Formulation and Nematicidal Efficiency of Some Alternative Pesticides against *Meloidogyne spp.*

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ABSTRACT

Citric acid was prepared as soluble powder formulation SP (C_3). The prepared formulation showed stability and passed successfully all phyisco chemical properties of soluble powder formulation. The nematicidal efficiency of citric acid SP 90 % (C_3) was compared with citric acid Sp 90 % (C_1) that prepared by EL-kady (2008), Salicylic acid EC 10 % (SA) that prepared by Abd-Alla (2011), nemanol (S) SL 10 %(NS) and commercial nematicide methomyl 90 % SP (ME) as reference.

Generally all tested compounds showed nematicidal activity of *Meloiidogyne spp.* (Second stage larvae and egg – masses). Salicylic acid EC 10 % showed the highest effect against second stage larvae followed by Methomyl, (C_1), (C_3) and nemanol S respectively. The EC_{50} values after 96 hours were 6.6, 12.5, 13.7, 39.96 and 46.8 ppm respectively, whereas the descending order of the same compounds according to their ET_{50} values at 50 ppm concentration were (SA), (C_1), (NS), (C_3). The ET_{50} values were 19, 49.9, 78.1 and 91 hours. From another point of view, except methomyl, egg–masses were more tolerant to the tested compounds than larvae. The descending order of tested compounds against egg–masses after seven days from treatment were methomyl, (SA),(C_1),(C_3) and (NS) their EC_{50} were 0.17, 151.4, 199.5, 316.2 and 1023 ppm respectively . Also the nematicidal effect of all tested compounds except (SA) increased as a result to increase in exposure periods.

Although (C_1) and (C_3) contain the same active ingredient they recorded different effect against second stage larvae and egg-masses.

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INTRODUCTION

The continuing world population pressure ensures the need to maximize the average yields of most major crops. Root-Knot nematodes. *Meloidogyne spp.*, are economically important plant pathogens and distributed world wide. They are obligate parasites and parasitize, thousands of different plants species including monocotyledons, dicotyledons, and herbaceous and woody plant. Species of *Meloidogyne* are pests of major food crops, vegetables, fruit and ornamental plants grown in tropical, subtropical, and temperate climates. They reduce the yields as well as the quality of product (Nickle, 1991 and Ashoub and Amara, 2010). In Egypt, Root-Knot nematodes. *Meloidogyne spp*.are becoming a real threat to almost all vegetable crops and they have been considered as limiting factors in the crop production (Ibrahim, 2011). Plant parasitic nematodes are controlled by cultural practices, chemical nematicides and the growing resistant cultivars. However nematicides use is slated for reduction to environmental problems, human and animal health concerns (xaki et al, 1982). Therefore alternative nematode control methods or less toxic nematicides need to be developed (Noling and Becker, 1994).

Salicylic acid (SA) is known to regulate both basal and R.gene-mediated defense response against pathogens and insects (Kim et al, 2008) EL-Kady. (2008) prepared citric acid as soluble powder SP formulation and tested it against second stage juveniles of *M.incognita* under laboratory conditions. The highest effect of the prepared formulation was noticed after 96 hours. On the other hand SP formulation was found to be more effective than its active ingredient by 88 %.

The successful use of an active ingredient depends on its correct formulation that can be applied for crop protection with safety to animal life and to the environment. In general formulation plays an important role in spreading an active ingredient over a very large area. Also it facilitates penetration of the active ingredient to reach its target and achieve its action (EL-kady et al, 2010).

The main aim of this work is to prepare citric acid in a SP formulation (C_3), and comparing the nematicidal efficiency of both soluble powder formulations 90 % (C_1,C_3), salicylic acid 10 % EC, Nemanol S 10 % SL and the commercial formulation methomyl 90 % SP as a reference.

MATERIALS AND METHODS

A. Tested chemicals

a. Citric acid (2-hydroxy propane 1,2,3 tricarboxylic acid-molecular weight 192.193 g/mol., that was supplied by EL-Gomhoria Co., Cairo, Egypt

b. Surface active agent, anionic surfactant, sodium lauryl sulphate (sls).

c. Citric acid 90 % SP (C_1) prepared according to the method described by EL-kady (2008).

d. Salicylic acid 10 % EC prepared according to the method described by Abd Alla (2011).

e. Nemanol S 10 % SL supplied by Amer fatoh abd elkany abo satot, this compound is recorded in the Egyptian scientific research academy under number 1719/2011 as preparatory step to get petancy .

f. The physical pattern of nemanol S SL formulation was measured under laboratory conditions, as follow:

1- Free acidity or alkalinity was measured according to WHO specification (1979)

2-PH was measured using Schoott Gerate PH- meter

3- Electrical conductivity and salinity measured by using water Quality Instrument YSI model 33 S.C.T meter m mohs is the unit of electrical conductivity measurement

4- Refractive index was measured according to ASTM D – 1218.

5- Viscosity was measured according to ASTM D – 2196.

6- Density was measured according to CIPAC MT3.

7- Specific gravity was measured according to CIPAC MT 3.2.

8- Surface tension was measured according to ASTM D- 1331.

Table (1): physico–chemical properties of Nemanol S SL Formulation

Free alkalinity as NaOH	PH	Conductivity M mhos	Salinity %	Refractive Index	Viscosity cm poise	Surface tension dyne/cm	Density g/cm^3	Specific gravity
0.09	5.94	39.9	25.3	1.356	9.9	40.5	1.054	1.0558

g. Methomyl (Lannate) 90 % SP (S.methyl carbamoyloxy) thio a cetimidate

B. Phsico-chemical properties of formulation constituents.

a. Surfactant.

1- Hydrophilic- Lipophilic balance (HLB):-

The solubility of surfactant in water is considered as approximate guide to its hydrophilic- lipophilic balance (HLB) (Lynch and Griffin, 1974).

2- Critical micelle concentration (CMC):-

CMC is the concentration at which the surface tension of the solution does not decrease with further increase in concentration of surfactant. CMC of the tested surfactant was determined according to (Osipow, 1964).

3- Surface tension: It was measured by DU-Nouy tensiometer for solutions containing 0.5% (w/v) active ingredient.

4- Free acidity or alkalinity: It was determined according to the method of WHO specifications (1979).

b. Active ingredient

1- Free acidity or alkalinity: It was determined a according to WHO specifications (1979) as mentioned before.

2- Solubility: It was determined by measuring the volume of distilled water, acetone and xylene for complete solubility or miscibility of one gram of active ingredient at 20 ^{0}C (Nelson and Fiero, 1954). The solubility was calculated according to equation:

% solubility = w/v * 100 (where; w: active ingredient weight: volume of solvent required for complete solubility).

C. Preparation of citric acid soluble powder formulation 90 % (C_3):

This formulation was prepared by mixing active ingredient with surfactant in three forms (95 % + 5 %), (92.5 % + 7.5 %) and (90 % +10%) respectively.

D. Physico-chemical properties for the prepared soluble powder formulation:

The following physico-chemical properties were determined for the above three mixtures of the prepared formulation before and after heat storage at (54 ± 2 ^{0}c) for three days.

1-Surface tension: as mentioned before.

2-Solubility: as mentioned before.

3-Free acidity or alkalinity: as mentioned before.

E. Bioassay:

All tests were carried out on root-knot nematode *Meloidogyne spp* treub, 1855 and chit wood 1949 propagated in pure culture in central Agricultural pesticides laboratory (CAPL) .

1-Toxicity of the tested formulations on second stage larvae of *Melodogyne SPP*.

These set of experiments were conducted to evaluate the toxicity of six compounds namely citric acid SP 90 % (C_1) , citric acid SP 90 % (C_3), salicylic acid EC 10 % (SA) , nemanol S SL 10 % (NS) and methomyl SP 90 % on second stage larvae of *Melodogyne spp* under laboratory conditions.

A concentration of each tested formulation was prepared in water and each concentration was replicated three times, the final volume of replicate was 10 ml in clean glass vial of 20 ml capacity. Control treatment was done by using water (10 ml).

Larvae of *Melodogyne spp* needed for these tests were obtained by incubating egg-masses collected from pure culture in distilled water. Newly hatched larvae were collected by using micropipette. About 1000 newly hatched larvae were used for each replicate .Mortality counts were tallied after 24, 48, 72 and 96 hours from treatment.

2- Ovicidal action of the tested compounds on *Meloidogyne.spp.* Egg. Masses under laboratory conditions.

Egg-masses of *Melodogyne spp* were isolated from infected tomato roots obtained form available pure culture. Six concentrations from the tested compounds were prepared in water to establish the LCP line, and each concentration was replicated three times. The final volume of replicate after dilution was 10 ml. ten egg masses of almost equal size were placed in Petri dish (10 cm) with tested concentration. Newly hatched larvae were counted after seven days of treatment unhatchability percentages were calculated.

F. Statistical analysis.

Inhibition percentages were corrected using Abbott's formula (1925) and the concentration. Inhibition regression lines were drawn according to the method of Finney (1952).

RESULTS

A. Preparation of citric acid as soluble powder formulation.

1- physico - chemical properties of active ingredient

Table (2): Physico-chemical properties of citric acid as active ingredient

Solubility at $25C^0$ (W/V) in			Free acidity as H_2SO_4
Water	Acetone	Xylene	
100	11.1	In soluble	45.6

Table (2) showed that citric acid as active ingredient was not soluble in xylene where as it showed a slight solubility in acetone (11.1%).On contrast their percentage solubility in water was 100 %. On the other hand it was acidic; the percentage of their acidity was 45.6 % as H_2SO_4. According to FAO, WHO Meeting (2002) on pesticides specification, the type of formulation is limited by pesticide solubility and hydrolytic properties therefore soluble powder formulation is the suitable form for the tested material.

Table (3): physico-chemical properties of sodium lauryl sulphate (SLS) as surface active agent

Type	Solubility W/V %			HLB	CMC%	Free alkalinity as NaOH	Surface tension (dyne/cm)
	xylene	Acetone	water				
Anionic	N.s	N.S	27.8	>13	%8	0.48	34.2

N.S: means not soluble

2- Physico-chemical properties of surface active agent:

According to data presented in table (3) anionic surfactant (sls) showed acceptable solubility in water 27.8 % and not soluble in acetone and xylene, also it have HLB values more than 13 whereas CMC value was 8 % and possess a low surface tension 34.2 dyne/cm. Surfactants applied to soluble powder formulations will reduce the surface tension of droplets that spread over treated surface providing more coverage for toxicant by decreasing contact angle of spray on solid surface (EL-Sisi, 1985).

Table (4): Preparation and physico-chemical properties of soluble powder formulations

Active ingredient	% of wetting agent	Surface tension	Solubility	sedimentation
Citric acid	0.0	75	Soluble	Nil
	2.5	64.8	Soluble	Nil
	5.0	60	Soluble	Nil
	10.0	53.46	soluble	Nil

3- physico- chemical properties of spray solution for the locally prepared SP formulation:

In table (4) the mixture of active ingredient (citric acid) and surface active agent (sls) gave low surface tension when diluted to 0.5 % in water.

On the other hand surface tension, decreased as a result to increase in the concentration of surfactant. The lowest surface tension was recorded when active ingredient was mixed with 10 % surface active agent. Also all prepared SP formulations were completely soluble in water without any sedimentation, and this SP gave clear solution when added to water.

Table (5): Effect of heat storage on physico-chemical properties of local SP formulation

Compound	Before storage			Heat storage		
	Solubility	Sedimentation	Free acidity as H_2SO_4	Solubility	Sedimentation	Free acidity as H_2SO_4
Citric acid 90% SP (C3)	Soluble	Nil	49	Soluble	Nil	46.27

4- Effect of heat storage on physico-chemical properties of local SP formulation.

As shown in table (5) no considerable changes were recorded in solubility, sedimentation and free acidity of the prepared SP formulation before and after heat storage.

Table (6): Effect of tested compounds on *Meloidgyne spp*. Larvae under laboratory conditions

compound		24 hr	48 hr	72 hr	96 hr
Citric acid	EC 50 ppm	394	199.5	89.3	12.5
90 % SP (C1)	EC 90 ppm	1175.9	112.7	3252	4571
	slope	2.7	1.7	0.82	0.9
Citric acid	EC 50 ppm	151.3	125.3	11.5	39.96
90 % SP (C3)	EC 90 ppm	574.1	407	199.5	48.8
	slope	2.2	2.5	1.07	14.6
Nemanol S	EC 50 ppm	63.1	52	50.1	46.8
10 % SL	EC 90 ppm	208.9	120.2	120.2	88
	slope	2.5	3.9	3.25	4.7
Salicylic acid	EC 50 ppm	39.8	12.5	22.5	6.6
10 % EC	EC 90 ppm	126	173	125.9	100
	slope	2.5	1.15	1.7	1.1
Methomyl	EC 50 ppm	29.5	19.1	14.1	13.7
90 % SP	EC 90 ppm	79.4	61.9	24.9	23.8
	slope	2.8	2.5	5.2	5.3

The nematicidal effect of the tested compounds against *Meloidgyne spp*. was studied. The relationship between tested concentration and percentage of inhibition after 24, 48, 72 and 96 hours was calculated as regration correlation. The obtained data was tabulated in table (6) Depending on EC_{50} values at the end of the experiment after 96 hours. Salicylic acid 10 % EC was the most effective compound followed by citric acid (C_1) 90 % SP, methomyl 90 % SP, citric acid (C_3) 90 % SP and Nemanol S SL 10 %. EC_{50} values were 6.6, 12.5, 13.7, 39.96 and 46.8 ppm. From a nother point of view citric acid (C_1) 90 % SP and methomyl 90 % SP recorded the closest EC_{50} values (12.5 and 13.7) respectively.

On contrast citric acid 90 % SP (C_1) and 90 % SP (C_3) that possessed the same active ingredient recorded different EC_{50} values (12.5 and 39.96 pp) respectively. According to slope values of the tested compounds after 96 hours from treatment. Citric acid 90 % sp (C_3) recorded the sharpest LCP line 14.6 whereas citric acid (C_1) showed the flattest one the descending order of tested compounds depending on slope values was citric acid (C_3) , methomyl, salicylic acid, nemanol S and citric acid (C_1). Generally except salicylic acid 10 % EC nematicidal effect of all tested compounds increased as a result to the increase in exposure periods.

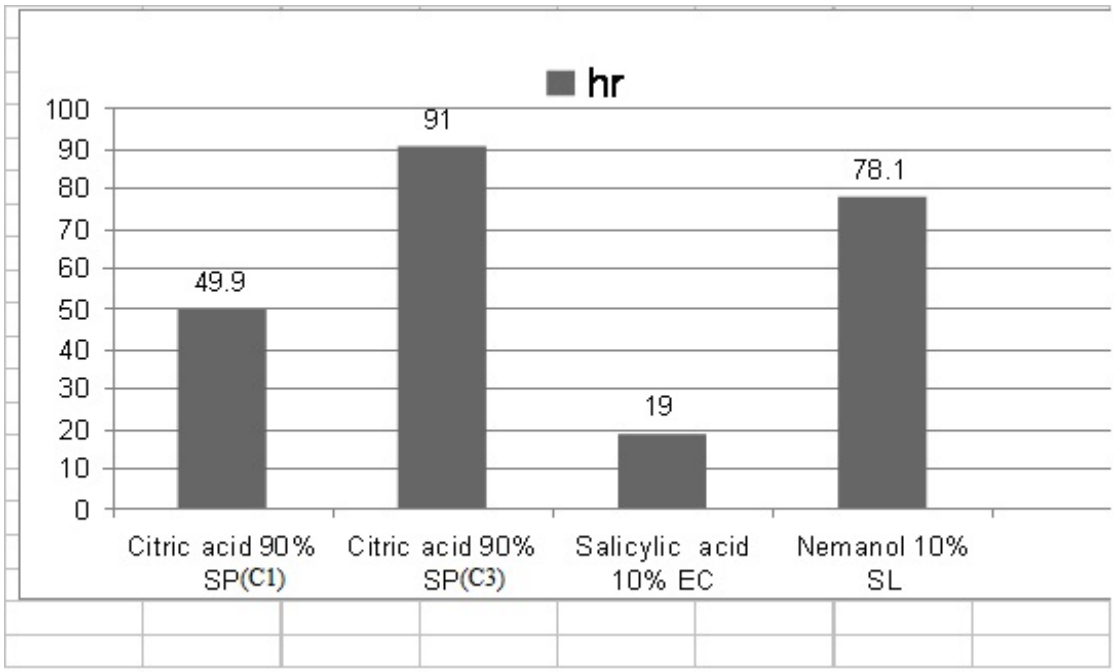

Fig. (1): the required time for each compound to inhibit second stage larva of *Meloidgyne spp* at 50 ppm under laboratory conditions.

The relationship between time and percentage inhibition was calculated as LTP line and graphically illustrated as histogram fig. (1) summarized results showed that the lowest ET_{50} values were recorded in case of salicylic acid 10 % (19 hr). On contrast the highest one was recorded in case of citric acid 90 % (C_3) (91 hr) whereas the ET_{50} of nemanol S 10 % SL and citric acid 90 % SP (C_1) showed a moderate values. On the other hand the ET_{50} value of methomyl did not calculate because their percentage inhibitions at 24 hours to 96 hours were between 84 to 99.8.

Table (7): Effect of tested compounds against *Meloidgyne spp* Egg hatching after one week from treatment

Concentration ppm	Citric acid 90% sp(C1)	Citric acid 90% sp(C3)	Nenanol S10% SL	Saliycacid EC 10%	Mothomyl 90% SP
2	-	-	-	-	37.6
4	-	-	-	-	44.7
8	-	-	-	-	52.6
16	-	-	-	-	59.3
32	-	-	-	-	66.9
125	38.3	15.9	-	35.6	-
150	-	-	-	54.8	-
250	56.o	39.0	-	86.5	-
500	72.6	68.1	-	99.5	-
900	-	-	20.4	-	-
1000	85.4	88.9	42.1	-	-
1300	-	-	87.7	-	-
1600	-	-	99.38	-	-
EC50 ppm	199.5	316.2	1023.3	151.4	0.17
EC 90 ppm	1258.9	1056.8	1258.9	251.2	0.37
Slope	1.5	2.5	13.6	4.9	6.6

-: none calculated

The effect of tested compounds on hatching of *Meloidgyne spp.* egg masses under laboratory conditions after one week from treatment table (7) regarding to EC_{50} values, methomyl was the most effective compound followed by salicylic acid 10 % EC, citric acid SP 90 % (C_3), citric acid SP 90 % (C_1) and nemanol S 10 % SL EC_{50} values were 0.17, 199.5, 316.2 and 1023.3 ppm respectively. On the other hand all tested compounds

recorded sharpe slope values. Their descending order were Nemanol S 10 % SL, metlomyl 90 % SP, salicylic acid 10 % EC, citric acid 90 % SP (C_3) and citric acid 90 % SP (C_1).

Table (8): physico-chemical properties of tested formulation spray solutions.

Formulation	Viscosity cm poise	Surface tension dyn/cm	Free acidity or alkalinity	conductivity	Foam cm	PH	TDS	Salinity
Nemanol S 10% SL	9.528	49.59	0.424	2340	-	9.18	1170	1.2
Methomyl 90% SP	8.940	66.57	0.536	700	0.1	8.08	335	0.3
Citric acid 90% SP C1	8.846	51.10	3.92	1531	3.O	2.8	739	0.8
Citric acid 90% SP C3	9.251	44.59	4.14	1573	1.1	2.44	771	0.8
Salycilic acid 10%EC	10.643	35.47	2.79	1573	-	3.17	751	0.8

Data in table (8) clearly showed that, the spray solution of salicylic acid 10 % EC showed the highest viscosity value 10.643 followed by nemanol S 10 % SL citric acid 90 % SP (C_3), , methomyl 90 % SP and citric acid 90 % SP (C_1) by 9.528, 9.251, 8.940 and 8.846 respectively. Also salicylic acid spray solution recorded the lowest surface tension 35.47 followed by citric acid (C_3), citric acid (C_1), nemanol S and methomyl by 35.47, 44.59, 49.59, 51.10 and 66.57 dyne/cm respectively.

On the other hand spray solution of salicylic acid (C_1) and (C_3) was acidic whereas nemanol S was alkaline. From another point of view salicylic acid (C_1) and (C_3) recorded very close conductivity values 1537, 1531 and 1573 m mhos respectively. Whereas the spray solution of nemanol S recorded the highest conductivity value 2340 m mhos and methomyl recorded the lowest one 700 m mhos.

According to the PH values salicylic acid, (C_1) and (C_3) recorded PH values lower than 7, on contrast nemanol S and methomyl recorded PH values higher than 7.

From the above results it could by concluded that the physico-chemical properties of spray solutions were compatible with the biological effect of these compounds for example, salicylic acid recorded the highest effect against second stage larva and the second order against egg masses, the highest viscosity value, the lowest surface tension, the highest conductivity and the lowest PH values. Increasing electrical conductivity and decreasing PH values of insecticide spray solution would lead to deionization of insecticide, increase the deposit and penetration in the tested surface, which will result in an increase in the tested surface, which will result in an increase in the insecticidal efficiency (Tawfik and EL-SISI, 1987 and El-attal et al 1984). The decrease of surface tension of pesticide spray solution give a prediction of increasing wettability and spreading on the treated surface and then increasing pesticide efficiency (Osipow,1964), increasing viscosity of spray solution cause reduction drift, increasing the retention sticking and insecticide efficiency (Richardson, 1974).

DISCUSSION

The prepared formulation citric acid 90 % SP (C_3) showed stability and passed successfully all physico-chemical properties for soluble powder formulations. This formulation completely soluble in water without any sedimentation and gave clear solution when added to water. Also no changes were noticed between the physic-chemical properties of these formulations before and after heat storage. According to FAO, WHO Meeting, (2002) soluble powder formulations, SP are the simplest formulation, Anonymous (1973) reported that no precipitation or flocculation should be occurred when SP diluted with water at field application rate.

Generally all tested compounds showed nematicidal activity against different stages of *Meloidogyne spp.* (Second stage larvae and egg masses). These observations could be discussed depending on the mode of action of these compounds as follow : salicylic acid recorded the highest effect against second stage larvae and second order against egg-mass also it need a lowest time to effect on 50 % from tested population at 50 ppm definite concentration (ET_{50} values was 19 hours) salicylic acid was known as inducer of plant

resistance to some pathogens (kessman et al 1994 a,b).Also it has been thought to play an important role in systemic acquired resistance because exogenous salicylic acid induces this resistance and accumulated in pathogen infected tissue (Malamy et al, 1990).

Citric acid 90 % SP (C_1) recorded the second order against second stage larvae (ET_{50} 49 hours). Whereas citric acid 90 % SP (C_3) showed nematicidal efficiency lower than (C_1) against second stage larvae (ET_{50} 78.1 hours) and lower then against egg masses. The mode of action of citric acid and their SP formulation was recorded by (EL-Kady, 2008) as stimulation in movement, but then gradually slows down, therefore nematode are partially paralyzed and the number of body undulations decreased, whereas abnormal stylet protrusions are made. All these reactions are concentration dependent. In high concentrations nematode become paralyzed and seems to be dead. Finally all body was as mummification.

Methomyl showed little lower activity against second stage larvae in comparison with (C_1) and (C_3) but it showed the highest inhibition against egg-masses .Methomyl was known as carbamate pesticide. These class of compounds acts as nerve poison. It works as a competitive inhibitors of a acetylcholine esterase, an enzyme essential in the breakdown of acetylcholine which is a transmitter for signals to muscular system, that results in convulsions, paralysis and finally death (Bunt. ; 1975). The same symptoms were noticed on second stage larvae in our experiment as a result to treatment with methomyl.

From another point of view except methomyl egg-masses were more tolerant than larvae. The above indication may be due to the morphological and behavior characteristics of each stage. Egg stage was sedentary stage and surrounded by egg shell and it exists in a gelatinous matrix which forms an egg-masses. Egg shell and gelatinous matrix may be act as defensive barriers, against tested compounds. On contrast larval stage is a mobility stage and covered with permeable cuticle. These characteristics may increase uptake of tested compounds from their solutions.

On the other hand except salicylic acid 10 % EC nematicidal effect of the tested compounds increased as a result to increase in exposure periods. these results were in agreement with data recorded by (EL- kady: 2008) who reported that the efficiency of citric acid and Alum 90 % SP against *Meloidogyne incognite* larvae under laboratory

conditions was noticed as a slight inhibition after 48 hours and increased to give the highest efficiency after 96 hours .

Although citric acid 90 % SP (C_1) and citric acid 90 % SP (C_3) contains the same active ingredient they recorded different effect against second stage larvae and egg-masses that could be discussed from the forgoing results depending on the type of additives that were used in preparation of each formulation. These results were in agreement with the data obtained by (EL-Kady, 2010).

Depending on ET_{50} values at 50 ppm concentration the descending order of the tested compounds was methomyl, salicylic acid, citric acid 90 % (SP) (C_1), nemanol S 10 % SL and citric acid 90 % SP (C_3). This order may be due to the mode of action of tested active ingredients, accumulation degree in organism tissues, type of formulation and nematode defense systems in breaking down compounds under investigation.

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