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EFFECT OF CERTAIN MEDICINAL PLANTS NATURAL PRODUCTS ON *Meloidogyne incognita* MANAGEMENT IN VITRO

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ABSTARCT

Under laboratory conditions, three aqueous seed extracts of *Sinapis alba, Ammi visnaga and Lepidium sativum* were tested against the root- knot nematode *Meloidogyne incognita* Exposure of juveniles and eggs to the tested plant water extract solutions for 72 hr , and 144hr separately reduced the number of active nematodes to J_2 immobility and egg hatching as well. Among tested materials, *Lepidium sativum* seed extract surpassed other seed extracts treatment in diminishing values of J_2 immobility and egg hatching by 94.6 and 5.3% at 72 hr of exposure as well as 63.5 and 1.4% at 144 hr of exposure, respectively. Moreover, treatments of *Ammi visnaga* and *Sinapis alba* seed extracts showed considerable percentage of reduction values of J_2 immobility and egg hatching that averaged 36.4 & 48.5%; and 63.5 & 51.4%; and 60.7 & 69.9% ; and 39.3 & 29.9% at 72 and 144 hr of exposure time, respectively. Combination of plant seed extracts were the most effective in J_2 mobility after 1 and 7 day comparing to control.

Keywords : laboratory, *Meloidogyne***,** exposure, nematode , aqueous extracts**.**

INTRODUCTION

Plant parasitic nematodes caused significant damage and losses to various agricultural crops in the tropical and sub-tropical (Luc *et al.,* 2005). For some crops, such as soybeans, nematodes are the most important pest or pathogen. However, many of the conventional nematicides that were used to control Plant-parasitic nematodes have been shown to contribute to ground water contamination and to be hazardouse to the health of humans and animals ,and have therefore been banned or restricted in use. Brassicaceae produce glucosinolates which are β-D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups). Glucosinolates, classified as aliphatic, aromatic or indole forms, occur in all parts of the plant and degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase, which is stored separately in the cell, to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs) (Fahey *et. al.,* 2001) .

The objective of the present investigation was to study the role of Brassicacous natural products on *Meloidogyne incognita* management in vitro

MATERIALS AND METHODS

1- Isolation of *Meloidogyne* spp. ;

Under compound microscope identified the different genera of plant parasitic nematodes according to perineal patterns. A total number of 4 different genera of nematodes were identified which were belong to the order Tylenchida*, Meloidogyne spp: M. javanica, M. arenaria, M. hapla,and M.incognita* .(Taylor *et. al*., 1955)

Eggs of *Meloidogyne incognita* were extracted from tomato (*Lycopersicon esculentum*) Mill cv. Castle Rock roots infected with the nematode using sodium hypochlorite solution (Hussey and Barker 1973).

Second-stage juveniles (J2) were collected daily from eggs and stored at 15°C. The juveniles used in the experiments were less than 5 days old.

2-Isothiocyanate extraction and analysis:

The plants and plant parts used for extraction of medicinal plants are shown in Table 1. Components were extracted from10g fresh medicinal plants seeds soaking for 48 hr in a 150 ml sterilized water in room temperature . Isolated medicinal plants extraction solutions were stored at 4 to 6°C. The chemical composition of the nematicidal activity of such isolation was determined with a G 1800 B GCD system with an electron ionization detector (Hewlett-Packard Co., Palo Alto, CA) for high-resolution gas chromatography-mass spectrometry (GC-MS) analysis (Ravid *et. al.,* 1985.). Plants extraction were diluted in hexane (1:100) and injected into the HP-5 fused silica capillary column (3.0 m × 0.25 mm inside diameter), at 1.0 ml/min helium (flow rate split ratio 100:1). Temperature was programmed to 4°C/min, from 70 to 200°C. MS were taken at 70 eV. The scanning range was 40 to 450 m/z. Essential oil components were identified by MS library match and by comparing retention times to those of authentic samples (Tables 2, 3 and 4).

3-In vitro tests:

Approximately 20-32 J2 and nematode eggs in 100µl of water were introduced into 100µl of medicinal plants extraction (*Sinapis alba, Ammi visnaga, Lepidium sativum*) in wells of 32-well plates (each medicinal plants extraction with serial water diulation (50 µl,100 µl,150 µl and 200 µl) respectively ,and incubated at 25°C. Percentages of immobile J 2 were recorded after 72hr and 144hr . In control: content 35 J2 and eggs in 100 µl of water in wells of 8-well plates replicates. Percentages of J2 immobility , hatching and natural products on mobility were recorded after 3 and 6 days, respectively.

4-Combination experiment:

Approximately 15-23 J2 and nematode eggs in 75µl of distalled water were introduced into 10 µl from each medicinal plants extraction(*Sinapis alba*+ *Ammi visnaga, Sinapis alba*+ *Lepidium sativum, Ammi visnaga*+ *Lepidium sativum* and *Sinapis alba*+ *Lepidium sativum*+ *Ammi visnaga*) in wells of 32-well plates replicates ,and incubated at 25°C. Percentages of mobile J 2 were recorded after 1day and 7days and compared to control .

***Percentages of mortality J2s;**

Percentage of nematode mortality was calculated according to Abbot's formula (1925).

Table(1); Plants used for isothiocyanates extraction.

Botanical name	Common name	Extraction part	Family
Sinapis alba	Masturd	Seed	Brassicaceae
Ammi visnaga	Khella	Seed	Apiaceae
Lepidium Sativum	garden cress	Seed	Brassicaceae

RESULTS AND DISCUSSION

1- Sensitivity of *Meloidogyne incognita*. to Isothiocyanates in laboratory Assays(In vitro tests):

Bioassay of plant extract (*Ammi visnaga, Sinapis alba ,and Lepidium sativum*) against root-knot nematode *Meloidogyne incognita***.** Average of J2 immobility and hatched of *Meloidogyne incognita* eggs in water controls after 3 days (72 hr) and 6 days (144 hr) was 32.5% ,20.7%, 67.5%and79.3% respectively. Plant extract(*Ammi visnaga, Sinapis alba ,and Lepidium sativum*)decreased *Meloidogyne incognita* hatch and J2 mobility relative to those of controls, especially at high concentrations from plants extraction. It was evident that an increase of J2 immobility and reductions in egg hatching were detected with values of 36.4% ,48.5%,63.5%,51.4%,60.7 %,69.9% ,39.3% ,29.9% ,and 94.6%,98.5% ,5.3%,1.4%,respectively), but in mobility was reduced as compared to controls with values of 63.5& 51.4% ; 39.4 & 29.9% ; and 5.3& 1.4% respectively, while control treatment averaged 67.5 % and 79.6% respectively

Table(2):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanates to *Sinapis alba* (nematicidal activity).

Major components of Isothiocyanate	Concentration PPm	Structure of side chain R	Molecular weight
Lucanine 2	14.3	C27H30O16	440
12-octadeca dienoic acid,(Z) -2,3-bis[trimethyl silyl) oxy] proplyl ester	12.7	C27H45O4SI2	498
15-Hexa deca methyl-octasiloxane	12.3	C16H50O7SI8	578
13-teradeca methyl-Hepta siloxane	10.4	C14H44O6SI7	504
11-Dodecamethyl-Hexa-siloxane	9.2	C12H38O5SI6	430
15- octadeca trienoic acid,2,3- bis(tri methyl silyl) propyl ester,(z)	8.5	C27H52O4SI2	496
Ethyl isoallocholate	8.4	C26H44O5	436

Table(3):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanate to *Ammi visnaga*(nematicidal activity).

Major components of Isothiocyanate	Concentration PPm	Structure of side chain R	Molecular weight
Ethyl t-5- carbomethxy-3- dichloro-t-2- methyl cyclo propane- r- thio carboxylate.	56.9	C11H16C12O S	298
8a' -Dicyano- 4,6- dimethyl-7-trioxa tetracyclo (10) dodecane	53.1	C13H14N2O3	246
7H-furo {3,2-g}[1]benzopyran-7-one,2,3- dihydro-2-(1- hydroxyl-1- methyl ethyl	49.2	C14H14O4	246
5H- furo{2-g}{1} benzopyran-5- one 4,a-dimethoxy-7- methyl-	43.8	C14H12O5	260
2- Pentanone,4-hydroxy-4-methyl	40.4	C6H12O2	116
Visnagin	39.3	C13H10O4	230
6 Dimethoxy-2- ethyl benzaldehyde	37	C11O14O3	194
Hydroxy--ói dimethoxy-3`-methyl – acetonaphthone	31.4	C15H16O4	260
2-Benzene dicarboxylic acid, bis(2-ethyl-hexyl) ester	28.9	C24H38O4	390

Table(4):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanates to *Lepidium Sativum* (nematicidal activity).

Major components of Isothiocyanate	Concentration PPm	Structure of side chain R	Molecular weight
4-Benzobicyclo (4.2.0) oct-3-en-2-one	54.2	C12H12O	*172*
Anhydro 6-(4Methyl phenyl)aminopyrido(1,2-d) quinazolin-7-ium hydroxide	51.1	C19H15N3	*285*
2- Pentanone, 4hydroxy-4- methyl	47.2	C6H12O2	116
8H-fura(3,4-d) dibenz(b,f) azepine	37.4	C16H11NO	233
10- hydroxyl-1,4,5,8- tetra- methylanthrone	37.4	C18H18O2	*266*
Dimethyl-bis (1-methyl-2- pyrrolyl) germane	34.6	C12H18GeN2	*264*
2'- Dihydro -1,2',3- triphenyl spiro(2) pyr azoline-4,3'(4', H)- Quinoline) -5one	32.3	C29H23N3O	429
6-Dimethyl-3-(methoxy methyl)-p- benzo-quinone	28.6	C10H12O3	180

Table (5); Effect of isothiocyantes content from mixed certian natural products on immobility and egg hatching of *Meloidogyne incognita* second-stage Juveniles and egg hatching at 72hrs and 144hrs .

Treatment / Time of exposure	Average % J2 immobility		Average % Egg hatching	
	72hrs	144hrs	72hrs	144hrs
Sinapis alba	60.7	69.9	39.3	29.9
Ammi visnaga	36.4	48.5	63.5	51.4
Lepidium sativum	94.6	98.5	5.3	1.4
Distilled water	32.5	20.7	67.5	79.3

2.2.Combination *assay* ;

Average of J2 mobility of *Meloidogyne incognita* in water controls after1 and 7 days was recorded to be 75% and 91.5% respectively.

*Plant extract(*Sinapis alba+ Ammi visnaga, Sinapis alba+ Lepidium sativum, Ammi visnaga+ Lepidium sativum* and *Sinapis alba+ Lepidium*

sativum+ Ammi visnaga) decreased *Meloidogyne incognita* J2 mobility relative to those of control that were detected with values of 58.3 % , 33.3 % , 50 % and 16.6 % respectively as shown in Table (6) Isothiocyanates are released through enzymatic degradation of glucosinolates produced by plants in the family Brassicaceae. Glucosinolate profiles differ among plant species and the isothiocyanate derivatives differ in their toxicity to nematodes. The nematicidal effects of plant isothiocyanates extracts against local populations of RKN. Extract of *Ammi visnaga, Sinapis alba ,and Lepidium sativum* caused a high percent mortality J2s and low in egg hatched of *M. incognita* The response of the nematodes varied with the type of plant, the extract concentration, and the exposure time.

Table(6);Effect of isothiocyantes content from mixed certian natural products on mobility of *Meloidogyne incognita* second-stage Juveniles at 72 hrs and 144hrsin combination experiment.

Treatment / Time of exposure	Average number of active J2		% mortality of J_2	
	1day	7day	1day	7day
Sinapis alba +Ammi visanga	15	0	58.3	100
Sinapis alba +Lepidium sativum	7	0	33.3	100
Ammi visnaga+Lepidium sativum	10	0	50.0	100
Sinapis alba+Ammi visanaga+Lepidium sativaum	5	5	16.6	16.6
Distilled water	20	18	75	91.5

Time of exposure to the extracts affected the mortality J2s and egg hatched of of root - knot nematodes and also affected on the mobility of J2 s was most lethal against *Meloidogyne incognita* . J2s after 72 and 144 h of incubation, while number of mobility of (PPN) in combination plant extracts are most effective already after (1 day) and (7 day) lead to decrease at long exposure time compared to control in combination experiment of medicinal plants extract to *M. incognita* . . The nematicidal activity of methanolic extracts from twenty Jordanian plant species against two species of root-knot nematodes *in vitro* was evaluated. Whole-plant extract of *Hypericum androsaemum* showed the highest activity (11% mortality) against *Meloidogyne javanica* after 24 h of incubation. However, leaf extract of *Origanum syriacum* also increased *M. javanica* mortality markedly a day later, reaching 59 and 82% after 48 and 72 h of exposure respectively. Against *M. incognita* the response of leaf extracts was somewhat different, with leaf extract of *Artemisia herba alba* the most effective causing 22, 51, 54% mortality after 24, 48 and 72 h of exposure respectively. With a tenfold concentration (200g ml-1) of those plant extracts thought to contain volatile oils, the second stage juveniles (J2) mortality of both nematodes increased after 24 and 72 h of incubation. Nematicidal tests of some volatile oils that are active ingredients of the plants tested revealed that geraniol, thymol, and camphor were the most effective against *M. javanica* J2s, with 91, 60, 56% mortality respectively after 72 hr of exposure. Cineole, menthol, and pinene were not effective against this nematode. Against *M. incognita* J2s, the most effective oil components were carvacol, thymol, and geraniol, with mortalities

of 100, 90, and 74% respectively after 72 h of exposure. Cineole was the least effective against *M. incognita*. Increasing the concentration of medicinal plant extracts to 200g ml-1increased the mortality and decreased hatched of eggs of both nematodes. also ,a higher concentration (1000 µl 1-1) of certain plant extracts led to an increase in the mortality of *M. javanica* J2s. Oka *et. al,* (2000) &Luma *et. al*,2003.

However, in our study seeds extract of *Ammi visnaga, Sinapis alba ,and Lepidium sativum are* as effective at the higher as at the higher concentration. Isothiocyanates *extracts of Lepidium sativum* were more effect on the mortality and egg hatched than, *Ammi visnaga and Sinapis alba* (Fahey, *et.al* , 2001) reported that, plants that should be evaluated include *B. hirta (Lepidium sativum)* which contains glucotropeolin and gluconasturtiin, the glucosinolate precursors to benzyl and 2-phenylethyl isothiocyanates, respectively, and *B. napus* L. or *B. juncea* (mustrad) both containing gluconasturtiin). Sinigrin-containing plants (the glucosinolate precursor to allyl isothiocyanate) warrant consideration for *T. semipenetrans* suppression although they may be less effective against *M. javanica*. *Brassica juncea* and *B. napus* contain significant concentrations of sinigrin. Ahmad Khan *et.al* ,2005 reported that the *applications* of *herbicide* (*Convolvulus arvensis, Carthamus oxyacantha, Avena fatua, Phalaris minor and Melilotus parviflora, Medicago denticulata and Ammi visnaga*) adoption of economical, feasible and effective weed control packages such as herbicides is encouraging in the wheat growing and increased yield of effective grass specific herbicides. Under laboratory conditions, five aqueous extracts, of basil leaves (*Ocimum basilicum*), marigold leaves (*Tagetes erecta*), pyrethrum leaves (*Chrysanthemum cinerariafolium*), neem seeds (*Azadirachta indica*) and China berry leaves (*Melia azedarach)* were tested against the root- knot nematode *Meloidogyne incognita* . All the tested materials affected the survival of the nematode juveniles depending on material property and concentration.(Noweer and Susan Hasabo ,2005).

Many of the plants tested are known to contain volatile oils as active ingredients. Several studies have reported that the volatile oils have a role in the nematicidal effect of these plants (Sangwan *et al*., 1985; Malik *et al.*, 1987; Saxena *et al*., 1987; Oka *et al*., 2000). In laboratory assays were conducted to determine lethal concentration (LC) values in sand of seven commercially available isothiocyanates against *Tylenchulus semipenetrans* and *Meloidogyne javanica*. The LC90 values were 0.01 and 0.03 µmol/ml for 2-phenylethyl isothiocyanate and 0.48 and 0.35 µmol/ml for phenyl isothiocyanate for *T. semipenetrans* and *M*. *javanica*, respectively. Brassicaceous sources of benzyl or 2- phenylethyl isothiocyanate and, to a lesser extent allyl isothiocyanate, are the most promising candidates for plant-parasitic nematode management. Zasada and Ferris 2003

The nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized 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).The present *in vitro* study found that some of these medicinal plants extract were

very effective against one or both nematodes at relatively low concentrations. the most promising extracts, their mode of action, and the effect of combinations of volatile oils. In vitro assay Isothiocyanates are released through enzymatic degradation of glucosinolates are effective on developmental stages of (RKN),and unaffected medicinal plant extracts on developmental of *M . incognita* shown data. Addition, field applications of promising extracts should be conducted to verify their nematicidal effectiveness.

REFERENCES

Abbott W. S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology.18:265–267.

Ahmad Khan , Muhammad Ilyas and Tajir Hussain (2005) Response of wheat to herbicides application and hand weeding under irrigated and non-irrigated conditions. Pak. J. Weed Sci. Res. 11(1-2): 1-9.

Fahey, J. W., Zalcmann, A. T., and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5-51.

Hussey, R. S., and K. R. Barker. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025–1028.

Knoblock, K., Weis, N., Weigant, R., (1989). Mechanism of antimicrobial activity of essential oils. 37th Ann. Cong. Med. Plant Res., Braunschweig, pp. 5–9.

Luc, M.; R. A. Sikora and J. Bridge (2005). Plant parasitic nematodes in subtropical and tropical Agriculture. 2nd edn. CAB international , Wallingford, Uk.

Luma, Al-banna, Rula M. Darwish and Talal Aburjai (2003) Effect of plant extracts and essential oils on root-knot nematode. *Phytopathol. Mediterr.* 42, 123–128.

Malik M.S., N.K. Sangwan, K.S. Dhindsa, K.K. Verman and D.S. Bhatti, (1987). Nematocidal efficacy of some monoterpenes and related derivatives. *Pesticides* 21, 30–32.

Noweer, E.M.A. and A. Susan-Hasabo (2005). Effect of different management practices for controlling root-knot nematode *Meloidogyne incognita* on squash. Egypt. J. Phytopatholo., 33: 73-81.

Oka, Y.; S. Necar; E. Putievesky; V. Ravid; Z. Yaniv and Y. Spiegel (2000). Nematicidal activity of essential oils and their components against the root-knot nematode. J. Phytopathol., 90(7): 710-715.

Ravid U, Putievsky E, Katzir I. (1994). Chiral GC analysis of (1R)(.)- pulegone with high enantiomeric purity in essential oils of some lamiaceae aromatic plants. Flavour and Fragrance Journal 9: 205±207.

Sangwan N.K., K.K. Verma, B.S. Verma, M.S. Malik and K.S. Dhindsa, (1985). Nematicidal activity of essential oils of Cymbopogon grasses. Nematologica 31, 93–99.

Saxena D.B., B.K. Goswami and S.S. Tomar, (1987). Nematicidal activity of some essential oils against *Meloidogyne incognita*. Indian Perfume 31, 150–154.

Taylor, A. L., V. H. Dropkin, and G. C. Martin (1955). Perineal patterns of root-knot nematodes. Phytopathology 45:26–34.

Zasada, I.A., Ferris, H., (2003). Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. Phytopathology 93, 747–750.

"تأثير المنتجات الطبيعية لبعض النباتات الطبية علي مكافحة نيماتودا تعقد الجذور مليدوجيني تحت ظروف المعمل"

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تم عزل أنواع من نيماتودا تعقد الجذور (الميلودوجينى) وتم دراسة تأثير المستخلصات النباتية الطبية لكل من الخردل وحب رشاد و الخلة الطبى تحت ظروف المعمل على نيماتودا تعقد الجذور (الميلودوجينى) بأخذ (100µl)ومن ثم يتم التخفيف بماء معقم كالتالى (50 µl,100 µl,150 µl and 200 µl) على التوالى. وقد اسفرت النتائج عن زيادة نسبة الموت فى الطور اليرقى الثانى (الطور المعدى) ونقص فى معدل فقس البيض بتدرج تركيزات المستخلصات النباتية وكانت اعلى نسبة للموت ونسبة فقس البيض للمستخلص حب رشاد بعد مرور ٧٢ساعة (94.6%)،(5.3%) وعند ١٤٤ساعة (98.5%) ،(1.4%) على التوالى اما بنسبة للمستخلصات النباتية الخردل والخلة الطبى عند٧٢ و ١٤٤ساعة كانت (63.5%)، (51.4%)،(39.3%)،(29.9%) و (36.4%) ،(48.5%)،(60.7 %)، (69.9%) على التوالى . دراسة تأثير خلط المستخلصات النباتية الطبية لكل من(الخردل وحب رشاد) و (الخردل و الخلة الطبى) و (حب رشاد و الخلة الطبى) و من ثم (حب رشاد و الخردل و الخلة الطبى) على يرقات نيماتودا تعقد الجذور (الميلودوجينى) حيث اعطت نتائج موثرة علي معدل موت اليرقات بعد مرور يوم واحد بمعدل : (58.3 %) ، (33.3 %) ، (50 %) و (16.6 %) علي التوالى أمابعد سبعة أيام كان معدل الموت بنسبة (١٠٠%) فيما عدا الخلطة الثلاثية .

قام بتحكيم البحث

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