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Efficacy of some Aqueous Plant Extracts on Egg Hatching and Juveniles Mortality of the Root Knot Nematode, *Meloidogyne incognita* under Laboratory Conditions

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ABSTRACT

Aqueous extracts of *Zingiber officinale* (Leaves), *Salvadora persica* (Leaves and stem), *Moringa oleifera* (Leaves), and *Origanum vulgare* (Leaves), at three concentrations (S, S/2, S/4) were assessed on egg hatching and juvenile mortality of the root knot nematode, *Meloidogyne incognita in vitro*. Data were recorded after 12, 24, 48 and 72 hours of application. Results showed that, some of the tested extracts significantly (p =0.05) inhibited egg hatching and increased juvenile mortality as compared to check treatment. The result noted that, the highest reduction in egg hatched and hatching inhibition percentage recorded with *Salvadora persica* leaves and in juveniles mortality and reduction mortality percentages were (17 and 58.53 %) (136 and 90.67%), respectively.

Keywords: Aqueous extracts; Root knot nematode, Egg hatching, Juvenile mortality.

INTRODUCTION

Root knot nematodes *Meloidogyne* spp. have a wide host range and are considered the greatest threat to global agricultural production (Dong *et al.,* 2001 and Anwar *et al*., 2007). Incorporation of plant parts/extracts into the soil alone or with biocontrol agents have been suggested as an alternative, safe and effective control method for the management of plant parasitic nematodes (Siddiqui & Alam, 1985; Kaur & Katoch, 2012 and Sowley *et al*., 2014). Aqueous extracts of ginger (*Zingiber officinale*), miswak (*Salvadora persica*), moringa (*Moringa oleifera*) and oregano, (*Origanum vulgare*) were tested against root knot nematode *Meloidogyne javanica in vitro* as suppressants of egg hatching and causing juveniles mortality with varying degrees at different concentrations. Amer & Javed, (2003) noted that the aqueous extract of ginger significantly inhibited egg hatching and caused mortality of *M. javanica.* Fewer eggs were hatched in the extract after 48 hours, also significantly greater mortality was observed at 48 h exposure to plant extract than at 24 hours. Devi, (2002) evaluated the effect of aqueous extract of moringa against root knot nematode *Meloidogyne incognita* egg hatching and showed that egg hatch inhibition was ranged between 40 % to 63.7 % in the extracts with respect to control with 0 %. Juvenile mortality in extracts was from 82 % - 93.8 % compared to the control with 25 %

The objective of this study was to test nematicidal activity of aqueous extracts representing four plant species for their potential toxicity against eggs and juveniles of the root knot nematode, *M. incognita in vitro*.

MATERIALS AND METHODS

Aqueous extracts of ginger (*Zingiber officinale*), miswak (*Salvadora persica*), moringa (*Moringa oleifera*) and oregano, (*Origanum vulgare*) were prepared by soaking 25 g leaves in one liter of water. Miswak was used as dry steam also. After 48 hours, extracts were purified by filtration to remove plant tissues and considered as 100% (S) concentration and diluted to 50% (S/2) and 25% (S/4).

The galled roots of eggplant *solanum melongena* were soaked in tap water one hour to remove adhering soil particle. Roots were cut into small pieces of 2-cm and placed in a 1000-ml container with 200 ml of 0.5% sodium hypochlorite solution. The tightly capped container was shaken vigorously forthree minutes. The liquid suspension was poured through a 200-mesh sieve nested up on a 500 mesh sieve. Eggs suspended in the agitated solution passed through the 200-mesh sieve which removed the root debris and were collected on the 500-mesh sieve. Eggs were mashed free the residual sodium hypochlorite solution under the slow stream of tap water (hussy and Barker 1973).

In vitro assay 500 eggs were immersed in 5 ml of three concentrations of tested aqueous extracts at S, S/2 and S/4 prepared in sterile distilled water. Each treatment was replicated three times .Cups were loosely covered which slightly permit aeration and reduce evaporation, at 28 ± 5 ˚C. Percentage of egg hatch inhibition was calculated using the following formula:

$$\text{Hatching inhibition \%} = \left(\frac{\text{Number of juveniles hatched in control} - \text{Number of juveniles hatched in treatment}}{\text{Number of juveniles hatched in control}}\right) X\ 100$$

A total of 150 freshly hatched second stage juveniles used for each treatment including the control. 1-ml of this juveniles suspension including 150 j_2 and poured in screw-capped test tubes which contained 5 ml of different concentrations of plant extracts incubated at 28±5 °C for three days and the numbers of dead juveniles were counted at 24,48 and 72 hours. Each treatment was replicated three times. The mortality percentage was calculated according to the following formula.

$$\text{Juveniles mortality \%} = \left(\frac{\text{Number of survied juveniles in treatment}}{\text{Number of survived juveniles in control}}\right) X\ 100$$

The randomized complete design was used in the experimental. Data were subjected to analysis of variance (ANOVA) using SPSS statistics. The means were compared according to Duncan's multiple range tests at P ≤0.05 (Duncan, 1955).

RESULTS

Water extracts of *Zingiber officinale* (Leaves), *Salvadora persica* (Leaves and steam), *Moringa oleifera* (Leaves), and *Origanum vulgare* (Leaves) at three concentrations (S, S/2, S/4) were assessed on egg hatching of the root knot nematode, *Meloidogyne incognita in vitro*. Numbers of hatched juveniles were recorded after 12, 24, 48 and 72 hours of application (Table 1). It was found the tested solutions significantly ≤ 0.05 inhibited egg hatching compared with check. After 24 h, a *Zingiber officinale* chieved best impact on mean number of juveniles hatched and hatching inhibition (8and 58.53%) flowed by *Origanum vulgare* (8 and 7.31 %) in least dilution (S/4)

whill in (S/2) *Salvadora persica* leaves recorded (9 and 58.53 %) flowed by *Origanum vulgare* (10 and 46.34 %) and with increase conc. to 100 % (S) *Moringa oleifera* recorded best inhibition (8 and 39.02 %) respectively, compared with control at this period. Increasing exposure time to 48 h proportional to the increase effect was noticed. *Moringa oleifera* achieved (9 and 39.02 %) respectively as best impact flowed by *Zingiber officinale* (16 and 19.51 %) respectively, in least dilution but in medium (S/2) and high dilutions (S) the impact was fairly close, compared with control at this period (23). *Salvadora persica* leaves recorded least dilution decrease in the effect of egg hatching and inhibition hatching percentage in concentrations (S/2 and S) (17 and 58.53 %) & (18 and 56.09 %) respectively, compared with control to this period (41) until end of the experiment (72 h).

Data table (2) showed that the tested extracts caused significantly reduction (P≤0.05) in all treatments, compared with check. The effect of S/4 and S/2 showed low number of juveniles dead and mortality percentage after 24 ad 48 h. However with increasing both of exposure time and dilution to 72 h and 100 % (S)

Table 1. Efficacy of aquatic extracts of four medicinal plants at three concentrations on egg hatching of *Meloidogyne incognita, in vitro*.

Materials	Conc.	Mean number of juveniles hatched after three intervals (hrs.)			Hatching inhibition
		24	48	72	(%)
Ginger	S/4	8 e	16 bcdef	33 abcd	19.51
Zingiber officinale	S/2	13 cde	21 abcd	28 cde	31.70
(Leaves)	S	22 ab	28 a	29 bcde	29.26
Miswak leaf	S/4	13 cde	21 abcd	23 de	43.90
Salvadora persica	S/2	9 de	7 g	17 e	58.53
(Leaves)	S	13 cde	11 efg	18 e	56.09
Miswak wood	S/4	13 cde	17 bcdef	29 bcde	29.26
Salvadora persica	S/2	24 a	24 ab	37 abc	9.75
(Stem)	S	17 bcd	26 a	25 cde	39.02
Moringa	S/4	9 de	9 fg	26 cde	36.58
Moringa oleifera	S/2	10 de	15 cdefg	26 cde	36.58
(Leaves)	S	8 e	9 fg	25 cde	39.02
Oregano	S/4	8 e	12 defg	44 a	7.31
Origanum vulgare	S/2	10 de	10 fg	22 de	46.34
(Leaves)	S	18 abc	20 abcde	21 de	48.78
Check		12 cde	23 abc	41 ab	-------

Values followed by the same letter are not statistically different according to Duncan's multiple-range test (P ≤0.05).

$$\text{Hatching inhibition } \% = \left(\frac{\text{Number of juveniles hatched in control} - \text{Number of juveniles hatched in treatment}}{\text{Number of juveniles hatched in control}}\right) X\ 100$$

Table 2. Efficacy of some water extracts of four medicinal plants at three concentrations on juveniles mortality of *Meloidogyne incognita, in vitro*.

Materials	Conc.	No. nematode mortality after hours.			Juveniles mortality
		24	48	72	(%)
Ginger *Zingiber officinale*	S/4	4 de	4 f	9 gh	6.00
	S/2	1 e	4 f	25 fgh	16.67
	S	4 de	1 f	35 efg	23.33
Miswak leaf *Salvadora persica*	S/4	7 de	25 e	37 ef	24.67
	S/2	120 a	129 a	136 a	90.67
	S	90 b	112 b	125 ab	83.33
Miswak wood *Salvadora persica*	S/4	13 de	61 cd	65 d	43.33
	S/2	17 d	53 d	87 cd	58.00
	S	44 c	76 c	80 cd	53.33
Moringa *Moringa oleifera*	S/4	2 e	2 f	3 g	2.00
	S/2	93 b	99 b	102 bc	68.00
	S	87 b	106 b	118 ab	78.67
Oregano *Origanum vulgare*	S/4	5 de	25 e	63 d	42.00
	S/2	17 d	24 e	60 de	40.00
	S	37 c	48 d	66 d	44.00
Check		1e	9 ef	13 fgh	0.00

Values followed by the same letter are not statistically different according to Duncan's multiple-range test (P ≤0.05).

The mortality percentage was calculated according to the Abbott's formula.

$$\text{Juveniles mortality } \% = \left(\frac{\text{Number of survived juveniles in treatment}}{\text{Number of survived juveniles in control}}\right) X\ 100$$

The tested extracts showed significantly effect. Since, *salvadora persica* showed best effect on mean number of juveniles dead and mortality percentage, (136 and 90.67 %) flowed by *Moringa oleifera* (102 and 68.00 %) ,(113 and 78.67 %) respectively , compared with control to this period (13).

DISCUSSION

All of the plant extracts tested were found to exhibit some level of toxicity toward juveniles of the root-knot nematode, *M. incognita*, but the control values varied.

Generally, the mortality of juveniles increased with

an increase in exposure time. All of the extracts exhibited higher mortality rates after 72 h of exposure as compared with 24 h.

This confirmed the report of Makkar and Becker (1996) who showed that, extract of some plants such as moringa, oregano, ginger and miswak contained the cytokinine group which effective in reducing nematode population in plants with a subsequent increase in plant growth and number of leaves. Also, obtained results confirmed with Guzman (1984) who reported that aqueous extract of moringa is as toxic to *Meloidogyne* spp. as standard pesticides. The nematicidal effect of the moringa could be attributed to its high content of certain oxygenated compounds which are characterised by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock *et al*. 1989). This property may also be responsible for the extract's efficacy in nematode management (Nchore *et al.,* 2011, Maina *et al* 2012*.,* and Pavaraj *et al.,* 2012).The mechanisms of plant extract action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*. 1994). According to Claudius-Cole *et al*. (2010), *M. oleifera* is a good inhibitor of nematode egg hatching and juvenile survival.

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تأثير المستخلصات المائية لبعض النباتات على فقس البيض وموت اليرقات لنيماتودا تعقد الجذور تحت الظروف المعملية

أنس فرج المسلمى

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في تجربة لتقدير كفاءة بعض المستخلصات المائية لنباتات الزنجبيل والمورينجا والزعتر والأراك (السواك) من خلال ثلاث تركيزات وهى 100و50 و25 % من المستخلص القياسي على معدل فقس البيض وموت يرقات الطور اليرقى الثاني من نيماتودا تعقد الجذور تحت ظروف المعمل . وأشارت النتائج الى كفاءة هذة المستخلصات بشكل معنوى فى التأثير على فقس البيض وموت اليرقات ، حيث سجل مستخلص أوراق الاراك أفضل النتائج على كلا من عدد أفراد الطور اليرقي الثاني التي نجحت في الخروج من البيض (الفقس) ونسبة تثبيط المستخلص لعملية الفقس بمعدلات 17 و58 % كما انه كان هناك تأثير إيجابي أيضا على كلاً من عدد الأطوار الميتة ونسبة الموت (136و 90.67 %)على التوالي مقارنة بالكنترول.