

EVALUATION OF NEMATICIDAL ACTIVITY FOR CERTAIN SOIL PESTICIDES TO *Meloidogyne javanica*

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ABSTRACT

The present investigation was undertaken to ascertain the efficacy of three soil pesticides viz., triazophos, methomyl and carboxin-thiram against egg-masses hatching of *Meloidogyne javanica* under laboratory conditions and gall formation as result to soil drench, foliar spray and bare-root dip treatment through using modified gall formation test.

The study revealed that under laboratory conditions, triazophos recorded high efficiency against egg-masses hatching followed by carboxin-thiram and methomyl. The descending order of ovicidal activity at EC₅₀ was 0.086, 0.620 and 6.5 ppm.

There are an obvious relationship between effectiveness of tested compounds against gall formation and methods of treatment with the same technique. So, triazophos and methomyl were more effective in case of gall formation under soil drench treatment than foliar spray treatment. Also foliar spray treatment indicated highly efficacy of carboxin-thiram against gall formation as comparing with soil drench. The respective EC₅₀ values were 13.8 and 21.4 ppm.

All tested compound showed effectiveness against root galling after different durations of bare-root dip treatment. Triazophos was most toxic compound followed by carboxin-thiram and then methomyl. The respective reduction in gall formation were between 29.8-100 %, 37-73.3 % and 41.9-60 % by treatment with 100-400 ppm. No significant effect was noticed between tested concentrations in case of triazophos also period of exposure was not significant with triazophos and carboxin-thiram.

Modified gall formation test appear to be adequate for screening large number of chemicals to detect nematocidal activity independent of mode of action through different methods of treatment (soil drench, foliar spray and bare-root dip).

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., are economically important plant pathogens and distributed world wide. They are obligate parasites and parasitize thousands of different plant species including monocotyledons, dicotyledons, herbaceous and woody plants. 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 quality of produce (Eisenback and Triantaphyllou, 1991). In Egypt as in many warm countries root-knot nematodes *Meloidogyne* spp. are serious pests to many field and vegetable crops (El-Morshedy *et al.*, 1993).

Many classes of chemicals namely fertilizer, herbicides, insecticides, fungicides, ... etc., are applied to the soil of a given crop during the growing season. There are limited information about the biological activity of these chemicals to nematode populations. The objective of this study to evaluate the effectiveness of two insecticides and one fungicide against *M. javanica*.

MATERIALS AND METHODS

Pesticides used were triazophos (Hostathion) 40 EC (O,O-diethyl O-1-phenyl-1H-1, 2, 4 triazol-3-yl phosphorothioate), methomyl (Lannate) 90 SP (S-methyl carbamoyloxy thioacetimidate) and carboxin + thiram (Vitavax-thiram) 37.5 + 37.5 WP (5, 6 dihydro-2-2 methyl-N-phenyl-1, 4-oxathiin-3-carboxamide) + (S- ((phenylmethyl) bis (1-methylpropyl) carbamothioate). The first two compounds are insecticides while the third one was fungicide.

Egg-masses of *M. javanica* were isolated from infected tomato roots obtained from pure culture and propagated in greenhouse.

The effect of tested pesticides on gall inducing ability of *M. javanica* was examined by gall index technique which described by (Bunt, 1975; Eric and Rich, 1987 and Birch et al., 1992). This technique was modified through two steps to improve estimating degree of galling with naked eye and to evaluate different application methods of tested pesticides (soil drench, foliar spray and bare root dip) as follow :

- 1- Use egg-masses of *Meloidogyne javanica* which need only to magnifying-glass for handling as test stage instead of larvae which need microscope examination.
- 2- Change the period between inoculation and galling examination from seven days in Bunt technique to 40 days in modified technique without missed the inoculated plants through irrigate each replicate as need with 2 ml artificial solution (0.8 ammonium sulfate 20.5 % + 0.1 gram potassium sulfate 47 % + 0.6 gram calcium superphosphate 15 %/one litre of water) after seven days from inoculation to guarantee the development of gall to size which can be estimated with naked eye or magnifying-glass.

Drench application test :

Two egg-masses of almost equal size were placed in brown glass vial (20 ml) containing 4 ml of the tested pesticides concentrations. 9.95 grams of washed, sieved, dried sand were added to each vial. After incubation at 25°C for 24 hours. A four-week old tomato seedlings (cv. Castle Rock) was planted in each vial. Seven days later, vials were irrigated as need with 2 ml artificial solution (0.8 gm ammonium sulfate 20.5 % + 0.1 gm potassium sulfate 47 % + 0.6 gm calcium superphosphate 15 %/one litre of water). After 40 days, the roots are washed free from sand and are rated for galling compared with control. Each treatment was replicated five times.

Foliar application tests :

Two egg-masses of almost equal size were placed in brown glass vial (20 ml) containing four ml of water. 9.95 gm of washed, sieved, dried sand were added to each vial. Four week old tomato seedling (cv. Castle Rock) was planted in each vial. Aluminum foil paper placed around the base of each seedling to protect the soil during foliar application. Each plant was carefully sprayed with ten ml of water or tested pesticides at 4, 8, 16, 32 ppm using air brush. Seven days later, vials were irrigated as need with 2 ml artificial solution (0.8 gm ammonium sulfate 20.5 % + 0.1 gm potassium sulfate 47 %

+ 0.6 gm calcium superphosphate 15 %/one litre). After 40 days, the roots are washed free from sand and are rated for galling, compared with control. Each treatment was replicated five times.

Dipping application test :

Two egg-masses of almost equal size were placed in brown glass vials (20 ml) containing 4 ml water. 9.95 gm of washed, sieved, dried sand were added to each vial. Four week old tomato seedlings (cv. Castle Rock) were dipped in water or tested pesticides at 100, 200 and 400 ppm for 30 and 60 second, then planted in each vials. Seven days later, vials were irrigated as need with 2 ml artificial solution (0.8 gm ammonium sulfate 20.5 %, 0.1 gm potassium sulfate 47 % + 0.6 gm calcium superphosphate 15 %/one litre). After 40 days, the roots are washed free from sand and are rated for galling compared with control. Each treatment was replicated five times.

Anti-hatching test :

To study the ovicidal action of the tested compounds, ten egg-masses of almost equal size were placed in Petri-dishes containing 10 ml of pesticide concentrations. Hatched larvae were counted 7 days after treatment and unhatch ability was recorded. Each treatment was replicated five times.

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).

The toxicity index method developed by Sun (1950) was used for measuring the relative nematocidal efficiency between tested pesticides. A correlation coefficient (r) was calculated for each data set assuming each model values to determine the significant of each regression at the $\alpha = 0.05$ level.

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

RESULTS

Ovicidal action :

According to correlation coefficient values, a significant succession linear relationships were found between tested concentration and inhibition percentage of hatching with all tested compounds (Table 1). On the other hand, triazophos was the most toxic compounds followed by carboxin-thiram and then methomyi. The respective EC_{50} values were 0.086, 0.62 and 6.5 ppm. the tested population showed equal slope values in case of methomyi and carboxin-thiram, whereas the slope value of triazophos was the highest one.

Table (1). Ovicidal action of tested pesticides against *M. javanica* egg-masses under laboratory conditions.

Concentration (ppm)	% Inhibition of		
	triazophos	methomyl	carboxin-thiram
0.0625	40.2	-	-
0.125	59.9	-	-
0.250	67.4	-	-
0.500	80.4	-	47.5
1.00	88.8	-	54.2
2.00	94.3	37.6	61.3
4.00	97.4	44.7	70.9
8.00	98.8	52.6	75.0
16.00	99.6	59.3	79.2
32.00	99.9	66.9	83.6
C.f. (r)	1***	1***	1***
EC ₅₀	0.086	6.50	0.62
Slope	1.20	0.62	0.57
Toxicity Index at EC ₅₀	100	1.32	13.9

- : not applied

C.f. (r) : Correlation coefficient

*** Highly significant

Drench application test :

Experiments demonstrated that inhibition percentage of root gall formation as result to drench application increased significantly with increasing concentrations in case of methomyl and carboxin-thiram. The respective correlation coefficient values were 0.921 and 1.00 (Table 2), whereas the above relationship was not recorded in case of triazophos which causes 100 % root galling inhibition with 2 ppm. So, it was considered the potent compound followed by methomyl (EC₅₀ : 7.3 ppm) and carboxin-thiram (EC₅₀ : 21.4 ppm).

Table (2). Effect of tested pesticides against gall formation as result to soil drench.

Concentration (ppm)	% Inhibition of		
	triazophos	methomyl	carboxin-thiram
2	100	9.1	34.5
4	100	20.5	39.1
8	100	34.9	43.0
16	100	52.4	47.0
32	100	70.5	52.6
64	100	84.6	57.3
128	100	93.2	61.9
C.f. (r)	-	0.921***	1***
EC ₅₀	-	7.3	21.4
Slope	-	1.5	0.38
Toxicity Index at EC ₅₀	-	100	34.3

- : not calculated

C.f. (r) : Correlation coefficient

*** Highly significant

Foliar application test :

Data presented in Table (3) indicated that correlation coefficient values were significant because of high succession correlation between tested concentrations and inhibition percentage of gall formation as shown by foliar application. Correlation coefficient values of all tested compounds were (1). Dependent on toxicity index, it is obvious that carboxin-thiram was the most potent compound followed by triazophos and methomyl. The respective EC₅₀ values were 13.8, 40.0 and 867.2 ppm.

Root-dip test :

According to data presented in Table (4), all tested concentrations of triazophos showed highly reduction effect to gall formation as a result to root dip technique with both exposure period, with a maximum of 100 % at 400 ppm.

Table (3). Effect of tested pesticides against gall formation as result to foliar application.

Concentration (ppm)	% Inhibition of		
	triazophos	methomyl	carboxin-thiram
2	0.0	21.4	21.7
4	0.0	24.0	30.0
8	1.0	27.0	41.3
16	10.6	30.0	55.1
32	32.9	33.3	63.5
64	74.7	36.6	89.0
128	95.2	40.2	-
256	99.7	44.3	-
C.f. (r)	1***	1***	1***
EC ₅₀	40.0	867.2	13.8
Slope	3.3	0.3	0.97
Toxicity index at EC ₅₀	34.5	1.6	100

- : not applied

C.f. (r) : Correlation coefficient

*** Highly significant

On the other hand, no significant effect was noticed between tested concentrations, exposure period and interaction between tested concentrations and exposure period.

Considering the general mean for exposure period (Table 5), data indicated that under methomyl conditions, 60 seconds exposure reduced significantly root galling, compared to that of 30 second exposure. Also tested concentrations of methomyl recorded significant effect against gall formation. L.S.D. was 6.9, moreover no significant effect was recorded with interaction between tested concentrations and exposure period.

According to general mean of concentrations (Table 6), number of gall/root system decreased significantly from 76 with untreated plants to 53.2, 26.2 and 20.3 in plants treated with 100, 200 and 400 ppm. On the other hand, no significant effect was recorded between both exposure period, while significant effect was noticed between tested concentrations and period of exposure.

Table (4). Effect of triazophos against gall formation as result to root dip technique.

Concentration (ppm)	Period of exposure				General mean effect of concentration*
	30 second		60 second		
	No. of gall/ root system*	% Reduction	No. of gall/ root system*	% Reduction	
0.0	50.2	0.0	50.2	0.0	50.2
100.0	4.0	92.0	3.2	93.6	3.6
200.0	0.2	99.3	0.0	100.0	0.1
400.0	0.0	100.0	0.0	100.0	0.0
General mean of exposure period	13.6	-	10.7	-	

L.S.D._{0.05} for :
 concentrations : N.S.
 time : N.S.
 Interaction : N.S.
 * : Average of five replicates

Table (5). Effect of methomyl against gall formation as result to root dip technique.

Concentration (ppm)	Period of exposure				General mean effect of concentration*
	30 second		60 second		
	No. of gall/ root system*	% Reduction	No. of gall/ root system*	% Reduction	
0.0	41.0	0	41.0	0.0	41.0
100.0	35.0	28	22.4	55.7	29.2
200.0	35.4	29	21.0	57.0	28.2
400.0	27.0	46	13.0	74.0	20.0
General mean of exposure period	27.9	-	19.5	-	

L.S.D._{0.05} for :
 concentrations : 6.9
 time : 3.2
 Interaction : N.S.
 * : Average of five replicates

Table (6). Effect of carboxin-thiram against gall formation as result to root dip technique.

Concentration (ppm)	Period of exposure				General mean effect of concentration*
	30 second		60 second		
	No. of gall/ root system*	% Reduction	No. of gall/ root system*	% Reduction	
0.0	76.6	0.0	76.0	0.0	76.0
100.0	63.0	17.0	43.4	57.0	53.2
200.0	33.0	56.6	19.4	74.5	26.2
400.0	22.4	70.5	18.2	76.1	20.3
General mean of exposure period	48.6	-	39.3	-	

L.S.D._{0.05} for :
 concentrations : 8.3
 time : N.S.
 Interaction : 31.8
 * : Average of five replicates

DISCUSSION

In general, all tested compounds showed effectiveness against hatching of egg-masses of *M. javanica* under laboratory conditions and gall formation applied to soil drench, foliar spray and root dip technique.

There are an obvious relationship between effectiveness of tested compounds and methods of application with the same technique, i.e. triazophos was the most effective compounds against root galling under conditions of soil drench followed by carboxin- thiram and methomyl. This arrangement changed to carboxin-thiram, triazophos and methomyl under condition of foliar application. Similar indication was recorded with Stephan and Trudgill (1983) who reported that similar amounts of oxamyl (3000 or 4000 ppm) applied as soil drench or as granules more effective than foliar sprays.

Triazophos was the most toxic compounds against egg-masses hatching under laboratory conditions and gall formation as result to (soil drench and root dip technique), whereas it showed a slight effect on gall formation when applied to foliar spray.

- 1- Regarding the foregoing results, it could be discussing as follow : 1- The effectiveness of triazophos against gall formation under soil drench may be due to highly ovicidal action which recorded under laboratory conditions as well as the mode of action of this compound which act as contact.
- 2- On the other hand, the inhibition effect of this compound against gall formation as respect to root dip technique and foliar application may be due to its ability to penetrates deeply into plant tissue.

Lewis (1997) indicated that triazophos are cholinesterase inhibitor, with contact and stomach action and it is non-systemic, but penetrates deeply into plant tissues. Several investigators had studied and confirmed the role of triazophos inhibition effect against development of *Meloidogyne* species (Bharal and Phukan, 1977 and Prasad and Narayana, 2000).

Carboxin-thiram was the most effective compounds against gall formation in case of foliar application EC_{50} was 13.8 ppm, whereas recorded second position between tested compounds against hatching EC_{50} was (0.62 ppm) and gall formation as result to root dip technique. On the other hand, it occupied third position in case of soil drench EC_{50} was 21.4 ppm.

With other view and depending on EC_{50} values, carboxin-thiram was more effective against hatching under laboratory condition followed by foliar application and soil drench.

From above result, we can deduce that, carboxin- thiram may be do not act as hatch inhibiting but act as hatch undutifuling. EC_{50} values increased from 0.62 ppm when egg-masses tested under laboratory conditions to 21.4 ppm when egg-masses treated as soil drench. This increasing in EC_{50} values may be due to effect of weather and soil conditions or to root exudates of tested plant which decreased untifuling effect through stimulating of egg-masses to hatch.

On the other hand, the effectiveness of carboxin- thiram against gall formation by foliar application and root dip technique conditions may be due

to its systemic action. These explanation agree with Lewis (1997) which indicated that carboxin possessed systemic action, whereas thiram act as contact fungicide.

The nematocidal activities of carboxin-thiram and thiram were studied and reported by Shahzad and Gaffar (1996) and Shina *et al.* (1994).

On the other hand, the tested population showed equal slope values in case of methomyl and carboxin- thiram against egg-mass hatching under laboratory conditions. This indication may be due to both carbamate compounds which act with the same mode of action.

Methomyl recorded third positions between tested compounds against hatching of egg-masses under laboratory conditions EC₅₀ value was 6.5 ppm and gall formation as result to (foliar application EC₅₀ was 867.2 ppm and root dip technique), however this position changed to second in case of soil drench with EC₅₀ 7.3 ppm.

On the other hand and according to EC₅₀ values, methomyl recorded close EC₅₀ values in case of egg- masses hatching under laboratory conditions and gall formation as result to soil drench. The respective EC₅₀ values were 6.5 ppm and 7.3 ppm whereas showed a slight effect against gall formation as result to foliar application with EC₅₀ value 867.2 ppm.

From going results, we could expected that the effective methomyl against gall as result to drench soil due to ovicidal action which recorded under laboratory conditions. On the other hand, methomyl don't act as systemic so it recorded high EC₅₀ value in case of foliar application (867.3 ppm).

Similar effects were observed by McLeod and Khair (1975), Abu El-Amayem *et al.* (1979), El-Shoura (1981), El-Kadi (1990) and Renu Sharma *et al.* (1997).

All tested compounds showed effectiveness against root galling as result to root dip technique. Triazophos was the most toxic compound followed by carboxin-thiram and then methomyl. Effectiveness increased with increasing of concentrations and exposure period.

Generally, gall formation inhibition as result to treatment with tested compounds may be due to effectiveness of this compound through inhibition of penetration, development and giant cell development or retardation of nematode growth and sexual differentiation.

Congruksa and David (1973) found that at 600 ppm of Lannate was retardation of nematode growth and sexual differentiation when applied to tomatoes in pots 0, 24, 48 or 72 hours after inoculation with *M. incognita* larvae.

Prasad and Rao (1977) indicated that oxamyl and fensulphothion inhibited giant cell development at nematode establishment sites.

The modification technique was suitable to evaluate different application methods (soil drench, root dip and foliar spray) of tested compounds.

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تقييم نشاط بعض مبيدات التربة كمبيدات نيماتودا ضد نيماتودا تعقد الجذور

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يستهدف هذا البحث دراسة فاعلية ثلاثة من مبيدات التربة (تراى أزوفوس، ميثوميل، كاربوكسين + ثيرام) من حيث قدرتها على إعاقه فقس بيض نيماتودا تعقد الجذور ميلودجينا جافانكا تحت ظروف المعمل وكذلك قدرتها على إعاقه تشكل العقد الجذرية بواسطة نفس النيماتودا على جذور نباتات الطماطم كنتيجة (لمعاملة التربة، رش الأوراق، غمر الجذور) باستخدام اختبار تشكل العقد الموصوف بواسطة بنت (1970) وذلك بعد تحويله.

أوضحت النتائج أن مبيد التراى أزوفوس تحت ظروف المعمل - سجل فعالية عالية من حيث قدرته على إعاقه فقس البيض وقد تلاه من حيث الفاعلية مبيد الكاربوكسين - ثيرام ثم الميثوميل حيث كانت القيم النصف مميئة 0.086، 0.62، 6.9 جزء في المليون على التوالي.

كما لوحظ أن هناك علاقة واضحة بين فاعلية المركبات المختبرة ضد تفقد الجذور وطرق المعاملة مع ثبات أسلوب الاختبار حيث كان تأثير التراى أزوفوس والميثوميل أكثر فاعلية في حالة معاملة التربة بالمقارنة برش الأوراق، بينما كانت فاعلية الكاربوكسين - ثيرام أعلى عندما رشت الأوراق عنها عندما تمت معاملة التربة.

وقد أظهرت كل المركبات المختبرة فاعلية ضد تعقد الجذور بعد فترات مختلفة من غمر الجذور في تراكيزات 100، 200، 400 جزء في المليون للمبيدات الثلاث المختبرة، حيث كان التراى أزوفوس أكثر المبيدات المختبرة فاعلية تلاه الكاربوكسين - ثيرام ثم الميثوميل، وقد تراوحت النسبة المئوية لتثبيت تكون العقد الجذرية ما بين 29.8 - 100%، 37 - 72.3%، 41.9 - 60% على التوالي. كما لم تكن هناك فروق معنوية بين التراكيزات المختبرة في حالة التراى أزوفوس وكذلك لم تلاحظ فروق معنوية بين فترات التعريض في كلا من التراى أزوفوس والكاربوكسين - ثيرام.

أظهر اختبار تشكل العقد الجذرية بواسطة نيماتودا تعقد الجذور الموصوف بواسطة بنت (1970) بعد تحويله صلاحية عالية لتقييم عدد كبير من المركبات الكيماوية لإكتشاف مدى فاعليتها كمبيدات نيماتودية بدون الإعتداع على طريقة فعلها، ومن خلال الطرق المختلفة للمعاملة سواء كانت معاملة تربة أو رش للأوراق أو غمر للجذور.