Egyptian Journal of Biological Pest Control, 25(1), 2015, 39-44

Extracts of Some Indigenous Plants Affecting Hatching and Mortality in the Root-Knot Nematode [*Meloidogyne javanica* (Treub) Chitwood]

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(*Received: December 6, 2014 and Accepted: January 12, 2015*)

ABSTRACT

The root-knot nematodes (*Meloidogyne* spp.) (RKNs) are one of the major pests of vegetables which cause yield losses due to galling and reduction in root development and shoot growth. Herein, effects of five different plants extracts; pepper, *Capsicum frutescents* (*Cf*); henbane, *Hyoscyamus niger* (*Hn*); bead-tree, *Melia azedarah* (*Ma*); common cocklebur, *Xanthium strumarium* (*Xs*); and yarrow, *Achillea wilhelmsii* (*Aw*) on eggs and second stage juveniles (J2s) of the RKN (*Meloidogyne javanica*) were evaluated *in vitro* tests. All plant extracts showed different levels of anti-nematode's activity. Plant extract concentrations of 3, 6 and 12% for *Hn*, *Xs* and *Ma* caused 100% inhibition of egg hatching, followed by *Cf* and *Aw*. *Ma* and *Xs* were more effective for inducing mortality among J2s than the other tested extracts.

Key words: Root-knot nematode, *Meloidogyne javanica*, plant extracts, hatching, mortality.

INTRODUCTION

Plant-parasitic nematodes, particularly root-knot nematodes (RKNs), *Meloidogyne* spp., are widely distributed and cause significant yield losses in a wide range of crops (Luc *et al*., 2005). Current management of nematodes has been attempted using plant resistance, crop rotation, cultural practices or using chemical nematicides (Chitwood, 2002).

Chemical nematicides are limited and largely based on synthetics so they present risk to non-target organisms. Nowadays, new strategies are needed as acceptable alternatives to chemicals such as exploiting nematode-antagonistic plants, crop rotations, root exudates or plant extracts (Chitwood, 2002). Linford *et al*. (1938) were the first to study the nematicidal effect of chopped pine-apple (*Annanas comosus*) leaves used as organic amendment against RKNs, 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 have been identified to contain nematicidal compounds (Sukul, 1992 and Andres *et al.,* 2012). In Turkey, the species *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* are the most commonly found (Kepenekci, 2012). Traditionally, synthetic nematicides are used to control RKNs. For this reason, production costs have been increased as well as their impacts on the environment and non-target organisms. Moreover, pests may develop resistance to conventional pesticides. Use of plant extracts as alternative pesticides to control RKNs has becoming important. In recent years, research on this approach has been increased rapidly in Mediterranean coast.

Therefore, the objective of this study was to evaluate the efficacy of some plant extracts as alternative nematicides, especially on hatching and mortality of *M. javanica* eggs and juvenile (J2s).

MATERIALS AND METHODS

The nematode

M. javanica egg masses were obtained from the roots of greenhouse-grown tomato plants (SC-2121 variety). The eggs were released using a 0.575% NaOCl and then were collected using the modified technique described by McClure *et al.* (1973). The eggs were washed by rinsing with tap water through a 75 µm sieves, collected on a 26 µm sieve and transferred into distilled water forming egg suspension. Eggs of *M. javanica* were placed on a filter paper in a Baemann funnel. Emerged J2's were collected daily for up to 4 days and incubated at 4 ºC until been used in experiments (approximately one week).

Plant materials

Five selected indigenous plants were collected from various ecological zones of Anatolia, Turkey (Table 1).

Extraction

Healthy 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 shaken for 48 hours. Soxhlet apparatus was used for extraction for 5-6 hours. The solution was filtered to remove solids, and the material was vacuumed and concentrated in a rotary evaporator at 50-60°C to obtain corresponding organic crude extracts; ethanol was eliminated (Brauer and Devkota, 1990). Prepared extracts were used immediately in laboratory tests.

Table (1): Tested indigenous plants, used as extracts, against *M. javanica*

Common name	Botanical name (abbreviation)	Families	Collected Regions of Turkey
Bead-tree	*Melia azedarach* L. (*Ma*)	Meliaceae	MR
Pepper	*Capsicum frutescens*L. (*Cf*)	Solanaceae	MR
Henbane	*Hyoscyamus niger* L. (*Hn*)	Solanaceae	CAR
Yarrow	*Achillea wilhelmsii* C. Koch (*Aw*)	Asteraceae	CAR
Common Cocklebur	*Xanthium strumarium*L. (*Xs*)	Asteraceae	CAR

MR: Mediterranean Region; CAR: Central Anatolia Region.

Suspensions of the concentrations of 0.5, 1, 2, 3, 6 and 12% were prepared by distilled water (Orisajo *et al*., 2007).

Effects of plant extract on egg hatching

Nematicidal effects of the plant extracts on *M. javanica* were evaluated under laboratory conditions. Eggs suspendedin distilled water were prepared. One ml of *M. javanica* eggs' suspension, containing 115±2 eggs ml^{-1}, was added to 1 ml of the abovementioned plant extract concentrations and 3 ml of distilled water in sterilized Petri dishes. Distilled water was served as a check. All treatments were kept at 28±2°C. After seven days of exposure, numbers of hatched eggs were counted using a low power (6×) stereomicroscope. Toxicity of plant extracts was assessed as mean percentage of the dead nematodes. Treatment was replicated 5 times. After 7 days of exposure, the number of juveniles hatched was counted with the aid of inverted microscope at magnification 40×.

Effect of plant extract on larval (J2) mortality

The test procedure was identical to egg hatching one but by using larvae suspended in distilled water [111.8±5.9 juvenile (J2) ml^{-1}]. One ml of suspended juvenile, 1 ml of extract and 3 ml of distilled water were transferred into sterilized Petri dishes in five replicates. Distilled water served as a check. All dishes were kept at 28±2°C. Nematode juveniles were considered dead if they did not move when probed with a fine needle (Abbasi *et al*., 2008).

Statistical analysis

One-way analysis of variance was carried out by the Statistical Package for the Social Sciences (SPSS). Means were compared at $P \leq 0.05$ level and Tukey's test was used to separate means (SPSS, 1999). Data are expressed as mean±SE.

RESULTS AND DISCUSSION

Effect of plant extracts on juveniles (J2) (Mortality test)

All plant extracts had a nematicidal effect on *M. javanica* (Fig. 1). Although *Aw* and *Cf* had the lowest level of mortality rates, *Hn, Ma* and *Xs* showed highest levels of mortality rate after 7 days. 100% reduction was obtained at the 6 or 1% concentration level. The juvenile mortality was increased by increase of plant extract concentration (Fig. 1).

Inhibition effect of extracts on egg hatchability

As shown in fig. (2), there was a gradual decrease in egg hatching with increase in plant extract concentration. The plant extracts; *Hn*, *Xs* and *Ma* were the most effective in reducing egg hatchability. 100% reduction was obtained 7 days post treatment at the extracts of 3, 6 or 12% concentration levels.

The *Cf* and *Aw* plant extracts were less effective than *Hn*, *Xs* and *Ma*. However increased concentrations of *Cf* and *Aw* inhibited also egg hatching. All plant extracts significantly reduced the hatching rate of the egg masses of *M. javanica* with different degrees ($P \leq 0.005$).

In the present study, results of *in vitro* experiments on nematicidal activities of *Cf*, *Hn*, *Ma*, *Xs* and *Aw* when evaluated on *M. javanica* egg hatching and mortality showed that the aqueous plant extracts were toxic to *M. javanica*. Nematoxic effects were found even at the relatively low concentrations used in these experiments. Active ingredients of extracts were effectively ensured to eggs and juveniles. Highest percentages of egg hatching and life activities of the nematode were recorded at the control. Yarrow is known as a poisonous plant and is used as an insecticide (Calmasur *et al.,* 2006; Erdoğan *et al*., 2010 and Khani and Asghari, 2012). Many studies reported that *Achillea* sp. had antibacterial properties (Barel *et al.,* 1991). *A. wilhelmsii* showed more effective as a nematicide than *A. millefolium* (Ardekani *et al.,* 2010). Ntallie *et al.* (2011) studied nematicidal activity of some plant essential oils against *M. incognita. A. millefolium* was reported as it had no nematicidal effect. Oka *et al.* (2000) stated that *A. fragrantissima* was not effective against *M. javanica* on tomato plants. This plant extract has not been demonstrated as a nematicide in Turkey. *In vitro* studies showed also that *A. wilhelmsii* had low nematicidal effect on root-knot nematodes Henbane, a poisonous plant, is used for medicinal purposes. Its dried leaves were used as a repellent against mice in closed areas (Coffey, 1993) and had not been reported as nematicide. Bead-tree is common in the Mediterranean region in Turkey. It is known that their leaves and fruits have been used as pesticides

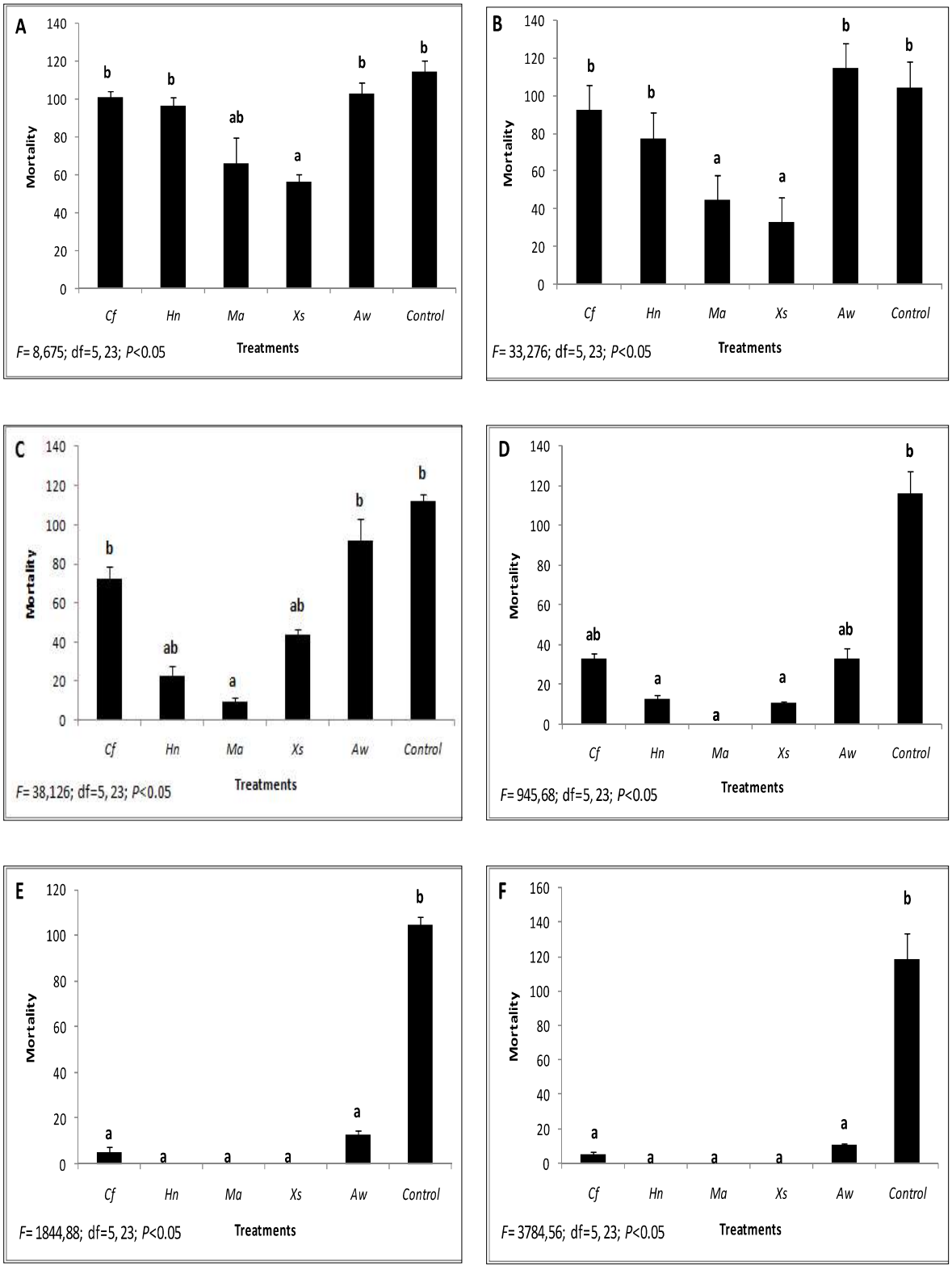

Fig. (1): Effect of certain concentrations of tested plant extracts on *Meloidogyne javanica* J2s (mortality test) under laboratory conditions. [pepper (*Capsicum frutescens*) (*Cf*), henbane (*Hyoscyamus niger*) (*Hn*), bead-tree (*Melia azedarach*) (*Ma*), common cocklebur (*Xanthium strumarium*) (*Xs*) and yarrow (*Achillea wilhelmsii*) (*Aw*)] [0,5 (A), 1 (B), 2 (C), 3 (D), 6 (E) and 12% (F)].

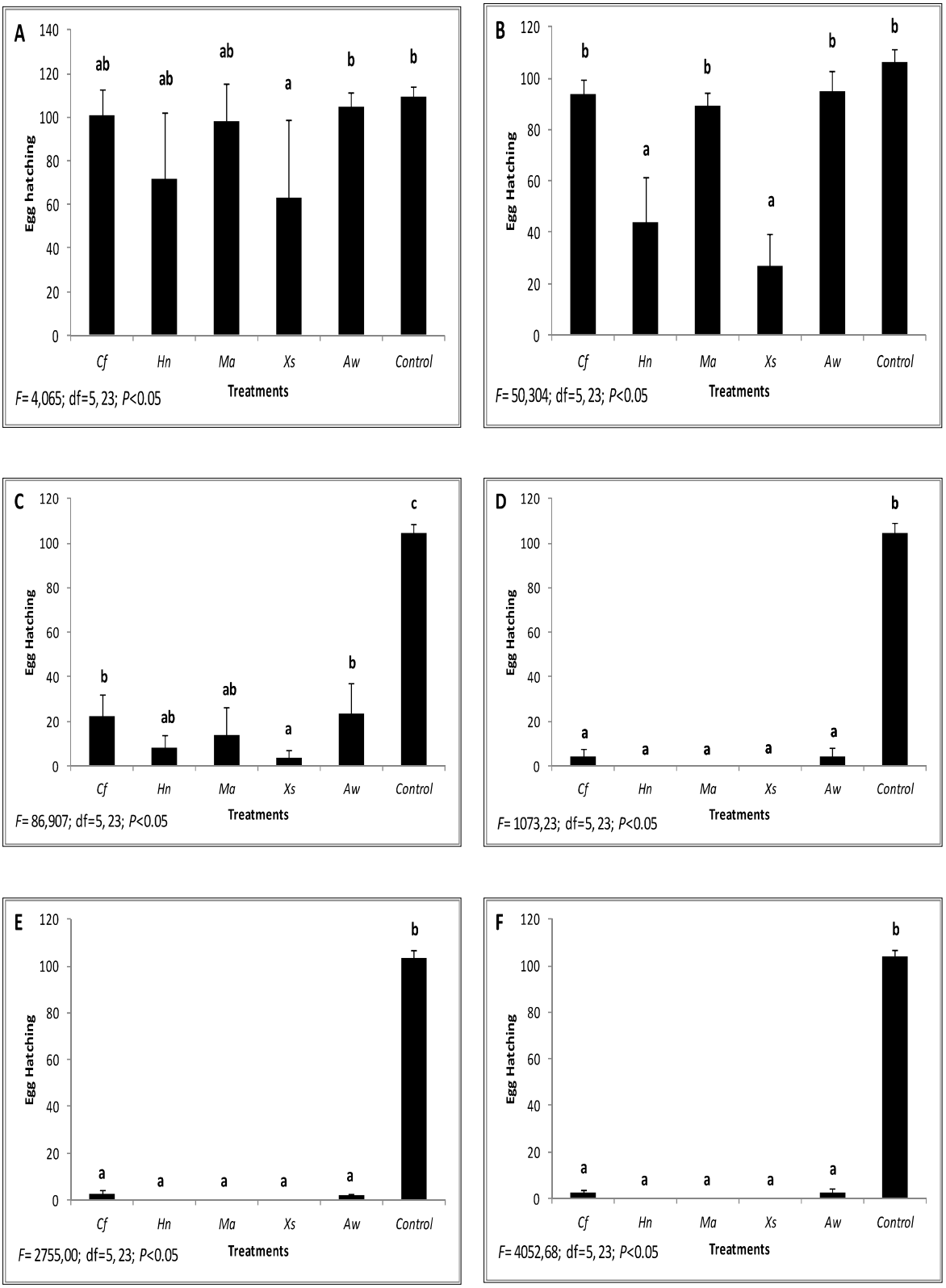

Fig. (2): Effect of certain concentrations of plant extracts on *Meloidogyne javanica* eggs (egg hatchability) under laboratory conditions. [pepper (*C. frutescens*) (*Cf*), henbane (*H. niger*) (*Hn*), bead-tree (*M. azedarach*) (*Ma*), common cocklebur (*X. strumarium*) (*Xs*) and yarrow (*A. wilhelmsii*) (*Aw*)] [0,5 (A), 1 (B), 2 (C), 3 (D), 6 (E) and 12% (F)].

(Erdoğan and Toros, 2005). *M. azedarach* has been widely studied and promising results as pesticide were obtained (Lee, 1990; Hasabo and Noweer, 2005; Maregiani *et al.,* 2010 and Rehman *et al*., 2012). Common cocklebur is widely distributed around the world. Many studies have been carried out on this plant in Turkey as well in other countries (Çetinsoy *et al.,* 1998 and Erdoğan and Toros, 2007). The tested extracts have been subjected to considerable nematological studies and showed positive results (Bala *et al.,* 1986; Nandal and Bhatti, 1986; Malik *et al.,* 1988 and Shaukat and Siddiqui, 2001). Some studies reported that *X. strumarium* extract inhibited egg hatching (Mennan *et al.,* 2000). Pepper contents such as capsaicin, capsainoids and allyl isothiocyanateare have been widely used as pesticides (Abbas *et al.,* 2009). *C. frutescens*, a common used plant in Turkey, showed negative effect towards the nematodes.

In the present study, *X. strumarium, M. azedarach* and *H. niger* proved to be good inhibitors of *M. javanica* egg hatching and juvenile survival. *H. niger* and *M. azedarach* extracts may possess ovicidal and larvacidal properties. *M. azedarach* was more effective than the other plant extracts, especially at the mortality test. These findings and further studies should be considered in greenhouse cultivation of vegetables in Turkey.

ACKNOWLEDGMENTS

The authors are grateful to Assoc. Prof. Dr. Z. Devran (Dept. of Plant Protection, Fac. of Agriculture, Antalya Univ., Antalya, Turkey) for supplying the root-knot nematode (*M. javanica*). Thanks also to the Plant Protection Central Research Institute, Ankara, Turkey for supplying the plant extracts. This study was supported by the Scientific and Technical Research Council of Turkey (TÜBITAK 111O784).

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