academicJournals

Vol. 8(2),pp.11-18, March 2016
DOI: 10.5897/JEN2015.0145
Article Number: CDC008958260
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Evaluation of some botanicals and *Trichoderma harzianum* against root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chit wood) in tomato

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Received 28 September, 2015, Accepted 19 February, 2016

Root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chit wood) is one of the major constraints for the successful cultivation of tomato (*Lycopersicon esculentum* Mill.) in Ethiopia. Among different management strategies, biological control is important in the light of increased awareness of environmental and human health hazards. Therefore, the present study was conducted to evaluate the effect of different locally available botanicals and an antagonistic fungus, *Trichoderma harzianum* to control root-knot nematode attacking tomato under *in vitro* condition. Leaf and seed extracts of four botanicals viz., Rape seed (*Brassica napus* L.), Lantana (*Lantana camara* L.), African marigold (*Tagetes erecta* L.) and Neem (*Azadirachta indica* L.) at two different concentrations (5 ml and 10 ml) and *Trichoderma harzianum* (5 ml) were tested. Plant extracts were more effective and significantly inhibited egg hatching and immobilizing the J_2 larval mortality of *M. incognita* than *T. harzianum.* Aqueous extracts of all the botanicals inhibited egg hatching of nematode and resulted in 84.67 - 100 % mortality of the second juveniles of *M. incognita* at the 10% concentration after 72 h of exposure time. Leaf extracts of *T. erecta and A. indica* exhibited 100% inhibition of egg hatch and larva mortality, while at similar concentration of *B. napus and L. camara* leaf extracts exhibited 92 and 93.2% inhibition of egg hatch and 62.1 and 73% larval mortality, respectively. Egg inhibition and larval mortality decreased with increase in the dilution (10 ml) of extracts. Juvenile mortality increased corresponding to an increased time of exposure. Aqueous seed extracts of *A. indica* more significantly inhibited egg hatching and larva mortality of the J_2 of *M. incognita* at 10% concentration and immobilized by 89, 93 and 100% after 24, 48 and 72 h of exposures, respectively, This study revealed that the test plants are readily available to farmers at no cost and able to reduce nematode population below economic threshold. There is a need for further studies in identifying new classes of bio-pesticides from natural plants to replace the synthetic chemicals used at present.

Key words: Botanical leaf extracts, egg hatching, larval mortality, root-knot nematode, *Trichoderma harzianum.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world and the third most cultivated vegetable next to potato and sweet potato (FAO, 2006).It is rich in minerals (potassium, magnesium, calcium, iron and zinc), proteins (essential amino acids), citric acid, sugars, dietary fibers (pectin) and high levels

of vitamin *C, lycopene,* and beta-carotene which are anti-oxidants against oxygen radicals that probably cause cancer, aging and arteriosclerosis (Naika et al., 2005). In Ethiopia, tomato is among the most important vegetable crops providing higher incomes to small scale farmers compared to other vegetable crops (Lemma et al., 1992). Most intensive production is done in the Rift Valley, mainly along Awash River Valley and around the lakes (Lemma, 1994). It is produced both during the rainy and dry seasons under supplemental irrigation (Lemma, 1994).

Tomato crops are more susceptible to several biotic stresses compared to other vegetables and cereals. Among the different biotic stresses, the root-knot nematode is one of the most destructive and widespread attacking tomato in Ethiopia. HARC (2005) reported of a high incidence of root-knot nematodes attack on tomato in major tomato producing areas of Ethiopia, particularly in Ambo and Toke Kutaye districts of West Showa. The most common diseases in tomato production fields are the root knot nematode, *M. incognita* which are the dominant disease in Rift Valley of Ethiopia (MoARD, 2009). Many workers have attempted to assess crop losses caused by plant parasitic nematode species in Ethiopia (Tadale and Mengistu, 2000; Wondirad and Tesfamariam, 2002). The yield of tomato suffered 2.3% loss due to *M. incognita* infestation at the rate of 3-4 larvae/g soil under field conditions in Ethiopia (Sikora and Fernández, 2005; Wesemael et al., 2011). Several methods known to manage the root-knot nematode include the use of nematicide, organic amendments, resistant cultivars, soil solarization and biological control, which have been used with different levels of success on tomatoes (Randhawa et al., 2001; Sakhuja and Jain, 2001). Although the application of chemical nematicide has been found as an effective measure for the control of nematodes, it has a high toxic residual effect on the environment and particularly on non-target organisms (Anastasiadis et al., 2008). In view of this, current research is focused on the development of alternative strategies that are environmently friendly and sustainable (Pinkerton et al., 2000; Mashela et al., 2008). Bio-control strategies appear to offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture. They also help beneficial microorganisms in the soil. The bio control efficiency depends on the nematode species, plant host and their root exudates, and other crops in rotation (Hallman et al., 2009). The beneficial effects of certain types of plants derived materials and microorganisms in soil have been attributed to a decrease in the population densities of plant-parasitic nematodes (Akhtar, 2000).

Several fungi have been identified and classified according to their nematophagous properties. They include trappers, endo-parasites, egg-parasites and toxin producers (Liu et al*.* 2009). Fungi that have toxic effects on nematodes include *Aspergillus* spp. and *Trichoderma* spp. *Trichoderma viride* which were reduced egg-hatching (Goswami and Mittal, 2004) and trade formulations have also proven to be efficacious in tropical greenhouse conditions (Cuadra et al*.,* 2008). Some species of *Trichoderma* have been used widely as bio-control agents against soil-borne plant diseases (Whipps, 2001) and also they have activity towards root-knot nematode (Meyer et al*.,* 2001; Sharon et al*.,* 2001). A number of *Trichoderma* isolates are now used commercially for the control of nematodes in the soil. It was found that the gelatinous matrix enables fungal attachment and enhances parasitic abilities of most isolates, which could also utilize it as a nutrient source (Sharon et al*.,* 2009). The conidia of *Trichoderma* attach to nematode cuticle or to egg shell and parasitize on them (Sharon et al., 2007). Al Kader (2008) reported a high nematicidal effect of the fungus *Paecilomyces lilacinus* culture filtrate on J_2 of *M. incognita*, with 99% of J2 immobilized after 2 days of treatment. *Trichoderma* spp. has been reported to produce chitinase into the culture (Chet and Baker, 1981), which might help in the inhibition of egg hatching. Botanicals, plant-based pesticide chemicals have found favor as alternatives to pesticides in recent times. When French marigold was planted immediately after the termination of a *Meloidogyne* susceptible host, bitter melon (*Momordica charantia* L.), and marigold suppressed approximately 50% of *M. incognita* compared to the bare ground treatment (Marahatta et al*.,* 2010). Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non-phytotoxic, unlike chemical fungicides (Alam et al., 2002). The fresh leaf extracts of *Azadirachta indica*, *Allium sativum* (Garlic) and *Tagetes erecta* (African marigold) were examined against *M. incognita* on tomato *in vitro* and *in vivo* conditions (Abo-Elyouser et al*.*, 2010). All treatments immobilized juveniles (J_2), the highest effect caused by neem leaves extract after 24 and 48 h of exposure. In soil, all treatments significantly reduced the root galling, nematode population, and enhanced the plant growth and yield (Abo-Elyousr et al*.*, 2010).In spite of the wide distribution of root-knot nematode on many crops in Ethiopia, little work has been done on the management of tomato root-knot nematode. So far, little efforts have been made to exploit locally available botanicals and antagonistic fungal organisms for the control of root-knot nematode on crops in Ethiopia. Even

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Table 1. List of botanicals used against root-knot nematode.

Common name of the botanicals	Botanical name	Parts used
Rape seed	*Brassica napus L.*	Leaf
Lantana	*Lantana camara L.*	Leaf
African marigold	*Tagetes erecta L.*	Leaf
Neem	*Azadirachta indica L.*	Leaf and seed

if few works were done by botanicals in Ethiopia, their combination with biological and their synergistic effect with antagonistic fungi are not studied.

In management of plant parasitic nematodes using plant products and their derivatives is gaining importance in the light of increased awareness of environmental and human health hazards associated with nematicidal chemicals, biodegradability, selective toxicity to target pests, safety to non target organisms. The plant protection scientists all over the world are aiming at non chemical means to tackle the pest and disease problems. Therefore, the present study was conducted to evaluate some locally available plant species and an antagonistic fungus, *Trichoderma harzianum* for the management of tomato root-knot nematode under *in vitro* conditions.

MATERIALS AND METHODS

Description of the study area

In vitro experiments were conducted at Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia in 2013-2014. The center is located at Ambo District, with an altitudes of 2100 m, latitude 8° 57' 58"N and longitude 37°5'33"E.

Collection of botanicals and preparation of extracts

Rapeseed, *Lantana* and marigold were collected from Ambo University campus, Ambo. Neem seeds and leaves were collected from Melkasa Research Center and *Trichoderma harzianum* (Jimma isolate) was obtained from Department of Mycology, APPRC, Ambo, Ethiopia.

The seeds of tomato cv. *Marglobe* were obtained from Melkassa Research Centre, Melkassa, Ethiopia. The test plants leaves and seeds were shade dried (Table 1) and were separately made powdered form using an electric grinder and 20 g powder of each plant powder was soaked separately in 100 ml of distilled water for 24 h in 500 ml Erlenmeyer flask. After 24 h of soaking, they were filtered through Whatmann No.1 filter papers and then the filtrate was centrifuged at 2000 rpm for 10 min for *in vitro* experiments. Each extract was considered as a standard solution "S" (100% concentration) and then kept in the refrigerator until use for further studies. Suspensions of the concentrations of 0, 5, and 10% were prepared with distilled water (Taye et al*.,* 2012; Tiwari and Mukhppadhyay, 2001). 5 ml and 10 ml of plant extracts were incorporated in to each pot with different treatments.

Extraction of root-knot nematode juveniles

Diseased root samples of tomato were collected from culture pots of APPRC green house during the month of October, 2013. Roots of tomato infested with root-knot nematode were thoroughly washed, cut into small pieces and stained with Acid Fuchsin in lacto phenol (Barker et al*.,* 1985). After cooling to normal temperature, they were keeping in lacto phenol overnight for partial de-staining (Seinhorst, 1998). Root pieces were dissected under stereo-microscope and adult females were taken out and placed in lacto phenol. The perineal region of females were cut with a sharp razor blade and adhering tissue clear off with a fine pick and the perineal sections were examined under microscope. The ten female patterns of root knot nematode were examined and estimated (Orisajo et al*.,* 2007).

Maintenance and multiplication of root knot nematode juveniles

Egg masses of *M. incognita* were picked up from pure culture pots of infected roots using forceps and needle and placed sterilized water and kept on laboratory benches at room temperature (20-23°C) for 3-6 days. Two weeks old transplanted seedlings of tomato cv. *Marglobe*, raised in sterilized soil were inoculated with the *M incognita* juveniles. Inoculation was done by removing top soil (1-2 cm) around the seedlings to expose the roots. The exposed roots were inoculated with 20 J_2 root-knot nematode juveniles. The removed soils were again placed on sides of the seedlings and watering was done.

Extraction and counting number of *M. incognita* juveniles

To obtain nematode inoculum for *in vitro* experiment, pure cultures of *M. incognita* were raised from single egg mass and maintain on tomato roots in wire house. Infected plants were uprooted from the soil and the root were dipped in water and washed gently to remove adhering soil particles. Egg masses of nematodes were picked up with the help of sterilized forceps, kept the eggs masses in small sieves and the sieves were placed in sterilized plastic plates and pour the water up to neck of the sieves and kept in the laboratory at room temperature. After 2 to 7 days, eggs were hatched and active juveniles cross the sieve and settle down in plastic plates. The juveniles were collected with the help of pipette for *in vitro* study. Second stage of juveniles was counted by eelworm nematode counting dish, for experimental study. Population densities of J_2 were determined from 3 replications of one ml aliquot of an inoculum suspension for *in vitro* culture experiments. 100 J_2 and 10 egg masses of *M. incognita* were used for each treatment in *in vitro* experimental study.

Raising and maintenance of tomato plants and inoculation with nematode

The seeds of tomato cv. *Marglobe* were axenized by NaOCl method (Koenning and Barker, 1985). About 100 seeds were placed in sterilized beaker containing a mixture of 95% ethanol and 5.25% NaOCl in the ratio of 1:1. The mixture was stirred gently and the

seeds were allowed to soak for about 10 min. The mixture was drained off and the seeds were rinsed thrice with distilled water. Seeds of cultivar *Marglobe* were sown on sterilized soil in plastic pots under greenhouse. Three leaf stage/ one month-old seedlings were transplanted to plastic pots (15 cm dia.) containing 3 kg of sterilized soil with 1:2:3 proportions of sand, compost and clay, respectively. Each pot was planted only one tomato seedling. Fresh roots of tomato were taken from pure culture developed in the wire house and brought to Plant Pathology laboratory. Egg masses were picked up by using sterile forceps and dissecting needle and placed to Petri dish having sterile water then kept on laboratory benches at room temperature (20-23°C) till hatching was completed. Appropriate suspension of nematode was prepared in a beaker and 3 ml was taken from the total suspension and placed on counting dish, then the number of juveniles in the suspension was determined under stereomicroscope at the magnification of 50X. The population of nematode per ml was calculated from 3 replications of one ml aliquot of an inoculum suspension for *in vitro* and *in vivo* experiments. Finally, seedlings of tomato were inoculated with the 2 ml suspension of *M. incognita* at 2000 juveniles/pot after one week of transplanting. For inoculation, 1-2cm of top soil was separated out and nematode suspension was poured around the plant. Each treatment has been replicated three times and the pots were arranged in randomized complete design. Un-inoculated set of plants served as control. The soil was replaced and watering was done.

Production of *Trichoderma harzianum*

Multiplication of *T. harzianum* was performed by the method described by Tiwari and Mukhopadhyay (2001). By inoculating sterilized sorghum seeds, sand and water with spore suspensions. Spore suspensions were obtained by adding 20 ml sterilized distilled water to three- week old cultures and scraping gently with spatula. The spore suspension of *T. harzianum* was inoculated into sterilized one litter jar containing sorghum seeds and transferred or inoculated to water medium and preserved at 20°C for 3 days. Pure cultures of *T. harzianum* were cultured on Potato Dextrose Agar (PDA) media and the PDA medium was poured in to sterilized Petri dishes (9 cm wide) with 20 ml each. 5 mm blocks of the 10-day old pure cultures of *T. harzianum* were placed upside down at the center of each plate. The block was cut with the help of a flame sterilized cork borer (5 mm diameter).

The inoculated Petri dishes were kept in the growth chamber or incubated at 22ºC temperature. After 10 days, an aliquot of 10 ml of distilled sterile water (DSW) was added to each plate and the mycelium was scraped with a spatula until the culture surface was free from mycelia and the suspension was collected in a 100 ml conical flask. Spores/conidial suspension were separated from mycelia by sieving through cheese cloth and the spore/conidial suspensions were then adjusted to the desired concentration (10^6spores/ml) after counting spore density using a haemocytometer (Niranjana et al*.,* 2009).

***In vitro* experimental study**

Test tube bio-assay was carried out to determine the effect of different concentrations of botanical extracts and *T. harzianum on* the hatching of *M. incognita* egg masses (Nitao et al*.,* 1999). Egg masses of *M. incognita* were picked up from the root using dissecting needle and forceps. Ten uniformly sized 500 egg masses of *M. incognita* were transferred separately into 5 ml and 10 ml of each concentration of plant extracts and 5 ml of *T. harzianum* alone and combined in sterilized test tubes. Egg-masses in equal volumes of distilled water served as control (Alam, 1985).

The experiment was laid out in completely randomized design (CRD) with three replications. All the test tubes containing the suspensions and the egg masses were kept at room temperature on laboratory bench for seven days to allow eggs hatching.

Juveniles (J2) bioassay

Two ml of water-juvenile suspensions which contain 100 J_2s were placed in test tube containing 5 ml and10 ml of each botanical and 5 ml of *T. harzianum* alone and combined with what. Each treatment was replicated three times. The number of dead J_2s was recorded every 24 h for three days. After 24, 48 and 72 h, active and inactive J_2s were counted in each test tube and sterilized distilled water (SDW) served as control (Zasada et al*.,* 2002). Juveniles were considered dead if they did not move when teased with fine needle and body become straight (Siddique and Shahkad, 2004). Percent J_2 mortality in a tube was calculated as:

%J2 mortality = [No. of inactive (dead) J2s] / Total J2s in a tube x 100

Data analysis

The data were subjected to an Analysis of Variance (ANOVA) procedures using Statistical Analysis system (version.9.1.3, SAS Institute Inc., Cary, NC, USA). All data were subjected to analysis of variance and Duncan's New Multiple Range Test used to separate means at 5% level of probability.

RESULTS AND DISCUSSION

Results of the study showed that plant extracts and *Trichoderma harzianum* applied individually and in combination were able to immobilized *M. incognita* J_2 after 24, 48 and 72 h of exposures (Table 2). There was a significant difference in the mortality rate of second stage juveniles of *M. incognita* treated with different concentrations of aqueous plant extracts and *Trichoderma harzianum* at 24 h. Neem seed extract at 10% concentration caused significant mortality of *M. incogita* J_2 24 h after treatment application when compared to all the other treatments. It was able to immobilized J_2 of *M incognita* by 89, 93 and 100% after 24, 48 and 72 h of exposure, respectively. Results of the study agrees with the findings of Agbenin, (2004) who reported of a 100% in mortality of root-knot nematode larvae after 24 h exposure to dry leaf neem extract. Parmar, (1987) also reported that aqueous extracts of leaf, flower, fruit, bark, root and gum of neem were highly toxic to nematodes egg or juveniles with fruit extract showing the most lethal activity followed by leaf extract. In the present study, at 5% concentration, the highest juvenile mortality of what % within 24 h was shown in neem seed and followed by *L. camara*, African marigold and neem leaf, respectively. After 48 h of application both at 5 and 10% concentrations, the highest mortality was shown in neem seed and the lowest mortality was shown in rape seed + *T. harzianum.* After 72 h treatment

Table 2. Percentage mortality of the J2 of *M. incogita* under *in vitro* test using botanicals and *T. harzianum.*

Percent mortality of J2 of *M. incogita*				
Treatments	**Con. %**	**24h**	**48h**	**72 h**
Rape seed leaf extract alone	5	72.33e	76.33e	78.67d
	10	77.67d	81.33d	84.67c
Lantana leaf extract alone	5	82.67bc	86.33bc	87.33c
	10	82.60bc	85.33bcd	96.00b
African marigold leaf extract alone	5	80.33cd	83.33cd	86.33c
	10	84.00b	88.00b	95.00b
Neem leaf extract alone	5	79.00d	82.00d	85.33c
	10	83.33bc	86.67bc	94.67b
Neem seed extract alone	5	84.33b	88.67b	94.00b
	10	89.00a	93.00a	100.00a
T. harzianum suspension alone	5	64.33f	70.00f	80.67d
Rape seed + *T.harzianum*	5	50.33i	53.00e	57.33g
Lantana + *T. harzianum*	5	58.67g	61.67g	65.67f
African marigold + *T. harzianum*	5	55.667gh	58.67gh	64.00f
Neem Leaf + *T. harzianum*	5	54.00h	56.00hi	59.00g
Neem Seed + *T. harzianum*	5	62.30f	67.00f	73.67e
Distilled Water (Control)	5	0.00j	0.00j	0.00h
LSD		3	3.69	3.54
CV (%)		2.68	3	2.78

Note: Means in each column followed by the same letter were not significantly different at ($P< 0.0001$), according to Duncan's Multiple Range Test (DMRT).

application the highest and the lowest percent mortality was found in neem seed and rape seed + *T. harzianum,* respectively. On the other hand, all botanicals which combined with *T. harzianum* and applied at both 5% and 10% concentrations showed less mortality of juveniles than individually applied within 24, 48 and 72 h. There were no significant differences among treatments of rape seed leaf at 10% concentration, *L. camara*, African marigold and neem leaf at 5% concentration within 72 h. Effects of all treatments and *T. harzianum* on J_2 mobility continued as exposure time increased, although the differences were not significant as such after 24 h (Table 2). Generally, the mortality rates of juveniles increased with an increase in exposure time. A similar result was reported by Elbadri et al*.* (2008).

There were significant differences between treatments in number of infective juveniles/egg mass of *M. incognita* (Table 3). Different botanicals applied at different concentrations and *T. harzianum* individually and in combination inhibited egg/juvenile hatching. Among botanicals applied, neem seed at concentration of 10 % inhibited egg mass hatching to juveniles, because this concentration had least number of infective juveniles per 10 egg masses, in comparison to 100 juveniles in control. On the other hand, there were no significant differences between *T. harzianum* which applied at 5%. In general botanicals applied at concentration of 10% was more effective than botanicals applied at 5% concentration on egg mass hatching than *T. harzianum* applied at 5% concentration. Neem seed, neem leaf and *L. camara* at both concentrations, African marigold at 10% concentrations reduce the hatching maximum (>90%) over the control. Both at 10 and 5% concentrations, the greatest percentage of hatching inhibition (96%) and (92%) was achieved by neem seed, neem leaf followed by *L. camara* and African marigold (90%). Among the botanicals, the least egg mass inhibition was obtained by rape seed leaf at both concentrations individually (88%) and combination with *T. harzianum*. Susan and Noweer, (2005) reported that the plant extracts of basil, marigold, pyrethrum, neem and china berry proved to be effective against *M. incognita*. Also, the inhibitory effect of the extracts might be due to the chemicals present in the extracts that possess ovicidal and larvicidal properties (Adegbite, 2003). These chemicals either affected the embryonic development or killed the eggs or even dissolved the egg masses. Similar results were reported that the extracts contained alkaloids, flavonoids, saponins, amides including benzamide and ketones that singly and in combination inhibit egg mass hatching

Table 3. Effect of aqueous extracts of botanicals and *T. harzianum* on eggs of *M incognita*

Treatments	Concentration	No. eggs hatched to J_2 after 7days	Z**
Rape Seed Leaf extract alone	5	36.00cdef	88
	10	33.60cdef	89
Lantana leaf extract alone	5	30.67cdef	90
	10	32.60cdef	91
African marigold leaf extract alone	5	33.00cdef	89
	10	27.00ef	91
Neem Leaf extract alone	5	33.33cdef	89
	10	29.00def	90
Neem Seed extract alone	5	25.00f	92
	10	10.67g	96
T.harzianum suspension alone	5	38.67cde	87
Rape seed + *T.harzianum*	5,5	56.33b	81
Lantana + *T. harzianum*	5,5	40.30cd	87
African marigold + *T. harzianum*	5,5	43.00c	86
Neem Leaf + *T. harzianum*	5,5	54.67b	82
Neem Seed + *T. harzianum*	5,5	39.33cde	87
Distilled Water (Control)	5	301.33a	
LSD		11	
(CV)%		13	

Means in column with the same letters are not significantly different ($P < 0.0001$) by DMRT Z** Hatching inhibition over the control in percent.

(Mousa et al*.,* 2011). Also, Salawu, (1992) reported that the neem seed extracts to inhibit egg hatch and juvenile activity. In the present study, the neem seed was acted as the highest in juvenile mortality and egg mass hatch inhibition by *in vitro*. Meira et al., (2006) reported that the soluble plant extracts were very effective in inhibiting egg-hatch and larval motility of nematodes. The active principles of neem viz. nimbidin and thionimone were reported to be highly active against nematodes. Fatema and Ahmad, (2005) have been reported that the extracts of neem leaf and garlic bulb completely inhibited hatching of egg masses of *M. incognita* and were lethal to larvae. In this study, the neem leaf extracts can inhibit 90% of egg hatching. The inability of the egg mass to hatch is as a result of ingress/entrance of plant extracts into the egg mass. Larvae in the egg mass were exposed to the toxic effect of the extract resulting first in reduced mobility and finally death or moribund state. Once this state is reached the larva cannot pierce through the wall with its stylet hence hatching ceases. The egg mass which is a part of the perineal region of the female in root-knot is permeable to the active ingredient in the extracts (Hirschmann, 1985). These compounds act by various mechanisms like blocking molting of larvae, disrupting mating and sexual communication of nematodes, reducing the motility of gut and by inhibiting the formation of chitin (Ramasamy, 2008). In this study, the botanicals used only they were effective but when they were used in combination they show less effective so it is evident that as extract was diluted, toxicity was decreased resulting in correspondent decrease in inhibition and any inhibition was observed in distilled water.

Conclusion

Water extract of all tested botanicals plants significantly inhibited egg hatching of root knot nematode and resulted in 100% mortality of the second juveniles of *M. incognita in vitro* after 72 h of exposure. Egg inhibition and larval mortality decreased with increase in dilution of all the extracts. Juvenile mortality increased with a corresponding increased in time of exposure. Thus this finding is important in the identification and development of alternative strategies in controlling root-knot nematodes. There is however the need for further studies in identifying new classes of bio-pesticides from natural plants to replace the synthetic dangerous and expensive chemicals used at present.

Conflict of Interests

The authors have not declared any conflict of interests.

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