"EFFECT OF ENTOMOPATHOGENIC NEMATODES AND BIOPESTICIDES IN CONTROLLING THE CABBAGE WORM, *PIERIS RAPAE*(L.) (LEPIDOPTERA: PIERIDAE) ON CAULIFLOWERS"

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Abstract

Laboratory and field tests were conducted to evaluate the impact of the beneficial nematodes and some biopesticides when used alone or together in controlling the cabbage worm *Peiris rapae* (L.) (Lepidoptera: Pieridae) in infested cauliflowers field as a new approach among integrated pest management (IPM). Larvae and pupae of *P. rapae* were highly susceptible to two nematode species, *Steinernema carpocapsal* (Sc) and *Heterorahabditis bacteriophora* (Hb) when used separately and there were ascending effect for the concentrations of nematodes. All tested concentrations were effective for both of the two species of nematodes. The biopesticides: spinosad and proclaim were more effective than nematodes when used separately, spinosad was more significant than proclaim. Mixing of nematodes and both spinosad and proclaim increased effectiveness and significance of mixtures under the laboratory and field conditions. The effects of nematodes and biopesticides when used alone or mixed in the laboratory was higher than in the field.

INTRODUCTION

Beneficial nematodes, particularly, Steinernematids and Heterorhabidtids are biological control agents that provide the basis of some bio-insecticides when used alone or mixing with pathogens. Biopesticides are certain types of pesticides derived from such natural sources as animals, plants, micro-organisms. They are an important group of pesticides that can reduce the side effects of chemical insecticides. Vegetable crops, especially cruciferous plants are attacked by several insect pests. The cabbage worm, *Pieris rapae* (L.) (Lepidoptera: Pieridae) causes large damage affecting the quantity and quality of the cauliflower plants (*Brassica oleraceae*). The cabbage worm *P. rapae* is found in the most Egyptian governorates wherever the cauliflowers cultivated. Entomopathogenic nematodes (Steinernematideae and Heterorhabditidae) are parasites of insects. Nematodes kill their hosts by the aid of bacteria found in the nematode's alimentary canal, steinernematides carry *Xenorhadus* spp whereas Heterorhabditids carry *Photorhabdus* spp. (Adams and Nguyen, 2002 and Poinar, 1990). These nematodes can be used as biological control agents to suppress a various number of economically important insect pests (Grewal *et al.*, 2005). Spinosad is a fermentation product produced by one or more chemical mutants of naturally occurring actinomycetes soil bacterium *Saccharopolyspora spinosa* (Book *et al.*, 1994). Spinosad has been applied to over 200 different crops. Spinosad used for control of lepidopterous pests e.g. *Ostrinia nubilalis, Helicoverpa zea, Trichoplusia* ni, *Plutella xylostella, Spodoptera* spp., *Heliothis* spp, *P. rapae*. Also used for urban pest control e.g. *Agrotis ipsilon*. Effectives as a bait for fruit flies *Ceratitis* spp. And *Bactrocera* spp. and some sucking lice and ants (Thompson *et al.*, 2000). Proclaim is a highly potent pesticide unique foliar insecticide that control lepidopteron pests (caterpillars and worm) in Cole corps, turnip greens in addition to leafy and fruiting vegetables. Proclaim effectively controls the larvae stages of many pests at low rates and increasing the crop's value. Proclaim contains the active ingredient emamectin benzoate, a semi – synthetic second generation a vermectin pesticides. Avermectins are gained from a naturally occurring soil bacterium *Streptomyces avermitilis*. Porcalim can be applied by ground air or ground only, giving the growers the flexibility needed for effective integrated pest management (IPM) programs (Jansson *et al.*, 1997). The present work was carried out to study the efficiency of the entomopathogenic nematodes and biopesticides when used sole or mixed on the cabbage worm, *P. rapae* under laboratory and field conditions.

MATERIALS AND METHODS

1.Laboratory bioassays

Infective third stage juveniles (IJs) of one steinernematid nematode *Steinernema carpocapae* (Sc) and one Heterorhabdit nematode *Heterorhabditis bacteriphora* (Hb) were used in this experiment. They were cultured on the last instar larvae of the wax moth, *Galleria mellonella* under laboratory conditions of 25±2°C and 65±2% RH (Dutky *et al.*, 1964). Fourth instar larvae and two days old pupae of *P. rapae* were treated in Petri dishes (fifteen larvae or pupae / dish) containing moistened filter paper with EPN, some cauliflower leaves put above it. Five concentrations of the nematodes Sc and Hb 250, 500, 1000, 2000 and 4000 IJs /ml were added. Control (untreated test) carried out in the same technique sprayed with water only. Treatments were replicated three times. Mortality of the larvae and pupae were calculated after 48 hours. Larval and pupal mortality were recorded daily and nematode development in cadavers of cabbage worm was ensured after seven days. The same technique was repeated using the biopesticides spinosad and proclaim at different five concentrations 0.0157, 0.0313, 0.0625, 0.1250 and 0.2500 mg/L, each replicated three times on the fourth instar larvae only. Control also repeated with spraying by water. Mortality resulted from application with nematodes and biopesticides were calculated and corrected according to Abott (1925). Log- probity lines for different concentrations were determined according to Finney (1971) using a detected software program. Relative toxicity between the nematodes and biopesticides determined by using the same software. Duncan's multiple range test (Duncan 1955) was used to differentiate between the means.

2. Field application:

Field trials were carried out in cauliflowers field at Qaha region, Qalubia governorate during Feb., 2009, cultivated area was one feddan (1 feddan = 4200 m^2) using portable sprayer, two liters capacity. The spray was directed towards to the top of cauliflowers in early morning. *P. rapae* larvae and pupae counts were randomly taken from samples of 50 plants (5 replicates x 10 plants) before spraying and 7 days after spraying with 2 liters of LC_{90} each nematode suspension 2622.72 IJs/ml for Sc and 1787.18 IJs/ml for Hb, respectively, and also with LC_{90s} for spinosad and proclaim 0.076 mg/L. and 0.114 mg/L., respectively, separately or in mixture from them with each of the nematodes (Sc and Hb). Control was treated with water only. Samples were taken from control and treated plots were counted before applications and one week after application. The percent of reduction in *P. rapae* population in relation to the sprayed nematodes, spinosad and proclaim suspensions were calculated according to Henderson and Tilton equation (1955).

3. Statistical analysis:

The data presented in percentage values in this offered study were normalized using arcsine transformation. The significance of the main effects was determined by analysis of variance ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P < 0.05$) (SAS Institute, 1988).

RESULTS AND DISSCUSSION

1. Laboratory bioassay:

1.1. Infectivity of nematodes against *Peiris rapae*:

Laboratory bioassay proved that the entomopathogenic nematodes (EPN) were highly virulent to *P. rapae* (Table 1). Mean parasitism followed by death by the

EPN (Sc) and (Hb) showed that both of the tested nematodes Sc and Hb were effective on the cabbage worm, while Hb nematode was more potent than Sc nematode and the percent of mortality ranging from (25.0 - 100%) and (17.7-100%) for larvae and pupae treated with Sc nematode, respectively, and ranging from (31.0-100%) and (25.3-100%) for larvae and pupae treated with Hb nematode, respectively. Further more there were a significant values appeared with different concentrations of the both using nematodes (Sc and Hb) on both of larvae and pupae of *P. rapae*. Generally there are significant differences among the tested concentrations (250, 500, 1000, 2000 and 4000 IJs/ ml) on larvae and pupae. The pupae of *P. rapae* were less susceptible to nematode infections than larvae because of the reduced number of portal entry (Kaya, 1984 and Azazy, 2001).

1.2. Effect of biopesticides on *Peiris rapae*:

Data showed in Table (2) reflect the effectiveness of two biopesticides (spinosad and proclaim) on the cabbage worm under laboratory conditions. Both of spinosad and proclaim were effective against the larvae of *P. rapae* and there were ascending significant effect appear i.e. the increasing in concentration resulted in increasing in the mortality percentages (dose-depended). The mortality percentages were 27.7% and 22.7% for the lowest concentration (0.0157 mg/L) and 100% for the highest one (0.2500 mg/L) for spinosad and proclaim, respectively. The results also indicated that spinosad was faster killer to *P. rapae* than proclaim and this is in the same trend with Salgado (1998). Spinosad has been applied to over 200 different crops. It has been used to control the caterpillars in cotton and the cabbage butterfly leaf miners in various crops (Thompson *et al.*, 2000). Proclaim is a highly potent pesticide unique foliar insecticide that controls lepidopteron pests on the leafy and fruiting vegetables. Proclaim effectively controls the larval stages of lepidopteron pests at low concentrations, giving the growers the flexibility needed for effective integrated pest management (IPM) programs (Jansson *et al.*, 1997).

1.3. Relative toxicity of various concentrations of the nematodes and biopesticides:

The calculated values of LC_{25}, LC_{50}, LC_{75} and LC_{90} in Table (3) indicated that LC_{25s} were 249.21 IJs/ml, 212.70 IJs/ml, 0.017 mg/L and 0.017mg/L, while values of LC_{50s} were 561.09 IJs/ml, 443.12 IJs/ml, 0.029 mg/L and 0.330mg/L, values of LC_{75s} 1263.29 IJs/ml, 923.15 IJs/ml, 0.048 mg/L and 0.063 mg/L, values of LC_{90s} were 2622.72 IJs/ml, 1787.18 IJs/ml, 0.076 mg/L and 0.114 mg/L for Sc & Hb nematodes and spinosad & proclaim, respectively.

2- Effect LC_{90} of nematodes and biopesticides sole or mixed on *P. rapae* under the laboratory conditions:

In the laboratory, data showed that using mixture of EPN and biopesticides in addition to using Hb alone achieved 100% morality, while mortality of 85% was reported for Sc nematode alone. Using both spinosad and proclaim alone resulted in mortality of 99% and 89%, respectively, (Table 4).

The results clearly show that the virulence of EPN is strongly affected by interactions between nematode species and the cabbage worm, the virulence of nematodes species relative to each other differed greatly among associated symbiosis bacteria (Dunphy and Webster, 1988) with natural products which are synthetic biopesticides spinosad was high effect more than proclaim because its effect work on neural system while proclaim work on digestive system. (Hill and Foster, 2003).

3. Effect of LC_{90} of nematodes and biopesticides (sole or mixed) on *Peiris rapae* under field conditions:

Mortality percentage of *P. rapae* due to application with LC_{90} Sc and Hb alone or mixed with spinosad and proclaim in the cauliflowers field are shown in (Table 4). The obtained data revealed that significant difference was observed between Sc and Hb nematodes, where Hb nematode was more significantly than Sc nematode. The mortality percentage under field conditions was 70% and 80% for Sc and Hb, respectively. But there were significant differences between all treatments and Sc nematode application alone. However, the mixture of EPN and spinosad and proclaim increased the significant effect than utilization of EPN alone and showed higher mortality rates (75 and 83%) and (80-90%), respectively, than EPN alone (70- 80% of Sc and Hb), while the spinosad and proclaim gave mortality percentages 90% and 86%, respectively, under field condition.

The results also indicated that spinosad was the faster killer of *P. rapae* followed by proclaim, while EPN came next with regard to host morality. This is largely due to its contact mode of entry (Salgado, 1998), rather than strict reliance on ingestion as was most traditional synthetic pesticides. Meanwhile, the proclaim controls larvae via some contact activity to most larvae but they should ingest it (Hill and Foster, 2003).

Table 1. Efficacy of the entomopathogenic nematodes on the fourth instar larvae and two days old pupae of the cabbage worm, *Peiris rapae*.

Nematodes concentration (IJs/ml)	Percent parasitism rate followed by death			
	Steinernema carpocapae		*Heterorahabditis bacteriophora*	
	larvae	pupae	larvae	pupae
Control	0.0±0.0 f (0-0)	0.0±0.0 f (0-0)	0.0±0.0 f (0-0)	0.0±0.0 f (0-0)
250	25.0±5.0 e (20-30)	17.7±2.5 e (15-20)	31.0±6.6 e (25-38)	25.3±2.5 e (23-28)
500	43.3±7.6 d (35-50)	41.0±3.6 d (38-45)	51.3 ±4.2 d (48-56)	51.3±2.5 d (40-45)
1000	69.0±16.5 c (52-85)	70.0±6.6 c (64-77)	80.0 ±5.0 c (75-85)	74.3±4.0 c (70-78)
2000	86.0±5.3 b (80-90)	82.3±2.5 b (80-85)	91.0 ±3.6 b (88-95)	91.0±2.1 b (88-92)
4000	100.0±0.0 a (100-100)	100.0±0.0 a (100-100)	100.0±0.0 a (100-100)	100.0±0.0 a (100-100)

The numbers with the same letter in the same column is not significantly different.
The numbers between the brackets refer to the range.

Table 2. Efficacy of the biopesticides on the fourth instar larvae of the cabbage worm, *Peiris rapae*.

Biopesticide concentration (mg/L)	% Mortality	
	Spinosad	Proclaim
Control	0.0±0.0 f (0-0)	0.0±0.0 f (0-0)
0.0157	27.7±2.5 e (25-30)	22.7±2.1 e (20-25)
0.0313	45.0±5.0 d (40-50)	44.7±3.3 d (40-48)
0.0625	85.7±6.0 c (80-92)	80.7±3.2 c (77-85)
0.1250	99.0±1.7 b (97-100)	89.0±0.8 b (88-90)
0.2500	100.0±0.0 a (100-100)	100.0±0.0 a (100-100)

The numbers with the same letter in the same column is not significantly different.
The numbers between the brackets refer to the range.

Table 3. The calculated values of LC_{25}, LC_{50}, LC_{75} and LC_{90} of the nematodes and biopesticides on *Peiris rapae* larvae.

Toxicity parameters	*Steinernema carpocapae* (IJs/ml)	*Heterorahabditis bacteriophora* (IJs/ml)	Spinosad (mg/L)	Proclaim (mg/L)
LC_{25} 95% FL	249.21 179.93-313.60	212.70 154.08-266.92	0.017 0.004-0.020	0.017 0.013-0.021
LC50 95% FL	561.09 470.37-658.33	443.12 369.57-516.42	0.029 0.013-0.045	0.330 0.028-0.038
LC75 95% FL	1263.29 1046.53-1626.08	923.15 787.12-1125.35	0.048 0.034-0.124	0.063 0.055-0.076
LC90 95% FL	2622.72 1970.28-4003.40	1787.18 1416-2489.40	0.076 0.067-0.631	0.114 0.092-0.153
Slope	1.914	2.116	3.027	2.373

Index compared with Hb for nematodes and spinosad for biopesticides.

Table 4. Effect LC_{90} of nematodes and biopesticides alone or mixed on *P. rapae* under laboratory and field conditions.

Treatment	Mortality %	
	Lab	Field
Steinernema carpocapae (Sc)	85.0±5.0d (78-90)	70.0±2.2f (68-73)
Heterorahabditis bacteriophora (Hb)	100.0±0.0a (100-100)	80.0±3.8d (76-85)
Spinosad	99.0±1.4b (97-100)	90.0±4.1a (85-95)
Proclaim	89.0±0.8c (88-90)	86.0±2.2b (83-88)
Sc+ Spinosad	100.0±0.0a (100-100)	83.0±2.5c (80-86)
Sc+ Proclaim	100.0±0.0a (100-100)	75.0±2.9e (72-79)
Hb+ Spinosad	100.0±0.0a (100-100)	90.0±1.6a (88-92)
Hb+ Proclaim	100.0±0.0a (100-100)	80.0±5.7d (75-88)

The numbers with the same letter are non significantly different in the same column.
The numbers between the brackets refer to the range.

REFERENCES

1. Abbott, W. S. 1925. Amethod for computing the effectiveness of insecticides. J. Econ. Ent. 18:265-267.
2. Adams, B. J. and Nguyen, K. B. 2002. Taxonomy and systematic. In: Gauglar, R. (Ed.), Entompathogenic Nematology. CABI, New York, NY, 1-34.
3. Azazy, A. M. 2001. Comparative studies between insect pathogenic nematodes and other nematodes in controlling some soil insect and fruit tree borers. Ph.D. Thesis, Fac. Mohstohor Agric. Zagazig, Univ. 200 P.
4. Book, L. D., C. Hang, T. E. Eaton, O. W. Godfrey, K. H. Michel, W. M. Nakatsukasa and R. C. Yao. 1994. Process for producing A 83543 compounds. US Patent No. 5.362, 624.
5. Duncan, D. B. 1955. Multiple range and multiple F. test. Biometeric, 11-145.
6. Dunphy, G. B. and J. M. Webster. 1988. Virulence mechanism of *Heterorhabdits heliothidis* and its bacterial associate in non- immune larvae of the greater wax moth, *Galleria mellonella*. International J. Parasitology. 18 (6) 729 – 737.
7. Dutky, S. R., J. V. Thompson and C. E. Cantwell. 1964. A technique for mass production of the DD-136 nematode. J. Insect Pathol., 6: 417-422.
8. Finney, D. J. 1971. Probit Analysis. 3rd edition Cambridge univ. Press Cambridge.
9. Grewal, P., R. U. Ehlers, and Shapiro - Ilan, D (Ed.) . 2005. Nematodes as Biological control Agents. CABI publishing, Walling ford. UK.
10. Henderson, C. F. and W. Tilton. 1955. Tests with Acaricides against the brown wheat mite. J. Econ. Ent., 48 (2) 157 – 161.
11. Hill, T. A. and R. E. Foster. 2003. Influence of selected insecticides on the population dynamics of diamondback moth (Lepidopter: Plutellidae) and its parasitoid *Diadegma insularae* in cabbage. J. Ent. Sci. 38, 59 – 71.
12. Jansson, R K., R. F. Peterson, P. K. Mooke, Jee, W. R. Halliday and R. A. Dybas. 1997.Development of novel soluble granule formulation of enamectin benzoate for control of lepidopterous pest. Florida Entomologists, 80: 425 – 450.
13. Kaya, H. K. 1984. Effect of the antomopathogenic nematode *Naplectana carpcapose* on the tachinid parasite *Compsieler concinnata* (Diptera: Tachinidae). J. Nematology. 16, 9-13.
14. Poinar, Jr., G. O. 1990. Biology and Taxonomy of Steinernematide and Heterorhabditidae. In: Gaugler, R. and Kaya, H. K. (Ed.), Entomopathogenic Nematodes in Biological control. CRC Press, Boca Raton, FL: 23-62.
15. SAS Institute. 1988. "SAS" User's Guide statistics. SAS Inst., Cary, Nc.
16. Salgado, V. L. 1998. Studies on the mode of action of spinosad: symptoms and physiological correlates. Pest. Bio-chem. Physiol. 60, 91-102.
17. Thompson, G. D., R. Dutton and T.C. Sparks .2000. Spinosad a case study: an example from a natural products discovery program. Pest Management Science 56: 696-702.

"تأثير النيماتودا الممرضة للحشرات والمبيدات الحيوية في مكافحة دودة الكرنب علي القنبيط"

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أجريت تجارب معملية وحقلية لمعرفة تأثير النيماتودا الممرضة للحشرات وبعض المبيدات الحيوية منفردة ومخلوطة علي دودة الكرنب *Pieris rapae* في حقول قنبيط مصابة بها كإتجاة جديد في برنامج المكافحة المتكاملة لها ، أظهرت كلا من اليرقات والعذاري حساسية عالية للإصابة بنوعين من النيماتودا الممرضة للحشرات هما شتينرنيما كاربوكابسي و هيتيروراهيبديتيس باكتروفورا ، وقد كان تأثير التركيزات المختلفة منهما تصاعديا أي أنه كلما زاد التركيز زادت نسب الموت، وكانت المبيدات الحيوية المستخدمة مثل الاسبينوساد والبروكلام أكثر فاعلية من النيماتودا عند إستخدامهما منفردين ، وقد كان الاسبينوساد أكثر فعالية من البروكلام ، وعند خلط النيماتودا مع كل من الاسبينوساد والبروكلام كلا علي حدة أظهرت هذه المخاليط تأثيراً عالياً في مكافحة دودة الكرنب، وعموماً كان تأثير جميع المعاملات في المعمل أكثر تأثيراً عنه في الحقل، وإن كانت جميع المعاملات لها تأثير فعال في الحقل مما يشجع علي إستخدامها في برامج المكافحة المتكاملة.