Efficacy of some *Trichoderma* species in the control of *Rotylenchulus reniformis* and *Meloidogyne javanica*

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Abstract

The environmental pollutions are one of the major problems in the world and the decrease of Agro-Chemicals pollutions like chemical nutrition and hormones and pesticides are very important for human health and environmental balances. Utilizing of certain biocontrol agents against plant pests are one of our targets in decreasing environmental pollutions. The efficacy of *Trichoderma* species in control of reniform nematode (*Rotylenchulus reniformis*) and root-knot nematode (*Meloidogyne javanica*) were studied in vitro and under greenhouse conditions. The effect of *Trichoderma harazianum*, *T. viride , T. koningii, T. reesei* and *T. hamatum* culture filtrate in controlling reniform nematode and root-knot nematode was studied in vitro in 14 cm Petri-dished during one week exposure and under greenhouse conditions on one month eggplant cv black beauty seedlings in 12 cm plastic pots containing one Kg sandy-loam (1:1 V/V) soil. All culture filtrate of *Trichoderma* species was highly significant in controlling both nematode genera on eggplant. *Trichoderma harazianum*, *T. hamatum* and *T. koningii* culture filtrates gave a significant reduction ($p \leq 0.01$) in vitro and decreased the female and egg-masses of reniform and root-knot nematodes. *Trichoderma* species led to inhibit the nematode activity and movements in vitro during one week exposure. *Trichoderma* culture filtrate was greatly significant on root-knot nematode (*Meloidogyne javanica*) egg than on larvae. It can be summarized the role of *Trichoderma* in control of both nematode genera into direct effect by toxic metabolites and inhibit nematode penetration and developments.

Keywords : biocontrol**,** Nematodes, *Trichoderma*, *T. harazianum, T. koningii, T. reesei, T. viride.*

Introduction

Parasitic nematodes considered the most important pest infected different plant crops under field, greenhouse and plastic tunnel conditions. Nematodes causes direct harm for plants as a parasites or due to its relation with other diseases agents like bacteria, fungi and viruses. Nematodes may be causes a diseases for plants through several ways. The nematodes gives the chances for the opportunistic bacteria and fungi, like *Fusarium* and *Meloidogyne* disease complex (Hagag & Amin, 2001; Haseeb et al., 2005). The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops (Barker et al., 1985; Al-Hazmi, 1992; Sikora & Greco, 1993, Husman, et al., 1996).The infection starts with root penetration of second stage juveniles (J2) hatched in soil from eggs encapsulated in egg masses laid by the females on the infected roots (Barker et al., 1985).

Eggplant is one of the most important vegetables in Saudi Arabia, huge amounts were produced for local consummation in open fields or using glass houses. The damages in the vegetables crops under the effect of nematodes were fluctuated between 10 to 70 % and may be reached to yield losses (Al-Hazmi, 1992).

Besides chemicals, various workers suggested other control measures in view of the need to replace highly toxic and potentially polluting chemicals used to control plant parasitic nematodes and fungi, with less dangerous chemicals or preferably with biological control agents and botanicals (Oostendrop & Sikora, 1989). Biological control of plant pathogens is a distinct possibility for future and it can be successfully exploited in modern agriculture. Biological control of plant diseases aims at reduction in inoculum density or pathogen activity. The discovery of new biocontrol agents and the demonstration of their value in reducing disease incidence and severity have opened new promising avenues for practical applications in agriculture and for promoting environmental safety (Boland, 1990). Some species of the genus *Trichoderma* have been used as biocontrol agents against soilborne phytopathogenic fungal pathogens (Chet, 1987). These fungi may also promote plant growth (Inbar et al., 1994). Several attempts have also been made to use *Trichoderma* spp. to control plant parasitic nematodes. Antibiotic production by bacteria, antagonistic to fungi is well recognized but there have been a limited number of observations of antibiotic production by *T. harazianum*. Several attempts have also been made to use

Trichoderma spp. for controling plant parasitic nematodes. Windham et al. (1989) reported reduced egg production in the root-knot nematode, *M. arenaria* after soil treatments with *T. harazianum* (T-12) and *T. koningii* (T-8) preparations. Dennis & Webster (1971) and Elad & Henis (1982) reported that *Trichoderma* spp. are capable of producing either antibiotics and or intracellular lytic enzymes are responsible for antagonism. *Trichoderma harazianum* could antagonize *Meloidogyne incognita* eggs by producing anti-nematodal compounds that directly affect nematodes or make the roots less attractive and thus limit nematode penetration. Different species of *Aspergillus, Penicillium* and *Trichoderma* are known to produce toxins and antibiotics like malformin, hadacidine, gliotoxin, viridian and penicillin (Subramanian, 1964). Shukla & Swarup (1971) obtained lethal effects of culture filtrates of *Sclerotium rolfsii* on *M. incognita* larvae. They visualized the presence of oxalic acid and other inhibitory substances synthesized by *S. rolfsii* in culture filtrate.

Therefore, the present study was carried out to determine the comparative efficacy of five *Trichoderma* (*T. hamatum* , *T. harazianum, T. koningii, T. reesei* and *T. viride*) species (will known as biological control agents against several plant pathogens without environmental hazardous effects) against *Rotylenchulus reniformis* and *Meloidogyne javanica.*

Material and methods

Nematodes cultures

A single egg mass of the *M. javanica* and *Rotylenchulus reniformis* picked by hand with fine forceps from infected eggplant root was surface sterilized in 1:500 (*V*/*V*) aqueous solution of "chlorax" (sodium hypochlorite) for 5 min. It was then transferred to a small coarse sieve lined with tissue paper, placed beforehand in a Petri plate containing sufficient amount of water. The Petri plates were incubated at room temperature (27±5) °C for 5 d (den Ouden, 1958). Seedlings of eggplant raised in autoclaved soil, were inoculated with the progeny of the single egg mass in order to get regular supply of the inoculum for the experiments.

Trichodema isolates

Trichoderma hamatum , *T. harazianum, T. koningii, T. reesei* and *T. viride* were isolated from some plants rhizosphere. Those *Trichoderma* species were isolated on PDA medium. *Trichoderma* species were cultured on molt extract broth medium for 15 days at 25°C. The mycelia were harvested by filtration in Watmann filter paper (11

cm). Filtrates of each species was added to same volume of the liquid of nematodes culture that contained 1000 nematodes.

Effect of *Trichoderma* species on *Rotylenchulus reniformis* and *Meloidogyne javanica* in vitro

Sixty Petri-dishes plates (14 cm) were used in this experiment, 30 plates for each genus of nematode. Five replicates were used for each treatment. Each plate contained 1000 of infective stage from un-swollen females of *Rotylenchulus reniformis* (or *Meloidogyne javanica*) in 5 ml water and completed with the same volume of the tested *Trichoderma* species filtrate. The control plates contained distilled water instead of the *Trichoderma* filtrate. The pates were incubated at 25 °C for one week. Every 24 h three replicates of one ml from each treatment was checked. The active (with normal movement), non moving and non active nematodes average numbers were encountered.

Effect of *Trichoderma* species on *Rotylenchulus reniformis* and *Meloidogyne javanica* under greenhouse conditions

Two weeks eggplant cv black beauty seedlings in 12 cm plastic pots containing one Kg sandy-loam (1:1 V/V) soil were used in this experiment. Each plastic pot received the containing of one Petri dish that containing *Trichoderma* filtrate and nematodes. The control pot received the containing of Petri dish that curtained nematodes and distilled water. The plots were kept under greenhouse conditions for 45 days at 30 ± 5 °C. Plants were harvested and its roots were wished thoroughly and kept in 5% formalin till the exanimation processes. The roots were checked and the immature stages, swollen females and egg lying females were encountered.

Statistical analysis

Data were analyzed by analysis of variance (Cochran & Cox, 1957) and significant differences among treatments were tested by the least significant difference test (LSD) at probability levels of 5% ($LSD_{0.05}$) and 1% ($LSD_{0.01}$).

Results

Table (1) and Figure (1) showed that , all of the tested *Trichoderma* species gave high significant effect on the reduction of *Rotylenchulus reniformis* movement after two days of treatment in Petri-dishes, while after one week the rate of effect was increased. *Trichoderma harazianum* , *T. hamatum* were the most effective species. The percentages of non active females of *Rotylenchulus reniformis* were 89% and

100%, 93% and 100% after two days and one week of *T. harazianum* and *T. hamatum* exposure, respectively. *Trichoderma harazianum* and *T. hamatum* were respectively followed with *T. koningii, T. viride* and *T. reesei* on the effect of *Rotylenchulus reniformis* activity.

Rotylenchulus reniformis that was treated with different Trichoderma species showed an ability to penetrate eggplant roots, hence it can not complete its life cycles and its reproduction (Table 2 and Figure 2). The most effective *Trichoderma* species were *T. harazianum* and *T. hamatum,* whereas no numbers of nematodes were recorded on the eggplant roots. *Trichoderma koningii, T. viride* and *T. reesei* ranked secondly without big differences (Table 2 and Figure 2).

Table (3) and figure (3) showed the ability of *Meloidogyne javanica* on hatching, penetrating roots, reproducing and growing on eggplant (Black Beauty cutivar) after one week treatment with some *Trichoderma* species. No galls, immature stages, females and egg-masses were recorded in the samples that were treated with *T. harazianum.* Two galls, one larval stage, one female and one egg-masses were recorded in the samples that were treated with *T. hamatum.*

Table (4) and Figure (4) confirmed the effectiveness of *Trichoderma harazianum* and *T. hamatum* against the tested nematodes. They followed by *Trichoderma koningii, T. viride* and *T. reesei* without significant differences. The numbers of galls were 4 and ; egg lying females were 3 and 5 for the samples that were treated with *T. harazianum* and *T. hamatum,* respectively.

Discussion

The tested fungi showed a highly significant effects on the fertility and activity of nematodes and its growth and pathogenic ability for host penetration. The greatest effect was recorded for eggs. The eggs failed in hatching and consequently the numbers of reniform nematodes , root-knot nematodes and larvae were reduced to be fluctuated between 0-62%, 0-1% and 3-18%, respectively. The results indicated that *T. harazianum* followed by *T. hamatum* were the most effective species against eggs, larvae of *Meloidogyne javanica* and immature female of *Rotylenchulus reniformis*. These results came in agreement with Stephan et al. (1996) results , who reported that *T. harazianum* gave the favorite results against growth and reproduction of *M. javanica* and consequently enhanced the growth of tomato and eggplants . Reddy et al. (1996) proved *T. harazianum* incorporated to oil cakes was effective in the yield

increasement and reducing the nematodes numbers in soil and roots. Other researchers (Siddiqui et al., 1999; Haggag & Amin, 2001; Haseeb et al., 2005) studied the effect of *Trichoderma* on the development and growth of parasitic nematodes Devi et al. (2002) studied the effect of *T. viride* and *T. harazianum* on *M. incognita* on tomato plants, they reported that the addition of *Trichoderma* improved the growth of plant seedling and the numbers of nematodes were significantly reduced. Stephan et al. (2002) reported that *T. harazianum* and animal's organic matters reduced the numbers of root-knot nematodes, also Farouk et al. (2002) indicated the effectiveness of *T. harazianum* on the biocontrol of root knot nematodes on tomato. Saifullah (1996a,b) showed the death of 100% of *Globodera rostochien*, *G. pallida* by using poisoning compound from *T. harazianum* on the medium after 24 h of exposure. It is will known that *T. harazianum* producing a several poisoning and antibiotic compounds (Di Pietro, 1995) that can protect plant from pathogenic organisms in soil (Wu & Wu, 1998). Several studies (Spiegel & Chet, 1998; Susan et al., 2000; Haggag & Amin, 2001; Sharon et al., 2001, Howell, 2003; Siddiqui & Shaukat, 2004, Santhosh et al., 2005) showed the using of *Trichoderma* for inhibiting the growth of plant parasitic nematodes. The secondary metabolites of *Trichoderma* includes chitinase enzyme considered that most effective component against pathogenic fungi. Chitinase enzymes degrades the fungal cell walls which composed of chitin (Lorito et al., 1993). Chitin composed the outer shell of nematode's eggs so the nematode's eggs affected greatly under *Trichoderma* species treatment (Haggag & Amin 2001; Jin et al., 2005).

Acknowledgments

The author expresses her gratitude to Dr. Amin W. Amin (Professor of nematology, Faculty of Agriculture , Cairo University, Egypt) not only for submitting the populations of nematodes, but also for his helpful discussion and critical reading of the manuscript.

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Table 1. Effect of the filtrate of five species from *Trichoderma* on the activities of *Rotylenchulus reniformis* in Petri-dishes

Treatments	Exposure for 48 h			Exposure for 168 h		
	Living active females	Non active females	% of non active females	Living active females	Non active females	% of non active females
Control	100.0	0.0	0.0	100.0	0.0	0.0
T.harzianum	11.0	89.0	89.0	0.0	100.0	100.
T. viride	31.0	69.0	69.0	14.0	86.0	86.0
T. koningii	23.0	77.0	77.0	6.0	94.0	94.0
T. reesei	43.0	57.0	57.0	23.0	77.0	77.0
T. hamatum	7.0	93.0	93.0	0.0	100.0	100.0
LSD less than 0.05	3.2	3.2	3.2	1.9	1.9	1.9
LSD less than 0.01	4.3	4.3	4.3	2.6	2.6	2.6

Table 2. Effect of the filtrate of five species from *Trichoderma* on the growth and reproduction of females of reniform nematodes on eggplants.

Treatments	Numbers of females on the roots	% of affected females	Number of egg-masses	% of affected egg-masses
Control	100.0	0.0	96.0	0.0
T. harzianum	0.0	100.0	0.0	100.0
T. viride	20.0	80.9	15.0	84.4
T. koningii	8.0	92.4	2.0	97.9
T. reesei	36.0	64.0	36.0	62.5
T. hamatum	0.0	100.0	0.0	100.0
LSD less than 0.05	20.6	-	6.9	-
LSD less than 0.01	29.6	-	9.5	-

Table 3. Effect of the filtrate of five species from *Trichoderma* on the activities of *Rotylenchulus reniformis* in Petri-dishes

Treatments	Number of galls	Developing stages	Number of females	Number of egg-masses
Control	109.0	40.0	98.0	71.0
T. harzianum	0.0	0.0	0.0	0.0
T. viride	9.0	4.0	6.0	5.0
T. koningii	3.0	0.0	1.0	1.0
T. reesei	11.0	2.0	9.0	8.0
T. hamatum	2.0	1.0	1.0	1.0
LSD less than 0.05	8.6	1.6	4.7	4.8
LSD less than 0.01	11.8	2.1	6.5	6.5

Table 4. Effect of the filtrate of five species from *Trichoderma* on the larvae of the activities of *Rotylenchulus reniformis* in Petri-dishes

Treatments	Number of galls	Developing stages	Number of females	Number of egg-masses
Control	100.0	45.0	94.0	74.0
T.harzianum	4.0	8.0	3.0	3.0
T. viride	12.0	10.0	10.0	10.0
T. koningii	10.0	8.0	8.0	7.0
T. reesei	33.0	22.0	18.0	18.0
T. hamatum	6.0	5.0	5.0	5.0
LSD less than 0.05	2.2	3.4	2.7	2.7
LSD less than 0.01	3.3	5.0	4.0	4.0

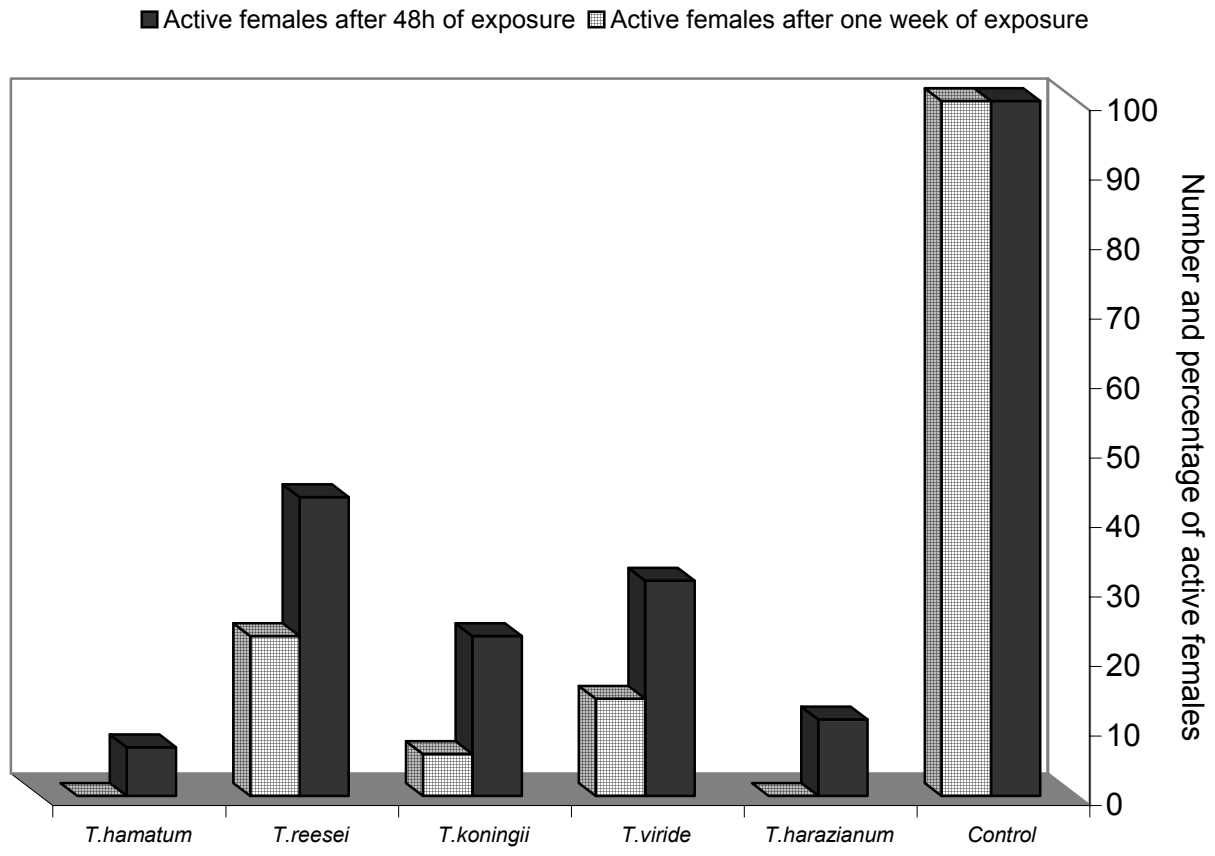

Figure1. Effect of the filtrate of five *Trichoderma* species on the activity of reniform nematodes.

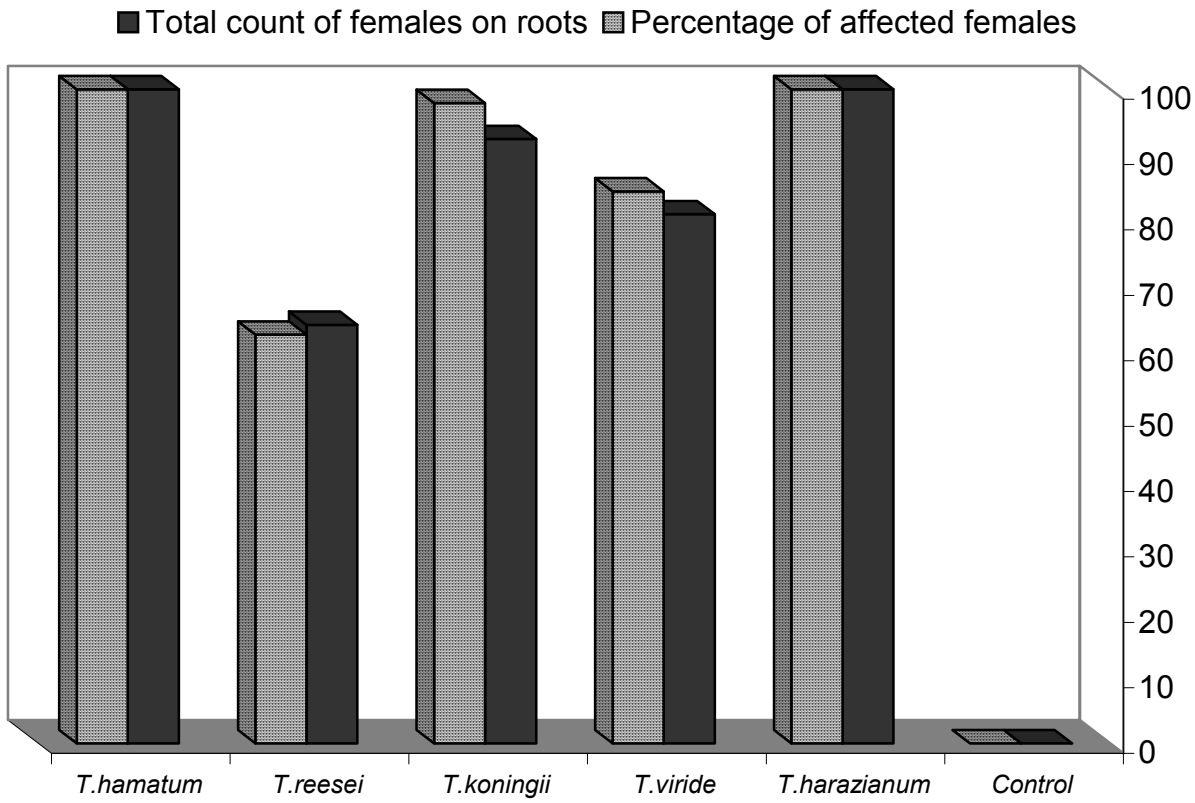

Figure 2. Effect of the filtrate of five *Trichoderma* species on the reproduction and egg-lying by nematodes on eggplant roots.

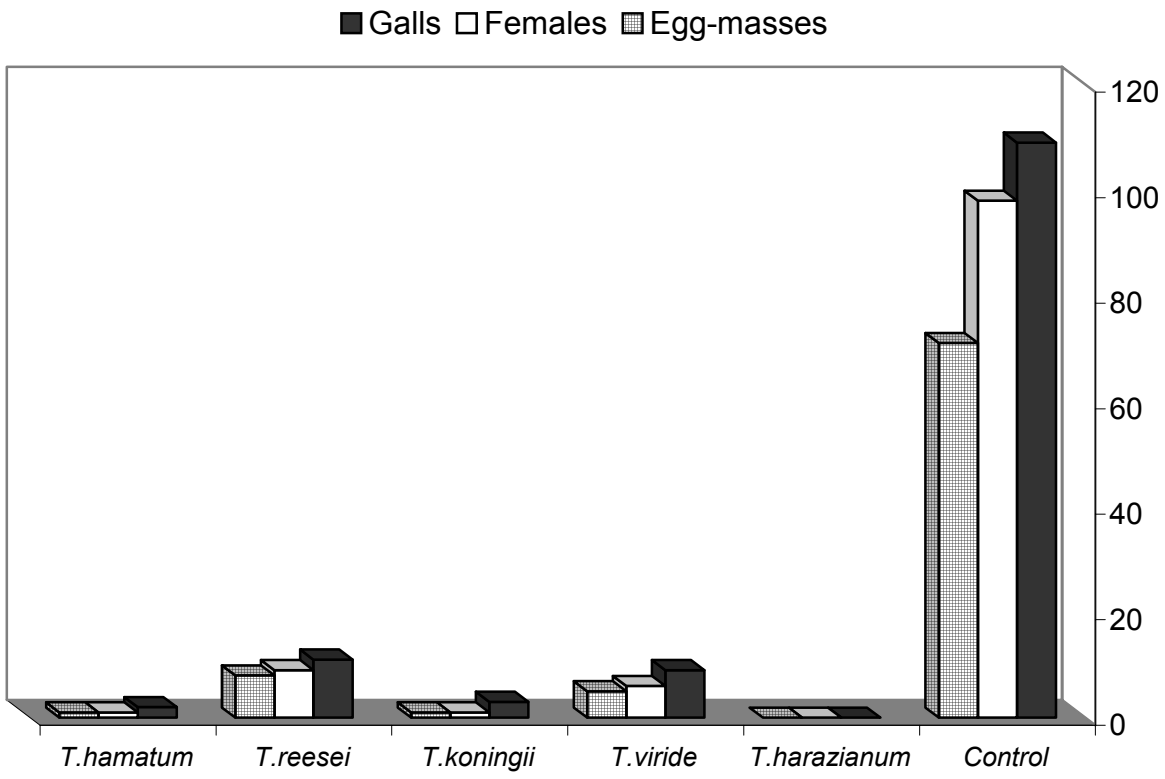

Figure 3. Exposure of root-knot nematodes for the filtrate of five *Trichoderma* species for five days, and its effect on hatching , penetrating and developing of nematodes on eggplant roots.

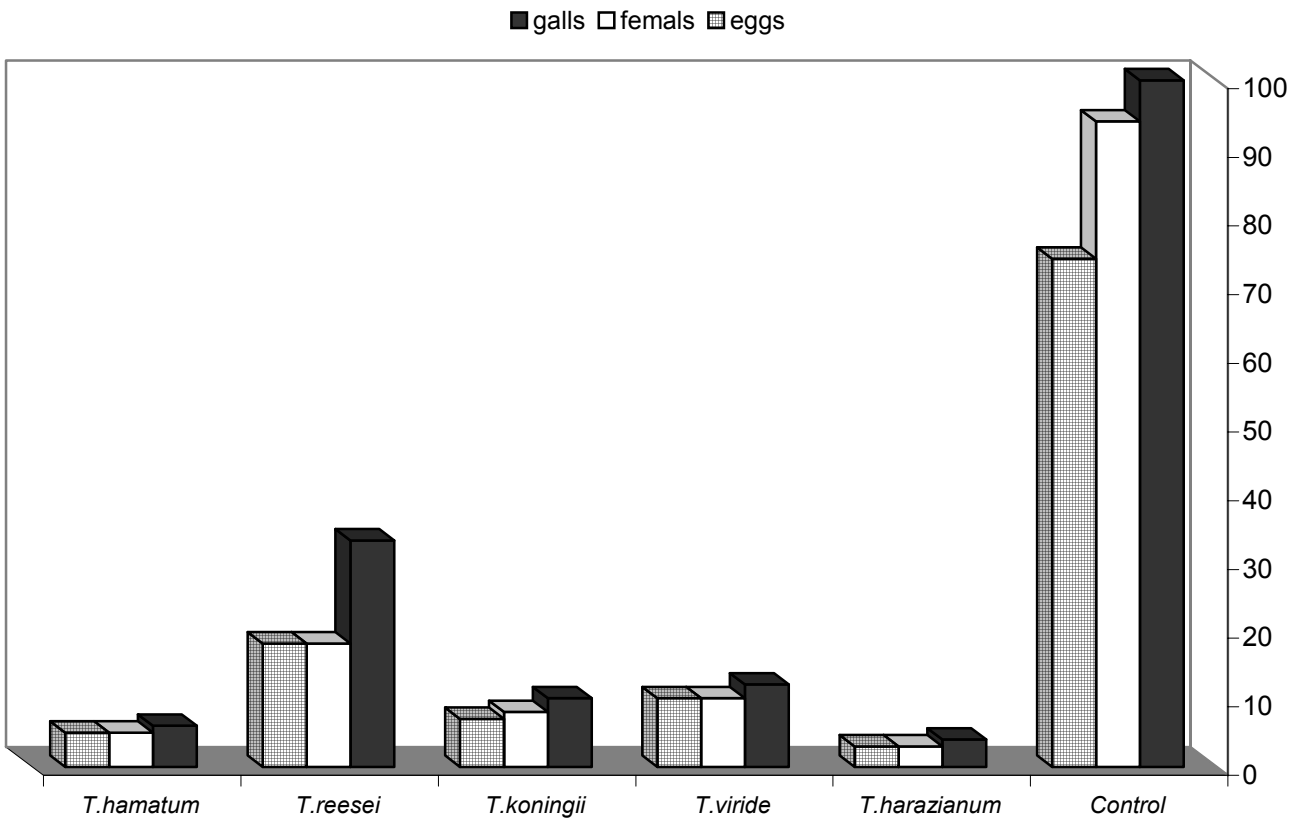

Figure 4. Exposure of root-knot nematodes larvae for the filtrate of five *Trichoderma* species for five days, and its effect on penetration, development and reproduction of nematodes on eggplant roots.