Research Article

Comparative Methods for Controlling Root Knot Nematode, *Meloidogyne javanica* under Laboratory and Greenhouse Conditions

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Abstract | Under laboratory conditions, aqueous, acetonic and methanolic extracts of *Solanum nigrum*, *Atropa belladonna*, *Hyoscyamus muticus*, *Capsicum frutescens*, *Datura innoxia* and *Withania somnifera* were tested against root-knot nematode, *Meloidogyne javanica*. All the tested materials affected the survival of the nematode juveniles and egg-masses hatched depending on materials property, concentrations and solvents used in extraction. The aqueous extract had the best percentage of mortality and inhibition of egg hatching compared to the acetonic and methanolic extracts. Whereas, aqueous extracts of deadly nightshade, winter cherry and chili pepper at concentration 500 ppm applied to the plants as foliar sprays alone, foliar sprays with chelates and soil drenches were tested against root-knot nematode *M. javanica* infecting tomato plants cv. Strain-B under greenhouse conditions. Almost tested materials have significantly reduced nematode parameters compared to Oxamyl 24% L. and the untreated plants (check). The degree of nematode reduction varied according to the method of applications and the type of materials. Adding the extract with the chelating nutrients improved the properties of the plant, but adding the extract as doses to the soil reduced the numbers of nematodes better than other methods of application.

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Introduction

Plant-parasitic nematodes have the greatest impact on crop productivity when they attack the roots of plants immediately after seed germination (Ploeg and Stapleton, 2001). Root-knot nematodes, *Meloidogyne* spp. are common pathogen that parasitizes vegetables and other crops and cause significant yield reductions worldwide (Sasser, 1980). Nematicides such as oxamyl, Thionazin, carbofuran are effective in controlling nematodes but are not ecofriendly for their serious threat to the ecological balance. The influenced of plant parts on controlling nematodes and consequently improving plant growth, voluminous studies had been done on several economic vegetable crops by many workers (Montasser *et al.*, 1999; Olanigi *et al.*, 2005; Sowley *et al.*, 2014). Solanaceous plants have nematicidal activity for management nematode parasites (Hussain *et al.*, 2011; Saeed *et al.*, 2015) and the materials used in extractions varied depending to workers (Khan *et al.*, 2008; Meena *et al.*, 2010; Nandakumar *et al.*, 2017a; Correia, 2014; Oplos *et al.*, 2018). Youssef *et al.* (2016) used garlic clove and acetylsalicylic acid (ASA) aqueous extracts by soil

drench and foliar spraying for controlling root-knot nematode, *Meloidogyne incognita* infecting sugar beet cv. Gazelle.

The work has summarized literatures on the use of plant extracts prepared as aqueous extracts or extracted with chemical solvents including methanol and acetone in the control of plant parasitic nematodes *M. javanica in vitro*. Whereas, the objectives of greenhouse study to determine the extracts against the infection of *M. javanica* and to compare between modes of applications, soil drenches, foliar spray alone and foliar spray with chelates.

Materials and Methods

Nematode inoculum

Pure culture of *M. javanica* was raised from single egg mass and maintained on tomato roots in greenhouse. Infected plants were uprooted from soil and the entire root system was dipped in water and washed gently to remove adhering soil. Egg-masses were picked with forceps and rinsed with sterile water then placed in 0.5% sodium hypochlorite (NaOCl) solution agitated for 4 minutes and rinsed with sterile water on a 26 µm sieve (Hussey and Barker, 1973). The eggs were incubated for 3-5 days using a modified Baermann funnel method (Southey, 1986) to obtain second stage juveniles (J_2) for in vitro and pots experiments.

Extraction

Fifty grams of each plants powder were extracted with 500 ml of distilled water for 48 hrs. The suspension was filtered with Whatman No. 1 filter paper, and a concentration of "S" was prepared for each plant extract and was considered as standard. In addition, fifty grams of the powder of each plant was suspended in 500 ml of methanol or Acetone in a 1-L flask for 48 hours in the dark on an orbital incubator shaker at 200 rpm. The suspension was filtered under vacuum with Whatman No. 1 filter paper, then evaporated to dryness in a rotatory evaporator at 45 °C, and the resulting extract (5g) was stored at 5 °C until use. (Nandakumar *et al.*, 2017b).

In vitro experiment

The evaluation was carried out in 5 cm clean Petri dishes in 3 replications for each treatment. The Petri dishes with distilled water were taken as control. One hundred second stage (J_2) of *M. javanica* were suspended in 10 ml of different extracts with concentrations 250, 500 and 1000 ppm in acetone and methanol extracts. However, other dilutions of aqueous extract S/16, S/8 and S /4 were prepared by adding 16, 8 and 4 ml of distilled water to 1 ml of stander concentration "S" for studying the juveniles mortality. After 72 hours incubation, all dead and alive J_2 were counted and the percentages of J2 mortality were calculated.

$$\text{Percent mortality} = \frac{\text{No. juveniles dead}}{\text{Total No. juveniles}} \times 100$$

Five egg-masses medium size handpicked from the galls of eggplant were placed in each of Petri dishes containing 5 ml of extracts with concentrations 250 and 500 ppm in acetone and methanol extracts, other dilutions of aqueous extract S/8 and S /4 were prepared by adding requisite amount of distilled water to determine the effect on egg-masses hatching. Egg-masses kept in distilled water served as control. Each treatment were replicated 3 times. After 7 days exposure, the number of juveniles hatched were counted and the percentage of juveniles hatched and inhibition were calculated.

$$\text{Percent of juveniles hatched} = \frac{\text{No. Juveniles hatched in the treatment}}{\text{No. Juveniles hatched in control}} \times 100$$

$$\text{Percent inhibition of egg hatching} = \frac{\text{No. juveniles hatched in control} - \text{No. juveniles hatched in treatment}}{\text{Number of Juveniles hatched in control}} \times 100$$

Greenhouse experiment

Two grams powder of *Atropa belladonna*, *Withania somnifera* leafs and *Capsicum frutescens* fruits were macerated in 100 ml of distilled water for 48 hrs. at 28° C (2% w/v) (Ardakani, 2012). The suspensions were filtered and used as standard concentrations (2%) for each plant and diluted with the appropriate amount of distilled water to obtain 500 ppm concentration. Also, the nematicide (Oxamyl) was applied according to the recommended dose with the same methods of applications. On the other hand, seeds of tomato var. Strain-B were cultured in seed boxes at the greenhouse and water daily for one month, then the slips were transferred gently at the pots filled with a 1:1 mixture of loamy sand soil. Two weeks after transplant, the tomato plants were inoculated with 2000 freshly newly hatched juveniles obtained from pure culture of *Meloidogyne javanica*.

Materials applied to plants as soil drenches, sprayed onto leaf surface alone or sprayed onto leaf surface with chelates1 g/L (Verdimix form EDTA) two days after inoculation (El-Eslamboly *et al.*, 2019). All

treatments including the check (untreated, nematode only, nematode and chelates and chelates only) were replicated four times. Forty-five days after inculcation the plants removed carefully from the soil, length and fresh weights of both shoots and roots were estimated. Also, the number of galls, egg-masses per root and eggs per egg-mass were counted. Nematode final population and rate of nematode reproduction were calculated. Data were analyzed according to Duncan's Multiple Range Tests (1955).

Results and Discussion

Aqueous extracts of solanaceous plants were found to be highly effective having nematicidal potential against J_2 of *M. javanica* in in vitro experiment, (Table 2). The nematode mortality was in the range of 54.33 to 100.0 % in concentrations S/16, S/8 and S /4 % compared to distilled water (1.33 %). Deadly nightshade and chili pepper extracts were more effective against second stage juveniles at concentration of S/4 followed by winter cherry, thorn apple, black nightshade and Egyptian henbane and had shown 100, 100, 99, 98.33, 93 and 75.67 %, respectively. Whereas, acetone extracts of deadly nightshade, Egyptian henbane, thorn apple and winter cherry were found to be highly toxic to juveniles of *M. javanica* and killed more than 75 % of the second stage juveniles. Methanolic extracts of deadly nightshade, Egyptian henbane and winter cherry with concentrations 250, 500 and 1000 ppm exhibited (82.00, 82.33 and 92.33%), (84.67, 92.00 and 95.67%) and (81.00, 83.33 and 99.00%) of larval mortality percentage after 72 hrs, respectively.

Concentration (S/8) of aqueous extracts gave the highest percentage hatch inhibition in winter cherry (97.63%) followed by Egyptian henbane, deadly nightshade, black nightshade, thorn apple and chili pepper with percentage inhibition of egg hatching (95.42, 94.60, 92.87, 79.61 and 67.97%), respectively. While, acetonic extracts showed inhibition in hatching at both lower and higher concentrations with winter cherry treatment as the best (93.45 and 98.88%) followed by black nightshade (87.56 and 90.66%) followed by thorn apple (78.72 and 83.36%).

Methanolic extracts of winter cherry and black nightshade were found to be most effective in reducing percentage of egg-masses hatching in concentrations 250 and 500 ppm with percentage inhibition of egg hatching (93.45and98.88%) and (87.65and 90.66%), respectively. Methanolic extract of chili pepper proved to be less effective against hatchability of egg-masses of *M. javanica* (Table 2).

Data presented in Table 3 showed that all applications suppressed populations of nematodes and fewer root galls per plant were formed of applications. Therefore, all applications of the tested extracts significantly reduced the values of root galls per root, nematode reproduction on roots of tomato plants cv. Strain-B when compared with untreated plants (check). On the other hand, aqueous extracts of deadly nightshade (foliar sprays) and chili pepper (foliar sprays and soil drenches) treatments caused the lowest gall numbers and rate of nematode reproductions (38 and 0.73 and 25 and 0.35) respectively, compared to the untreated plants and plants treated with chemical control (Oxamyl). While, good nematode control was also achieved with aqueous extracts of deadly nightshade (soil drenches) and winter cherry (foliar sprays and soil drenches) with number of galls and rate of nematode reproductions (58, 1.88, 56, 1.99, 41 and 1.49), respectively.

Plant growths were inversely related to the level of nematode population resulting from the different treatments. No significant differences in tomato cv. Strain-B shoots and roots weights were observed among methods of application with all tested materials. Highly significant differences were observed in shoots and roots lengths. The greatest increase of plant length and weight (75.40-19.89 %) was observed in tomato treated with chili pepper applied as foliar spray with chelates followed by treatments with aqueous extract of winter cherry applied foliar spray with chelates (65.39 – 66.59%), respectively.

The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several authors (Adegbite and Adesiyan, 2005; Orisajo *et al.*, 2007; Abbasi *et al.*, 2008) who reported that, aqueous extracts of tested plant leaves showed nematicidal effect against *Meloidogyne* ssp., Root-knot nematode reduced hatching of egg-masses, increased mortality of juveniles with an increase in exposure of the time. Phytochemical analysis revealed that plants are rich in alkaloids: atropine, meteloidine, nicotine, scopolamine, hyoscyamine, terpenoids and flavonoids, which have high rate of nematicidal activity (Shahwar *et al.*, 1995; Pavela, 2004). The alkaloids killed 90 to 100% of *Hoplolaimus indicus*, *Helicotylenchus multicinctus*, and *M. incognita* (Qamar *et al.*, 1995).

Table 1: *Solanaceous plants used in the experiment.*

	Common name	Scientific name	Parts used	Collected from
1	Black nightshade	Solanum nigrum	leaves	Univ. farms and gardens
2	Deadly nightshade	Atropa belladonna	leaves	Univ. farms and gardens
3	Egyptian henbane	Hyoscyamus muticus	leaves	New Valley governorate
4	Thorn apple	Datura innoxia	leaves	Univ. farms and gardens
5	Winter cherry	Withania somnifera	leaves	Univ. farms and gardens
6	Chili pepper	Capsicum frutescens	fruits	New Valley governorate

Plant parts were washed thoroughly under running tap water, cut into small pieces, shade dried and used for extraction. Dried plant materials homogenized to a fine powder then stored in airtight bottles.

Table 2: *Evaluation of aqueous, acetonic and methanolic extracts of some solanaceous plants on larval mortality and egg masses hatching of M. javanica at different concentrations in vitro.*

Materials	Aqueous extract			Chemical extracts			
	Conc.	No. larval mortality after 72 hrs.	Inhibition of egg hatched %	Conc. ppm	Acetone		Methanol	
					No. larval mortality after 72 hrs.	Inhibition of egg hatched %	No. larval mortality after 72 hrs.	Inhibition of egg hatched %
Black nightshade (*Solanum nigrum*)	S/16	65.33 cd	78.64	250	29.00 e	87.56	27.67 e	70.87
	S/8	75.00 bc	92.87	500	73.67 d	90.66	34.67 e	73.41
	S/4	93.00 a	------	1000	83.67abcd	------	56.67 d	------
Deadly nightshade (*Atropa belladonna*)	S/16	97.33 a	92.06	250	83.67abcd	59.49	82.00 ab	44.65
	S/8	98.33 a	94.60	500	95.67 ab	86.82	82.33 ab	51.33
	S/4	100.00 a	------	1000	100.00 a	-------	92.33 a	-------
Egyptian henbane (*Hyoscyamus muticus*)	S/16	54.33 d	84.42	250	75.33 cd	62.46	84.67 ab	31.48
	S/8	66.67 cd	95.42	500	80.67 bcd	85.96	92.00 ab	59.43
	S/4	75.67 bc	-------	1000	91.00 abc	-------	95.67 a	------
Chili pepper (*Capsicum frutescens*)	S/16	89.00 ab	69.22	250	4.67f	5.17	3.33 f	57.86
	S/8	99.00 a	67.97	500	6.33f	60.01	11.00 f	34.20
	S/4	100.00 a	-------	1000	10.33f	-------	5.00 f	-------
Thorn apple (*Datura innoxia*)	S/16	94.33 a	72.23	250	80.67 bcd	78.72	61.67 cd	17.87
	S/8	96.00 a	79.61	500	79.67 bcd	83.36	74.33 bc	71.43
	S/4	98.33 a	-------	1000	84.67abcd		88.67 ab	-------
Winter cherry (*Withania somnifera*)	S/16	88.00 ab	88.21	250	91.00 abc	93.45	81.00 ab	54.79
	S/8	93.33 a	97.63	500	99.00 a	98.88	83.33 ab	64.63
	S/4	99.00 a	-------	1000	100.00 a	-------	99.00 a	-------
Distilled water (Control)		1.33 e	0.00		1.33 g	00	1.33 f	0.00
Mean		88.31 a	84.41 a		83.18 b	74.38b	75.73 c	52.66 c

Means in each column followed by the same letters are not significantly different by (P=0.05) according to Duncan's multiple range test.

The study relates to control plant parasitic nematodes using systemic nematicides by applying to plants a non-toxic nematicides effective amount of organic plants extracts. It has been recognized that an ideal solution might reside in the use of materials that could be applied to the foliage or stems of growing plants in such conventional forms as sprays, dusts, pastes. In accordance with this work, we have found to treat plants infected by nematodes with that organic plants extract which were used alone, or with chelating agents. Chelates are a class of compounds which when applied to the foliage and stems of growing plants are absorbed into said plants. Thus, it is possible to carry the used materials and systemically translocate to areas where nematodes choose to attack, primarily the roots, and thereby repel and/or eventually kill nematodes attacking plants. Data in the present study showed that, almost the plants sprayed by chelates were significantly the best in improving plant growth. Absence of zinc increased nematode density in soil and

Table 3: *Effect of some materials applied as soil drenches and foliar surface against root-knot nematode, M. javanica infecting tomato cv. Strain-B under greenhouse condition.*

Materials		Nematode parameters				Plant growth	
	Applications	No. Galls	(P_f)	(P_f/P_i)	Redaction %	Increase in plant length %	Increase in plant weight %
Deadly nightshade *(Atropa belladonna)*	Foliar	32g	1632	0.82	88.60	24.39	13.28
	Foliar +Chelates	66de	7974	3.99	44.51	44.4	22.2
	Soil drenches	58def	3766	1.88	73.85	53.07	6.52
Chili pepper *(Capsicum frutescens)*	Foliar	38g	1470	0.73	89.85	40.78	14.64
	Foliar +Chelates	69cde	6619	3.31	53.96	75.48	19.89
	Soil drenches	25g	709	0.35	95.13	26.68	6.22
Winter cherry (*Withania somnifera)*	Foliar	56def	3986	1.99	72.32	17.16	15.07
	Foliar +Chelates	78bcd	10427	5.21	27.54	65.39	66.59
	Soil drenches	41fg	2979	1.49	79.28	19.35	0.37
Oxmyle	Foliar	26g	376	0.19	97.36	57.95	16.54
	Foliar +Chelates	33g	289	0.14	98.05	65.76	25.72
	Soil drenches	25g	245	0.12	98.33	71.66	14.42
Untreated (control)		---	---	---	---	---	---
Nematode only		98a	14374	7.19	---	---	---
Nematode + Chelates		101a	14833	7.42	---	44.11	42.30
Chelates only		---	---	---	---	52.63	30.91

Means in each column followed by the same letters are not significantly different by (P=0.05) according to Duncan's multiple range test. P_f: Nematode final population; P_f/P_i= Rate of nematode reproduction.

reduced plant growth (Haque and Mukhopadhyaya, 1975; Siddique *et al*., 2002). The involvement of minerals especially Fe, Mg, Zn and Ca in the formation of enzymes (Graham *et al*., 1988; Auld, 2001), may explain their role in the acquired systemic resistance by increasing the antioxidant enzymes included in the defense mechanisms that resulted in reducing nematode populations. Systemic acquired resistance can be enhanced by applying materials of different sources which suppress nematode populations and improve the growth of treated plants either directly by their effects on nematodes or by enhancing resistance of treated plants (Farahat *et al*., 2015). Abamectin has been evaluated in soil applications and foliar sprays for potential control of plant parasitic nematodes in several crops (Sasser *et al*., 1982; Cayrol *et al*., 1993; Jansson and Rabatin, 1997).

Novelty Statement

Developing effective methods for using natural extracts in the soil to reduce nematode populations and boost plant productivity.

Author's Contribution

Mohammed Abdel-Mageed Abdel-Aziz Abdel-Mageed: A laboratory evaluation *Meloidogyne javanica*, a root knot nematode, was treated with aqueous, acetonic, and methanolic extracts.

Eman Alsayed Hammad: Corresponding author, evaluated Under greenhouse conditions, aqueous extracts of deadly nightshade, winter cherry, and chilli pepper applied to the plants as foliar sprays alone, foliar sprays with chelates and soil drenches against *M. javanica* were infecting tomato plants cv. Strain-B.

Nashaat Abdel-Aziz Mahmoud: Data statistical analysis.

Anas Farag El-Mesalamy: Extraction for tested material.

Conflict of interest

The authors have declared no conflict of interest.

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