Preliminary Nematicidal Activity of Some Plant Extracts on A Field Root-knot Nematode (*Meloidogyne incognita*) Species

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ABSTRACT: Lethal effects of bitter wood, thyme and myrrh aqueous extracts were evaluated against Meloidogyne incognita at concentrations of 20, 40, 60, 80 and 100% after 24h. and 48h. The results revealed that the mortality percent of *M. incognita* tended to with increasing the concentration. The effect of the three tested aqueous extracts slightly changed from 24 to 48 hours exposure. The probit analysis revealed that the heterogeneity of nematode response to myrrh was slightly higher than thyme and bitterwood. According to LC_{95}, LC_{50} and LC_{05} thyme achieved 147.7, 44.75 and 13.57%, respectively after 24 and 48 hours, no significant differences was observed between LC_{95} of thyme (107.4%) and bitterwood (122.7%) but both differed significantly from myrrh (182.3%).

Keywords: Aqueous extracts, nematicidal activity, *Quassia amara, Commiphora molmol*, *Thymus vulgaris*.

INTRODUCTION

Using of synthetic pesticides in crop production resulted in disturbance in the environment, pest resurgence, pest resistance and lethal and sub-lethal effects on non-target biota, including humans (Prakash and Rao, 1997). At the same time, increases in plant parasitic nematode populations lead to use greater quantities of pesticides, which increases the environmental problems (Abd-Elgawad and Mohamed, 2006). Although the chemical nematicides hold major promise in nematode control, their high costs, hazards as environmental pollutants discourage most potential users. These disadvantages have stimulated research on alternative nematode management practices for plant parasitic nematodes.

The use of environmentally friendly bio-nematicides, organic soil amendments, cropping systems and biological control agents have been reported efficiently against nematodes (Abd-Elgawad and Aboul-Eid, 2005; Delfosse, 2005; Gohar, 2003; Maareg, 1984; Youssef *et al.,* 2008). Several plants are good sources for naturally occurring nematicides (Chitwood, 2002; Gommers, 1981) including neem (*Azadirachta indica*), garlic (*Allium sativium* L.), castor bean (*Ricinus communis*) and marigolds (*Tagetes* spp.). Studies on the identification and use of local plant materials for control of nematodes or integrated pest management are current areas of research in plant nematology. This study aimed to evaluate the lethal effects of some plant aqueous extracts against the second stage juveniles (J2s) of *M. incognita.*

These extracts are bitterwood (*Quassia amara*), Myrrh (*Commiphora myrrh*) and Thyme (*Thymus vulgaris*) as it has an deadly impact against some other pests.

MATERIALS AND METHODS

Plant materials:

The medicinal plant species were (bitterwood tree, *Quassia amara*; myrrh, *Commiphora myrrh* and thyme, *Thymus vulgaris*) this plant species were purchased from a perfumery shop in the market.

Tested root-knot nematode:

Females and egg masses of *Meloidogyne incognita* were isolated from infected eggplant (*Solanum melongena*) roots collected from the West Nubaryia region (Mohamed Abdel Wahab Village). The culture of this nematode was obtained from a single egg mass of adult females previously identified by the morphological characteristics of the female patterns (Taylor and Sasser, 1978). The culture was reared on eggplant cv. Black Beauty growing in earthen pots filled with steam sterilized soil consisted of clay and sand (1 : 2) in volume in a greenhouse.

Meloidogyne incognita isolates were maintained on eggplant roots in pot cultures. Inocula of freshly hatched second stage juveniles (J2s) were obtained from egg masses in distilled water. Only, the J2s that hatched within 24 hr period were used.

Preparation of the tested aqueous plant extracts:

Different parts of the tested plants were taken to prepare their extracts. The chosen parts were stem of bitterwood, crude of myrrh (gum, resins) and flowers and leaves of thyme. These plant origins were washed with distilled water to remove any dust and air dried in shade. The dried plant materials were powdered and passed through a 50 mesh sieve. Samples of plant powders were homogenized with a laboratory blender used at 50 g from each powders in one liter of distilled water for 10 min., and then left in dark glass bottles for 72 hr for tissue maceration. The extracts were filtered through muslin cloth, followed by whatman filter paper No. 1 to get the clear extract. The final extracts were collected separately in dark glass bottles and stored in refrigerator at 5°C until use. Each extract was arbitrarily termed as a standard solution.

Contact toxicity bioassay measurements:

A direct-contact bioassay test was used to evaluate the biological performance of the tested aqueous extracts against the second stage juveniles (J2s) of root-knot nematode, *M. Incognita.* Aqueous nematode suspension (aprox. 50 freshly hatched J2s ml^{-1}) was prepared from a standard nematode suspension. Five concentrations of each tested aqueous plant extract (20, 40, 60, 80 and 100%) were prepared from the standard extract. The assessment was carried out in 5 cm Petri plates containing 5 ml of each plant material concentratios. Nematode suspension carrying one ml (50 J2s) was added. One

petri plate containing juveniles in water was kept as control. Five replicates were considered as one treatment. Dead larvae were counted and the dishes were covered with lids and held at the same conditions (incubated at 28°C). Mortality percents were determined after 24 and 48h. exposure under binocular microscope and corrected (Abbott, 1925).

Statistical analysis:

Statistical analysis was performed using Costat program (1988) with LSD at 5% probability. The mortality percents treated of the nematode were corrected (Abbott, 1925). LC_{50}, LC_{95} values and the regression line-slope were calculated using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Lethality effects of the tsted plant extracts:

The used tested plant extracts as shown in Tables (1 and 2) killed the treated nematode, *M. incognita* in a concentration and exposure time dependant effect. The untreated nematode population was naturally killed with 4.02% after 24 hours. The tested, Thyme (*Thymus vulgaris*) extract caused 17.29, 37.90, 63.87, 79.92 and 88.94% mortality at concentrations of 20, 40, 60, 80 and 100% of the original aqueous extract solution achieving 147.7, 44.75 and 13.57% for LC_{95}, LC_{50} and LC_{05} values, respectively. The bitterwood aqueous extract revealed its lethal effect against the treated animal population systematically with increasing the tested concentration giving 151, 48.85 and 15.74% LC_{95}, LC_{50} and LC_{05} values, respectively with no significant differences between their aqueous extract at the tested concentration range. Both Thyme and bitterwood aqueous extracts exceeded Myrrh in their mortal effect significantly at the used concentration range as it caused LC_{95}, LC_{50} and LC_{05} values of 13.99, 60.39 and 260.7% respectively. Worth mentioning, Myrrh achieved good effect at the lowest concentration nearly similar to bitterwood at 20% of the original aqueous extract after 24 hours exposure (Table 2).

Table (1). The tested plant species.

Common names	Scientific name	Major components	Extracted origin
Bitterwood tree	*Quassia amara*	Quassinoids (quassin and neoquassin)	Stem (wood)
Myrrh	*Commiphora molmol*	Oles - gum - resins - terpenoids	Gum - resins
Thyme	*Thymus vulgaris*	Thymol - phenols - carvacrol	Flowers - leaves

Table (2). Mortality effects of the tested aqueousTable (2). Mortality effects of the tested aqueous extracts on *Melodogine incognita* after 24 hours.

Tested Extracts	Mortality (%) at different concentrations of the standard aqueous extract (%)						LC_{95}	LC_{50}	LC_{05}	χ^2	p
	0	20	40	60	80	100					
Thyme	4.022 ± .29	17.29 ±0.24	37.90 ±0.56	63.87 ±0.30	79.92 ± 0.17	88.94 ±0.36	147.7 [b] (137.7-158.1)*	44.75 [c] (43.3 - 46.5)	13.57 [a] (12.4 - 14.9)	3.99	0.399
Bitter wood	4.022 ± 0.29	11.02 ±0.63	36.20 ±1.62	61.83 ±0.93	77.01 ±0.93	85.35 ±1.05	151 [b] (141.6-162.2)	48.85 [b] (47.4 - 50.4)	15.74 [a] (14.5 - 17.1)	4.24	0.526
Myrrh	4.022 ±0.29	11.59 ±0.46	31.63 ±0.68	47.30 ±0.51	63.84 ±1.42	72.03 ±0.86	260.7 [a] (231.7-293.5)	60.39 [a] (58.2 - 62.7)	13.99 [a] (12.5 - 15.7)	3.96	0.389

* Confidence limits; **P,** Probability; χ^2**,** Chi Square; **DF,** Dgree of freedom = 4

All the tested plant extracts increased their mortal effect against the treated animal population after 48 hours exposure at all the tested concentrations (Table 3). Both Thyme and bitterwood aqueous extracts overcomedd the myrrh aqueous extract in their nematicidal effects significantly after 48 hours exposure also as the achieved 107.4, 122.7 and 182.3 LC_{95} values comparing with 38.54, 42.01 and 49.99 LC_{50} values and 13.84, 14.38 and 13.71 LC_{05} values for thyme, bitterwood and myrrh aqueous extracts, respectively. The obtained results agreed with Salazar-Antón and Guzmán-Hernández (**2014)** as they found that *in vitro* treatment of *M. incognita* with 10% *Quassia amara* extract caused 78% mortality of its juveniles after 48 hours exposure. Korayem *et al.* (1993) added *Thymus vulgaris* shoot powder killed all the treated juveniles after 72 hours exposure. On the other hand, Soler-Serratosa *et al.* (1995) proved that LC_{90} value of thymol against *M. arenarea* in soil was 161 ppm and its activity was enhanced when combined with benzaldehyde as an essential oil of almond. From the results in Tables (2 and 3), it was obvious that the estimated probability values of the aqueous extracts were considered to be reliable and acceptable, whereas, it ranged between (0.683 - 0.917). Hence, Chapman (1985) mentioned that one line with a probability of less than 0.01 was a result of poor replication at lower doses.

Table (3): Mortality effect of the tested aqueous extracts on *Melodogine incognita* after 48 hours.

Tested Extracts	Mortality (%) at different concentrations of the standard aqueous extract (%)						LC95	LC50	LC05	χ^2	p
	0	20	40	60	80	100					
Thyme	9.02 ±0.47	17.48 ±1.20	48.71 ±1.54	73.84 ±1.26	91.16 ± 0.72	93.59 ±0.66	107.4 b (101.8- 113.3)*	38.54 c (37.2- 39.9)	13.84 a (12.7-15.1)	22.68	0.433
Bitter wood	9.02 ±0.47	15.69 ±0.80	40.74 ±0.63	74.15 ±1.66	83.95 ±1.31	90.88 ±1.96	122.7 b (115.6- 130.3)	42.01b (40.6- 51.8)	14.38 a (13.1- 15.7)	26.77	0.415
Myrrh	9.02 ±0.47	15.33 ±1.08	39.78 ±0.95	50.34 ±1.29	72.04 ± 1.08	86.42 ±1.02	182.3 a (166.5- 199.6)	49.99 a (48.2- 51.8)	13.71 a (12.3- 15.3)	55.57	0.372

* Confidence limits; **P,** Probability; χ^2**,** Chi Square; **DF,** Dgree of freedom = 4

Comparing between the tested intervals, the slopes of the tested plant extracts were slightly higher after 48h than 24h. The highest variation in the regression line slope of thyme (0.53) resulted from subtraction between 24 hours (3.17) and 48 hours (3.7) was observed followed by myrrh (0.34) that subtracted from 24 hours (2.59) and 48 hours (2.93). While, this difference was nearly neglected for bitterwood which was (0.18) in variance between 24 hours (3.35) and 48 hours (3.53). This observation revealed that the behaviour of myrrh did not differ than 48h. On the other hand, the behaviour of thyme altered after 24 hours than 48 hours. While, Bitterwood behaviour slightly changed after 24 hours. than 48 hours.

The slope of the tested aqueous extracts after 48 hours exposure of bitterwood (3.53) and thyme (3.70) were almost the same and higher than myrrh (2.93). Therefore, the heterogeneity of response of the treated nematode to thyme and bitterwood were slightly higher than myrrh after both bioassay intervals. Differences in heterogeneity maybe, due to the differences of their active ingredients and/or their mode of action. The regression and slope of statistical analysis were shown in Table (4).

Table (4): Regression of N.E.D response (Y) on log dose

Plant species	(Y=a + bX) after 24 hrs	(Y=a + bX) after 24 hrs
Thyme	Y =-5.24 + 3.17 X	Y =-5.86 + 3.70 X
Bitterwood	Y =-5.65 + 3.35 X	Y =-5.74 + 3.53 X
Myrrh	Y =-4.61 + 2.59 X	Y =-4.97 + 2.93 X

* Regression of normal equivalent deviation (N.E.D); **y**, log dose; **x**

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الملخص العربى

النشاط الإبادى المبدئ لبعض المستخلصات النباتية على نيماتودا تعقد الجذور (ميلودوجينى إنكوجنيتا) سلالة حقلية

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تم إجراء المستخلص المائى لثلاثة مصادر نباتية (خشب المر، الزعتر، صمغ المر) لتقييم تاثيرها على نيماتودا تعقد الجذور (ميلودوجينى إنكوجنيتا) معمليا. تم عمل خمسة تركيزات من التركيز الاصلى (٥٠ جرام مسحوق نباتى لكل لتر ماء) هى (٢٠، ٤٠، ٦٠، ٨٠، ١٠٠%) من المستخلصات النباتية لتقييم تأثيرها بعد ٢٤ ساعة و ٤٨ ساعة. ظهر تغير طفيف فى تأثير المستخلصات المائية الثلاثة خلال ٢٤ ساعة و ٤٨ ساعة من المعاملة. التركيز الأقل والأعلى (٢٠ و ١٠٠%) للزعتر حقق أعلى نسبة موت هى ١٧.٢٩ و ٨٨.٩٤% بعد ٢٤ ساعة وحقق ١٧.٤٨ و ٩٣.٥٩% بعد ٤٨ ساعة من المعاملة. اظهرت الأنحدارات البيانية أن عدم التجانس فى إستجابة النيماتودا لصمغ المر كان أكثر قليلا من إستجابتها للزعتر وخشب المر. حقق الزعتر أعلى نتيجة حيث أعطى أقل قيم للتركيزات المميتة (LC_{95}, LC_{50} and LC_{05}) وهى ١٤٧.٧ و ٤٤.٧٥ و ١٣.٥٧% على التوالى بعد ٢٤ ساعة من المعاملة. بعد ٤٨ ساعة من المعاملة لوحظ أن ليس هناك فروق معنوية للتركيز المميت LC_{95} للزعتر وخشب المر ولكنهما يختلفان معنويا بين صمغ المر. التركيز المميت LC_{50} للزعتر ٣٨.٥٤% له أعلى تأثير يلية خشب المر ٤٢.٠١% بينما صمغ المر لة أقل تأثير ٤٩.٩٩%. ليس هناك فروق معنوية بين المستخلصات الثلاثة فى التركيز المميت LC_{05} حيث تتراوح النتيجة بين ١٣.٧١% لصمغ المر و ١٤.٣٨% لخشب المر.