Research Article

Eco-Friendly Biocontrol of Root-Knot Nematode, *Meloidogyne incognita in* Cowpea using Fresh Leaves and Flowers of Khella (*Ammi majus*)

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Abstract | Under *in vitro* conditions, aqueous extracts of Khella (*Ammi majus)* mashed fresh leaves and flowers at concentrations of 2.5, 5.0 and 10.0 % were bioassayed against root-knot nematode, *Meloidogyne incognita* second stage juveniles (J_2s). The obtained results revealed that the tested extracts at 10% caused 100% nematode mortality at 72 h of exposure followed by the other concentrations with the percentages of net mortality ranging from 49 at the lowest concentration of mashed leaves to 100% at the highest concentration of mashed leaves and flowers. Under screen house conditions, the same plant parts of khella as residues at the rates of 5.0 and 10.0 g or their aqueous extracts at concentrations of 5.0 and 10.0 % were treated to pots (5kg soil) planted to cowpea cv. Baladi infected with *M.incognita*. The greatest percentages of nematode reductions, 84.8 and 84.0% caused by mashed leaf and flower residues on cowpea at their highest rate (10g), respectively followed by other rates. In addition, the maximum percentages of nematode reductions, 78.2 and 86.1%, occurred at the highest concentration (10%) from the respective mashed leaf and flower extracts. Number of galls was reduced coinciding with the increasing the tested rates or concentrations. Consequently, plant growth and yield parameters increased coinciding with the tested rates for residues and concentrations for extracts of khella.

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Introduction

The main methods for controlling plant-parasitic nematodes are chemical pesticides or soil fumigants rather than other approaches. One of the most limiting factors that affects commercial production of vegetables and causes from 15% to 60% yield loss is root-knot nematode, *Meloidogyne incognita* (Krishnappa *et al*., 1992). The application of medicinal plant parts, their extracts and plant products are considered one of the most effective approaches for nematode control, because they are cheap, easy to apply, causing no threats to environment and human health and improving soil fertility (Pakeerathan *et al*.,

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2009; Ghazalbash and Abdollahi, 2013; Youssef and Lashein, 2013; El-Nagdi *et al*., 2014; Youssef *et al*., 2015; Müller and Mioranza, 2016; El-Nagdi *et al*., 2017). Using organic amendments and extracts of some medicinal plants can reduce *M. incognita*, which consequently increase the growth and yield of the plant (Zareena and Das, 2014; El-Nagdi and Youssef, 2021). Aatrilal or bishop's weed or greater ammi, *Ammi majus* is a medicinal herb, belonging to the family, Apiaceae. Furanocoumarins and xanthotoxin are considered the major chemical constituents of this plant (Zafar *et al*., 2018) that may act as toxic substances against nematodes. Few studies carried out on effect of khella residues on root-knot nematode, *Meloidogyne* spp. Amin and Youssef (1997) used *A. majus* as powdered dry and chopped fresh leaves for controlling *M. javanica* and *Rotylenchulus reniformis* on sunflower. They found that its tested residues decreased significantly nematode parameters in soil and roots and consequently increased plant growth criteria and flowering disc weights.

Therefore, the purpose of this research was to study effect of Khella plant as mashed fresh leaf and flower residues and their aqueous extracts on root- knot nematode, *M. incognita* infecting cowpea under *in vitro* and screen house conditions.

Materials and Methods

Source of the test plant

The cowpea cv. Baladi seeds were provided by Vegetative Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Egyptian environment is rich in the tested plants.

Preparation of aqueous extracts of the plant residues

Khella residues weighing 2.5, 5.0 and 10.0 g in the forms of mashed fresh leaves and flowers were prepared. Each weight was thoroughly soaked in 100-ml distilled water and left for 72 hr. Then, filtered through Whatman's filter paper no. 1 to form aqueous concentrations of 2.5, 5.0 and 10.0% to be used *in vitro* and *in vivo* conditions.

Identification and pure culture of root-knot nematode inoculum

A single egg mass of root-knot nematode was used to make pure culture on susceptible tomato cultivar in a screen house. The tested species of root-knot nematode was identified from nematode adult females as *M. incognita* depending upon their perineal patterns (Taylor and Sasser, 1978). Newly hatched J_2s of *M. incognita* in soil used as inoculum were extracted according to Barker (1985). For extraction J_2s from roots, tomato roots with galls bearing egg masses were washed thoroughly and incubated in tap water according to Young (1954) and J_2s were collected every 24 hrs to be used *in vitro* and *in vivo* experiments.

In vitro bioassay

This bioassay was performed by using three concentrations, 2.5, 5, and 10% from each material to be easily mixed with solution of nematodes. Concentrations were tested to nematodes by adding 1 ml distilled water containing 300 individuals in plastic capsule with 9 ml of each filtrate. Equal number of juveniles without treatment also served as control. Under light microscope, numbers of dead and live juveniles per each treatment were determined at 24, 48 and 72 hrs after treatment. To ascertain that the J_2s were dead after treatment, they did not move when probed by a fine needle. The percentages of nematode mortality were calculated according to Abbott's Formula as cited by Finney (1971) as follows:

$$Juvenile\ mortality\ (\%) = (m - n)/ (100 - n) \times 100$$

Where the dead juveniles percentages in the treatment and control were expressed by m and n, respectively. Net mortality was calculated by subtracting nematode recovery (live or survived juveniles) percent in distilled water from total mortality percent at 72 hrs exposure

In vivo experiment

Under screen house conditions, cowpea (*Vigna unguiculata* (L.) Walp.) cv.Baladi seeds were sown at the rate of 1 seedlings in each pot of 30-cm in diameter containig 5kg solarized sandy: loam (1:1) soil in April 5,2022. Two weeks after seed emergence, plantlets in each pot were inoculated with 3,000 newly hatched nematode juveniles (J_2s) by adding suspension of nematode in 4 holes around roots in each pot. Before sowing, mashed fresh leaves and flowers of khella (*Ammi majus*), each was added at the rates of 10 and 5g and thoroghly mixed with soil in each pot in five replicates for each treatment. Each aqueous extract of these materials at concentrations of 5.0 and 10.0% was added separately at the rate (volume) of 20-ml per pot in 5 replicates. Equal number of untreated pots with nematodes only served as control.

A completely randomized design was used to arrange pots on a bench under screen house conditions maintained at 30±5 °C. Then, the plants were irrigated as needed. Three months of nematode inoculation (Harvest stage of cowpea), plants of cowpea were carefully uprooted and roots were washed thoroughly with running tap water to discard debris. Numbers of J_2s in roots examined by root teasing, egg masses as well as number of galls were counted in one half of roots. Then, number of J_2s in another half of roots was extracted by incubation method described by Young (1954). Soil samples were treated by sieving and decanting technique (Barker, 1985) to extract number of J_2 per pot and counted.

Simultaneously, shoot length (cm), fresh and dry weights (g) and fresh and dry weights of roots (g) as indicators of plant growth parameters were measured. In addition, number and weight of pods (g), number and weight of seeds per pod and weight of 100 seeds as indicators of yield attributes were recorded. Mean percentages of nematode reduction, plant growth and yield increases were used to compare among treatments. To calculate this parameter, sum percentages of all parameters of each treatment was divided on number of these parameters.

Statistical analysis

Analysis of variance (ANOVA) procedures was performed in this experiment. For comparing among treatments, Duncan's Multiple Range Test as reported by Snedecor and Cochran (1989) was used at probability 5% by Computer Statistical (COSTAT) software.

Results and Discussion

Impact on mortality % of second stage juveniles (J_2s) of M. incognita

From Table 1, the greatest concentration (10%) of mashed leaf and flower extracts exhibited 100% mortality at 72 hr of exposure with net mortality 100% too. Mashed leaves extract at other concentrations, 2.5 and 5% exhibited net mortalities of 49 and 84%, respectively after 72 hr. However, mashed flowers extract exhibited net mortalities 95 and 100%, respectively. In general, mashed flowers extract seemed to be more efficient in reducing nematode juveniles than mashed leaves extract.

Table 1: *Effects of leaf or flower extracts of khella on %mortality of the second-stage juveniles (J_2s) of Meloidogyne incognita in vitro bioassay.*

Treatments	Concen-tration (%)	% Juvenile mortality (in hours)			% Net mortality
		24h	48h	72h	
Mashed fresh Leaves extract	2.5	95	97	60	49
	5.0	97	97	91	84
	10.0	100	100	100	100
Mashed fresh flowers extract	2.5	92	97	98	95
	5.0	98	98	100	100
	10.0	100	100	100	100
Untreated (control)	Distilled water	-	-	-	-

Values are means of 5 replicates.

Impact on nematode parameters

Data in Table 2 illustrated the effect mashed leaf and flower residues of khella at two rates, 5 and 10 g on reproductive parameters and galls of root-knot nematode. M. incognita on cowpea soil and roots. The averages of total percentages nematode reduction proved that the maximum reduction was achieved by the highest rate of the tested materials and vice versa as follows: The mashed leaves at 10g recorded the highest average percentages nematode reduction (84.8%) followed by mashed leaves at 5g (73.6%). Also, mashed flowers at 10g recorded almost the same average percentage reduction (84.0%) more than mashed flowers at 5g (72.5%). Number of galls behaved the same trend. Generally, the mashed leaves residue was as effective as mashed flowers residue in reducing nematode parameters (Table 2). As for extracts of plant parts, the greatest average total percentage nematode reduction (86.1%) was achieved by using the highest rate of fresh flowers extract (10%) followed by 83.7% a by the same extract at 5%. Also, mashed fresh leaves extract achieved average percentages (78.2%) by the highest rate followed by the lowest rate (66.9%). In addition, effect of extracts of mashed leaves at the two concentrations on number of galls behaved the same trend (Table 3). In general, it was seemed that fresh flowers extract was more effective than fresh leaves extract.

Impact on plant growth and yield parameters

Plant growth and yield parameters of cowpea treated with *A. majus* residues and extracts were similar in their response, as they increased by the tested materials at the highest rates and concentrations more than those by the lowest ones (Tables 4 and 5). However, mashed

Table 2: *Effect of fresh leaf or flower residues of khella on root-knot nematode, Meloidogyne incognita infecting cowpea under screen house conditions.*

Treatments	Rate (g)/pot	Reproductive nematode parameters						% Average total percentages nematode reduction	Galls	
		J_2s in soil/pot	% Reduction	J_2s in roots	% Reduction	Egg masses	% Reduction		No.	% Reduction
Mashed fresh leaves residue	5	2600b	85.9	80b	66.7	14bc	68.2	73.6	19bc	70.8
	10	2000c	89.1	45cd	81.3	7d	84.1	84.8	13c	80.0
Mashed fresh flowers residue	5	1600c	91.3	68c	71.7	20b	54.5	72.5	23b	64.6
	10	1200c	93.5	45d	81.3	10cd	77.3	84.0	15c	76.9
Untreated (control)	-	18400a	-	240a	-	44a	-	-	65a	-

Values are means of 5 replicates. Means in the same column followed by the same letter(s) do not significantly (p ≤ 0.05) differ based on Duncan's Multiple Range Test. Reduction= Control-Treated/Control x100.

Table 3: *Effect of fresh leaf or flower extracts of khella on root-knot nematode, Meloidogyne incognita infecting cowpea under screen house conditions.*

Treatments	Concentration (%)	Reproductive nematode parameters						% Average of total percentages nematode reduction	Galls	
		J_2s in soil/pot	% Reduction	J_2s in roots	% Reduction	Egg masses	% Reduction		No.	% Reduction
Mashed fresh Leaves extract	5	2200b	88.0	100 b	58.3	20 b	54.5	66.9	27 b	58.5
	10	1600c	91.3	60 c	75.0	14 c	68.2	78.2	19 c	70.8
Mashed fresh flowers extract	5	1400cd	92.4	45 cd	81.3	10 cd	77.3	83.7	17 c	73.8
	10	1000 d	94.6	38 d	84.2	9 d	79.5	86.1	15 c	76.9
Untreated (control)	-	18400 a	-	240 a	-	44 a	-	-	65 a	-

Values are means of 5 replicates. Means in the same column followed by the same letter(s) do not significantly (p ≤ 0.05) differ based on Duncan's Multiple Range Test.

Table 4: *Effect of Khella fresh leaf or flower residues on vegetative and yield parameters of cowpea infected by root-knot nematode, Meloidogyne incognita under screen house conditions.*

Treatments	Rate (g)/pot	Shoot			Root		Pod		Seed			%Average total percenta-ges of plant growth and yield increases
		Length (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	No.	Weight (g)	No./pod	Weight/pod (g)	Weightof 100 seeds (g)	
Mashed fresh leaves residue	5	57a (0.0)	110.6a (73.1)	20.4b (106.1)	9.5a (93.9)	2.4a (50.0)	15a (114.3)	10.8b (58.8)	6b (0)	0.71c (6.7)	9.80c (18.6)	- 52.2
	10	61a (2.0)	115.1a (80.1)	24.7a (149.5)	10.3a (110.2)	2.5a (56.3)	16a (129.6)	13.2a (94.1)	8a (33.3)	1.28a (91.0)	11.70a (41.6)	- 78.8
Mashed fresh flowers residue	5	56a (0.0)	66.1c (3.4)	12.6c (27.3)	8.9a (81.6)	1.9b (18.8)	9b (28.6)	7.3c (7.4)	7b (16.7)	0.84b (25.4)	10.60b (28.3)	- 23.8
	10	56a (0.0)	77.4b (21.1)	13.0c (31.3)	10.7a (118.4)	2.3a (43.8)	10b (42.9)	7.9d (16.2)	8a (33.3)	1.25a (86.6)	11.10b (34.4)	- 42.8
Untreated (control)		60a (-)	63.9c (-)	9.9d (-)	4.9b (-)	1.6c (-)	7b (-)	6.8e (-)	6b (-)	0.67c (-)	8.26d (-)	- (-)

Values are means of 5 replicates. Means in the same column followed by the same letter(s) do not significantly (p ≤ 0.05) differ based on Duncan's Multiple Range Test. Values between brackets indicate the percentages of increase.

fresh leaves proved to be more superior to mashed flowers in increasing plant growth in terms of shoot length, fresh and dry weights and root fresh and dry weights and yield parameters in terms of number and weight of pods, number and weight of seeds/pod and weight of 100 seeds.

Results indicated that percentages of mortality of J_2s were concentration- and exposure period- dependent as the percentage of mortality increased with increasing filtrate concentrations of khella flower and leaf extracts and time of exposure. The greatest nematode mortality (100%) occurred by the highest concentration (10%) of each of the previous extracts.

Table 5: *Effect of Khella fresh leaf or flower extracts on vegetative and yield parameters of cowpea infected by root-knot nematode, Meloidogyne incognita under screen house conditions.*

Treat-ments	% Con-centra-tion	Shoot			Root		Pod		Seed			%Average total percent-ages plant growth and yield increase
		Length (cm)	Fresh weight (g)	Dry weight (g)	fresh weight (g)	dry weight (g)	No.	Weight (g)	No./ pod	Weight/ pod (g)	Weight of 100 seeds (g)	
Mashed fresh leaves extract	5	56 b (-)	65.2 d (2.0)	12.2 b (23.2)	7.0 b (1.4)	2.0 a (25.0)	9bc (28.6)	8.2 b (20.6)	7bc (16.7)	0.71 b (6.7)	10.03 b (21.4)	- 14.6
	10	61ab (2.0)	69.1 c (8.0)	15.8 a (59.6)	8.9 a (29.0)	2.1 a (31.3)	12 a (42.9)	9.6 a (41.2)	7bc (16.7)	0.75 b (11.9)	10.17 b (23.1)	- 24.3
Mashed fresh flowers extract	5	60 b (0.0)	76.1 b (19.1)	12.9 b (30.3)	6.7 b (36.7)	1.8ab (12.5)	11ab (57.1)	8.9 b (30.9)	8 b (33.3)	1.15 a (71.6)	10.98 a (32.9)	- 32.4
	10	66 a (10.0)	93.0 a (45.5)	15.0 a (51.5)	6.9 b (40.8)	1.8ab (12.5)	11ab (57.1)	10.7 a (57.4)	10 a (66.7)	1.21 a (80.6)	11.69 a (41.5)	- 46.4
Untreated (control)	-	60 b (-)	63.9 d (-)	9.9 c (-)	4.9 c (-)	1.6 b (-)	7 c (-)	6.8 b (-)	6 c (-)	0.67 b (-)	8.26 c (-)	- (-)

Values are means of 5 replicates. Means in the same column followed by the same letter(s) do not significantly ($p \leq 0.05$) differ based on Duncan's Multiple Range Test. Values between brackets indicate the percentages of increase.

In the present study, the potentiality of the mentioned extracts to kill and immobilize second stage juveniles of root-knot nematode at different degrees was proved which might be due to effect of their toxic contents, Furanocoumarins and Xanthotoxin against the tested nematode. Consistently, as cited by Pakeerathan *et al*. (2009), that leaf extract of medicinal plant, neem inhibited egg emergence and increased juvenile mortality percent of *M. incognita* up to 60%, which may be due to ethanol chemical compound present in the leaf extract (Aderbite and Adesiyan, 2005). Also, using extract of dry leaves of rosemary induced the greatest percentage of root-knot reduction (El-Nagdi and Youssef, 2021) which may attributed to the results obtained by Brand *et al*. (2010) who showed that using aqueous extract at 3% of dry leaves of rosemary induced phaseolin and phytoalexines as toxic materials in the hypocotyl of beans infected by fungus, *Colletotrichum lindemuthianu*.

Results showed that khella as mashed fresh leaf and flower residues, and their extracts significantly reduced number of egg masses and galls of root-knot nematode, *M. incognita* on cowpea in roots and number of J_2s in soil and roots according to the tested materials. In addition, the same materials increased plant growth and yield of cowpea that may refer to that reduction in nematode favored the development of plants as shown by Wille *et al*. (2019). Sowley *et al*. (2013), cited that essential oils produced by the medicinal plants contain high contents of certain oxygenated substances with lipophilic properties which dissolve the cytoplasmic membrane of nematode cells and their functional groups. The enzyme of protein structure of nematode may be influenced by these substances (Knobloch *et al*., 1989). Another explanation that the nematode reduction by the tested materials may be referred to their direct contact with root system and inoculated nematode juveniles, when they were added to rhizosphere soil in our experiment. In contrast, in another study, Müller and Mioranz (2016) explained the reduction in number of galls on soybean by the systemic response of defense mechanisms in plant, when the extract was sprayed only on top parts and not in direct contact with root system. In addition, the tested mashed residues, when used as organic amendments, may be influenced by their decomposition in soil, as they produce toxic products against nematodes or they provide beneficial ingredients for plant growth (Mahmood and Saxena,1992). In addition, one of the most important factors is carbon/nitrogen (C/N) ratio of the amendment that affects on the degree of its decomposition and subsequently affecting on nematodes (Stirling, 1991) and that could influence the present results. The effect of khella residues and extracts, used in this study, in reducing the nematode population agreed with the results of Amin and Youssef (1997).

Conclusions and Recommendations

The tested khella parts at the highest rate (10g) and concentration (10%) achieved the highest percentages of nematode reduction on cowpea followed by other ones. Consequently, plant growth and yield

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parameters increased coinciding with the tested rates and concentrations of khella. Some factors as chemical contents or C/N ratio could inflence the effect of khella residues and extracts. Therefore, further studies are needed to ensure their efficacy on nematodes.

Novelty Statement

The tested residues and their aquatic extracts of khella plant used in this experiment are tested almost for the first time as few reports were found in the literture about their effects on plant parasitic nematodes. These materials, especially at the greatest rates or concentrations achieved the highest percentages nematode reduction on cowpea. Consequently, plant growth and yield parameters increased coinciding with the tested treatments.

Author's Contribution

Mahmoud M.A. Youssef suggested the idea, wrote the experiment and executed this work.
Wafaa M.A. El-Nagdi carried out this experiment in the laboratory and screen house and statistically analyzed data. The two authors read and approved this manuscript.

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The authors have declared no conflict of interest.

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