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Effect of some plant extracts on larval mortality against the stem nematode (*Ditylenchusdipsaci*) and compared with synthetic pesticides.

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Abstract: Studies were carried out in 2014,in department of plant protection-Damascus University, to determine the nematicidal effect of leaves of *Inulaviscosa*, dry fruits of *Meliaazedaracht*, whole plant of *Tagetespatula* and leaves of *Eucalyptus camaldulensis* on the stem nematode **(***Ditylenchusdipsaci*Kühn, 1857**)**and compared with synthetic pesticides (carbofuran, methomyl, carbaryl and dimethoate) in vitro.The ethanol extracts of the tested plants were the most toxic against *D. dipsaci*, followed by water extracts. Ethanol extracts of *Inulaviscosa*had the highest effect on fourth-stage juveniles (J4)the corrected mortality %, (78%&82%), followed by ethanol extract of *Meliaazedaracht*which gave the corrected mortality %, (73%& 77%) after 24 and 48 hrs. of exposer, respectively.The concentration of 100 mg/kg^{-1}of carbofuran, methomyl, carbaryl and dimethoate gave the corrected mortality %,:94%, 89%, 43% and 65 % of the treated juveniles (J4) nematodes after 48 hrs. Carbofuran and methomyl had better activity as compared to water and methanol extracts of tested plants. When the dead nematodes were studied under the microscope it became apparent that they had either one of four very distinct shapes, namely: straight (I-shape), bent (banana- shape), sigmoid (∑-shape), or curly (∞-shape). We can arranged the effect of plant extraction and pesticides at corrected mortality %: carbofuran>methomyl> *Inulaviscosa*> *Meliaazedarach*> dimethoate>*Tagetespatula*>carbaryl>*Eucalyptus camaldulensis*.In the consequence, Ethanol and water extracts of leaves of *Inulaviscosa* and dry fruits of *Meliaazedaracht*showed nematicidal activity against *Ditylenchusdipsaci* under laboratory condition.
Keywords: plant extracts, *Ditylenchusdipsaci,* pesticides, nematode mortality.

Introduction

Nematodes, are present in virtually all soil types. World-wide annual losses caused by plant-parasitic nematodes are estimated at approximately US 100 $ billion. They attack a wide range of economically important crops of horticultural and field crops as well as forest systems[1]. *Ditylenchusdipsaci*(Kühn, 1857) Filipjev, 1936 is among the plant-parasitic nematodes of greatest economic impact worldwide and widely distributed mainly in temperate areas. Almost 500 different plant species are known as hosts for *D. dipsaci*but the different biological races of this nematode each have limited host-ranges. *D. dipsaci*lives mostly as an endoparasite in aerial parts of plants (stems, leaves, flowers), but also attacks bulbs, tubers and rhizomes. *D. dipsaci*readily withstands desiccation and can be isolated even from completely dry plant material after moistening (resistant stage = fourth-stage juveniles)[2,3].The population of plant-parasitic nematodes in the field can be minimized through several approaches such as using natural enemies, enhancing cultural practices, cultivating resistant cultivars and applying pesticides[1,4]. Since the 1950s, however, farmers have relied mainly on synthetic pesticides rather than on other approaches. This sometimes results in excessive and unsafe use of

synthetic pesticides[5]. The use of various parts of indigenous plants as botanical extracts has become important in pest management in recent years following the environmental hazards caused by chemical control measures[6]. Neem is available in simple homemade formulations like seed powder, seed kernel powder, seed cake powder, dry leaf powder and aqueous extracts made from them[7]. The isolated bioactive compounds from neem have been divided into two major classes: isoprenoids and non isoprenoids[8]. Neem products have revealed that some of them are effective against insects and nematodes[9,10]. Neem (*Azadirachtaindica*L.) is the best example of plant with nematicidal properties and is available commercially in some parts of the world[11].A significantly increased mortality of *D. dipsaci* was obtained by exposure to essential oils of *Eugenia caryophyllata*, *Origanumcompactum*, *Origanumvulgare*, *Thymus vulgaris* and *T. matschiana*, with which only the concentrations of 5000 and 7500 ppm were effective[12].*Azadirachtaindica*, *Vernoniaamygdalina*and *Moringaoleifera*were evaluated for their effect on pathogenicity of *Meloidogyne incognita*. Eggs and juveniles of *M. incognita* were exposed to water extracts from leaves of these indigenous plants for ten days. Egg hatch inhibition ranged from 40% - 63.7% in the extracts compared to the control with 0%. Juvenile mortality in extracts was from 82% - 93.8% compared to the control with 25%[13].In the other study different parts of *Eucalyptus* sp., viz., leaves, stem, bark and fruit used as aqueous and ethanol extracts showed nematicidal effect against *Meloidogynejavanica*root knot nematode, reduced hatching of eggs, increased mortality of juveniles with an increase in exposure of time *Eucalyptus* species are known to have essential oils which are composed of mixture of volatile compounds. Presumably the parts of *Eucalyptus* compounds were lethal to root knot nematode[14]. Theeffects of extracts of *Tageteserecta*plants cultured onmodified Murashige and Skoog medium (MS+0.1mg/l NAA), using ethyl or methyl alcohol or petroleum ether or chloroform or hexane extracts on *Meloidogyne incognita* second stage juveniles (J2) were measured *in vitro*. Their mortality at standard (100%) concentration of the extracts was 100,74,87,34 and 49%, respectively. Ethanolic extracts of *T. erecta*calli were prepared from seed, leaf, stem or root grown on Ms- medium supplemented with three combinations of the two growth regulators namely; naphthalene acetic acid (NAA) and 6-benzylamine purine (BAP) and cultured for 8 weeks under dark or light conditions. The percentage mortality of *M. incognita* J2 treated with extracts from these tissues was studied under laboratory conditions. Generally, the net mortality was positively correlated with the concentrations of all callus extracts[15]. Also,reported that various species of the genus *Tagetes*are well known for their insecticidal properties. *T. minuta*contains large amounts of thiophenes[16]. Recently, reported that, the roots of *Tageteserecta*, *T. patula*and *T. minuta*, extracted by petroleum ether and chloroform were highly potent against the reniform nematode, *Rotylenchulusreniformis*. The chloroform extract of *Tageteserecta*roots produced a higher mortality rate than the individual component isolated by column and purified on preparative thin layer plates[17].*Inulaviscosa* is a perennial plant that is widely distributed in Mediterranean countries. Formulations of *I. viscosa* extracts were tested for their effectiveness in control of *Meloidogynejavanica* in laboratory, The plant extracts have potential as a natural nematicide, although the formulations need improvement[18].

The effect of Fertinemakil-Plus and Carbofuran was investigated on the population densities of three nematodes and yield of chilli. The population density of all three nematodes namely *Meloidogyne incognita*, *Longidoruselongatus* and *Rotylenchuscapsicumi* was adversely affected by Carbofuranwhile Fertinemakil-Plus showed lesser but significant difference ($p < 0.001$) over the controls[19].Abamectin, in certain doses, were effective against *Ditylenchusdipsaci* in garlic, which decreased the nematodes per cm2 of tissue[20].

The aim of this study:

Nematicidal activity of plants against *Ditylenchusdipsaci*has only been reported in a few studies. The aim of the present study was to evaluate Nematicidal activity of plant extracts of *Inula viscos* L.,*Meliaazedarach* L., *Tagetespatula*L.,*Eucalyptuscamaldulensis*Dehnh.in comparison to synthetic some pesticides againstfourth-stage juveniles*D. dipsaci* (J4)in vitro.

Materials And Methods

The investigation was carried out during 2014at the laboratories of biological control in Faculty of Agriculture, Damascus University. The present study was undertaken to evaluate the comparative performance of water and ethanol extracts of *Inula viscos* L. (Inula),*Meliaazedarach* L. (Chinaberry), *Tagetespatula*L. (Marigold), *Eucalyptus camaldulensis* Dehnh.(RiverRidGum) in comparison to synthetic pesticides (Carbofuran, Methomyl, Carbaryl,and Dimethoate) againstjuveniles*D. dipsaci* (J4)in vitroand consequent effect on nematode's shape.

Preparation of Plant Extracts:

Leaves of *Inula viscos* L. and *Eucalyptus camaldulensis* Dehnh.,dry fruits of *Meliaazedarach* L. and whole plant of *Tagetespatula* L. were collected from Damascus Government, Syria. These samples were air dried in the laboratory and milled into powder with a coffee mill. Water or ethanol extracts were prepared by soaking 100 g of powder in 1000 ml of water or ethanol for 48 hours, in the dark on an orbital shaker at 150 rpm and then filtering through a Whatman® No. 1 filter paper. The filtrate was the stock solution of 100,000 mg/ kg^{-1} concentration[21]. The filtrate ethanol extracts were evaporated to dryness in a rotatory evaporator at 50°C, then resolution with mixture of water: ethanol (9:1) and adjust the volume to the equal volume of water extract . One hundred milliliters (100 ml) of the stock extracts of 100,000 mg/ kg^{-1} was diluted with distilled water at a ratio of 1:3 to obtain extracts of 25,000 mg/kg $^{-1}$.

Synthetic pesticides:

The synthetic pesticides: three Carbamate pesticides (Methomyl: Lannate, SP 90% ,Carbaryl: Sevin, 85%WP and Carbofuran: Brun, 10% G) and oneOrganophosphate pesticides (Dimethoate: Roger, 40% SL). were tested. Stock solutions of the pesticides were prepared in distilled water: acetone (95:5). The rate used against *D. dipsaci* (J4)at concentrations of 100 mg L^{-1}.

Extraction and preparation the nematodes:

The nematodes *Ditylenchusdipsaci*juvenile(J4) were extracted from infested tissues of garlic bulb by a modified Baermann's funnel method[22,23,24].Extraction from plant tissue *D. dipsaci* can be detected by placing plant tissue with suspected infestation into water. Any plant material to be tested is cut into pieces or sliced and placed on a Baermann funnel on a sieve covered with soft filter paper (e.g. cotton wool filter). These nematode species are very mobile and will usually emerge from the tissues within 2 to 4 h; the water from the bottom of the funnel can then checked by microscope for the presence of nematodes.

***In- vitro* assay:**

One hundred (100) µl nematode concentrate (ca 200 J4) of *D. dipsaci* were placed in labeled sterilized petri dish (9 cm) containing five mls of the 25,000 mg/kg^{-1} extract concentration or the 100 mg./L^{-1} concentration of tested pesticides. Water was used as a control. Each treatment was replicated three times and laid out in a completely randomized design (CRD). Petri dishes were kept at room temperature (22–26°C) in darkness. The number of dead and living juveniles in the test solutions was observed at 24 and 48 hours. Inactive nematodes were noted as 'dead' when they assumed characteristic death position and failed to react to touch with a handling needle. Juveniles that appeared dead were removed from the glass blocks and placed in distilled water for a few minutes for confirmation.

The corrected nematode mortality percent was calculated according to the Schneider Orelli's formula[25]:
Corrected % =

{[mortality % in treatment − mortality % in control]/[100 − mortality % in control]} × 100

All statistical analyses were carried out using spss. 20 software was used for data analysis. A *p*-value <0.05 was considered statistically significant.

In the pilot experiment the dead nematodes were found to have a specific shape, defined as either straight (I-shape), bent (banana-shape), sigmoid (∑-shape), and curly (∞-shape) which can be used to determine the type effect of plant extracts and pesticides on morphologically of death nematodes.

Result and Discussion

In our laboratory study the average background mortality of the nematodes in the control treatment was about 5%, indicating good starting conditions. Water and ethanol extractsof leaves (*Inulaviscosa*),dry fruits (*Meliaazedarach*), whole plant (*Tagetespatula*) and leaves (*Eucalyptus camaldulensis*)showed variation in mortality of *D. dipsaci*(J4)(Table 1). Nematode survival was significantly affected by type of plant extracts or synthetic pesticides. The results showed that *D. dipsaci*was quite sensitive to all plant extracts and the tested pesticides. Generally, length of exposure did not affect nematode mortality remarkably, although 48 h exposure to ethanol extracts from leaves *I.viscosa* and dry fruits of *M.azedaracht* (25000 mg/kg^{-1}), revealed a significant

mortality in comparison to 24 h exposure time. The substances in the plant extracts that are active against nematodes are generally grouped as alkaloids, flavonoids, saponins, amides, benzamide and ketones which act singly and in combination[26].

Table 1.The corrected mortality% of *D. dipsaci*after 24 &48 hrs.of exposure to plant extracts and synthetic pesticides.

Corrected mortality %	Corrected mortality %	Tested Compounds	
Incubation 48 h	**Incubation 24 h**	**Plant Extracts**	
82	**78**	**Ethanol**	***Inulaviscosa***
79	**75**	**Water**	
77	**73**	**Ethanol**	***Meliaazedarach***
75	**71**	**Water**	
56	**55**	**Ethanol**	***Tagetespatula***
51	**51**	**Water**	
35	**33**	**Ethanol**	***Eucalyptus camaldulensis***
26	**25**	**Water**	
		Synthetic pesticides	
94	**88**	**Carbofuran**	
89	**83**	**Methomyl**	
65	**61**	**Dimethoate**	
43	**39**	**Carbaryl**	

Each value is an average of three replications.

L.S.D.($p \leq 0.05$): Between treatments: 1.79, L.S.D.($p \leq 0.05$):Between type extracts:1.53, L.S.D.($p \leq 0.05$): Between length of exposure:3.14.

In our studies the effect of exposure of the nematode for 24 and 48 h. to plant extracts from other plant species significantly differ from the untreated control.The larvae exposed to 25000mg/kg $^{-1}$ concentration of the leaves *Inulaviscosa* and dry fruit*Meliaazedarach*ethanol extracts showed 78 % and 73% corrected mortality percentage within 24 hours of exposure, respectively. By 48 hours the corrected mortality percentage had reached 82% and 77%, respectively, in contrast to about 8% mortality recorded in the control after 48 hours. The harmful of *Meliaazedarach* influence on mode of action azadirachtin, these resultssupported by[27]Azadirachtin, a complex tetranortriterpenoidlimonoid from the neem seeds, is the main component responsible for both antifeedant and toxic effects in insects. Neem products have revealed that some of them are effective against insects and nematodes[10].All ethanol extracts were found more effective on *D. dipsaci* (J4) after 24 and 48hrs ($p < 0.005$) compared with water extracts.However, the water extract of leaf *Eucalyptus ecamaldulensis* gave the lowermortality of *D. dipsaci* (J4). The correct mortality percentage ranged from 25 % to 26 % during 24 and 48 hrs. Similarly in previous studies nematicidal property by a number of plants has been investigated for nematode control in agricultural crops[28,29].Our findings was the opposite of the results obtained[30]The fresh and dry leaf extracts of *Eucalyptus citriodora* standard solution "S" caused the highest net mortality percentage of 100% after 72 hrs. of exposure nematode *Meloidogyne incognita*. A high-performance liquid chromatography analysis showed that the following acids; caffeic, ferulic, coumaric, benzoic, vanelic, chlorogenic, and hydroxybenzoic were present in *Eucalyptus* extracts. This is possibly due to the difference in the kind nematode and the tested concentrate. In the other hand, the water extracts of leaves *Inulaviscosa* showed maximum larva mortality were The correct mortality percentage reached: 75% and 79% at 24 and 48hrs. incubation, respectively. Similar results were supported by[12]A significantly increased mortality of *D. dipsaci* was obtained by exposure to essential oils of *Eugenia caryophyllata*, *Origanum compactum*, *Origanumvulgare*, *Thymus vulgaris* and *T. matschiana*,

In case the tested pesticides, our results showed differences in the efficacy. Significant differences were observed among all pesticides after 24hrs.& 48 hrs. The carbaryl (carbamate) showed the lowest correct mortality percentage (39 % &43%), while the highest correct mortality percentage of infective juveniles was observed in carbofuran (carbamate) (88% &94%), followed by Methomyl (carbamate) the correct mortality

percentage reached (83% &89%) after 24& 48 hrs, respectively. The dimethoate (organophosphate) pesticides caused an almost 61% &65 % increase in the nematode mortality over the observed in control at 24 and 48 hrs. Also, a significant differences were found between the times 24 and 48 hrs between pesticides. In the earlier studies it was proved thatneem leaf extracts compared with carbofuran in reducing nematode population in infected cowpea plant with an accompanied yield increase[31].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[32]. The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation [33].

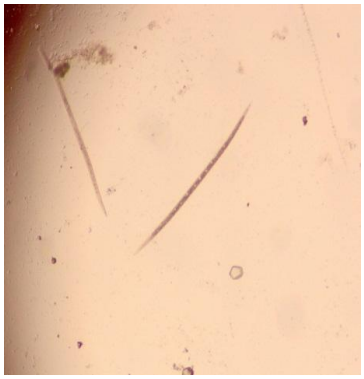
Impact of the tested extracts can be arranged in descending order according to their impact on *D. dipsaci* as follows: carbofuran>methomyl> *Inulaviscosa*>*Meliaazedarach*> dimethoate>*Tagetespatula*> carbaryl> *Eucalyptus camaldulensis*.Based on these findings, these plant extracts and pesticides were divided into 3 main groups i.e. highly toxic (>71% mortality), consisting of carbofuran ,methomyl,*Inulaviscosa* and *Meliaazedarach* ,morality toxic (51- 70 %) consisting of dimethoateand Tagetes, slightly toxic (10-50% mortality) consisting of carbaryl and Eucalyptus .

Table 2. Relative occurrence (%) of characteristic shapes and percentage of relative occurrence among dead nematodes,*Ditylenchusdipsaci* (J4) after 48 hrs. exposure to plant extracts or synthetic pesticides

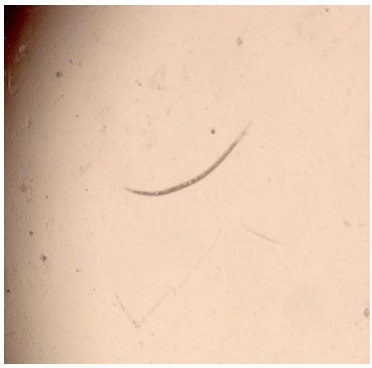
Shapes of Dead Nematodes (%) 48hrs.				**Tested Compounds**	
Straight (I-shape)	**Bent (Banana-shape)**	**Sigmoid (∑-shape)**	**Curled (∞-shape)**	**Plant Extracts**	
13	27	35	25	**Ethanol**	***Inulaviscosa***
22	29	30	19	**Water**	
80	17	3	0	**Ethanol**	***Meliaazedarach***
80	20	0	0	**Water**	
14	31	32	23	**Ethanol**	***Tagetespatula***
17	34	27	22	**Water**	
31	65	4	0	**Ethanol**	***Eucalyptus camaldulensis***
33	67	0	0	**Water**	
				Synthetic pesticides	
10	20	25	45	**Carbofuran**	
11	22	27	40	**Methomyl**	
8	14	21	57	**Dimethoate**	
13	25	21	41	**Carbaryl**	
77	23	0	0	**Control**	

When the dead nematodes were studied under the microscope it became apparent that they had either one of four very distinct shapes, namely: straight (I-shape), bent (banana- shape), sigmoid (∑-shape), or curly (∞-shape) (Table **2**, Fig.1-**4**). The dead nematodes from the control group mostly was straight (I shape) with only very few showing a bent (banana) shape. The characteristic shape of nematodes killed by *Inulaviscosa*and *Tagetespatula*weresigmoid (∑-shape),curly (∞-shape) andbent (banana- shape)with some (I-shape) shapes, which was similar to those killed by the cholinesterase inhibitors carbofuran,carbaryl, methomyl and dimethoate. The efficacy of extracts of*Inulaviscosa* and *Tagetespatula*, can be explained by the high content of thiophene group, α-terthienyl and other naturally compounds, which have an impact cholinesterase activities. The appearances of the nematodes killed by other plant extracts mostly followed straight or bent shapes, similar to those killed by the control. Results of this study are in general agreement with reports on the biological activities of plant extracts. Isolated and described some active principles from Tagetes plants, these chemicals belong to a group of heterocyclic sulphur-containing compounds, the thiophenes[34]. *Tagetes*species contain biocidal compounds of the thiophene group as non polar products of secondary metabolites[35,36]. Many investigators identified several strong nematicidal compounds such as α-terthienyl and some unstable derivatives in marigold plants and these compounds seemed to be activated by illumination. The photo-toxins,

α-terthienyl and other naturally occurring acetylenes seem to havepotential pesticidal activity[37]. While, [38]reported that marigold (*T. patula*) hairy roots induced by infection with *Agrobacterium rhizogenes* produced α-terthienyl when grown in darkness, and an n-hexane extract of the roots had a nematicidal activity.α-Terthienyl and related compounds were isolated from *Tagetes* spp. and have been shown to be nematicidal at low concentrations in vitro[39]. Recently, elecampane (*Inulaviscosa*, syn. *Cupulariaviscosa*, *Dittrichiaviscosa*) (Asteraceae), a widespread plant in Mediterranean countries, has been found to have nematicidal activity in the shoot [40]. Our finding showed that the nematodes killed by the cholinesterase inhibitors, carbofuran, methomyl, carbaryl, and dimethoate mostly had a curled shape (40- 57%),flowed sigmoidshape (21-27%),whilefew of them had bent (14- 25%) and straight shapes (8-13%).The 4 Distinguished Shapes are shown in Fig. (1-4)

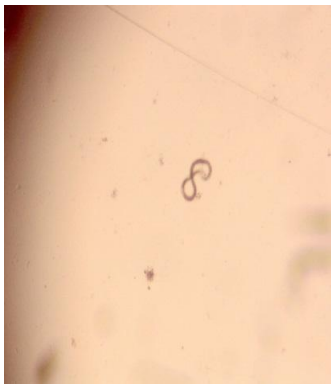


Straight (I-shape)



Bent (banana-shape)

Sigmoid (∑-shape)



Curly (∞-shape)

Fig. (1-4). Characteristic shapes of dead nematodes

Conclusion:

In summary, at the standard concentration (25000mg/kg^{-1}) all the extracts showed nematicidal activity for *Ditylenchusdipsaci* after 24 and 48hrs ander laboratory conditions. These data support previous reports of nematicidal activity by some of other plants against these plant parasitic nematode[12].

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