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Orijinal araştırma (Original article)

Effects of some plant extracts on root-knot nematodes *in vitro* and *in vivo* conditions[1]

Bazı bitki ekstraktlarının kök-ur nematodlarına karşı etkinliğinin *in vitro* ve *in vivo* koşullarda araştırılması

İlker KEPENEKÇİ[2,3*] **Dolunay ERDOĞUŞ[4]** **Pervin ERDOĞAN[4]**

Summary

One of the major pests of the vegetables, root knot nematodes (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (RKNs) can cause economic losses by forming knots on the host plant roots. RKNs are more prevalent in the greenhouse vegetable growing areas of the coastal regions. In this study, the effects of plant extracts from five different plants; *Capsicum frutescens*, *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), *Xanthium strumarium* and *Achillea wilhelmsii* (Asteraceae) were evaluated against RKNs. In the first studies; the effects of plant extracts (0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% concentrations) on eggs, egg masses and second stage juveniles (J2s) of the *Meloidogyne incognita* were evaluated in laboratory conditions (*in vitro* tests). Concentrations of 3.0, 6.0 and 12.0% for *H. niger*, *X. strumarium* and *M. azedarach* caused 100% inhibition of egg hatching and J2s mortality. Effects of the same plant extracts against *M. javanica* on egg mass of nematode (I), the plant height (II), the plant age (III) plant dry weight (IV), root fresh weight (V) and root dry weight (VI) were also evaluated in greenhouse-pot studies. Result of pot trials, 12.0% of *H. niger* and *X. strumarium* has shown high effect on hatching studies. In the toxicity studies, 6.0% and 12.0% of *X. strumarium* and *M. azedarach* were found effective.Three plant extracts (*X. strumarium, H. niger* and *M. azedarach*), against mixed populations of *M. incognita* and *M. javanica* on tomatoes under natural greenhouse conditions (*in vivo*) were evaluated. Treatmets were repeated 4 times by watering for each pot (extract were applied 1 ml plant^{-1}). In greenhouse trials, root gal indicies (root knot) and crop yield (tomatoes) (kg plant^{-1}) values and effect of root gal indicies and yield (%) were evaluated. *In vivo* studies, it could be concluded that, only the *M. azedarach* has effected the galling indicies and consequentelly crop yield significantly.

Keywords: Root knot nematode, *Meloidogyne incognita*, *M. javanica,* plant extracts, tomato

Özet

Sebzelerin önemli zararlılarından biri olan ve bitki köklerinde urlar meydana getirerek ekonomik bağlamda ürün kayıplarına neden olan kök-ur nematodları (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (KUN) kıyı bölgelerimiz başta olmak üzere örtü altı sebze yetiştiriciliği yapılan tüm alanlarda yaygın olarak görülmektedir. Bu çalışmada, *Capsicum frutescens*, *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), *Xanthium strumarium* ve *Achillea wilhelmsii* (Asteraceae) bitkilerinden elde edilen bitkisel ekstraktların KUN'lere karşı etkileri araştırılmıştır. İlk çalışmalar laboratuvar-petri (*in vitro*) denemleri olarak yürütülmüştür. Denemlerde *Meloidogyne incognita* kültüründen elde edilen yumurta, yumurta paketleri ve 2. dönem larvalar (L2)'ına karşı ekstraktlar 6 farklı konsantrasyonda (0.5, 1.0, 1.5, 3.0, 6.0 ve %12.0) kullanılmıştır. *H. niger*, *X. strumarium* ve *M. azedarach'nın* 3.0, 6.0 ve %12.0 konsantrasyonları yumurta açılımına ve L2 (larvaya toksisite)'ye %100 etkili bulunmuştur. Diğer çalışmalar olan sera-saksı denemelerinde *M. javanica* kullanılmış; her bir bitki kökündeki yumurta paketi sayısı (I), bitkinin boyu (II), bitkinin yaş (III) ve kuru ağırlığı (IV), kök yaş (V) ve kuru ağırlığı (VI) parametreleri değerlendirilmiştir. Sera-saksı denemelerinden elde edilen sonuçlarına göre; kök-ur nematodu yumurta açılımına etkisi çalışmalarında, *H. niger* ve *X. strumarium*'un %12'lik konsantrasyonu yüksek etki göstermiştir. Nematod larvalarına karşı olan toksisite çalışmalarında ise *X. strumarium* ve *M. azedarach*'ın %6.0 ve 12.0'lik konsantrasyonları etkili bulunmuştur. Son çalışmalar doğa-sera çalışmaları (*in vivo*) olarak yürütülmüştür. Üç bitki ekstraktı (*X. strumarium, H. niger* ve *M. azedarach*) *M. incognita* ve *M. javanica* ile karışık popülasyonlar olarak bulaşık olduğu tespit edilen seralarda uygulanmıştır. Parsellere 1 ml bitki^{-1} olacak şekilde süzgeçli kovayla uygulanmıştır. Çalışmalar; kök indeks ve verim (kg bitki^{-1}) değerleri ile kök ur indeksi etki ve verim artışı (%) açısından değerlendirilmiştir. Doğa-sera çalışmaları sonucu, bitkisel ekstraktlardan sadece *M. azedarach*'nın köklerde meydana gelen urlanmalar ve verim açısından etkili olduğu söylenebilir.

Anahtar sözcükler: Kök ur nematodu, *Meloidogyne incognita*, *M. javanica,* bitkisel ekstraktlar, domates

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[2] Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, Turkey
[3] Kyrgyz Turkish Manas University, Faculty of Agriculture, Department of Plant Protection, Bishkek, Kyrgyzstan
[4] Plant Protection Central Research Institute, Department of Entomology, Ankara, Turkey
* Sorumlu yazar (Corresponding author) e-mail: kepenekci@gmail.com
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Introduction

Plant-parasitic nematodes are major pests in many countries, particularly in the tropics and subtropics, where they are recognized as the cause of serious yield losses on a wide range of crops (Luc et al., 2005; Sasser & Freckman, 1987). Among all plant-parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) (Tylenchina: Meloidogynidae) (RKNs), are economically the most important and agriculture productivity and quality limiting pathogens (Javed et al., 2006). The most destructive species is *Meloidogyne incognita* (Kofoid & White) Chitwood (Tylenchina: Meloidogynidae), which causes serious problems to tremendous number of economically important crops (Tsay et al., 2004). Nematode control is largely based on synthetic nematicides, which is expensive and potential risk to environment, consequentely non-target organisms. For more acceptable alternatives to chemicals, the possibilities are being investigated of exploiting nematode-antagonistic plants for the management of plant parasitic nematodes (Chitwood, 2002; Akhtar, 2004). Current management of nematodes are focused on plant resistance, crop rotation, cultural practices or chemical nematicides (Chitwood, 2002). Because of these disadvanteges, scientists found natural product with nematicidal activity such as plant extract, root exudates, plant volatiles etc. Linford et al., (1938) were the first to study the nematicidal effect of chopped pine-apple [*Annanas comosus* (L.) Merr. (Poales:Bromeliaceae)] leaves used as organic amendment against *Meloidogyne* spp., while a review of phytochemical strategies for the control of nematodes was given by Chitwood (2002). Numerous plant species, representing 57 families including Lamiaceae, Asteraceae, Myrtaceae, Rutaceae, Lauraceae, can contain nematicidal compounds (Sukul, 1992; Andres et al., 2012).

In Turkey, *M. incognita*, *M. arenaria* Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood are the most commonly found RKN species, with *M. incognita* being the most pathogenic and widespread (Kepenekci, 2012). The use of plant extracts as an alternative to synthetic pesticides for control of RKNs is becoming important. In recent years, research on this topic has increased rapidly in the Mediterranean coast (Ntallie et al., 2011; Andres et al., 2012).

The objective of current study was to determine the efficacy of plant extracts derived from *Capsicum frutescens* L., *Hyoscyamus niger* L. (Solanaceae), *Melia azedarach* L. (Meliaceae), *Xanthium strumarium* L. and *Achillea wilhelmsii* C.Koch (Asteraceae) as alternative to chemical nematicides. The effects of five different plants extracts on mortality of RKNs were investigated *in vitro* and *vivo* conditions.

Material and Methods

Plant material

Five indigenous plants namely; pepper (*Capsicum frutescens*), henbane (*Hyoscyamus niger*) (Solanaceae), bead-tree (*Melia azedarah*) (Meliaceae), common cocklebur (*Xanthium strumarium*) and yarrow (*Achillea wilhelmsii*) (Asteraceae) were collected from various ecological zones of Anatolia, Turkey.

Extraction

Plant leaves were plucked from their branches and spread on polythene sheets on benches in the laboratory for ten days to air dry. Then plants were dried at 80°C for 3-4 days. The dried materials were ground to fine particles using a blender. Ethanol was added to the ground plant material and shakenon arotary in a shaker at 120 rpm for 48 hours. The solution was filtered and the material was vacuumed in a rotary evaporator at 50-60°C to obtain organic crude extracts (ethanol is eliminated) (Brauer & Davkota, 1990). Each plant extract was prepared in 200 g/200 ml and were used immediately in all tests. Concentrations of 0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% was prepared with distilled water (Orisajo et al., 2007).

Culture root-knot nematodes

SC-2121 varieties of tomato [*Solanum lycopersicum* L. (Sloanaceae)] are known to be susceptible to RKNs (*Meloidogyne incognita* for labarotory-petri dish studies and *M. javanica* for greenhouse-pot studies), were sown into pots containing steriled soil and sand in greenhouse conditions at 25±1ºC temperature. Roots were well washed and were cut 1 centimeter in length and were shaken for 3.0-3.5 minutes in 1 liter of 0.525% NaOCl (Sodium hypochlorite) solution to extract nematodes eggs. Optained solution was sieved through 75 and 26 µm (200 and 500 mesh) sieves and nematode eggs were retained on 500 mesh opening sieve were collected in 100 ml size glass beakers (Hussey & Barker, 1973). The egg suspension was poured on to a cotton-wool filter and incubated at 26±2ºC. Emerged second stage juveniles (J2s) were collected daily for up to 4 days and stored fridge (4ºC) until used for experiment. To collect egg masses for labarotory-petri studies, tomato plants infected with a RKN (*M. incognita*) were carefully washed by tap water and egg-masses were hand-picked into Petri dishes containing distilled water.

Laboratory-petri dish studies (*in vitro*)

Nematicidal effect of plant extracts was evaluated on *M. incognita* under laboratory conditions. Plant suspensions of concentrations of 0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% were prepared with distilled water. A suspension of eggs, medium size 5 egg masses and J2s in distilled water was prepared.

Effects of plant extract on egg hatching**,** one ml of RKN eggs suspension containing 101–123 (110±5.5) eggs ml^{-1} added to 1 ml of selected plant extract and 3 ml of distilled water were transferred to sterilized Petri dishes. Distilled water used as a control. All treatments were kept at 28±2°C. After seven days of exposure, the numbers of hatched eggs were counted. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Medium sizes of 5 egg masses [1712–1794 (1768.3±25.3) J2s 5 egg masses ml^{-1}] added to 1 ml of the selected plant extract and 3 ml of distilled water were transferred to sterilized Petri dishes. Egg masses kept in distilled water as control. Each treatment was replicated 5 times. After 7 days exposure, the number of juveniles hatched was counted with the aid of inverted microscope at magnification 40×.

Effects of plant extracts (0.5, 1.0, 1.5, 3.0, 6.0 and 12.0% concentrations) on eggs and J2s of the *M. incognita* were evaluated in laboratory conditions. Suspensions of J2s [97-115 (103.7±4.1) J2s ml^{-1}] and eggs [101-123 (110±5.5) eggs ml^{-1}] in distilled water were prepared. One ml of J2s or eggs suspension, 1 ml of extract and 3 ml of distilled water was transferred in sterilized Petri dishes in five replicates while, distilled water used as a control and kept at 28±2°C. After 7 days of exposure, the numbers of dead RKN was counted. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Abbasi et al., 2008).

Greenhouse-pot studies

Tomato seeds (SC-2121 variety) were planted in 80 ml (5 cm diam. 4 cm height) pots filled with autoclaved peat. Two-four leaves, tomatoes seedlings were transferred individually to the approximately 340 ml plastic pots (7 cm diam. 7 cm height) containing 320 g sterilized loamy sand soil (80% sand, 15% silt and 5% clay). After 2 weeks, tomato seedlings (approximately 10 cm height) were inoculated with 3000 *M. javanica* eggs or 1000 J2s in 1 ml suspension (egg hatching and mortality test) (Adekunle & Akinlua, 2007; Liman et al., 2010). Seedlings which showed either development disorders or larger than average size were not used in the experiments. *Meloidogyne javanica* eggs or J2s were applied with a pipet to 2 cm deep holes around the seedling roots. Plant extracts and nematodes were applied the same time of holes explained above with the concentration of 3.0, 6.0 and 12.0% 1 ml extract per plant or pot. The control pots received only water (- control) and only nematodes (+ control) containing [negative (-)

control, water only (no nematodes were applied); positive (+) control, (nematodes only, 3000 eggs or 1000 J2s)]. The experiments contained five replicates (pots) for each treatment. The parameters; total number of egg masses (I), plant height (II), fresh (III) and dry weight of the green parts of plants (IV) and fresh (V) and roots dry weight (VI).

The experiment contained five replicates (pots) for each treatment, arranged in a randomized block design (blocked by row on the greenhouse bench in controlled conditions). Temperature [13.32-33.59ºC (22.02ºC ±4.14)] and humidity [23.40-77.10% (36.34%±9.25)] was monitored throughout the experimental periods in the trial. Bioassays were conducted in a greenhouse between October 2012 and May 2013.

Nine weeks after treatments, the plants were harvested, plants were cut one cm above the soil level to divide the roots from the above-ground portion of the plants and each section was weighed, then dried at 70°C for 48 hours and weighed again (Mohammad et al., 2007). To count egg masses, plant roots were stained red with phloxine B (Fenner, 1962; Dickson & Struble, 1965; Holbrook et al., 1983). Roots with nematod galls were placed in an aqueous solution of phloxine B (0.15 g L tap water^{-1}) for 15-20 min (Daykin & Hussey, 1985). Root systems were rinsed in tap water to remove residual stain from the roots, and egg masses were counted under a dissecting microscope or a magnifying glass with light (8×).

Nature-greenhouse studies (*in vivo*)

The results obtained from the greenhouse-pot studies caaried out in two different areas of Mediterranean (Kepez-Antalya) and Aegean (Fethiye-Muğla) Regions of Turkey. These regions where known to have planty of greenhouse crop production. All the studies conducted in the greenhouses were based on the “Standart Pesticide Trial Methods” (Anonymous, 2009).

Due to the non-homogenius distribution of the nematodes in nature, the trials were conducted as four replicates for each treatment and arranged in a randomized complete block designs in a naturally mixed populations of *M. incognita* and *M. javanica* occurring greenhouse. The characteristics of the trials were formed by plant extracts which had given promising results after greenhouse-pot studies, one of which has (+) control [nematicide, *Quillaja saponaria* (QL Agri ®), had been applied to this extract as comparison pesticide] and (-) control (only periodically upkept and watered).

All applications were performed with the filtered bucket. The trial plots were 8.0 m in length1.5 m in width and containing total of two rows and thirty-two plants. Two trial plots were seperated by a 2.5 m border line. Plant extracts (12% concentration *of M. azedarach, X. strumarium* and *H. niger*'s) were applied 4 times (early planting, with planting, 15th day after planting, 30th day after planting) with watering pot (extracts were applied 1 ml plant^{-1}). After application, drip irrigation was opened approximately 1 hour for diffusing the biopreparats in the soil.

In the greenhouses, Care F1 (in Kepez) and Ilgın F1 (in Fethiye) tomato cultivars had been used. In the nature-greenhouse applications; during the growing period of the tomato plant, the mature tomatoes had been weighed and the effects of the applications to the efficiency had been evaluated. At the end, twenty tomato plants were removed for each parcel and, the root galling of plants were evaluated according to the 0-10 Zeck scale (Zeck, 1971).

Regarding the effect of the applications to the efficiency the weighings had been started on the 22nd of September, 2014 in Kepez and on the 11th of January, 2015 the last weighing were recorded. The same day the tomato plants had been removed and evaluated for the root knots. In Fethiye the tomatoes that had become mature between the dates 12th of September, 2014 and 08th of January, 2015 had been weighed and 2 days after the last weighing the plants had been removed and had been evaluated regarding the root knots. For both greenhouses, not only the weighing data were recorded 8 to 10 times during the growing season but also in the intermittent periods the markatable tomatoes were recorded.

The galling index (I), the percentage effect (%) (II), efficiency (yields) (kg plant^{-1}) (III) and efficiency increase percentage (%) (IV) points of view were statistically evaluated.

During the trials, growing temperature and the humidity conditions in the greenhouse were recorded using HOBO (the temperatue and the humidity recorder) [Kepez, 14.46-42.22ºC (27.28ºC ±7.71) and 62.26-84.12% (76.57%±5.13); Fethiye, 15.57-43.12ºC (24.85ºC ±6.88) and 67.33-96.97% (86.24%±8.70)].

Nematodes diagnosis studies

Morphological and molecular diagnosis of nematodes taken from infected roots of tomatoes from natural greenhouses –from Kepez (Antalya), Mediterranean Region and Fethiye (Muğla), Aegean Region– were performed. Our result indicated that both greenhouse locations were contaminated with mixed population of *M. incognita* and *M. javanica*. Greenhouses in Fethiye were sustained mostly *M. incognita* and in Kepez however, sustained mostly *M. javanica*.

Statistical analysis

Analyses of varians were applied to the obtained data by using SPSS (1999) software. The found effects were compared to the controls and means of data groups were separated by Duncan multiple range test.

Results and Discussion

In this study, the nematicidal activities of pepper (*Capsicum frutescens*), henbane (*Hyoscyamus niger*), bead-tree (*Melia azedarach*), common cocklebur (*Xanthium strumarium*) and yarrow (*Achillea wilhelmsii*) were evaluated by root-knot nematodes (*Meloidogyne* spp.) (RKNs) hatching and mortality test.

Laboratory-petri dish studies (*in vitro*): In trials performed at concentrations of 0.5%, 1.0% and 1.5%, the effect of treatments against the J2s (larva toxicity to second stage juveniles) was determined to be too low. The effect was stronger when the concentrations were raised to 3%, 6% and 12%. Compared with the other plant extracts, the *A. wilhelmsii* and *C. frutescens* were found to be less effective in terms of killing capacity. The 6% and 12% concentrations of *H. niger*, *M. azedarach* and *X. strumarium* had the highest killing capacity by killing all applied J2s. Higher concentrations were associated with higher death rates (Table 1). Similar results were observed with the egg hatching trial rate, and the 6% and 12% concentrations of *H. niger*, *M. azedarach* and *X. strumarium* as well as the 3% concentrations were found to be 100% effective on egg hatching (no J2s hatching) (Table 1). According to these results of laboratory-petri dish studies, we decided to use 3%, 6% and 12% concentrations for all the plant extracts in the greenhouse-pot trials (Table 1).

Greenhouse-pot studies: The effect to the egg hatching and J2 mortality of nematodes in the greenhouse-pot trials were evaluated separately. When we evaluate the number of egg masses on the roots of the tomato; regarding the egg hatching; with the maximum concentration (12%) of *X. strumarium* has the maximum effect (10.4 egg masses per plant) and followed by *H. niger* (19.8 egg masses per plant). Although the effect to the larval toxicity of the *M. azedarach* was great, it has low effect on the egg hatching. There counted 102.2 egg masses per plant in the 12% concentration of *M. azedarach* application. In the control group there determined 196.2 egg masses per plant (Figure 1A). Regarding the larval toxicity the 12% concentration of *M. azedarach* has the maximum effect with 3.6 egg masses. The 12% concentration of *X. strumarium* and 6% *M. azedarach* followed by 33.8 and 38.0 egg masses per plant. *H.n*, which is effective to the egg hatching has low effect for the larval toxicity. In the control group 166.2 average number of egg masses had been determined ($F= 11.92$; df: 16.68; $P<0.05$) (Figure 1 A).

Table 1. Effect of various concentrations of some plant extracts *Melia azedarach, Xanthium strumarium, Hyoscyamus niger, Achillea wilhelmsii* and *Capsicum frutescens* on *Meloidogyne incognita* survival under laboratory conditions. Data are expressed as mean±SD

Treatment		Hatch inhibition and mortality of J2s after 7 days Tested concentration					
		0.5%	1%	1.5%	3%	6%	12%
C. frutescens	E*	108±4.3 b	97±5.2 b	22.6±8.9 b	8±5.2 a	3.6±1.1 a	2.8±1.6 a
	EM**	1737±62.1 *c*	1546.4±71.8 *c*	66.2±27 *c*	8.6±5.4 *a*	4.4±3.7 *a*	3.8±2.4 *a*
	J2s***	104.4±4.8 B	98.4±5.3B	36.6±16.3 B	12.4±3.2 ab	9.2±2.6 a	5.2±1.9 a
H. niger	E	42±9.13 a	50.8±12.1 ab	16±11.8 ab	0 a	0 a	0 a
	EM	1212.2±5.8 *b*	853±94.1 *b*	8.6±5.4 *a*	0 *a*	0 *a*	0 *a*
	J2s	49.2±11.7 A	38.6±11.1 AB	13.4±7.8 AB	0 A	0 A	0 A
M. azedarach	E	99±12.2 b	80.4±1.36 ab	14.4±6.4 ab	0 a	0 a	0 a
	EM	1695.4±61.5 *c*	1007.8±103.3 *b*	24±13.7 *ab*	0 *a*	0 *a*	0 *a*
	J2s	86.8±16 AB	36.4±4.9 AB	7.2±3 A	0 A	0 A	0 A
X. strumarium	E	46±19.3 a	18.4±7.8 a	4.6±4.5 a	0 a	0 a	0 a
	EM	1153.8±51.3 *a*	608.2±171.2 *a*	9.8±5.9 *a*	0 *a*	0 *a*	0 *a*
	J2s	53.4±13.7 A	18.8±8.3 A	8.6±5.8 A	0 A	0 A	0 A
A. wilhelmsii	E	107.8±5.4 b	85±6.6 ab	27±10.1 b	7±5.3 a	3.8±1.3 a	3.4±1.5 a
	EM	1730±32 *c*	1518.4±122 *c*	40.4±13.1 *bc*	12.2±4.8 *a*	7.6±4.5 *a*	4.4±2.5 *a*
	J2s	103.2±3.8 B	90.8±7 B	38.6±4.5 B	15.6±3.3 AB	10.6±2.9 AB	5.4±2.1 A
Control (water only)	E	114.2±2.58 b	109.4±5.2 b	111.4±6.9 c	108.4±6 b	107.4±4.9 b	109.2±6.3 a
	EM	1758.8±28.9 *c*	1766.6±28.3 *d*	1777.8±17.4 *d*	1763.8±29.8 *b*	1775.8±21.7 *b*	1767±31.7 *b*
	J2s	106.8±7 B	103.2±4.2 B	104.4±4.9 B	102.4±1.6 B	103.2±3 B	102.2±1.7 B

* Eggs ml^{-1} (lower case letters indicate significant, P<0.05), eggs suspension [101-123 (110±5.5) eggs ml^{-1}] (E)
** 5 egg masses which J2s out of ml^{-1} (italicized letters indicate significant differences, P<0.05), medium size 5 egg masses [1712-17.94 (1768.3±25.3] juvenile 5 egg masses ml^{-1}) (EM)
*** Second stage juveniles (upper case letters indicate significant differences, P<0.05), juvenile suspension [97-115 (103.7±4.1) juvenile ml^{-1}] (J2s).

Regarding the plant length, from the egg hatching point of view the best effect comes from the 12% concentration of *X. strumarium* which is same with the negative control (44.8 cm). The same concentration of *H. niger* followed this with 42.3 cm but had taken place in a different group with the control group statistically. In the same trials the applications which had been under positive control (33.6 cm) which are *C. frutescens* at 3%, 6%; *M. azedarach* at 3%; *X. strumarium* at 3%, 6%; and *A. wilhelmsii* at 6% concentrations (28.6, 29.1, 28, 32.2, 26.2, 24.6 and 28.8 cm respectively) (F=7.98; df:16.68; P<0.05) (Figure 1B). In the larval toxicity trials; although the maximum effect had come from 12% concentration of *M. azedarach* with 42.5 cm. It statistically did not take place in the same group with the -control 45.2 cm. All the plants in the other applications were found out that they are under the +control (40.6 cm.) (F= 3.77; df:16.68; P<0.05) (Figure 1B).

Regarding the egg hatching, between the plant upper parts fresh weight and dry weight (Figure 1C, D) and root dry weight (Figure 1F) parameters no difference had been found statistically except the plant upper parts fresh weight and the fresh root weight in the larval toxicity trials (P>0.05).

The effect to the larval toxicity trials, the maximum effect about fresh root weight was found out for the 3, 6 and 12% concentrations of *M. azedarach* as 15.11, 15.74 and 15.76 g. Among these applications the minimum concentration application 3% is under –control (15.5 g). In all other applications, except the all concentrations of *X. strumarium* (13.79, 14.4 and 14.39 g) were under +control (13.34 g) (F=12.10; df:16.68; P<0.05) (Figure 1 E).

For the “egg hatching” point of view, the maximum effect (1.84 g) for the plant upper parts dry weight comes from the 12% concentration of *X. strumarium* applications which was higher than the -control (1.78 g). Except 12% *H. niger* and 3% *X. strumarium* applications (1.64 and 1.62 g) all the other applications were under +control (1.60 g) ($F=4.84$; df: 16.68; $P<0.05$) (Figure 1 F). When the trials were evaluated regarding the root weight terms; for the fresh root weight the maximum effect had come from 3% concentration of *X. strumarium* with 15.2 g. In the applications the –control had 14.3 g and the +control had 16.4 g root weight ($F=2.55$; df:16.68; $P<0.05$) (Figure 1 E). Regarding the root dry weight terms, although the two applications (for 12% concentration of *H. niger* it is 1.84 g and for 12% *X. strumarium* 1.86 g) had the maximum effect, they had been under the –control value of 2.08 g of root weight. All the other applications were under +control value of 1.78 g of root weight ($F=4.24$; df:16.68; $P<0.05$) (Figure 1 F).

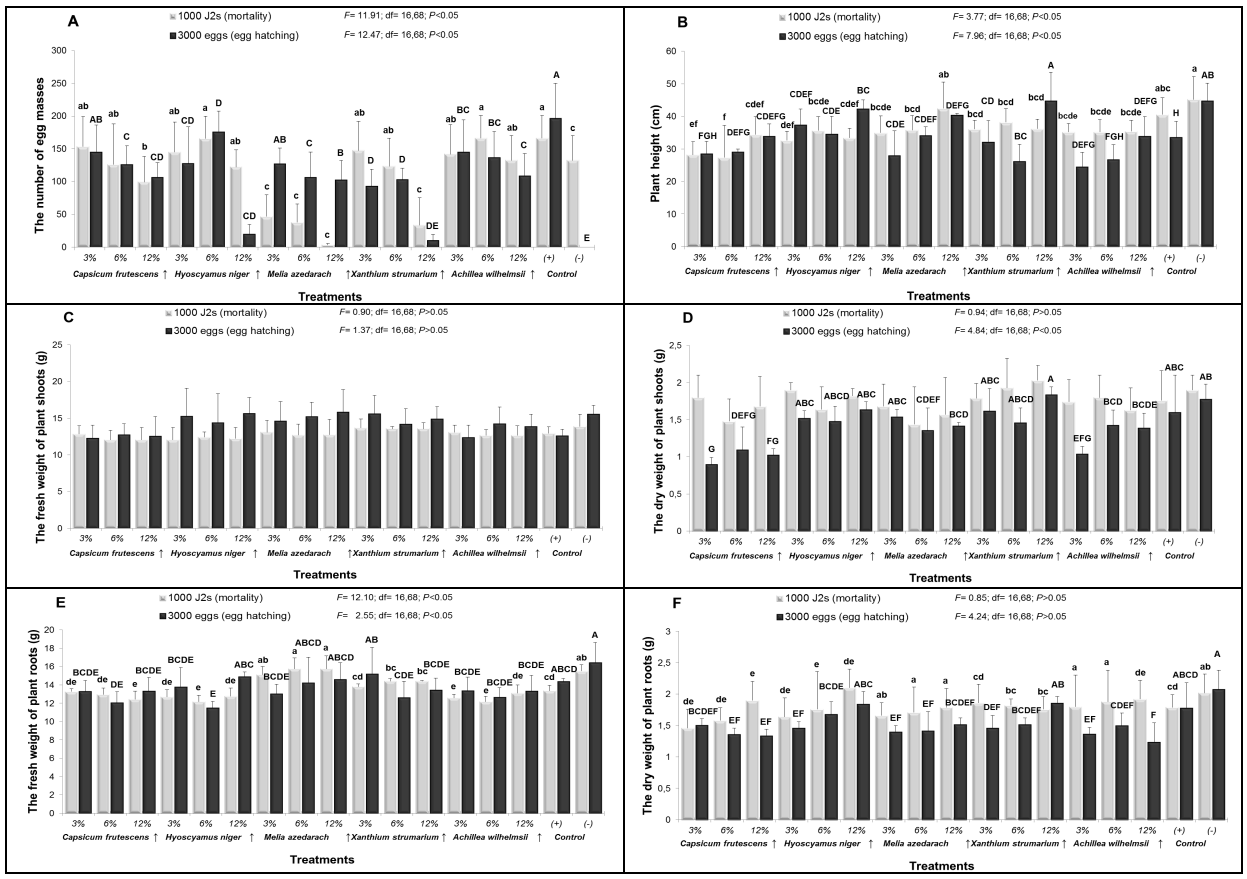

Figure 1. Effect of selected concentrations (3%, 6% and 12%) of five indigenous plant extracts (*Melia azedarach, Xanthium strumarium, Hyoscyamus niger, Achillea wilhelmsii* and *Capsicum frutescens*) on *Meloidogyne javanica* reproduction (egg hatching test, 3000 eggs were applied and mortality test, 1000 J2s were applied) under greenhouse tomatoes (SC-2121 variety) [negative (-) control, water only (no *M. javanica* eggs were applied); positive (+) control, (nematodes only, 3000 eggs or 1000 J2s)] [(A) total number of egg masses, (B) plant height, (C) fresh and (D) dry weight of the upper parts of plants and (E) fresh and (F) dry roots weight].

As a significant results of all greenhouse-pot experiments (both of all trials, egg hatching and larval toxicity); the number of egg masses found in the roots of tomato plants were evaluated; plant extracts of *X. strumarium* at 12% for egg hatching trials and *M. azedarach* at 12% for larval toxicity trials were reduced the nematode reproduction (10.4 and 3.6 egg masses per plant respectively) on tomato roots compared to control groups (196.2 and 166.2 egg masses per plant respectively).

Nature-greenhouse studies (*in vivo*): Nature-greenhouse trials were evaluated separately regarding the root index and efficiency. In the Fethiye trials (Figure 2A, B); the *M. azedarach*, which had

the maximum effect and which had the minimum root index or which caused the least galls on the tomatoes roots with a 2.97 root index was taken place statistically in the same group with a registered and +control [nematicide, *Quillaja saponaria* (QL Agri ®)]. *X. strumarium* and *H. niger* (7.35 and 7.53) had quite low effects and were taken place in the –control (8.01) groups (negative control was only applied water by drip irrigation) in the parcels-where no application were made and only watered- which were contaminated with the nematodes (mixed populations of *M. incognita* and *M. javanica* on tomatoes under natural greenhouse conditions) ($F=17.307$; df:4.19; $P<0.05$) (Figure 2 A). The *M. azedarach* applications had showed the maximum effect (62.54%) as being the closest to the value of +control (72.45%) root knot index effect (%). The applications that had high effects statistically were taken place in the same +control groups. The *H. niger* and *X. strumarium* applications (6.08 and 7.59%) that takes place in the same group with –control seems to have the quite low effect ($F=21.823$; df:4.19; $P<0.05$) (Figure 2 B). When the trials were evaluated regarding the efficiency terms, the maximum tomato efficiency (yields of tomatoes) were provided by the *M. azedarach* applications (3.90 g per plant). This application were statistically taken place in a different group and determined different than the +control (4.50 g per plant). The *H. niger* and *X. strumarium* applications (3.62 and 3.12 g per plant) had provided low efficiency and the *X. strumarium* from these applications were statistically taken place in the same group with the –control groups (2.95 g per plant) ($F=8.331$; df: 4.19; $P<0.05$) (Figure 2 A). When the trials were evaluated regarding the efficiency increase terms there were no applications found that could take place in the same group with +control (63.06%). The efficiency increase in the whole three applications were found low and they statistically were taken place in the same group (11.46, 31.30 and 39.41%) ($F=1.697$; df: 4.19; $P<0.05$) (Figure 2 B). When the Kepez (Antalya, Turkey) trials (Figure 2 C, D) were evaluated regarding the root index terms, the maximum effect from *M. azedarach* applications (3.56) and this *M. azedarach* applications were statistically taken place in the same group with +control (2.21). In the trials that were set up, the *X. strumarium* and *H. niger* (7.53 and 7.62) had quite low effect and were taken place in the same group with -control (8.40) ($F=11.764$; df: 4.19; $P<0.05$) (Figure 2 C). The *M. azedarach* application (45.05%) had the maximum effect and had the closest value to the +control (73.69) root knot index effect (%). The *H. niger* and *X. strumarium* applications (9.04 and 10.28%) determined to have the quite low effect ($F=11.764$; df:4.19; $P<0.05$) (Figure 2 D). When the trials evaluated regarding the efficiency, the highest tomato efficiency comes from the *M. azedarach* applications (2.94 kg per plant). This application had been statistically taken place in the same group with +control (2.95 kg per plant). Low efficiency was obtained from the *H. niger* and *X. strumarium* applications (2.39 and 2.65 kg per plant). In the –control group in this study 2.11 kg plant^{-1} efficiency was obtained ($F=8.614$; df:4.19; $P<0.05$) (Figure 2 C). When the trials were evaluated regarding the efficiency increase (%) terms, the *M. azedarach* applications had the highest efficiency increase with a value of 39.82% and had statistically taken place in the same group with the +control applications (40.19%) ($F=4.042$; df:4.19; $P<0.05$) (Figure 2 D).

The effect of *M. azedarach* on root galling was the highest in Fethiye compared to remaining *X. strumarium* and *H. niger* treatments in nature-greenhouse studies. Also, in Kepez *M. azedarach* sustained the highest effect and fall into the same group with + controls (nematicide). When the nature-greenhouse studies were evaluated together with the all trials those had been set up in Kepez and Fethiye; only *M. azedarach* had effect on the root knots and regarding the efficiency terms had effect on the root-knot nematodes (Figure 2).

Evaluated the +control parcels of the both greenhouses, the efficiency of the trials in Kepez were lower than the Fethiye. In Fethiye, sustained a quite high efficiency than those of in Kepez. In the control parcels of both greenhouses it had been determined 4.50 kg plant^{-1} for Fethiye and 2.95 kg plant^{-1} for Kepez. Besides the greenhouses were quite similar, regarding the root knot index [control without nematicide, only water used for (-control)] the tomato plant roots in Kepez had more knots than the other greenhouse (Fethiye). In the control parcels index values were found out as 8.01 for Fethiye and 8.40 for Kepez (Figure 2).

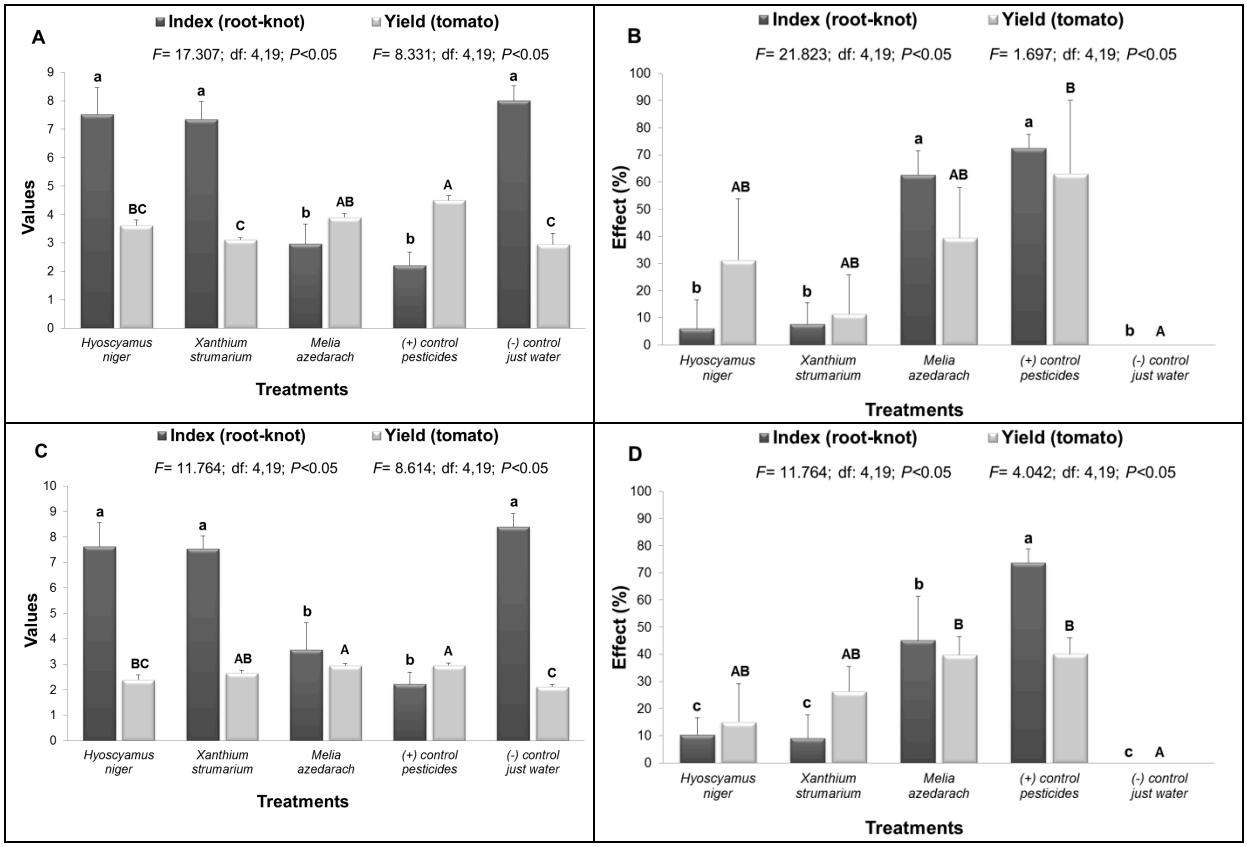

Figure 2. Effect of three indigenous plant extracts (*Melia azedarach, Xanthium strumarium* and *Hyoscyamus niger*) is naturally contaminated mixed populations of *Meloidogyne incognita* and *M. javanica* at Aegean (Fethiye-Muğla) (A, B) and Mediterranean (Kepez-Antalya) (C, D) regions where greenhouse products are widespread [negative (-) control, only periodically upkept and applied water by drip irrigation; positive (+) control, nematicide, *Quillaja saponaria* (QL Agri ®), had been applied to this extract as comparison pesticide] [the index (root knot) values, the index percentage effect (%), efficiency (yields) (kg plant^{-1}) and efficiency increase percentage (%)].

The result of this experiment showed that aquatic plant extract of *C. frutescens, H. niger, M. azedarach, X. strumarium* and *A. wilhelmsii* were toxic to *M. javanica in-vitro* conditions. Nematoxic effects were found even at the relatively low concentrations used in these experiments. Active ingredients of extract were effectively ensured to nematodes. Highest percentages of egg hatch and life activities of nematodes were monitored by control. *A. wilhelmsii* which is known poisonous plants use as an insecticides (Baytop, 1997; Çalmaşur et al*.,* 2006; Erdoğan et al., 2010; Khani & Asghari, 2012). Many studies were showed that *Achillea* sp. has antibacterial properties (Barel et al., 1991). *Achillea wilhelmsii* shows more effective nematicide effect then *Artemisia millefolium* L. (Asteraceae) (Dias et al., 2000; Ardekani et al., 2010). Ntallie et al. (2011) was studied some plant essential oils nematicidal activities against *M. incognita* so *A. millefolium* were not found to be nematicidal effect. Oka et al*.* (2000) showed that *A. fragrantissima* (Forssk.) Sch.Bip. (Asteraceae) was not effective against *M. javanica* on tomatoes plants. This plant extract was not demonstrated as a nematicide in our country. This study was showed that *A. wilhelmsii* has a low nematicide effect on root-knot nematodes. *H. niger*, a poisonous plant is used for medicinal purposes. Dried leaves of *H. niger* in enclose area used as a repellent against mice (Coffey, 1994). There were undesignated as nematicide in any study of *H. niger* was found to be effective in our experiment against *M. javanica*. Bead-tree is common in the Mediterranean region in Turkey. It was known that their leaves and fruits were used as pesticides in many years (Yelekçi et al., 1981; Erdoğan & Toros, 2005). *M. azedarach* was widely studied and successful results were obtained (Lee, 1990; Hasabo & Noweer, 2005; Maregiani et al., 2010; Rehman et al., 2012). In this study, the results have been promising. In particular has been demonstrated to be effective against the root-knot nematodes. Common

cocklebur is widely distributed all around the world. Many studies have been done with this plant in our country and other countries (Çetinsoy et al., 1998; Erdoğan & Toros, 2007). On this plant extracts have been done considerable nematological study so far and got effective results (Bala et al*.,* 1986; Nandal & Bhatti, 1986; Malik et al*.,* 1988; Shaukat & Siddiqui, 2001). Some study show that this plant extract effects egg hatch (Mennan et al., 2000). With this study, *X. strumarium* extract has been found to be successful inhibit egg hatching. *C. frutescens*, have been content many chemicals such as capsaicin, capsainoids and allyl isothiocyanate, are widely used as pesticide and their capsaicin and its analogues content have shown inhibitory activities towards the pests (Abbas et al., 2009; Mackeen et al., 1997). *C. frutescens* which is common used plant in Turkey has not been found any studies against nematodes. This study showed that *C. frutescens* plant extract didn't affect nematodes so much as similar effect show up *A. wilhlmsii*.

According to *X. strumarium, M. azedarach* and *H. niger* are a good inhibitor of nematode egg hatching and juvenile survival in this study. *X. strumarium, M. azedarach* and *H. niger* were found highly effective against RKNs. *H. niger* and *M. azedarach* extracts may be due to possessing ovicidal and larvicidal properties. *M. azedarach* is demonstrated more effective than other plant extracts and necessities has been arisen work on it and evaluate their results. This is a first time known that the effect of *H. niger* against nematode were revealed in the world. The use of *M. azedarach* extracts are suggested as a potential substitute for synthetic nematicides used in the management of RKNs in the greenhouse vegetable growing areas of the coastal regions.

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