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# Effect of Some Botanicals and *Trichoderma Harzianum* against Root-Knot Nematode *Meloidogyne Incognita*, *Infecting* Tomato under Green House

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**Root-knot nematode, *Meloidogyne incognita,* causes significant economic losses to tomato production. Hence, the present study was conducted to evaluate the effect of 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 5% and 10% concentrations and *T. harzianum* at 5% plus control were tested against root-knot nematode development and their role on plant growth parameters of tomato under *in vivo* condition. The effect of different botanicals and *T. harzianum* singly and in combination were studied for the management of tomato root-knot nematode under greenhouse condition. There was a significant difference in the reduction of root-knot nematode incidence, root-knot nematode population, nematode reproduction rate (NRR), number of galls and egg masses per plant were recorded. In pot culture condition, the application of leaf extract of individual plant in the presence of the nematode significantly enhanced the growth of tomato seedlings in comparison to the control. A significant increase in plant height, shoot weight and root weight of the seedlings were observed at the 10% concentration of leaf extracts in comparison to control. There was a significant difference in the reduction of root-knot nematode population, nematode reduction rate, number of galls and egg masses per plant of *L. camara* combined with *T. harzianum*. The mean fruit weight and total yield were observed highest in the combination treatment of *L. camara* combined with *T. harzianum*. This study results 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.**

**Keywords:** Botanical leaf; Seed extracts; Root-knot nematode; Growth; egg mass

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## 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., 2002). Most intensive production is done in the Rift Valley, mainly along Awash River Valley and around the lakes. It is produced both during the rainy and dry seasons under supplemental irrigation (Lemma, 2004). 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 biocontrol 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 nonphytotoxic, 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 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, and 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 vivo* conditions.

## MATERIALS AND METHODS

### *Description of the study area*

*In vivo* experiments were conducted at Ambo Agricultural Research Center (AmARC), 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, and 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. 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 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.

### 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 *vivo* experiments.

**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 |

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 ($10^6$spores/ml) after counting spore density using a haemocytometer (Niranjana et al*.,* 2009).

***In vivo* experimental study**:

20 cm wide plastic pots were filled with 3 kg/pot of sterilized mixed soil (sandy clay loam, sand and compost as 2:1:1 (v/v). Seeds of susceptible tomato cultivar were sown at germination pot and after 21 days, seedlings were transplanted to the green house pots. One seedling per pot was maintained at the center. The experiments were laid out in Complete Randomized Design (CRD) with three replications. Tomato potted plant soils were inoculated with 2 ml suspension of 2000 freshly hatched second stage juveniles (J2) of *M. incognita* and also infested with 10 ml of each botanical and 20 ml of *T. harzianum* suspension(Elbadri GAA, Lee DW, Park JC, Choo HY, 2009). Then proper watering was provided and the pots were kept at 20ºC ± 2ºC. Applications of botanicals and *T. harzianum* were also repeated after once in 20 days (Taye W, Sakhuja PK, Tefera T, 2012).

**In vivo experiment consisted of the following thirteen treatments:**

T1- Application of Rape seed leaf extract alone,
T2- Application of *Lantana* leaf extract alone,
T3- Application of African marigold leaf extract alone,
T4- Application of neem leaf extract alone,
T5- Application of neem seed extract alone,
T6- *T. harzianum* alone*,*
T7- Rapeseed+*T. harzianum,*
T8- Lantana+*T. harzianum,*
T9- Marigold+*T. harzianum*,
T10- Neem leaf+*T. harzianum*,
T11-Neemseed+*T.harzianum*
T12- Un inoculated control and
T13- Nematode only inoculated control.

After 90 days of the growth, the plants were uprooted, thoroughly washed and then the plant height, fresh and dry weight of shoot and roots, root-knot nematode population, nematode reproduction rate (NRR), number of galls/plant and egg masses per plant were recorded. The number of fruits per pot was counted. The galling index and the number of egg masses (gall) per plant in each pot were determined using a scale following the rating scale described by Taylor and Sasser (1978) and Colyer et al. (2008). Scale 0=0, 1=1-2; 2=3-10; 3=11-30; 4=31- 100; 5=>100. Galling index: 0=no galls, 1=slight infection, 2=moderate infection, 3=moderately severe, 4=severe, 5=very severe. The numbers of egg masses per plant on infected roots were counted after staining with Phloxin B (1984). The nematode population was

recorded in soils of each treatment separately, after 90 days. The final population density of nematode was determined based on Cobb's sieving and decanting method (1986). The number of nematodes per pot was counted using counting dish. The reproduction factor (RF) was calculated by the formula (Zhang F, Schmitt DP, 1994). RF =PF/PI Where Pf is the final population and Pi is the initial population.

**Data analysis:**

Data on plant height, fresh shoot weight, fresh root weight and dry shoot weight, number of galls, egg mass/root, and final nematode population / pot were statistically analyzed as described by Gomez and Gomez (1984). The data were subjected to an Analysis of Variance (ANOVA) procedures using Statistical Analysis system (2002) (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

***In vivo e□ect of botanicals and Trichoderma harzianum against M. incognita Plant height:***

The treatments did not showed any negative e□ects on plant growth. There were significant di□erences in the height of tomato plants treated with aqueous plant extracts and *T. harzianum* over inoculated control plants (Table 2). The highest plant height was observed in pots treated with combination of *L. camara* and *T. harzianum* followed by neem seed and neem leaf with *T. harzianum* over inoculated control. The lowest height of plants was recorded in pots treated with rape seed leaf. The highest plant height was 160% increase in pots treated with combination of *L. camara* and *T. harzianum* over inoculated control. Pots treated with combination of botanicals and *T. harzianum* showed more height than botanicals applied alone or without fungus. The addition of botanicals to soil leads to a better environment for the growth of the roots. This enhances the utilization of soil nutrients, as a consequence of which the nematode damage might have been markedly reduced (Abubakar U, Adamu T, Manga SB, 2004). These botanicals may be act as substrate for the growth and multiplication of *T. harzianum*. Some *Trichoderma* isolates were reported to do both enhanced plant growth and reduced root-knot nematode damage (Meyer SLF, Huettel RN, Liu XZ, Humber RA, Juba J, et al., 2004). It has been reported that *Trichoderma* has not only been proved to parasitize nematodes and inactive pathogen enzymes but also help in tolerance to stress condition by enhanced root development. It participates in solubilization of inorganic nutrients (Sharma P, Pandey R, 2009). The shortness' of the plant height might be due to the stunting action of *M. incognita*. Jinfa et al. (2006)] also reported that this kind of height reduction caused by root-knot nematode. In inoculated control, the lowest growth performances by the control plants could be as result of the combined e□ect of nematodes and availability of nutrients (Jinfa Z, Waddell C, Sengupta GC, Potenza C, Cantrell RG, 2006). The galls on the root system might disturb important root functions like uptake and transport of water and nutrients (Sikora RA, Fernandez E, Bridge J, Luc M, 2005).

**Fresh and dry shoot weight:**

The higher fresh shoot weight was signifcantly obtained in seedlings treated with aqueous plant extracts and *T. harzianum* over inoculated control (Table 1). The highest and the lowest shoot fresh biomass was observed in plants treated with the combination of *T. harzianum* with *L. camara* and rape seed leaf, 146 and 80 g, respectively, when compared with inoculated control. The results of the present experimental study was not agree with Agbenin et al. (2004) Neem seed powder increased root and shoot weights and heights and decreased root galling index and presence of mycelium on root. Generally, *T. harzianum* individually and combination with botanicals showed more e□ective on plant fresh shoot weight than botanicals. Dry shoot weight of plants after 90 days were significantly lower in inoculated control plants than inoculated treated plants (Table 1). There were no signifcance di□erence between pots treated with all botanicals applied individually, rape seed and neem leaf with combination of *T. harzianum* and un inoculated control but they were signifcant di□erence when compared with inoculated control. The maximum total plant shoot dry weights were recorded in pots treated with combination of *L. camara* and *T. harzianum* followed by *T. harzianum* with combination of neem seed and neem leaf over inoculated control. The lowest dry shoot weight was observed in pants treated with rape seed than other treatments (Table 2).

**Fresh root weight:**

There were highly signifcance di□erences among recorded fresh root weight between pots treated with aqueous plant extracts and *T. harzianum* when compared with inoculated control (Table 2). The highest fresh root weight was recorded by plants grown on pots with inoculated control followed by neem leaf with *T. harzianum* and African marigold jointly with *T.harzianum*. The lowest weight was recorded in pots with un inoculated control or negative control when compared with inoculated control. *Trichoderma* spp. found in close association with roots contributes as plant growth

**Table 2.** Effect of aqueous plant extracts and Trichoderma harzianum on growth of tomato plants against root-knot nematode infested soil under greenhouse condition.

| Treatment | Conc. | Plant height (cm) | Z** | Fresh shoot weight (g) | z** | Dry shoot weight(g) | Z** | Fresh root weight(g) |
|---|---|---|---|---|---|---|---|---|
| Rape seed leaf extract alone | 10 | 68.00d | 50 | 80.00d | 46 | 23.17e | 71 | 41.00cd |
| Lantana leaf extract alone | 10 | 80.0bcd | 66 | 109.00c | 88 | 26.00e | 86 | 29.33efg |
| African marigold leaf extract alone | 10 | 80.bcd | 66 | 108.00c | 86 | 25.50e | 82 | 27.00fg |
| Neem leaf extract alone | 10 | 73.33cd | 52 | 86.00d | 48 | 25.00e | 78 | 24.00gh |
| Neem seed leaf extract alone | 10 | 72.33cd | 41 | 85.00d | 38 | 24.00e | 64 | 20.00h |
| *T.harzianm* suspension only | 20 | 83.0bcd | 73 | 110.00c | 89 | 27.00e | 93 | 30.00efg |
| Rape seed + *T.harzianum* | 10+10 | 88.0bcd | 83 | 120.00bc | 106 | 30.00de | 114 | 35.00de |
| Lantana + *T. harzianum* | 10+10 | 125.00a | 160 | 146.47a | 151 | 62.00a | 342 | 10.00i |
| African marigold + *T. harzianum* | 10+10 | 89.00bc | 85 | 121.00bc | 108 | 38.00cd | 171 | 45.00bc |
| Neem Leaf + *T. harzianum* | 10+10 | 91.67bc | 90 | 128.00b | 120 | 40.00bc | 185 | 48.00b |
| Neem Seed + *T. harzianum* | 10+10 | 95.00b | 98 | 130.00b | 124 | 48.00b | 242 | 32.00ef |
| UC | - | 84.67bcd | | 111.00c | | 28.00e | | 19.00h |
| IC<br>CV (%) | -<br>12.82 | 48.00e<br>8.2 | 16 | 58.00e | 12.7 | 14.00f | | 55.00a |
| LSD | 17.84 | 14.76 | 8.5 | | 6.8 | | | |

**Note**: means in column with the same letter are not significantly different (P<0.0001) DMRT.
Z** increase over inoculated the control in percent.
Values are averages of three replicates.
Significance is given compared to positive controls (inoculated control).
Control positive = control in conjunction with inoculation of *M. incognita* juveniles.
Control negative = control without inoculation of *M. incognita* juveniles

stimulators. In the present study, the root weight of inoculated control was greater than that of un inoculated weight. Wong and Mai (1973) reported that di erences in root weight may be explained by gall development, gall mass being heavier than an equivalent linear length of similar non-galled roots. Perry et al. (2009) reported similar results that root weight increased in untreated infected plants compared with those amended with herbal powder due to the formation of galls and giant cells.

**Number of galls per root system:**

The number of galls per root system was observed significantly reduced between the pots treated with aqueous plant extracts and *T. harzianum* over inoculated control (Table 3). Maximum inhibition of gall formation was observed in pots treated with combination of lantana and *T. harzianum* and followed by neem seed with *T. harzianum* .Te highest and lowest reduction of number of galls per root system was observed in pots treated with combination of *L. camara* with *T. harzianum* and rape seed leaf because they reduced number of galls by 88 and 37% over inoculated control, respectively. Combination of *T. harzianum* with neem seed, neem leaf and the fungus only also showed gall reduction next to combination of neem seed with *T. harzianum* that shows gall reduction 83, 79 and 75%, respectively. Generally the highest reductions in number of galls per root were

**Table 3.** Effect of aqueous plant extracts and *Trichoderma harzianum* on nematode population, gall and egg mass on tomato plants (cv. Marglobe) in root-knot nematode infested soil under green house.

| Treatment | Conc. | Gall/root | X** | Eggmass/ root | X** | Final Nematode population./ pot | X** | Reproduction factor(R=PF/P) |
|---|---|---|---|---|---|---|---|---|
| Rape Seed leaf extract alone | 10 | 203.00b | 37 | 175.00b | 39 | 650.00b | 79 | 0.325b |
| Lantana leaf extract alone | 10 | 115.00ef | 64 | 91.00de | 68 | 513.00c | 83 | 0.26de |
| A. marigold leaf extract alone | 10 | 138.00d | 57 | 128.00c | 55 | 548.00c | 82 | 0.27c |
| Neem leaf extract alone | 10 | 158.00c | 51 | 129.00c | 55 | 560.00c | 81 | 0.275c |
| Neem seed extract alone | 10 | 120.00de | 63 | 96.00d | 66 | 521.00c | 83 | 0.261cde |
| *T.harzianum* suspension alone | 20 | 79.00hi | 75 | 62.00g | 78 | 532.00c | 82 | 0.266f |
| Rape seed+*T.harzianum* | 10+10 | 99.00fg | 69 | 83.00ef | 71 | 509.00c | 83 | 0.25de |
| Lantana+*T.harzianum* | 10+10 | 39.00k | 88 | 35.00i | 87 | 303.00d | 90 | 0.15g |
| African margold+*T.harzianum* | 10+10 | 90.00gh | 72 | 78.00f | 73 | 490.00c | 84 | 0.245ef |
| Neem leaf+*T.harzianum* | 10+10 | 68.00ij | 79 | 53.00h | 81 | 463.33c | 85 | 0.23cd |
| Neem seed+*T.harzianum* | 10+10 | 55.00jk | 83 | 42.00i | 85 | 341.00d | 89 | 0.17h |
| UC | - | 0.00l | | 0.00j | | 0.00e | | 0.00e |
| IC | - | 325.00a | | 290.00a | | 3080.00a | | 1.54a |
| CV (%) | | 9.81 | | 5.44 | | 8.2 | | |
| LSD | | 18.85 | | 8.86 | | 89.8 | | |

**Note**: means in column with the same letter are not significantly different (<0.0001) PDMRT
Each pot contains 3000 cc sterilized soil.
X**: Reduction over inoculated control in percent

observed in pots treated with combination of botanicals and *T. harzianum* than botanicals treated individually. The lowest growth rate, high galling due to nematode activity at root zone resulting in giant cell formation, high population of nematodes because the nematodes larvae were able to penetrate roots freely and reproduce without any inhibition. A reduction in root knot development could be attributed to poor penetration of the second stage juveniles and later retardation in their activities, for example feeding and /or reproduction as suggested by Abdi M (1996).

**Number of egg masses per root system:**

There were significant di□erences between treatments on egg mass reduction over inoculated control (Table 3). Similarly all the treatments were found to be highly e□ective in their ability to reduce egg mass per root system when compared with inoculated control/untreated plants. The highest and the lowest egg mass reduction was observed in pots treated with *L. camara* combined with *T. harzianum* and botanical rape seed leaf over inoculated control. The highest percentage of egg mass

reduction was observed with pots treated with combination of *L. camara* and *T. harzianum* (87%) and followed by combination of neem seed with *T. harzianum* (85%) and *T. harzianum alone* (80%). Concerning the e□ect of rape seed on nematodes it is true with results reported by Johnson et al. (1992).

**Final nematode population and reproduction factor:**

The suppressive effect of aqueous plant extracts and *T. harzianum* was recorded as the nematode population in the soil at the end of the experiment 90 days after nematode inoculation. Signifcantly, the less number of parasitic nematodes was observed in the soil samples obtained from pots treated with *L. camara* with *T. harzianum* as compared to the control. Among treatments, pots treated with *L. camara* and *T. harzianum* showed more effective in reducing the final nematode population over inoculated control. For this reason it is suggested that the use of plants residue too would be more efficient against nematodes when used in combination with other management practices that are currently available. Except rape seed there were no significant difference between pots treated with botanicals each other. The maximum and minimum final nematode population was recorded from combination of rape seed leaves and *T. harzianum* and *Lantana* leaves with *T. harzianum,* respectively, (Table 3). The highest percentage (90%) of fnal nematode population reduction was shown in pots treated with combination of *L. camara* combination with *T. harzianum* followed by combination of neem seed with *T. harzianum* (89%) over inoculated control. The lowest nematode population reduction was observed in pots treated with rape seed leaf when compared with other treatments (Table 3). A reduction in root-knot development could be attributed to poor penetration of the second stage juveniles and later retardation in their activities, for example feeding and/or reproduction as suggested by Abdi [1996]. Nematotoxic compounds especially the Azadirachtin released through gradual decomposition of the neem seeds (Sharon et al., 2001) and suppress nematode populations throughout the whole period of the nursery stage. Nematode population in nematode + fungus treatment was 532 but *L. camara* has decreased population to 303. Except rape seed leaf extract, there were no significant differences between the pots treated with all botanicals including *T. harzianum* which applied individually. In this study, African marigold and neem seed treated pots were reduced nematode population in the soil 82 and 83%, respectively. Similarly, (Hasabo and Noweer, 2005) found that the soil treatment with aqueous extracts of marigold leaves and neem seeds significantly reduced *M. incognita* J2 in soil and roots of egg plants. The J2 population in roots was reduced by 90% and 75% respectively, 4 months after treatments, applied at 50 ml/plant as soil drench. Begum et al. (2005) and Qamar et al. (2005) observed that isolated chemical constituents such as lantanoside, lantanone, camaric acid and oleanolic acid from aerial parts of *L. camara*, possessing nematicidal activity against *M. incognita*. Ahmad et al. (2010) also noted that various concentrations of leaf extract of *L. camara* were deleterious to *M. incognita*.

**Reproduction factor:**

The reproduction rate of *M. incognita* was significantly suppressed by all the treatments as compared to untreated inoculated plants (Table 3). Reproduction rate of *M. incognita* was 0.23 in nematode+ fungus but its decrease to 0.15 by *L. camara*. Nematode reproduction factor was reduced in pots treated with combination of lantana and *T. harzianum* followed by pots treated with neem seed and *T .harzianum* when compared with inoculated control than other treatments. The highest nematode reproduction factor was observed in pots treated with rape seed leaf aqueous extracts than other treatments. This is because we suggest that *L. camara* act as substrate for the growth and multiplication of *T. harzianum*. Decomposed leaves have been found to support greater sporulation and multiplication of *T. harzianum* and *P. chlamydosporia* (Khan MR, Khan N, Khan SM (2001).Several authors have been shown the potential of using plant extracts in the control of plant parasitic nematodes (Okeniyi MO, Fademi OA, Orisajo SB, Adio SO, Otunoye AH, et al., 2010). The reduction in population of *M. incognita* in this investigation may be due to the accumulation of nematicidal components and/or to increase host resistance. This significant reduction on the final nematode population density in the soil could be due to the chemicals present in the extracts that possess ovicidal or larvicidal properties resulting in inhibition of its multiplication. *T. harzianum* and *L. camara* not only could decrease nematode population but also increase growth parameters of tomato.

**Fruit number per pot:**

Highest number of tomato fruit was found in pots treated with lantana camara + *T. harzianum* followed by neem seed+ *T. harzianum* over inoculated control (Table 4). Among all treatment the lowest number of fruit was recorded from pots treated with rape seed leaves. There were no significance di□erence between pots treated with only botanicals but they were significant di□erence from inoculated control. The inability of the control plants to flower and fruit is probably due to the combined action of the nematode and inadequate availability of nutrients.

**Yield of tomato per hectare:**

Application of aqueous plant extract and *T. harzianum* on

**Table 4.** Effect of aqueous plant extracts and *Trichoderma harzianum* on yield of tomato plants (cv. Marglobe) in root-knot nematode infested soil under green house.

| Treatment | Con | Total no. of fruits/pot | Kg/Pot | t/he |
|---|---|---|---|---|
| Rape Seed leaf extract alone | 10 | 7.50h | 0.75ef | 14.00f |
| Lantana leaf extract alone | 10 | 10.00fgh | 0.60fg | 18.00def |
| A. marigold leaf extract alone | 10 | 9.00gh | 0.90def | 16.00ef |
| Neem leaf extract alone | 10 | 8.00h | 0.80ef | 15.00ef |
| Neem seed extract alone | 10 | 11.00e-h | 1.10cde | 22.00cdef |
| *T.harzianum* suspension alone | 20 | 14.00edf | 1.15cde | 23.00cdef |
| Rape seed+*T.harzianum* | 10+10 | 15.00cde | 1.20cde | 24.00cde |
| Lantana+*T.harzianum* | 10+10 | 34.00a | 2.2a | 44.00a |
| African margold+*T.harzianum* | 10+10 | 17.33cd | 1.30bcd | 26.00bcd |
| Neem leaf+*T.harzianum* | 10+10 | 19.00bc | 1. 5bc | 30.00bc |
| Neem seed+*T.harzianum* | 10+10 | 22.00b | 1.70b | 34.00b |
| UC | - | 13.00d-g | 1.12cde | 22.40cdef |
| IC | - | 3.00i | 0.20g | 4.00g |
| CV (%) | | 17.5 | 22.6 | 22.1 |
| LSD | | 4.13 | 0.4 | 8.35 |

**Note**: means in column with the same letter are not significantly different (P<0.05) by DMRT

**Table 5.** Correlation of plant height, fresh shoot weight, dry shoot weight, fresh root weight, yield/tons/hectare, egg masses, final population, and number of gall Meloidogyne incognita in tomato plant under green house.

| | HT | FSW | DSW | FRW | G.root | E.Mass | N.po | N.Fruit | Kg/pot | Ton/ha |
|---|---|---|---|---|---|---|---|---|---|---|
| HT | 1 | 0.83 | 0.81 | -0.48 | -0.69 | -0.70 | -0.60 | 0.85 | 0.77 | 0.75 |
| FSW | | 1 | 0.80 | -0.35 | -0.79 | -0.79 | -0.65 | 0.81 | 0.79 | 0.78 |
| DSW | | | 1 | -0.31 | -0.62 | -0.61 | -0.46 | -0.90 | -0.83 | -0.81 |
| FRW | | | | 1 | 0.54 | 0.55 | 0.59 | -0.38 | -0.42 | -0.43 |
| G.root | | | | | 1 | 0.99 | 0.86 | -0.68 | -0.75 | -0.73 |
| E.Mass | | | | | | 1 | 0.88 | -0.67 | -0.74 | -0.73 |
| N.po | | | | | | | 1 | -0.47 | -0.57 | -0.57 |
| N.Fruit | | | | | | | | 1 | 0.86 | 0.87 |
| Kg/pot | | | | | | | | | 1 | 0.97 |
| Ton/ha | | | | | | | | | | 1 |

** Correlation is significant at the 0.0001 level * Correlation is significant at the 0.05 level. Where HT=Height; FSW=Fresh dry weight; DSW=Dry shoot weight; FRW=Fresh root weight; G. root=Number of gall per root system; E.mass=number of egg mass per root system,; N.pop=Final nematode population; N. fruit=Number of fruit per plant; Kg/pot=kilogram per pot; Ton/ha=ton per hectare

root knot nematodes; *Meloidogyne incognita* infested pot shows significant di□erence (P 0.0001) on yield of tomato over control (Table 5). The highest yield of tomato was observed in pots treated with combination of *Lantana camara* with *T. harzianum* and followed by neem seed with *T. harzianum* over inoculated control, respectively. The lowest yield was observed in pots treated with aqueous rape seed leaf extracts. The presence of nematode on tomato plants significantly a□ected their yield, un inoculated plants had 82% yield higher (P 0.0001) than inoculated plants. Root colonization by *Trichoderma* spp. frequently enhances root growth and development, crop productivity, resistance to a biotic stresses and uptake and use of nutrients (Harman GE1, Howell CR, Viterbo A, Chet I, Lorito M, 2004). Cuevas VC (2006) showed that the presence of the fungus in the soil in sufficient population resulted in the uptake of more mineral nutrients especially P and Zn available for plant use that increased crop growth and yield in the screen house and farmers' field.

## CONCLUSION AND RECOMMENDATIONS

For its management, di□erent plant species (botanicals) and an antagonistic fungus, *T. harzianum* were being tried in di□erent forms as an alternative to nematicide. Similarly a prominent reduction in final nematode population density, egg mass, galls per root system and a significant increase yield per plant and total yields of tomato were observed from plants treated with the combination of *T. harzianum* with *L. camara* and neem seed extracts compared to any other treatments. These results suggest that in green house combination of *T. harzianum* jointly with *Lantana* leaf and neem seed would be a good alternative to manage root-knot nematode populations in tomato production. These combinations not only reduce nematode infestation and population buildup on tomato but also increase soil fertility. Therefore, bio-control is suggested to be a safer solution. Botanicals are more e□ective jointly with fungus than applied individually in green house because some botanicals act as a substrate for the growth and multiplication of *T. harzianum*. Non chemicals and eco-friendly management such as bio-control management system by using *T. harzianum* and botanicals mentioned above were gaining importance and also greater attention which are easily available, less cost e□ective with no pollution hazards.

## RECOMMENDATIONS

At this time most of Ethiopian farmers not know plant parasitic nematodes so, Awareness creation activities should be done with stakeholders (farmers, Developmental agents, etc.) on its effect on plants specially vegetables and fruits. Non chemicals and eco-friendly management such as bio-control management system by using *T. harzianum* and botanicals mentioned above were gaining importance. All parts of the effective botanicals should be tested for efficacy. In this study there was a significant difference in the reduction of root-knot nematode Population, nematode reduction rate, number of galls and egg masses per plant of *L. camara* combined with *T. harzianum*.

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