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ANTI-NEMATODE ACTIVITY OF SOME PLANT OLEORESINS AGAINST *MELOIDOGYNE INCOGNITA*

BY

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ABSTRACT

Anti-nematode activity of oleoresins of cardamon (*Elettaria cardamomum*). thyme (*thymus vulgaris*). clove (*Eugenia caryophyllata*) and melissa (*Melissa officinalis*) against *Meloidogyne incognita* infecting cucumber at the concentrations of 250, 500 or 750 µL/ml was evaluated in two application times. All the applied concentrations showed highly nematicidal activities Gall formation in roots and counts of soil nematodes were greatly affected due to oleoresins application. Such effect was correlated with the type and concentration of the tested oleoresins.

Accordingly, the rate of the nematode build up was greatly affected by the oleoresin type and its concentration level.

Moreover, application of oleoresins did improve growth of the cucumber plants, regardless, to type of oleoresins and their concentration levels. The oleoresins were more effective when applied simultaneously with the nematode inocula than when they added one week after nematode inoculation

In general, oleoresin of cardamon was the most effective followed by thyme. clove and finally Melissa against the nematode The volatile fractions of the oleoresins were chemically analyzed by GC-MS The dominant components in cardamon were trans-caryophyllene (7.38%), nonanol (6.02%), α-terpinene (4.06%). sabinene (4.06%) and 1,8 cineol (6.84). On contrast. thymol (9.31%), β-thujone (6.39%). P-cymene (5.54%) and 1,8 cineole (4.36%) were the dominant constituents in thyme oleoresin. Clove oleoresin had high contents of geraniol (6.67%), B-cymene (0.47%), thymol (9.53%), carene (4.07%). eugenol (3.78%). carvacrol (5.89%) and citral (2%). As for melissa oleoresin, data ascertained high contents of citronellol (6.86%). β-cubebene (5.08%). citronellal (12.01%). eugenol (6.01%). citral (8.92%) and thymol (1.30%).

Key Words: *Meloidogyne incognita*, plant oleoresins. cardamon. thyme. clove. melissa. anti-nematode activity

INTRODUCTION

Myriad compounds, in nature, have the ability to inhibit several microorganisms Essential oils and their major constituents derived from commonly available plants have proven to possess fungicidal, bactericidal. insecticidal. antifeedant. antigonadal. repellent or attractant activities (Singh *et* *al.*, 1980; Shaaya *et al.*, 1991; Müller-Riebau *et al.*, 1995; Wilson *et al.*, 1997. Isman 1999; Srivastava *et al.*, 1999; Gurdip-Singh & Singh-G, 1999 and Demirci *et al.*, 2000). Essential oils and their constituents have been reported to be a potent source of environmentally safe biocides (botanical pesticides) that could be explored for commercial application. However, only a few essential oils and their components have been evaluated for their nematicidal effects (Sangwan *et al.*, 1990; Leela *et al.*, 1992; Walker & Melin 1996; Oka *et al.*, 2000; Al-Shalaby & Ali, 2001 and Ali & Al-Shalaby, 2004).

Since, oleoresins are more concentrated than essential oils (Health, 1981), they considered the true essence of the spice and consist mainly of the volatile essential oils and the non volatile resinous fractions (Mazza *et al.*, 1993).

Also, efficacy of oleoresins extracted from different plant species have been evaluated against many pests (Cook, 1992; Reed and Ramaswamy, 1995; Mayeux, 1996 and Miller, 2001).

The efficacy of oleoresins extracted from capsicum, paprika and white pepper were evaluated against the root-knot nematode (Al-Shalaby, 2004).

Many reports have been documented about the antimicrobial activities of cardamon, clove, thyme and melissa in essential oil form or oleoresin form (Marth, 1966; Bullerman *et al.*, 1977; Conner, 1983 and Zaika, 1988). Also, Lawless (1992) reported that clove has anthelmintic and antibiotic activity. Thyme has been considered as one of the most earliest medicinal plants employed throughout the Mediteranean region. Furthermore, it has a wide range of uses such as anthelmintic, antibiotic and antimicrobial activities.

This work was designed to evaluate the nematicidal activity of the oleoresin froms of four different plants against *M. incognita*. The tested oleoresins were also subjected to chemical analysis to find out their main constituents by GC-MS technique.

MATERIALS AND METHODS

Source of botanical materials:

Leaves and flowering tops of thyme (*Thymus vulgaris* Fam., Lamiaceae) and melissa (*Melissa officinalis* L. Fam., Labialae) were harvested and collected in March, 2004 from medicinal Farm of faculty of Agric, Cairo. Univ., Giza, Egypt. Fruits of clove (*Eugenia caryophyllata*, Thunb., Fam., Myrtaceae) and cardamon (*Elettaria cardamomum* Fam., Zingiberaceae) were purchased from the local markets, Giza, Egypt. The plant materials were blended to be in fine powdered form.

Extraction of oleoresins:

The four materials were extracted by acetone for 48 hr at 30°C, then the resulted extracts were filtrated and concentrated at 55°C in reduced pressure using a rotary evaporator. Likewise, drying was made with N_2 in a 55°C water bath to obtain oleoresins as described by Dapkevicius *et al.*. (1998).

GC-MS analysis of oleoresins:

The volatile fractions of oleoresins were obtained by using the head space method in which aliquots of 5 g. of the oleoresins were put into 10 ml sealed tubes. The tubes were heated for 1 hr. in a thermostitically controlled water bath at 60°C. The volatile fractions were removed with chromatographic syrings for quantification and identification by GC-MS as described by Guadayol *et al.* (1997).

Anti-nematode activity of the oleoresins:

Seeds of cucumber, *Cucumis satives* cv Sweet crunch were planted in 12 cm diam. Pots containing 1kg autoclaved sandy clay soil (2:1, v:v). After germination, plants were thinned to one seedling per pot and divided to three sets.

In the first set, plants were inoculated with 1000 newly hatched juveniles of *M. incognita*/pot. After one week, pots received 1ml/pot of the oleoresins diluted in ethanol 90% at the concentration rates of 250, 500 or 750 µl/ml of the tested oleoresins.

In the second set, both nematode and oleoresins were simultaneously applied at the same levels of the oleoresins concentrations and nematode inoculation.

Plants of the third set received different concentrations of the oleoresins without adding nematode inocula to detect the possible effect of such oleoresins on the plant growth. Besides, two comparable check treatments were involved including a separate treatment of inoculated plants provided with only solvent and the other of only inoculated plants without oleoresins. Each treatment was replicated five times.

All pots were kept in a greenhouse bench and distributed as a randomized block design. After 45 days, the experiment was ended, lengths and weights of the shoots and roots were recorded. Number of galls per root system and nematode counts in soil and roots were counted.

Statistical analysis:

Data were subjected to analysis of variance (ANOVA), and means were separated according to L.S.D. ($P > 0.05$) (Snedecor and Cochran, 1980).

RESULTS

1- The role of the oleoresins in nematode inhibition and plant growth improvement:

a- Simultaneous application of oleoresins and nematode infection:

The nematicidal activities of the oleoresins extracted from cardamon, thyme, clove and melissa are presented in Table (1) and Fig. (1). Gall formation of *M. incognita* in roots of cucumber was adversely affected due to oleoresins application. Namely, a negative correlation was detected between counts of galls and the applied concentration.

However, oleoresin of cardamon was the mostly effective followed by thyme, clove and then melissa (Fig. 1).

Likewise, all tested oleoresins significantly reduced counts of the nematodes in soil and roots of cucumber comparing with those of both check treatments.

All applied concentrations of the oleoresins significantly reduced the nematode developmental stages, number of females, number of egg-laying females in roots and J_2 in soil.

Collectively the nematode final population was therefore, reduced and less than those of the check treatments. Successive increase of the concentration level of the oleoresins caused gradual decrease in counts of nematode developmental stages in roots and soil. Evidently, variable effects among the tested oleoresins were noticed, regardless, to the concentration levels. Oleoresins of cardamon caused remarkable effect on the nematode counts in roots and soil, followed by thyme, clove and then melissa.

Therefore, the rate of the nematode build-up was greatly affected by the oleoresin type and its concentration level. Comparatively, rates of the nematode build-up in the treatments of the high concentration level were 0.082, 0.132, 0.262 and 0.453 for cardamon, thyme, clove and melissa respectively; while it was 2.2 or 2.1 for those of the check treatments.

Table (1): Effect of some plant oleoresins applied simultaneously with *M. incognita* on development and reproduction of the nematode.

Oleoresins (µL/ml.)	Counts of Nematodes in Roots			Counts of Nematodes in soil (J_2)	Final* pop. (pf)	Rate of build up (pf/pi)
	Develop-mental stages	Adult females	Egg-masses			
Cardamon						
250	16^{ef}	48^{def}	27^{efg}	81^{fg}	172^{def}	0.172
500	10^{f}	34^{ef}	19^{fg}	65^{fg}	128^{ef}	0.128
750	7^{f}	22^{f}	11^{g}	42^{g}	82^{f}	0.082
Thyme						
250	31^{cd}	78^{cd}	48^{de}	167^{def}	324^{def}	0.324
500	28^{cde}	43^{def}	27^{efg}	91^{efg}	189^{def}	0.189
750	18^{def}	36^{ef}	18^{fg}	60^{fg}	132^{ef}	0.132
Clove						
250	27^{de}	94^{c}	70^{c}	223^{cd}	414^{cde}	0.414
500	20^{def}	67^{cde}	39^{ef}	202^{cde}	328^{def}	0.328
750	16^{ef}	52^{def}	34^{ef}	160^{def}	262^{def}	0.262
Melissa						
250	60^{b}	141^{b}	115^{b}	488^{b}	804^{b}	0.804
500	42^{c}	97^{c}	75^{c}	499^{b}	713^{bc}	0.713
750	29^{cde}	71^{cvde}	61^{cd}	292^{c}	453^{cd}	0.453
Check 1 (Solvent only)	108^{a}	280^{a}	362^{a}	1369^{a}	2119^{a}	2.119
Check 2 (Nematode only)	116^{a}	292^{a}	378^{a}	1394^{a}	2180^{a}	2.18

Means which are not significantly different are followed by the same letters

* Final pop. = Final population

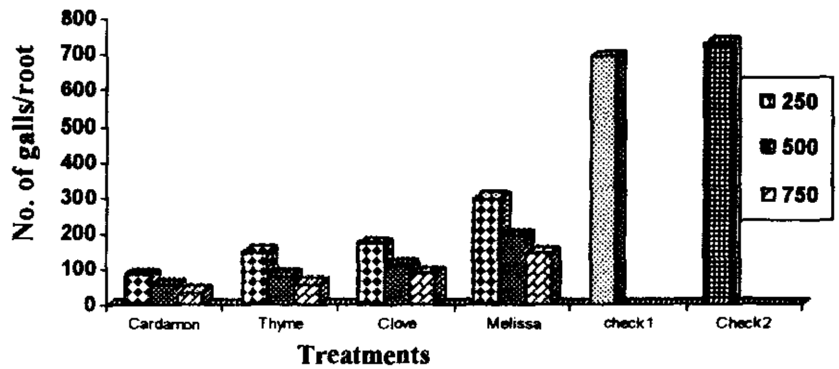

Fig. (1): Effect of some plant oleoresins on gall formation when applied simultaneously with *M. incognita* inoculation.

Growth parameters of cucumber plants were also affected due to oleoresins application (Table 2). Oleoresins of thyme, clove and melissa, at all concentration levels, had no adverse effects on growth of non-nematized plants when compared with those of the check treatments. Also, no significant effects have been noticed on growth parameters of cucumber plants and even those of the check treatments. Application of thyme and melissa caused remarkable increase in root length than in shoot length, especially with the highest concentration level.

b- Application of oleoresins one week after nematode infection:

Data illustrated in Fig. (2) demonstrate that gall formation of *M. incognita* on cucumber roots was also affected by the tested oleoresins even when they were applied one week after nematode inoculation. Oleoresin of cardamon had the greatest effect followed by those of clove, thyme and melissa.

Effect of the tested oleoresins on development and reproduction of *M. incognita* when applied one week after nematode inoculation is presented in Table (3).

Oleoresins at all used concentrations achieved significant reduction in counts of egg masses, and developmental stages in roots as well as counts of (J_2) in soil.

Accordingly, the nematode final population and rates of build up were decreased in comparing to those of the check treatments. Efficiency of the oleoresins were positively correlated with the material concentration; namely, the effect was increased by increasing the concentration rate. Oleoresin of cardamon was the most effective when compared with those of the other treatments. The least rates of build up were obtained by the application of the highest concentration level of cardamon, thyme or clove oleoresins with 0.30, 0.37 or 0.38, respectively. Oleoresin of melissa achieved the least effect on nematode reduction, specially with the lowest concentration level (1.16).

Table (2): Effect of some plant oleoresins on growth parameters of cucumber when simultaneously applied with *M. incognita* inoculation.

Oleoresins (µl/ml.)	Shoot					
	Length (cm)			Weight (g)		
	Non* inf.	Inf.**	Increase %	Non inf.	Inf.	Increase %
Cardamon						
250	50^{cde}	40^{c}	-	39^{cd}	39^{a}	-
500	48^{de}	41^{bc}	-	40^{bcd}	41^{a}	0
750	43^{e}	44^{abc}	-	37^{d}	42^{a}	2.4
Thyme						
250	52^{bcde}	48^{abc}	0	44^{abc}	42^{a}	2.4
500	55^{abcd}	49^{abc}	2.1	45^{ab}	42^{a}	2.4
750	60^{abc}	50^{ab}	4.2	46^{ab}	43^{a}	4.9
Clove						
250	56^{abcd}	49^{abc}	2.1	44^{abc}	43^{a}	4.9
500	58^{abc}	50^{ab}	4.2	45^{ab}	42^{a}	2.4
750	61^{ab}	52^{a}	8.3	46^{ab}	42^{a}	2.4
Melissa						
250	64^{a}	50^{ab}	4.2	48^{a}	43^{a}	4.9
500	60^{abc}	50^{ab}	4.2	46^{a}	42^{a}	2.4
750	57^{abcd}	46^{abc}	-	44^{abc}	42^{a}	2.4
Check 1 (solvent only)	57^{abcd}	49^{abc}	2.1	45^{ab}	42^{a}	2.4
Check 2 (Nematode only)	59^{abc}	48^{abc}	-	46^{ab}	41^{a}	-
	Root					
Cardamon						
250	24^{d}	28^{a}	-	11^{c}	10^{a}	11.1
500	26^{cd}	29^{a}	0	12^{bc}	9^{a}	0
750	23^{d}	30^{a}	3.4	10^{c}	9^{a}	0
Thyme						
250	32^{abc}	30^{a}	3.4	14^{abc}	10^{a}	11.1
500	35^{ab}	31^{a}	6.9	14^{abc}	11^{a}	22.2
750	36^{ab}	32^{a}	10.3	15^{ab}	11^{a}	22.2
Clove						
250	32^{abc}	30^{a}	3.4	15^{ab}	10^{a}	11.1
500	34^{ab}	30^{a}	3.4	15^{ab}	10^{a}	11.1
750	38^{a}	29^{a}	0	16^{a}	11^{a}	22.2
Melissa						
250	37^{ab}	30^{a}	3.4	16^{a}	10^{a}	11.1
500	34^{ab}	31^{a}	6.9	15^{ab}	11^{a}	22.2
750	32^{abc}	31^{a}	6.9	14^{ab}	11^{a}	22.2
Check 1 (solvent only)	31^{bc}	30^{a}	3.4	12^{bc}	9^{a}	0
Check 2 (Nematode only)	32^{abc}	29^{a}	-	12^{bc}	9^{a}	-

Means which are not significantly different are followed by the same letters

* Non inf = Non infected ** inf = infected

Table (3): Effect of some plant oleoresins when applied one week after *M. incognita* inoculation on development and reproduction of the nematodes.

Oleoresins (μL/ml).	Counts of Nematodes in Roots			Counts of Nematodes in soil (J_2)	Final* pop. (pf)	Rate of build up (pf/pi)
	Develop-mental stages	Adult females	Egg-masses			
Cardamon						
25	20^{ghi}	100^{efg}	87^{def}	392^{cd}	599^{de}	0.599
500	15^{hi}	90^{fg}	71^{efgh}	338^{de}	514^{ef}	0.514
750	11^{i}	55^{g}	42^{h}	196^{e}	304^{f}	0.304
Thyme						
250	47^{cd}	151^{cd}	127^{c}	578^{b}	903^{c}	0.90
500	33^{defg}	97^{fg}	84^{defg}	402^{cd}	616^{de}	0.62
750	29^{efgh}	64^{g}	47^{gh}	228^{e}	368^{ef}	0.37
Clove						
250	38^{def}	119^{def}	97^{cde}	541^{bc}	795^{cd}	0.795
500	30^{efgh}	78^{fg}	62^{efgh}	296^{de}	466^{ef}	0.466
750	26^{fghi}	68^{g}	50^{fgh}	237^{e}	381^{ef}	0.381
Melissa						
250	75^{b}	228^{b}	193^{b}	661^{b}	1157^{b}	1.157
500	57^{c}	191^{bc}	168^{b}	632^{b}	1048^{bc}	1.048
750	43^{cde}	143^{de}	120^{cd}	578^{b}	884^{c}	0.884
Check 1 (Solvent only)	114^{a}	288^{a}	370^{a}	1380^{a}	2152^{a}	2.15
Check 2 (Nematode only)	116^{a}	292^{a}	378^{a}	1394^{a}	2180^{a}	2.18

Means which are not significantly different are followed by the same letters

* Final pop. = Final populaion

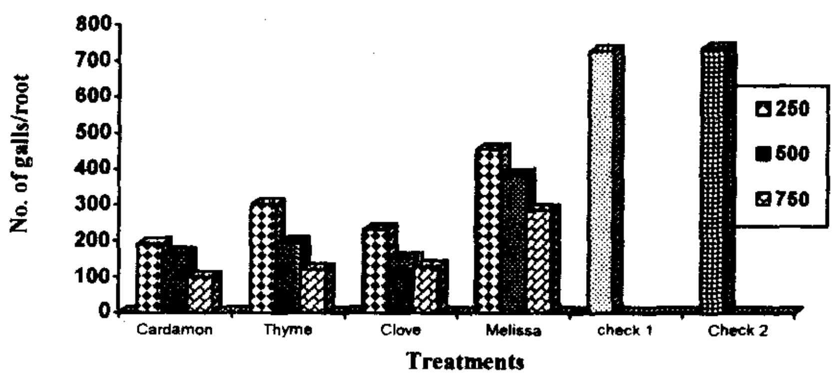

Fig. (2): Effect of some plant oleoresins on gall formation when applied one week after *M. incognita* inoculation.

Regarding to the effect of the oleoresins on cucumber growth. data manifested in Table (4) reveal that they all had no or little effect on root parameters.

Statistically, no significant increase was obtained either in shoot weight or root length and weight amongst the oleoresin treatments and though the check ones. Unexpectedly, pronounced effect was noticed on shoot length of plants treated with melissa oleoresin at the lowest concentration and on root weight of plants treated with clove oleoresin at 500 and 750 µl/ml concentrations.

2- GC-MS analyses of cardamon, thyme, clove and melissa:

Analyses of cardamon, thyme, clove and melissa were carried out and listed in tables (5-8). Constituents of cardamon oleoresin (Table 5) are identified as fourty-one components involving 98.7% of the material. while only one component (1.3) was not identified. Terpenes components are dominant such as sabinene (4.06%), delta-carene (2.38%), hexyl furan (4.64%), limonene (3.13%). Cyclohexane (3.43%) trans caryophyllene (7.38%), α terpinene (4.06%). hexadecene (3.79%), and Ciscyclohexandiamine (4.18%).

Meanwhile, alcoholic components represent a second group like 1,8 cineole (6.84%), Linalool (2.06%), 1-tetradecanol (1.64%), heptadecanol (2.41%), Nonanol (6.02%), decanol (2.83%) and Cis-Farnesol (1.59%). Esters, as a third group contain 1-p-menthene acetate (2.65%), geranyl acetate (3.48%), Linalyl hexanoate (2.87%). While the ketones represent the last one. These results are in agree with those reported by Lawless (1992).

When thyme oleoresin was fractionated it contain twenty two components (Table, 6). The fractions are thymole (9.31%), α-Thujene (5.00%), 1.8 cineole (4.36%) and P-cymene (5.54%).

Fractionation of the volatile fractions of clove oleoresins revealed the presence of fourty two volatile components (Table. 7).

The oxygenated compounds are dominant where thymol (9.53%), geraniol (6.67%), carvacrol (5.89%), hexanal (2.14%). anethole (1.28%) and chavicol (1.09%).

Terpene compounds are less one like hexa-butyl distannousoxane (10.3%). delta guaiene (2.88%), α-copaene (1.29%) and caryophyllene (1.96%).

GC/MS analyses of melissa oleoresins are presented in Table (8). Fourty two components were identified. The identified components attain 93.90%. They are classified to 6 main groups; hydrocarbons, aldehydes & ketones. alcohols. phenols. esters and oxides.

The main compounds are citronellol (6.86%). β-caryophyllene (3.26%). β-cubebene (5.08%), citral (8.92%). citronellal (12.01%). Eugenol (6.01%) and thymol (1.30%).

Table (4): Effect of some plant oleoresins on growth parameters of cucumber when applied one week after *M. incognita* inoculation.

Oleoresins (µl/ml.)	Shoot					
	Length (cm)			Weight (g)		
	Non* inf.	Inf.**	Increase %	Non inf.	Inf.	Increase %
Cardamon						
250	50^{cde}	43^{d}	-	39^{cd}	41^{a}	0
500	48^{de}	45^{cd}	-	40^{bcd}	41^{a}	0
750	43^{e}	46^{bcd}	-	37^{d}	42^{a}	2.4
Thyme						
250	52^{bcde}	50^{abcd}	4.2	44^{abc}	43^{a}	4.9
500	55^{abcd}	50^{abcd}	4.2	45^{ab}	43^{a}	4.9
750	60^{abc}	54^{abcd}	12.5	46^{ab}	45^{a}	9.8
Clove						
250	56^{abcd}	54^{abcd}	12.5	44^{abc}	43^{a}	4.9
500	58^{abc}	55^{abc}	14.6	45^{ab}	46^{a}	12.2
750	61^{ab}	57^{ab}	18.8	46^{ab}	45^{a}	9.8
Melissa						
250	64^{a}	62^{a}	29.2	48^{a}	47^{a}	14.6
500	60^{abc}	57^{abc}	18.8	46^{a}	44^{a}	7.3
750	57^{abcd}	53^{abcd}	10.4	44^{abc}	42^{a}	2.4
Check 1 (solvent only)	57^{abcd}	47^{bcd}	-	45^{ab}	40^{a}	-
Check 2 (Nematode only)	59^{abc}	48^{bcd}	-	46^{ab}	41^{a}	-
	Root					
Cardamon						
250	24^{d}	24^{bc}	-	11^{c}	10^{d}	11.11
500	26^{cd}	27^{abc}	-	12^{bc}	11^{bcd}	22.2
750	23^{d}	23^{c}	-	10^{c}	10^{cd}	11.1
Thyme						
250	32^{abc}	30^{abe}	3.4	14^{abc}	12^{abcd}	33.3
500	35^{ab}	32^{a}	10.3	14^{abc}	13^{abcd}	44.4
750	36^{ab}	34^{a}	17.2	15^{ab}	13^{abcd}	44.4
Clove						
250	32^{abc}	29^{abe}	0	15^{ab}	13^{abcd}	44.4
500	34^{ab}	31^{a}	6.9	15^{ab}	14^{abc}	55.6
750	38^{a}	33^{a}	13.8	16^{a}	15^{a}	66.7
Melissa						
250	37^{ab}	32^{a}	10.3	16^{a}	15^{ab}	66.7
500	34^{ab}	32^{a}	10.3	15^{ab}	13^{abcd}	44.4
750	32^{abc}	30^{ab}	3.4	14^{ab}	12^{abcd}	33.3
Check 1 (solvent only)	31^{bc}	28^{abc}	-	12^{bc}	9^{d}	0
Check 2 (Nematode only)	32^{abe}	29^{abc}	-	12^{bc}	9^{d}	-

Means which are not significantly different are followed by the same letters

* Non inf. = Non infected ** inf. = Infected

Table (5): GC/MS analysis of cardamon oleoresin.

Components	%	Components	%
(Hydrocarbons)		1-Heneicosylformate	1.03
Sabinene	4.06	Total	13.01
β-pinene	1.26	**(Alcoholes)**	
α-thujene	1.11	1,8 cineole	6.84
β-phellandrene	1.13	Linalool	2.06
Delta-carene	2.38	1,4 Terpineol	1.52
Limonene	3.13	1-α terpineol	1.53
Cyclohexane	3.43	Decanol	2.83
Hexylfuran	4.64	1-tetradecanol	1.64
α-Fenchene	1.14	Cis Farnesol	1.59
α-terpinene	4.06	Nonanol	6.02
α-myrcene	0.77	Heptadecanol	2.41
Tridecane	0.73	Nerolidol	0.90
Ciscyclohexandiamine	4.18	Dodecanol	0.83
Eicosane	1.04	Nerol	2.86
Rishitin	0.84	Total	31.03
Trans caryophyllene	7.38	**(Aldehydes & ketones)**	
Hexadecene	3.79	2,4-decadienal	1.87
Total	45.07	Cyclohexanone	1.90
(Esters)		Roemerialinone	2.76
Ethyl-2-pyridylacelate	1.06	Thujone	1.44
Linalyl acetate	0.82	Humulone	1.62
Linalyl hexanoate	2.87	Total	9.59
1-p-menthene acetate	2.65	Total identified	98.7%
Geranyl acetate	3.48	Non-identified	1.3
Neryl acetate	1.10		

Table (6): GC/MS analysis of thyme oleoresin.

Components	%	Components	%
(Hydrocarbons)		Linalool	2.48
2-Nonene	13.10	Borneol	2.02
α-thujene	5.00	Jeraniol	1.51
α-pinene	1.59	Isomenthol	1.79
Camphene	2.91	Stigmastanol	1.54
Sabinene	3.02	Total	13.7
β-pinene	1.67	**(Esters)**	
P-cymene	5.54	Geranyl acetate	2.60
β-caryophyllene	3.04	α-terpenyl propionate	2.96
γ-terpenene	1.66	Total	5.56
Total	37.53	**(Phenols)**	
(Aldehydes & Ketones)		Carvacrol	1.70
Menthone	6.16	Thymol	9.31
Isomenthone	3.34	Total	11.01
β-thujone	6.39		
Total	15.89	Total identified	83.69
(Alcohol)		Non-identified	16.31
1.8 cineol	4.36		

Table (7): GC/MS analysis of clove oleoresin.

Components	%	Components	%
(Hydrocarbons)		**(Aldehydes & ketones)**	
Pentane	4.32	2-pentadecanone	0.55
Trans B-ocimene	0.45	Acetatdehyde	0.52
Limonene	1.61	Carvone	0.65
α-morphinane	0.79	Thujone	1.47
Hexa butyl dislannousoxane	10.30	Jasmone	1.33
Cyclo butane	0.47	Hexanal	2.14
P-cymene	0.47	Citral	2.00
Sabinene	0.62	Total	8.66
Carene	4.07	**(Phenols)**	
β-myrcene	6.55	Eugenol	3.78
α-fenchene	1.07	Carvacrol	5.89
Caryophyllene	1.96	Thymol	9.53
α-copaene	1.29	Total	19.2
Cyclononyne	1.69	**(Esters)**	
β-salinene	1.90	Ethyl acetate	0.91
Delta guaiene	2.88	Linalyl acetate	0.48
Total	40.44	Borenyl acetate	0.58
(Alcohol)		Linalyl-2-methyl propanoate	6.54
1,8 cineol	1.19	Methyl salycatate	2.76
Elemol	1.09	Hexade canoate	1.04
Chavicol	1.09	Eugenol acetate	0.78
Anethol	1.28	Total	13.09
Borneol	0.44	**(Oxides)**	
Cyclohexanol	0.56	Caryophyllene oxide	0.61
Indenol	0.85	Total	0.61
Geraniol	6.67	Total identified	95.17
Total	13.17	Total unidentified	4.83

Table (8): GC/MS analysis of Melissa oleoresin.

Components	%	Components	%
(Hydrocarbons)		Total	15.02
α-pinene	4.40	**(Aldehydes & ketones)**	
Delta-carene	0.22	2-propanone	0.74
Phellandrene	2.18	Acetone	1.31
β-pinene	6.92	Hexanone	0.97
Camphene	0.27	Carvone	2.10
Limonene	3.54	Butanone	2.01
Trans-caryophyllene	3.01	Citral	8.92
Germacrene	1.04	Hexanal	0.72
β-caryophyllene	3.26	Citronellal	12.01
α-copanene	0.25	Geranial	0.33
Myrcene	1.84	Total	29.11
β-ocimene	1.00	**(Phenols)**	
γ-Elemenene	3.50	Anethole	0.72
α-Terpinene	1.30	Eugenol	6.01
β-Cubebene	5.08	Methyl eugenol	0.21
Bisaboline	1.55	Carvacrol	0.50
α-Farnesene	0.27	Thymol	1.30
Total	39.63	Total	8.74
(Alcohole)		**(Esters)**	
α-Terpineol	0.44	Linalyl acetate	0.27
Citronellol	6.86	Geranyl acetate	1.00
Linalool	2.10	Total	1.27
α-cadinol	1.70	**(Oxides)**	
Estragol	0.22	1,8 cineol	0.11
Geraniol	2.91	Total	0.11
Gingerol	0.52	Total identified	93.88
Nerolidol	0.27	Total unidentified	6.12

DISCUSSION

Oleoresins have been known as safe compounds for humans, animals and environment. Chemically, the oleoresins divided into two parts; volatile and non volatile fractions. Both contain several compounds including phenols, alcohols, esters, ketones, aldehydes and others.

The volatile components have in general, several biological activity. They may possess antibacterial, antifungal and/or antioxidants properties (Deans and Water, 1993). Moreover, they were successfully used against many insect species (Cook, 1992; Reed & Ramaswamy, 1995; Mayeux, 1996 and Miller, 2001).

Derivatives of oleoresins like essential oils and their major components have been evaluated for their nematicidal activities (Sangwan *et al.*, 1990; Walker and Melin, 1996; Oka *et al.*, 2000; Al-Shalaby & Ali, 2001 and Ali & Al-

Shalaby, 2004). When oleoresins of the tested plant species, i.e., cardamon, thyme, clove and melissa were used in simultaneous application with *M. incognita* inocula on cucumber plants; or used one week after inoculation, they showed nematicidal activity against the nematode. Regardless to application time, all concentrations of the oleoresins significantly reduced the nematode counts in roots and soil as well as its rates of build up when compared with those of the check treatments.

However, the oleoresins were more efficient when applied simultaneously with the nematode than when used in post-infected plants. The attributable explanation of such finding is due to the possible contact action of the oleoresins on the nematode. Systemic ability of these compounds may be less than their contact action. Oleoresins of cardamon achieved pronounced effect on the nematode counts in roots and soil, followed by thyme, clove and melissa when the oleoresins applied in the same time with nematodes. But when they were applied one week after nematode inoculation, the oleoresin of cardamon was more effective on the nematode followed by clove, thyme and then melissa.

It seems that nematicidal activities of the previous oleoresins are matching their chemical constituents. Cardamon oleoresin contains many active components such as Delta-Carene, 1,8 cineole, and monterpenes at high concentrations (2.38%, 6.84 and 45.07%). Packiyasothy and Kyle (2002) reported that carene exhibited strong antibacterial and antifungal activity against some bacterial or fungal strains at concentrations of 20 and 50 mg/ml, respectively.

Meanwhile, Gustafson, *et al.* (1998) proved the antimicrobial activity of 1,8 cineol. Furthermore, Lis-Balchin and Deans (1997) found that monoterpenes had strong antibacterial activity.

Beta-caryophyllene had, also such effect as mentioned by Mimica-Dukic *et al.*, 2004. Antinematode potential of clove oleoresin, could be attributed to the presence of hydrocarbons like, carene and caryophyllene. Also, the high contents of thymol, geraniol, carvacrol and citral in clove oil (9.53, 6.67, 5.89 and 2.00%, respectively), could enabling it to act as an antibacterial compound (Kim, *et al.*, 1995 and Rhayour, *et al.*, 2003).

Likewise, Lis-Balchin and Deans (1997) reported that eugenol and thymol had strong antimicrobial activity.

Many reports have been concerned with the antimicrobial activity of thyme oleoresin (Ozcan, 1998,Beales, 2002 and Ozkan, *et al.*, 2003).This may be due to the high concentration contents of α-pinene, β-Binene and β-caryophyllene (1.59, 1.67 and 3.04%, respectively).

Also, the highly content of phenols such as thymol and carvacrol (9.31% and 1.70%, respectively) could play an effective role.

Moreover, Packiyasothy and Kyle (2002) claimed that borneol and cymene exhibited antimicrobial activity. These two compounds constituted 2.02 and 5.54% (Table, 6).

Concerning, the effect of melissa, Kedzia *et al.* (1994) and Mimicia-Dukie *et al.* (2004) reported that its essential oil had high antibacterial activity. They also added that citronellal, beta-caryophyllene, thymol, carvacrol and citronellal were the most active essential oil component against microorganisms. Another studies reported that eugenol and thymol were the most effective components against some microorganisms (Rhayour, *et al.*, 2003). Also, citral (aldehyde compound) exhibited antibacterial activity (Deans, *et al.* 1992). The antimicrobial activity of essential oil has been interpreted by Eklund, (1985) and Davidson *et al.* (1993) where they showed that the mode of action was due to phenolic compounds which thought to involve interference with functions of cytoplasmic membrane including proton motive force and active transport. Otherwise, Juven *et al.* (1994) hypothesized that inhibition by thymol was due to a reaction with proteins in the cytoplasmic membrane of microorganism that caused changes in membrane permeability.

Therefore, it could be concluded in general, that the tested materials have antinematode activities; and they could be utilized in nematode management.

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التأثير المضاد لبعض الزيوت الراتنجية على نيماتودا تعقد الجذور ميلودوجين انكوجنيتا

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تم اختبار تأثير أربعة زيوت راتنجية مستخلصة من نبات الحبهان، الزعتر، القرنفل والميلسا فى مكافحة نيماتودا تعقد الجذور "ميلودوجين انكوجنيتا" على جذور نباتات الخيار بثلاث تركيزات هى ٢٥٠، ٥٠٠، ٧٥٠ ميكروليتر/ مللى مذابة فى كحول أيثايل ٩٥%، وتمت إضافتها إلى التربة الموجودة بالأصص البلاستيكية فى ثقوب حول سيقان النباتات فى ميعادين الأول أضيفت فيه الزيوت الراتنجية مع النيماتودا فى نفس اليوم والثانى أضيفت فيه الزيوت الراتنجية بعد عدوى النباتات بالنيماتودا بأسبوع وذلك بمعدل ١ مللى لكل أصيص فى الحالتين.

وأتضح من النتائج أن كل الزيوت الراتنجية المستخدمة بكل تركيزاتها كان لها تأثير مضاد للنيماتودا وقد أدى استخدام هذه التركيزات إلى خفض عدد العقد الجذرية المتكونة وعدد كتل البيض وعدد الأطوار المختلفة داخل الجذر وأيضا عدد النيماتودا فى التربة وبالتالى التعداد النهائى وبناءا عليه أنخفض معدل التكاثر مقارنة بالكنترول حيث أنعكس هذا إيجابيا على النمو الخضرى والجذرى مقارنة بالنباتات المقارنة. ووجد أن الكحول كوسط مذاب فيه الزيوت الراتنجية لا يؤثر على نمو النباتات أو النيماتودا. وأتضح من النتائج أن أفضل ميعاد لاستخدام الزيوت الراتنجية فى مكافحة النيماتودا هو الميعاد الأول الذى أضيفت فيه الزيوت الراتنجية مع النيماتودا فى نفس اليوم. وعموما كانت أفضل الزيوت الراتنجية هى المستخلصة من الحبهان يليها الزعتر ثم القرنفل ثم نبات الميلسا.

وقد تم إجراء تحليل لهذه الزيوت الراتنجية بواسطة GC-MS لمعرفة المكونات الطيارة وكان هناك العديد من المركبات أهمها والذى ربما يعزى إليها التأثير المضاد للنيماتودا – ترانس كاريوفلين (٧,٣٨%)- نونانول (٦,٠٢%)، الفا-تربينين (٤,٠٦%)، صابونين (٤,٠٦%)، ٨،١ سينيول (٦,٨٤%) وذلك فى نبات الحبهان أما فى نبات الزعتر فكان هناك الثيمول (٩,٣١%)، بيتاثيجون (٦,٣٩%)، ب ثيمين (٥,٥٤%)، ٨،١ سينيول (٤,٣٦%).

وأحتوى الزيت الراتنجى لنبات القرنفل على المركبات التالية جيرانول (٦,٦٧%)، ب ثيمين (٠,٤٧%)، ثيمول (٩,٥٣%)، كارين (٤,٠٧%)، ايوجينول (٣,٧٨%)، كارفاكرول (٥,٨٩%) سترال (٢%)، أما فى نبات الميلسا فكان هناك سترونيلول (٦,٨٦%)، بيتاكيوبين (٥,٠٨)، سترونيلال (١٢,٠١%)، أيوجينول (٦,٠١%)، سترال (٨,٩٢%) وأخيرا ثيمول (١,٣٠%). لذلك يمكن استخدام تلك المركبات فى مكافحة النيماتودا على المحاصيل المختلفة