Egypt. J. Agric. Res., 80 (3), 2002.

PRELIMINARY INVESTIGATION ON THE UTILIZATION OF ENTOMOPATHOGENIC NEMATODES AS BIOLOGICAL CONTROL AGENTS AGAINST THE PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) [DIPTERA : TEPHRITIDAE]

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(Manuscript received October 2001)

Abstract

The infectivity two steinernematid of entomopathogenic nematodes species, namely *Steinernema carpocapsae* All and *Steinernema riobravae* (Weiser 1955) Poinar1995 and two native isolates of *Heterorhabditis bacteriophora* Poinar 1975 (AS1 and Eg-1) to prepupae and pupae of peach fruit fly, *Bactrocera zonata*(Saunders)(Diptera Tephritidae)was evaluated . The data obtained indecated that , *Bactrocera zonata* prepupae are very susceptible to nematode infection, low differents between pupae and prepupae ,the mortality ranged between 16.67 to 93.70% and 28.72 to 91.49%, respectivaly.The two strains belonging to genus *Heterorhabditis* which seem to be infective to the pupal stage than those belonging to genus *Steinernema*. The opposite was true in case of the prepupal stage, where steinernematid species were more infective than heterorhabditid strains.

Key Words: Entomopathogenic nematodes; biological control, *Bactrocera zonata*

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* is native to South and South-East Asia, where it attacks a wide range variety of host plants soft fruits, *e.g.*, peach, guava, and mango (White and Elson-Harris, 1994; Allwood *et al.*, 1999) and is commonly known as the peach fruit fly. It is not known exactly when it has been spreaded in the Middle East Agroecosystem , but there is a record from Saudi Arabia dated 1982 and recently from Oman (CABI, 1996). By late 1990, it was well established in Egypt. There is also an old record from Egypt (Efflatoun, 1924), but that appears to have been based on a quarantine interception. In Egypt, few studies had been carried out throughout the last years on the biology and morphology of *B. zonata* (El-Minshawy *et al.*, 1999). Studies concerning method of biological control against this pest are very scanty or even com-

pletely not available .

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) have emerged as excellent insect biological control agents. Recent advances in mass-production and formulation technology and the discovery of numerous isolates/strains, together with the desirability of reducing pesticide usage, have resulted in a surge of scientific and commercial interest in these insect-killing nematodes (Gaugler and Kaya, 1990). Successful parasitization in nature by *Steinernema* and *Heterorhabditis* depends mainly upon host-nematode contact. Thus, there must be a period in the insect's life cycle when it contacts habitats favourable for nematode survival, such as borer galleries in bushes and trees and damp soil (Woodring and Kaya, 1988).

B. zonata larvae are known to pupate in the soil, few reports were given concerning the infectivity of entomopathogenic nematodes (EPN) to dipterous insect that pupate in the soil (*e.g.*, El-Sadawy, 1994; Zayed *et al.*, 1997). The present investigation aimed at evaluating four entomopathogenic nematodes as biological control agents against pupae of *B. zonata* under laboratory conditions.

MATERIALS AND METHODS

Host Insect : A laboratory culture of the peach fly, *B. zonata* was established from naturally occurring infestation in guava orchard in (Kerdasa , Giza Governorate). Larvae were reared on wheat bran medium at 25±2°C and 60-80% R.H., while adults were fed on sugar, protein hydrolyzate and water (Vargas *et al.*, 1993).

Nematode Source : Two steinernematid species, *i.e., Steinernema carpocapsae* All and *S. riobravae* and two native isolates of *Heterorhabditis bacteriophora* (AS1 and Eg-1) (Randa M. Abd El-Rahman, Unpublished), were used in the present study. The nematodes were kindly offered by Dr. M.H. El-Bishry, Plant Protection Research Institute. Nematodes were cultured on the last instar larvae of the wax moth, *Galleria mellonella* according to Dutky *et al.* (1964).

Experimental Design : Prepupae and pupae of *B. zonata* were subjected to nematode infection in small plastic cups (5x6x6 cm), each contained 20 cm 10% w/w moist sandy clay soil. Each cup contained 25 insects. Serial ascending concentrations of 250,

500, 1000 and 2000 infective juveniles (IJs)/cup (*i.e.*, 10, 20, 40 and 80 IJs/insect), were tested for each nematode isolate. Four replicates were made for each concentration, in addition to a control treatment, which received tap water only.

All cups were kept at 26±1°C and 80% R.H., after 4 days, insects were transferred to new cups containing clean sandy soil. Daily inspection was carried out for two weeks for monitoring insect mortality and adult emergence. Number of adults in each treatment was recorded and dead individuals were dissected to detect nematode infection. Mortality records due to nematode infection were calculated and corrected according to Abbott's formula (Abbott, 1925). Data were subjected to probit analysis according to Finney (1956), and LD_{50}, LD_{90} and slope values were calculated. Significance between obtained results of the different nematode species was determined according to the overlapping of the confidence limits at 95% probability.

RESULTS AND DISCUSSION

From the data presented in Table 1, it is evident that *B. zonata* prepupae are very susceptible to nematode infection. After 4-days exposure period, mortality ranged between 16.67 and 93.70%. Mortality was dose-dependent, *i.e.*, increasing nematode is accompanied by increased mortality. Based on the LD_{50} values derived from log-dose probit lines, Fig. 1, the two steinernematids: *S. riobravae* and *S. carpocapsae* were more virulent than the two-heterorhabditid nematodes (Eg-1 and AS-1). Significant difference was yielded between *S. riobravae* and Eg-1 and AS-1 and between *S. carpocapsae* (All). At the LD_{90} level *S. riobravae* and *S. carpocapsae* (All) were significantly more virulent than *H. bacteriophora* Eg-1 and AS-1.

From the data presented in Table 1,it is obvious that *B. zonata* pupae are susceptible enough to the tested entomopathogenic nematode strains. Mortality ranged between 28.72 and 91.49% after a four-day exposure period to nematode infection in sandy clay soil. Similar to the prepupal stage, mortality is dose-dependent. Based on the LD_{50} values derived from log-dose probit lines ,Fig. 2 , the tested strains can arranged descendingly according to their infectivity as follows: Eg-1, AS-1, *S. riobravae* and finally *S. carpocapsae* (All). However, Eg-1 significantly recorded the lowest LD_{50}, while insignificant differences were evident the other three nematode strains. The case

at the LD_{90} level, where significant differences were observed among the four tested nematode strains.

Generally, the two strains belonging to genus *Heterorhabditis* were much more infective to the pupal stage than those belonging to the genus *Steinernema*. The phenomenon can be attributed to the ability and capability of heterorhabditid nematodes in penetrating the host's cuticle using their teeth found in the labial region (Woodring and Kaya, 1988). Also, members of this genus are known to adopt a cruising behaviour for infecting a potential host and they are much more effective against sedentary insect stages (Gaugler *et al.*, 1989). The opposite was true in case of the prepupal stage, where steinernematid strains were more infective than heterorhabditid strains. The explanation may lie in the fact that, members of this species are known to adopt a sit and wait strategy for infection , hence, they termed "ambushers" and they are more effective against soil dwelling insects (Kaya and Gaugler, 1993). Other factors may also be responsible for the difference in the isolates infectivity, such as their ability to penetrate the host, the differences in toxins production by the nematode-bacteria complex and number of bacteria per nematode juvenile (Dunphy and Webester, 1986).

Some previous reports support our findings that EPN can be included in the integrated pest control of dipterous pests. El-Sadawy *et al.* (1997) and Zayed *et al.* (1997) reported that the DD-136 strain of *Steinernema feltiae* and two *Heterorhabditis* species (HP88 and Ht) are quite virulent to *Chrysomyia albiceps* and *Gastrophilus intestinalis*, pupae, respectively. Schroeder *et al.* (1996) reported that using of four entomopathogenic nematodes under greenhouse and field conditions could successfully control the cabbage maggot. The number of formed pupae was very much minimized in plots treated with nematodes.

CONCLUSION

The entomopathogenic nematodes can be used in IPM program to control the peach friut fly *B. zonata* which infests many fruit trees in Egypt and some other countries .

Table 1. LD_{50}, LD_{90} and slope values of four entomopathogenic nematode strains against prepupae and pupae of *B. zonata*.

Nematode strain	% Mortality				LD_{50}	LD_{90}	Slope	Index*
	10 IJs	20 IJs	40 IJs	80 IJs				
Prepupae								
S. riobravae	34.38	60.42	73.96	93.70	18.83a	100.86a	1.98	100
S. carpocapsae (All)	27.08	52.08	80.21	91.67	21.28ab	90.76a	2.28	86.28
H. bacteriophora (Eg-1)	26.04	43.75	68.75	81.25	27.74bc	209.79b	1.75	68.20
H. bacteriophora (AS-1)	16.67	45.83	57.29	82.29	32.61c	197.27b	1.95	57.28
Pupae								
H. bacteriophora (Eg-1)	39.36	69.15	79.79	91.49	12.74a	67.16a	1.78	100
H. bacteriophora (AS-1)	34.04	61.70	79.79	86.17	15.24a	85.15b	1.72	83.56
S. riobravae	31.91	65.96	76.60	78.72	15.31a	125.82c	1.40	83.21
S. carpocapsae (All)	28.72	47.87	63.83	74.47	23.77b	210.75d	1.35	53.58

• Based on the LD_{50} of the *S. riobravae* strain for prepupae and Eg-1 strain for pupae.

ACKNOWLEDGEMENT

We wish to thank Dr. M.H. El-Bishry, Plant Protection Research Institute, for offering the nematodes used in the present study and for his revision of an early version of the manuscript.

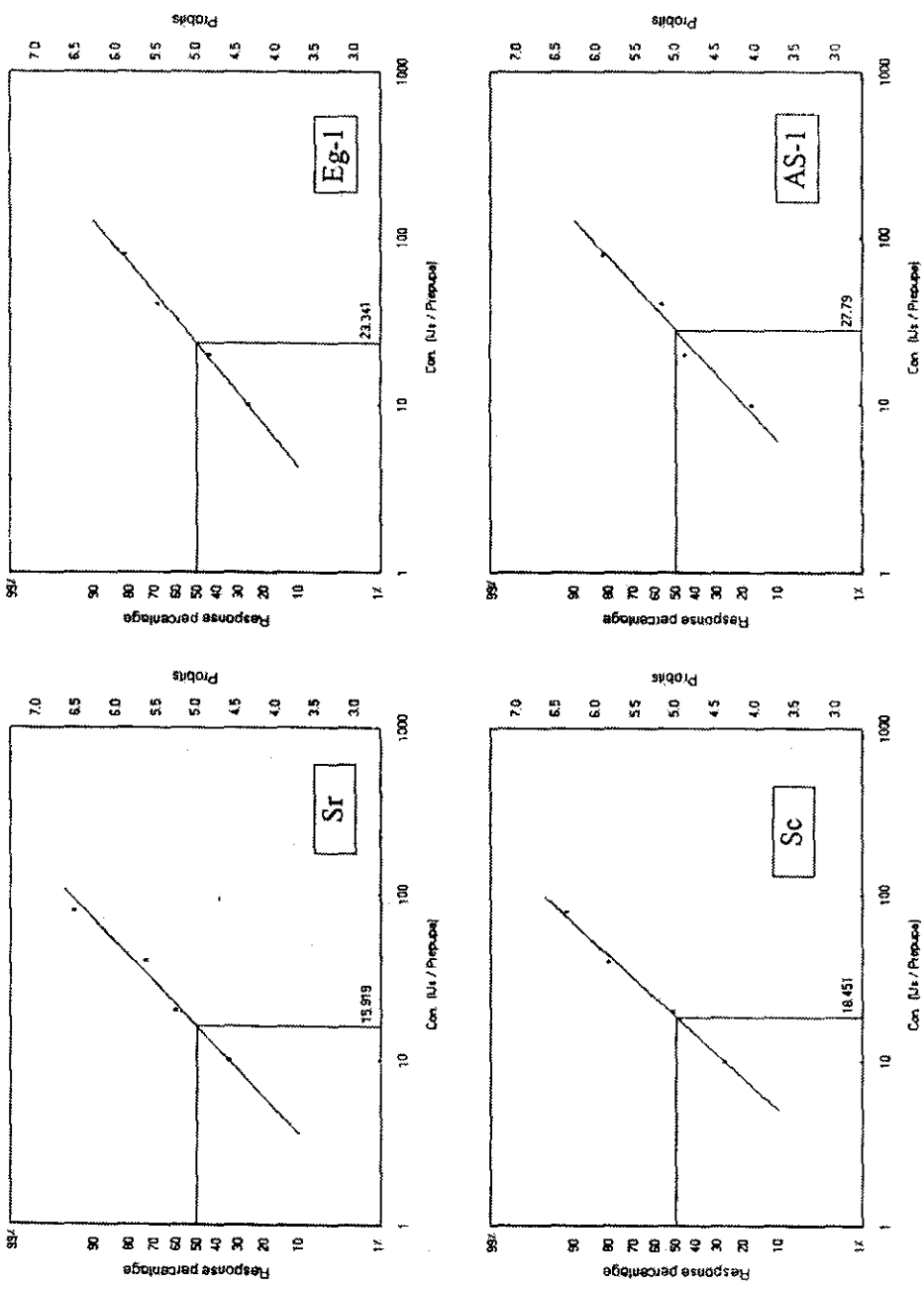

Fig. 1. Log-dose probit lines of four entomopathogenic nematode strains against *B. zonata* prepupae in sandy clay soil.

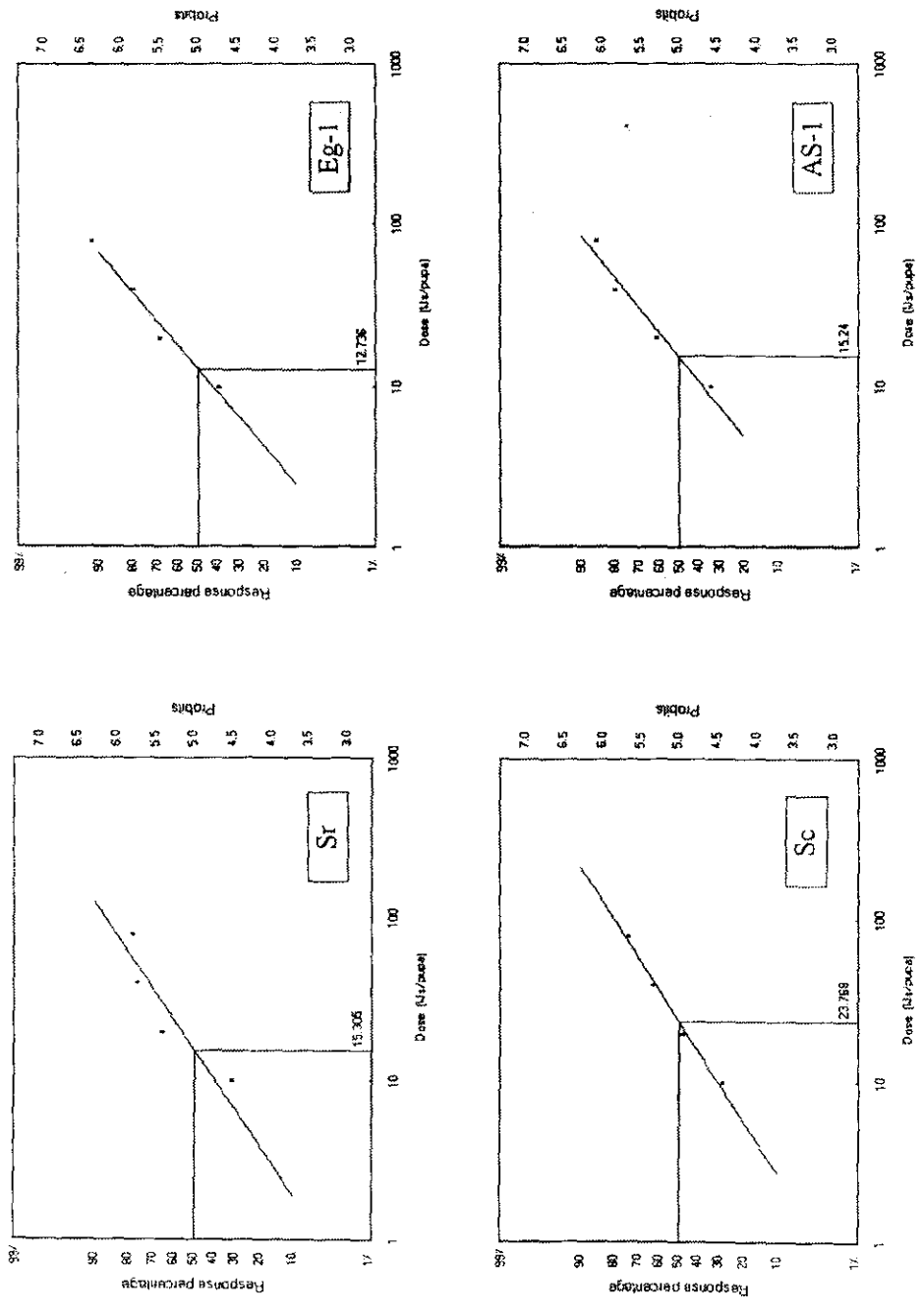

Fig. 2. Log-dose probit lines of four entomopathogenic nematode strains against *B. zonata* pupae in sandy clay soil.

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استخدام النيماتودا الممرضة للحشرات كوسيلة لمكافحة ذبابة ثمار الخوخ (بكتيروسيرا زوناتا) بيولوجياً

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تم دراسة فاعلية سلالتين من جنس ستشيرنيما وعزلتين من هيتيروربتيتدس ضد طور ما قبل العذراء وطور العذراء من حشرة ذبابة ثمار الخوخ (بكتيروسيرا زوناتا) حيث أشارت النتائج الي أن طور ما قبل العذراء كان اكثر حساسية للمعاملة بالنيماتودا وتراوحت نسبة الموت مابين ١٦,٦٧-٩٣,٧٠و ٢٨,٧٢-٩١,٤٩ في طور ما قبل العذراء والعذراء علي التوالي وأيضا كان جنس هتيروهبتيتس اكثر تأثيراً على طور العذراء عنه في جنس ستشيرنيما وعلى العكس من ذلك كان طور ما قبل العذراء حيث كان جنس ستشيرنيما اكثر تأثيراً عليه.