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Nematicidal potential of some botanicals against *Meloidogyne Incognita in vitro* and *in vivo*

Manar M. Refaat*, Mostafa E. Mahrous, Ramadan M. El-Ashry and Amr M. El-Marzoky

Plant Prot. Dept., Fac. Agric., Zagazig University**, Egypt**

***Correspondence: manarrefaat@zu.edu.eg** **Received 15-12-2019, Revised: 15-01-2020, Accepted: 22-01-2020 e-Published: 23-01-2020**

Five fresh and uniform size egg-masses or 100 infective juveniles of the root-knot nematode, *Meloidogyne incognita* were separately exposed to aqueous leaf extracts of jojoba (*Simmondsia chinensis*), moringa (*Moringa oleifera*) and water hyacinth *(Eichhornia crassipes*) at three concentrations (S, S/2 and S/4). Results showed that egg hatching and juvenile mortality were obviously influenced by plant species, concentration and exposure time. All the tested extracts significantly ($P \leq 0.05$) inhibited egg hatching and increased juvenile mortality *in vitro*. Undiluted leaf extracts of jojoba and moringa gave the highest inhibition of egg hatching (89.1 and 87.9%) and juvenile mortality (89.8 and 84.1%) after 6 and 7 days of exposure, respectively. However, water hyacinth showed a relatively lower effect on egg hatching and juvenile mortality. Incorporation of leaf powder of jojoba, moringa and water hyacinth at rate of 10g/plant under greenhouse conditions, significantly ($P \leq 0.05$) suppressed galling and reproduction of *M. incognita* and enhanced plant growth of *Luffa aegyptiaca* as indicated by length and fresh weight of shoots. Jojoba and moringa (after oxamyl) gave the best results.

Keywords: *Meloidogyne incognita*, plants extracts, jojoba, moringa, water hyacinth, egg hatching, juveniles motility, *Luffa aegyptiaca* .

INTRODUCTION

Members of the root- knot nematodes (*Meloidogyne* spp.) are among the most damaging nematodes in agriculture, causing an estimated $100 billion loss/year worldwide (Oka et al., 2000). On the other hand, control of such nematodes is difficult partly because they have high reproductive potential, sedentary endoparasites and therefore protected in plant tissues and have extensive host range. Nearly one hundred valid species are in the genus *Meloidogyne* (Trinh et al.,2019).The most destructive species is *M. incognita* (Kofoid and White) Chitwood which cause serious damage in most agricultural crops worldwide (Moens et al.,2009). In Egypt this species is one of the most common and economically important root-knot nematodes cause considerable damage to majority of crops including sponge gourd, *Luffa aegyptiaca* L. especially in localities with sandy soils (Ibrahim, 1985 and Hilal et al., 2001).

The nematicides have been applied widely to control plant-parasitic nematodes with fast–acting and considerable results. However, they are both expensive and environmentally unfriendly method. Since there are many reports concerned with contamination of ground water and different food types with nematicides. For example, concentration of aldicarb residues exceeded the reference doses in orange fruits at Sharkia governorate, Egypt (Tchounwou et al., 2002). Most of the nematicides are banned because of their harmful effect on human and environment. The

recent European legislation has deeply revised and restricted their use on agricultural crops. This procedure stimulated scientists to find new alternative control strategies that are environmentally safe and economically convenient at the same time. Therefore, research on alternatives to chemical nematicides has received a strong impulse and considered a wide range of options including botanical nematicides. Since, a wide variety of plant species, representing 57 families have been shown to have nematicidal compounds such as alkaloids , isothiocyanates , phenols ,glycosides , thiophenics, tannins, saponins and fatty acids (Gommer,1981; Akhtar 2000 ;Chitwood,2002**;** Ntalli and Caboni, 2012). These plant extracts have no negative effect on non-target organisms, humans and animals and are environment friendly. On the other hand, many authors reported the nematicidal effect of jojoba (Abd-Aziz et al.,1996;El-Nagdi,2005;Ismail et al.,2011) moringa (Claudius-Cole et al.2010; Sowley et al. ,2014;Medeiros et al.,2018) and water hyacinth (Pathak et al.,1988;Umar and Mohammed,2013 ;Mehta et al., 2015). Therefore, the main view of the present experiments was designed to evaluate the nematicidal effect of aqueous leaf extracts of the aforementioned plants on egg hatching and juvenile mortality of *M.incognita in vitro* and the impact of leaf powder of these plants on the same nematode infecting sponge gourd under greenhouse conditions *in vivo*.

MATERIALS AND METHODS

Culturing of the root knot nematode, *M. incognita* and preparation of nematode inoculum

Pure culture of *M. incognita*, was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for using as source of inoculum. A single egg-mass was used to establish a nematode population. Species identification was based on juvenile measurements and examination of perineal pattern system of adult females according to Eisenback *et al*., (1981) and Jepson, (1987). Infected tomato roots were cut into pieces of 2-cm long and placed in a 600 –ml flask with 200 ml of 0.5% sodium hypochlorite (180 ml water + 20 ml Clorox). The tightly capped flack was shaken for 3 minutes. The shaking partially dissolved the gelatinous matrix and thus freeing eggs from egg-masses (Hussey and Barker,1973). The liquid suspension of eggs was poured through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs collected on the 500-mesh sieve were immediately washed free of residual sodium hypochlorite solution under a slow stream of tap water and incubated in Petri dishes at 25±1°C until hatching. Newly hatched juveniles were collected by using a micropipette.

Egg-masses of equal size needed to study the effect of the tested extracts on egg hatching of *M.incognita* were hand-picked with fine forceps from small galls on the infected tomato roots obtained from previously maintained pure culture. The collected egg-masses were surface sterilized in 1:500(v/v) aqueous solution of sodium hypochlorite (Clorox) for 5min (Haseeb et al.,2005).

Preparation of aqueous plant extracts

Leaves of jojoba , *Simmondsia chinensis* (Link) Schneid.,(family: Simmondsiaceae), moringa , *Moringa oleifera* Lam.(family: Moringaceae) and water hyacinth , *Eichhornia crassipes* (Mart.) Solms (family: Pontederiaceae) were obtained from their natural habitats at Sharkia Governorate .The collected leaves were washed, dried in a vacuum oven (45˚C.) and ground to a fine powder in a mill. Milled leaves were extracted with distilled water using magnetic stirrer at room temperature (*ca*. 25˚C) then by filtration via filter paper (Whattman No.1). The extraction ratio was 1 W: 10 V (plant: solvent). The extraction period was 2 hours. The filtration was stored at -20˚C for further usage. Each extract was arbitrarily termed as a standard solution (S). Other dilutions i.e., S/2 and S/4 were prepared by adding distilled water.

Effect of leaf extracts of jojoba, moringa and water hyacinth on egg hatching and juvenile mortality of *M. incognita in vitro*

A-Effect on egg hatching

Five fresh and uniform size egg-masses were transferred to 9-cm diameter Petri dishes contained 10 ml of each concentration i.e., S (100%), S/2 and S/4.Control treatment was prepared using distilled water. Each treatment was replicated five times. All treatments were left under room temperature 25 ±3˚C. Numbers of hatched juveniles were counted after 2,6,10 days of treatment using a research microscope (100X magnification). Percentage of hatching inhibition was calculated in comparison with the control treatment, according to the following equation:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{Control-treatment}}{\text{Control}} \times 100$$

Effect on juvenile mortality

The suspension concentration of second-stage juveniles (J_2) was adjusted to1000 infective juveniles per ml. About 100 juveniles in 0.1 ml of the suspension was added to final volume of 5 ml of prepared concentrations (S, S/2 and S/4) for each plant extract in 5 cm diameter Petri dishes. Control treatment was done using only distilled water. All treatments were left under room temperature (25±3˚C) to assess the effect of these treatments on juvenile mortality. Each treatment was replicated five times. Juvenile mortality was entailed after 1, 3, and 7 days post treatment. Second-stage juveniles showing inactive straight posture or did not show any movement after prodding were considered dead (Elizabeth et al., 2003). Mortality counts were observed under 100 X magnification in 1 ml over the specified periods. The mortality percentages were calculated according the following equation:

$$\text{Mortality } (\%) = \frac{\text{Dead juveniles}}{\text{Total number of juveniles}} \times 100$$

Efficacy of dry leaf powder of jojoba, moringa and water hyacinth on galling and reproduction of *M.incognita* infecting *Luffa aegyptiaca* L. under greenhouse conditions

Pot experiment was maintained in the greenhouse, Faculty of Agriculture, Zagazig University, Egypt on the sponge gourd (*Luffa aegyptiaca* L.) as a host plant. Seeds of sponge gourd (Local cultivar) were soaked in distilled water in Petri dishes and kept in an incubator at 27 ±1°C. After 48 hours, seeds were sown in formalin sterilized 15-cm diameter plastic pots filled with about 1600 g steam sterilized soil (2:1 v/v sandy soil: clay soil) mixed with 60 g compost. Three weeks after sowing, seedlings were thinned to one plant per pot. Ten grams of dry leaf powder of jojoba, moringa and water hyacinth were incorporated with the upper 3cm of soil around each plant. Pots were kept moist for a week before nematode inoculation to allow the degradation of the tested materials. All plants were inoculated with 1000 newly hatched infective juveniles of *M.incognita* by pipetting 2 ml of the inoculum suspension into three holes around the root system and directly covered with moist soil after inoculation. The chemical pesticide oxamyl (Vydate 24%EC), methyl 2-(dimethylamino)-N-[(methylcarbamoyl) oxy]-2-oxoethanimidothioate ,was applied to sponge gourd seedlings at the rate of 0.3 ml/plant. Plants of the control treatment were inoculated with *M. incognita* alone. Each treatment was replicated five times. All pots were arranged in a randomized complete design in the greenhouse at 28 ± 4°C., and received similar horticultural treatments. Forty -five days after inoculation, plants were removed carefully from pots and data on plant growth as indicated by length and fresh weight of shoots were recorded. An aliquot samples of 100 g soil were processed for nematode extraction using a combination of sieving and Baermann trays technique (Hopper *et al*., 2005).Roots were soaked in tape water for one hour to facilitate removing adhering soil and to keep egg -masses on root surface. During the steps of evaluation, roots were wrapped in tissue paper to prevent drying out and numbers of galls and egg masses were counted per root system.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using MSTAT VERSION 4 (1987). Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS

***In vitro* experiments**

A. Ovicidal activity of leaf extracts on egg hatching of *M. incognita*

Aqueous leaf extracts of jojoba, moringa and water hyacinth at three concentrations (S, S/2 and S/4) significantly ($P \leq 0.05$) inhibited egg hatching of *M. incognita*. The inhibitory effect varied according to plant species, concentration and exposure time. The highest values were detected with jojoba and moringa at the standard solution (S) after the sixth day post treatment with percent inhibition reached 89.1 and 87.9%, respectively, while the lowest value was observed with water hyacinth at S/4 dilution after the second day post treatment with percent reduction of 0.2 % . As dilutions of the tested botanicals were increased, numbers of emerged juvenile were obviously increased. For instance, number of emerged juveniles in treatments of jojoba and moringa at S, S/2 and S/4 concentrations, two days after treatment were 11.6 (12.8) , 33.6 (39.2) and 41.6(48.8) juveniles with percent inhibition of 84.6 (83.1) ,55.6 (48.2) and 45.1(35.6) % respectively. Likewise, numbers of emerged juvenile in water hyacinth treatment, at S,S/2 and S/4 after the same time were 60.8 , 61.6 and 75.6 juveniles with percent inhibition of 19.7 ,18.7 and 0.2 %, respectively . The same trend was found after 6

and 10 days post treatment. Generally, the rate of egg hatching inhibition was inversely proportion to the dilution of the extract. On the other hand, as exposure time increased from 2 to 6 days after treatments, ovicidal effect of the tested extracts was obviously increased at all the tested concentrations. However, at the tenth day after treatment, the inhibitory effect showed reversible trend. It was true with the three tested leaf extracts at the three dilutions. The distilled water treatment (control) had significantly higher numbers of eggs that hatched compared to other treatments. Ten days after treatment, mean hatch per egg mass was 214.3 eggs in distilled water treatment compared to 49.0 eggs per egg- mass in treatment of jojoba at the hundred percent concentration (S).

Larvicidal activity of leaf extracts on infective juveniles of *M. incognita*

Similarly, the water leaf extracts of the tested botanicals were found to be significantly ($P \leq 0.05$) effective against infective juveniles of *M. incognita* (Table 2). Among which, jojoba was the most effective one followed by moringa while water hyacinth was the lowest effective one. The highest juvenile mortality was found with jojoba at the standard solution after 7 days of exposure (89.8%) followed by moringa (84.1%) at the same concentration and exposure time while the least mortality was obtained at S/4 concentration after one day of exposure (1.0%). Generally, the nematicidal effect of the tested leaf extracts on *M. incognita* juveniles was directly proportion to concentration and exposure time. Standard dilution of jojoba, moringa and water hyacinth caused 89.8, 84.1 and 25.8 % after 7 days while half and quarter dilutions after the same period gave 84.4 (75.0), 72.8(64.8) and 21.8 (19.0) % respectively.

Table 1: Effect of leaf extracts of jojoba, moringa and water hyacinth on egg hatching of *M.incognita in vitro*

Treatments	Concentrations	Mean number of juveniles emerged after			Mean hatch per egg mass
		2 days	6 days	10 days	
Jojoba	S	11.6 d (84.6)	79.6 d (89.1)	245.2 e (77.1)	49.0
	S/2	33.6 d (55.6)	238.8 d (67.4)	617.2 de (42.4)	123.4
	S/4	41.6 c (45.1)	328.4 d (55.2)	694.4 e (35.1)	138.8
Moringa	S	12.8 d (83.1)	88.4 d (87.9)	309.6 d (71.1)	61.9
	S/2	39.2 d (48.2)	353.6 c (51.8)	664.4d (37.9)	132.8
	S/4	48.8 c (35.6)	391.4 c (46.7)	760.0 d (29.0)	152.0
Water hyacinth	S	60.8 b (19.7)	295.6 b (59.7)	694.4 b (35.1)	138.8
	S/2	61.6 b (18.7)	449.2 b (38.8)	856.0 b (20.1)	171.2
	S/4	75.6 a (0.2)	575.2 b (21.6)	984.8 b (8.1)	196.9
Distilled water		75.8 a	734.4a	1071.6 a	214.3

*Reported numbers represent means of 5 replicate.

**Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water.

***Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test. .

Pot experiment

The effect of three grinded leaves of jojoba, moringa and water hyacinth compared to oxamyl on *M. incognita* infected sponge gourd (*Luffa aegyptiaca*) under greenhouse conditions is presented in Table(3) . The obtained results revealed that all treatments significantly ($P \leq 0.05$) reduced galling (as indicated by number of galls) and reproduction (as indicated by number of egg-masses on roots and number of juveniles in soil) and enhanced growth of sponge gourd (as indicated by length and fresh weight of shoots) compared to plants inoculated with *M. incognita* alone. Pots treated with oxamyl overwhelmed those treated with grinded leaves. Among the tested plants, jojoba gave the best results followed by moringa while water hyacinth showed the lowest nematicidal effect.

Number of galls, egg-masses and juveniles in soil in treatments of jojoba and moringa were 12.2 (17.8), 3.4(7.0) and 315.0 (276.0), respectively. However, these values in treatment of water hyacinth and control were 23.4(32.8), 7.8(18.2) and 351.2(456.0), respectively. Percent reduction in galls, egg- masses and soil juvenile number in treatments of jojoba, moringa and water hyacinth were 62.8 ,81.3, 30.9; 45.7, 61.5, 39.4and 28.6,57.1 , 22.9% , respectively. For plant growth parameters, it was clear that all tested treatments ameliorated growth of the sponge gourd to certain extend as compared to plants inoculated with *M. incognita* alone.

Table 2: Effect of leaf extracts jojoba, moringa and water hyacinth on juvenile mortality of *M.incognita in vitro*

Treatments	Concentrations	Mean number of dead juveniles after		
		1 day	3 days	7 days
Jojoba	S	24.7 a	73.0 a	89.8 a
	S/2	16.0 c	67.4 b	84.4 b
	S/4	16.2 c	46.4 e	75.0 c
Moringa	S	20.0 b	61.8 c	84.1 b
	S/2	11.8 d	52.5 d	72.8 c
	S/4	9.0 e	29.7 f	64.8 d
Water hyacinth	S	2.8 f	20.2 g	25.8 e
	S/2	1.7 fg	16.5 h	21.8 f
	S/4	1.0 gh	11.7 i	19.0 g
Distilled water		0.1 h	1.8 j	3.7 h

*Reported numbers represent means of 5 replicates..

**Different letter(s) in the same column represent significant differences ($P \leq 0.05$) according to Duncan`s multiple range test.

Table 3: Effect of leaf powder of jojoba, moringa and water hyacinth in comparison with oxamyl on galling and reproduction of *Meloidogyne incognita* in relation to growth of *Luffa aegyptiaca* under the greenhouse conditions

Treatments	Shoot fresh weight (g)	Shoot length (cm)	Number of galls per root system	Number of Egg-masses per root system	Number of juveniles per 100 g soil
	(Increase %)	(Increase %)	(Reduction %)	(Reduction %)	(Reduction %)
***M. incognita* alone**	7.0 d	28.6 c	32.8 a	18.2 a	456.0 a
***M.incognita* + jojoba**	9.6 b (37.1)	38.0 b (32.8)	12.2 d (62.8)	3.4 c (81.3)	315.0 c (30.9)
***M.incognita* + moringa**	8.3 c (18.5)	36.0 b (25.8)	17.8 c (45.7)	7.0 b (61.5)	276.0 d (39.4)
***M.incognita* + water hyacinth**	7.1 d (1.4)	29.8 c (4.1)	23.4 b (28.6)	7.8 b (57.1)	351.2 b (22.9)
***M.incognita* + oxamyl**	10.5 a (50.0)	56.0 a (95.8)	2.8 e (91.4)	0.6 d (96.7)	27.8 e(93.9)

*Reported numbers represent means of 5 replicate.

*Different letter in the same column represent significant differences ($P \leq 0.05$) according to Duncan`s multiple range test. .

$$\text{Increase } (\%) = \frac{\text{Treated - Control}}{\text{Control}} \times 100 \qquad \text{Reduction } (\%) = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

Generally, jojoba and moringa surpassed water hyacinth in improving plant growth parameters. Percent increase in shoot fresh weight and shoot length in treatments of jojoba, moringa and water hyacinth were 37.1(32.8), 18.5 (25.8), 1.4 (4.1) %, respectively. The parallel values in oxamyl treatment were 50.0 and 95.8%, respectively.

DISCUSSION

Control of root-knot nematodes, Meloidogyne spp. with synthetic nematicides is expensive and cause many problems to environment and human health .On the other hand , as organic agriculture increased ,new alternative control methods need to be developed because chemical nematicides are not acceptable in organic farms. Nowadays, nematologists are resorting to the use of botanicals for the management of nematodes, since they are eco-friendly, ease to apply, not expensive and are available to farmers (Oka et al., 2000; chitwood, 2002; Prasad et al. 2002). The potential of applying botanicals in management of root –knot nematodes has been reported by many authors (Goswami and Vijayalakshmi, 1986; Akhtar and Mahmood, 1994; Asif et al., 2013).

This study has shown that the leaf extracts of jojoba, moring and water hyacinth showed inhibitory effect on egg hatching an increased juvenile mortality of *M.incognita in vitro*. Jojoba and moringa gave best results compared to water hyacinth. These findings corroborate the results obtained by Jidere and Oluwantayo (2018) who showed that leaf extract of moringa decreased egg hatch and increased juvenile mortality of *M.incognita in vitro*. However ,our results are not completely confirmed with those reported by Umar and Mohammed (2013),since they showed that crude extract of water hyacinth gave 100% mortality one day after treatment ,while in our study standard solution (S) of water hyacinth leaf extract gave 2.8 , 20.2 and 25.8% juvenile mortality after 1, 3 and 7 days of exposure .

Incorporation of leaf powder of the tested botanicals into the soil significantly suppressed galling and reproduction of *M.incognita* and consequently enhanced the growth of *L. aegyptica*. Better growth of sponge gourd in pots amended with 10g leaf powder of the tested plants appears to be due to suppression of galling and reproductions of *M.incognita* as well as due to their manural effect. Moreover, application of plant powders increase the tolerance and resistance of host plant against *M.incognita* .This result is in agreement with finding of Sowley *et al*.,(2014) ; El-saedy *et al*., (2015) ; Mostafa *et al*., (2017). Generally, inhibition of egg hatching and increasing juvenile mortality of *M.incognita in vitro* as well as reduction of galling and reproduction of such nematode *in vivo* strongly suggest the presence of compounds that possess ovicidal and larvicidal properities. Further studies are needed to identify and characterize these compounds as well as to ascertain the nematicidal potential to control *Meloidogyne* spp. under field conditions.

CONCLUSION

Leaf extracts and dry leaf powders of jojoba and moringa showed significantly higher nematicidal effect compared to water hyacinth against *M.incognita in vitro* and *in vivo* experiments.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example WEP designed and performleed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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