ORIGINAL ARTICLE

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Infectivity prospects of both nematodes and bacterial symbionts against cotton leafworm, *Spodoptera littoralis* (Biosduval) (Lepidoptera: Noctuidae)

Abstract A study was made on the susceptibility of the cotton leafworm, *Spodoptera littoralis* (Biosd.), to the nematode, *Steinernema carpocapsae* (All) and *Heterorhabditis bacteriophora*. Three concentrations of each nematode species were used (75, 150, and 300 infective juveniles) for each treatment. The nematode, *H. bacteriophora*, gave 100% mortality 96, 90, and 48 h, respectively, post-treatment of *S. littoralis* larvae with 75, 150, and 300 infective juveniles. On the other hand, *S. carpocapsae* (All) gave 100% mortality 120, 90, and 56 h post-treatment, respectively. Therefore, *H. bacteriophora* was more potent against cotton leafworm than *S. carpocapsae*. Studies on the infestation intensity of the nematode species against the cotton leafworm showed the predominance of *H. bacteriophora* over *S. carpocapsae*, while in studies of the reproductive rate those of *S. carpocapsae* predominate. The bacterial symbionts of each nematode species were isolated and tested alone against cotton leafworm at concentrations ranging between 1×10^2 and 5×10^3 bacterial cells/larva. The results showed a higher activity of *Photorhabdus luminescens* than that of *Xenorhabdu nematophilus*.

Keywords Cotton leafworm · Entomopathogenic nematodes · Bacterial symbionts · Infestation intensity · Production rate

Introduction

Agriculture is one of the major sectors of Egyptian economy. Egyptian cotton is the most important crop, playing a great role in this sector. This crop faces many problems with insect pests, of which cotton leafworm is

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the major pest (Hammad 1974; Hosny et al. 1976). The use of biological control agents against this pest in Egypt (Salama et al. 1981; Rizk et al. 1977) faced many problems due to the indiscriminate use of chemical insecticides. In spite of these problems, there is still considerable research interest in the subject. Efforts continue to be made in the augmentation and introduction of natural enemies. However, these biological controls are sensitive to the common chemical insecticides used by farmers.

The development of entomopathogenic nematodes and their bacterial symbionts as biological control agents against insect pests is receiving a great deal of attention for the following reasons: they provide environmentally safe insect control; they can be engineered genetically, thereby changing their pathogenicity and persistence (Bonning and Hammock 1996); some of the protinacious toxins they produce can be transferred to and expressed in crop plants or other microorganisms (Raichon et al. 1994); or they can be used as inundative or inoculative biological control agents. Like most chemical insecticides, it kills a wide spectrum of insects species rapidly (Morris 1985); the insect host is killed by septicemia induced by symbiotic bacteria released from the guts of infective juveniles when they invades the hemocoel of the insect (Poinar 1983).

The bacterial symbionts create a favorable environment for the nematode to propagate by suppressing the immune protein of the insect (Gotz et al. 1981) and provide nutrition for the nematode to develop and reproduce (Poinar 1983). The mutualistic association between the bacteria and the nematode makes it difficult for the insect to develop resistance against the parasite. There is a paucity of published information concerning the effect of bacterial symbionts alone on insect pests. The recent studies of Abdel-Razek (Abdel-Razek and Gowen 2002; Abdel-Razek 2003) reported laboratory tests which showed the capability of the bacterial symbionts to infect the larvae of *Galleria mellonella* (L.) and larvae and pupae of *Plutella xylostella* (L.).

This paper reports on the evaluation of the effects of *S. carpocapsae* All, *H. bacteriophora*, and their bacterial symbionts *Xenorhabdus nematophilus* and *Photorhabdus luminescens* with their two phases (I and II) separately against the cotton leafworm, *S. littoralis* (Biosd.), which occupies a foliar habitat, has developed multiple resistance to most insecticides and is difficult to control.

Materials and methods

Insects

The cotton leafworms were obtained from a laboratory pure culture maintained on *Ricinus* plant leaves at the National Research Centre. The greater wax moth larvae used in nematode culturing were raised in the laboratory on a semi-artificial diet as described by Grewel (1992). The insect cultures were maintained at $28 \pm 2°C$, $60 \pm 5\%$ RH and 16:18 (L:D) photoperiod.

Nematodes

The entomopathogenic nematodes, *S. carpocapsae*, strain All and *H. bacteriophora* were obtained from Dr. Gown, Department of Agriculture, Reading University and maintained at the laboratory of microbial control of insect pests, National Research Centre. Nematodes were propagated by passage through last instar larvae of *G. mellonella* (L.), adopting the method of (Dutky et al. 1964).

The infective juveniles (IJs) were harvested using White traps (White 1927). The IJs of each species were maintained in deionized distilled water at 5 –7°C for *S. carpocapsae* and 15°C for *H. bacteriophora*.

Isolation of symbiotic bacteria

The bacterial symbionts for each nematode species were isolated as described by Abdel-Razek (2003). Pure cultures of primary bacteria were obtained after 48-h incubation at 25°C. Culture purity was tested by subculturing on triphenultetrazolium chloride medium. All bacterial cultures were incubated at 25°C.

Infectivity tests with nematodes

Three different concentrations (75, 150, and 300 IJ/ml distilled water) of each nematode species were counted and prepared as described by Woodring and Kaya (1988). The infectivity of the two nematode species for *S. littoralis* larvae was determined by the filter paper method outlined by Kondo and Ishibashi (1986). Individual cotton leafworm larvae were placed in 9-cm diameter Petri dishes lined with Whatman No. 1 filter paper. The larvae were left overnight in Petri dishes to defecate before the respective IJs for each nematode species were added. The dishes were examined at time intervals of 12, 24, 48, 56, 72, 90, 96, and 120 h. The dead larvae were counted, collected, and washed in 0.1% formalin and each placed in a sterilized 3-cm diameter Petri dish with a strip of wetted filter paper. One group was tested after 4 days for the calculation of the infestation intensity. Another group was used for the calculation of the number of IJs that will emerge from the cadaver, following the methods described by (Glazer and Lewis 2000).

Infectivity tests with symbiotic bacteria

The last instar larvae of the cotton leafworm, *S. littoralis*, were surface sterilized with 70% alcohol and rinsed in sterile distilled water at the injection site, which is the posterior lateral part of the larvae. The larvae were then injected under aseptic conditions with 10 µl of the bacterial strains in an autoclaved phosphate buffer solution containing 5×10^3, 1×10^3, 5×10^2, 1×10^2, bacterial cells/larvae. A similar volume of sterile phosphate buffer was injected into larvae as control. After injection, the treated and control larvae were placed in Petri dishes (9-cm diameter) with a strip of moist filter paper and incubated at $28 \pm 2°C$.

Each treatment involved ten larvae and was done in three replicates. Larval mortality and color change were recorded for 4 days.

Statistical analysis

The percentage mortalities of cotton leafworm were taken for 5 days at different intervals, i.e., at 12, 24, 48, 65, 72, 90, 96, and 120 h. The dead larvae were dissected and examined for the presence of nematodes and for productive rate and a corrected mortality percentage applied at analysis. A probit analysis of the dose response for the treatments with bacterial strains was performed using the computer program, Stat1.

Results

Infectivity tests with nematodes

Table 1 shows that the treatment of the cotton leafworm with 300 IJ/ml killed almost all larvae within 48 and 56 h of treatment with *H. bacteriophora* and *S. carpocapsae*, respectively. At an inoculum level of 150 IJ per dish, more than 50% were killed; after 48 and 56 h, it was 70 and 80% for *H. bacteriophora* and *S. carpocapsae*, where as 100% mortality for this dose occurred after 90-h exposure.

On the other hand, the 100% mortality for the dose of 75 IJ/ml was reached after 96 and 120 h of exposure to *H. bacteriophora* and *S. carpocapsae*, respectively.

Table 1 Percent mortality of *S. littoralis* larvae infected with the infective juveniles (IJ) of the different nematode species

Time (h)	*H. bacteriophora*			*S. carpocapsae*		
	Dose (No. IJ/ml)			Dose (No. IJ/ml)		
	75	150	300	75	150	300
12	–	–	25	–	–	15
24	30	50	75.4	25	30	43
48	45	70	100	56	43	85
56	72	90		70	80	100
72	85	95		75	96	
90	96	100		90	100	
96	100			90		
120				100		

The results showed that *H. bacteriophora* kill *S. littoralis* larvae more rapidly than with *S. carpocapsae*..

Results of Table 2 show the infestation intensity of the cotton leafworm by each nematode species. This was calculated by counting all the IJs, fourth stage and all the males and females of the first generation after infecting the larvae. The results showed that the degrees of infestation intensity were significantly greater in the case of infection with *H. bacteriophora* than with *S. carpocapsae*. This was 19.3, 35.7, and 64.5 as compared to 13.5, 25.4, and 55.9 for the concentrations 75, 150, and 300 IJ/ml water, respectively.

Table 2 shows also that the rate of infective juvenile production from dead larvae was greater in infection with *S. carpocapsae* than with that in infection with *H. bacteriophora*. This was 30,000, 45,500, 64,300 as compared to 12,100, 32,000 and 49,500 for the concentrations 75, 150, and 300 IJ/ml water, respectively.

Infectivity tests with symbiotic bacteria

Data in Table 3 show that the injection of phase I of *P. luminescens* gave a significantly low LD_{50} value compared with that for injection with *X. nematophilus*.

The same trend was also observed for the injection with phase II of the bacterial symbionts.

The results show the predominant effect of phase I of both bacterial symbiont species compared with that of phase II against the cotton leafworm larvae.

The LD_{50} for phase I was 25.80 ± 1.03 and 48.55 ± 1.03 cells/larvae for *P. luminescens* and *X. nematophilus*, respectively. This is compared to 68.04 ± 1.90 and 75.64 ± 3.84 cells/larvae for phase II of *P. luminescens* and *X. nematophilus*, respectively.

Discussion

Entomopathogenic nematodes show great variation in their pathogenicity to insects; some of the strains are highly specific (Georgis and Manweiler 1994). In this study *H. bacteriophora* proved to be highly pathogenic to *S. littoralis* compared to *S. carpocapsae*. It gave a 100% mortality to larvae of *S. littoralis* within 48 h, while *S.carpocapsae* gave 100% mortality 56 h after infection. This may result either from some of the difficulties of *S. carpocapsae* to locate the host or from the lesser effects of the proteolytic enzymes produced by the symbiotic bacteria, *X. nematophilus*, compared to that of enzymes produced by the symbiotic bacterium, *P. luminescens*, which is associated with the nematode, *H. bacteriophora* (Homminick and Reid 1990; Dunphy and Webster 1986).

It is possible that the increase in infestation intensity by *H. bacteriophora* into *S. littoralis* is due to the ability of the nematode to enter directly through the cuticle (Mracek et al. 1988) or possibly the infected cadaver is very attractive to IJs through the period of exposure.

The data obtained demonstrated that the greater infestation intensity of *S. littoralis* is positively correlated with the dose of the nematode applied.

Also, this study showed a positive correlation between the rate of productivity of the nematode applied and the dosage used against the cotton leafworm. These results are in great agreement with those of Poinar and Thomas (1967), Kaya and Brown (1986), and Reardon et al. (1986). So, these nematodes need to be produced successfully in fermenters to be exploited commercially to compete with the insecticides used against the lepidopterous pests of the cash crop, cotton.

The pathogenesis of hetrorhabditid and steinernematid nematodes is due in large part to their symbiotic bacteria of the genus *Photorhabdus* and *Xenorhabdus*, which are carried in the alimentary tract of the IJs (Thomas and Poinar 1979). The symbiotic bacteria, *P. luminescens*, proved to be highly pathogenic when

Table 2 Mean infestation intensity of *S. littoralis* infected with the different nematodes and the mean reproductive rate of nematodes in insect cadavers

Dose (IJ/ml)	*H. bacteriophora*		*S. carpocapsae*	
	Mean infestation intensity/larva	Mean IJ/ cadaver	Mean infestation intensity/larva	Mean IJ/ cadaver
75	19.3	12,100	13.5	30,000
150	35.7	32,000	25.4	45,500
300	64.5	49,500	55.9	64,300

Table 3 Comparative effects of phases I and II of the bacterial symbionts of the different nematodes expressed as LD $_{50}$ to the last instar larvae of *S. littoralis*

Bacterial phase	*Photorhabdus luminescense*		*Xenorhabdus nematophilus*	
	LD$_{50}$ (cells/larve) (95% conf. limits)	Slope $\pm$ SE	LD$_{50}$ (cells/larve) (95% conf. limits)	Slope $\pm$ SE
Phase I	25.80 (22.90 – 29.30)	4.20 $\pm$ 0.86	48.55 (38.00 – 60.40)	1.65 $\pm$ 1.03
Phase II	68.04 (50.99 – 83.01)	1.80 $\pm$ 1.90	75.64 (67.99 – 89.03)	2.68 $\pm$ 3.84

injected into the hemocoel of *S. littoralis* as compared to the pathogenicity of *X. nematophilus*, which was much lower. These observations are in great agreement with earlier studies of Forst et al. (1997), Akhurst (1986), and Boemare and Givaudan (1998). These results however, showed the importance of both phases I and II of the bacterial symbionts of the different nematodes in the toxicity and control of the cotton leafworm in a short time, but this depends mainly on the treatment, as also observed by Akhurst (1986) and Clarke and Dowds (1991).

These results showed that the manner of introducing the toxin and the growth potential of the cells both contribute towards pathogenicity and rate of killing of the larvae of cotton leafworm, which also depend mainly on the immunity virulence towards the bacterial symbionts.

Most symbiotic bacteria occur in two phenotypic forms, phase I and phase II cells. Those of phase I are larger and produce greater amounts of exoenzymes, toxins, and antibiotics than phase II forms. The differential toxicity of the two bacterial symbionts under study at both phases could be attributed to the produced exoenzymes that effect the virulence of the bacteria towards their host.

Akhurst (1985) found that *X. poinarii*, which has slight lipase and no lecithinase activity, kills *G. mellonella* at only 0.01–0.1 times the rate of other species of *Xenorhabdus* and *Photorhabdus*.

The reason for the difference in the effects of viable cells of phases I and II of the bacterial symbionts may be because they could survive the immune response of the host insect in different degrees and present the toxic components on its surface. Lipopolysaccharides (LPS) are components of the outer membrane of the bacterial cells and have been shown to damage the hemocyte and inhibit activation of the humoral immune system (Dunphy and Webster 1988, 1991).

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