

## **EFFECTS OF SOME INSECTICIDES AND NEMATICIDES ON PENETRATION, INFECTIVITY AND REPRODUCTION OF THE ENTOMOPATHOGENIC NEMATODES**

**Anany, A. E.\*; A. F. El-Mesalamy\*\*; A.A. Deabes\*\*\* and A. M. Kella\*\*\*\***

\* **Agric. Zoology and Nematology Dept. Fac. of Agric., Al-Azhar Univ.**

\*\* **Agric. Zoology and Nematology Dept. Faculty of Agriculture Al-Azhar University at Assiut**

\*\*\* **Central Laboratory of Pesticides. Plant protection Institute. Agricultural Research Centre**

\*\*\*\* **Nematology Dept., Plant Diseases Inst., Agric. Research Centre**

### **ABSTRACT**

Laboratory bioassays were conducted to determine the effects of seven insecticides and three nematicides on penetration, infectivity and reproduction of the infective juveniles (IJs) of entomopathogenic nematodes *Heterorhabditis bacteriophora* and *H. indica* nematode species. In penetration assay, results of tested seven insecticides i.e. abamectin, *Bacillus thuringiensis* B.t. as bioinsecticides, chlorpyrifos, chlorpyrifos-methyl, esfenvalerate, methomyl and profenfos as chemical insecticides on both of entomopathogenic nematodes, indicate that the more pesticides events for reduction in average numbers of IJs nematodes penetrating last instar larvae of *Spodoptera littoralis* was chlorpyrifos-methyl and methomyl at 100% reduction comparing with control. While the more pesticides compatible with tested nematode species are abamectin and B.t on the three tested rates of application. In infectivity and reproduction assays, three nematicides has been tested i.e. aldicarb, ethoprophos and cadusafos at three rates of application after four periods of exposure soil to tested nematicides at one day, one week, two weeks, three weeks. Results show that, the most compatible nematicides with *H. bacteriophora* are cadusafos in both assays production and efficiency against *Galleria mellonella* larvae comparing with control, while other pesticides tested is not compatible with this nematode where the aldicarb at percentage 100% reduction in events insect larvae death, as well as test production by day, week, 2 weeks. Overall, results indicate the feasibility of an integrated use of these nematode species and chemical pesticides in crop protection.

**Keywords:** Entomopathogenic nematodes, insecticides, nematicides, penetration, infectivity, reproduction, *Heterorhabditis bacteriophora*, *H.indica*, *Spodoptera littoralis*.

### **INTRODUCTION**

Entomopathogenic nematodes (EPNs) are considered as one of the most promising non-chemical control alternatives for insect pests. In developing integrated pest management (IPM) strategies involving the use of entomopathogenic nematodes (Fam. :Steinernematidae and Heterorhabditidae) and chemical pesticides, it is important to ascertain the degree to which these nematodes may be affected by the chemicals involved.

Many studies have addressed the consequences of direct exposure to solutions of insecticides on the behavior and infectivity of selected entomopathogenic nematode species (Hara and Kaya, 1982, 1983; Rovesti

*et al.*, 1988; Zhang *et al.*, 1994; Gordon *et al.*, 1996) and detrimental effects reducing infectivity and motility have been recorded for several compounds (Rovesti and Deseo, 1990; Gaugler and Campbell, 1991). However it has also been suggested that exposure to certain chemicals may stimulate nematode movement and enhance host finding behaviour and penetration of the host (Ishibashi and Takii, 1993).. Patel and Wright(1996) studied the exposure *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev to two neuroactive pesticides i.e. oxamyl and fenamiphos, widely used against plant parasitic nematodes, and found that oxamyl ( $50 \mu\text{g ml}^{-1}$ ) initially stimulated non-sinusoidal movement in *S. feltiae* but had no appreciable effect on IJs of *S. carpocapsae*, while fenamiphos ( $10$  and  $50 \mu\text{g ml}^{-1}$ ) increased and sustained non-sinusoidal movement in both species over an observation period of 48 h. Moreover the two pesticides reduced the infectivity of both nematode species against late instar larvae of the wax moth, *Galleria mellonella* L. Elizabeth *et al.* (2003) found that *S. feltiae* is compatible with the majority of the tested formulations (17 different pesticides and three plant growth regulators) with no loss in viability and infectivity up to 24 h of exposure. Three pesticide formulations, Prostar (1%), Gnatrol (9%), and Terrazole (10%) decreased the viability of *S. feltiae* significantly within 24 h compared to the control. However, during the 24 to 72 hr incubation period, eight pesticides affected the viability of *S. feltiae* with Gnatrol and Terrazole causing the highest decrease (17% and 15%, respectively). Only Terrazole decreased the infectivity of *S. feltiae* to *Galleria mellonella* larvae compared to the control when tested after 24 h exposure. At 72 hr, Orthene and Terrazole caused significant decrease in the infectivity of *S. feltiae* (10% and 15%, respectively)

The present study was done to determine the effect of selected insecticides and nematicides on infectivity, penetration and reproduction of the entomopathogenic nematodes using *Galleria mellonella* and *Spodoptera littoralis* larvae as insect target.

## **MATERIALS AND METHODS**

**Nematodes.** Nematodes were routinely cultured in greater wax moth *Galleria mellonella* L. (Woodring and Kaya , 1988) entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *H. indica* were used in bioassays which collected at Behira Governorate (Anany,2005). Nematodes were stored at 15 °C. as aqueous suspensions in tissue culture flasks 250 ml, no longer than one week before use in bioassay.

**Insects.** The cotton leaf worm, *Spodoptera littoralis* was used as a model to test the effect of insecticides on penetration of entomopathogenic nematodes. The culture of the cotton leaf worm was reared under laboratory conditions of 26°C and 65 % R.H. The colony was reared for several generation before using the larvae in the bioassay experiments . The egg mass were allowed to hatch in clean jars provided with leaves castor plants (*Ricinus communis*). The larvae continued their development till pupation .The pupae were collected in separate jars until adult emergence . Moths were fed on 10 sugar solution.

The greater wax moth *Galleria mellonella* L. larvae were reared in the laboratory on artificial diet at a constant temperature of  $27\pm 1^{\circ}\text{C}$  and  $65\pm 5\%$  R.H.

**Pesticides.** All tested insecticides and nematicides were obtained from Central Laboratory of Pesticides, Plant Protection Institute – Agricultural Research Centre.

**Penetration assay.** Experiments were conducted in cylindrical plastic cups (4.5 cm height x 2.5 cm diameter) filled with sterilized sandy soil. The tested insecticides were added at three tested rates of application per feddan e.g. abamactin 1.8% (50, 60 and 80 ml/ f.), B.t. *Bacillus thuringiensis* 2X (150, 175 and 200 g / f.), chloropyrifos 48% (700, 850 and 1000 ml/ f.), chloropyrifos-methyl 50% (700, 850 and 1000 ml/ f.), esfenvalerate 5% (400, 500 and 600 ml/ f.), methomyl 90% (150, 200 and 300 g / f.) and profenfos 72% (500, 600 and 750 ml/ f.). Also the insecticides pipette on to the dry soil so that final moisture was standardized at field capacity (60% humidity). Approximately 100 IJs of both insect nematode species *H. bacteriophora* or *H. indica* were applied to each cup. Three replicates per each treatment, were incubated at  $25^{\circ}\text{C}$ . After 24 hrs. of nematode exposure to insecticides, one last instar larvae of *Spodoptera littoralis* was added to each cup. After 3 – 4 more days, cadavers were dissected and the number of penetrating nematode were counted.

**Infectivity assay.** Cylindrical plastic cups (7 x 6 cm diameter) filled with 50 gm sterilized sandy soil. Each used nematicide were added at three rates i.e. the half of recommended, the recommended and two fold. So, the rates of each aldicarb, ethoprophos and cadusafos were at (0.034, 0.017 and 0.0085 gm/ 1 kg soil), (0.08, 0.04 and 0.02 gm/ 1 kg soil) and (0.048, 0.024 and 0.012 gm/ 1 kg soil), respectively. Each rate of a nematicide was solved in 60 ml of sterilized water and incorporated with 1kg mixture of soil so that final moisture was standardized at field capacity (60% humidity). Three replicates per each treatment were incubated at  $25^{\circ}\text{C}$ . After one day, one week, two weeks and three weeks of soil to nematicides, 200 IJs of *H. bacteriophora* nematodes were applied to each cup. After 24 hours of nematode exposure to insecticides, five last instar larvae of *G.melonella* was added to each cup. Insect mortality was determined four days after infection.

**Reproduction assays.** This experiment was carried out as described in the infectivity bioassay except the use of plastic cups (4.5 x2.5 cm) by using the nematode concentration 50 IJs/ *G.melonella* larva. The infect cadavers were removed from the sand, rinsed, transferred to water traps and inoculated at  $25^{\circ}\text{C}$ . All emerging IJs from a single larva were recovered over a period of 10 days and stored in culture flasks 250 ml. The content of each flask (nematode suspension from individual cadavers) was mixed thoroughly. Eight samples of 10  $\mu$  from each suspension were examined under a stereomicroscope and the total number of IJs nematode per cadaver was calculated (Susurluk, 2006).

**Statistical analyses.** Data were then analyzed according to Duncan's (1955).

## RESULTS

### 1- Exposure effects of *H. bacteriophora* and *H.indica* to seven insecticides on nematode penetration.

The results reveal that all tested insecticides at different rates of application were found to suppress penetration rate of IJs in both tested nematode species on the last instar larvae of *Spodoptera littoralis*. Meanwhile they, significantly reduced (  $P = 0.05$  ) penetration of both entomopathogenic nematodes species as compared with the control. Data presented in Table (1) and Fig . (1) showed that, the highest percentage reduction of *H. bacteriophora* and *H.indica* penetration to insect host larvae was obtained by exposure to chlorpyrifos-methyl and methomyl at all tested rates of application(100% reduction), followed by exposure to chlorpyrifos, profenfos and esfenvalerate, while the lowest percentage reduction of nematode penetration were observed by exposure IJs of *H. bacteriophora* to B.t. and abamactin against IJs of *H.indica* at averages(52.66, 46.66 and 42.0 IJs/cadaver) and (38.33, 29.33 and 20.33 IJs/cadaver) at three levels of application, respectively.

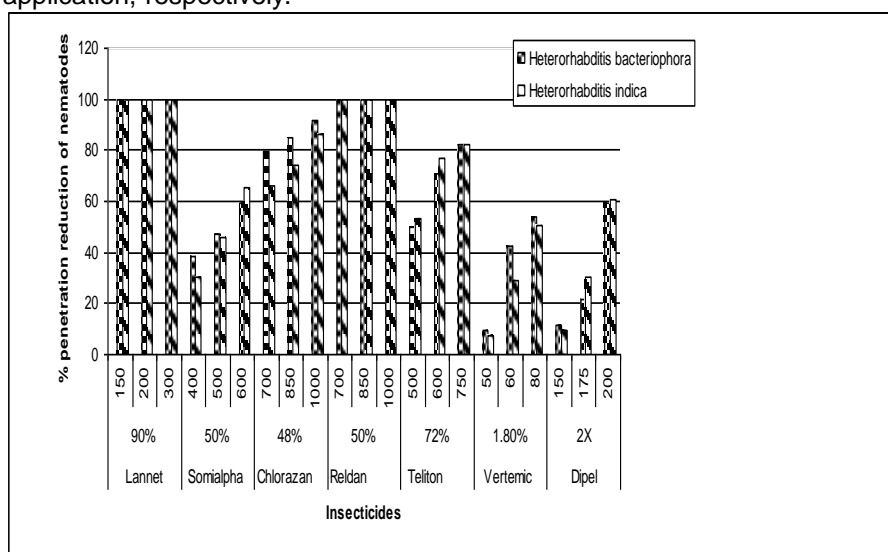


Fig. (1): Influence of seven insecticides on penetration of *Heterorhabditis* nematodes in last instar larvae of *Spodoptera littoralis*.

### 2- The effect of three nematicides on infectivity of *H. bacteriophora* against *G. mellonella* larvae.

There was a significantly differences in the infectivity of IJs of *H. bacteriophora* after exposure to aldicarb or ethoprophos nematicides at one day, one week, two weeks or three weeks as exposure periods of the treated soil to tested nematicides when compared with the control .

**T1**

Data indicate that *H. bacteriophora* did not kill larvae of *G. mellonella* when the nematicides aldicarb or ethoprophos were applied in soil at all tested exposure periods except that of ethoprophos after three weeks from application at 0.33 average mortality in *G. mellonella* larvae (Table 2 and Fig . 2).

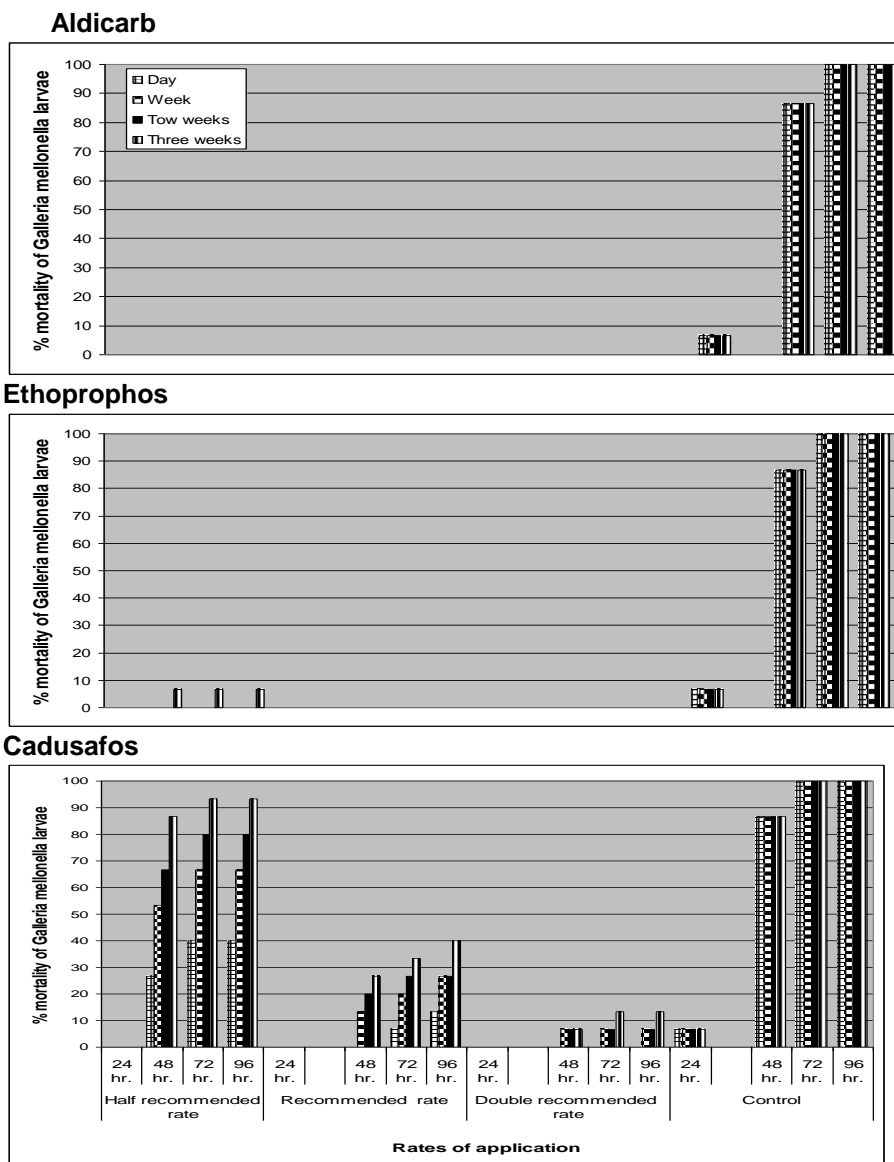


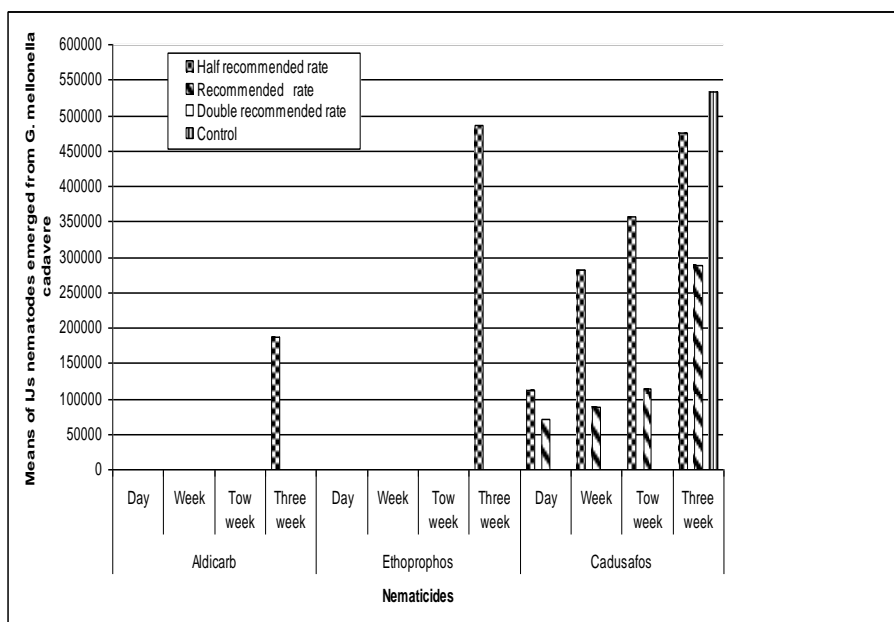
Fig.(2): Effect of three nematicides on mortality of *G. mellonella* larvae by IJs of *H. bacteriophora* nematodes.



Either after exposure the soil to cadusafos nematicide, the opening of mortality in *G. mellonella* caused after 48 hr. from exposure to nematodes all exposure periods. After one day, one week or two weeks as exposure periods the treated soil to cadusafos significantly different at all exposure times of insect larvae to *H. bacteriophora* nematodes at averages mortality in *G. mellonella* (0.0, 1.33, 2.0 and 2.0),(0.0, 2.66, 3.33 and 3.33)and (0.0, 3.33, 4.0 and 4.0) at three exposure periods, respectively. Not significantly different after three weeks from application with cadusafos (4.33, 4.66 and 4.66) at 48, 72 and 96 hr. after exposure the insect larvae to nematodes , respectively.

**3- Effect of three nematicides on reproduction of *H. bacteriophora* in *G. mellonella* larvae.**

After exposure to aldicarb or ethoprophos nematicides at all tested levels of application significantly reduced (  $P = 0.05$  ) reproduction of *H. bacteriophora* nematodes as compared with the control . Results in Table (3) and Fig . (3) indicate that *H. bacteriophora* did not develop and produce in larvae of *G. mellonella* when aldicarb or ethoprophos were applied in soil at all tested exposure periods and at all tested levels of application except after three weeks from application at half recommended level with averages 485300 and 188166 IJs/ cadaver, respectively. Whereas cadusafos nematicides significantly reduced nematode reproduction at all tested exposure periods and at all tested levels of application after three weeks except the half recommended level when compared with the control.



**Fig.(3): Effect of three nematicides on reproduction of *Heterorhabditis bacteriophora* nematodes in *Galleria mellonella* cadavers.**



The highest percentage reduction in reproduction of nematodes was obtained at two fold, the recommended level (100% reduction), followed by the recommended level (70333, 89183, 113000 and 289150 IJs/ cadaver) after the four tested exposure periods, respectively. But at half recommended level of application, the lowest percentage reduction in reproduction of nematodes was recorded after three weeks from exposure the treated soil to cadusafos.

**Table (3): The influence of tested nematicides on reproduction of *Heterorhabditis bacteriophora* in *Galleria mellonella* larvae.**

Nematicides	Rates of application	* Means of IJs nematodes emerged from <i>G. mellonella</i> cadavere			
		Day	Week	Tow week	Three week
Aldicarb	Half recommended rate	0.0 d	0.0 d	0.0 d	188166 d
	Recommended rate	0.0 d	0.0 d	0.0 d	0.0 e
	Double recommended rate	0.0 d	0.0 d	0.0 d	0.0 e
Ethoprophos	Half recommended rate	0.0 d	0.0 d	0.0 d	485300 b
	Recommended rate	0.0 d	0.0 d	0.0 d	0.0 e
	Double recommended rate	0.0 d	0.0 d	0.0 d	0.0 e
Cadusafos	Half recommended rate	112150 b(D)	281666 b(C)	357300 b(B)	474938 b(A)
	Recommended rate	70333 c(D)	89183 c(C)	113000 c(B)	289150 c(A)
	Double recommended rate	0.0 d	0.0 d	0.0 d	0.0 e
Control		532500 a			

\* Means followed by the same letters within a column are not significantly different ( $P \leq 0.05$ ) according to Duncan's multiple range test.

## DISCUSSION

This study shows that the presence of tested insecticides and nematicides in soil adversely affects the ability of *Heterorhabditis bacteriophora* and *H.indica* to penetrate, infect and reproduce an insect host.

Our results agree with (Hara and Kaya, 1982, 1983; Rovesti *et al.*, 1988; Rovesti and Deseo, 1990; Zhang *et al.*, 1994; Gordon *et al.*, 1996). They generally accepted the mode of action of nematicides which is not the direct killing of the nematodes, but the adverse effects on various aspects of behavior and development. Some of the behavioral effects include inhibition of motility, dispersion, attraction of plant hosts, and attraction of males to females (Greco and Thomason, 1980; Marban-Mendoza and Viglierchio, 1980 a , b). Direct developmental effects include inhibition of egg production, hatching, and molting (Hough and Thomason, 1975; Marban-Mendoza and Viglierchio, 1980 c). Hara and Kaya (1982) suggest that *S. feltiae* can be used before the pesticide or the pesticide and nematode applications can be spaced apart to allow for pesticide degradation. Their results showed also some of the effects of organophosphates and carbamates are similar to plant-parasitic nematodes. Reduced reproduction, or reproductive failure, may be explained in part by the disruption of the nematode's mating behavior. Lack of juvenile development may be due to the inhibition of molting by the organophosphates and carbanlates. Followed studies by Petersen *et al.* (1986) showed that fenamiphos was found at detectable levels in turfgrass

clippings and thatch two months after application and in soil six months after application. Prolonged exposure to this pesticide, however, may reduce the efficiency of the nematodes to infect the insect host or may cause nematode mortality (Kaya and Burlando, 1989). Movement of *S. feltiae* was slightly reduced by exposure to deltamethrin (Rovesti and Deseo, 1990) and trichlorfon was found to reduce *S. carpocapsae* infectivity to unacceptable levels (Zhang *et al.*, 1994). Head *et al.* (2000) reported that a limited range of insecticides can be applied simultaneously with *S. feltiae*, alternatively high levels of control of leafminer larvae can be achieved by the application of *S. feltiae* to vegetable foliage previously treated with insecticides.

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## تأثير بعض المبيدات الحشرية و النيماتودية على إختراق وكفاءة وإنتاج النيماتودا المتطفلة على الحشرات

عبد المنعم السعيد عناني\*, أنس فرج المسلمي\*\*, أحمد عبد المحسن دعيس\*\*\* و عاطف محروس كيلة\*\*\*\*

- \* قسم الحيوان الزراعي والنيماتودا - كلية الزراعة - جامعة الأزهر
- \*\* قسم الحيوان الزراعي والنيماتودا - كلية الزراعة - جامعة الأزهر - فرع أسبوط
- \*\*\* المعمل المركزى للمبيدات- معهد وقاية النباتات- مركز البحوث الزراعية
- \*\*\*\* قسم النيماتودا - معهد أمراض النبات - مركز البحوث الزراعية\*

إستهدفت هذه الدراسة تقييم تأثير سبعة من المبيدات الحشرية وثلاثة من المبيدات النيماتودية على إختراق وكفاءة وإنتاج الأطوار اليرقية المعدية لنوعين من النيماتودا المتطفلة على الحشرات هما هيتيرورابديتيس بكتريوفورا و هيتيرورابديتيس إنديكا . فى إختبار الإختراق تم اختبار سبعة مبيدات حشرية منهم إثنان من المبيدات الحيوية هما أبامكتين, بتى(باسيلاس ثيرينو -جينسيس) وخمسة من المبيدات الكيماوية وهم كلوروبيريغوس, كلوروبيريغوس- ميثيل, إيثيفينفيرات, ميثوميل, بروفينوفوس على كلا النوعين من نيماتودا الحشرات واثبتت النتائج ان أكثر المبيدات احداثا للخفض فى متوسط أعداد النيماتودا المخترفة ليرقات العمر الأخير لحشرة دودة ورق القطن هو المبيد كلوروبيريغوس- ميثيل و ميثوميل وذلك بنسبة مئوية 100% خفض بينما كان أكثر المبيدات توافقا وأمانا مع نيماتودا الحشرات المختبره هما المبيدان الحيويين أبامكتين, بتى وذلك على الثلاث التركيزات المختبرة لكل مبيد.

أما فى كلا من إختبار القوة المرضية والإنتاج تم إختبار ثلاثة مبيدات نيماتودية وهى الأديكارب, الإيثوبروفوس, الكادوفوس على ثلاث معدلات للتطبيق وهى نصف المعدل الموصى و المعدل الموصى وضعف المعدل الموصى وذلك بعد أربعة أوقات لتعريض المبيد للتربة وهى يوم واسبوع واسبوعين وثلاثة اسابيع واثبتت النتائج ان أكثر المبيدات توافقا مع النيماتودا المختبره هو مبيد الكادوفوس وذلك فى كلا من إختبار القوة المرضية والإنتاج عند المقارنة بالكنترول فى حين كان أكثر المبيدات عدم توافقا مع النيماتودا هو مبيد الأديكارب بنسبة مئوية 100% خفض فى إحداث النيماتودا لموت يرقات دودة الشمع الكبيره وكذلك إختبار الإنتاج بعد يوم واسبوع واسبوعين وثلاثة اسابيع.

وعموما تشير النتائج إلى جدوى استخدام هذه الأنواع من النيماتودا المتطفلة على الحشرات فى نظم المكافحه المتكاملة مع المبيدات المختبرة فى الحماية من الآفات الحشرية.

قام بتحكيم البحث

أ. د/ أحمد جمال الشريف

أ. د/ مصطفى عبد اللطيف مصطفى

كلية الزراعة – جامعة المنصورة

كلية الزراعة – جامعة الأزهر

**Table (1):Influence of seven insecticides on penetration of Heterorhabdits nematodes last instar *Spodoptera littoralis* larvae.**

Insecticides	* Means of Heterorhabdits nematodes penetrated to <i>Spodoptera littoralis</i> larvae																					
	Abamactin 1.8 %			B.t. 2X			Chloropyrifos 48%			Chloropyrifos- methyl 50%			Esfenvalerate 5%			methomyl 90%			Profenfos 72%			control
Nematode species	50 ml/F.	60 ml/F.	80 ml/F.	150 g/ F.	175 g/ F.	200 g/ F.	700 ml/F.	850 ml/F.	1000 ml/F.	700 ml/F.	850 ml/F.	1000 ml/F.	400 ml/F.	500 ml/F.	600 ml/F.	150 g/ F.	200 g/ F.	300 g/ F.	500 ml/F.	600 ml/F.	750 ml/F.	
Heterorhabdits bacteriophora	53.7	34.0	27.3	52.7	46.7	42.0	12.0	9.0	5.0	0.00	0.00	0.00	36.3	31.3	24.0	0.00	0.00	0.00	29.7	17.3	10.3	59.3
Heterorhabdits indica	38.3	29.3	20.3	37.3	28.7	16.3	14.0	10.7	5.7	0.00	0.00	0.00	28.7	22.3	14.3	0.00	0.00	0.00	19.3	9.7	7.3	41.3

\* Means followed by the same letters within a column are not significantly different ( $P \leq 0.05$ ) according to Duncan's multiple range test.

Table (2): Effect of three nematicides on infectivity of *Heterorhabditis bacteriophora* against *Galleria mellonella* larvae.

Rates of application	Exposure time	* Means of mortality of <i>Galleria mellonella</i> larvae												
		Aldicarb				Ethoprophos				Cadusafos				
		Day	Week	Tow weeks	Three weeks	Day	Week	Tow weeks	Three weeks	Day	Week	Tow weeks	Three weeks	
Half recommended rate	24 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.00 de	0.00 ef	0.00 d
	48 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.33 c	1.33 bc	2.66 c	3.33 c	4.33 a
	72 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.33 c	2 b	3.33 bc	4 bc	4.66 a
	96 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.33 c	2 b	3.33 bc	4 bc	4.66 a
Recommended rate	24 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.00 de	0.00 ef	0.00 d
	48 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.66 de	1.00 de	1.33 bc
	72 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.33 d	1.00 de	1.33 d	1.66 b
	96 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.66 cd	1.33 d	1.33 d	2.00 b
Double recommended rate	24 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.00 de	0.00 ef	0.00 d
	48 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.33 de	0.33 ef	0.33 d
	72 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.33 de	0.33 ef	0.66 cd
	96 hr.	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 d	0.33 de	0.33 ef	0.66 cd
Control	24 hr.	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c	0.33 c
	48 hr.	4.33 b	4.33 b	4.33 b	4.33 b	4.33 b	4.33 b	4.33 b	4.33 b	4.33 b	4.33 a	4.33 ab	4.33 a	4.33 a
	72 hr.	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a
	96 hr.	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a

\*Means followed by the same letters within a column are not significantly different ( $P \leq 0.05$ ) according to Duncan's multiple range test.

