Population Density of Root Knot Nematode, *Meloidogyne incognita* Infecting Date Palm under Stress of Aqueous Extracts of Some Botanicals and a Commercial Bacterial Byproduct

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**ABSTRACT**

Under screen house conditions, certain plant aqueous extracts were added to pots planted with date palm cv. Sewi inoculated with root knot nematode *Meloidogyne incognita*. These plants, *Hyphaene thebaica* as mashed fruit pomace, *Punica granatum* as mashed fruit peel, *Stevia rebaudiana* as mashed dry leaves, and *Moringa oleifera* as mashed dry leaves were used as 5% concentration at the rate of 30 ml per pot and the commercial byproduct, micronema (containing strains of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *B. thuringiensis*) was used at the rate of 0.5 ml/pot. The influence of the tested botanical aqueous extracts and the commercial product on root knot nematode was shown as evidenced by the percentages reduction of number of galls, eggmasses and hatched juveniles on roots of date palm seedlings. Also, plant growth criteria were enhanced at different degrees.

**Key words:** Botanical aqueous extracts, micronema, root knot nematode, date palm

**Introduction**

Date palm, *Phoenix dactylifera* L., is dioecious and artificially pollinated by man and that 90% of all the dates of the world are grown in Iraq, Saudi Arabia and United Arab Emirates. Suitable climatic conditions occur in the dry parts where the palm has been successfully grown on a commercial scale. In Egypt, there are about 12 millions date palm trees grown in 99,867 feddans (fed.=4200m²). Total production is 1,352,954 tons f. 111.104 kg/tree (Anonymous, 2011). Several nematode pests were recorded as parasites to date-palm roots causing losses in the early stage of growth (Nurseries). The root-knot nematode, *Meloidogyne* spp. is one of the most common wide-spread nematodes all-over the world (Sasser, 1989). Griffith and Koshy (1990) stated that young seedlings of 50 date palm cultivars were susceptible to infection by the root knot nematode. More than 90% of the tested seedlings were killed prior to emergence when seedlings were planted in heavily nematode-infested soil. Since nematicides are very expensive and cause serious environmental pollution, control strategies are today directed towards the use of natural products. The use of botanical extracts for controlling the root knot nematodes is becoming appealing because of the growing problems of environmental pollution arising from the use of persistent pesticides. There has been a de-registration of some hazardous nematicides. Increasing cost is on farmers to use non-chemical pest control methods safety the environment. Efficacy of different plant extracts in nematode control has been studied (Khan et al., 2011; Mousa et al., 2011). Murslain et al. (2013) reported that Efficacy of *Moringa oleifera* at various concentrations (S, S/2, S/4) was assessed on the invasion and development of root-knot nematode, *Meloidogyne javanica* on eggplant. Minimum numbers of nematodes reproduced in standard concentration of *Moringa oleifera* extracts. While maximum occurred in S/4 concentration. Untreated control showed highest number of females due to pathogenic effect of nematodes. Leaf extract of *Moringa oleifera* has been reported to be lethal to *Radopholus similis* (Jasy and Koshy, 1992). Mohamed et al. (2010) determined the levels of phenolic, flavonoid, B-carotene and lycopene compounds in doum palm, *Hyphaena thebaica* and its extracts were evaluated for their antioxidant activity. The antimicrobial activity of doum palm extract was evaluated against a panel of microorganisms which was more active against gram-positive bacteria. Stevia is a sweetener and sugar substitute made from the leaves of the plant. Stevia is composed of several natural, heat-stable ent-kaurene glycosides (Steviol glycosides) whose intensities of sweetness and flavour profiles differ from each other and vary according to concentration and environment (Geuns, 2003). The two main alkaloids being stevioside (ST) and rebaudioside A(R-A) are the sweetest compounds and tasting about 300 and 450 times sweeter than sucrose, respectively (Tanaka, 1982; Geuns, 2003). Stevia, also, contains significant quantities of chlorogenic acid, which has hypoglycemic effects (Khramov and Dmitrienko, 2000; Gregersen et al., 2004). No researches were done about the effect of stevia on plant parasitic nematodes. Middleton et al. (2000) reported that *Punica granatum* is rich in antioxidant of polyphenolic class including tannins and flavonoids. Antioxidant activity has been proposed to play a vital role in various pharmacological activities such as anti-aging, anti-inflammatory aging, anti-atherosclerosis and anti-activities. El-Nagdi and Abd-El-Khair (2008) reported that the commercial product micronema protected...
eggplant from attack of Meloidogyne incognita and Rhizoctonia solani, increased yield of eggplant and affected populations of soil mycoflora differently.

The objective of this research is to evaluate effect of aqueous extracts of some medicinal plants and a commercial product containing some bacterial isolates for managing root knot nematode infecting date palm cv. Sewi under screen house conditions.

Materials and Methods

For this investigation, Seeds of date palm cv. Sewi were soaked in renewable tap water for one week then incubated in wetted cloth for two weeks. Under greenhouse conditions, sprouted seeds were planted in 20 cm diameter clay pots filled with sterilized sandy loam soil. Two months later, five replicates from each treatment were inoculated with 500 newly hatched juveniles of M. incognita juveniles/pot. Other five non-inoculated pots were kept as untreated control. At the same time of inoculation, certain plant aqueous extracts were added to pots inoculated with root knot nematode. These plants were doum palm, Hyphaene thebaica as mashed fruit pomace, pomegranate, Punica granatum as mashed fruit peel, stevia, Stevia reibndiana as mashed dry leaves, and moringa, Moringa oleifera as mashed dry leaves and used at 5% concentration. The commercial product micronema (containing strains of Serratia sp., Pseudomonas sp., Azotobacter sp., Bacillus circulans and B.thuringiensis) obtained from the Agricultural Research Center (Giza, Egypt) was used at the rate of 0.5 ml/pot.

The aqueous plant extracts were prepared by thoroughly mixing 10g of each plant material in 200ml distilled water. The resultant mixture from each material was left for 72hr. at lab. temperature and then, passed through 15 mm diam. Whatman No.1 filter paper. Obtained filtrates were used at the rate of 30ml per pot and were added as mentioned before. The pots were watered regularly. After four months from inoculation time, the experiment was terminated in May 14, 2014 and plants were uprooted and cleaned by tap water to avoid adhering soil. Numbers of galls and egg masses on roots of date palm were counted. Hatched juveniles from eggmasses on roots were extracted by method of Young (1954). Plant growth parameters including lengths of shoots and fresh weights of shoots and roots were recorded.

Results

Date in Table (1) clarified the significant (p≤0.05) influence of the tested botanical aqueous extracts and a commercial byproduct on root knot nematode, M. incognita as evidenced by the percentages reduction of number of galls, eggmasses and hatched juveniles on roots of date palm seedlings. It was noticed that the highest percentage gall reduction (75%) was achieved by using micronema followed by aqueous extract of Hyphaene thebaica (62.5%) and Stevia reibndiana (50%). While the lowest one (25%) occurred by Moringa oleifera. On the contrary, the highest percentage reduction of the hatched juveniles on roots was achieved by Moringa oleifera (83.3%) followed by micronema (80.7%) and Stevia reibndiana (77.7%). The rest treatments were nearly equal in their percentages reduction as they were 72.3% for Hyphaene thebaica and 73.3% for Punica granatum. The percentages reduction of eggmasses were equal at different treatments (66.6%) except moringa as it caused the lowest one (33.3%).

Table 1. Population density of root knot nematode, Meloidogyne incognita on date palm cv. Sewi as influenced by different botanical aqueous extracts and a commercial byproduct

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of galls</th>
<th>Reduction %</th>
<th>No. of egg masses</th>
<th>Reduction %</th>
<th>No. of Hatched juveniles on roots</th>
<th>Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyphaene thebaica (Doum palm)</td>
<td>30 bc</td>
<td>62.5</td>
<td>10 c</td>
<td>66.7</td>
<td>83 b</td>
<td>72.3</td>
</tr>
<tr>
<td>Moringa oleifera (Moringa)</td>
<td>60a</td>
<td>25.0</td>
<td>20 b</td>
<td>33.3</td>
<td>50 b</td>
<td>83.3</td>
</tr>
<tr>
<td>Punica granatum (Pomegranate)</td>
<td>50 b</td>
<td>37.5</td>
<td>10 c</td>
<td>66.7</td>
<td>80 b</td>
<td>73.3</td>
</tr>
<tr>
<td>Stevia reibndiana (Stevia)</td>
<td>40 bc</td>
<td>50.0</td>
<td>10 c</td>
<td>66.7</td>
<td>67 b</td>
<td>77.7</td>
</tr>
<tr>
<td>Micronema</td>
<td>20 c</td>
<td>75.0</td>
<td>10 c</td>
<td>66.7</td>
<td>58 b</td>
<td>80.7</td>
</tr>
<tr>
<td>Untreated check</td>
<td>80a</td>
<td></td>
<td>30 a</td>
<td></td>
<td>300a</td>
<td></td>
</tr>
</tbody>
</table>

*Values are averages of 4 replicates.

+Values with the same letter(s) are not significantly different according to Duncan’ Multiple Range Test at p≤0.05.

As for plant growth, all treatments significantly (p≤0.05) improved shoot length and weight and weight of roots compared to untreated check. It was noticed that aqueous extract of doum palm was the first in increasing length of date palm shoots (44.7%) followed by the commercial byproduct, micronema (42.1%). Regarding the weight of shoots, it was increased by aqueous extract of Punica granatum (113.5%) followed by doum palm (92.1%). The least percentage increase in weight of shoots (73.0%) occurred by micronema. Moringa was the first in increasing weight of roots (90.2%) followed by micronema (70.6%).The least increase in weight of roots (31.4%) was caused by Punica granatum (Table 2).
Discussion

Biological control of plant parasitic nematodes has become one of the most feasible alternatives to the nematicides. It would be the most enviable because most of the successful nematicides have been banned in agriculture because of high risk to human health and environment (Vermis and Roberts, 1996). The results proved that botanical extracts suppressed root knot nematode and enhanced plant growth criteria which may due to that these botanical extracts have nematicidal activity in different forms of compounds. Moringa aqueous extract in this study was found to be good inhibitor to root knot nematode as it reduced number of hatched juveniles with a subsequent increase in plant growth criteria which agree with the results of Guzman (1969) who found that water extract of moringa was toxic to M. incognita as standard pesticide. Sowley et al. (2014) reported that sweet pepper plants treated with 80g/L of moringa leaf powder per plot recorded the highest mean value of plant height, number of leaves, number of fruits per plant, fruit weight per plant total yield per plot and the thickest plant girth. Similarly, the plants treated the same had the lowest infection index (root gall) and nematode population. The present results conform to those of Middleton et al. (2000) regarding effect of Punica granatum and Mohamed et al. (2010) on their work with Hyphaena thebaica extract on root knot nematode. The effect of the commercial product, micromena on M. incognita may be due to that it contains some bacterial isolates of Serratia sp., Pseudomonas sp., Azotobacter sp., Bacillus circulans and B. thuringiensis which are well known to suppress nematodes as follows: El- Haddad et.al. (2011) stated that some beneficial bacteria including the nitrogen fixing bacteria (of which Azotobacter sp.), and the potassium solubilizing bacteria (of which Bacillus circulans) significantly reduced the nematode multiplication which was more obvious after 60 days after inoculation. These results are in a line with those reported by Zavaleta-Mejia and Van Gundy (1989) who reported potentiality of Serratia marcescens to suppress root-knot larvae of Meloidogyne incognita. They attributed this effect to the volatile substances produced during its metabolic activity. In the agreement with the present results, some investigators have reported the nematicidal activity of S. marcescens against M. incognita (Ali, 1996 and El-Sherif et al., 1999). Eklund (1970) confirmed that Pseudomonads are natural inhabitants on the root surface and primary consumers of root exudates rich in amino acids which are converted to ammonia along the root to maintain a micro-zone around the growing roots that would be suppressive to pathogens. The reduction of root galls number may be due to the failure of majority of the encumbered juveniles to penetrate the host root. These results agree with those reported by Stirling and Sharma (1990) and El-Nagar et al., (1998) who reported that bacterium, Pasteurella penetrans not only prevented reproduction of root-knot nematodes, but also reduced the infectivity of the juveniles. As reported by Noweer and Hasabo (2005), the commercial product, agentin (containing strains of Bacillus thuringiensis) had also adverse effect as it reduced number of the second stage juveniles of M. incognita in soil and galls on roots of squash which conform to the present study. The mode of action of B. thuringiensis toxins is mainly inhibition of protein and nucleic acid synthesis (Sebesta et al., 1969).

References


Table 2. Plant growth response of date palm cv. Sewi infected by Meloidogyne incognita as influenced by different botanical aqueous extracts and a commercial bacterial byproduct.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot length (cm)</th>
<th>Increase (%)</th>
<th>Shoot weight (g)</th>
<th>Increase (%)</th>
<th>Root weight (g)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyphaena thebaica (Doum palm)</td>
<td>27.5a</td>
<td>44.7</td>
<td>1.71ab</td>
<td>92.1</td>
<td>0.86ab</td>
<td>68.6</td>
</tr>
<tr>
<td>Moringa oleifera (Moringa)</td>
<td>24.0a</td>
<td>26.3</td>
<td>1.63b</td>
<td>83.1</td>
<td>0.97a</td>
<td>90.2</td>
</tr>
<tr>
<td>Punica granatum (Pomegranate)</td>
<td>24.3a</td>
<td>27.9</td>
<td>1.90a</td>
<td>113.5</td>
<td>0.67c</td>
<td>31.4</td>
</tr>
<tr>
<td>Selvia rehmliana (Selvia)</td>
<td>25.8a</td>
<td>36.3</td>
<td>1.70ab</td>
<td>91.0</td>
<td>0.75bc</td>
<td>43.1</td>
</tr>
<tr>
<td>Micromena</td>
<td>27.0a</td>
<td>42.1</td>
<td>1.54b</td>
<td>73.0</td>
<td>0.87ab</td>
<td>70.6</td>
</tr>
<tr>
<td>Untreated check</td>
<td>19.0 b</td>
<td>-</td>
<td>0.89c</td>
<td>-</td>
<td>0.51 d</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are averages of 4 replicates.
*Values with the same letter(s) are not significantly different according to Duncan’s Multiple Range Test at p≤0.05.


