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EFFECT OF CRUDE EXTRACT GARNEW ON PEACH AND GRAPE INFECTION WITH ROOT KNOT NEMATODE

Moatamed, A. M. H.[1] and Hanaa S. Zawam[2]
1-Hortculture Research Institute
2- Plant Pathology Research Institute Agric. Res. Center, Giza-Egypt

ABSTRACT

A pot experiment were carried out during two seasons of 2009/2010 and 2010/2011 under greenhouse conditions in the nursery of Hortculture Research Institute. Garnew crude extract of (Artemisia, Garlic, Chrysanthemum, Menthe and Marjoram)

was tested to control the nematode infection of *Meloidogyne incognita* and *Meloidogyne javanica* on peach and grape .Three concentrations of Garnew were used (0.5% ,5% and10%) to control the both nematode species on peach and grape. The most effective concentration of Garnew was 10%. Whereas the nematicide ethoprop decreased the nematode soil population by 98.8% at the recommended dose and oxamyl decreased it by 98.1% at the recommended dose. Garnew decreased the number of galls ,egg-masses and the developmental stages of the both nematode species on peach and grape by the same level of reduction. Results of plant growth parameters indicated that the highest concentration of Garnew (10%) has appositive effect on plant growth. Mineral accumulation in the leaves or roots of both cultivars of peach and grape was differed according to the concentration of the treatment, but generally increased than the untreated plants. The total protein electrophoresed on one dimension SDS-PAGE revealed differences in the intensity of the same protein bands between the treated and untreated plants.

Keywords: Non fumigant nematicides, crude extract (Garnew), *Meloidogyne* spp., Peach, Grape, nutrient uptake, one dimension SDS-PAGE, total protein electrophoreses

INTRODUCTION

Nematodes are important pests in grapes and peach trees around the world, and these soil-borne pests can be particularly problematic in Egypt on peach and grape. Two factors intensify the impact of nematodes : the high value of grapes and of vineyard land costs. These factors force growers to ignore the steps of leaving land fallow and rotating crops, both of which reduce nematode build up and delay the selection of adapted strains. Nematicides and fumigants help in control nematodes, but the use of these pesticides has been greatly restricted. Plants are an important source of naturally occurring pesticides. Many compounds with nematicidal activity have been found in plants, including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls; (Gommers, 1981; Chitwood, 2002). Many compounds with nematicidal activity have been isolated from species in the family Asteraceae(Gommers, 1981; Chitwood, 2002). Also, Allicin (an active nematicidal principle in garlic) has been isolated by Gupta and Sharmaj, 1993 and tested against *Meloidogyne incognita* infesting tomato, they found that

juvenile mortality of 87–100% at 2.5–5.0 ppm allicin was recorded within 72 h. Essential oils of some plants and/or their components have been tested for nematicidal activity *in vitro* and in soil(Chatterjee *et al.*,1982; Soler-Serratosa *et al.*, 1996; Oka *et al.*, 2000). Recently, the antifungal and insecticidal activity of the essential oil of *Chrysanthemum coronarium* flowerheads has been reported (Perez and Pascual-Villalobos, 1999; Alvarez-Castellanos *et al.*, 2001). the essential oil from *Mentha spicata* with a high content of carvacrol and thymol, was effective against *M. javanica* (Oka *et al.* 2000).

The aim of the proposed study included;

Evaluate the efficacy of the crude extract compound to reduce the number of nematode in soil and roots of plants

MATERIALS AND METHODS

The present study revealed the comparative efficacy of Garnew compound as crude extract comparing with nematicides (Ethoprop and Oxamyl) with two addition rates.

Table (1) : List of compounds tested in this investigation

Compound	Structure	Application rates
Ethoprop	Organophosphate	40 Kg /feddan(1) 4 Kg /feddan(2)
Oxamyl	Oximcarbamate	8 L / feddan(1) 800 Cm^3/feddan(2)
Garnew	Crude extract of Artemisia, Garlic Chrysanthemum, Menthe and Marjoram	8 L / feddan (1) 800Cm^3/ feddan (2)

Three experiments were carried out to study the effect of crude extract on plant parasitic nematode *Meloidogyne javanica*, and *Meloidogyne incognita*. The first experiment was conducted to test the dosage rates using on peach cultivar (Mit Gammer) and grape cultivar (Superior) seedlings under green house conditions

(25-± 2°C) to control the root-knot nematode .The experimental soil was collected from the ARC farm, Giza, Egypt. The soil texture was sandy clay . Black plastic bags 20 cm in diameter were used for our test filled with steam sterilized soil about five kilo per bag

Nematode stock culture

Nematode populations were maintained on tomato plants cv. Castle rock under greenhouse conditions . Plants were infected at 2-3 leaves stage by adding egg-masses to roots (one egg-mass per one plant for making pure culture from *M. incognita* and *M. javanica* in plastic cups) then covered with soil. After 60 days nematode egg-masses collected from each root by a needle, put in Petri dishes and put it in incubator for hatching at 25±2°C for a week. The hatched juveniles were collected daily.

To study the effect of Garnew on development of *Meloidogyne* sp. on peach cultivar Mit Gammer seedling ,forty plastic bags were cultivated by one year

old seedlings of peach in steam sterilized sandy clay soil . Twenty plastic bags were inoculated by 2000 newly hatched larvae of *M. incognita* by boring the nematode suspension in holes around the roots of the peach seedlings. Other twenty plastic bags were inoculated by the same method by 2000 newly hatched larvae of *M.javanica.* The twenty plastic bags which inoculated by *M.incognita* were divided into four groups 3 of them treated by Garnew compound by the dosage of (0.5%, 5% and 10%) and the other 5 bags left without treatment of Garnew and served as inoculated control. The same treatment were made by the plastic bags of *M.javanica* This experiment was repeated on grape cultivar Superior of one year old seedlings. After 90 days the plants were uprooted and the roots were washed free from the adhering soil particles. Number of galls, number of egg-masses per 5gm roots and number of nematodes in 250 cm^3 soil and also the developmental stages inside the roots were determined.

Another pot experiment was conducted to explore the effectiveness of the crude compound Garnew to reduce *Meloidogyne incognit* on peach cultivar Mit Gammer and grape cultivar Superior seedlings comparing with two nematicides (ethoprop and oxamyl) by using two concentrations from each.

Forty plastic bags were cultivated by one year old seedlings of peach in steam sterilized sandy clay soil . The plastic bags were divided into 8 groups, 7 of them inoculated by 2000 newly hatched larvae of *M. incognita* by boring the nematode suspension in holes around the roots of the peach seedlings, and one group left without inoculation and served as control. Other 7 groups were divided into :

1-two groups treated by ethoprop by the recommended dose and 1/10 of the recommended .
2-two groups treated by oxamyl by the recommended dose and 1/10 of the recommended .
3- two groups treated by Garnew by the recommended dose and 1/10 of the recommended .
4- one group left without treatment and served as inoculated control.

This experiment was repeated on grape cultivar Superior of one year old seedlings.

After 90 days the plants were uprooted and the roots were washed free from the adhering soil particles. Number of galls, number of egg-masses per 5gm roots and number of nematodes in 250 cm^3 soil and also the developmental stages inside the roots were determined. Also, plant growth parameters ,shoot length, shoot weight and root weight were determind for both peach and grape cultivars. Also, the number of new branches for peach was recorded . The plant analysis and total protein electrophoresis from this experiment determined according the following methods:

Plant analysis:

Samples of the fourth top leaves and secondary roots were taken and oven dried at 70° C for 48hrs and kept for chemical analysis.

A wet digested according to the methods of Tomas *et al.*, (1967). In the digest solution N,P,K,Fe and Mn were analyzed according to the following methods :

1- Total nitrogen % was determined by the distillation in a macrokjeldahl apparatus (Helrich, K. 1990)
2- Phosphorus % was calorimetrically determined as described by Ranganna (1979
3- Potassium % was determined photometrically using flame photometer, as described by Ranganna(1979)
4- Ca, Fe, Mg, Mn, Na and Zn were determined using Atomic Absorption Spectrophotometer PERKIN ELEMER 3300 according to Chapman and Pratt (1981)

All treatments of greenhouse experiments were statistically arranged in a complete randomize design according to Snedecor and Cochran (1989), where mean values were compared using L.S.D. at 5% level.

Electrophoresis studies:

Total protein analysis

Three grams of plant root samples were ground in precooled mortar and pestle with liquid nitrogen to a fine powder then 0.7 ml of extraction buffer (0.6 ml 1 M Tris HCl pH 6.8, 5 ml 50% glycerol , 2 ml 10% SDS , 0.5 ml β-mercaptoethanol and 0.9 ml H_2O) was added and the extracts were clarified by centrifugation at 14000xg for 15 minutes under cooling. The supernatants were transferred in fresh ependorf tubes and stored at -20°C. Supernatants containing soluble proteins fractions were transferred to clean tubes and stored at- 20°C. Protein content was estimated according to the methods of Bradford (1976) using Bovine Serum Albumin (BSA) as a standard. Protein content was adjusted to 2 mg / ml per sample. SDS was added to the sample at the rate of 4 mg SDS / 1 mg protein, then 50 µl, ß- mercaptoethanol were added. The mixture was boiled at 100°C in a water bath for 3-5 min. Vertical slab (18x16 cm) gel electrophoresis apparatus was used as marketed by Hoofer (Hoofer SE 600 series Pharmacia). 20 µl of this crude protein solution were resolved on 11 % SDS – PAGE using molecular weight protein marker as a standard. Electrophoresis was carried out at 2 mille ampere per sample at 10 °C for 3 hrs.Gels were stained by silver staining method for protein as described by Sammons *et al* (1981) . This method of staining is sensitive and detects as little as 2 ng of protein in a single band. Gels were scanned for estimation molecular weight by using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6C, Fullerton CA, USA 92631). The different molecular weights of bands were determined against protein standard (Peqlab) marker.

RESULTS AND DISCUSSION

Plant-parasitic nematodes feed on grapevine roots and cause malformations or necrosis. This leads to destruction of physiologically active roots and an overall reduction in water and nutrient uptake. Above-ground parts of grapevines show no specific visual symptoms on leaves, shoots or fruits, but there is a general reduction in vigour. Similar symptoms could be due to, and confused with, other conditions

such as poor physical characteristics of the soil, mineral excess or deficiency, water stress, or other soil-borne pests and diseases . Soil amended with crude extract Garnew offers a satisfactory and environmentally friendly compound for the control of root-knot nematode

Table (2) : Effect of Garnew on development of *Meloidogyne* sp. on peach Mit Gammer seedlings.

Treatments		Nematode parameters (*M.incognita*) /root		
	J2/250cm³	No.galls/root	No.egg-masses	No.D.S/root
M.incognita	4200	2300	1700	1300
Garnew0.5%	3600*	1900*	1200*	980*
Garnew 5%	900*	470*	300*	240*
Garnew 10 %	350*	160*	70*	45*
LSD 5%	249.737	197.508	95.946	144.847
		Nematode parameters (*M.javanica*) /root		
M.javanica	3090	1730	1360	990
Garnew0.5%	2700*	1500*	1080*	830*
Garnew 5%	850*	420*	340*	210*
Garnew 10 %	330*	180*	92*	53*
LSD 5%	75.804	36.930	159.131	29.503

*** means there is a significant effect at 5% level**

Results in Table (2) indicated that there was a significant effect of the crude extract on all nematode parameters estimated with either *M. incognita* or *M. javanica*.

Table (3) : Effect of Garnew on development of *Meloidogyne sp.* on grape Superior seedlings.

Treatments		Nematode parameters (*M.incognita*) /root		
	J2/250cm³	No.galls/root	No.egg-masses	No.D.S/root
M.incognita	5100	3250	2570	2400
Garnew0.5%	3800*	2350*	1860*	990*
Garnew 5%	890*	380*	430*	320*
Garnew 10 %	370*	210*	65*	38*
LSD 5%	127.798	166.757	162.329	198.862
		Nematode parameters (*M.javanica*) /root		
M.javanica	3600	1340	1210	1040
Garnew0.5%	2400*	900*	950*	720*
Garnew 5%	720*	330*	230*	110*
Garnew 10 %	230*	130*	60*	55*
LSD 5%	178.394	66.763	91.373	18.855

*** means there is a significant effect at 5% level**

The same trend has been shown in Table (3) the reduction of juveniles in soil numberes of galls, egg-masses and different stages imbedded in the roots was gradually decreased as the concentration of the crude compound Garnew increased either for *M. incognita* or *M. javanica* .
The effectivness of the crude compound Garnew was studied by comparing with the nematicides ethoprop and oxamyl using two doses on peach as showen in Table 4

Table (4) : Effect of some treatments on development of *Meloidogyne incognita* in Peach (Mit Gammer) seedlings.

Treatments	J2/250cm³ soil	R%	No. Galls/root	R%	egg-masses /root	R%	No. D.S /root	R%
Ethoprop1	48	98.8	25	98.3	23	97.6	41	95
Ethoprop2	2620	35.6	1152	23.2	500	48.5	113	86.2
Oxamyl1	76	98.1	42	97.2	31	96.8	50	93.9
Oxamyl2	2700	33.7	1400	6.67	620	36.1	217	73.5
Garnew 1	450	88.9	220	85.3	192	80.2	134	83.7
Garnew 2	700	82.8	340	77.3	221	77.2	161	80.4
Infected plant	4070		1500		970		820	
LSD 5%	900.99		85.58		87.58		6.724	

J2: number of second stage juvenile in soil
D.S: number of developmental stages inside the roots
Reduction: R%= Nematode number in control_-_nematode number in treatment
Nematode number in control
1:recommended dose of the compound 2: 1/10 of the recommended dose

Data in Table (4) showed that highest effect refer to the treatment with ethoprop at recommended dose followed by oxamyl .Also, the crude compound Garnew gave a decrease in root knot nematode on peach seedling compared with the treatment of ethoprop and oxamyl at low concentration (1/10 recommended dose) and untreated control .

Table (5) : Effect of some treatments on development of *Meloidogyne incognita* on grapevine (Superior seedlings.

Treatments	J2/250cm³ soil	R%	No. Galls/ root	R%	egg-masses/ root	R%	No. D.S /root	R%
Ethoprop1	200	83.3	300	72.7	321	65.5	117	81.06
Ethoprop2	500	58.3	370	66.4	352	62.2	169	72.7
Oxamyl1	280	76.7	341	69.0	372	60	92	85.1
Oxamyl2	520	56.7	380	65.5	416	55.3	131	78.8
Garnew 1	500	58.3	410	62.7	432	53.5	141	77.2
Garnew 2	600	50	460	58.2	466	49.9	154	75.1
Nematode	1200		1100		930		618	
LSD 5%	97.671		60.516		6.751		6.634	

J2: number of second stage juvenile in soil
D.S: number of developmental stages inside the roots
Reduction : R%= Nematode number in control-nematode number in treatment
Nematode number in control
1:recommended dose of the compound 2: 1/10 of the recommended dose

The nematicide protects the roots from nematode invasion which resulted in sharp reduction in the number of galls, egg-masses in roots, and juveniles in soil. The present results in Table (5) emphasized that the crude extract exhibited potential nematicidal activity against the root-knot nematode and improved growth criteria of vineyard and peach even at low concentrations.

All the tested materials significantly suppressed root-galling, the number of egg-masses and subsequently the final population. However, the natural compound Gar- new seemed to have toxic action

The nematicidal effect of the tested natural compound may possibly be attributed to 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 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(Konstantopoulou *et al.*, 1994).

Table (6) : Effect of Garnew and two nematicides on some growth parameters of Mit Gammer peach cultivar.

Treatments	Shoot length Cm	Shoot weight gm	Root weight gm	No.of new branches
Infected plant +N+ Ethoprop1	88.3	53.7	40.1	3
Infected plant +N+ Ethoprop2	78.7	45.3	35.7	3
Infected plant +N+ Oxamyl1	78.3	50.1	38.3	4
Infected plant +N+ Oxamyl2	69.7	39.3	28.7	3
Infected plant +N+ Garnew1	96.3	60.3	43.7	7
Infected plant+N+ Garnew2	89.1	49.7	39.3	7
Infected nematode plant	45.1	21.7	13.3	2
Non-infected plant	47.3	30.3	19.7	3
LSD 5%	5.6	3.4	4.5	1.6

It is clear from the data in Table (6) that applying the nature compound Garnew at high rate (8 L/feddan) recorded the highest shoot length and shoot and root weights. Also the number of new branches significantly was increased by 2.5% as compared with untreated plants. The lowest values of vegetative growth were associated with the treatment of nematode only without any treatment. Increasing ethoprop and oxamyl doses increased significantly root and shoot criteria. Nyczepir *et al* 2000 found that plant growth of peach cultivar Lovell was suppressed by both *M. incognita* and *M. javanica*.

Table (7) : Effect of Garnew and two nematicides on some growth parameters of Superior grape cultivar.

Treatments	Shoot length Cm	Shoot weight gm	Root weight gm
Infected plant +N+ Ethoprop1	58.6	30.5	20.3
Infected plant +N+ Ethoprop2	46.6	28.7	18.1
Infected plant +N+ Oxamyl1	60.2	30.7	20.7
Infected plant +N+ Oxamyl2	45.0	25.3	17.3
Infected plant +N+ Garnew1	87.7	43.2	31.3
Infected plant+N+ Garnew2	75.2	40.7	29.1
Infected nematode plant	33.7	12.3	15.3
Non-infected plant	36.3	23.7	13.7
LSD 5%	2.23	4.8	4.16

Data in Table (7) showed that the same trend of increasing in shoot length ,shoot and root weights of grape. The highest values recorded from Garnew treatments followed by ethoprop and oxamyl at the dose 1 and finaly the lowest treatment values was recorded with the ethoprop and oxamyl at the dose two.

Table (8) : Effect of Garnew and two nematicides on mineral accumulation of Leaf samples from peach cultivar Mit Gammer inoculaled with *Meloidogyne incognita.*

Treatments	Concentration% concentration ppm								
	N	P	K	Mg	Ca	Na	Zn	Mn	Fe
Infected plant +N+ Ethoprop1	2.61	0.16	3.20	908.09	0.182	0.250	36.93	8.37	296.3
Infected plant +N+ Ethoprop2	2.53	0.15	2.20	905.68	0.126	0.241	25.81	7.69	282.1
Infected plant +N+ Oxamyl1	2.76	0.23	3.00	908.09	0.172	0.259	25.54	7.63	297.2
Infected plant +N+ Oxamyl2	2.73	0.22	2.77	901.56	0.144	0.213	25.40	7.48	280.3
Infected plant +N+ Garnew1	2.57	0.16	4.15	1206.5	0.247	0.286	86.40	10.8	347.3
Infected plant+N+ Garnew2	2.45	0.18	3.08	909.43	0.209	0.259	26.80	8.77	315.4
Infected nematode plant	2.20	0.15	2.89	806.84	0.107	0.222	24.62	6.73	252.7
Non-infected plant	2.30	0.15	2.89	901.15	0.182	0.227	24.73	7.0	277.3
LSD 5%	0.41	0.01	0.43	15.1	0.08	0.006	2.62	1.2	2.883

The obtained results in Table (8) showed that the uptake and accumulation of minerals by Mit Gammer leaves that reflected the improvement of plants according to the treatments comparing with nematode treated plants in some minerals

Table (9):Effect of Garnew and two nematicides on mineral accumulation of root samples from peach cultivar Mit Gammer inoculaled with *Meloidogyne incognita.*

Treatments	Concentration%						concentration ppm		
	N	P	K	Mg	Ca	Na	Zn	Mn	Fe
Infected plant +N+ Ethoprop1	1.76	0.17	0.71	808.45	0.182	0.232	28.17	28.09	352.1
Infected plant +N+ Ethoprop2	1.59	0.16	0.60	800.97	0.135	0.222	23.59	29.05	317.0
Infected plant +N+ Oxamyl1	1.67	0.19	0.71	706.73	0.228	0.222	32.17	53.60	322.7
Infected plant +N+ Oxamyl2	1.59	0.16	0.57	706.73	0.135	0.204	26.65	24.82	303.5
Infected plant +N+ Garnew1	1.82	0.22	0.67	1200.6	0.219	0.305	27.78	28.93	355.3
Infected plant+N+ Garnew2	1.70	0.19	0.54	1106.4	0.219	0.277	26.56	25.72	336.4
Infected nematode plant	1.16	0.14	0.37	701.11	0.163	0.204	24.03	44.18	218.7
Non-infected plant	1.56	0.15	0.44	704.40	0.228	0.222	26.36	20.7	265.1
LSD 5%	0.08	0.012	0.02	12.2	0.003	0.003	3.31	3.2	2.955

Our data in Table (9) showed that the roots of treated plants accumulated more N, P ,K and small or minor elements than the untreated controls. Since the treated roots were heavier than those of the controls, this higher amount of nutrients is probably a consequence of an increased root

system absorbing surface, although gall formation would have contributed significantly to the final root mass.

Table (10): Effect of Garnew and two nematicides on mineral accumulation of Leaf samples from grape cultivar Superior inoculated with *Meloidogyne incognita*.

Treatments	Concentration %						concentration ppm		
	N	P	K	Mg	Ca	Na	Zn	Mn	Fe
Infected plant +N+ Ethoprop1	1.73	0.29	3.86	703.93	0.246	0.250	28.27	9.03	285.1
Infected plant +N+ Ethoprop2	1.66	0.27	3.07	604.05	0.246	0.241	25.44	8.80	272.3
Infected plant +N+ Oxamyl1	1.78	0.30	3.02	609.11	0.225	0.259	26.73	7.97	281.9
Infected plant +N+ Oxamyl2	1.65	0.28	2.55	508.24	0.219	0.213	23.13	7.89	275.7
Infected plant +N+ Garnew1	1.78	0.35	2.34	708.66	0.274	0.286	42.42	8.73	336.5
Infected plant+N+ Garnew2	1.65	0.32	2.34	562.87	0.246	0.259	36.77	8.38	307.2
Infected nematode plant	1.41	0.20	2.17	400.17	0.163	0.222	24.42	6.40	243.6
Non-infected plant	1.60	0.32	2.57	405.94	0.181	0.247	24.67	6.55	265.7
LSD 5%	0.09	0.06	0.33	13.42	0.02	0.006	1.36	0.79	0.920

Most elements were within sufficiency levels (Jones *el al.*, 1991). Leaves of plants inoculated with the nematode alone were low in N and Fe. The addition of nematicides and biofertilizer resulted in increased plant growth and gave the highest Ca, Fe, Mg, Mn and Na values. Also, P and K increased with the addition of defferent treatments with some exceptions

Table (11) : Effect of Garnew and two nematicides on mineral accumulation of root samples from grape cultivar Superior inoculated with *Meloidogyne incognita*.

Treatments	Concentration %						concentration ppm		
	N	P	K	Mg	Ca	Na	Zn	Mn	Fe
Infected plant +N+ Ethoprop1	0.95	0.26	1.21	804.8	0.219	0.289	40.03	20.95	335.4
Infected plant +N+ Ethoprop2	0.85	0.24	1.10	709.59	0.209	0.286	38.98	20.58	297.3
Infected plant +N+ Oxamyl1	0.95	0.24	1.14	701.85	0.217	0.286	39.85	20.86	315.3
Infected plant +N+ Oxamyl2	0.94	0.21	0.78	690.85	0.200	0.277	31.02	19.59	289.7
Infected plant +N+ Garnew1	1.25	0.33	1.65	902.11	0.293	0.350	70.3	26.06	344.5
Infected plant+N+ Garnew2	1.09	0.30	1.50	802.72	0.228	0.323	40.88	22.31	327.1
Infected nematode plant	0.67	0.21	0.75	409.5	0.172	0.236	27.01	16.94	226.3
Non-infected plant	0.83	0.24	1.00	505.39	0.200	0.268	30.75	17.39	255.1
LSD 5%	0.06	0.06	0.15	15.36	0.33	0.04	1.93	2.49	1.264

Our findings in Table (11) indicated that some nutrient elements decrease(N and Fe) while others increase notably in leaf tissues (Mg, Mn, Zn, and Na) in nematode inoculated treatrnents. In the first case, absorption and transport of Fe and N to aerial parts would seem to be impaired by the destruction of the root cortical tissues caused by the nematode probably due to the loss of the capaciry for differential permeabiliry which reduces nutrient element transport (Kirkpatrick, 1964). In contrast, Mg, Mn, Zn and Na, seem to be absorbed continuously and accumulate in leaf tissues as a result of reduced growth, thus their increasing concentration. The lower concentrations in leaf tissues of these same elements in treatment without the nematode is explained by a growth dilution effect (Kleinschmidt & Gerdemann, 1972; Granger *et al.,* 1983).

A similar pattern for these elements (increase in Zn, Mg, and Mn and reduction in Fe and Cu in foliar apple **(Pinochet *el al.,* 1993 *a)*** in plants infected with nematode.

Protein profile of peach and vineyard infected with *Meloidogyne* spp.:

To find the biochemical differences between the infected and treated plants with Gar new and nematicides, total protein was extracted and electrophoresed on one dimension Sodium dodecyle sulphate, polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE analysis of infected plants revealed a clear differences in the intensity of the same protein bands between the infected and treated plants.

Data presented in Fig. (1& 2) showed the protein profile of plants infected with *Meloidogyne incognita* and treated with biofertelizer Gar new and nonfumegant nematicides ethoprop and oxamyl this reflected the possible physiological differences among the treatments . The present results are in harmony with those of Farahat *et al* 2012, who reported that treating infected plants with fertilizers improve the performance of infected plants by enabling them to recompense root losses of soluble sugars and total carbohydrate and brought phenol contents back to be almost near to those in untreated healthy plants, raising tannins content, diminishing root contents of amino acids to be around those in healthy plants.

This study was designed to evaluate the nematicidal activity of the organic compound from several family species of the Acteraceae on the root-knot nematode *M. incognita* in planta experiments clearly demonstrated that J_2 survival and reproduction rate of the nematode were significantly reduced on grape and peach compared to the nonamended treatment. other researchers found that the population density of *Meloidogyne* spp. was reduced when host plants were grown in soil amended with *Chrysanthemum spp.* or *Artemisia, Mentha, Garlic* and / or *Marjoram* the results of the present study regarding the effects of nematode parasitism on plant growth under artificial conditions may be are not in agreement with the results of other researchers under field conditions. Differences in the susceptibility of the plant cultivars or differences in environmental conditions could be responsible for this. Essential oils from several plant species have been shown to have nematicidal activity on root-knot nematodes *in vitro* and in soil This compound is easily used into organic, conventional and integrated control

growing system. Given obvious benefits and government may consider it as a promoting practice

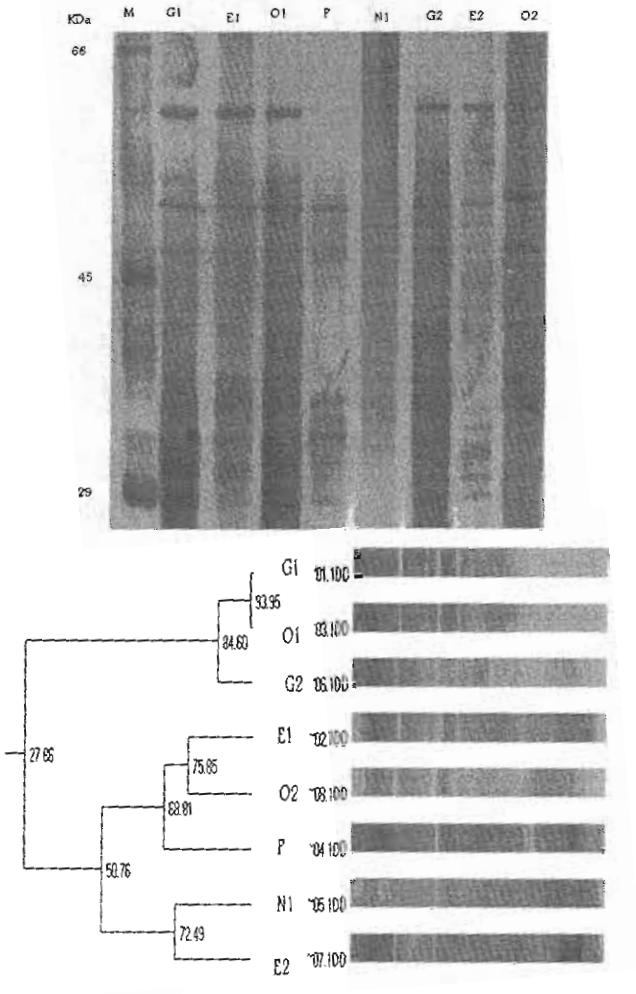

Fig. (1). Protein profile analysis and dendrogram of peach plants infected with *Meloidogyne incognita* using SDS-polyacrylamide gel electrophoresis stained with silver nitrate.

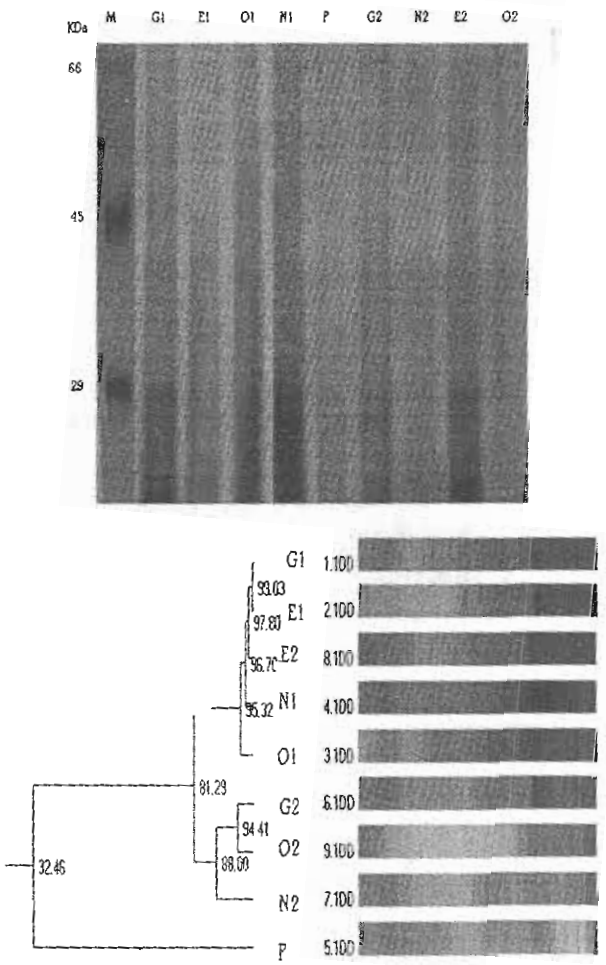

Fig. (2) :Protein profile analysis and dendrogram of grape plants infected with *Meloidogyne* incognita using SDS-polyacrylamide gel electrophoresis stained with silver nitrate.

REFERENCES

Alvarez-Castellanos P.P.; C.D. Bishop and M.J .Pascual- Villalobos , 2001. Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* 57, 99–102.

Bradford, M. 1976 "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" *Anal. Biochem.* 72:248-254.

Chapman, H. D. and F, Pratt (1981) Method of analysis for soil. Plant and Water Calif.Univ.USA.

Chatterjee A, Sukul NC, Laskar S, Ghoshma Jumdar S, 1982. Nematicidal principles from two species of Lamiaceae. *Journal of Nematology* 14, 118–20.

Chitwood DJ, 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40, 221–49.

Farahat,A.A.; A. A. ALsayed; H. S. EL-beltagi; N.M. Mahfoud(2012) Impact of Organic and Inorganic Fertilizers on Nematode Reproduction and Biochemical Alterations on Tomato. Notulae Scientia Biologicae 4(1):48-55

Gommers FJ, 1981. Biochemical interactions between nematodes and plants and the irrelevance to control: a review.*Helminthological Abstracts (B)* 50, 9–24.

Granger, R. L.; C. Plenchette and J.A.Fortin (1983). Effecr of vesicular-arbuscular (VA) endomycorrhizal fungus*(Glomus epigaeum)* on the growth and leaf mineral content of two apple clones propagated *in vitro. Canad.* J. *Pl. Sei.*, 63: 551-555.

Gupta, R. and Sharmaj, N.K. (1993). A study of the nematicidal activity of allicin—an active principle in garlic, *Allium sativum* L., against root-knot nematode, *Meloidogyne incognita.* International Journal of Pest Management Volume 39, Issue 4, pages 390-392

Helrich, K. (ED).(1990) Official methods of analysis of the association of official analytical chemists. 2volumes Arlington: AOAC, 1990. 15th ed. 2 volumes. 28 cm. Book number: 7027

Jones,J. B., Wolf, B. and Mills, H. A. (1991). *Plant analysis* handbook. 1. Methods of planl analysis and Interpretation. Athens, GA, USA, Micro-Macro Pub!., 213 p

Kirkpatrick,]. D. (1964). Interrelationships of plant nutrition, growth and plant parasitic nemarodes. *In* : Reuther, W. (Ed.). *Plant analysis and fertilizer problems. Volume IV,*American Institute of Biological Sciences,Washington DC, USA:189-225.

Kleinschmidt, G. D. and J. W. Gerdemann, (1972). Stunting of cirrus seedlings in fumigared nursery soils relared to the absence of endomycorrhizae. *Phytopathology,* 62 : 14471453

Knoblock, K.; Weis, N. and Wergant, R. 1989. Mechanism of antimicrobial activity of essential oils. 37th Ann. Cong. Med. Plant Res. Braunschweig, pp. 5-9.

Konstantopoulou, I.; Vassilopoulou, L.; Mawogantisi, Pidou P. and Scouras, G.

1994. Insecticidal effect of essential oils, A study of essential oils extracted from eleven Greek aromatic plants on *Drosophila auroria. Experientia,* 48: 616-619.

Nyczepir, A.P. ;T.G. Beckman and G.L. Reighard (2000): Field evaluation of 'GUARDIAN'™ PEACH ROOTSTOCK TO DIFFERENT ROOT-KNOT NEMATODE SPECIES ISHS Acta Horticulturae 713: VI International Peach Symposium

Oka ,Y.; S. Nacar ; E. Putievsky ; U. Ravid ; Z. Yaniv and Y. Spiegel (2000): Nematicidal activity of essential oils and their components against the root -knot nematode. Phytopathology, 90: 710–715.

Pérez MP, Pascual-Villalobos MJ, 1999. Efectos del aceite esencial de inflorescencias de *Chrysanthemum coronarium* L. en mosca blanca y plagas de almacén. *Investigación Agraria: Producción y Protección Vegetal* 14, 249-58

Pinochet,J.; A. Cambrvbi and C.Calvet, (1993 *a).* Effects of the root-lesion nematode *Pralylenchus vulnus* and the mycorrhizal fungus *Glomlls mosseae* on the growth of EMLA-26 apple rootstocks. *Mycorrhiza,* 4 : 79-83.

Ranganna, S (1979) Manual analysis of fruit and vegetable products. Data Magrow Hill Publishing Company Limited New Delhi 634p.

Sammons W. D.; L. D. Adams and E. E. Nishizawa (1981) Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels ELECTROPHORESIS Volume 2, Issue 3, pages 135–141, February 1981

Snedecor, G.W. and W.G. Cochran (1989): Statistical Methods. Eighth Ed. Iowa: Blackwell Publishing.

Soler-Serratosa A, Kokalis-Burelle N, Rodríguez-Kábana R, Weaver C.F. and King P.S., 1996. Allelochemicals for control of plant-parasitic nematodes. 1. *In vivo* nematicidal efficacy of thymol and thymol/benzaldehyde combinations. *Nematropica* 26, 57–71.

Tomas, R. L.; Shearel, R. W. and Z. R. Mayer (1967) Comparison of conventional and automated producers for nitrogen, phosphorus and potassium analysis of plant material using single digestion . Agroni. J. 59: 240-243.

تأثيرالمستخلص الطبيعى جارنيو على اصابة الخوخ والعنب بنيماتودا تعقد الجذور

عاطف معتمد حسين معتمد[1] و هناء سيدهم زوام[2]

١- معهد بحوث البساتين

٢- معهد بحوث امراض النباتات - مركزالبحوث الزراعية - جيزة

تمت هذه الدراسة تحت ظروف الصوبة لدراسة تأثيرالمستخلص الطبيعى جارنيو (مستخلص مخلوط مجموعة نباتات الاقحوان والنعناع والثوم والدمسيسة) بتركيزات ٠,٥%و٥%و١٠%على نوعى نيماتودا تعقد الجذور ميلوديجينا انكوجنيتا وميلوديجينا جافانيكا على كل من الخوخ ميت غمر والعنب صنف سبيريور وقد اظهرت النتائج انه بزيادة تركيز المستخلص يزداد الانخفاض فى اعداد الطور اليرقى الثانى فى التربة وكذلك اعداد العقد النيماتودية وأكياس البيض على الجذور كما قلت بوضوح اعداد الاطوار بداخل لجذور لكل من نوعى النيماتودا على كلا النباتين

وفى تجربة لمقارنة تأثير المستخلص بمبيدين نيماتوديين هما الايثوبروب والاوكساميل بتركيزات ٨ لتر / للفدان و ٨٠٠سم^{٣}/ للفدان للمستخلص و ٤٠ كجم /فدان و ٤كجم /فدان للايثوبروب و٨ لتر/فدان و ٨٠٠سم^{٣}/فدان للاوكساميل لنوع النيماتودا ميلوديجينا انكوجنيتا على الخوخ والعنب اظهرت النتائج نسبة انخفاض لأعداد اليرقات فى التربة تراوحت بين ٩٨٫٨% – ٩٨٫١%للتركيزات المرتفعة لكل من الايثوبروب والأوكساميل فى حين كانت نسبة الانخفاض ٨٨٫٩% للتركيز المرتفع من المستخلص وذلك مقارنة بالكنترول المعدى بالنيماتودا فقط وصارت نسبة الانخفاض فى اعداد العقد النيماتودية وأكياس البيض والاطوار بداخل الجذور حول نفس المعدل تقريباً وذلك عند معاملة الخوخ ميت غمر

أما فى حالة صنف العنب سبيريور فقد انخفضت أعداد اليرقات فى التربة الى ٨٣٫٣% و٧٦٫٧%و٥٨٫٣% لكل من الايثوبروب والأوكساميل والمستخلص جارنيو على الترتيب فى التركيزات المرتفعة وتفوق الأوكساميل على الايثوبروب والجارنيو فى التأثير على الاطوار بداخل الجذور حيث كانت نسبة الانخفاض ٨٥٫١%و٨١٫٠٦%و٧٧٫٢% للمركبات الثلاثة على الترتيب مقارنة بالكنترول المعدى بالنيماتودا فقط

كما أوضحت القياسات النباتية تأثير المركبات الثلاثة المحفز للنمو سواء للخوخ او العنب كما زاد عدد النموات الحديثة فى الخوخ المعامل بالمستخلص النباتى الطبيعى عن المبيدات النيماتودية مقارنة بالكنترول المعدى بالنيماتودا فقط كما زادت النسبة المئوية لتركيزات العناصر الكبرى والمتوسطة وكذلك التركيزات بالجزء فى المليون للعناصر الصغرى سواء فى الأوراق او الجذور لكلا النوعين النباتيين

كذلك اوضح التفريد الكهربى للبروتين المستخلص من نباتات الخوخ والعنب المصابة بنيماتودا تعقد الجذور والمعاملة بالإيثوبروب والأوكساميل والجارنيو بتركيزين ان هناك اختلافات بيوكيميائية تظهر من خلال عدد الباندات وكذلك كثافتها باختلاف المعاملة والتركيز .

قام بتحكيم البحث

أ.د / عبد البديع عبد الحميد غانم	**كلية الزراعة – جامعة المنصورة**
أ.د / محمود حسن الحموى	**مركز البحوث الزراعية**