

Effects of Some Indigenous Plant Extracts on *Meloidogyne javanica* Infesting Eggplant and Pepper under Greenhouse Condition

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ABSTRACT

Among the major pests of vegetables are Root-Knot Nematodes (*Meloidogyne* spp.) (RKNs), which cause loss of production due to galling and reduction in root development and shoot growth. Herein, the efficacy of plant extracts of *Capsicum frutescens*, *Hyoscyamus niger*, *Melia azedarach*, *Xanthium strumarium* and *Achillea wilhelmsii* were evaluated at 3 concentrations (3, 6, and 12%) against *Meloidogyne javanica* on pepper and eggplant. Experiments were carried in pots under greenhouse condition, using pepper cv. Charleston and eggplant cv. Kemer as assay plants that are commonly cultivated in Turkey. Approximately 3,000 eggs of *M. javanica* were used for hatching test and 1,000 J2 of *M. javanica* were used for mortality test. Approximately, 5 mL of plant extracts were added by a syringe into the soil. Each experiment was arranged in a Randomized Block Design (RBD) with 5 replications. The control (+) pots received water containing *M. javanica* and the control (-) pots received only water. At the end of the experiment, plants heights and weights were measured. According to the results, all plant extracts showed a different level of nematicidal activity at 3, 6, and 12% concentrations. The plant extracts of *H. niger*, and *X. strumarium* at 12% concentration were found more efficient than *M. azedarach*, *C. frutescens* and *A. wilhelmsii* extract on egg hatching, on both pepper and eggplant plants. J2s mortality experiments showed that 12% concentration of *H. niger*, *M. azedarach* and *X. strumarium* were more effective against *M. javanica* than the other treatments, on both pepper and eggplant plants. In general, there was no significant difference was found among nematode mortality and growth parameters (such as plant height, the fresh and dry weights of the above-ground parts of the plants, fresh and dry weights of roots of both pepper and eggplant plants). Accordingly, using *H. niger* and *X. strumarium* plant extracts can provide effective methods of *M. javanica* control.

Keywords: Hatching test, Eggplant, Nematicidal effect, Pepper.

INTRODUCTION

Plant parasitic nematodes, especially Root-Knot Nematodes (RKNs) from the genus *Meloidogyne*, are widely distributed and cause significant yield losses in a wide range of crops (Davis, 2005; Luc *et al.*, 2005). *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* are the most commonly found root-

knot nematode species in Turkey (Kepenkci, 2012). Current management of nematodes has been attempted using plant resistance, crop rotation, cultural practices, biological control, or using chemical nematicides (Chitwood, 2002; Khan *et al.*, 2007, 2008, 2012). Traditionally, synthetic nematicides are used to control *Meloidogyne* spp. These synthetic nematicides increase production costs and have potentially

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negative impacts on the human health and the environment including on non-target organisms. New strategies are needed to substitute traditional chemicals such as antagonistic plants or plant extracts against plant-parasitic nematodes (Chitwood, 2002; Akhtar, 2004). Many plants including Brassicaceae, Asteraceae, Myrtaceae and Rutaceae families' member plants contain nematicidal compounds (Sukul, 1992; Andres *et al.*, 2012). The use of plant extracts as an alternative to synthetic pesticides for control of RKNs is becoming important and, in recent years, studies on plant extracts have accelerated (Lee, 2011, Ntalli *et al.*, 2011; Andres *et al.*, 2012; Oka, 2012). *Azadirachta indica* is well known as a pesticide and controlling insect, mite, nematode, and plant diseases (Agbenin *et al.* 2005; Bashir, 2013; Anwar, 2015; Benelli, 2015). *Tagetes* spp. includes a-Terthienyl and this content shows highly nematicidal activities against plant-parasitic nematodes, especially *Meloidogyne* spp. (Ploeg, 1999). Nematicidal effect of garlic has been studied against *Meloidogyne* spp. (Bekhiet *et al.*, 2010; El-Nagdi and Youssef, 2013). Therefore, the objective of this study was to determine the efficacy of plant extracts from *Capsicum frutescens*, *Hyoscyamus niger*, *Melia azedarah*, *Xanthium strumarium* and *Achillea wilhelmsii* as alternative nematicides against *Meloidogyne javanica* on pepper and eggplant under greenhouse condition.

MATERIALS AND METHODS

Nematodes

The egg masses of *M. javanica* were collected from tomato roots infected with the nematode (SC-2121 variety susceptible to RKNs). RKNs eggs were extracted from roots using a 0.575% NaOCl solution and the eggs were collected using the modified technique described by McClure *et al.* (1973). Eggs were washed by rinsing with tap water through a 75 μm sieves, collected

on a 26 μm sieve and transferred into the distilled water. The egg suspension was poured on to a cotton-wool filter and incubated at $26\pm 2^\circ\text{C}$. Emerged J2s were collected daily for up to 4 days and stored at 4°C until used for the experiment. The population density of J2 and eggs of *M. javanica* were determined from 3 replications of one mL subsample of an inoculum suspension. A total number of J2 and eggs were calculated by multiplying the mean number of nematodes per subsamples by the number of subsamples in the total volume.

Plant Material

Five selected indigenous plants were collected from different regions of Anatolia, Turkey. These were *Capsicum frutescens* (Solanaceae), *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), *Xanthium strumarium* (Asteraceae) and *Achillea wilhelmsii* (Asteraceae).

Preparation of Plant Extracts

Plants leaves were picked from their branches and spread on polythene in the laboratory for ten days to air dry. After that, they were dried at 80°C for 3-4 days. The dried plants parts were ground to fine particles using a blender. Ethanol was added to the ground plant powder and shaken on a rotary shaker at 120 rpm for 48 hours. The solution was filtered to remove solids and the material was vacuum concentrated in a rotary evaporator at $50-60^\circ\text{C}$ to obtain the corresponding organic crude extracts (Brauer and Davkota, 1990; Lee *et al.*, 2008). Each plant extract was prepared in $200\text{ g } 200\text{ mL}^{-1}$ and was used immediately in greenhouse tests. Suspensions of concentrations of 3, 6, and 12% were prepared with distilled water (Orisajo *et al.*, 2007).

Greenhouse Pot Experiments

According to treatment, approximately 3,000 eggs (egg hatching test) and 1,000 J2s (mortality test) of *M.javanica* were applied to the root of pepper and eggplants (Adekunle and Akinlua, 2007; Liman *et al.*, 2010). The whole plant extracts were applied at the same time with *M. javanica* on pepper (*C. annuum*, Charleston variety) and eggplant (*Solanum melongena*, Kemer variety) roots. Each experimental unit consisted of the plastic pot (10×10 cm) containing 800 cm³ sterilized loamy sand/pot (sterile moist loamy soil, 80% sand, 15% silt, and 5% clay) and a seedling of pepper or eggplant. Eggs or J2s of *M. javanica* were applied to 2 cm depth of the soil surface. Approximately 5 mL of extract was added by a syringe into the soil. The control (+) pots received water containing *M. javanica* and the control (-) pots received only water. Each experiment was arranged in a Randomized Block Design (RBD) with 5 replications. Greenhouse conditions were recorded by a data logger (HOBO-Onset computer cooperation, USA). During the experiment, the average temperature and the humidity were recorded at 25.04±4 °C and 30.14±10%, respectively. Nine weeks after inoculation, plants were uprooted and their roots gently washed with tap water. Galled roots were placed in an aqueous solution of phloxine B (0.15 g L⁻¹ tap water) for 15-20 minutes. After weighing, egg masses were counted. To facilitate counting of egg masses, they were stained red with phloxine B (Fenner, 1962; Dickson and Struble, 1965; Holbrook *et al.*, 1983). Root systems were rinsed in tap water to remove the residual stain from the roots, and egg masses were counted under a dissecting microscope. Plant height was measured from the base to the terminal bud. Fresh roots were weighed, then dried at 70°C for 48 hours in the incubator and weighed again. Data recorded included the total number of egg masses for each plant, plant height, the fresh and dry weight of the plant shoots and roots.

Statistical Analysis

Data were analyzed by analysis of variance, and means were compared using Duncan's multiple range test (SPSS, 1999). Differences were reported at $P \leq 0.05$.

RESULTS

Egg Hatching Test

The concentrations of 3, 6, and 12% of plant extracts displayed varied nematicidal effects on egg hatching of *M. javanica*. On eggplant, 12% concentration of *X.strumarium* showed the highest nematicidal effect (11.13±1.3). This was followed by 12% of *H. niger* on eggplant (15.53±2.4), 12% *X. strumarium* on pepper (17.83±1.3) and 12% of *H. niger* on pepper (19.53±3.4) (Figure 1-A). Their differences were found statistically significant ($P \leq 0.05$). Also, 3 and 6% concentrations of *C.frutescens* plant extracts influenced *M.javanica* both on pepper and eggplant, although this effect was not statistically important ($P \leq 0.05$). Although the low effect was observed in 3% and 6% concentrations of *H.niger*, the high effect was observed in 12% concentration. The 3, 6, and 12% concentrations of *M.azedarach* plant extract affected nematode both on pepper and eggplant, although these effects were not statistically important ($P \leq 0.05$). Treatments 3, 6, and 12% concentrations of *A. wilhelmsii* plant extracts had less influence on *M. javanica* on both pepper and eggplant (Figure 1-A). The concentration of 12% of *X. strumarium* and *H. niger* were highly effective against *M. javanica* compared with the other extracts. As the concentration increased, the nematicidal effects also increased against *M. javanica* on pepper and eggplant.

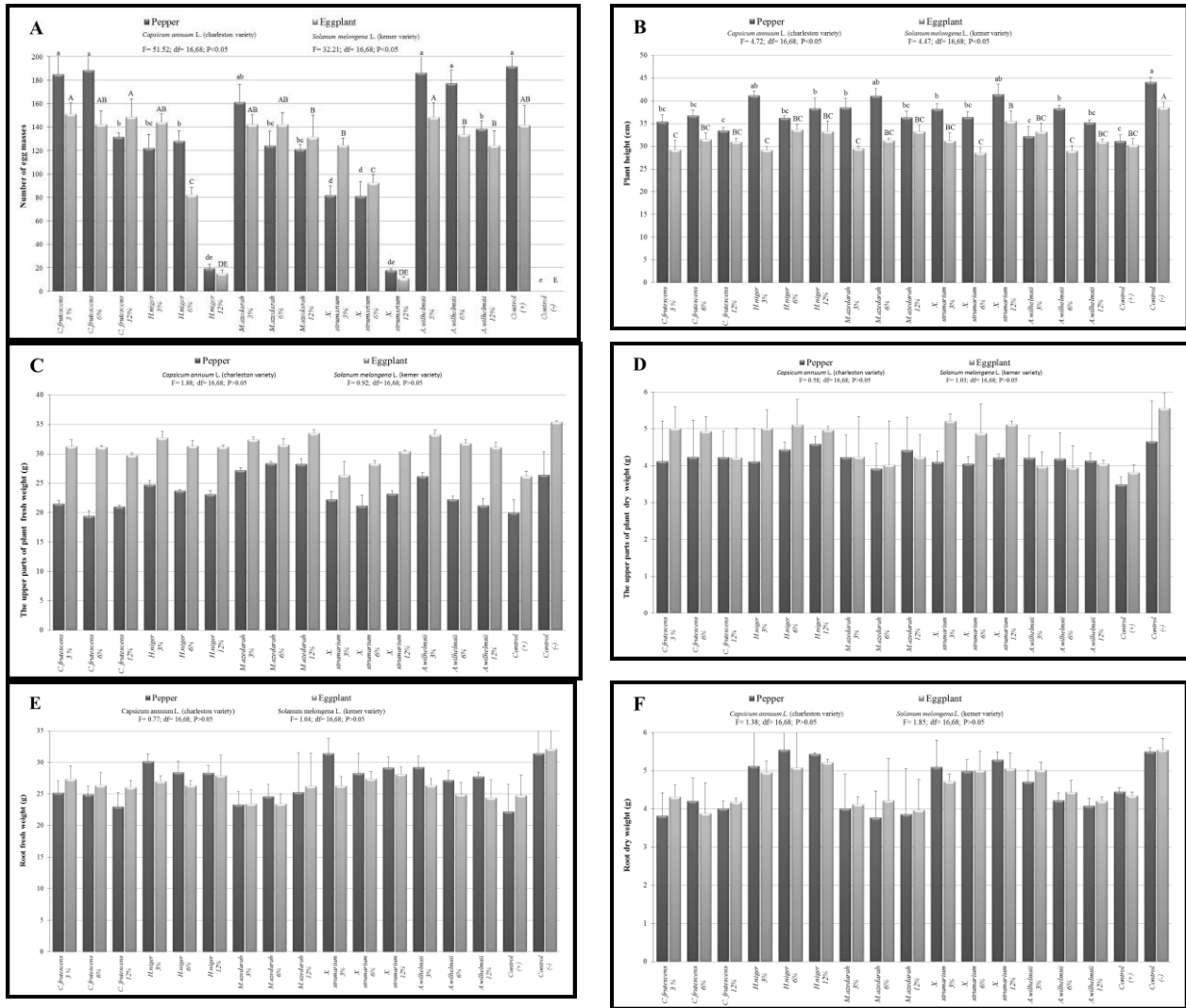


Figure 1. Effect of selected concentrations (3, 6 and 12%) of some indigenous plant extracts on *Meloidogyne javanica* reproduction in greenhouse pepper and eggplants. [(A) Total number of egg masses; (B) Plant height; (C) Fresh; (D) Dry weight of the aboveground parts of plants; (E) Fresh, and (F) Dry weights of roots].

J2 Mortality Test

Different effects were observed in 3, 6 and 12% concentrations of all plant extracts against 2. stage of *M.javanica*. The highest effects were observed at the concentration of 12% of *X. strumarium* (19.3±2.3) on eggplant. This was followed by 12% of *M. azedarach* (21.2±2.2) on pepper, 12% of *H. niger* (22.5±2.1) on eggplant, 12% of *M. azedarach* (33.3±3.8) on eggplant and 12% of *X. strumarium* (42.3±2.1) on pepper,

respectively (Figure 2-A). Their differences were statistically significant (P< 0.05). The concentrations of 3, 6, and 12% of *C. frutescens* and *A. wilhelmsii* plant extracts were less effective against *M. javanica* on both pepper and eggplant. Their extracts displayed lower nematicidal effect, therefore, this impact was not significantly important. Additionally, the 3% concentration of *H. niger* had a low nematicidal effect on both pepper and eggplant besides that 6% concentration of *H.niger* had lower effect against *M. javanica*

on eggplant (Figure 2-A). The increase in *M. azedarach* and *X. strumarium* concentrations led to increase in the nematocidal effect against *M. javanica* on pepper and eggplant.

Effect of Plant Extracts on Plant Growth

At the end of the egg hatching and J2

mortality test experiments, growth of pepper and eggplant plants were measured on plant height, the fresh and dry weight of the aboveground plant parts, and fresh and dry weight of roots. There was no significant difference between the control and plant extract treated plants in parameters such as plant height, the fresh and dry weight of the aboveground parts of plants, and fresh and dry weight of roots, both in pepper and

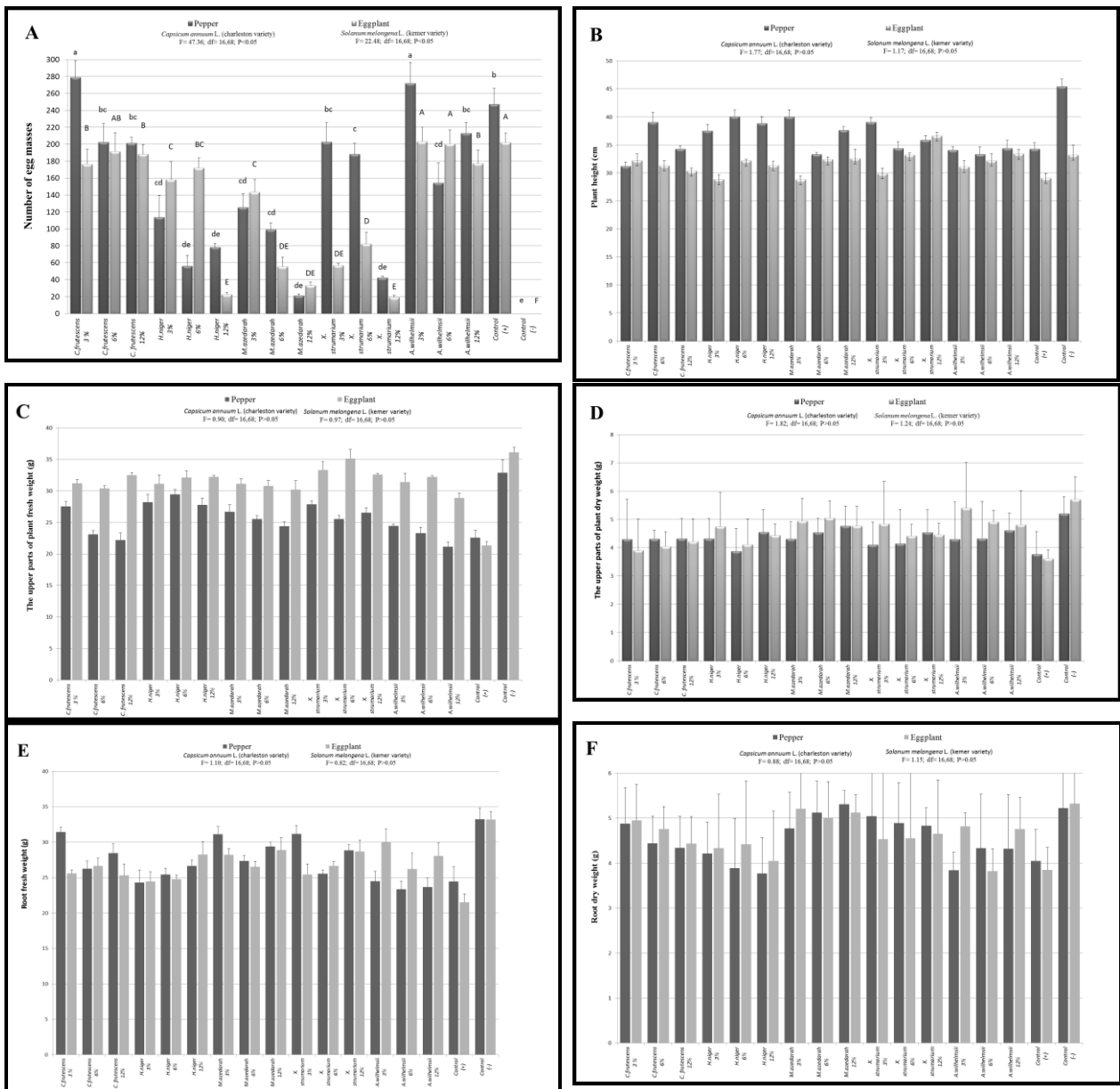


Figure 2. Effect of selected concentrations (3, 6 and 12%) of some indigenous plant extracts on *Meloidogyne javanica* reproduction (mortality test, 1000 J2s were applied) in greenhouse pepper and eggplants. Symbols A to F are defined in Figure 1.



eggplant plants from both egg hatching and mortality tests [Figures 1 (B-F) and 2 (B-F)] ($P < 0.05$). The 12% concentration of *X. strumarium* and *H. niger* plants extracts caused a significant reduction in root galling and nematode population on pepper and eggplant, even though the plant height, the fresh and dry weight of the aboveground plant parts, and fresh and dry weight of roots were not increased by using plant extracts [Figures 1 (B-F) and 2 (B-F)] ($P < 0.05$).

DISCUSSION

In this study, the nematicidal activities of *C. frutescens*, *H. niger*, *M. azedarach*, *X. strumarium* and *A. wilhelmsii* were evaluated on egg hatching and J2 mortality of *M. javanica* in pots of pepper and eggplant plants under greenhouse condition. The plant extracts used in our study showed a different level of nematicidal effect in a concentration-dependent manner. *C. frutescens* contains phytochemicals such as capsaicin and capsaicinoids, which are used to control plant diseases (*Aspergillus flavus*, *A. niger*, *Penicillium* sp. and *Rhizopus* sp.) (Soumya and Nair, 2012) and storage insects (*Callosobruchus maculatus* and *Sitophilus zeamais*) (Oni, 2011) and were found highly effective to them. In this study, *C. frutescens* was identified as having low nematicidal activities against *M. javanica* on pepper and eggplant plants. Kepenekci *et al.* (2016) applied *C. frutescens* extracts against *M. javanica* on tomato and found low nematicidal activity against nematode. Our study supports this finding. *H. niger* is known to be rich in tropane alkaloids. Tropane alkaloids of hyoscyamine and scopolamine show the insecticidal effect by affecting the activity of the neurotransmitter acetylcholine (Roddick, 1991; Shonle and Bergelson, 2000). Acetylcholine mechanism is enzymatic and inhibition of acetylcholinesterase is the target for the control of plant-parasitic nematodes by tropane alkaloids. The concentration 12% of *H. niger* showed higher nematicidal effect

on eggplant and pepper infected by *M. javanica*. Kepenekci *et al.* (2016) also reported similar results on tomatoes infected by *M. javanica* both *in vivo* and *in vitro* studies. *M. azedarach* has potent active ingredient azadirachtin. Azadirachtin is used for controlling insects, diseases, and weeds effectively. (Isman, 2006; El-Ghany *et al.*, 2015; Phuagphong *et al.*, 2015). Salgado *et al.* (2003) reported that 3 different (methanol, chloroform, and ethyl acetate) extracts of *M. azedarach* against *Meloidogyne exigua* were used and methanol and chloroform extracts of *M. azedarach* caused 94.3 and 82.5% mortality, while this rate was 48.7% for ethyl acetate. Ntalli *et al.* (2010) stated that melia methanol extracts higher than 0.08% showed higher nematicidal activity. In our study, the concentration of 6 and 12% of *M. azedarach* impact on mortality of J2 of *M. javanica* was higher both on eggplant and pepper plants. In contrast, all concentrations of *M. azedarach* extracts were had a lower effect on egg hatching on *M. javanica*. Chaudhary *et al.* (2013) reported that the nematicidal activities of ethanol and aqueous extracts of *X. strumarium* were tested against egg hatching and mortality of J2 of *M. incognita* and hot water and ethanol extracts showed lower effects on nematode, while Kepenekci *et al.* (2016) reported that *X. strumarium* demonstrated highly active nematicidal activities on tomato against *M. javanica*. The extract of *A. wilhelmsii* is known as an insecticide. It shows fumigant toxicity against stored pests (Asghari *et al.*, 2010). All concentration of *A. wilhelmsii* extracts displayed lower nematicidal activities in both egg hatching and mortality experiment on eggplant and pepper. According to the results of this study, *X. strumarium*, *M. azedarach* and *H. niger* present good inhibitory effect on nematode egg hatching and juvenile reproduction. The use of *M. azedarach*, *H. niger* and *X. strumarium* extract are suggested as potential substitutes for synthetic nematicides used in the management of RKNs disease in the greenhouse vegetable production. Further

studies will be conducted in the field conditions for determining the nematocidal ability of plant extracts. Therefore, the use of indigenous plant extracts should be considered in integrated pest management strategies. It is suggested that further trials be conducted in the field on the basis of the promising results from these studies. It is interesting to note that high concentration of the plant extracts showed a greater effect on egg hatching. Hence, we suggest that high concentrations of plants extracts are required for effective nematode control.

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اثرات عصاره های برخی گیاهان بومی روی *Meloidogyne javanica* آلوده کننده بادمجان و فلفل در شرایط گلخانه

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چکیده

نماتدهای گره ریشه (RKNs) (*Meloidogyne* spp.) از شمار آفات عمده سبزیجات است که می تواند به لحاظ ایجاد گال (galling) و کاهش توسعه ریشه و رشد اندام هوایی منجر به تلف شدن تولید شود. در این پژوهش، موثر بودن عصاره گیاهی *Hyoscyamus*، *Capsicum frutescens*، *Achillea wilhelmsii* و *Xanthium strumarium*، *Melia azedarach niger* در ۳ غلظت (۳٪، ۶٪ و ۱۲٪) بر علیه *Meloidogyne javanica* روی گیاه فلفل و بادمجان ارزیابی شد. آزمایش ها در گلدان در شرایط گلخانه روی فلفل کولتیوار چارلستون و بادمجان کولتیوار *Kemer* که در ترکیه به طور گسترده کشت می شود اجرا شد. برای آزمون از تخم بیرون آمدن (*hatching*) تقریباً ۳۰۰۰ تخم *M. javanica* و برای آزمون مرگ و میر (*mortality*)، *J2* ۱۰۰۰ از *M. javanica* استفاده شد. حدود ۵ میلی لیتر از عصاره گیاهی با سرنگ به خاک افزوده شد. طرح هر آزمایش بلوک های تصادفی (RBD) با ۵ تکرار بود. به گلدان های شاهد (+) آب حاوی *M. javanica* و به گلدان های شاهد (-) فقط آب داده شد. در آخر آزمون، بلندی و وزن گیاه اندازه گیری شد. بر اساس نتایج، همه عصاره های گیاهی در غلظت های ۳، ۶ و ۱۲٪ سطح متفاوتی از فعالیت ضد نماتی نشان دادند. در آزمون بیرون آمدن از تخم در هر دو گیاه فلفل و بادمجان، اثر عصاره های گیاهی *H. niger* و *X.*



A. wilhelmsii , *C. frutescens* *M. azedarach* در غلظت ۱۲٪ بیشتر از *strumarium* بود. نیز، در آزمون مرگ و میر *J2* در هر دو گیاه فلفل و بادمجان، عصاره *M. H. niger* و *azedarach* و *X. strumarium* در غلظت ۱۲٪ بر علیه *M. javanica* موثر تر از دیگر تیمارها بود. به طور کلی، هیچ اختلاف معناداری بین مرگ و میر نماتدها و پارامترهای رشد مانند بلندی گیاه، وزن تر و خشک اندام هوایی گیاهان و وزن تر و خشک ریشه های فلفل و بادمجان وجود نداشت. به این قرار، استفاده از عصاره گیاهان *H. niger* و *X. strumarium* می تواند روش موثری برای کنترل *M. javanica* فراهم کند.