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Nematicidal Potential of Some Botanical Products Against *Meloidogyne incognita* Infecting Eggplant

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ABSTRACT: Work experiments were designed to verify the efficacy of nine medicinal plant extracts against *Meloidogyne incognita* (root-knot nematode) infecting eggplant (*Solanum melongena* L.). The tested plants were, Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* Roscoe), Peganum (*Peganum harmala* L.), Thyme (*Thymus vulgaris* L.), Bitter Melon (*Citrullus colocynthis* L.), Artemisia (*Artemisia absinthium* L.), Alpinia (*Alpinia officinarum* Hance), Thuja (*Thuja orientalis* L.) and Capsicum (*Capsicum annuum* L.). Results showed that nematode population and eggplant growth parameters were affected when different plant extracts under different concentrations 1, 2 and 3% were applied. Examination of root system showed that egg-masses, galls and females affected markedly. The *P. harmala* at 3% gave the highest reduction with second stage juveniles (J2S)/250 cc soil, galls and egg-masses/root system comparing to other treatments applied and nematode alone. No phytotoxicity to eggplant plants appeared at used application rates.

Keywords: Botanical products, *Meloidogyne incognita*, eggplant, control.

Eggplant (*Solanum melongena* L.) is a member of family *Solanaceae* and widely cultivated vegetable crop in Egypt. It has a huge domestic and export demand. Nowadays it is widely grown in different seasons in open fields and under plastic house conditions especially in newly reclaimed lands. According to the report of the Food and Agriculture Organization (FAO,2016), the total cultivated area of eggplant in Egypt reached 48556 ha which yielded 1194315 tons, with an average of 245967 g/ha. High eggplant yield loss may occur when grown in sandy soils infested with high nematode population, especially in the summer season.

Plant-parasitic nematodes (PPN) are exceedingly notoriously difficult plant pathogens to control. International annual loss in eggplant due to nematodes were 16.9 % (Sasser, 1987). Root-knot nematodes are considered damaging nematode pests of many host plants (Bakr, 2014). Annual world losses occurred because of *Meloidogyne* spp. are about USD$ 100 billion (Brand *et al*., 2010). Heavy infected eggplant, melon and tomato plants give yield losses over 30% (Sikora and Fernandez, 2005). The damage level influenced by a lot of factors for example, nematode specie, level of soil infestation, host cultivar and environmental conditions.

Use of botanical pesticides is a promising method in plant and environment protection from pesticides pollution (Manju and Sankari Meena, 2015; Akhter and Khan 2018). Many plants have been obviously used as an effective means in plant nematode control when used as a soil amendment, plant extracts, grown in rotation, intercropping within susceptible crops (Olaniyi, 2015). These higher medicinal plants have some advantages as antagonistic over synthetic nematicides. They contain active components such as:, isothiocyanates, cyanogenic glycosides, lipids, alkaloids, diterpenoids, polyacetylenes, terpenoids, glucosinolates, polythienyls, quassinoids, steroids, sesquiterpenoids, triterpenoids, phenolics, and other compounds (Bakr *et al*., 2015; Khan *et al*., 2017).

The botanicals may contain no resistance history compounds by the pests, fast biodegradable in cultivated soil or plant, narrow pre-harvest intervals (PHI) and renewable resources originally (Chitwood, 2002; Nikoletta and Menkissoglu-Spiroudi, 2011). Nowadays, botanicals use is gaining prime importance in the integrated nematode management (INM) methods because these plants produce secondary exudates of volatile or nonvolatile nature by stem, root, flower and leaf (Khalil, 2014; Bakr 2018).

The objective of the study was designed to evaluate some selected plants for ecofriendly controlling of *Meloidogyne incognita* infecting eggplant under greenhouse conditions.

MATERIAL AND METHODS

Dried materials of selected plants based on their traditional usage for medicinal purposes. Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* Roscoe), Peganum (*Peganum harmala* L.), Thyme (*Thymus vulgaris* L.), Bitter Melon (*Citrullus colocynthis* L.), Artemisia (*Artemisia absinthium* L.), Alpinia (*Alpinia officinarum* Hance), Thuja (*Thuja orientalis* L.) and Capsicum (*Capsicum annuum* L.) were obtained from the market and ground into fine powder using a Micro-hammer-mill (KINEMATICA AG, PX-MFC). Then stored in air-dried containers until required for usage.

Multiplication of *M. incognita*

For obtaining a pure culture of *M. incognita*, one egg mass was taken from infected plant and then identified by observation of perineal pattern (Hartman and Sasser, 1985) and was added to infect the black nightshade (*Solanum nigrum*) plants grown in plastic pots 30 cm diameter filled with sterilized sand-clay soil (2:1 v/v) under controlled conditions (25 ± 3 ºC with relative humidity 85%) at the experimental greenhouse, Agricultural Botany Dept., Faculty of Agriculture, Menoufia University, Egypt.

Preparation of *M. incognita* inocula

Two months old, heavily galled black nightshade roots infested by *M. incognita*, used to prepare nematode inoculum. Heavily infested roots washed gently using tap water to take out the attached soil particles. Roots divided into small pieces and then macerated using blender (Monlinex) for 10 seconds each at high-speed two times. Then root solution placed in a jar containing sodium hypochlorite (NaOCl) under concentration 0.5% according to (Hussey and Barker, 1973). The solution in the jar vigorously shaken for 3 min. to allow NaOCl removing gelatin matrix and release the eggs from the egg masses. Root tissues separated by transfer the solution through different size sieves. Eggs were collected on the 20ìm sieve and washed several times to remove residual NaOCl. Eggs then collected in a flask containing tap water. The number of eggs / ml was estimated by counting 4 samples of 1 ml using a counting dish under a stereomicroscope (Bel photonics, Biological Microscope, Bio 1-B) at 100x.

Greenhouse experiment achieved to study the effect of selected plants (Table 1) on *M. incognita*. Eggplant seedlings cv. Balady four weeks old were transplanted into plastic pots 15 cm in diameter. Dried materials of nine medicinal plants were mixed with soil at ratio 1, 2 and 3% (w/w) at time of transplanting. Each plant inoculated with 3000 eggs of *M. incognita* around the young hairy roots. Plants in pots without amendments used as a control treatment. Pots arrange on a bench in the greenhouse in a completely randomized design. Treatments repeated 4 times. Plants removed after 2 months from inoculation, and then roots washed carefully by running tap water. Plant growth and nematode parameters were recorded as follow: Root length (cm), Plant height (cm), Root fresh weight (g), Shoot fresh weight (g), Shoot dry weight (g), Number of galls /root system, number of egg masses /root system. For counting of egg-masses, roots stained as described by (Daykin and Hussey, 1985) by dipping the roots in 0.015% Phloxine-B solution for 20 min., number of females /root system was determined (Mahdy, 2002), number of second stage juveniles (J2s) /250g soil.

Statistical analysis

Data statistically analyzed using Duncan's Multiple Range test (*P=0.05*) using Costat 6.3 version program.

Table 1. Scientific and common names, families and used parts of tested plants.

Scientific name	Common name	Family	Used part
Curcuma longa	Turmeric	*Zingiberaceae*	rhizomes
Zingiber officinale	Ginger	*Zingiberaceae*	rhizomes
Peganum harmala	Peganum	*Nitrariaceae*	seed
Thymus vulgaris	Thyme	*Lamiaceae*	shoots
Citrullus colocynthis	Bitter Melon	*Cucurbitaceae*	fruits
Artemisia absinthium	Artemisia	*Asteraceae*	shoots
Alpinia officinarum	Alpinia	*Zingiberaceae*	rhizomes
Thuja orientalis	Thuja	*Cupressaceae*	fruits
Capsicum annuum	Capsicum	*Solanaceae*	fruits

RESULTS

Treated soil with the selected plant materials, in general, gave a significant suppression of nematodes parameters i.e. galls, egg masses, females and developed stages/root system of eggplant plants infected with *M. incognita* and nematode density in soil compared to non-treated soil under controlled conditions of greenhouse. The reduction in number of J2s/250 g soil varied from treated and non-treated pots as the results cleared that the most effective treatment was *P. harmala* at 3% by 29 J2s/250 g soil followed by *A. absinthium* at 3% by 33 J2s/250 g soil while *A. officinarum* at 1% recorded less effective by 105 J2s/250 g soil. There was a gradual decrease in number of J2s/250 g soil with increase in concentration of each treatment (Fig. 1).

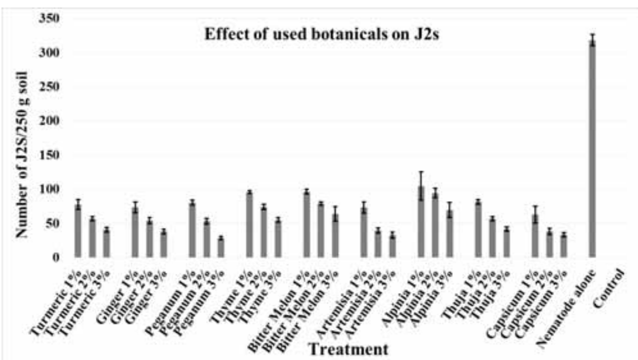

Fig. 1. Effect of used botanicals on number of J2s /250 g soil

Examination of eggplant root system showed that the number of nematode galls/root system was affected markedly by treatments. The results indicated that soils treated with *P. harmala* 3% have a very high reduction of galls /root system (89.3 % reduction) followed by *C. annum* 3% and *A. absinthium* at 3% by 87.6 and 87.1 % respectively while the least reduction was recorded with *A. officinarum* at 1% by 55.4 % compared with the control treatment (Fig. 2).

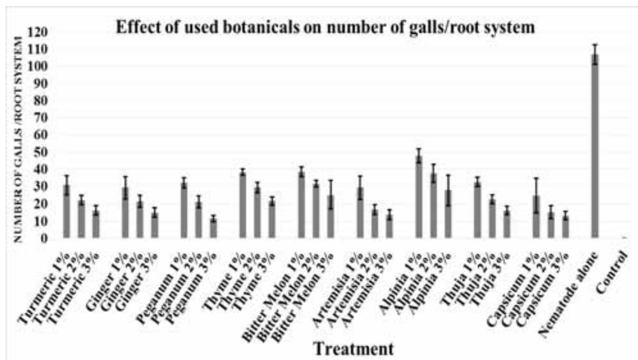

Fig. 2. Effect of used botanicals on number of galls /root system

Results also indicated that application of treatments at the different doses were effective in reducing the number of egg masses/root system compared with plants treated with nematode alone. The highest reduction was also recorded in plants treated by *P. harmala* 3% while *Z. officinale* 3% came in the second but the third one was *A. absinthium* at 3%, meanwhile *A. officinarum* at 1% came in the last compared with the control treatment (Fig. 3).

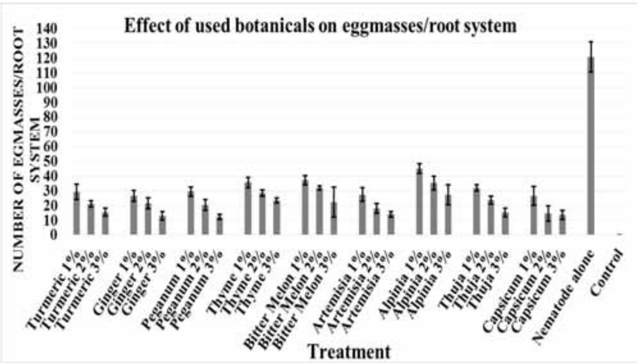

Fig. 3. Effect of used botanicals on number of eggmasses / root system

Observation of root system showed that number of females /root system was markedly affected by using the different plant materials (Fig. 4). *P. harmala* 3% was the effective treatment in reducing number of females followed by *Z. officinale* 3% while the least effective one was *A. officinarum* at 1% compared with the nematode alone.

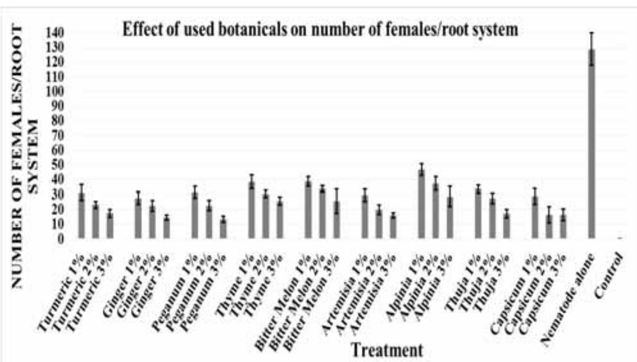

Fig. 4. Effect of used botanicals on number of females /root system

The reduction in nematode criteria participated in enhancement the plant growth parameters in treated plants by botanical materials compared to plants infested by nematode alone. Treatments at different rates generally resulted in an improvement of plant height and root length (cm) more than untreated plants as presented in Fig. (5) and Fig. (6) which revealed that the most effective treatment was *P. harmala* at 3% followed by *A. absinthium* at 3% and *C. annuum* 3%. The least plant height recorded with plants treated with *A. officinarum* at 1% compared to the control treatment (nematode alone).

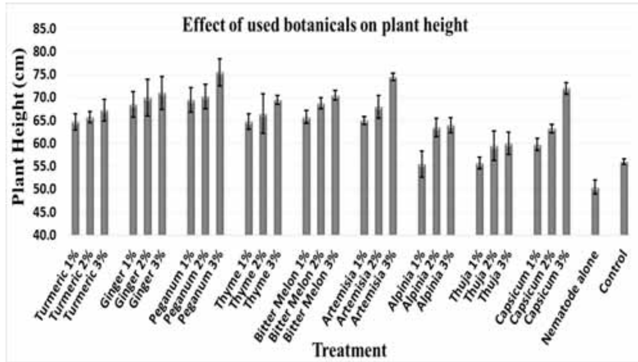

Fig. 5. Effect of used botanicals on plant height of eggplant plants

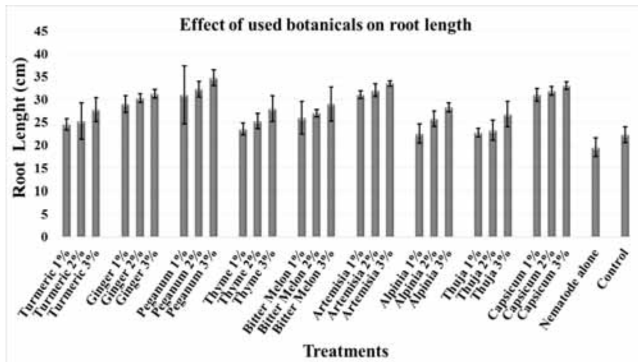

Fig. 6. Effect of different used plants on root length of eggplant plants

Obtained results proved that treated plants with the different treatments affected the root fresh weight of eggplant plants. The *P. harmala* 3% high followed by *C. annuum* 3% and *A. absinthium* at 3% respectively, while the least showed with *A. officinarum* at 1% (Fig. 7).

Results cleared that all treatments affected the eggplant fresh and dry shoot weight. Plants treated *P. harmala* 3% recorded highest fresh and shoot dry weight followed by *C. annuum* 3% and *A. absinthium* at 3% respectively, while plants treated with *A. officinarum* at 1% were the lighter (Figs. 8 and 9). Investigation of eggplant plants general status revealed that there is no phytotoxicity to the eggplant plants due to use of any treatment at the different concentration especially in shoot system, leaf color and area.

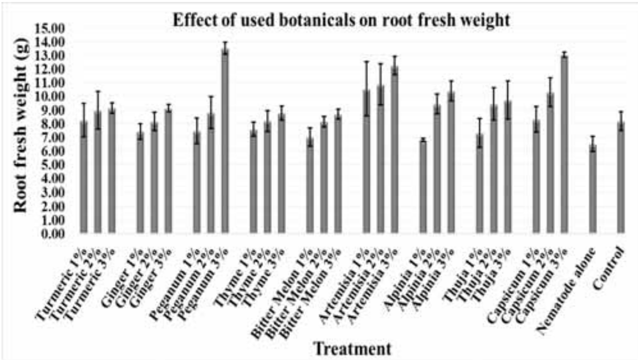

Fig. 7. Effect of used botanicals on root fresh weight of eggplant plant

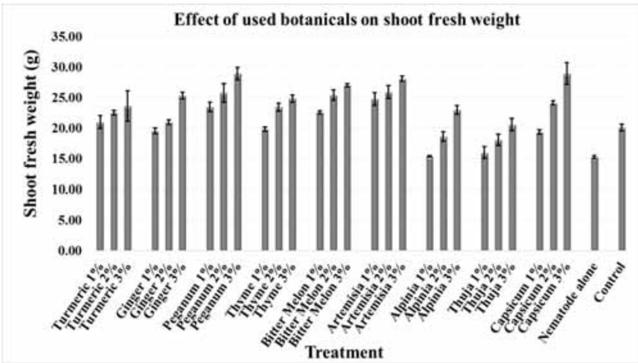

Fig. 8. Effect of used botanicals on shoot fresh weight of eggplant plants

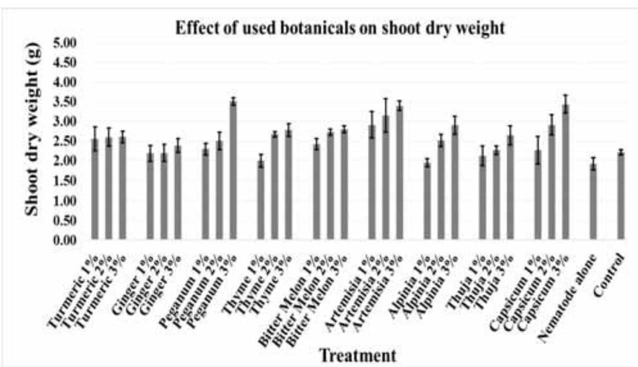

Fig. 9. Effect of used botanicals on shoot dry weight of eggplant plants

DISCUSSION

Results of the current study cleared that all treatments were superior over control. *Curcuma longa* showed satisfactory effect in nematode control. These results were in agreement with those obtained by (Youssef *et al*., 2015), who stated that turmeric showed nematicidal activity against *M. incognita* by decreased number of juveniles in soil, egg masses and galls, hatched juveniles on roots, and enhanced the eggplant plant growth parameters i.e. fresh shoot and root weight, shoots height and dry shoots and roots weights. Abbas *et al*., (2009); Neeraj *et al*., (2017) reported that *C. longa* significantly inhibited egg hatching of *M. javanica* and *M. incognita*. The nematode inhibitory activity may be refer to Curcumin and other derivatives from *Curcuma longa* (diacetyl curcumin sodium curcuminate, triethyl curcumin, tetrahydro curcumin, Bisdemethoxy curcumin, Methylcurcumin, Demethoxy curcumin and Sodium curcuminate) with biological activities as nematicidal compounds (Araujo and Leon, 2001). *Curcuma longa* is cultivated worldwide throughout subtropical and tropical regions. Its rhizomes exhibit some antioxidant effects, nematocidal and anti-bacteria activities (Youssef *et al*., 2015). The curcumin nematicidal potential may revealed to inhibit the activity of glutathione-S-transferase enzyme of *M. incognita*, responsible for survival of nematode in the host plants and the detoxification of *M. incognita* by antioxidant activity (Babu *et al*., 2012).

Zingiber officinale gave satisfied results in reducing nematode parameters. Plant extracts of ginger on the micro plots suppressed the root galling and population of *M. javanica* and *M. incognita* in tomato (Zasada, 2002). Results observed in the current research, have been also substantiated by some researchers (Abbas *et al*., 2009; Salim *et al*.,(2017); Ibrahim and Hussein, 2017). *Zingiber officinale* completely (100%) prevented attack and hatching of *M. incognita* eggs and destroyed juveniles at 1,000ppm concentrations (Bawa *et al*., 2014). *Zingiber officinale* also showed nematicidal activity on *M. incognita* infecting eggplant by decreasing nematode criteria in roots and soil (Youssef *et al*., 2015). The anthelmintic potential of *Z. officinale* may be revealed to the synergetic effect of active phyto constituents i.e. terpenes, alkaloids, steroids, saponins and flavonoids (Singh *et al*., 2011).

Peganum harmala showed the highest effect in reducing number of root galls, juveniles in soil, developmental stages and egg masses of *M. incognita* at the different dosage rates in comparison to control treatment and these results were similar to the findings of (Radwan *et al*., 2012; Saeed *et al*., 2015; Sholevarfard and Moosavi, 2015). The nematicidal properties could be attributed to some contents like those that harmal's alkaloids including carboline as harmine, harmaline, harmalol, harmol and Harman (Moloudizargari *et al*., 2013) which are considered as strong nematicides (El-Hassan *et al*., 2013). In addition, some of this component dissolve the nematode cell cytoplasmic membrane and the effective functional groups interfering with nematodes enzyme protein structures (Asif *et al*., 2017b).

Thymus vulgaris is a native flowering plant in Mediterranean Region. Thyme oil consists of carvacrol, thymol and other different monoterpenoids (Kubeczka and Formáèek, 2002). *Thymus vulgaris* had good nematicidal activity toward root-knot nematode *M. incognita* Race 2 (Nour El-Deen *et al*., 2016).

Findings cleared that *C. colocynthis* markedly affect plant growth and nematode parameters which is in accordance with (Chaudhary and Kaul, 2013; Nour El-Deen *et al*., 2016) who recorded similar results against *M. incognita* infecting Chilli pepper (*Capsicum annuum* L.), Pomegranate (*Punica granatum*) and *Vigna ungiculata* respectively. Rizvi and Shahina (2014) reported that fruit extract of *C. colocynthis* give 80% of *Meloidogyne* spp. larvae mortality after 72 h of exposure. The nematicidal effect of *C. colocynthis* may be due to its antioxidant activity according to the rich amounts of flavonoids and phenolics (Kumar *et al*., 2008) which may be possessing nematicidal properties such as: Colocynthin and Colocynthetin, Cucurbitacin A, B, C, D, E and α-aelaterin, and other constituents (Tannin-Spitz *et al*., 2007; Torkey *et al*., 2009). Early accumulation of reactive oxygen species and up regulation of plant antioxidants may play an essential act in resistance process to various plant pathogens (Ketta, 2015; Ketta *et al*., 2017).

Results established that *A. absinthium* affected root and shoot fresh weight and shoot dry weight and decreased number of galls, eggmasses and females /root system. This report is similar to those by (Sellami *et al*., 2010) who reported that *A. herba alta* oils suppressed larvae mortality and egg hatching of *M. incognita* from 27.7 to 100% and 18.2-62.8% respectively. The anthelmintic effect of *A. absinthium* may be due to richness of *Artemisia* genus by flavonoids and sesquiterpene lactones such as Artemisinin with nematicidal activity with no toxicity to mammalians (Kerboeuf *et al*., 2008). Artemisinin act by tow mode of action first, by alkylation the parasite specific protein and second by production of free radicals (O'neill *et al*., 2010).

Application of tomato plants by defferent concentrations of *A. officinarum* showed satisfactory effect in plant growth and nematode parameters. These data support previous reports by El-Sherbiny and Al-Yahya, (2011) of nematicidal activity of *A. officinarum* against root-knot nematodes, *M. incognita* on tomato. The nematicical activity of *A. officinarum* may be due to the rhizome main constituents identified such as: α-terpineol , 1,8-cineole, α-pinene β-pinene and terpinen-4-ol, camphor and α-fenchyl acetate (Raina *et al*., 2014). A nematicidal effect against *M. hapla* juveniles and eggs by *A. galanga* rhizome which contain the similar contents was recorded by Jeon *et al*. (2016).

In the current study, also *T. orientalis* effectively reduced the root-knot infection of eggplant and reduce root galling, nematode reproduction and enhancing the plant growth characters. This nematicidal activity of plants against *M. incognita* has been reported by Rather *et al*., (2008), who found that *T. orientalis* reduce root galling and significantly improve the tomato plant growth due to the application of these organic additives. Aqueous extract of *T. orientalis* affected the larvae mortality and egg hatching of *M. incognita* (Kavita *et al*., 2011). The inhibitory effect might be related to the chemical component of *T. orientalis* such as Thujone (Kyo *et al*., 1990). The higher contents of *T. orientalis* of certain oxygenated compounds with lipophilic properties may lysis the nematode cytoplasmic cell membranes and their functional groups connected with structure of enzyme protein (Knoblock *et al*., 1989).

Adding of *C. annuum* powder to eggplant plants undergreen house condition at the three concentrations showed a great reduction of *M. incognita* and inhanced plant growth. This result was in the same line by (Patel *et al*., 1993), who reported that chili pepper suppressed the population *M. javanica* and *M. incognita* in tomato. *Capsicum annuum* reducing the number of galls and eggs / system of tomato plants infected by *M. javanica* (Neves *et al*., 2009). Ethanol extract of *C. annuum* significantly inhibited egg hatching of *M. javanica* (Abbas *et al*., 2009) and *M. incognita* (Bawa *et al*., 2014). The observed increment in growth in tomato plants may be attributed to the effective nematode control. The larvicidal principles of *C. annuum* may be related to the compound piperolein alkaloid, pipernonaline. Piperamides such as capsaicin which belong to *Capsicum* genus has nematicidal properties (Neves *et al*., 2009).

Therefore, the use of these plants showsed a promise role to suppress root-knot nematode populations and may provide an alternative environmentally safe and economical method for nematode controlling. From current study, the nematicidal effect may be due to one /or more active ingredient so that, further studies under open field conditions is necessary to evaluate the feasibility of insertion these plants as a part of an integrated nematode management strategy. Also, developing formulation, propagation, stimulating mechanism to improve their efficacy and stability is recommended.

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