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Nematicidal Properties of Three Adjuvants for Management of Southern Root-Knot Nematode, *Meloidogyne incognita In Vitro* and under Greenhouse Conditions

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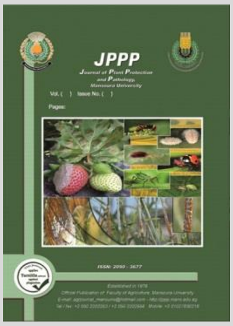

ABSTRACT

Lettuce and French bean production are severely infected by root-knot nematode, *Meloidogyne incognita* which causes critical damage to economic plants. Therefore, the farmers have to use chemical nematicides with or without adding adjuvants to enhance the active ingredient delivery to the target nematode. A current study was conducted to assess the direct effect of three adjuvants namely Silwet L-77, Sylgard 309 and Agrimax 3H comparing with three formulations of oxamyl, fosthiazate and fenamiphos on root-knot nematode *Meloidogyne incognita*. *In vitro* experiments, ovicidal effect of Silwet L-77, Sylgard 309 and Agrimax 3H on eggs hatching of root-knot nematode was close to the lowest efficient nematicide fenamiphos when the adjuvants were applied at double recommended rate but their effect does not rise to the level of oxamyl or fosthiazate. On the other hand, mortality percentages of second-stage juveniles resulted from Silwet L-77, Sylgard 309 and Agrimax 3H at the recommended rate were 37.33, 50.00 and 42.19 %, respectively. Whereas, double recommended rate of such materials induced mortality percentages reached 46.66, 47.83 and 48.66 %, respectively comparing with 90.83,74.33 and 45.83% resulted from the tested nematicides oxamyl, fosthiazate and fenamiphos, respectively. On the other hand, controlling the root-knot nematode infecting lettuce and French bean plants under greenhouse conditions revealed that adjuvants have weak or limited nematicidal effect with minor potency in reduction nematode development incomparable with the tested standard nematicides, and does not rise to the lowest effect of a tested nematicide fenamiphos. However, both tested plants showed no visual phytotoxicity symptoms.

Keywords: Root-knot nematode, oxamyl, fosthiazate, fenamiphos, Silwet L-77, Sylgard 309, Agrimax 3H

INTRODUCTION

Root-knot nematodes are an economically important polyphagous pests of highly adopted obligate plant parasites (Moens *et al*., 2009). Root-knot nematodes are considered as a soil-borne disease, after removing the affected plants and the soil retain inoculum many years due to the long survival of phytonematodes in deep layers of the soil (McKenry,1999). More than 2000 plants are susceptible to root-knot nematodes infection which are responsible for approximately 5% of universal crop losses (Eisenback and Triantaphyllou,1991) and raised to 12% (Ferraz and Brown,2002). The high reproduction rate of root-knot nematode, *Meloidogyne incognita* results in large quantities of eggs accumulated in the soil which is considered a major constraint facing vegetable production. In Egypt, *Meloidogyne* species became real threats for most vegetable crops and classified as limiting factors in crop production (Ibrahim *et al*., 2011). Application of chemical nematicides has disadvantages like high expensive beside human health and environment hazards.

Generally, adjuvants are an additional inert material combined with toxic active ingredient for enhancing the pesticidal efficacy and inadvertently the non-target effects of the active ingredient (Mullin *et al*.,2015).

Nowadays, pesticide formulations particularly in combinations, are often used proprietary spray adjuvants to achieve high efficacy for targeted pests (Green and Beestman,2007), subsequently, enhancing the performance with raising the stability of active ingredients (Hazen,2000). An adjuvant is added to the pesticide formulation called premix or a tank mix to ensure that the formulation spreads out and making thin film resulted from enhancing coverage of sprayed plants (Coret and Chamel,1993; Kirkwood,1999). Adjuvant recommendations may change with the changes in pesticide formulations, newly labeled tank mixes and premixes, and changes in application technology and procedures based on label recommendations. Spray adjuvants are safe and effective in pest control when used based on label recommended. Although a single adjuvant may perform more than one function, but, no single product can solve every problem of active ingredient.

The pesticide formulations are formulated combinations of surfactants, penetrant enhancers, activators, spreaders, stickers, cosolvents, wetting agents, pH modifiers, defoaming agents, drift retardants, nutrients, etc., depending on their proposed utility. Usually, adjuvants are much less expensive than formulated active ingredients and can reduce the active ingredient rate needed (Green,2000; Ryckaert *et al*.,2008). Newer agrochemical technologies include spray adjuvants such as organosilicone polyethoxylates (Mullin *et al*.,2015) but organosilicone surfactants are the most potent adjuvants and super-penetrants available to growers although spray adjuvants are largely assumed to be biologically inert and are not registered by the USA EPA (Mullin *et al*., 2016). Therefore, the present study aimed to determine the effect of three pesticide adjuvants *in vitro* and *in vivo* against *M.*

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incognita on lettuce and French bean plants infected with nematodes.

MATERIALS AND METHODS

Pesticides used:

Three commercial formulations of nematicides registered and available in market applied against different nematodes pests in Egypt including oxamyl (Vaydate® 24 % SL), fosthiazate (Nemathorin® 10% WG) and fenamiphos (Fenatode® 10% GR) were applied at the rates of 0.2 ml/plant,12.5 kg/fed and 20 kg/Fed, respectively (recommended rates). The tested chemical nematicides were obtained from the Central Laboratory of Pesticides, Dokki, Giza, Egypt.

The tested Adjuvants include:

1. Silwet L-77® (organosilicone nonionic surfactant) distributed by Loveland (Greeley, CO, USA).
2. Sylgard 309® (organosilicone nonionic surfactant) distributed by Wilbur-Ellis (Fresno, CA, USA)
3. Agrimax-3H® (excellent adhesive) alkylated vinyl pyrrolidone polymers which alkyl groups are grafted surfactant and polymer properties combined polymeric surfactant.

The three tested adjuvants were applied at the rates 5 ml/100 l, 250 ml/100 l and 15 ml /100 l, respectively (recommended rates).

Maintenance and identification of *Meloidogyne incognita*:

Meloidogyne incognita culture was maintained on the tomato susceptible cultivar Super Strain B in the greenhouse as a source of inoculum. Perineal pattern region of adult females and infective juveniles (IJs) measurements were used to verify and identify the root-knot nematode, *Meloidogyne incognita* according to Jepson,1987. The root-knot nematode eggs extraction was done using 0.5% NaOCl technique (Hussey and Barker,1973) from the roots of a pure culture of *M. incognita* on tomato and purified using two different size sieves (60 and 500 mesh). Extracted eggs were washed softly with distilled water to eliminate sodium hypochlorite and incubated in Petri dishes at 25±1°C until hatching and newly hatched juveniles were collected using a micropipette.

***In vitro* Treatments:**

Nematicides and adjuvants effects on individual eggs hatching:

The extracted eggs of *M. incognita* from galled roots of tomato were suspended in water and counted using a counting slide under a stereomicroscope. The number of eggs per ml was adjusted to about 1000 by concentrating the suspension using the centrifuge. Approximately 100 nematode eggs in 0.1 ml were exposed to 10 ml of the above mentioned concentrations of pesticides and adjuvants into Petri dishes (5 cm diameter) kept at 25 ±3˚C for 10 days. The number of hatched J_2 was expressed as a cumulative number of viable J2. Percentages of hatching inhibition were calculated in comparison with the control treatment, according to the following equation:

$$\textbf{Egg hatching inhibition (\%)} = \frac{\textbf{No. of hatched in control - No. of hatched treatment}}{\textbf{No. of hatched in control}} \times 100$$

The nematicidal effect of tested pesticides and adjuvant on nematode juveniles (J2):

The suspension concentration of second-stage juveniles (J_2) was adjusted to 1000 infective juveniles per ml, then 0.1 ml of J_2 suspension containing about 100 juveniles in each one was added to final volume 5 ml to prepare concentrations (recommended rate and double recommended rate) for each pesticide or adjuvant in 5 cm diameter Petri dishes. Control treatment was used only distilled water. All treatments were left under room temperature (25±3˚C) to assess the effect of these materials on juveniles mortality and each of them was replicated four times. The treatments were observed daily for J_2 mortality until 14 days. Second-stage juveniles showing motionless straight posture after prodding were considered dead (Ishibashi and Takii,1993). Immobile J2 were collected then washed with distilled water and allowed to recover in distilled water for 5 h. Mortality was observed under 100 X magnification in 1 ml over the specified periods and mortality percentages were calculated from the following equation:

$$\textbf{Mortality (\%)} = \frac{\textbf{Dead juveniles}}{\textbf{Total number of juveniles}} \times 100$$

Pot treatments under greenhouse conditions:

Seeds of the tested lettuce plant (*Lactuca sativa* L.) c.v. local, and french bean (*Phaseolus vulgaris*) local cultivar were soaked in sterile distilled water in Petri dishes and kept in an incubator at 26±1°C. After 48 hours, seeds were sown in plastic pots 15 cm diameter sterilized with formalin solution 5%, and then filled with steam 1.7 kg sterilized soil (95.7% sand; 1.2% silt and 3.1% clay) amended with 80 g compost. At the 3:4 leaf stage (after two weeks), seedlings (approximately 10 cm in height) were thinned to one plant per pot. Every plant seedling was inoculated with 1000 newly hatched infective juveniles (IJs) of *M. incognita*. The second-stage juveniles were added by pipetting 2 ml of the inoculum suspension into four holes around the root system.

The treatments of lettuce and French bean seedlings were done according to the following scheme:

Control treatments (Healthy plants and *M. incognita* alone) as positive and negative controls; *M. incognita* IJs + oxamyl; *M. incognita* IJs + fosthiazate; *M. incognita* IJs + fenamiphos, *M. incognita* IJs + Silwet L-77, *M. incognita* IJs + Sylgard 309 and *M. incognita* IJs +Agrimax 3H. The nematicides and adjuvants were applied at recommended rates. Each treatment was replicated five times in the greenhouse at 28 ± 4°C. All treatments received the same horticultural treatments. Forty-five days after inoculation, plants were removed carefully from pots and data on plant growth (fresh root weight (g), fresh shoot weight (g), shoot diameter (cm), root length (cm), root galls/root) were recorded. Roots and surrounding soil in the pots were soaked in clean water for one hour to facilitate removing adhering soil and keep egg masses on the root surface. During the steps of evaluation, roots were wrapped in tissue paper to prevent drying out and numbers of galls and egg masses were counted per root system under a stereomicroscope. Root-knot index was assessed using 0-5 scale 0 of: No galling;1: 1- 2 galls; 2: 3- 10 galls; 3: 11- 30 galls; 4: 31- 100 galls and 5: more than 100 galls (Taylor and Sasser, 1978).

Statistical analysis

The completely randomized design was applied for laboratory experiments, while, experimental units arranged in a randomized complete block design in the greenhouse experiment. Data were subjected to statistical analysis using MSTAT version 4 (1987), where, analysis of variance and means were compared using Duncan's multiple range test at P ≤0.05 probability.

RESULTS AND DISCUSSION

Data in Table (1) showed the effect of three nematicides included oxamyl, fosthiazate and fenamiphos used as reference treatments for comparison with three adjuvants included Silwet L-77, Sylgard 309 and Agrimax 3H applied at recommended and double recommended rate to judge the ovicidal effect of the adjuvants on egg nematodes *Meloidogyne incognita*. After one day of exposure, oxamyl at recommended rate showed a significant superior ovicidal effect recording 86.46 % egg inhibition followed by fosthiazate (55 %) and fenamiphos (42.79 %). The tested adjuvants at recommended rates showed limited ovicidal effects 33.97 and 15.44% reduction for Silwet L-77 and Agrimax 3H while Sylgard 309 recorded 29.85 % reduction. Double recommended rate of adjuvants raised the inhibition effect to 48.82, 49.41 and 41.91 % for Silwet L-77, Sylgard 309 and Agrimax 3H, respectively. The second-day exposure, all tested chemicals showed a little reduction in egg hatching inhibition except fosthiazate and fenamiphos showed a least raising in egg hatching inhibition effect but all tested adjuvants at recommended rate showed a high drop in egg hatching inhibition especially, Agrimax 3H which occupied the second significant rank after control treatment. The long exposure period the tested chemical exhibited fluctuated response with narrow range with the specific nematicides; this narrow range was least with oxamyl while all tested concentration of the adjuvants showed a broad range of fluctuated ovicidal effect. After 10 days of exposure, the best significant ovicidal effect noticed with oxamyl recorded 84.72% reduction in nematodes egg hatching, followed by fosthiazate and fenamiphos recorded significant reduction 44.73 and 44.31%, respectively. While tested adjuvants at different concentrations caused a convergent egg hatching reduction doesn't achieve the economic feasibility of doubling the rate although presence significant difference between the concentrations and adjuvants. The general mean of reduction in nematode egg hatching treated with adjuvants (Silwet L-77, Sylgard 309 and Agrimax 3H) at double recommended rate showed 47.09, 45.20 and 41.51% reduction equal with fenamiphos that showed lowest ovicidal effect between tested nematicides and recorded 47.27 % egg hatching reduction, as shown in Fig. (1).

Table 1. Mean hatched juveniles and reduction percentages in *Meloidogyne incognita* egg hatching treated with nematicides and adjuvants after exposure to different periods.

Exposure time (days)	Control	Egg-hatching inhibition (%)								
		Nematicides			Adjuvants					
		Recommended rate			Recommended rate			Double recommended rate		
		Oxamyl	Fosthiazate	Fenamiphos	Silwet L-77	Sylgard 309	Agrimax 3H	Silwet L-77	Sylgard 309	Agrimax 3H
1	226.67 ^{a}	30.67 ^{g} (86.46)	102.00 ^{f} (55.00)	129.67 ^{de} (42.79)	149.67 ^{cd} (33.97)	159.00 ^{c} (29.85)	191.67 ^{b} (15.44)	116.00 ^{ef} (48.82)	114.67 ^{ef} (49.41)	131.67 ^{de} (41.91)
2	433.33 ^{a}	62.00 ^{g} (85.69)	159.33 ^{f} (63.23)	191.00 ^{ef} (55.92)	351.00 ^{c} (18.99)	364.67 ^{b} (15.84)	431.00 ^{b} (0.53)	226.67 ^{e} (47.69)	228.33 ^{e} (47.30)	277.33 ^{d} (36.00)
3	596.67 ^{a}	70.33 ^{f} (88.21)	265.00 ^{e} (55.58)	415.00 ^{cd} (47.20)	478.00 ^{bc} (19.72)	507.33 ^{ab} (14.97)	567.00 ^{ab} (4.97)	338.67 ^{de} (43.32)	348.67 ^{cde} (41.56)	346.67 ^{cde} (41.89)
4	930.00 ^{a}	91.00 ^{f} (90.21)	517.00 ^{d} (56.91)	528.00 ^{d} (53.97)	681.33 ^{c} (26.73)	694.3 ^{c} (25.34)	758.33 ^{b} (18.45)	381.67 ^{e} (58.96)	374.00 ^{e} (59.78)	390.00 ^{e} (58.06)
7	1193.7 ^{a}	150.3 ^{f} (87.40)	620.7 ^{e} (48.00)	723.0 ^{cde} (39.43)	756.0 ^{cd} (36.66)	825.3 ^{bc} (30.86)	888.3 ^{b} (25.58)	687.0 ^{de} (42.44)	754.7 ^{cd} (36.77)	746.0 ^{cd} (37.50)
10	1346.3 ^{a}	205.7 ^{f} (84.72)	744.0 ^{e} (44.73)	749.7 ^{e} (44.31)	889.7 ^{c} (33.91)	925.3 ^{bc} (31.21)	993.0 ^{b} (26.24)	788.7 ^{de} (41.41)	856.7 ^{cd} (36.37)	893.0 ^{c} (33.67)

Number between parentheses refer to the reduction percentage resulted from treatment calculated from the number of hatched juveniles after define incubation period; The same letter (s) in each row indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test.

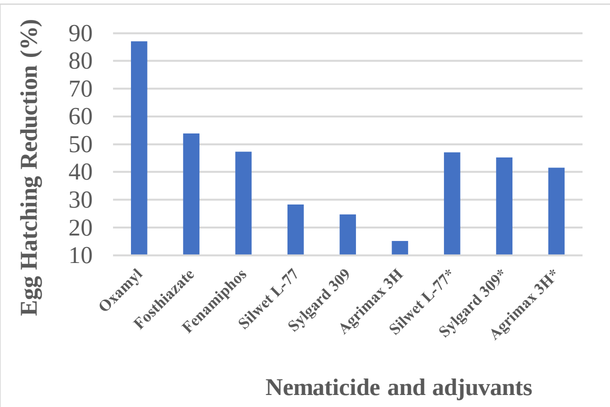

Figure 1. Egg hatching reduction percentages of *Meloidogyne incognita* treated with the recommended rate of nematicides and recommended and double recommended rates of adjuvants.

Data in Table 2 represented the effect of three nematicides included oxamyl, fosthiazate and fenamiphos used as reference treatments for comparison with three adjuvants included Silwet L-77, Sylgard 309 and Agrimax 3H applied at recommended and double recommended rate to judge the larvicidal effect of the adjuvants on infective juveniles of *M. incognita*. The IJs mortality after one-day exposure to the tested chemicals showed highest significant mortality with oxamyl (39.83 %) followed by fosthiazate (19.16%) and fenamiphos (16.16 %) while, all adjuvants with both tested concentrations induced mortality ranged from 6 to 9 % with no significant difference between all adjuvants. After 2 days of exposure, the tested nematicides showed non-significant mortality with closer range with the retention of oxamyl superiority over other nematicides.

In the long incubation period in the third and fourth day, accumulative mortality percentage showed increasing difference between treatments certainly after 7 days exposure to show a significant difference between the tested nematicides and adjuvants different treatments. Data retained the same trend until the experimental endpoint after 2 weeks showing the highly significant difference between different treatments, the highest significant mortality was recorded with oxamyl (90.83%) followed by fosthiazate and fenamiphos in the second significant rank recording 74.33 and 45.83% mortality, respectively in contrast with different adjuvant treatments with both tested concentrations occupied the third significant rank in causing mortality of second-stage juveniles of root-knot nematode, *M. incognita,* ranged from 37.33 to 48.66% as shown in Fig. 2.

Table 2. Mortality percentages of the second-stage juveniles of root-knot nematode, *Meloidogyne incognita* treated with nematicides and adjuvants after exposure to different periods.

Exposure time (days)	Mortality of IJs								
	Nematicides			Adjuvants					
	Recommended rate			Recommended rate			Double recommended rate		
	Oxamyl	Fosthiazate	Fenamiphos	Silwet L-77	Sylgard 309	Agrimax 3H	Silwet L-77	Sylgard 309	Agrimax 3H
1	79.67 [a] (39.83)	38.33 [b] (19.16)	32.33 [b] (16.16)	13.67 [c] (6.83)	14.00 [c] (7.00)	12.00 [c] (6.00)	16.67 [c] (8.33)	18.00 [c] (9.00)	15.33 [c] (7.66)
2	99.00 [a] (49.50)	91.33 [a] (45.66)	91.00 [a] (45.50)	29.33 [cd] (14.66)	29.00 [d] (14.50)	32.67 [bcd] (16.33)	37.00 [bcd] (18.50)	40.33 [bc] (20.16)	43.00 [b] (21.50)
3	121 [a] (60.50)	[b] 107 (53.50)	102.33 [b] (51.16)	33 [d] (27.16)	58.667 [cd] (29.33)	54.667 [d] (27.33)	62.67 [cd] (31.33)	68.00 [c] (34.00)	66.67 [c] (33.33)
4	133.00 [a] (66.51)	117.67 [ab] (58.83)	103.67 [b] (51.83)	72.67 [cde] (36.33)	62.33 [de] (31.16)	60.00 [e] (30.00)	77 [cd] (38.50)	74.67 [cde] (37.33)	83 [c] (41.50)
7	155.67 [a] (77.92)	124.33 [b] (62.16)	119.67 [b] (38.83)	77.67 [c] (43.50)	87.00 [c] (43.00)	86.00 [c] (38.60)	77.00 [c] (38.50)	74.67 [c] (37.33)	83.00 [c] (41.50)
10	157.00 [a] (78.50)	132.00 [b] (66.00)	131.00 [b] (43.00)	86.00 [c] (45.83)	91.67 [c] (47.00)	94.00 [c] (41.97)	87.00 [c] (43.50)	91.67 [c] (45.83)	92.33 [c] (46.16)
14	181.67 [a] (90.83)	148.67 [b] (74.33)	140.33 [b] (45.83)	91.67 [c] (37.33)	94.67 [c] (50.00)	100.00 [c] (42.19)	93.33 [c] (46.66)	95.67 [c] (47.83)	97.33 [c] (48.66)

Numbers between parentheses refer to the reduction percentages resulted from treatment; The same letter (s) in each row indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test.

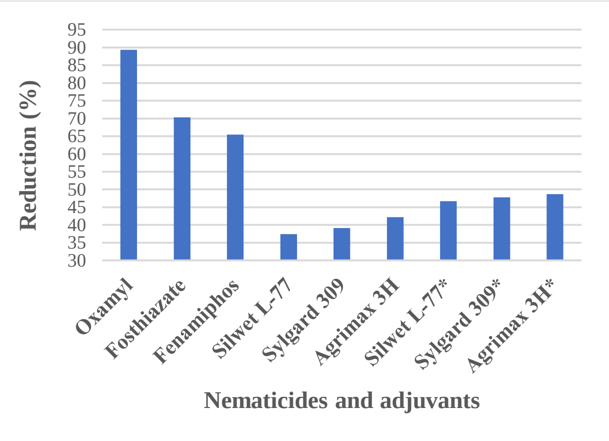

Figure 2. Accumulative mortality percentages of infective juveniles of *Meloidogyne incognita* treated with nematicides and recommended and double recommended rate of adjuvants.

The tested nematicides included fosthiazate, fenamiphos and oxamyl, chemically classified based on mode of action as carbamate compounds (Ntalli and Caboni,2012) are well known as acetylcholinesterase inhibitors. The EC_{50} for oxamyl, fenamiphos, fenamiphos sulfoxide, fenamiphos sulfone were 0.3, 0.2, 0.2 and 0.3 µg/ml, respectively for galling on tomato plants caused by *M. incognita,* whereas their nematicide concentration that inhibited acetylcholinesterase activity by 50% were 0.17, 555, 58.2 and 22.5 $1\times10^{6-}$ M, respectively (Nordmeyer and Dickson, 1990). No aging of AchE is possible with carbamate insecticides. Although the half-life of AChE inhibition by carbamates is shorter than that of organophosphorus nematicides, the time is long enough for clinical signs and sometimes death to occur (Peterson and Talcott, 2013). The corresponding value for the suppression of the second-stage juveniles (J2) was 91.2% with oxamyl (Radwan *et al*., 2011)

Silwet L-77 and Sylgard 309 are silicone-based surfactants (Greene, 2006). The toxicity of a non-ionic surfactant (Silwet L-77) on green peach aphid (*Myzus persicae*) was evaluated at different humidity in the laboratory recorded mortality reach to 99.28% at 90% RH, but only 33.89 and 23.78% at 60 and 30% RH, respectively (Imai *et al*., 1995) which concluded the role of adjuvant on changing the physical characteristics of water in plant sap and related to filter chamber in aphis gut leading to failure extraction of amino acid from plant sap subsequently aphis death. The obtained results ascertained with *M. incognita* eggs exposed to oxamyl at different concentrations recorded to notice decreasing egg hatching and increasing mortality of juveniles with increasing concentration of nematicides (Haq *et al*., 1983).

Data in Table (3) showed different treatments of nematicides (oxamyl, fosthiazate, and fenamiphos) and adjuvants (Silwet L-77, Sylgard 309 and Agrimax 3H) for controlling root-knot nematode *M. incognita* infecting lettuce plants. The measured parameters included botanical parameters and root-gall formation parameters. Healthy plants showed a vigor growth with the absence of root-gall formation, in contrast with the treatment of *M. incognita* alone which recorded the lowest growth parameter as well as highest values with root-gall formation parameters. Oxamyl treatment occupied the best second significant rank after healthy plants recorded 5.1 g of fresh root weight with increasing 80.85% comparing to root-knot nematode alone treatment. While, the fosthiazate treatment recorded 4.82 g of such previous criteria with increasing 70.67%, but, with a significant difference between oxamyl or fenamiphos treatments. On the other hand, fenamiphos caused increasing 61.13% in fresh root weight close to Sylgard 309 which recorded 59.21% with no significant difference with fenamiphos or Silwet L-77 treatment which recorded 43.26% increase in fresh root weight. Finally, Agrimax 3H treatment recorded the lowest significant difference of 21.27% in fresh root weight. While, fresh shoot weight was the least effect with nematode infection, where, healthy plants and oxamyl (97.36%) treatments recorded the highest significant of lettuce fresh root weight followed by Silwet L-77 (32.25%) then fosthiazate (29.91%) and fenamiphos (19.97%).

Table 3. Lettuce growth parameters, reproduction and root-gall formation of *Meloidogyne incognita* treated with three nematicides and three adjuvants.

Treatments	Shoot diameter (mm)	Fresh shoot weight (g)	Fresh root weight (g)	Root galls /Root	Egg masses /Root	Gall diameter		
						≥ 4 mm	< 4-2 mm	< 2 mm
Healthy plants	8.9 ^{a}	14.85 ^{a}	6.91 ^{a}	0.00 ^{e}	0.00 ^{e}	0 ^{c}	0 ^{c}	0 ^{d}
M. incognita alone	5.6 ^{f}	6.82 ^{cd}	2.83 ^{f}	57.33 ^{a}	69.67 ^{a}	3.00 ^{a}	23.0 ^{a}	34.67 ^{a}
M. incognita IJs + oxamyl	7.9 ^{b} (41.07)	13.46 ^{a} (97.36)	5.10 ^{b} (80.85)	13.67 ^{d} (76.15)	27.00 ^{d} (61.24)	0.00 ^{c}	3.67 ^{c}	13.0 ^{cd}
M. incognita IJs + fosthiazate	7.8 ^{bc} (39.28)	8.86 ^{b} (29.91)	4.82 ^{bc} (70.67)	37.00 ^{c} (35.46)	54.00 ^{b} (22.49)	1.33 ^{b}	14.0 ^{b}	22.0 ^{abc}
M. incognita IJs + fenamiphos	7.7 ^{c} (37.50)	8.17 ^{bc} (19.97)	4.56 ^{c} (61.13)	40.00 ^{bc} (30.22)	39.00 ^{c} (44.02)	1.33 ^{b}	21.0 ^{ab}	21.0 ^{bc}
M. incognita IJs + Silwet L-77	6.9 ^{d} (23.21)	9.02 ^{b} (32.25)	4.04 ^{d} (43.26)	42.67 ^{bc} (25.57)	37.67 ^{c} (45.93)	1.67 ^{b}	16.67 ^{ab}	24.33 ^{abc}
M. incognita IJs + Sylgard 309	6.8 ^{de} (21.42)	7.47 ^{bc} (9.53)	4.50 ^{cd} (59.21)	49.00 ^{abc} (14.52)	53.00 ^{b} (23.92)	2.67 ^{a}	22.0 ^{ab}	24.0 ^{abc}
M. incognita IJs + Agrimax 3H	3.42 ^{e} (-38.92.)	6.87 ^{c} (0.73)	3.42 ^{e} (21.27)	50.67 ^{ab} (11.61)	55.67 ^{b} (20.09)	3.00 ^{a}	20.0 ^{ab}	27.67 ^{ab}

The same letter (s) in each column indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test; Numbers between parentheses refer to the percentages resulted from treatment; Percentages (%) of botanical parameter represented increasing %, while, root-gall formation parameters represented reduction % based on the treatment *M. incognita* alone.

Shoot diameter gave best results with healthy lettuce plants recorded 8.9 mm followed by oxamyl treatment which significantly recorded 7.9 with 41.07% increasing, then, fenamiphos recorded significant increase 37.50% (7.7mm), while, fosthiazate mediate significance between oxamyl and fenamiphos recorded 7.8 mm shoot diameter with increasing 39.28% comparing infected lettuce plants with nematode. The highest shoot diameter resulted from adjuvant treatments was performed by Silwet L-77 which significantly recorded 6.9mm with increasing 23.21% followed by Sylgard 309 caused 6.8 mm with increasing 21.42%. However, Agrimax 3H showed a negative effect on shoot diameter recording 6.7 mm with decreasing 38.32% compared to *M.incognita* only.

In contrast with the botanical parameters with depend on heathy plants, diseased plants treatment showed the highest values in root-knot nematode reproduction-related parameters to record 57.33 galls /root followed by Agrimax 3H recorded 50.67 galls /root with reduction 11.61% then Silwet L-77, Sylgard 309 and fenamiphos recorded 14.52, 25.57 and 30.22% reduction, respectively. Finally, fosthiazate caused a significant reduction of 35.46% which the highest significant reduction resulted in oxamyl treatment recorded 76.15% reduction.

Egg masses per root recorded 69.67% in infected plants with nematode, Agrimax 3H (20.09%) recorded the least significant reduction as well as fosthiazate (22.49%) and Sylgard 309 (23.92%) with no significance between these treatments. Whereas, Silwet L-77 and fenamiphos recorded reduction 44.02% and 45.93 %, respectively. Oxamyl induced the high significant reduction (61.24%).

Root gall index showed the highest value with disease lettuce treatment recorded 4.0, while, adjuvants Sylgard 309 and Agrimax 3H recorded 3.3 then Silwet L-77 recorded 3.0 On the other hand, the root gall index with the tested nematicides were 2.6 and 2.3 for fosthiazate and fenamiphos treatments, respectively. While the best efficient nematicide oxamyl resulted in 2.3 as shown in Fig. (3).

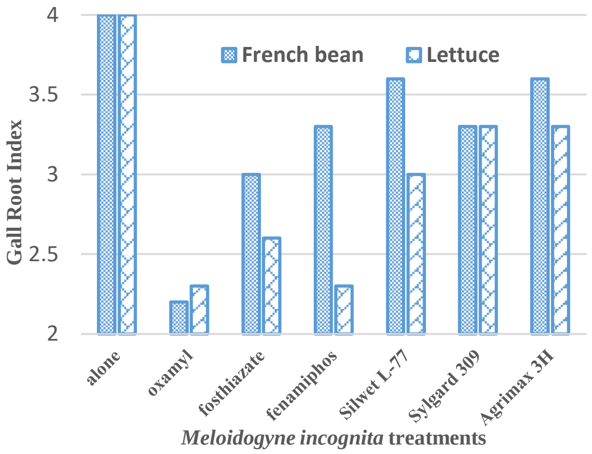

Figure 3. Root gall index for different tested nematicides and adjuvants on French bean and lettuce plants.

Root gall diameter resulted from nematode infection elucidate the disease progress and nematicidal efficiency, where gall diameter greater than 4mm indicated old successful infection while, galls diameter 2-4mm showed a short-term infection whereas increasing galls diameter lower than 2mm showed epidemic disease as shown with root-knot nematode only treatment, , while, tested material treatments increase the number of galls diameter lower than 2mm indicate to quick dissipation or diluted with irrigation water. Gall diameters with all measures were higher in the positive control treatment infected with nematode. In contrast with healthy plants and oxamyl resulted null especially with galls diameter higher than 4 which ensure the initial nematicidal effected on old infection although it didn't stop the disease progress due to persistence and behavior in soil, while, other treatments showed range from 1.33 to3. With galls diameter 2:4mm, incomparable value for oxamyl recorded 3.67 although the noticeable reduction efficacy in reduction gall number lower than 2 mm diameter or newly infection.

Table 4. French bean growth parameters, reproduction and root-gall formation of *Meloidogyne incognita* treated with three nematicides and three adjuvants.

Treatments	Shoot Diameter (mm)	Fresh shoot weight (g)	Fresh root Weight (g)	Root Length (cm)	Root galls /Root	Egg masses /Root	Gall diameter ≥4mm	Gall diameter 2-4 mm	Gall diameter <2 mm	Root gall-index
Healthy plants	15.69 ^{a}	15.69 ^{a}	9.81 ^{a}	21.27 ^{a}	0 ^{e}	0 ^{e}	0 ^{c}	0 ^{c}	0 ^{d}	0.0
M. incognita alone	8.17 ^{bc}	6.43 ^{b}	3.76 ^{d}	11.43 d	61.0 ^{a}	81.67 ^{a}	6.00^{a}	33.67 ^{a}	21.33 a	4.0
M. incognita IJs + oxamyl	13.46 ^{a} (64.75)	12.46 ^{a} (93.77)	5.10 ^{b} (35.63)	16.17 ^{b} (41.38)	13.67 ^{c} (77.59)	21.33 ^{d} (73.88)	0.00^{c}	3.67 c	9.67 ^{bc}	2.2
M. incognita IJs + fosthiazate	8.86 ^{bc} (8.45)	9.02^{b} (40.27)	5.11 ^{b} ((35.90)	13.53 ^{c} (33.50)	37.0 ^{bc} (39.34)	54.0 ^{bc} (33.88)	1.33^{b}	14.00^{b}	21.67 ^{ab}	3.0
M. incognita IJs + fenamiphos	8.17 ^{bc} (0)	8.85 ^{b} (37.63)	4.55 ^{bc} (19.68)	11.86 ^{cd} (3.76)	40.0 ^{b} (34.42)	53.0 ^{bc} (35.10)	1.33^{b}	20.0 ^{ab}	18.67 ^{bc}	3.3
M. incognita IJs + Silwet L-77	9.02 ^{b} (10.40)	8.17 ^{bc} (27.06)	4.04 ^{cd} (7.44)	12.23 ^{cd} (6.99)	42.67 ^{b} (30.04)	50.67 ^{bc} (37.95)	1.67^{b}	16.67^{ab}	24.33^{abc}	3.6
M. incognita IJs + Sylgard 309	7.77 ^{bc} (4.89)	7.46 ^{bc} (16.01)	4.49 ^{bc} (19.41)	15.26 ^{b} (18.37)	49.00 ^{a} (19.67)	55.67 ^{b} (31.83)	2.67^{a}	22.0 ^{ab}	24.0 ^{abc}	3.3
M. incognita IJs + Agrimax 3H	7.74 ^{c} (5.26)	8.17 ^{bc} (27.06)	4.82 ^{b} (28.19)	15.46 ^{b} (35.25)	50.67 ^{ab} (16.93)	57.67 ^{b} (29.38)	3.0 ^{a}	20.0 ^{ab}	27.67^{ab}	3.6

The same letter (s) in each column indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test; Number between parentheses refer to the percentages resulted from treatment; Percentages (%) of botanical parameter represented increasing %, while, root-gall formation parameters represented reduction % based on the treatment *M. incognita* alone.

Data in Table (4) showed different treatments of nematicides (oxamyl, fosthiazate, and fenamiphos) and adjuvants (Silwet L-77, Sylgard 309 and Agrimax 3H) for controlling root-knot nematode *M. incognita* infested French bean plants. The measured parameters included botanical parameters and root-gall formation parameters. Healthy plants showed a vigor growth with the absence of root-gall formation, in contrast with the treatment of *M. incognita* alone which recorded the lowest growth parameter as well as the highest values with galling formation. Shoot diameter in healthy plants treatment significantly recorded 15.69mm close to oxamyl treatment which recorded 13.46 mm with significant increase 64.75% comparing the infected plants with nematode, while, the adjuvant Silwet L-77 caused significant increase to 10.40% followed by Agrimax 3H (5.26 %) then Sylgard 309 (4.89%) whereas other treatments occurred a slight increase or reduction with no significance difference. On the other hand, fresh shoot weights of French bean plants treated with the tested nematicides and adjuvants exhibited highest weight with the healthy plants recorded 15.69, while, oxamyl (93.77%) and fosthiazate (40.27%) treatments occupied the second significant rank, on contrast with Sylgard 309 (16.01%) resulted the lowest significant shoot weight. The shoot weight is the least affect plant part may be due to long distance from infection site in root or short infection period of root-knot nematode which target the root system, so, the symptoms observed on the shoot system appeared in late stage of infection of nematode.

Fresh root weight is considered the closest botanical parameter to infection site of nematode and doesn't consider a vital parameter for judging root health or successful control method because of nematode infection which causes hypertrophy and hyperplasia on root resulted gall formation with consider an additional weight on root comparing roots of healthy plants, so, root weight is not crucial parameter. Weight root was 9.81 g with healthy plants and 3.76 in infected plants. The lowest root weight resulted in Silwet L-77 application significantly recorded a 7.44% increase while other adjuvant treatments were more effective. Root length was highly significant in healthy plants treatment recorded 21.27 cm followed by plants treated with oxamyl significantly recorded 16.17 cm and close to Sylgard 309 and Agrimax 3H with no different significance with oxamyl and fosthiazate.

The number of root galls per root was null in healthy plants infected plants occupied the last significant rank with 61 galls in pots treated with root-knot nematode alone followed by Agrimax 3H with 50.67 galls recorded 16.93% reduction, Sylgard 309 with 49.00 galls and Silwet L-77 with 42.67 galls, respectively. A significant reduction in gall numbers was recorded with nematicides (oxamyl, fosthiazate and fenamiphos) and the most significant reduction resulted in oxamyl treatment recorded 77.59% reduction.

The number of egg masses per root was null in healthy plants. On the other hand, the adjuvants were least effective in reducing the number of egg masses while nematicides were more effective than those pots treated with adjuvants. Therefore, oxamyl was the most effective nematicide recorded significant reduction 73.88% while, other treatments recorded reduction range from 29.38 to 37.95%.

Gall diameter was classified into three classes as an indicator for age and progression of infection. Gall diameter greater than 4mm reached null in oxamyl as an indicator for high potency nematicidal effect. In contrast with infected plants, Silwet L-77 (1.67) Agrimax 3H (2.67) and Sylgard 309 (3.0) recorded the highest enumeration, while, other treatments recorded 1.33. Gall diameter 2-4 mm showed different categories among screened nematicides and adjuvants. Oxamyl (3.67) and Silwet L-77 (16.67) showed the least gall diameter 2.4 mm

On the other hand, galls diameter lower than 2 mm showed complicated difference but significant difference was indicated only in French beans plants infected with nematode and healthy plants, while, others treatment range was 9.67- 27.67.

Gall indicator was arranged based on efficiency as follows: Oxamyl, fosthiazate, fenamiphos, Sylgard 309, Agrimax 3H, Silwet L-77 and infected plants with nematode (Fig. 3).

In vitro experiments involve evaluating the selected substances on tested organism directly without involving another factors e.g. host plant, fauna, flora or environmental condition e.g. temperature, humidity, solarization, soil, etc. which interact negatively or positively with target organism, so, it is important to elevate laboratory experiments to the greenhouse experiments.

Botanical parameters of lettuce plants showed a clear improvement with nematicide treatments but showed a marked decrease in adjuvant treatments to reach it's lowest in shoot diameter parameter in Agrimax 3H treatment recorded 38.92% reduction or limited increase, while, adjuvant effects on fresh root weight raised to nematicide treatments level with limited increase with Agrimax 3H. On the other hand, the number of root galls or egg masses per plant root showed the lowest reduction percent in Agrimax 3H treatment followed by Sylgard 309 then Silwet L-77 and tested nematicides, ascendingly.

French bean plants showed a relatively different response, where, shoot diameter parameter recorded the least improvement in Agrimax 3H and Sylgard 309 treatments compared with the treatment of nematode. Also, the fresh weight of French bean plants exhibited the same trend but with the equality of Silwet L-77 treatment with infected treatment with nematode. Whereas, fresh root weight exhibited a significant indication with all tested adjuvants and nematicides on contrast root length parameters were increased in all treatments without exception. As for the pathological criteria, the root gall reduction was arranged ascendingly as follows: Agrimax 3H, Sylgard 309, Silwet L-77, fenamiphos, fosthiazate, and oxamyl. Meanwhile, the reduction of egg masses number per root was also arranged ascendingly as follows: Agrimax 3H, Sylgard 309, fosthiazate, fenamiphos, Silwet L-77, and oxamyl.

Oxamyl showed noticeable reduction percentages for parameters related to nematode infection (El-Sherif *et al*.,2010). Many studies indicated that oxamyl was the most effective treatment in reducing root galls and egg masses of the nematode (Khalil *et al*.,2012; Elsebae,1996) reached 88.3% reductions in root gall (Radwan *et al*.,2011). While, other studies elucidated that fenamiphos and oxamyl were proved to be the highest chemical compositions that decreased galls by 91.73 and 89.53% and egg-masses by 90.80 and 88.65%, respectively. Meanwhile, oxamyl achieved the greatest reduction for eggs per egg-mass by 63.17% (Saad *et al*., 2017). It was observed similarity on the effectiveness of fenamiphos and oxamyl in reducing nematode populations (Mani and Al-Hinai,1996). Also, based on the number of eggs and juveniles, oxamyl was equally effective in controlling root-knot nematodes; fenamiphos was less effective. For all nematicides, control potency increased with the increase of application rate and applied nematicide solution, but decreased with the increase of nematode infection depth (Lamberti *et al*.,2002). Eradication infestations of *M. incognita* on coffee seedlings using oxamyl and fenamiphos were ineffective(Jaehn *et al*., 1984). But, the nematicides potency depend on many factors involved application time, so, it recommended to apply fenamiphos and fosthiazate before transplanting (Russo *et al*.,2003). While applying oxamyl to cucumber roots soon after infection was more effective than later application in reducing the numbers of adult females which developed and the proportion of these which had egg masses. The number of eggs/egg mass and the size of the young adult females was reduced significantly by all oxamyl treatments but the effect was greater with the earlier application (Wright and Rowland,1982).

On the other hand, the soil types (clay, sandy loam, and loamy sand) influence the application rates of oxamyl, fenamiphos, for the control of *M. incognita* on tomato (Abu Elamayem *et al*.,1984). As well as, the soil content of free minor element elucidated by raising oxamyl toxicity to *M. incognita* increased 11.3 times in combination with $Fe_2(SO4)_3$ and 4.1 times after mixing with $ZnSO_4$ (Korayem and El-Sisi,1989).

The rapid degradation for fenamiphos by soil microorganisms perhaps responsible for inadequate nematode control and need enhancement with small rates every 3 weeks. While, efficiency was mainly attributed to its long soil persistence, whereas, oxamyl provided adequate nematode control for the first 7-8 weeks after application, regardless of the application method used, although its rapidly dissipated field (Giannakou *et al*., 2005).

The plant uptake factor is a parameter measures the ability of plants to take up substances from the water soil via root systems. The uptake amount of substance is not leached into groundwater (Kuppe *et al*., 2016). Also, root system type, age, size, length, density and surface area (Feddes and Raats,2004) are vital parameters, so, differentiation response between lettuce and French bean plant to tested nematicides and adjuvants are related to growth parameters, materials effectiveness and depend on depth of nematode burial (Lamberti *et al*.,2002). Finally, the differential sensitivity among *Meloidogyne* spp. and races (Nordmeyer and Dickson, 1989) and the resistance development to non-fumigant nematicides under field conditions does not require continuous monthly stressing (Yamashita and Viglierchio,1987).

No observed signs of phytotoxicity for nematicides (Russo *et al*.,2003). Also, all the tested nematicides significantly affected plant growth parameters include: shoot length, fresh shoot weight, and root length compared to the untreated inoculated control, but, all of these nematicides significantly decreased root fresh weight except oxamyl (Radwan *et al*.,2012), while, significant reduction in root fresh weight was observed with Fosthiazate (Radwan *et al*.,2012).

Although the great difference of surfactants in their physical and chemical properties and their side effects on non-target organisms (Stevens *et al*., 1988; Stevens and Baker 1987), but thus expected to differ in their potential to improve the efficacy of fungicides or biological control agents. Furthermore, since soil dryness in areas colonized by the nematodes may be partly responsible for plant death, it is possible that surfactants alone could partially relieve disease symptoms by increasing soil water content, so, the experiment confirms that there is a weak or limited nematicidal effect among the evaluated adjuvants.

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الخصائص الإبادية لثلاثة مواد مساعدة (إضافية) لمكافحة نيماتودا تعقد الجذور معمليا وتحت ظروف الصوبة

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تتعرض نباتات الخس والفاصوليا للإصابة بنيماتودا تعقد الجذور *Meloidogyne incognita* وتتسبب في حدوث أضرار كبيرة. ولهذا يلجأ المزارعين الى استخدام مبيدات نيماتودية كيميائية مميتة مع أو بدون إضافة مواد مساعدة من أجل تعزيز توصيل المادة الفعالة بكمية أكبر. لذلك أجريت الدراسة لتحديد التأثير المباشر لثلاثة مواد إضافية مقارنة بثلاث تجهيزات لمبيدات نيماتودية أوكساميل (فايديت 24% مركز قابل لذوبان) وفوسثيايزيت (نيماثورين 10% محببات قابلة للانتشار) والفيناميفوس (فيناتود 10% محببات) على نيماتودا تعقد الجذور تحت ظروف الصوبة. خلصت التجارب المعملية إلى أن معاملة البيض بالجرعة المضاعفة لكل منSilwet L-77 وSylgard 309 وAgrimax 3H لها تأثير يقارب تأثير مبيد الفينأميفوس الأقل فعالية لكن تأثيرها لا يرتفع لمستوى الأوكساميل أو الفوسثيايزيت. في حين موت الطور الثاني المعدي الناتج عن المواد المساعدة المختبرة بالمعدل الموصي به هو 37.33 ، 50.0 و42.19٪، وبمضاعفة معدل التطبيق نتجت نسبة موت مقدارها 46.66، 47.83و48.66٪ مقارنة مع مبيدات النيماتودا المختبرة الأوكساميل وفوسثيايزيت والفينأميفوس والتي أحدثت نسبة موت مقدارها 90.83 ، 74.33 و 45.83٪. من ناحية أخرى، أظهرت تجارب الصوبة لقياس كفاءة المواد المساعدة الإضافية لمكافحة نيماتودا تعقد الجذور على نباتات الخس والفاصوليا، أظهرت وجود تأثيرًا مميتًا ضعيفًا أو محدودًا للمواد المساعدة المختبرة مع فاعلية طفيفة في الحد من تطور النيماتودا لا يضاهى أي من المبيدات النيماتودية المختبرة ولا ترقي في تأثيرها لأضعف مبيد نيماتودي مختبر وهو الفيناميفوس. ولم تلاحظ أي أعراض سمية نباتية علي كلا النباتات المعاملة عند معاملتها بأي من المواد الإضافية المختبرة.