The efficiency of some natural alternatives in root-knot nematode control

Abstract

Plant extracts are, nowadays, extensive used as environment friendly ways for biological control of parasitic pests, including the root-knot nematodes, instead of using chemical pesticides. Therefore, the aim of this study was to analyze leaf and root extracts nematicidal activities of four selected medicinal plants (i.e., Azadirachta indica, Moringaoleifera, Lantana camara, and Glycyrhizaglabra) against the root-knot nematode; Meloidogyne spp. Roots of G. glabra and leaves of A. indica, M. oleifera, and L. camara were collected from different sites in Fayoum Governorate. Roots and leaves were air-dried, powdered and then extracted by ethanol 95% for L. camara and G. glabra or by petroleum ether for A. indica and M. oleifera. The nematode eggs were exposed to the different extracts at different concentrations (i.e., 500, 1000, 2000, 4000ppm) for 24, 48 and 72h. Results showed that all four plant extracts caused significant decreases in egg hatching, but to varying degrees. A. indica extract was the most effective in preventing egg hatching, followed by M. oleifera extract. There was a gradual decrease in egg hatching with increasing the extract concentration and the duration of exposure. As the most effective, the crude extract of A. indica was analyzed by using GC/MS for the effective ingredients and found to be included alkaloids, flavonoids, saponins, amides including benzamide and ketones, and others, which showed effectiveness in preventing the egg hatching of the root-knot nematode; Meloidogyne incognita.

Keywords: extract, Meloidogyne spp., egg hatchability, mortality, GC/MS

Introduction

Nematodes are found in a wide variety of habitats. Free-living nematodes live in the soil, in freshwater, marine sands and muds. In soil, they are important components of nutrient turnover. Other nematodes are parasites of almost every species of animal, humans, plant and they cause enormous social and economic damage.1 Phytoparasitic nematodes parasitize plants to seek suitable food. This food source is basically planted cell contents. Thus a plant response to parasitism is the reaction to the cellular feeding of the nematode.2 Most phytoparasitic nematodes infect plant roots and some species have evolved sophisticated interactive relationships with host cells to sustain a sedentary parasitic habit.3 Plants carry a wide range of microorganisms in their phyllosphere and Rhizosphere which not only cause a large variety of diseases but also control of pathogens.4 Nematodes have an important niche in agro-ecosystem, causing a reduction in plant productivity and growth. Root-knot nematodes (Meloidogyne spp.) are very common and the most important nematode species of greenhouse-growing plants. Indiscriminate use of chemical nematicides to control nematode causes great injuries to human being, animal, vegetation and to the environment as a whole due to their non-target effect, hazardous nature besides they are expensive. So with the increasing awareness of possible deleterious effects of the chemicals, biological controls of plants pathogen have received considerable attention.5 The management of these nematode-parasites has little chance of success and is uneconomical because they live in the soil and feed on the internal plant tissues. Preventing the introduction of nematodes with planting material, seeds, or soil, using rotation and mixed cropping with the poor host, using nematode resistant varieties or rootstocks, and lowering nematode populations through nematicides are some of the most frequently used strategies.6 Until recently, methyl bromide was widely used to manage nematodes and other soil-borne pathogens in high-value horticultural crops. However, concerns on its impact on environment necessitate the ban or revoke of this methyl bromide in 2005 for its gas emission and global warming. Although nematicides are effective in nematode management, it discourages users because of their high costs, non-availability at the time of need, the hazards they pose on human as well as on non-target organisms.7 Other options for the management of root-knot nematodes become imperative and there is an increasing interest in non-chemical nematode management strategies.8 Extract from certain plants is used to control certain nematode because environmental consideration and costs of nematicides dictate that other methods of control may be investigated, on alternative method is the use of antagonistic plants in rotation with or inter planted with crop plants. Certain medicinal plant extracts and their constituent were experimentally used for such aim.9-11 The current study was designed to evaluate the potential beneficial effects of some plant extracts such as lantana (Lantana camara), neem (Azadirachta indica), moringa (Moringaoleifera), and liquorices (Glycyrhizaglabra) on controlling the root-knot nematode (Meloidogyne spp.) through their toxic effects on egg hatchability.

Material and methods

Plant material used in the experiment

As shown in (Table 1), plant materials of Moringaoleifera and neem were collected from mature plants grown at Demo Experimental Farm of Faculty of Agriculture, Fayoum University, and Fayoum, Egypt. In addition, Lantana camara leaves were collected from gardens of Faculty of Agriculture, Fayoum University, and Fayoum, Egypt. However, liquorice roots were collected from Anonymous fields located in Ashshawi district, Fayoum Governorate, Egypt.
Preparation of plant extracts

Plant leaves were plucked from their branches and spread on polythene sheets on benches in the laboratory for ten days to air dry. The dried materials were ground to fine particles using a blender. An amount of 400ml ethanol (95%) (L. camara and G. glabra) and Petroleum Ether (A. indica and M. oleifera) were added to 100g of ground plant material and shaken on a rotary in a shaker at 120rpm for 24 hours. The solution was filtered through muslin cloths then through Whatman No. 1 filter paper and the material was vacuumed in a rotary evaporator at 40°C to obtain organic crude extracts (solvent is eliminated). Extracts were used at 4000, 2000, 1000 and 500ppm concentration that obtained by the dilution with distilled water.

Extraction of nematode eggs

Eggs were obtained from a culture of nematode infected roots of tomato. root pieces containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% Clorox (sodium hypochlorite, NaOCI) solution shaken vigorously by hand for 4 min. This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sieves, 200- mesh (75μm) and 500mesh (25μm). Eggs in the 500 mesh sieve were washed free of NaOCI solution with a slow stream of cold tap water into a container previously marked to contain 1 L. The cut roots in the original container were washed twice with water to obtain additional eggs. The collected eggs were topped with water to obtain the egg-water suspension for in vitro studies.

Counting of root-knot nematodes eggs

Number of eggs in aqueous suspension was determined by using a stereo microscope. One milliliters of the egg-water suspension was pipette after bubbling air through the suspension for homogeneity and dispensed into a counting tray. Counting was done two times and the mean number of eggs/ml estimated.

Hatchability test

Eggs were collected by the method of Hussey and Barker. A suspension of eggs in water was prepared. 1 ml of egg suspension (100±10 eggs/ml) and 5 ml of leaf or root extract was transferred in Petri dishes and kept at room temperature. Each treatment was 3-time replicated. The Petri dishes containing 1 ml egg suspension and 5 ml water served as control. After 24, 48, 72 hours of exposure, the number of hatching eggs was counted under an inverted microscope.

Gas chromatography-mass spectrometry (GC/MS) analysis

The GC column was a 30m (0.25mm i.d., film thickness 0.25μm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 50°C for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70eV ionization energy. The sector mass analyzer was set to scan from 40 to 400amu for 5s. These data were obtained from environmental and food pollutants laboratory at Faculty of Agriculture, Fayoum University.

Results

Effect of exposure time and inhibition concentration (IC)

Regarding the effect of some plant extracts on egg hatching of root-knot nematode after 72h, data in (Table 2) and (Figure1) (Figure2) show that toxicity of extract IC<sub>50</sub> (Inhibition Concentration, 50%), IC<sub>90</sub> and slope value was calculated. It shows that neem extract is highly effective against egg hatching being the IC<sub>50</sub> scored 202.55ppm followed by moringa extract IC<sub>50</sub> conferred 497.55ppm, while the least effective extract against egg hatching was liquorices extract IC<sub>50</sub> granted 1479.15ppm. Consequently, neem extract caused 64, 76, 84 and 89% inhibition of egg hatching on root-knot nematode at the concentrations of 500, 1000, 2000 and 4000ppm, respectively. In contrast, the liquorices extract at the concentrations of 500, 1000, 2000 and 4000ppm caused the inhibition % of egg hatching of 15, 44, 63 and 74%, respectively at 72h.

Table 1: Information about the four plant species used in the present study

| English Name | Scientific Name | Family | Plant part used | Reference of previous use |
|--------------|----------------|--------|----------------|--------------------------|
| Lantana      | Lantana camara | Verbenaceae | Leaves         | Tayeet al.,<sup>30</sup> |
| Neem         | Azadirachta indica | Meliaceae | Leaves         | Tayeet al.,<sup>30</sup> |
| Moringa      | Moringaoleifera | Moringaceae | Leaves         | Sowleyet al.,<sup>19</sup> |
| Liquorice    | Glycyrrhiza glabra | Fabaceae | Roots          | Sardariet al.,<sup>18</sup> |

Table 2: Effect of some plant extracts on egg hatchling (%) of root-knot nematode (Meloidogyne spp.) after 72 hours exposure to the extracts.

| Extract | Concentration (ppm) | IC<sub>50</sub> (ppm) | 95% Confidence limits | IC<sub>90</sub> (ppm) | Slope ± SE |
|---------|---------------------|-----------------------|-----------------------|-----------------------|------------|
| Neem    | 500                 | 64<sup>*</sup> 76 | 84 89                  | 202.6                 | 46.3       | 374.7      | 4183.2 | 0.97±0.22 |
|         | 1000                | 49 66 77             | 86 97.6               | 268.3                 | 686.2      | 5600.5     | 1.22 ±0.21 |
| Moringa | 2000                | 33 55 70 79         | 920.8                 | 693.5                 | 1145.8     | 7741.2     | 1.39 ±0.20 |
|         | 4000                | 15 44 63 74         | 1479.2                 | 651.1                 | 3547.9     | 7621.5     | 1.80± 0.21 |

*Inhibition of egg hatchability (%)
The efficiency of some natural alternatives in root-knot nematode control

Effect of plant extract, concentration and exposure time

The mean performance of plant extract and the effect of the extract concentration and exposure time to extract on egg hatching of root-knot nematode are shown data in (Table 3). Data show that the egg hatching (%) was recorded the highest value with the extract of *Glycyrrhiza glabra* followed by *Lantana camara* extract, then *Moringa oleifera* extract, and the lowest value was recorded with the extract of *Azadirachta indica*. The egg hatching (%) was progressively reduced with increasing the extract concentration from 500 to 4000 ppm. In contrast, the egg hatching (%) was progressively increased with increasing the exposure time from 24 to 72h.

Table 3 Mean performance (± SE) of plant extract, concentration and time of egg hatching on Meloidogyne spp.

| Plant               | Means(%)±SE | Conc.(ppm) | Means(%)±SE | Time (h) | Means(%)±SE |
|---------------------|-------------|------------|-------------|----------|-------------|
| *Azadirachta indica* | 32.1±2.9 d  | 0          | 80.8±2.1 a  | 24       | 34.3±1.7c   |
| *Moringa oleifera*  | 38.5±2.7 c  | 500        | 48.3±2.2 b  | 48       | 44.7±2.5b   |
| *Lantana camara*    | 46.0±2.6 b  | 1000       | 36.3±1.4 c  | 72       | 48.7±2.9a   |
| *Glycyrrhiza glabra*| 53.6±2.7 a  | 2000       | 24. ±1.0 d  | -        | -           |
| -                   | -           | 4000       | 16.5±0.7 e  | -        | -           |

Interactive effect of plant extract and its concentration

The interactive effect of plant extract and its concentration on egg hatching of root-knot nematode is presented in (Table 4) and (Figure 3). Neem extract significantly decreased egg hatching of root-knot nematode. The percentages of reductions of egg hatching were 59.01, 73.03, 81.83 and 87.34% by application of neem extract (*Azadirachta indica*) at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively compared to the control (water). Egg hatching of root-knot nematode was significantly decreased egg hatching by moringa extract (*Moringa oleifera*) application. The percentages of reductions of egg hatching were 42.36, 61.48, 74.27 and 83.77% of the moringa extract at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively compared to the control. Lantana extract (*Lantana camara*) significantly decreased egg hatching of root-knot nematode. The percentages of reductions of egg hatching were 23.38, 49.24, 66.16 and 76.47% by application of lantana extract at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively compared to the control. Egg hatching of root-knot nematode was significantly decreased egg hatching by liquorices extract (*Glycyrrhiza glabra*) application. The percentages of reductions of egg hatching were 3.71, 36.45, 57.36 and 70.69% by application of liquorices extract at the concentrations of 500, 1000, 2000 and 4000 ppm, respectively compared to the control.

Citation: Haroon SA, Hassan BAA, Hamad FMI, et al. The efficiency of some natural alternatives in root-knot nematode control. *Adv Plants Agric Res.* 2018;8(4):355–362. DOI: 10.15406/apar.2018.08.00337
The efficiency of some natural alternatives in root-knot nematode control

Table 4 Mean performance (±SE) of interaction between plant extract and concentration on egg hatching of Meloidogyne spp.

| Plant             | Concentration(ppm) | Means(%)±SE |
|-------------------|-------------------|-------------|
| **Azadirachta indica** |                  |             |
| 0                 | 80.8±4.4a         |             |
| 500               | 33.1±1.3f         |             |
| 1000              | 21.8±0.9hi        |             |
| 2000              | 14.7±0.6j         |             |
| 4000              | 10.2±0.4k         |             |
| **Moringa oleifera** |                  |             |
| 0                 | 80.8±4.4a         |             |
| 500               | 46.6±1.3d         |             |
| 1000              | 31.1±0.8f         |             |
| 2000              | 20.8±0.7hi        |             |
| 4000              | 13.1±0.4j         |             |
| **Lantana camara** |                  |             |
| 0                 | 80.8±4.4a         |             |
| 500               | 61.9±1.4b         |             |
| 1000              | 41.0±1.0e         |             |
| 2000              | 27.3±0.7g         |             |
| 4000              | 19.0±0.5i         |             |
| **Glycyrrhiza glabra** |                |             |
| 0                 | 80.8±4.4a         |             |
| 500               | 77.8±2.7a         |             |
| 1000              | 51.3±1.9c         |             |
| 2000              | 34.4±1.2f         |             |
| 4000              | 23.7±0.9h         |             |

*Inhibition of egg hatchability (%)

Interactive effect of plant extract and exposure time:

(Table 5) (Figure 4) illustrate the interactive effect of plant extract and exposure time on egg hatching of root-knot nematode. The most effective extract at decreasing the egg hatching is *Azadirachta indica* followed by *Moringa oleifera*, and the lowest effective extract at inhibiting the egg hatching is *Glycyrrhiza glabra*.

Table 5 Mean performance and S.E. of interaction between plant extract and time of egg hatching on Meloidogyne spp.

| Plant             | Time (h) | Means(%)±SE |
|-------------------|----------|-------------|
| **Azadirachta indica** | 24       | 25.1±3.3i   |
|                   | 48       | 33.9±5.2h   |
|                   | 72       | 37.3±6.1g   |
| **Moringa oleifera** | 24       | 30.7±3.3i   |
|                   | 48       | 40.5±4.9f   |
|                   | 72       | 44.3±5.7e   |
| **Lantana camara** | 24       | 37.3±3.0g   |
|                   | 48       | 48.2±4.6d   |
|                   | 72       | 52.5±5.3c   |
| **Glycyrrhiza glabra** | 24       | 43.9±3.3e   |
|                   | 48       | 56.2±5.6b   |
|                   | 72       | 60.7±5.3a   |

*Inhibition of egg hatchability (%)

Interactive effect of plant extract, concentration and exposure time:

(Table 6) (Figure 5) reveal the interactive effect of plant extract, concentration and exposure time on nematode egg hatching. Under the application of plant extract concentration for different periods, the lowest effective plant extract concentration was 500ppm, which gave the least inhibition, followed by 1000ppm. The highest effective extract concentration was 4000ppm, conferring the lowest egg hatching.

Table 6 Mean performance (± SE) of interaction between concentration and time of egg hatching on Meloidogyne spp.

| Concentration(ppm) | Time(h) | Means(%)±SE |
|--------------------|---------|-------------|
| 0                  | 24      | 56.7±0.9d   |
| 500                | 24      | 47.7±3.2e   |
| 1000               | 24      | 31.6±2.1g   |
| 2000               | 24      | 21.1±1.5i   |
| 4000               | 24      | 14.3±1.0k   |
| 0                  | 48      | 57.2±3.7c   |
| 500                | 48      | 47.7±3.2e   |
| 1000               | 48      | 37.9±2.5f   |
| 2000               | 48      | 26.4±1.8h   |
| 4000               | 48      | 17.3±1.2j   |

*Inhibition of egg hatchability (%)

Figure 4 Mean performance of interaction between plant extract and time of egg hatching on Meloidogyne spp.

Interactive effect of plant extract, concentration and exposure time:

(Table 6) (Figure 5) reveal the interactive effect of plant extract, concentration and exposure time on nematode egg hatching. Under the application of plant extract concentration for different periods, the lowest effective plant extract concentration was 500ppm, which gave the least inhibition, followed by 1000ppm. The highest effective extract concentration was 4000ppm, conferring the lowest egg hatching.
The efficiency of some natural alternatives in root-knot nematode control

Effect of plant extract at different periods:

(Table 7) (Figures 6–8) show the effect of plant extract on hatching of root-knot nematode at different periods. The four plants; neem, moringa, lantana and liquorices were tested at different concentrations (500, 1000, 2000 and 4000ppm) for egg hatching of root-knot nematode. The results show a gradual decrease in egg hatching with increasing the concentration of each extract. The increase in exposure period and an increase of the concentration also decrease of egg hatching. After 24 h application of the extracts on root-knot nematode eggs, the mean egg hatching ranged from 8.67 to 67.67%. The highest egg hatching was observed in the G. glabra, whilst the lowest was observed with the A. indica. At 48 h, mean egg hatching was ranged between 11.00 and 85.67%. The highest egg hatching was found in the control, whilst the least hatch was in the A. indica extract. In addition, at 72 h mean egg hatching was ranged between 11.00 to 100 %. The highest egg hatching was observed in the control treatment, whilst the lowest hatching was found in the A. indica extract. The most effective plant extract inhibition of egg hatching was A. indica extract at the concentration of 4000ppm, which conferred the lowest egg hatching.

Table 7 Effect of some plant extracts on hatching of Meloidogynes spp. egg at different periods.

| Treatment             | Conc.(ppm) | Mean number of egg hatching (%) |
|-----------------------|------------|---------------------------------|
|                       |            | 24h                             | 48h                             | 72h                             |
| Azadirachtaindica     | 500        | 28.7±1.6n_q                     | 34.7±1.9l_r                     | 36.0 ±2.1jk                     |
|                       | 1000       | 19.0±1.1_t_s                    | 22.7±1.4r_u                     | 23.7 ±1.4u                       |
|                       | 2000       | 12.7±1.0a_c                     | 15.3±1.0x_b                     | 16.0 ±1.1w_b                     |
|                       | 4000       | 8.7±0.5c                        | 11.0±0.7bc                      | 11.0 ±0.7bc                      |
| Moringaoleifera       | 500        | 40.0±1.1hij                     | 48.7±1.6l_g                     | 50.7 ±1.6ef                     |
|                       | 1000       | 27.0±0.7o_r                     | 32.7±0.9k_o                     | 33.7 ±0.9k_n                     |
|                       | 2000       | 18.0±0.7u_a                     | 21.7±0.9r_w                     | 22.7 ±0.9r_u                     |
|                       | 4000       | 11.3±0.5bc                      | 13.7±0.2z_c                     | 14.3 ±0.5y_c                     |
| Lantana camara        | 500        | 54.0±0.5d_f                     | 64.3±0.7c                       | 67.3 ±0.7c                       |
|                       | 1000       | 35.7±0.3j_l                     | 42.7±0.3hi                      | 44.7 ±0.3gh                      |
|                       | 2000       | 23.7±0.3q_u                     | 28.7±0.3n_q                     | 29.3 ±0.3m_p                     |
|                       | 4000       | 16.7±0.3w_b                     | 19.7±0.3t_y                     | 20.7 ±0.3s_x                     |
| Glycyrrhizaglabra     | 500        | 67.7±3.4c                       | 81.0±3.8b                       | 84.7 ±4.1b                       |
|                       | 1000       | 44.7±2.5gh                      | 53.7±2.9d_f                     | 55.7 ±2.9de                      |
|                       | 2000       | 30.0±1.6L_p                     | 36.0±1.6L_k                     | 37.3 ±1.8I_k                     |
|                       | 4000       | 20.7±1.0s_x                     | 24.7±1.4p_t                     | 25.7 ±1.4p_s                     |
| Control               | 0          | 56.7±2.2d                       | 85.7±1.5b                       | 100.0 ±0.0a                      |

Data are means ±S.E. different lower or upper letters in a column indicate significant differences between the treatments at $P \leq 0.05$.

Citation: Haroon SA, Hassan BAA, Hamad FMI, et al. The efficiency of some natural alternatives in root-knot nematode control. Adv Plants Agric Res. 2018;8(4):355–362. DOI: 10.15406/apar.2018.08.00337
The efficiency of some natural alternatives in root-knot nematode control

Copyright: ©2018 Haroon et al.

Citation: Haroon SA, Hassan BAA, Hamad FMI, et al. The efficiency of some natural alternatives in root-knot nematode control. Adv Plants Agric Res. 2018;8(4):355‒362. DOI: 10.15406/apar.2018.08.00337

Figure 6 Effect of some plant extract on hatching of Meloidogyne spp. egg at 24h.

Figure 7 Effect of some plant extract on hatching of Meloidogyne spp. egg at 48h.

Figure 8 Effect of some plant extract on hatching of Meloidogyne spp. egg at 72h.

Active chemical compounds of neem leaf extract

As the most effective extract inhibiting nematode egg hatching, the GC/MS analysis (Figure 9) of the leaf extract of neem showed the following active chemical compounds: Hexane, 2,4-dimethyl; Hexane, 2,2,5-trimethyl; Cyclohexane, 2,4-diethyl-1-methyl-; Methylbicyclo [4.2.0] octane; Methallylcyclohexane; Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2- methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl; Undecane; Undecane, 2-methyl-; Dodecane; -; o-Xylene; Mesitylene; and Naphthalene, decahydro-, trans.

Figure 9 Chromatogram obtained from the GC-MS with the extract of Azadirachta indica leaves.

Discussion

The recent approach in nematode control is direct method towards the possibility of reducing populations of plant-parasitic nematodes in soil by using natural substances extracted from some plants. Such methods don’t lead to the disturbance of the biological balance of nature. Utilization of antagonistic plants or their byproducts is of common use all-over the world for avoiding hazards of the traditional chemical nematicides. The use of certain plant extracts for controlling plant-parasitic nematodes has been increased in the recent years. The plant extracts which tested in this study found in most cases to have an antagonistic action and a higher nematicidal activity against root-knot nematode. So, they undoubtedly contain natural nematotoxic constituents that able to inhibit the nematode egg hatching. In a study conducted by Hussaini et al., it has been reported that leaf extracts of 11 plant species inhibited egg hatching and caused 90% larval mortality in *M. incognita*, *M. javanica*, and *M. arenaria*. Our results are in parallel line with the results of Hussaini et al., In addition, results of the current study are in agreement with the results of Nandal & Bhatti who have reported that some of the plant extracts showed significant nematicidal properties. According to Khan, many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant-parasitic nematodes. The results of the study showed that neem extract had a toxic effect on the root-knot nematode in vitro by inhibiting the egg hatching at different concentrations of the extract. It was also observed that inhibition of egg hatching increased with increasing the concentration of the extract with the highest score that was recorded with the extract concentration of 4000ppm. This observation agrees with the findings of Adegbite and Adesiyan working with root extracts of *Azadirachta indica*, *Chromolaena odorata*, *Ricinus communis* and *Jatropha curcas* and recorded the gradual increase in inhibition of egg hatching with increasing the concentration of the extract. A similar
The efficiency of some natural alternatives in root-knot nematode control

National Symposium on...21
25
32
from rice roots. Adam et al.,...33
31
36
24

Citation: proteinaceous acetate extract caused 64% inactivity in...partially, with the others hatching of...discovered in the extract of our study and showed toxic effects on egg...on against root-knot nematode, Pseudomonas jessenii...1,2-benzenedicarboxylic acid were identified as main compounds which had been detected in petroleum ether extract of Neem;...Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Benzene, 1-ethyl-2-methyl-; Benzene, 1,3-dimethyl-; Cyclohexane ethyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyl-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-; cis-; Benzene (1-methylcyclohexyl); Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2-methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; 2,4- di-ter-butyl-phenol showed antioxidant activity. Many of the compounds found in the extract of Azadirachtaindica also remained unidentified in GC–MS analysis due to similar fragmentation pattern of many compounds with different retention times. It has been concluded from the results of the present study that the leaf extract of Azadirachtaindica has the ability to inhibit the egg hatchability of root-knot nematode. Thus, this finding is important in the identification and development of alternative strategies in controlling the root-knot nematodes. There is, however, further work is needed to identify some of these main compounds after purification. This study confirms the presence of nematicidal compounds in petroleum ether fractions of Azadirachtaindica, which were responsible for the prevention of egg hatching of root-knot nematodes at some concentrations, especially 4000 ppm.

Acknowledgements
None.

Conflict of interest
The author declares there is no conflict of interest.

References
1. Perry RN. Understanding the survival strategies of nematodes. Animal Science Reviews.2011;99–102.
2. Ahmad F, Rather MA, Siddiqui MA. Nematicidal activities of leaf extract from Lantana camara L. against Meloidogyne incognita (Kofoid and White) Chitwood and its use to manage root infection of Solanum melongena L. Brazilian Archives of Biology and Technology.2010;53(3):543–548.
3. Davis EL, Hussey RS, Baum TJ. Getting to the roots of parasitism by nematodes. Trends Parasitol.2004;20(3):134–142.
4. Elekcioglu IH, Ohnesorge B, Lung G, et al. Plant-parasitic nematodes in the Mediterranean region of Turkey. Nematologica Mediterranea.1994;22(1):59–63.
5. Garima G, Singh A, Trivedi PC. Bacteria: A potential bioagent against root-knot nematode, Meloidogyne incognita. National Symposium on Recent Advances and Research Priorities in Indian Nematology; IARI, New Delhi.2005:14–20.
6. Ploeg A. Biofumigation to manage plant-parasitic nematodes. In: Ciancio, et al. editors. Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer Vegetable and Grain Crops Nematodes, Netherlands.2008:239–248.
7. Nagaraju N, Karemegam N, Kadalmani B. Eco-friendly management of root-knot nematode, Meloidogyne incognita using organic amendments on tomato. International Journal of Research Pharmacological Science.2010;1:530–532.
8. Kerry BR. An assessment of progress toward microbial control of plant-parasitic nematode. J Nematol.1990;22(4):621–631.

Citation: Haroon SA, Hassan BAA, Hamad FMI, et al. The efficiency of some natural alternatives in root-knot nematode control. Adv Plants Agric Res. 2018;8(4):355–362. DOI: 10.15406/apar.2018.08.00337

Finding was reported by Ameer-Zareen et al., on root-knot nematode eggs in vitro when they have used the aqueous extract of ginger (Zingiber officinale). This study also agrees with the results of Barker et al. that nematode egg hatching was influenced by the exudates from its environment. In addition, egg hatching inhibition was increased with increase in exposure time, and this result also agrees with the results of Jommati et al. The inhibitory effect of plant extracts on egg hatching of nematode according to Adebrite & Adesiyan, might be due to the properties of the chemical compounds present in the extract that possess ovicidal properties. It was also suggested that botanicals with nematicidal properties affect the embryonic development or kill the eggs. Presumably, these properties found to increase with an increase in time, hence, the inhibition of egg hatching tend to increase with increasing the exposure period to the extract. These active chemicals either affect the embryonic development or kill the eggs or even dissolve the egg masses. It has been reported by Adebrite, Goswami et al., and Hackney et al., that extracts (i.e., Siam weed; Chromolaenaaodorata L., Neem; Azadirachtaindica A. Jass, Castor bean; Ricinuscommunis L., and Lemon grass; Cymbopogoncitratrus DC.) that contained alkaloids, flavonoids, saponins, amides including benzamide and ketones in a single form or in a combination inhibited nematode egg hatching. From the results of the present study, it has been found that neem extract was recorded the best results regarding inhibition of egg hatching compared to moringa, lantana, and liquorices extracts. Therefore, neem extract was exposed to GC/MS analysis to find out the active chemical compounds (Figure 9) that caused the toxicity effect on nematodes. The active chemical compounds found in this outbalanced extract and identified it as a best effective extract in inhibition of egg hatching are alkaloids, flavonoids, saponins, amides including benzamide and ketones, and others as follows: Hexane, 2, 4-dimethyl; Hexane, 2,2,5-trimethyl-; Cyclohexane, 2,4-diethyl-1-methyl-; Methylbicyclo [4.2.0] octane; Methallylcyclohexane; Cyclohexane ethyl-; Heptane, 2,6-dimethyl-; Cyclohexane, 1,2,4-trimethyl-, (1α,2β,4β)-; Trans-1,2-Diethyl cyclopentane; Octane, 4- methyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyl-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-; cis-; Benzene (1-methylcyclohexyl); Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2-methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; 2,4- di-ter-butyl-phenol showed antioxidant activity. Many of the compounds found in the extract of Azadirachtaindica also remained unidentified in GC–MS analysis due to similar fragmentation pattern of many compounds with different retention times. It has been concluded from the results of the present study that the leaf extract of Azadirachtaindica has the ability to inhibit the egg hatchability of root-knot nematode. Thus, this finding is important in the identification and development of alternative strategies in controlling the root-knot nematodes. There is, however, further work is needed to identify some of these main compounds after purification. This study confirms the presence of nematicidal compounds in petroleum ether fractions of Azadirachtaindica, which were responsible for the prevention of egg hatching of root-knot nematodes at some concentrations, especially 4000 ppm.

Conflict of interest
The author declares there is no conflict of interest.
9. Jayaprakash K, Ayyaran M, Geetha KN, et al. Traditional uses of medicinal plants among the tribal people in Theni district (Western Ghats), South India. Asian Pacific Journal of Tropical Biomedicine. 2011;1(1):S20–25.

10. Kadam PV, Kavita NY, Ramesh SD, et al. Mimusopselengi: a review on ethnobotany, phytochemical and pharmacological profile. Journal of Pharmacognosy and Phytochemistry. 2012;1(3):71–78.

11. Azhagumurugan C, Ranjan MK. Effect of leaf extract of nilkumil, (Gmelinaasiatica) against the root-knot nematode, (Meloidogyne incognita). Research Journal of Recent Sciences. 2014;3:264–266.

12. Brauer M, Devkota B. Control of Thaumatopoeiacocampa (Dem. & Schiff) by extracts of Melia azedarach L. (Meliaceae). Journal of Applied Entomology. 1990;110:128–135.

13. Hussey RS, Baker RR. A comparison of methods of collecting inocula of Meloidogyne spp. Including a new technique. Plant Disease Reporter. 1973;57:1025–1028.

14. Pandey RC, Dwivedi BK. Comparative study of different plant extracts for their nematicidal potential. Current Nematology. 2000;10(1):1:29–43.

15. Dias CR, Schwam AV, Ezeiquel DP, et al. Effect of aqueous extracts of some medicinal plants on the survival of Meloidogyne incognita juveniles. Nematologica Brasileira. 2000;24(2):203–210.

16. Insunza V, Aballay E, Macaya J. In vitro nematicidal activity of aqueous plant extracts on Chilean populations of Xiphinema americanum sensu lato. Nematropica. 2001;31(1):47–54.

17. Rakesh P, Alok K, Neetu K, et al. Nematicidal activity in flower of some medicinal and aromatic plants. Indian Journal of Nematology. 2001;31(1):96–98.

18. Hussaini SS, Rao RVVP, Pandu HK. Toxicity of water soluble leaf extracts against larvae and egg masses of three Meloidogyne species. Indian Journal of Nematology. 1996;26(1):23–31.

19. Nandal SN, Bhatti DS. Preliminary screening of some weeds shrubs for their nematicidal activity. Indian Journal of Nematology. 1983;28(2):253–255.

20. Khan AF. Nematicidal potential of some naturally growing plants against Pratylenchus zeae. Revenue Nematology. 1990;13(4):463–465.

21. Adegbite AA, Adesiyun SO. Root extracts of plants to control root-knot nematode on edible soybean. World Journal of Agricultural Science. 2005;1(1):18–21.

22. Ameer-Zareen, Zaki JM, Javed N. Nematicidal activity of ginger and its effect on the efficacy Pasteuria penetrans for the control of root-knot nematodes. Asian Journal of Plant Science. 2003;2(11):858–860.

23. Barker ADP. Novel approaches to potato cyst nematode control. In Abstracts Conference on Potato Production - Living with Pesticide Resistance. KwaZulu-Natal, South Africa. Trop J Pharma Res. 2012;11(5):729–737.

24. Choi SJ, Kim JK, Kim HK, et al. 2,4-Di-tert-butyphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. J Med Food. 2013;16(11):977–983.

25. Adegbite AA. Comparative effects of carbofuran and water extract of chromolaenaodora on growth, yield and food component of root-knot nematodes infested soybean (Glycine max L. Merrill) Ph.D. Diss., University of Ibadan, Ibadan, Nigeria. J Veg Sci. 2003;12:5–12.

26. Goswami BK, Vijayalakshmi V. Nematicidal properties of some indigenous plant materials against root-knot nematode Meloidogyne incognita on tomato. Indian Journal of Nematology. 1986;16(1):65–68.

27. Hackney RW, Dickerson OJ. Marigold, castor bean and Chrysanthemum control of Meloidogyne incognita and Pratylenchusalleni. J Nematol. 1975;7(1):84–90.

28. Sharma IP, Sharma AK, Prashad L, et al. Natural bacterial cell-free extracts with powerful nematicidal activity on root-knot nematode. Rhizosphere. 2018;5:67–70.

29. Siddiqui IA, Qureshi SA, Sultana V, et al. Biological control of root rot - root knot disease complex of tomato. Plant Soil. 2000;227(1-2):163–169.

30. Oliveira DF, Campos VP, Amaral DR, et al. Selection of rhizobacteria able to produce metabolitesactiveve against Meloidogyne exigua. European Journal of Plant Pathology. 2007;119(4):477–479.

31. Padgham JL, Sikora RA. Biological control potential and modes of action of Bacillus megaterium against Meloidogyne graminicola on rice. Crop Prot. 2007;26(7):971–977.

32. Adam M, Heuer H, Hallmann J. Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. PLOS One. 2014;9(2):1–8.

33. Rajeswari G, Murugan M, Mohan VR. GC-MS analysis of bioactive components of Hagoniayammustax L. (Linaceae). Res J Pharma Biol Chem Sci. 2012;3:301–308.

34. Boussadaa O, Saidana D, Chriaa J, et al. Chemical composition and antmicrobial activity of volatile components of Scorzonera undulate. J Essen Oil Res. 2008;20:1–5.

35. Yuan J, Raza W, Shen Q, et al. Antifungal activity of bacillus amyloliquefaciens NIN-6 volatile compounds against fusariumoxy sporum f. sp. Cubenese. Appl Environ Microbiol. 2012;78(16):5942–5944.

36. Okudoh VI, Wallis FM. Enhanced recovery and identification of a trypamine related antibiotic produced by intrasporangium N8 from KwaZulu-Natal, South Africa. Trop J Pharma Res. 2012;11(5):729–737.

37. Choi SJ, Kim JK, Kim HK, et al. 2,4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. J Med Food. 2013;16(11):977–983.

38. Sardari AA, HojatJalili AA, Bahraminejad S, et al. Effect of plant extracts on the mortality of root-knot nematodes’ J2, Meloidogyne javanica. Archives of Phyto pathology and Plant Protection. 2015;48(4):365–375.

39. Sowley ENK, Kankam F, Adomako J. Management of root-knot nematode (Meloidogyne f. sp. Cubenese) on sweet pepper (Capsicum annuum L.) with moringa (Moringaoleifera Lam.) leaf powder. Archives of Phyto pathology And Plant Protection. 2014;13:1531–1538.

40. Taye W, Sakhuja PK, Tefera T. Root-knot nematode (Meloidogyneincognita) management using botanicals in tomato (Lycopersiconesculentum). Academia Journal of Agricultural Research. 2014;1(1):9–16.