Evaluation of Two Nematode Strains Against the Egyptian Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in the Laboratory

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ABSTRACT

Efficacy of the two nematode strains; *Steinernema riobravae* and *Heterorhabditis* sp. (ISK-2) (Egyptian isolates) on the fourth larval instars of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) was studied under laboratory conditions. Data indicated that *S. riobravae* was more effective than *Heterorhabtidies* sp. (ISK2) and the concentration of 75 IJs /l gave highest mortality percentages compared with the other concentrations tested. The infectivity of *S. riobravae* at the concentration of 15 IJs/l was tested against the prepupae, pupa and the adult stages of *S. littoralis*. Data indicated that the pupae were less susceptible to nematodes' infection in soil than prepupae and adults. Mortality of pupae exposed to nematode was decreased from 38 to 29 % by increasing the exposure time from 3 to 7 days after the prepupae browning into the soil. In addition, there was a malformation of adults emerged treated pupae. Adults of *S. littoralis* were susceptible to nematodes' infection as they emerged from the soil. The majority of nematode induced mortality within 24 hr after emergence and many abnormal adults were observed; they could not mate or lay eggs. Data indicated also that, the appropriate stage for controlling *S. littoralis* by *S. riopravae* nematode is the prepupa, where 73-93% of the population was killed.

Key Words: *Spodoptera littoralis,* Nematode, Malformation, *Steinernema riobravae, Heterorhabditis* sp.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is well known as one of the most economic and destructive phytophagous pests on the majority of field and vegetable crops in Egypt, (Willcocks, 1937 and Shamseldean *et al.*, 1999). The Egyptian cotton leaf worm attacks all parts of plant of more than 70 cultivated crops, (El-Bermawy *et al.*, 1992). *S. littoralis* has acquired resistance to certain insecticides commonly used on various crops in Egypt (El-Guindy *et al.*, 1989; El-Sayed *et al.*, 1989 and Rashwan *et al.*, 1992). The need to reduce resistance to conventional insecticides as well as to find out environmentally safe insecticides has increased the urgency to develop alternative solutions (Hebert & Harper, 1987).

Entomopathogenic nematodes have recently received considerable attention as biological insecticides, particularly because of their availability for testing, ability to search for and kill hosts rapidly, ease of application and safety to mammals and plants (Gaugler, 1988). These nematodes kill insects with the aid of mutualistic bacterium, carried in their intestine; *Xenorhabdus* spp. and *Photorhabdus* spp. are associated with Steinernematid and Heterorhabditid nematode species, respectively (Boemare, 2002). Entomopathogenic nematodes are useful for managing pests that dwell in the soil because delivery of chemical pesticides to below-ground sites is difficult and because of the environmental concerns associated with application of traditional pesticides. Nematodes occur in the soil (Kaya, 1990) and have been used successfully against some soil-dwelling insect pests (Klein, 1990).

Entomopathogenic nematodes of the families *Steinernematidae* and *Heterorhabditidae*, are among the most suitable biological control agents for controlling *S. littoralis* and are considered potent biological control agents; Poinar (1986) and Adams and Ngyuine (2002). The susceptibility of *S. littoralis* developmental stages to *Steinernema carpocapsae* infection was reported by Ghaly *et al.* (1991). Reyad (2001) tested different inoculum levels of *S. carpocapsae* and *Heterorhabditis bacteriophora* against the larval instars of *S. littoralis* and stated that the level of 40 IJs/ml distilled water caused 100% mortality of the host.

In the present study, two nematode strains *Heterorhabditis* sp. (ISK-2) and *Steinernema riobravae* (Egyptian isolates) were used to test their efficacy against the cotton leaf worm *S. littoralis* under laboratory conditions.

MATERIALS AND METHODES

Heterorhabditis sp. (ISK-2), isolated from a soil sample taken from a newly reclaimed land at the eastern desert of Al-kassassin district,

Ismailia Governorate, Egypt and *S. riobravae* were cultured on the last instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Dutky *et al.*, 1964) Emerged infective juveniles (IJs) were harvested using the white traps and stored in distilled water at 15 °C for *Heterorhabditis* sp. (ISK-2) and at 4 °C for *S. riobravae*. Effect of the two strains, in the first experiment, was tested against the fourth larval instars of *S. littoralis* using plastic cups (15 x10 cm) filled with 150cc of sterilized soil mixture (2 parts sifted loam + 3 parts sifted sand) and moisted with 20 ml of distilled water each. The soil surface was moisted with 2 ml of distilled water containing the nematode species at the different concentrations; 0, 25, 50, 75 and 100 IJs/ 2 ml of distilled water, each was repeated three times. Five *S. littoralis* larvae were placed on the soil surface of each cup then supplied with a fresh clover bouquet as a source of food. All cups were covered by aluminum foil, kept at 25 ± 2 °C and checked daily to record dead larvae.

In another experiment, infectivity of *S. riobravae* against *S. littoralis* prepupae, pupae, and emerged adults was tested also at 25±2 °C. The same previous technique was followed but with newly formed *S. littoralis* prepupae. Five prepupae were placed in each cup and examined daily to record the time at which they entered the soil to pupate. Two ml of the nematode solution at the concentrations of 0 and 75 IJs/2ml (table 2) were evenly distributed on the soil surface four times, five days before placing the prepupae, at the same day of placing (against prepupae), 3 and 7 days after placing (against pupae). Pupae in all cups were carefully inspected, after 7 days of entering into the soil, to record dead individuals, also were dissected and examined for the presence of adult nematodes. Percent mortalities, in all experiments, were corrected with Abbot's formula, when it was necessary. In the tests on pupae, the criteria of nematode infection were based on successful adult emergence and the dissection, as described before, for un-emerged pupae. Adults emerged 10-12 days (after the prepupae entered the soil), were divided into two groups, the first was held in a glass jar with a sugar solution and observed to record the dead moths after 72 hr. which were dissected and examined for the nematodes' effect. The second group was kept for mating and laying eggs.

Statistical analysis

Statistical analysis was performed using the Analyze-it software (Analyze-it, Leeds, UK) according to the method of Maxwell and Delaney (1989).

RESULTS AND DISCUSSION

Fourth larval stage

Data presented in Table (1) show that the fourth instar larvae were more susceptible to *S. riobravae* than *Heterorhabditis* sp. (ISK-2) at all the tested concentrations. The data indicated also, that mortality percentage increased when nematode concentration of both strains was raised. Highest mortality percentage was obtained when the *S. riobravae* was used at the concentration of 75 IJs/2ml of distilled water. The analysis of variance presented in Table (2) indicates that the two nematode strains differed significantly and also the differences among the nematode concentrations were highly significant. This is in accordance with the findings of Sikora *et al.* (1979) who stated that, most developmental stages of *S. littoralis* were highly susceptible to *S. carpocapsae* infection, and the mortality was positively correlated with the parasite density. Similar findings were also reported by several authors, and Ghaly (1991) and Ghaly *et al.* (1992) who found that, the rate of development of *S. feltiae* was faster and the rate of reproduction was higher in *S. littoralis* than in *Musca domestica*. Also, Hatsukade and Grey (1996) showed a higher infectivity of *S. carpocapsae* to *S. littoralis* larvae. Khlibsuwan and Wirot-Khlibsuwan (1996) recorded a relationship between *S. carpocapsae* concentration and the number of nematodes invading *S. littoralis* larvae, also percentage of invasion increased with longer time of exposure.

Table (1): Corrected mortalities (%) of *S. littoralis* fourth instar larvae treated with different concentrations of *S. riobravae* and *Heterorhabtidies* sp.

Nematode sp.	Concentrations (IJs/2ml Distilled water)			
	25	50	75	100
S.riobravae	66	80	93	93
Heterorhabtidies sp.	26	40	66	93
$LSD_{0.05}$	28.7	28.3	19	14.1

Table (2): Analysis of variances of the efficiency of *S. riobravae* and *Heterorhabtidies* sp. (ISK-2) at different concentrations against fourth larval instar of *S. littoralis*.

S.V	d.f.	SS	MS	F	$F_{0.05}$
Nematode strain	1	612.9	1612.9	11.7**	7.7
Nematode concentrations	4	9013.6	2253.4	16.3**	6.38
Error	4	551.6	137.9		
$LSD_{0.05}$		28.3	19		

** Highly significant

Table (3): Corrected mortality percentages of *S. littoralis* prepupae and pupae in a soil treated with *S. riobravae* at different times from placing the prepupae.

Time of adding nematode* (treatments)	Stage	No of IJs/ 2 ml DW	Dead insects		Mortality %		No. of emerged adults	Normal adults %	Malformed adults %
			Pre- pupa	Pupa	Pre- pupa	Pupa			
5 days before	Pre	0	0	-	-	-	-	-	-
		75	11	-	73	-	-	-	-
Same day	Pre	0	0	-	-	-	-	-	-
		75	14	-	93	-	-	-	-
3 days after	Pupa	0	-	0	-	-	15	100	-
		75	-	5	-	33	10	40	60
	Pupa	0	-	0	-	-	15	100	-
		75	-	3	-	20	12	50	50

* Before / after placing *S. littoralis* prepupae. N = 15

Prepupal, pupal and adult stages

The nematode *S. riobravae,* was applied to the soil surface at: 5 days before adding *S. littoralis* prepupa, the same day and 3 and 7 days after adding the prepupa. Data shown in table (3) and fig. (1), indicated that the highest mortality percentage (93.0%) was obtained only when the nematode was added at the same day with the prepupae followed by prepupal, pupal and adult stages. The nematode *S. riobravae,* was applied to the soil surface at: 5 days before adding *S. littoralis* prepupa, the same day and 3 and 7 days after adding the prepupa. Data shown in table (3) and fig. (1), indicated that the highest mortality percentage (93.0%) was obtained only when the nematode was added at the same day with the prepupae followed by the treatment of 5 days before addition (73.0%), while the lowest mortality (20.0%) was recorded when the nematode was added 7 days after the prepupae. *S. littoralis* pupae were less susceptible to the nematode infections than the prepupae (Table 3). However, the mortality results showed differences based on length of exposure time and nematode concentration. Within the 3 and 7 days of pupal treatments, there was a significant difference in pupal survival. The dead pupae had a greenish-yellow hemocoel which was a condition atypical to a Steinernematid infection.

Adults of *S. littoralis* were susceptible to the infection by the nematodes as they emerged from the soil (Table 3). Most adult mortalities were recorded within 24 hr of adult emergence. Among the emerged adults 33 and 20 % pupal mortality were recorded by nematodes' applications at 3 and 7 days treatments, respectively (Table 3). Normal adults could mate and lay eggs ranged 40-50 % while 50-60 % was abnormal and failed to mate and lay eggs (Table 3) and (Fig 2). This was in agreement with the early reported data by Fry and McAdm (1972).

S. littoralis prepupae in soil and emerged adults were highly susceptible to *S. riobravae.* Because pupae in general are not susceptible to nematodes' infection in the soil, the susceptibility of emerged adults to these nematodes offers a new dimension for insect control.

The current data revealed that, although adults emerged in the nematode treatment, high mortality usually occurred within 24 hr after emergence, particularly at the higher nematodes' concentration

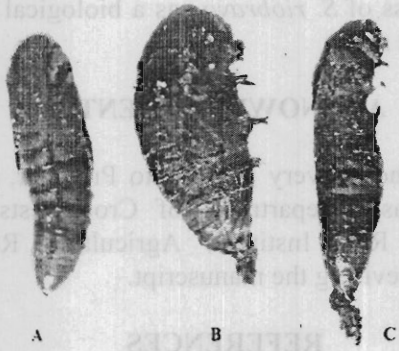

Fig (1): Malformation of pupal stage as results of nematodes' infection (A): Normal pupa, (B) & (C): Prepupae infected with *S. riopravae.*

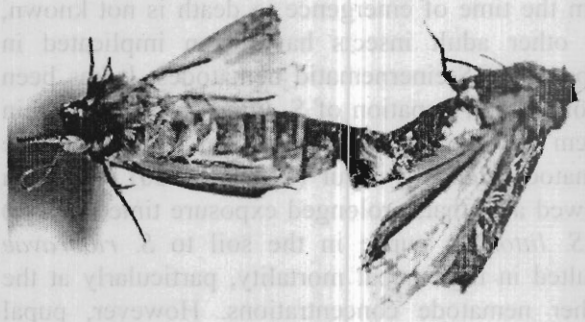

Fig (2): Malformed adults failed to mate and lay eggs due to nematodes' infection

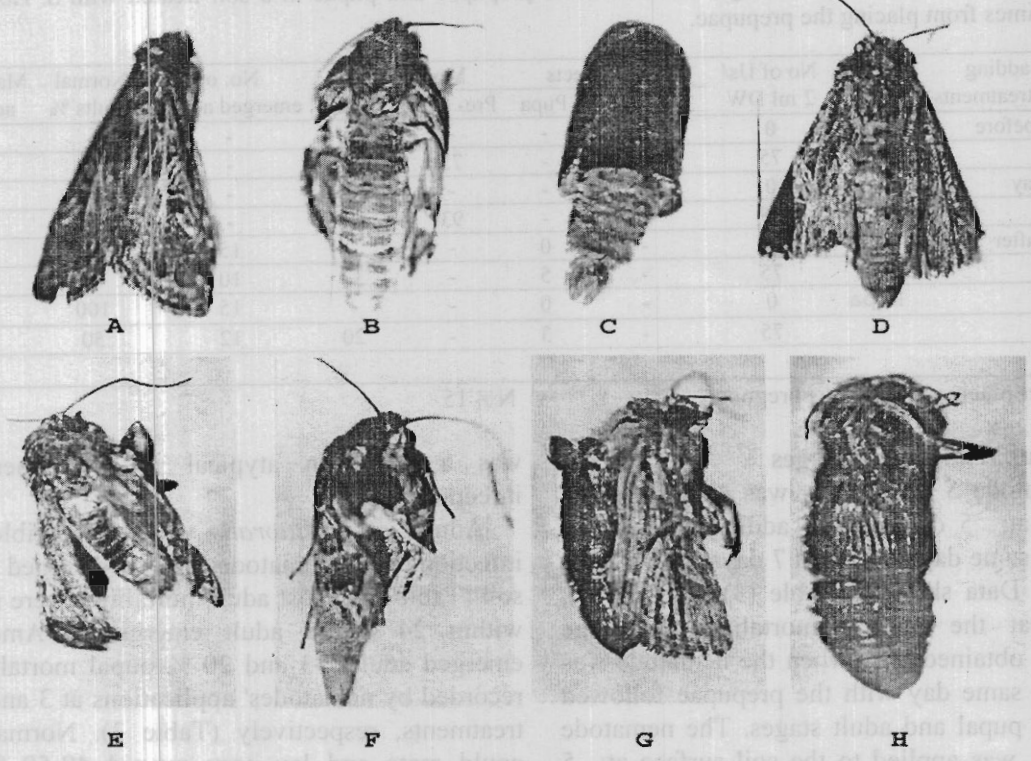

Fig (3): Malformed emerged adults. (A): Normal adult. (B) An adult failed to come out from pupal skin (C), shorting in wings (D) , dwarfing in adult size (E) ,wingless adult (F) , normal insect but the pupal skin stuck to the body (G) and the most obvious observation is that half of the body is an adult and the other half is still in pupal stage .

(75 IJs/ 2 ml of distilled water). These adults did not produce progeny because they could not lay eggs up to 3 days after emergence. Similar results were reported by Fry and McAdm (1972). Infected adults may aid nematodes' dispersal. As illustrated in Fig. (3), the adult malformations as a result of nematodes' infection. The majority of infected adults had nematode progeny, demonstrating that more than one infective nematode have entered the hosts. If the hosts die in a favorable location (*i.e.*, high moisture), the nematodes could reproduce and give a new generation. However, mobility of infected adults from the time of emergence to death is not known, but other adult insects have been implicated in dispersal of Steinernematid nematodes. It has been reported that, pupation of *S. littoralis* occurs within 1 cm of the soil surface where most of the nematodes tend to occur (Moyle, 1980). The data showed also that, prolonged exposure time (7 days) of *S. littoralis* pupae in the soil to *S. riobravae* resulted in high pupal mortality, particularly at the higher nematode concentrations. However, pupal mortality was not high in the present study as the mortality reported by Kaya and Hara (1980). Although data are not comparable because of different concentrations and techniques used, one of the reasons for the high pupal mortality in the earlier findings of Kaya and Hara (1980) was probably related to the continuous contact of the nematode with the pupae in Petri dishes.

The present study indicates that, the application of *S. riobravae* to soil pupating lepidopterous insects seems feasible. The prepupal and adult stages are the most susceptible to the nematodes, but some pupae are infected. Thus, the combined prepupal, pupal, and adult mortalities of *S. littoralis* increase the effectiveness of *S. riobravae* as a biological control agent in the soil.

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