CONTROL OF THE DIAMONDBACK MOTH USING ENTOMOGENOUS NEMATODES

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ABSTRACT

The efficacy of three Steinernematid nematodes, *Steinernema carpocapsae* All, *Steinernema riobravis*, and *Steinernema feltiae*,, and one isolate of *Heterorhabditis* sp. was tested against the diamondback moth. The "All" isolate of *S. carpocapsae* was the most virulent giving a LD_{50} of 1.9 (1.4-2.4). Spray application of this nematode on Chinese cabbage gave a 64.5% control of the insect. Mixing nematodes with *Bacillus thuriengiensis* did not enhance the efficacy. The common pesticides used on cabbage did not cause any adverse effect on the nematode efficacy; in fact, certain chemicals enhanced the nematode efficacy.

KEY WORDS: Biological control, Diamondback moth, Heterorhabditids, Pesticides, Steinernematids

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* L. has been known as a pest of cruciferous crops since 1746 (Harcourt, 1962). It attacks and severely damages various cruciferous crops grown worldwide. Though chemical control has been the most reliable method in controlling the pest, build-up of resistance to conventional insecticides (Sun *et al.*, 1986) has led to the consideration of other alternative methods such as biological control.

Entomogenous nematodes (Steinernematidae and Heterorhabditidae) have been used as alternatives for the control of insect pests particularly in the soil (Gaugler, 1981; Kaya, 1985). The nematode infective juveniles (IJ) carries a bacterium either of the genus *Xenorhabdus* (Steinernematids) or *Photorhabdus* (Heterorhabditids) which kills the insect once the IJ has penetrated the haemocoele. The host range of these nematode is very wide (Poinar, 1979) but there is considerable variation in the virulence of different nematode isolates (Bedding *et al.*, 1983; Wright *et al.*, 1988) to different insect hosts. Ratnasinghe and Hague (1995; 1997; 1998) reported virulency of certain Steinernematid and Heterorhabditid nematode isolates against DBM. The present study was aimed to compare the efficacy of four nematode isolates against DBM and their effectiveness following field application.

MATERIALS AND METHODS

The DBM was cultured in the laboratory on Chinese cabbage cv. Wong-bok. The nematodes used were: *Steinernema carpocapsae* "All" isolate from Georgia, USA; *S. riobravis* from Texas, USA; *S. feltiae* UK isolate (Nemasys) and a *Heterorhabditis* sp. (Fargo,UK). Nematodes were cultured on late instar larvae of the greater wax moth, *Galleria mellonella* L, and the IJs were extracted on a modified White trap. They were stored at 6°C and were less than two weeks old when used in experiments.

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Determination of the efficacy of nematode isolates

Petri-dishes (5 cm) were lined with Whatman #1 filter paper and inoculated with each nematode isolate at 0, 10, 20, 40, 80, and 160 IJs in 0.25 ml distilled water. Five 4th instar DBM larvae were placed in each Petri-dish which was sealed with parafilm. Replication was twelve fold. After 48 h exposure period at 23-24°C, mortality was recorded and larvae dissected to confirm infectivity. The LD_{50} and LD_{90} values at the 95% fiducial limits (FL) were calculated using probit analysis (SAS).

Application of nematodes on potted cabbage plants

One month old Chinese cabbage seedlings (cv. Wong-bok) were transplanted into 30 plastic pots (25x35cm) and kept in a glasshouse. When they were two months old, plants were infested with 20 late instar DBM larvae. After 6h, plants were sprayed with 15-20 ml of the each six treatments (Table 2), containing approximately 1000 IJs/ ml, using a hand sprayer. Replication was five-fold in a completely randomized design. Insects were collected 24 h after spray application and were placed in Petri-dishes lined with moistened filter paper. Mortality of the DBM larvae and number of nematodes found in each larvae were recorded after a 48 h incubation period at 22-23°C.

Application of nematodes under field conditions

The trial was conducted at the Regional Agricultural Research and Development Centre, Bandarawela. One month old seedlings of common cabbage (cv. AS) were transplanted in 2.5x2m^2 plots at 50x40 cm spacing. Fertilizer application and other cultural practices were done according to the recommendations made by the Department of Agriculture. The six treatments (Table 3) were made at weekly intervals using a back pack knapsack sprayer, commencing one month after planting. The rate of application was 20,000 to 30,000 IJs per plant. Replication was three-fold in randomized complete block design. Number of insect larvae found on nine plants (net plot) was recorded three days after each application. Percentage of leaf damage and weather data was also recorded.

Effect of commonly used pesticides on nematodes

The effect of commonly used pesticides on the efficacy and survival of nematodes were tested. Nematode suspension containing 5000-6000 IJs in 1 ml was added to 2 ml of each pesticide solution (Table 3). A sample of 0.05 ml was removed after 1 and 6 h, and observed under the dissecting microscope to record the number of surviving and dead nematodes; replication was five-fold. During the same time, a sample of 0.05 ml (containing approximately 100 IJs) was withdrawn from the treated solutions and put into 5 cm Petri-dishes lined with filter paper. Distilled water (0.2ml) was also added to provide sufficient moisture on filter paper and to further dilute the pesticide. Five late instar DBM larvae were introduced into each dish, sealed with parafilm and kept in an incubator at 22-23°C. Replication was five-fold. DBM larvae were dissected 48 h after infection and records were made on mortality of insect larvae and number of nematodes found in each insect.

RESULTS

All the nematode isolates tested were lethal to late instar larvae of DBM and *S. carpocapsae* was the most virulent. The LD_{50} values for the Steinernematids were significantly lower than for the Heterorhabditid tested (Table 1). The significant difference ($P<0.05$) for the LD_{50} and LD_{90} values were considered when there was no overlap in 95% fiducial limit. The difference between LD_{50} and LD_{90} was least as 3.8 for *S carpocapsae,* followed by 9.6 and 18.3 for *S. riobravis* and *S. feltiae*, respectively.

Table 1. Dose-mortality response of DBM larvae to different entomogenous nematodes

Nematode	*LD_{50} (95% FL)*[a]	*LD_{90} (95% FL)*[a]	*Slope SE*[b]
S. carpocapsae	1.9 (1.4-2.4)	5.7 (4.7-7.8)	2.72±0.41
S. feltiae	3.6 (2.6-4.6)	21.9 (15.8-36.5)	1.64±0.21
S. riobravis	2.5 (1.8-3.2)	12.1 (9.3-17.9)	1.87±0.25
Heterorhabditis sp.	28.5 (20.9-45.9)	198.6 (100.8-654.8)	1.52±0.22

[a] LD_{50} and LD_{90} expressed as infective juveniles per insect; [b] SE at DF=1; Figures within parenthesis refer to range

There was a significant difference ($P<0.05$) in the mortality of DBM larvae among the treatments tested on potted cabbage plants (Table 2). The highest mortality was achieved when *S. carpocapsae* was applied with 0.5% of the antidesiccant T45. However, there was no significant difference among the number of *S. carpocapsae* juveniles recovered. All anti-desiccants has increased the efficacy of nematodes. Mixing nematodes with commercial preparation of *Bacillus thuriengiensis* (Delfin) did not enhance the efficacy of nematodes.

Table 2. Efficacy of nematodes against DBM larvae feedings on cabbage foliage

Treatment	*% mortality due to nematodes*	*No. of nematodes/ insect larvae*
S. carpocapsae	36.7 c	3.5a
S. carpocapsae +Lyle water(wetting agent)	53.6 ab	2.8a
S. carpocapsae +Lyle water +*B. thuriengiensis*	41.6 bc	3.0a
S. carpocapsae +T45(wetting agent)	64.5 a	3.4a
S. riobravis +Lyle water	19.4d	1.6b
Control	0.0 e	0.0c

Means within a column followed by the same letter are not significantly different at 5% level according to DMRT

The insect pest infestation was very low in the field trial conducted at Bandarawela. However, slight infestation of DBM and cabbage semi-looper (*Chrysodeixis eriosoma*) were observed in that trial. Only three foliar applications of nematodes were made as and when necessary due to the low infestation. Though, there

was no significant difference (P<0.05) in semi-looper population, damage index was significantly different during second application, but not in DBM (Table 3).

Table 3. Observation on cabbage plants treated with nematodes under field conditions

Treatments	*3DA1A*			*3DA2A*			*3DA3A*		
	Mean no. of loopers/ 9 plants	*Mean no. of DBM/9 plants*	*Damage index*	*Mean no.of loopers/ 9 plants*	*Mean no.of DBM/ 9 plants*	*Damage index*	*Mean no.of loopers/ 9 plants*	*Mean no.of DBM/ 9 plants*	*Damage index*
S. carpocapsae	2.0	1.3	1.0	2.0^{ab}	2.0	1.0^{b}	1.7	2.3	1.0
S. carpocapsae +LW(MA)	3.0	4.7	1.0	2.7^{a}	3.0	1.0^{b}	0.7	1.7	1.0
S. carpocapsae +LW(EA)	2.0	1.3	1.0	0.7^{bc}	1.3	1.0^{b}	0.0	1.3	1.0
S. riobravis +LW(MA)	2.7	2.0	1.0	2.0^{ab}	2.3	1.0^{b}	0.3	3.3	1.3
S. riobravis +LW(EA)	5.7	1.3	1.3	2.0^{ab}	2.3	1.7^{a}	1.0	2.0	1.0
Chlorfluazuron	1.3	1.7	1.0	0.0^{c}	2.0	1.0^{b}	0.0	1.7	1.0
Control	2.7	1.3	1.3	2.3^{a}	3.7	2.0^{a}	2.0	3.7	1.7
	ns	ns	ns		ns		ns	ns	ns

Means within a column followed by the same letter are not significantly different at 5% level according to DMRT; Scale for damage index: 0= No damage; 1= Less than 10% leaf damage; 2=10-25% leaf damage; 3=25-50% leaf damage; 4=More than 50% leaf damage; 3DAIA = 3 days after 1st application; 3DA2A = 3 days after 2nd application; 3DA3A = 3 days after 3 rd application; MA =Morning application; EA= Evening application; LW = Lyle wetter

None of the pesticides tested caused any adverse effect on the survival of nematodes when IJs were in pesticide solutions for up to 6 h (Table 4). The survival rate was almost 100% with all the treatments but they did affect the infectivity of nematodes. It is interesting to see that Ethofenprox, Chlorpyrifos and Bitertanol increased the efficacy of nematodes resulting in the recovery of more nematodes from the insects.

Table 4: Effect of pesticides on nematodes and their efficacy

Treatment	*% Survival of IJs*		*% Mortality of insects*		*Nematodes recovered*	
	1h	*6h*	*1h*	*6h*	*1h*	*6h*
Chlorfluazuron 1.5 ml/l	100.0^{a}	100.0^{a}	76.0^{b}	76.0^{b}	22.0^{b}	22.0^{b}
	(0.0)	(0.2)	(9.8)	(4.0)	(2.8)	(2.3)
Ethofenprox 2.25 ml/l	99.6^{a}	99.8^{a}	92.0^{a}	80.0^{b}	52.2^{a}	42.0^{a}
	(0.0)	(0.2)	(4.9)	(6.3)	(12.0)	(2.8)
Prothiophos 2.7 ml/l	99.6^{a}	99.8^{a}	100.0^{a}	44.0^{c}	22.2^{b}	7.0^{b}
	(0.0)	(0.2)	(4.9)	(7.5)	(3.1)	(1.3)
Chlorpyrifos 3.0 ml/l	98.6^{a}	99.6^{a}	100.0^{a}	84.0^{a}	58.0^{a}	28.8^{a}
	(0.6)	(1.5)	(7.4)	(6.3)	(3.7)	(1.1)
Bitertanol 1.5 ml/l	99.6^{a}	99.6^{a}	100.0^{a}	84.0^{a}	58.0^{a}	28.8^{a}
	(0.2)	(0.4)	(0.0)	(11.0)	(9.1)	(5.6)
Control	99.6^{a}	99.6^{a}	100.0^{a}	88.0^{a}	27.2^{b}	25.8^{a}
	(0.2)	(0.4)	(0.0)	(4.9)	(2.1)	(4.7)

Means within a column followed by the same letter are not significantly different at 5% level according to DMRT; Figure within parenthesis refers to standard error

DISCUSSION

The results of the present experiments indicate that the "All" isolate of *S. carpocapsae* is the most virulent against DBM larvae. Glazer (1992) suggested that *H. bacteriophora* (HP88) is relatively ineffective against Lepidoptera larvae. Though *S. feltiae* was also effective, it is considered to be a nematode adapted to cooler temperatures (Hominik and Briscoe, 1990). On the other hand, *S. riobravis* has been isolated from Texas (Raulston *et al.*,1992) which has a very high temperature profile (Grewal *et al.*,1994). Ratnasinghe and Hague (1995; 1997) reported that virulency and potential of *S. carpocapsae* is greater than the other two Steinernematids, against DBM.

Foliar application of entomogenous nematodes on potted plants has successfully reduced DBM population to an acceptable level. Harris *et al.* (1990) found that foliar application of *S. carpocapsae* in the greenhouse provided significant control of *Liriomyza trifolii* on chrysanthemum. Glazer and Navon (1990) reported a 75-90% mortality of *Heliothis armigera* when *S. carpocapsae* was applied on foliage with anti-desiccant. However, data obtained from the present field experiment is not sufficient to discuss about the success in field application. Glazer *et al.*(1992) recommended an application of *S. carpocapsae* in water at a rate of 500-1000 IJs/ml for the control of *Earias insulana* and *Spodoptera litoralis* on bean which caused more than 85% mortality. Though *S. riobravis* adapted to high temperature, Ratnasinghe and Hague (Unpubl. data,1999) found that the very low persistence on foliage could be due to the higher metabolic activities. Since tropical environmental conditions adversely affect persistence of nematode on foliage, they may be more efficient under overcast and moist conditions. Further, the present study revealed that commonly used chemical pesticides do not adversely affect nematodes. Therefore, they are compatible with most pesticides. Ishibashi (1987) reported that various chemicals induce the activity of nematodes as found in the present experiment.

CONCLUSIONS

Although the control of insects biologically with entomogenous nematodes is universally desired, the technology for production and field application is still relatively new. Therefore, selection of appropriate strains, correct timing and application procedures are necessary for successful control programmes. The present study shows that Steinernematids, especially *Steinernema carpocapsae* "All" isolate is a very effective entomogenous nematode against DBM. Although its efficacy has been proven under laboratory and glasshouse conditions, further research are needed to test its suitability under field conditions.

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