ABOUT JEN

The Journal of Entomology and Nematology (JEN) (ISSN: 2006-9855) is published monthly (one volume per year) by Academic Journals.

Journal of Entomology and Nematology (JEN) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as applications of entomology in solving crimes, taxonomy and control of insects and arachnids, changes in the spectrum of mosquito-borne diseases etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JEN are peer-reviewed.

Contact Us

Editorial Office: jen@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://www.academicjournals.org/journal/JEN
Submit manuscript online http://ms.academicjournals.me/
Mehdi Esfandiari  
Department of Plant Protection  
College of Agriculture,  
Shahid Chamran University of Ahvaz,  
Ahvaz, Iran

Prof. Dr. Mahfouz M. M. Abd-Elgawad  
Nematology Laboratory  
Department of Phytopathology  
National Research Center El-Tahrir St., Dokki 12622,  
Giza,  
Egypt

Matthew S. Lehnert  
Department of Entomology, Soils, & Plant Sciences  
Clemson University, Clemson,  
United States

Wenjing Pang  
3318 SE 23rd Avenue  
Gainesville, FL 32641  
Agronomy and Biotechnological College,  
China Agricultural University, Beijing,  
China

Dr. G. Shyam Prasad  
Directorate of Sorghum Research (DSR),  
Rajendranagar, Hyderabad 500030, AP,  
INDIA

Dr. Rashid Mumtaz  
Date Palm Research  
Plant Protection Department  
Food & Agricultural Sciences  
King Saud University, Riyadh  
Kingdom of Saudi Arabia

Editorial Board

Godwin Fuseini  
International SOS Ghana,  
Newmont Ghana Gold,  
Ahafo mine,  
Ghana.

Dr. Waqas Wakil  
Department of Agriculture Entomology,  
University of Agriculture, Faisalabad,  
Pakistan

Gilberto Santos Andrade  
Universidade Federal de Viçosa  
Avenida Peter Henry Rolfs, s/n Campus Universitário  
36570-000  
Viçosa - MG - Brazil

Ricardo Botero Trujillo  
Calle 117 D # 58-50 apto. 515  
Pontificia Universidad Javeriana, Bogotá,  
Colombia

Dr. D. N. Kambrekar  
Regional Agricultural Research Station,  
UAS Campus, PB. No. 18,  
Bijapur-586 101 Karnataka-INDIA  
India

Dr. P. Pretheep Kumar  
Department of Forest Biology  
Forest College & Research Institute  
Tamil Nadu Agricultural University  
Mettupalayam – 641 301  
Tamil Nadu, India

Dr. Raman Chandrasekar  
College of Agriculture Entomology  
S-225, Agriculture Science Center  
University of Kentucky  
Lexington, KY 40546-0091  
USA.

Dr. Rajesh Kumar  
Central Muga Eri Research and Training Institute  
Lahdoigarh, Jorhat-785700, Assam,  
India
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prof. Ding Yang</strong></td>
<td>Department of Entomology, China Agricultural University, 2 yuanmingyuan West Road, Haidian, Beijing 100193, China</td>
</tr>
<tr>
<td><strong>Dr. Harsimran Gill</strong></td>
<td>University of Florida, 970 Natural Area Drive, PO Box 110620, Gainesville, Florida - 32611</td>
</tr>
<tr>
<td><strong>Dr. Mehdi Gheibi</strong></td>
<td>Department of Plant Protection, College of Agriculture, Shiraz Islamic Azad University, Shiraz, Iran</td>
</tr>
<tr>
<td><strong>Dr. Nidhi KakKar</strong></td>
<td>University College, Kurukshetra University, Kurukshetra, Haryana, India</td>
</tr>
<tr>
<td><strong>Dr. Marianna I. Zhukovskaya</strong></td>
<td>Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 44 Thorez Ave, 194223, Saint-Petersburg, Russia</td>
</tr>
<tr>
<td><strong>Gaurav Goyal</strong></td>
<td>University of Florida, 282#14 Corry village, Gainesville, Fl. 32603, USA</td>
</tr>
<tr>
<td><strong>Gilberto Santos Andrade</strong></td>
<td>Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n Campus Universitario, 36570-000 Vicosa - MG - Brazil</td>
</tr>
<tr>
<td><strong>Joshi Yadav Prasad</strong></td>
<td>Gyanashwor Kathmandu, Nepal, G P O Box: 8975 EPC: 5519, Kathmandu, Nepal, India</td>
</tr>
<tr>
<td><strong>Baoli Qiu</strong></td>
<td>Department of Entomology, South China Agricultural University, No 483, Wushan Road, Tianhe, Guangzhou, PR China 510640</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prof. Ding Yang</strong></td>
<td>Department of Entomology, China Agricultural University, 2 yuanmingyuan West Road, Haidian, Beijing 100193, China</td>
</tr>
<tr>
<td><strong>Dr. Harsimran Gill</strong></td>
<td>University of Florida, 970 Natural Area Drive, PO Box 110620, Gainesville, Florida - 32611</td>
</tr>
<tr>
<td><strong>Dr. Mehdi Gheibi</strong></td>
<td>Department of Plant Protection, College of Agriculture, Shiraz Islamic Azad University, Shiraz, Iran</td>
</tr>
<tr>
<td><strong>Dr. Nidhi KakKar</strong></td>
<td>University College, Kurukshetra University, Kurukshetra, Haryana, India</td>
</tr>
<tr>
<td><strong>Dr. Marianna I. Zhukovskaya</strong></td>
<td>Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 44 Thorez Ave, 194223, Saint-Petersburg, Russia</td>
</tr>
<tr>
<td><strong>Gaurav Goyal</strong></td>
<td>University of Florida, 282#14 Corry village, Gainesville, Fl. 32603, USA</td>
</tr>
<tr>
<td><strong>Gilberto Santos Andrade</strong></td>
<td>Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n Campus Universitario, 36570-000 Vicosa - MG - Brazil</td>
</tr>
<tr>
<td><strong>Joshi Yadav Prasad</strong></td>
<td>Gyanashwor Kathmandu, Nepal, G P O Box: 8975 EPC: 5519, Kathmandu, Nepal, India</td>
</tr>
<tr>
<td><strong>Baoli Qiu</strong></td>
<td>Department of Entomology, South China Agricultural University, No 483, Wushan Road, Tianhe, Guangzhou, PR China 510640</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. Ramasubramanian</strong></td>
<td>Central Research Institute for Jute and Allied Fibres (Indian Council of Agricultural Research), Barrackpore, Kolkata – 700 120, India</td>
</tr>
<tr>
<td><strong>Hasan Celal Akgul</strong></td>
<td>Istanbul Plant Quarantine Service, Nematology Laboratory, Halkali Merkez Mahallesi, Halkali Caddesi, No:2, 34140 Halkali, Kucukcekmec-Istanbul/Turkey</td>
</tr>
<tr>
<td><strong>J. Stanley</strong></td>
<td>Vivekananda Institute of Hill Agriculture, Indian Council of Agricultural Research, Almora– 263601, Uttarakhand, India</td>
</tr>
<tr>
<td><strong>Atef Sayed Abdel-Razek</strong></td>
<td>National Research Centre, Dept. of Plant Protection, El-Tahrir Street, Dokki, Cairo, Egypt</td>
</tr>
</tbody>
</table>
Evaluation of some botanicals and Trichoderma harzianum against root-knot nematode (Meloidogyne incognita (Kofoi and White) Chit wood) in tomato
Belay Feyisa, Alemu Lencho, Thangavel Selvaraj and Gezehegne Getaneh
Full Length Research Paper

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

Belay Feyisa¹, Alemu Lencho¹*, Thangavel Selvaraj¹ and Gezehgne Getaneh²

¹Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, P. O. Box 19, Ethiopia.
²Department of Nematology, Ambo Plant Protection Research Center, P. O. Box 27, Ethiopia.

*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 J₂ larva 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 J₂ of *M. incognita* at 10% concentration and immobilized by 89, 93 and 100% after 24, 48 and 72 h of exposure, 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 antioxidants 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 environmentally 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 J2 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 (J2), 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-Elyouser 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.

*Corresponding author. E-mail: alemulencho@yahoo.com

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
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° 53'33"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 Melkassa 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 Mukhpadhyay, 2001). 5 ml and 10 ml of plant extracts were incorporated in to each pot with different treatments.

<table>
<thead>
<tr>
<th>Common name of the botanicals</th>
<th>Botanical name</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rape seed</td>
<td>Brassica napus L.</td>
<td>Leaf</td>
</tr>
<tr>
<td>Lantana</td>
<td>Lantana camara L.</td>
<td>Leaf</td>
</tr>
<tr>
<td>African marigold</td>
<td>Tagetes erecta L.</td>
<td>Leaf</td>
</tr>
<tr>
<td>Neem</td>
<td>Azadirachta indica L.</td>
<td>Leaf and seed</td>
</tr>
</tbody>
</table>

**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 lactic pheno (Barker et al., 1985). After cooling to normal temperature, they were keeping in lactic pheno overnight for partial de-staining (Seinhorst, 1998). Root pieces were dissected under stereomicroscope 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 J₂ 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 were picked up from the sieves 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 J₂ were determined from 3 replications of one ml aliquot of inoculum suspension for *in vitro* culture experiments. 100 J₂ 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
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 22°C 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
(10³ spores/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*
only 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 J2s were
placed in test tube containing 5 ml and 10 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 J2s was
recorded every 24 h for three days. After 24, 48 and 72 h, active
and inactive J2s 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 J2 mortality in a tube was calculated as:

\[
\text{%J2 mortality} = \frac{\text{No. of inactive (dead) J2s}}{\text{Total J2s in a tube}} \times 100
\]

**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* J2
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.
incognita* J2 24 h after treatment application when
compared to all the other treatments. It was able to immobilize J2 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. incognita* under *in vitro* test using botanicals and *T. harzianum*.

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

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 J2 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*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration</th>
<th>No. eggs hatched to J₂ after 7days</th>
<th>Z**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rape Seed Leaf extract alone</td>
<td>5</td>
<td>36.00cdef</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>33.60cdef</td>
<td>89</td>
</tr>
<tr>
<td>Lantana leaf extract alone</td>
<td>5</td>
<td>30.67cdef</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32.60cdef</td>
<td>91</td>
</tr>
<tr>
<td>African marigold leaf extract alone</td>
<td>5</td>
<td>33.00cdef</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27.00ef</td>
<td>91</td>
</tr>
<tr>
<td>Neem Leaf extract alone</td>
<td>5</td>
<td>33.33cdef</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>29.00def</td>
<td>90</td>
</tr>
<tr>
<td>Neem Seed extract alone</td>
<td>5</td>
<td>25.00f</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.67g</td>
<td>96</td>
</tr>
<tr>
<td><em>T. harzianum</em> suspension alone</td>
<td>5</td>
<td>38.67cde</td>
<td>87</td>
</tr>
<tr>
<td>Rape seed + <em>T. harzianum</em></td>
<td>5,5</td>
<td>56.33b</td>
<td>81</td>
</tr>
<tr>
<td>Lantana + <em>T. harzianum</em></td>
<td>5,5</td>
<td>40.30cd</td>
<td>87</td>
</tr>
<tr>
<td>African marigold + <em>T. harzianum</em></td>
<td>5,5</td>
<td>43.00c</td>
<td>86</td>
</tr>
<tr>
<td>Neem Leaf + <em>T. harzianum</em></td>
<td>5,5</td>
<td>54.67b</td>
<td>82</td>
</tr>
<tr>
<td>Neem Seed + <em>T. harzianum</em></td>
<td>5,5</td>
<td>39.33cde</td>
<td>87</td>
</tr>
<tr>
<td>Distilled Water (Control)</td>
<td>5</td>
<td>301.33a</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>(CV)%</td>
<td></td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

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.
REFERENCES


Journal of Entomology and Nematology

Related Journals Published by Academic Journals

- Biotechnology and Molecular Biology Reviews
- African Journal of Microbiology Research
- African Journal of Biochemistry Research
- African Journal of Environmental Science and Technology
- African Journal of Food Science
- African Journal of Plant Science
- Journal of Bioinformatics and Sequence Analysis
- International Journal of Biodiversity and Conservation