Formulating Salicylic Acid as (Emulsifiable Concentrate, Wettable Granule) and Study Their Nematicidal Efficiency on Root-Knot Nematode.

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Abstract: Salicylic acid was prepared in two different formulation types, emulsifiable concentrate (EC) and wettable granules (WG). Both formulations passed successfully all the tests specified by different organizations related to pesticides like WHO and FAO. The nematicidal efficiency of both formulations was evaluated against root-knot nematode on egg plant under green house conditions. Also their effect on growth parameters of egg plant was studied. Generally no significant variations were found between both formulations on all pathogenicity parameters of root-knot nematode and growth parameters of egg plant that inoculated by second stage larvae of root-knot nematode before treatment by 24 hours and after treatment by week. Most tested concentrations of both formulations improved growth parameters of egg plant compared to control. On the other hand the tested concentrations of (WG) formulation before treatment and both formulations after treatment decreased the number of galls / gram root compared to control but this decreasing was not significant in case of first treatment (before infection by 24 h) and highly significant in second treatment (after treatment by week) . So egg masses / root and second stage larvae / 240 gram soil disappeared with all concentrations of both formulations under the two methods of treatment.

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1. Introduction:

The Root-Knot nematodes *Meloidogyne Spp.* are obligate parasites and very damaging plant pests limiting agricultural productivity. Most cultivated plant species are susceptible to root-knot nematode infection. In Egypt previous nematological studies revealed that, such nematodes are considered a limiting factor in vegetable crops production particularly in localities with high sandy soil (new reclaimed soil) (Kassab, 1980; Sasser and Carter; 1985) Control of root- knot nematode is difficult to accomplish by cultural methods such as follow, flooding, corporation, time of planting…etc because of their extensive host range and nematodes ability to survive in diverse conditions. Therefore the most commonly used methods for control are soil application with nematicides or the use of resistant cultivars. Radwan-Neveen, (2006) although chemical nematicides are primary means for the control of plant parasitic nematodes, but their residual effect and health hazardous nature led to a total ban or restricts (Mashewari and Anwar, 1990).

In literature salicylic acid (SA) has been reported as endogenous signal for the activation of certain plant defense responses including PR gene expression and enhanced resistance to pathogens (Greenberg *et al.,* 1994) . Salicylic acid (SA) and jasmonic acid (JA) are well known to regulate both basal and R.gene-mediated defense responses against pathogens and insects (Kim *et al.,* 2008) emulsifiable concentrate (EC 10 %) of salicylic acid showed nematicidal effect against second stage larvae and egg-masses of root-knot nematode *Meloidogyne spp*. Under laboratory conditions, their EC_{50} value was 6.6 ppm in case of second stage larvae and 0.17 ppm in case of egg-masses whereas their ET_{50} values at 50 ppm was 19 hours (Abd-alla *et al.,* 2013).

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 environment. In general formulation plays an important role in spreading an active ingredient over a very large area. Also it facilitates penetration of active ingredient to reach its target and achieve its action (El-Kady *et al.,* 2010).

The physical properties of water-dispersible granules are sensitive to the formulation ingredients used and the method of manufacture. Clearly this would be expected with different types of granules. However the properties are also sensitive to the processing and conditions used (Gordon A. Bell, 1990).

The main aim of this work is to prepare salicylic acid in water-dispersible granule (WG) and comparing their nematicidal efficiency with salicylic acid emulsifiable concentrate EC 10 % that prepared by (Abd-alla, 2011) under green house conditions.

2. Material and Methods:

A. Tested chemicals

1) Salicylic acid (2-hydroxy benzoic acid $C_7H_6O_3$ (molecular weight 138.12 g/mol.) that was supplied by EL-Gomhoria Co., Cairo, Egypt.

2) Surface active agents: Tween 20, Tween 80, sodium lauryl sulphate (sls), poly ethylene glycole 600 diolate (PEG 600 diolate), that was supplied by EL-Gomhoria Co., Cairo, Egypt .

3) Carries: Asownly clay supplied by Sinaa Company for manganese.

B. The physico-chemical properties of the basic formulation ingredients:

1) Active ingredient (Salicylic acid):-

a) Solubility: It was determined by measuring the volume of distilled water, acetone, DMF 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 the following equation:

$$\% \text{ solubility} = w/v * 100$$

(Where; w: active ingredient weight V: volume of solvent required for complete solubility).

b) Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

c) Melting Point: It was determined by using electro thermal melting point apparatus 9200A.

2) Surface active agents:

a) Solubility: It was determined as mentioned before.

b) Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

c) Hydrophilic-Lipophilic balance (HLB):

The solubility of surfactant in water was considered as approximate guide to their HLB and usefulness (Lynch and Griffin, 1974).

d) Critical micelle concentration (CMC):

The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentrations, (CMC) of the tested surfactants was determined according to Osipow (1964).

e) Surface tension: It was determined by using Cole-Parmer surface tension 21 for solutions containing 0.5 % (W/V) surfactant according to ASTM- 1331 (2001).

3- Carriers:

a) Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

b) Wettability: It was determined according to CIPAC MT 53.3 (2002).

3- Bulk density: This property was determined according to CIPAC MT 33 (2002)

c) Surface activity (PKa) : Hammet indicators were used to estimate surface activity of the crude mineral carriers according to Malina *et al.,* (1956) phenyl azo diphenyl dye was used to determine the Pka at 1.5 level (yellow = safe and purple = unsafe and active) and dimethyl amine azobenzene dye for Pka at 3.3 level (yellow = safe and red = unsafe and active).

C- Preparation of salicylic acid as water dispersible granules (WG):

This type of formulation is suitable for the active ingredients that did not soluble in water or xylene; several trials were carried out as follow:

Different weights from active ingredient were added to other different weights from carrier then mixed together to make a homogenous powder and wetting or dispersing agent single or mixed together with different percentages was added to the mixtures and stirred well using glass rod to ensure homogeneity. After drying, the mixtures were sieving through 590 my sieve to ensure that all particles have the same size. Suspensibilty test was carried out according to CIPAC (MT 185) for all prepared formulations to judge on the success of formulation.

D- Determination of the physico-chemical properties of the local formulated dispersible granules:

1- Flowability: It was determined according to CIPAC MT 172.
2- Dustiness: It was determined according to CIPAC MT 171.
3- Suspensibility: It was determined according to CIPAC MT 184.
4- Wet sieve test: It was determined according to CIPAC MT 185.
5- Wattability: It was determined according to CIPAC MT 53.3
6- Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

E- Determination the physico-chemical properties of the spray solution of the local formulation at the field dilution rate:

1- Surface tension: It was determined by using Cole-Parmer surface tensiometer 21, where dyne/cm is the unit of surface tension measurements.

2- Viscosity: It was determined by using Brookfield viscometer model DV Π + Pro, where centipoise is the unit of viscosity.

3- PH value: It was determined by using Cole-Parmer pH Conductivity meter 1484-44.

4- Electrical conductivity: It was determined by using Cole-Parmer pH Conductivity meter 1484-44, where μ mohs is the unit of electrical conductivity measurement.

F- Bioassay:

42 Plastic cups each of (6.5) cm in diameter were filled with (240 grams) sterilized sandy soil.

One eggplant seedling at the two leaf stage was transplanted to each cup. The cups were divided to two groups each one consisted of 21 cup. The first group was inoculated before treatment by 24 hours whereas the second group was inoculated after treatment by weak. Each seedling was inoculated with 1000 newly hatched larvae of *Meloidogyne Spp*. The nematode suspension was pipette into holes around the base of each seedling. Inoculations were obtained from available pure culture. Water dispersible granules WG 65 % and emulsifiable concentrate EC 10 % of salicylic acid was used at 200, 800 and 1600 ppm in 5 ml as soil treatment. Each treatment was replicated three times. Three cups were saved without treatment as control for each group. The cups were irrigated regularly as needed. After seven weeks from treatment, the number of galls / gram root, number of egg-masses/root system, means of hatchability/egg- masses and the population density of second stage larvae in 240 gram soil were determined. Shoot length, fresh weight and dry weight of the plants were also determined.

G-Statistical analysis:

The results obtained were subjected to statistical analysis according to Snedecor (1966) and the least significant differences between treatments were calculated.

3. Results and Discussion

1-Physico-chemical properties for the constituents of local formulation:

A) Physico-chemical properties of salicylic acid as active ingredient**:**

Data in table (1) indicated that salicylic acid was insoluble in water and xylene but soluble in acetone and DMF, the solubility percentage was 60 and 100 respectively. This result gives a prediction that salicylic acid could be prepared as wettable granule formulation. On the other hand it has a weak acidic property, so it requires a slight acidic to slightly alkaline adjuvant when it is prepared as formulation to avoid increasing acidity or alkalinity that will cause phyotoxicity to treated plant. Also the melting point range of salicylic acid was moderate 163-165ºC.

Table (1): physico-chemical properties of salicylic acid as an active ingredient:

Solubility % (W / V)				Acidity % as H_2SO_4	Alkalinity% as NaOH	Melting point ^{0}C
Water	Acetone	DMF	Xylene			
Insoluble	60	100	Insoluble	0.196	__	163-165

B) Physico-chemical properties of surface active agents:

The physico-chemical properties for four surface active agents namely tween 80, tween 20, sodium lauryl sulfate and polyethylene glycol 600 diolate was studied to determine the suitable one to prepare salicylic acid as wettable granule. Table (2) showed that all tested surface active agents showed solubility in the tested solvents except sodium lauryl sulfate with acetone and xylene. Tween 80 and tween 20 recorded complete solubility in acetone, DMF and xylene. On the other hand they recorded low to moderate solubility in water 20 and 50 % respectively. The lowest solubility percentages were noticed in case of sodium lauryl sulfate with water and DMF 5 and 2.3 % respectively. The solubility percentages of polyethylene glycol 600 diolate were 50, 33, 20.5 and 16.7 in xylene, water, DMF and acetone respectively.

According to HLB values tween 20, tween 80 and sodium lauryl sulfate were considered as detergents, their HLB values were greater than 13 Whereas poly ethylene glycol 600 diolate was considered as emulsifying agent (HLB value 8-10).

The descending order of the tested surface active agents depending on critical micelle concentration (CMC) value was polyethylene glycol 600 diolate, tween 80, sodium lauryl sulfate and tween 20 where their (CMC) values were 0.9, 0.49, 0.3 and 0.2 respectively. (CMC) value was determined to make sure that the applied concentration is less than (CMC) to avoid any micelle formation.

According to free acidity or alkalinity value, all tested surface active agents showed weak acidic nature except sodium lauryl sulfate that showed a weakly alkaline nature. The free acidity or alkalinity of surface active agent must be compatible with the free acidity or alkalinity of the active ingredient that required acidic medium for maximum chemical stability. On the other hand all tested surface active agents decreased the surface tension of water from 72 to 39.2, 36, 34.2 and 35.8 in case of tween 80, tween 20, sodium lauryl sulfate and P.E.G 600 diolate respectively.

Table (2): physico-chemical properties of the tested surface agents:

Surface active agents	Solubility % (W / V)				HLB	CMC	Acidity as H_2SO_4	Alkalinity as NaOH	Surface tension
	Water	Acetone	DMF	Xylene					
Tween 80	20	100	100	100	> 13	0.5	0.49	__	39.2
Tween 20	50	100	100	100	> 13	0.2	0.196	__	36
Sodium Lauyrl sulfate	5	N.S	2.3	N.S	> 13	0.3	__	0.48	34.2
P.E.G Do 600	33.3	16.7	20.5	50	8-10	0.9	0.196	_	35.8

P.E.G Do 600: poly ethylene glycol 600 diolate. N.S: Non soluble

C) Physico-chemical properties of the tested carriers:

As shown in table (3) Asoanly clay was acidic and it has the ability for wetting in water whereas their density before compacting was 1.0243 g/cm^3 and after compacting was 1.348g/cm^3.

Table (3): physico-chemical properties of asoanly clay as tested carrier:

Free acidity as H_2SO_4	Free alkalinity as NaOH	Wettability	Bulk density g/cm^3		Pka
			Befor compacting	After compacting	
0.098	__	16.3	1.024312	1.3477789	>1.5 < 3.5

1-Local formulation of salicylic acid as 65 % WG:

Many trials were conducted to formulate salicylic acid as 65 % WG by using asoanly clay as carrier with two non-ionic surfactants (poly ethylene glycol 600 diolate and tween 80) and anionic surfactant (sodium layrul sulphate). Anionic and non anionic surfactants were mixed in different ratios by weight/weight, and then each mixture passed through sieve 965 mesh to take granules form, then the suspensibility test was carried out in soft and hard water to determine the successful WGs. One trial only from 35 passed successfully the suspensibility test before and after accelerated storage.

According to data in table (4) no big changes were found in flowability, suspensibility, free acidity of (SA) local formulation (35) before and after accelerated storage for three days. On the other hand changes in the values of dustiness %, weit sieve test and wettability / second after storage were acceptable.

Table (4): The physico-chemical properties of local formulations of salicylic acid as wettable granules before and after hot storage:

Local formulation Salicylic acid (35)	Before storage	Flowability	Dustiness % through		Suspensibility		Weit sieve test	Wettabilitty/ second	Free acidity as H_2SO_4
			60* mesh	100* mesh	Soft water	Hard water			
		pass	0.576	0.016	64.25	61.52	3.182%	1.18	2.058
	After storage	pass	0.286	0.024	66.2	62.8	1.192%	2.28	2.204

* means : Sieve pores.

The physico-chemical properties of spray solution of local formulation of salicylic acid as 65 % wettable granules at field dilution rate:

As found in table (5) local formulation of salicylic acid 65 % WG possessed low surface tension values, high viscosity values , high conductivity values and low PH values (Osipow, 1964) indicating that, the decrease of surface tension of pesticide spray solution give a prediction of increasing wettability and spreading on the treated surface then increasing pesticidal efficiency. Richardson (1974) stated that, increasing viscosity of spray solution cause reduction drift and increasing the retention sticking and insecticidal efficiency. (Twifik and El-Sisi, 1987) reported that, the increase of electrical conductivity and the decrease in PH values of insecticidal spray solution would lead to deionization of insecticides and increase its deposit and penetration in the tested surface with a consequence increase the insecticidal efficiency.

Table (5): The physico-chemical properties of spray solution of salicylic acid WG 65 % at the field dilution rate:

Local formulation	Surface tension			Viscosity cm / poise			Conductivity			pH value		
	Nile water	Hard water	Soft water	Nile water	Hard water	Soft water	Nile water	Hard water	Soft water	Nile water	Hard water	Soft water
Sal 35	40	39.04	42.63	9.3525	8.212	8.357	817	1048	886	2.72	2.63	2.6

Biological study:

Evaluation of the local formulations of salicylic acid (EC and WG) against root-knot nematodes on egg plant under green house conditions:

Two experiments were conducted to evaluate the efficiency of local formulations (EC and WG) of salicylic acid under green house conditions. The first one was inoculated with both formulations before treatment by 24 hours**,** whereas the second was inoculated after treatment by week with the same formulations. The effect of both tested formulations on growth parameters of egg plant and the pathogenicity of root-knot nematode were studied tables (6-9)

1- Evaluation of the local formulations of salicylic acid (EC and WG) on egg plant growth parameters under green house conditions:

1.1 In case of inoculation before treatment by 24 hours:

According to data in table (6) no significant variations were found between both tested formulations on all growth parameters of eggplant. Generally most tested concentrations of both formulations increased all growth parameters of egg plant compared with control whereas this notification was significant only in case of fresh weight of shoot and dry weight of shoot and root compared to control, the respective L.S.D values were 0.123 and 0.077. On the other hand there is a negative relationship between tested concentrations and the increase in fresh and dry weight of shoots and roots with EC formulation and fresh weight of shoot and root and dry weight of root with (WG) formulation.

Table (6): Effect of salicylic acid formulations on growth parameters of eggplant under greenhouse conditions on treatment before infection by 24 hours:

Formulation	Concentration	Length of		Fresh weight of		Dry weight of	
		Shoot±SD	Root±SD	Shoot±SD	Root±SD	Shoot±SD	Root±SD
WG	0	19.5±3.9	13±1.1	1.2±0.5	1.20±0.3	0.11±0.02	0.05±0.004
	200	19.6±3	14.9±2.3	2.4±1.1	2.40±0.6	0.40±0.1	0.15±0.09
	800	19.6±3.5	11.2±2.3	2.3±1.4	1.98±1.2	0.40±0.06	0.16±0.03
	1600	19.1±3.4	14.1±4.4	2.2±0.8	1.90±0.8	0.40±0.11	0.17±0.02
EC	0	19.5±3.9	13±1.1	1.2±0.5	1.20±0.3	0.11±0.02	0.05±0.004
	200	18.6±4.3	13.4±3.8	2.0±1.3	1.80±1.3	0.30±0.2	0.18±0.02
	800	17.3±2.6	10.6±2.2	1.8±0.6	1.70±0.3	0.22±0.09	0.14±0.07
	1600	18.2±4.5	14.8±0.8	1.6±1.1	1.30±0.6	0.17±0.06	0.10±0.01
L.S.D	Formulation	N.S	N.S	N.S	N.S	N.S	N.S
	Concentration	N.S	N.S	0.391**	N.S	0.123	0.077**
	Interaction	N.S	N.S	N.S	1.1	N.S	N.S

- : Data are means three replicates. *: Significant. **: Highly significant N.S: Not significant.

1.2 In case of inoculation after treatment by week:

Data in table (7) indicated that no significant differences were found between both tested formulations on growth parameters of egg plant. On the other hand most concentrations of both tested formulations improved growth parameters of egg plant compared to control. This improvement was highly significant in case of length of shoot and fresh weight of root. From another point of view the lowest growth parameters was recorded with high concentration 1600 ppm in case of (WG) formulation. In contrast the high increase in growth parameters was recorded with high tested concentration (1600 ppm) in case of EC formulation whereas the root showed the lowest growth in all parameters with the second concentration (800 ppm) with the same formulation.

Table (7): Effect of salicylic acid formulations on growth parameters of eggplant under greenhouse conditions on treatment after infection by week:

Formulation	Concentration ppm	Length of		Fresh weight of		Dry weight of	
		Shoot±SD	Root±SD	Shoot±SD	Root±SD	Shoot±SD	Root±SD
WG	0	17.2±4.8	16.2±3.6	1.04±0.3	1.10±0.2	0.096±0.08	0.060±0.03
	200	19.0±2.7	17.2±3.1	1.60±0.5	2.25±0.2	0.385±0.2	0.178±0.03
	800	19.1±3.2	19.1±2.4	1.60±0.3	1.79±0.2	0.247±0.09	0.149±0.04
	1600	16.5±3.0	15.6±1.2	1.44±0.7	1.67±0.98	0.142±0.13	0.115±0.08
EC	0	17.2±4.8	16.2±3.6	1.04±0.3	1.10±0.2	0.096±0.08	0.60±0.03
	200	19.5±2.1	17.1±2.1	1.40±0.2	1.83±0.06	0.175±0.14	0.162±0.04
	800	17.4±7.2	9.7±5.5	1.53±1.3	1.35±1.1	0.297±0.28	0.154±0.12
	1600	21.1±1.5	17.2±2.2	2.11±0.4	2.74±0.3	0.390±0.07	0.617±0.04
L.S.D	Formulation	N.S	N.S	N.S	N.S	N.S	N.S
	Concentration	1.3**	N.S	N.S	0.64**	N.S	N.S
	Interaction	N.S	N.S	N.S	N.S	N.S	N.S

- : Data are means three replicates. *: Significant. **: Highly significant. N.S: Not significant

2- Effect of local formulations of salicylic acid (EC and WG) on the pathogenicity of *Melodogyne Spp.* Infecting egg plant under green house conditions:

2.1 In case of inoculation before treatment by 24 hours:

Data in table (8) indicated that no significant differences were found between both formulations. Tested concentrations of (WG) formulations decreased the number of galls/gram root compared to control. The relationship between the tested concentrations and the decrease in gall formation was positive. In contrast the number of galls/gram root was increased upon control with 200 and 800 ppm in case of (EC) formulation. On the other hand no egg-masses were found on root as a result to the treatment with both formulations except with the second concentration (800 ppm). In case of (EC) formulation all tested concentrations of both formulations prevent significantly root-knot females from laying the egg-masses, so the number of egg-masses/root, means of hatchability / egg-masses and the number of larvae / 240 gram soil were zero.

Table (8): Effect of salicylic acid formulations on pathogenicity parameters on egg plant before infection by 24 hours.

Formulation	Concentration	No of gall/gram root	No of Egg-masses/root	Mean of hatchability Egg-mass	No of larvae/240 gram soil
WG	0	156.7	29.3	536.9	1266.7
	200	144.2	00.0	000.0	0000.0
	800	116.6	00.0	000.0	0000.0
	1600	18.7	00.0	000.0	0000.0
EC	0	156.7	29.3	536.9	1266.7
	200	170.9	0.00	000.0	0000.0
	800	166.7	2.30	00.0	0000.0
	1600	155.9	0.00	00.0	0000.0
L.S.D	Formulation	N.S	N.S	N.S	N.S
	Concentration	N.S	16**	386**	768.7**
	Interaction	73.6**	N.S	N.S	N.S

- : Data are means three replicates. *: Significant. **: Highly significant. N.S: Not significant

2.2 In case of inoculation after treatment by week;

Data in table (9) showed, no significant effect was found between both formulations on all pathogenicity parameters of root-knot nematode. On the other hand both formulations decreased significantly the number of galls/gram root compared to control. From another point of view a positive relationship was found between tested concentration of both formulations and the decrease in gall formation generally.

Also egg-masses were disappeared in all roots treated with both tested formulations with all tested concentrations compared with control. So, no second stage larvae were found in soil at the end of experiment. The effect of the tested concentration was highly significant in case of number of egg-masses / root and number of larvae / 240 grams soil.

According to the obtained results no significant effect was found between both formulations on all growth parameters of egg plant that inoculated by second stage larvae of root-knot nematodes before treatment by 24 hours and after treatment by week. Also the same indication was noticed on all pathogenicity parameters of root-knot nematodes under the same conditions.

Generally most tested concentrations of both formulations showed an increase in all growth parameters of egg plant compared to control with both treatments whereas this indication was highly significant in fresh weight of shoot and dry weight of shoot and root in case of first method of treatment (inoculation before 24 hours). In contrast the increase in growth parameters was highly significant in case of shoot length and fresh weight of shoot in case of second method of treatment (inoculation after treatment by week).

Nandi *et al.,* 2002 reported that salicylic acid enhances resistance in cowpea against *Meloidogyne incognita* and increased growth of inoculated plants in terms of shoot length, shoot weight and root as compared with inoculated unsprayed plants. Also (Osman *et al.,* 2012) reported that salicylic acid at concentration of 5000 ppm improved egg plant growth compared to the whole plant inoculated with root-knot nematode only.

Generally no significant differences were found between both tested formulations on pathogenicity parameters of root-knot nematode with the two methods of treatment on egg plant. Tested concentration of (WG) before treatment by 24 hours and both formulations after treatment by week decreased the number of galls/ gram root compared to control but this decreasing was not significant in case of first case and highly significant in the second one On the other hand egg-masses/root disappeared with all concentrations of both formulations under the two methods of treatment. So, the second stage larvae were disappeared from the soils of trials cup.

Nandi *et al.,* 2002 indicated that root gall number and number of eggs in roots of cowpea were significantly greater in inoculated untreated plants with salicylic acid.

Osman *et al.,* 2012 recorded that salicylic acid at the concentration of 5000 ppm reduced nematode parameters in another half of root compared to the whole plant inoculated with the root-knot nematode only.

Hedin *et al.,* 1995 showed that salicylic acid reduced egg content of *Meloidogyne incognita* on cotton plant roots.

The role of salicylic acid in decreasing the pathogenicity parameters of root-knot nematode was discussed by several reports depended on their effect as plant defense signal and has induced resistance in several plant species through activation of certain plant defense responses by expression of genes for pathogensis related protein (PR-1) and enhances resistance to pathogens (Yang *et al.,* 1997 and Nandi *et al.,* 2003).

Table (9): Effect of salicylic acid formulations on pathogenicity parameters of root-knot nematode on egg plant after infection by week:

Formulation	Concentration	No of gall/gram root	No of Egg-masses/root	Mean of hatchability Egg-mass	No of larvae/240 gram soil
WG	0	192.0	40.0	982.8	663.3
	200	127.9	00.00	000.0	000.0
	800	106.6	00.00	000.0	000.0
	1600	61.3	00.00	000.0	000.0
EC	0	192.0	40.0	982.8	663.3
	1	145.5	00.0	000.0	000.0
	2	84.8	00.0	000.0	000.0
	3	93.8	00.0	000.0	000.0
L.S.D	Formulation	N.S	N.S	N.S	N.S
	Concentration	30.5**	12.4**	497.5**	185.6**
	Interaction	43.1*	N.S	703.6**	N.S

- : Data are means three replicates. *: Significant. **: Highly significant. N.S: Not significant

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