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Nematicidal effect of plant extracts against *M. incognita* associated with eggplant plants under greenhouse conditions.

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ABSTRACT:

Plant pathogenic nematodes, which are more than 4100 species, severely infect nearly all types of crops causing serious diseases .The efficacy of some aqueous extract for some plants, on eggplant plants, the effectiveness of wormwood (*Artemisia herba- alba* L.), mustard (*Brassica juncea* L.), pot marigold (*Calendula officinalis* L.), globe amaranth (*Gomphrena globosa* L.), and oleander (*Nerium oleander* L.) was compared to that of oxamyl, a common nematicide. Wormwood extract achieved the highest reduction (69.08%), which was followed by Oleander, Globe Amaranth, and Pot Marigold with reduction rates of 68.66, 63.02, and 57.39%, respectively. Mustard, on the other hand, showed the least amount of eggs per gram of root, resulting in a 64.53% reduction. Oleander achieved the greatest reduction in the amount of J2 in the soil (75%), followed by wormwood (73.33%) and pot marigold (68.66%). On the other hand, mustard had the least impact on the amount of J2 in the soil, resulting in a 43.33% drop.

Key words: control, *Meloidogyne incognita*, extract, oxamyl, eggplant.

INTRODUCTION

More than 4100 different species of plant pathogenic nematodes severely harm almost all types of crops, resulting in devastating illnesses [1]. [2] demonstrated the effectiveness of *Eucalyptus* sp. in controlling *Meloidogyne javanica* root knot nematode on mung bean and chickpea plants by demonstrating the nematodal effects of different parts of the tree, including leaves, stems, bark, and fruit, when used as aqueous and ethanol extracts against the nematode. Where soil was amended with leaves, stems, bark, and fruit of Eucalyptus sp., employed at concentrations of 0.1, 1 and 5% w/w, significant increases in shoot length, shoot weight, root length, and root weight were seen. Knots were substantially fewer as well.

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Eucalyptus species' entire plant was more efficient by 5% weight to weight. [3] investigated the effects of neem (Azadirachta indica), marigold (Tagetes erecta), eucalyptus (Eucalyptus chamadulonsis), garlic (Allium sativium), and essential oils on the inhibition of the root-knot worm *Meloidogyne incognita* in greenhouse and field settings. After 24 and 48 hours of exposure, all tested therapies in an in vitro investigation demonstrated nematicidal effects on nematode juveniles. Neem extract (65.4%), essential oils (64.4%), and marigold extract (60.5%) produced the highest percentage of nematode death, followed by garlic and eucalyptus extracts (38.7-39.5%). Neem and essential oil treatments provided tomato plants with the greatest protection against the root-knot nematode in field studies, 44.2 and 32.6%, respectively.

Therefore, the purpose of the current study was to determine the effectiveness of plant extract formulations against the root-knot nematode (*M. incognita*) that infects eggplant plants grown in greenhouses. The study looked at the effects of Wormwood, Mustard, Pot Marigold, Globe Amaranth, and Oleander..

MATERIALS AND METHODS

1-Preparation of Plant Aqueous Extracts: -

The leaves of the following five plants were gathered from nearby markets: wormwood (*Artemisia herba- alba* L.), mustard (*Brassica juncea* L.), pot marigold (Calendula officinalis L.), globe amaranth (*Gomphrena globosa* L.), and oleander (*Nerium oleander* L.). To create powder, some of the leaves were processed through a grinder. The procedure used to prepare the aqueous extracts by [4] was followed. These plant materials were cleaned with running water, let to air dry at room temperature, and then baked at 50 degrees. Plant materials were finely powdered in an electric blender. To 1000 ml of distilled water, 100 g of each powder was added, and the mixture was then allowed to sit for 72 hours. Over a water bath, heating was conducted for a full hour.. Whitman filter paper No. 1 was used to filter the extract once it had cooled. Each extract was given a standard solution designation at random. This solution had an initial concentration of 100,000 mg/L.

2. Nematode inoculum:

Roots of Solanum (*Solanum nigrum* L.) that were infested with *Meloidogyne incognita* (Kofoid & White) chitwood eggs were collected from El-Nubaria region, EL-Beheira Governorate. In order to dissolve the gelathineous matrix and remove free nematode eggs from the mass matrix, galls roots were rinsed by running water from the adhering soil particles and then chopped into small pieces [5].A 200-mesh sieve

nested inside of a 400-mesh sieve was used to filter the suspension. Before inoculation, eggs were rinsed with a gentle stream of tap water to remove any leftovers.

3. Egg-masses staining and counting: -

M. incognita egg masses were dyed by soaking them for 20 to 30 minutes in an aqueous solution of Phloxine B (0.15g per litre tap water). To get rid of any remaining stains on the roots, root systems were cleaned in tap water. Specifically, the gelatinous egg sac and the bare viable eggs are stained by phloxine B. [6]

4- Nematode extraction:-

Soil sample of 250 g was successively wet-sieved through 100, 200 and 325 mesh-sieves. The active nematode present in the fine sieve were extracted by Baermann-plate technique **[7]**The final volume of nematode extract solution was adjusted to a known volume, the second stage juveniles (J_2) of *M. incognita* in each sample were counted microscopically using a counting slide.

5. Greenhouse experiment: -

One kg of a 1:1 autoclaved sand:clay mixture was placed in plastic pots (10 cm in diameter and 15 cm in depth). Each pot was simultaneously inoculated with a suspension containing about 2000 eggs and newly hatching second stage juveniles around the plant stem after one week of rowing or transplanting plants, along with two control groups: untreated inoculated control with M. incognita and non-inoculated control.. All pots were arranged in completely randomized design (CRD) with four pots per treatment in a greenhouse. After 60 days of inoculation, Plants were carefully removed, and soil clinging to the roots was thoroughly rinsed with running water. In addition to shoot length, the fresh and dry weights of the shoots and roots were calculated. Egg masses, galls per root system, eggs per g of root, and juveniles per 250 g of soil were also assessed. Five aqueous extracts were tested for their nematicidal efficacy against M. incognita, which infected aubergine plants.50 ml of each extract, at two different strengths (50-100 mg/ml), were administered to each pot as a soil drench at the same time as the inoculation.. All treatments were compared with the synthetic nematicide; Oxamyl (0.3 g/kg) which applied to the soil after ten days from transplanting time.

The reduction of nematode parameters was calculated according to [8]:

$$\%\text{Reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \text{x100}$$

Statistical analysis:

Statistically, the obtained data were subjected to analysis of variance (ANOVA) (**Gomez and Gomez, 1984**) followed by Duncan's multiple range tests to compare means. [9]

RESULTS AND DISCUSSION

On eggplant plants, the effectiveness of various aqueous extracts against M. incognita was assessed. These plants included wormwood (*Artemisia herba- alba* L.), mustard (*Brassica juncea* L.), pot marigold (Calendula officinalis L.), globe amaranth (*Gomphrena globosa* L.), and oleander (*Nerium oleander* L.). Under greenhouse circumstances, soil drenches containing two quantities of each of the evaluated substances were used. After inoculating nematodes for 60 days, the experiment came to an end. Plants were harvested, and information was recorded and displayed in Tables (1&2) including the number of galls, egg masses, eggs per g, and ultimate nematode juveniles., as well as some eggplant growth parameters were recorded and presented in **Tables (1&2**).

Data in Table (1) showed that compared to the untreated inoculation control, all of the tested aqueous plant extracts at the tested two doses and the synthetic nematicide Oxamyl (92.89%) considerably (p 0.05) decreased the number of galls.. All aqueous extracts were less significantly than oxamyl (92.89 %). The highest reduction was obtained by Wormwood extract (69.08%), followed by Oleander, Globe amaranth, and Pot marigold with % of reduction of 68.66, 63.02 and 57.39%, respectively. As opposed to that, Mustard exhibited the lowest effect on galls formation, which gave 39.55 % reduction. Concerning the effects of different aqueous extracts on reduction of egg-masses, all of the tested extracts and the synthetic nematicide; In comparison to the untreated inoculated control that was not treated with oxamyl, there were considerably less egg masses. All aqueous extracts were less significantly than oxamyl (93.14%). The highest reduction was obtained by wormwood (71.81%), followed by Oleander (68.66%), Globe amaranth (63.07%) and Pot marigold (55.59%) On contrary, Mustard exhibited the lowest effect on number of egg-masses, which gave 37 % reduction.

Table (1): Effect of some aqueous plant extracts on the number of galls and egg-mass formation on eggplant plants infected with *M. incognita* under greenhouse conditions.

Treatments	Application rate mg/ml	Mean of galls/ root system	Reduction (%)	Egg-mass/ root system	Reduction (%)
Untreated inoculated control	-	478.67^{a}	---	423.33^{a}	-
Wormwood	50	200^{d}	58.21	182.67^{de}	56.88
	100	148^{f}	69.08	119.33^{h}	71.81
Mustard	50	300.33^{b}	37.25	276^{b}	34.8
	100	289.33^{b}	39.55	266.67^{b}	37
Pot marigold	50	250^{c}	47.77	266.67^{b}	37
	100	201.33^{d}	57.39	188d^{e}	55.59
Globe amaranth	50	230^{c}	51.95	202^{d}	52.28
	100	177de	63.02	156.33^{fg}	63.07
Oleander	50	185^{d}	61.35	168,33^{ef}	60.23
	100	150^{ef}	68.66	132.67^{gh}	68.66
Oxamyl10% G	0.12 g/kg	34^{g}	92.89	29^{i}	93.14

According to Duncan multiple range test at 0.05 level of probability, values in each column that are preceded by the same letter (s) are not statistically distinct.

Table (2) contains information on the reduction of eggs per 1g of root caused by various aqueous extracts. When compared to the untreated inoculation control, all of the studied aqueous extracts considerably (p 0.05) decreased the number of eggs per gramme of root. All aqueous extracts were less significantly than the synthetic nematicide oxamyl (93.39%). The highest percentage of reduction was obtained by Oleander extract (77.99%), Wormwood (71.72%), followed by Pot marigold (70.44%) and globe amaranth (70.44%). On the other hand, Mustard exhibited the lowest effect number of eggs/ g root, which gave 64.53 % reduction. Also, all the tested aqueous extracts significantly ($p \leq 0.05$) reduced the number of J_2 in the soil as compared with untreated inoculated control. All aqueous extracts were less significantly than oxamyl (93.11%). The highest reduction was obtained by oleander (75%), followed by wormwood (73.33%) and pot marigold (68.66%). In contrary, mustard exhibited the lowest effect on number of J_2 in the soil, which gave 43.33 % reduction. In general, all the tested aqueous plant extracts at the tested two concentrations and the synthetic nematicide caused significant reduction in nematode root galls, egg-masses, eggs per g root and number of larvae / 250 g of soil compared to untreated inoculated control. Oleander and wormwood extract had the highest nematicidal effect against root galls formation, egg-masses and number of eggs per g root. While oleander extract had the highest nematicidal effect against juveniles / 250 g of soil. On the other hand, mustard extract proved to be the lowest effective extract.

Table (2): Effect of some aqueous plant extracts on the number of eggs/g root and 2nd juveniles on eggplant plants infected with *M. incognita* under greenhouse conditions.

Treatments	Application rate mg/ml	Eggs/g root	Reduction (%)	Mean juveniles 2nd/ 250 g of soil	Reduction (%)
Untreated inoculated control	-	338.33^{a}	-	300^{a}	-
Wormwood	50	154.67^{c}	54.28	100.66^{e}	66.44
	100	95.67^{ef}	71.72	80g^{f}	73.33
Mustard	50	200^{b}	40.88	200^{b}	33.33
	100	120de	64.53	170^{c}	43.33
Pot marigold	50	150^{cd}	55.66	135.66^{d}	54.78
	100	100^{ef}	70.44	94^{ef}	68.66
Globe amaranth	50	165.67^{c}	51.03	140.33^{d}	53.22
	100	100^{ef}	70.44	145.33^{d}	48.55
Oleander	50	120de	64.53	98.33^{e}	67.22
	100	74.44^{f}	77.99	75^{g}	75
Oxamyl	0.12 g/kg	22.33^{g}	93.39	20.66^{h}	93.11

According to Duncan multiple range test at 0.05 level of probability, values in each column that are preceded by the same letter (s) are not statistically distinct.

Results in Table (3) revealed that all of the tested aqueous plant extracts and oxamyl significantly ($p \leq 0.05$) increased the shoot fresh weight as compared with untreated inoculated control, which gave 34.82 g/ shoot. At rate of 100 mg/ml, pot marigold, globe amaranth, mustard, and oleander cloves extracts were significantly similar to non-inoculated control (56.84 g/ shoot). Concerning shoot dry weight, all the tested aqueous plant extracts and oxamyl significantly ($p \leq 0.05$) increased the shoot dry weight compared to untreated inoculated control which gave 10.67 g/ plant. All treatments at two concentrations significantly equal to non-inoculated control, which gave 13.67 g/ shoot. All the tested aqueous plant extracts and oxamyl significantly ($p \leq 0.05$) increased the plant height as compared with untreated inoculated control, which resulted in a plant height (35.64) cm/ plant.

Table (3): Effect of some aqueous plant extracts on some plant growth parameters of eggplant plants infected with *M. incognita* under greenhouse conditions.

Treatments	Application rate mg/ml	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot hight (cm)
Untreated inoculated control	-	34.82^{f}	10.67^{e}	35.64^{d}
Non inoculated control	-	56.84^{a}	13.67^{abcd}	60.86^{a}
Wormwood	50	46.81^{de}	12.73^{cd}	46.6^{c}
	100	51.38^{bcd}	13.42^{abcd}	56.2^{ab}
Mustard	50	47.86^{de}	13.08^{bcd}	43.56^{c}
	100	54.44^{abc}	14.5^{abc}	55.5^{ab}
Pot marigold	50	45.33^{e}	12.23^{d}	48.33^{c}
	100	55.67^{ab}	14.6^{abc}	57.33^{ab}
Globe amaranth	50	49.04^{de}	13.94^{abcd}	46.6^{c}
	100	54.8^{abc}	14.4^{abc}	54.83^{b}
Oleander	50	50.52^{cd}	14^{abcd}	46^{c}
	100	54.26^{abc}	15.3^{a}	56.83^{ab}
Oxamyl 10 %G	0.12 g/kg	50.01^{abc}	14.89^{ab}	55.5^{ab}

According to Duncan multiple range test at 0.05 level of probability, values in each column that are preceded by the same letter (s) are not statistically distinct.

Data in Table (4) showed that all of the tested aqueous plant extracts significantly ($p \leq 0.05$) increased the root fresh weight compared to untreated inoculated control which gave 15.93 g/ root. At rate of 100 mg/ml wormwood, pot marigold, globe amaranth, mustard and oleander were significantly similar to non-inoculated control, which resulted in 25.66 g/ root. Concerning root dry weight all of tested aqueous plant extracts and Oxamyl significantly ($p \leq 0.05$) increased the root dry weight as compared with untreated inoculated control, which gave 2.16 g/ root.

Table (4): Effect of some aqueous plant extracts on root fresh and dry weights of eggplant plants infected with *M. incognita* under greenhouse conditions.

Treatments	Application rate mg/ml	Root fresh weight (g)	Root dry weight (g)
Untreated inoculated control	-	15.93^{f}	2.16^{d}
Non inoculated control	-	25.66^{a}	5.14^{a}
Wormwood	50	19.66^{e}.	3.87^{cd}
	100	24.71^{a}	4.8^{ab}
Mustard	50	19.91^{e}	4.28^{abc}
	100	23.33^{abcd}	4.6^{abc}
Pot marigold	50	20.59^{cde}	4.24^{bc}
	100	23.66^{abcd}	4.75^{abc}
Globe amaranth	50	21.06^{bcde}	4.2^{bc}
	100	23.79^{abc}	4.69^{abc}
Oleander	50	20.46^{de}	4.07^{bc}
	100	23.95^{ab}	4.68^{abc}
Oxamyl 10% G	0.12 g/kg	23.66^{abcd}	4.79^{ab}

According to Duncan multiple range test at 0.05 level of probability, values in each column that are preceded by the same letter (s) are not statistically distinct.

Controlling nematodes is essential for minimising crop losses and ensuring self-sufficiency in the needs for food and industrial raw materials. The use of insecticides with a plant origin is one of the many ecologically oriented strategies for nematode management [10,11,12]. In the present study, the tested aqueous extracts and the nematicide; oxamyl significantly ($p \leq 0.05$) reduced the number of galls, egg-masses, eggs per g root and J_2 in the soil as compared with the control with nematode. Oleander and Wormwood gave the highest potential effects. **[13]** found that in vitro aqueous extract of *Cumine cyminum* (100 % w/v) significantly reduced egg hatching of *M. javanica Oleander* has been found to have nematocidal characteristics that are effective against root-knot nematodes [14,15,16]. **[17]** found that neem leaf and oleander bulb extracts killed larva and decreased egg mass hatching. Additionally, [18 treatment with oleander cloves reduced root galls and nematode egg masses by 89.9–93.8%, respectively. Furthermore, [19] in pot trials, nematode populations decreased in seedlings that were treated with *A. sativum*, *C. frutescents*, and *F. vulgare* compared to control sample. In laboratory experiments, extracts of *A. sativum* and *C. frutescents*, *D. innoxia*, and *F. vulgare* were each more effective than the control sample.. Also, **[20]** tested fresh leaf extracts of *Azadirachta indica* (neem), *Allium sativum* (Oleander) and *Tagetes erecta* African marigold) and bacterial suspensions of *Pseudomonas fluorescens* and *P. aeruginosa* against *M. incognita* on eggplant under in vitro, pots and field conditions. Oleander showed the best management among the plant species, reducing root galls by 57% in pots under greenhouse conditions and by 33% in fields, while also boosting fruit output by 47%. In comparison to the nematicide and untreated plants,

botanical extracts were more efficient and significantly (p 0.05) reduced nematode criteria, including the number of galls and egg-masses on aubergine roots and the number of juveniles in roots and soil. In general, a high dilution of the studied compounds led to greater gains in shoot length and weight as well as fruit number and weight than a low dilution did. **[21]** A suppressive effect of *M. incognita* development was obtained by the Globe amaranth plant (*Lawsoniai nermis*) as reported by [22]. Also, Globe amaranth reduced eggplant root gall numbers, number of the egg-laying females and rate of the nematode reproduction, when eggplant and Globe amaranth were grown together.

Summary

The efficacy of some aqueous extract for some plants, wormwood (*Artemisia herba- alba* L.), mustard *(Brassica juncea* L.); Pot marigold (*Calendula officinalis* L.); globe amaranth (*Gomphrena globosa* L.) and oleander (*Nerium oleander* L.) compared with oxamyl as a standard nematicide was evaluated against *M. incognita* on eggplant plants.

REFERENCES

[1] Nicol, J.M.; Turner, S.J. & Coyne, D.L. (2011). Current nematode threats to world agriculture', In: Jones J., Gheysen, G., Fenoll, C., editors. Genomics and molecular genetics of plant-nematode interactions. Berlin: Springer" p. 21–43.

[2] Dawar, S., Younus, S. M. & Zaki, M. J. (2007). Use of *Eucalyptus* Sp., in the control of *Meloidogyne javanica* root knot nematode.pak. J. Bot., 39(6), 2209-2214.

[3] Abo-Elyousr, K. A.; Khan, Z.; El-Morsi Award, M. A. & Abedel-Moneim, M. F. (2010). Evaluation of plant extracts and *Pseudomonas* spp. For control of root-knot nematode, *Meloidogyneincognita* on tomato". Nematropica, 40(2), 289-299.

[4] Claudius-Cole, A. O., Aminu, A.E. & Fawole, B. (2010). Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea [*Vigna unguiculata* (L) Walp]. Mycopath 8(2), 53-60

[5] Hussey, R., S. & Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Pl. Dis. Reptr., 57: 1925-1928.

[6] Barker, K. R., (1985). Nematode extractions and bioassays. Pp. 19-35 in Barker, K. R., C. C. Carter, and. N. Sasser, Eds. An Advanced Treatise on *Meloidogyne*, Methodology, (Vol II). Raleigh, North Carolina State University Graphics, USA.

[7] Goodey, J. B. (1963). Laboratory methods for work with plant and soil nematodes. Laboratory methods for work with plant and soil nematodes., (2;4th edit).

[8] Raddy, H. M., Fouad, A. F. Montasser, S. A. Abdel-Lateef, M. F. & EL-Samadisy, A. M. (2013). Efficacy of six nematicides and six commercial bioproducts against root-knot nematode, *Meloidogyne incognita* on tomato. Journal of Applied Sciences Research, 9(7), 4410-4417.

[9] Duncan, D.B. (1955). Multiple ranges and multiple F test. Biometrics. 11, 1-42.

[10] Adesiyan, S. O.; Adeniji, M. O.; Caveness, F. E. & Fawole, B. (1990). Nematode Pest of Tropical Crops Heinemann Educational Books Nigeria Ltd. 114pp.

[11] Olowe, T. (1992). Economic importance of nematode on legume and cereal crops in Nigeria. In: proceedings of the first regional symposium on the biology and control of nematode pests of food crop in Africa, held in Ibadan.

[12] Mangala, R. & Mauria, S. (2006). Handbook of Agriculture Fact and figure for teacher, students and all interested farmers. Indian Council of Agricultural Research, New Delhi 1346pp.

[13] Abbas, S. S.; Dawar, M. T. & Zaki, M. J. (2009). Nematicidal activity of spices against *Meloidogyne javanica* (Treub) chitwood. Pak. J. Bot., 41(5), 2625-2632 .2009.

[14] Sukul, N. C. (1992). Plants antagonistic to plant-parasitic nematodes. Indian Review of Life Sciences", 12, 23-52.

[15] Agbenin, N. O., Emechebe, A. M. Marley, P. S. & Akpa, A. D. (2005). Evaluation of Nematicidal Action of Some Botanicals on *Meloidogyne*

[16] Zasada, I. A.; Ferris, H. & Zheng L. (2002). Plant sources of Chinese herbal remedies: Laboratory efficacy, suppression of *Meloidogyne javanica* in soil, and phytotoxicity assays. J. Nemato.", 34, 124-129.

[17] Agbenin, N. O.; Emechebe, A. M. Marley, P. S. & Akpa, A. D. (2005). Evaluation of Nematicidal Action of Some Botanicals on *Meloidogyne incognita* In Vivo and In Vitro. Journal of Agriculture and Rural Development in the Tropics and Subtropics", 106 (1), 29–39.

[18] Ibrahim, I. K. A.; El-Saedy, M. A. M & Mokbel, A. A. (2007). Control of the root-knot nematode *Meloidogyne incognita* on sunflower plants with certain organic plant materials and biocontrol agents. Egyptian J. Phytopathol". 35(1), 13-24.

[19] Ayazpour, K.; Hasanzadeh, H. & Arabzadegan, M. S. (2010). Evaluation of the control of citrus nematode (*Tylenchulus semipenetrans*) by leaf extracts of many plants and their effects on plant growth". J. of Agri. Res., 5(14), 1876-1880.

[20] Abo-Elyousr, K. A., Khan, Z. El-Morsi, Award, M. A. & Abedel-Moneim, M., F. (2010). Evaluation of plant extracts and *Pseudomonas* spp. For control of root-knot nematode, *Meloidogyneincognita* on tomato. Nematropica, 40(2), 289-299.

[21] El-Nagdi, W. M. A. & Abd-El-Khair, H. (2014). Biological control of *Meloidogyne incognita*

and *Fusarium solani* in dry common bean in the field. Arch.Phytopathol. Plant. Protect., 47(4), 388-397.

[22] Korayem, A. M. & Osma, H. A. (1992). Nematicidal potential of the Henna plant *Lawsoniainermis* against the root knot nematode *Meloidogyne incognita*. Anzeiger fuerSchaedlingskunde, Pflanzenschutz, umweltschutz. (Germany, F.R.), 56(1), 14-16.