Management of Root-Knot Nematode (*Meloidogyne incognita*) on Cucumber with the Extract and Oil of Nematicidal Plants

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**Abstract:** One of the methods for the control of cucumber root-knot nematode is the use of plant extracts, that have nematicidal effect. In this research the anti-nematode activity of some of native plants of Iran have been investigated against cucumber root-knot nematode (*Meloidogyne incognita*) in laboratory and greenhouse. Experiments were carried out with extract, oil, concentration and time level using Randomized completely design in laboratory condition and greenhouse. For this purpose, the effect of alcoholic extract of leaf and seed oil of Castor bean, Chinaberry and Rapseed with concentration of 0, 50, 100, 200, 300, 400, 500 and 1000 ppm on the percentage of immobility of second stage juveniles and hatching of eggs were evaluated. Then, the investigation these activity was held in greenhouse with adding of extract and oil of plants into pots having infested cucumber seedlings to nematode. The results indicated that all of the plants had anti-nematode activity. Overall alcoholic extracts and oil of Chinaberry and Castor bean had the most effect on immobility of second stage juvenile and unhatching eggs of nematode in laboratory conditions. The greenhouse experiments showed that the alcoholic extract and oil of Chinaberry and Castor bean, although reduced the number of galls and population of nematode in soil, also caused the longitudinal growth of plant.

**Key words:** Root-knot nematode, *Meloidogyne incognita*, control nematode, leaf alcoholic extract, seed oil

**INTRODUCTION**

Plant-parasitic nematodes are recognized as the cause of serious yield losses on a wide range of crops (Javad et al., 2006). The most destructive species is *Meloidogyne incognita* which cause serious problem and number of economically important agriculture and greenhouse crops. Therefore, there are several ways to manage and control this nematode included use of chemical toxic, resistance cultivar, crop rotation and biological control and sometime mixture this methods. Chemical control in expensive and is economically viable only for high value crops and create a potential hazard to the environment and human health (Tsay et al., 2004). Because of these inconveniences scientist identified natural produce with nematicidal activity such as plant extract, root exudates, plant volatile and etc. Linford et al. (1938) were the first to study the nematicidal effect of chopped

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pine-apple (*Annanas comosus* L.) leaves used as organic amendment against *Meloidogyne* sp. Some of the most plant species antagonistic to *Meloidogyne* sp. are leaves and flower of Marigolds (*Tagetes* sp.), leaves, root and seed of Neem (*Azadirachta indica*), leaves and seed of Chinaberry (*Melia azedarach*) (Azhar *et al.*, 2007). Essential oil plant of Sweet wormwood (*Artemisia absinthium*), Thyme (*Thymus vulgaris*), Peppermint (*Mentha spicata*), Fennel (*Foeniculum vulgare*), Garlic (*Allium sativum*) and Eucalyptus sp. (Ibrahim *et al.*, 2006). Among these plants, marigold (*Tagetes* sp.) is the most commonly studied. As marigold belongs to the Asteraceae, it is possible that other members of the family also may possess antagonistic properties against plant-parasitic nematodes (Tsay *et al.*, 2004). Azadirachtin with commercial name of Neem (*Azadirachta indica*) in Asteraceae family is one of the most nematicidal plant that was recognized by the middle of the 20-th century. Neem is available in simple home-made products like neem seed powder, neem seed kernel powder, neem seed cake powder, dry neem leaves powder and the appropriate aqueous extracts made from them (Javadi *et al.*, 2006). The produce of azadirachtin is common in more of species of Asteraceae family (Chitwood, 2002).

In this research, we studied effect of some native plant of Iran such as Castor bean (*Ricinus communis* L.), Chinaberry (*Melia azedarach* L.) and Rape seed (*Brassica napus* L.) for controlling of *Meloidogyne incognita*, cucumber root-knot nematode, in laboratory and greenhouse.

**MATERIALS AND METHODS**

**Extraction**

Leaves and seeds of plants of Castor bean (*Ricinus communis* L.), Chinaberry (*Melia azedarach* L.) and Rape seed (*Brassica napus* L.) collected and washing then dried, then triturated. The leaves triturated (20 g) was macerated with ethanol 70% (300 mL) three time at room temperature for 48 h, each time. The solvent was separated by filtration and the material was vacuum-concentrated in a rotary evaporator at 40°C (Cristobal-Alejo *et al.*, 2006). Fore extracted seed oils used of soxhelt with Petroleum ether solvent in 8 h each day (Hosseinejad, 2004).

**Culture of Root-Knot Nematodes, Meloidogyne incognita**

The root-knot nematode was culture on cucumber plants in a greenhouse. Galled roots with egg mass were washed free of soil and cut into 2 cm pieces. After placing in 0.5% sodium hypochlorite they were triturated for 30 sec at maximum speed in a two-speed blender. Eggs were separated from debris by pouring the suspension over a series of sieves and collecting them on a 38 μm-pore mesh. After washing, the egg suspension was poured on to a cotton-wool filter and incubated at 26±2°C. The hatched second stage juveniles (J2) were collected daily. Only freshly hatched J2 collected within 48 h were used for experiments.

**Nematicidal Assay**

**In vitro**

100 J2 and 100 eggs of nematode associated distilled water were placed in 0, 50, 100, 200, 300, 400, 500 and 1000 ppm of alcoholic extract and seed oil in petri dishes. Then, Tween 20 concentration 0.05 ppm was added to oil Petri-dishes for monotonous. Control treatments were distilled water instead of plant extract. Percentage of J2 immobility after 24, 48, 72 h and egg hatching after 7 days were recorded. Treatments replicated three times and incubated at 26±2°C in Randomized Completely Design as Factorial.
Pot Experiments

Cucumber seedlings were two leaves transplanted in pots, 5-7 days after transplanting, 2000±10 J2 were added to pots around the root seedling, after 2 days, 20 mL of alcoholic extract and seed oil adding to pots separately, adding alcoholic extract and seed oil was repeated after two days. So that, 40 mL of alcoholic extract and seed oil were added to each pots. Rugby were added 1 g per kg polluted soil as a control with 2000±10 J2 and cucumber seedlings of transplanted these pots, after two days. Treatment replicated three times in greenhouse conditions to Randomized Completely Design. After 60 days, recorded growth parameters and reproduction factor and then compare.

Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at p<0.05 using LSD test by SASS.

RESULTS

In vitro

The results of immobility J2 and egg hatching on the alcoholic extract and seed oil showed significant differences (p<0.05) and three factors: alcoholic extract or seed oil, concentration and time significant differences (p<0.05) were shown.

The results indicated all treatments had nematicidal activity and seed oil had more immobility effect than alcoholic extract. The most immobility in all treatments showed in more concentration at 1000 ppm after 72 h. These result indicated more immobility on Chinaberry oil with 76.33% and after on, respectively, Castor bean oil 71.33%, Chinaberry alcoholic extract 68.66%, Castor bean alcoholic extract 61.33%, Rapseed oil 53.66% and Castor bean alcoholic extract 47% in 1000 ppm concentration after 72 h (Fig. 1). Result for hatching eggs showed all treatments had effect and less hatching showed on Chinaberry oil 13.67%, Castor bean oil 16%, Rapseed oil 20%, Chinaberry alcoholic extract 26%, Castor bean alcoholic extract 21.67 and Castor bean alcoholic extract 25.67% in 1000 ppm concentration after 72 h (Fig. 2).

Pot Experiments

The results of all the treatments showed different plant growth and reproduction parameters were significant differences (p<0.05) in pots which treated with alcoholic extract and seed oil. The results showed seed oil had more nematicidal activity than alcoholic extract. However these produce could decrease number of galls and egg mass but nematicides rugby
Fig. 2: Effect of alcoholic extract and seed oil on egg hatching after 7 days

Table 1: Effect of alcoholic extract and seed oil on growth parameters and nematocidal against *Meloidogyne incognita* on cucumber

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Length (cm)</th>
<th>No. of galls</th>
<th>Reprod. rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oil</td>
<td>Extract</td>
<td>Oil</td>
<td>Extract</td>
<td>Oil</td>
</tr>
<tr>
<td>Untreated</td>
<td>0/00d</td>
<td>0/00e</td>
<td>0/00f</td>
<td>0/00f</td>
<td>170/32a</td>
</tr>
<tr>
<td>uninoculated</td>
<td>2/02a</td>
<td>2/02a</td>
<td>163/33a</td>
<td>163/33a</td>
<td>115/32d</td>
</tr>
<tr>
<td>Rugby</td>
<td>0/17c</td>
<td>0/17b</td>
<td>3/66c</td>
<td>3/66c</td>
<td>144/09c</td>
</tr>
<tr>
<td>Castor bean</td>
<td>0/17c</td>
<td>0/38c</td>
<td>5/33d</td>
<td>23/33c</td>
<td>166/63a</td>
</tr>
<tr>
<td>Chinaberry</td>
<td>0/19c</td>
<td>0/22d</td>
<td>7/60c</td>
<td>11/60d</td>
<td>169/16a</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>0/34b</td>
<td>0/437</td>
<td>9/33b</td>
<td>28/66d</td>
<td>159/76</td>
</tr>
</tbody>
</table>

had highest reduction in reproduction parameters. The results indicated Castor bean oil and Chinaberry oil had highest of gall reduction. Despite of alcoholic extract of Chinaberry and castor bean reduced the number of galls and egg mass in roots, they increased the longitudinal growth and weight of the plant. The lowest nematode reduction occurred by alcoholic extract of Rapeseed (Table 1).

**DISCUSSION**

The results of these survey showed that the leaf extract and seed oil of all plants and all of concentrations had nematocidal activity. The experiments indicated the seed oil of Castor bean and Chinaberry had the most anti-nematode activity. This experiment showed several family of plants had nematocidal and agreement with many naturally occurring compound are known to possess nematocidal activity (Chitwood, 2002). Polythienyl in *Tagetes sp.* (Kyo et al., 1990), isothiocyanates and glucosinolat from Brassicaceae (Brown and Morra, 1997), polyacetylen from Asteraceae (Kogiso et al., 1976) have been reported to possess nematocidal activity. In our study, plant species of leaf alcoholic extract and seed oil had nematocidal activity against *Meloidogyne incognita* causing root-knot disease and agreement with those obtained by Cristobal-Alejo et al. (2006), that showed anti-nematode activity against *M. incognita* on extract of Euphorbiaceae, Asteraceae, Meliaceae and Fabaceae of 20 native Yucaean plant in Mexico. Greenhouse results indicated reduce of number of galls and egg mass that agreement with those obtained by Azhar and Sediqu (2007), also agreement with experiment of Susan and Noweer (2005), that showed plant extract of basil, marigold, pyrethrum, neem and china berry proved to be effective against *M. incognita*. These results are accorded with Abd-Elgawed and Omer (1995), Tsay et al. (2004), Adegbite and Adefeyian (2005) and Ahmad et al. (2008). The nematocidal effect of the
tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock et al., 1989).

REFERENCES