaved and non-autoclaved) significantly reduced growth and dry weight of *Fusarium solani* (26% and 33%) and (15% and 23%), respectively, and *Rhizoctonia solani* (10% and 30%) as well as (24% and 54%), respectively.

Papavizas *et al.* (1982) found that *T. harzianum* produced compounds affected and non-affected by autoclaved filtrates (heat resistant). This means that *Trichoderma* spp. produced compounds with a broad spectrum of activity on fungi and nematodes. Heat

resistant metabolites were found to affect the nematode's egg hatching and mobility in our experiment *in vitro* bioassays with *Trichoderma*-treated soil extracts (Figure 5). This explains the ability of the bioagent to produce toxic products (nematicidal and protolytic activity) in soil with an effect on nematodes. Sharon *et al*. (2001) found that *Trichoderma*- derived soil extracts were active in J2 mortality and egg hatching and suggested that the main anti-nematode activity caused by *Trichoderma* spp. takes place in the soil and not within the roots. The results lead to a conclusion that the compounds of *Trichoderma* isolate filtrates and soil-treated extracts were highly active against egg hatching and J2 mobility.

In conclusion, the Jordanian isolates of *Trichoderma harzianum* were found to improve plant growth and decrease losses in plant growth in the infested soil with *M. javanica*. The biocontrol agent was successful in controlling *M. javanica* by reducing the number of root galling, reproductive factor and the ability of their product compounds in reducing egg hatching and J2 mobility. Future studies are needed on the efficiency of *T. harzianum* isolates to control nematodes under field conditions in Jordan.

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تأثير ثلاث عزلات أردنية من *Trichoderma harzianum* في نيماتودا تعقد الجذور *Meloidogyne javanica* في نبات البندورة

نوفل سليمان العميري*

ملخـص

تمت دراسة تأثير ثلاث عزلات أردنية من المقاوم البيولوجي *Trichodema harizanum* تحت ظروف البيت البلاستيكي ومخبرياً في النيماتودا المسببة لتعقد الجذور *Meloidogyne javanica* في نبات البندورة. أظهرت النتائج قدرة العزلات المستخدمة وحدها على زيادة كل من الوزن والطول للنباتات زيادة معنوية. وحدثت زيادة غير معنوية مع النيماتودا مقارنة مع النيماتودا وحدها، ماعدا العزلة رقم (1)، في ارتفاع النبات، وكذلك حدث خفض معنوي في العقد الجذرية ومعامل التكاثر للنيماتودا مقارنة مع معاملة النيماتودا وحدها تحت ظروف البيت البلاستيكي. وأظهرت النتائج المخبرية أن تعريض كل من أكياس البيض أو الطور اليرقي الثاني إلى مستخلص مزارع هذه العزلات أو مستخلص التربة المعداة بالمقاوم وبمستويات متعددة (صفر(شاهد)، 25%، 50%، 100%) أدى إلى انخفاض معنوي في كل من أعداد البيض الفاقس وأعداد اليرقات الحية سواء كان هذا المستخلص معقما (مزارع المقاوم ومن التربة المعداة بالمقاوم) أو غير معقم (مزارع الفطر)، وازداد هذا التأثير بزيادة التركيز، وتبين أن المستخلص المعقم أقل تأثيرا من المستخلص غير المعقم. وسجلت العزلة(1) فروقا معنوية عن بقية العزلات مع التركيزين 50% و100% لمستخلص التربة المعقم على نسبة وفيات الطور اليرقي الثاني لنيماتودا التعقد.

الكلمات الدالة: البندورة، *Meloidogyne javanica*، *Trichoderma harzianum* ، عزلات أردنية، مكافحة حيوية.

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