PATHOGENIC AND LETHAL EFFECTS OF THE ENTOMOPATHOGENIC NEMATODES ON THE PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) AND THE CUCURBIT FRUIT FLY, *DACUS CILIATUS* (LOEW) (DIPTERA : TEPHRITIDAE)

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Abstract

Laboratory experiments were performed to evaluate the pathogenic and lethal effects of the entomopathogenic nematodes Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strains) on the full grown larvae, newly formed pupae and seven days old adults of the peach fruit fly, *Bactrocera zonata* and the cucurbit fly, *Dacus ciliatus*. Mortality rates ranged from 9.3 to 42.7%, 12.7 to 52.3 % for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by Sc nematode, respectively, and from 67.3 to 100%, from 46.3 to 100% for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by Hb nematode, respectively, whereas mortality rates of pupae ranged from 2.7 to 32.7% for the pupae of *B. zonata* treated by Sc nematode, from 1.7 to 23.3% for the pupae of *D. ciliatus* treated by Sc nematode , from 12.7 to 51.7 % for the pupae of *B. zonata* treated by Hb nematode and from 6.3 to 39.3 % for the pupae of *B. zonata* treated by Hb nematode. Furthermore, the mortality rates varied from 35.0 to 78.7% , 7.7 to 50.3% for 7 days old adults of both *B. zonata* and *D. ciliatus* treated by Sc nematode, respectively, 41.7 to 90.3 and 17.0 to 67.7 % for 7 days old adults both *B. zonata* and *D. ciliatus* treated by Hb nematode, respectively. LC_{50} and LC_{90} values were 325.3, 286.2, 1718.6 and 1650.0 IJs/ cm^2 for larvae of *B. zonata* and *D. ciliatus* treated with Sc nematode, 28.8, 56.7, 167.2 and 156.1 IJs/cm^2 for larvae of *B. zonata* and *D. ciliatus* treated with Hb nematode, 540.2, 447.4, 1785.4 and 2009.8 IJs/ cm^2 for pupae of *B. zonata* and *D. ciliatus* treated with Sc nematode, 235.0, 420.8, 1167.0 and 1941.5 IJs/ cm^2 for pupae of *B. zonata* and *D. ciliatus* treated with Hb nematode, 116.8, 261.6, 319.3 and 375.0 IJs/ cm^2 for adults of *B. zonata* and *D. ciliatus* treated with Sc nematode and 77.3, 196.7, 290.8 and 253.7 IJs/ cm^2 for adults of *B. zonata* and *D. ciliatus* treated with Hb nematode, respectively. From the obtained results we can conclude that the entomopathogenic nematodes: Hb *Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strains) were effective on the different stages of *B. zonata* and *D. ciliatus* , Hb nematode was more virulent than Sc nematode and the larvae and adults of *B. zonata* and *D. ciliatus* were more susceptible to the nematodes infection than the pupae.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) is one of the most harmful insect species of family Tephritidae. It is a polyphagous species, but is particularly a pest of peach, mango and guava. It also, infests some vegetables as a secondary pest. It is a significant pest in many countries such as India, Pakistan, Indonesia, Sri-Lanka, Vietnam, Thailand, Burma, Nepal and Bangladesh. Publications from Pakistan show that it is possibly more important than *B. dorsalis* (Kapoor, 1993). In Egypt, *B. zonata* is now established and widespread. In Egypt, the cucurbit fly, *Dacus ciliatus* (Loew) was recorded for the first time by Azab and Kira (1954) as a serious pest on cucurbitaceous fruits , continued till 1980 and disappeared , then reappeared again after nearly 23 years (Fetoh, 2006). Current control methods of these pests rely heavily on the aerial application of malathion, bait sprays, or ground cover sprays of potent organophosphorus pesticides. These methods have a negative impact on the environment, and specifically on the phytoparasitic populations of beneficial organisms. Thus, environmentally friendly methods of control are much in need (Roessler, 1989). Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae have been shown to be pathogenic to a wide range of agricultural important pests and are useful alternatives to chemical insecticides for insect control (Gaugler and Kaya, 1990). The infective juveniles of entomopathogenic nematodes enter their host through natural openings (Steinernematidae and Heterorhabditidae) and rarely through the direct penetration of host cuticle (Heterorhabditidae) (Shapiro and Lewis 1999). Entomopathogenic nematodes kill their hosts though the association with the mutualistic bacteria, i.e. *Xenorhabdus* spp. in steinernematids and *Photorhabdus* spp. in heterorhabitids. These mutualistic bacteria release toxins or metabolites or proteases that finally kill the host within 2-3 days. The aim of the present work is estimating the pathogenic and lethal effects of the entomopathogenic nematodes, Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strains) on different stages of the *B. zonata* and *D. ciliatus* under the laboratory conditions.

MATERIALS AND METHODS

A culture of adult flies of *B. zonata* and *D. ciliatus* was maintained in the laboratory in Vegetable, Medicinal, Ornamental and Aromatic Insect Pests Researches Department, Plant Protection Research Institute, at 25±2°C, 80±10% R.H. and L12:D12 photoperiod. Larvae of *B. zonata* were reared on bran diet described by Tanaka *et al*., (1969). Larvae of *D. ciliatus* were reared on small marrow fruits

according to Fetoh (2006). Pupae were obtained by sieving the sandy layers at the bottom of rearing containers.

The nematodes, Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strains) were reared and multiplied on the greater wax moth larvae, *Galleria mellonella* L. according to the method described by Kaya and Stock (1997).

Nematode hosts exposure were carried out in Petri dishes (10cm) containing 30 gm of clean and dry sand. Different concentrations of EPN were prepared at: 50, 100, 150, 200 and 250 IJs/cm2 of both nematodes species and added to Petri dishes. 100 of full grown larvae (third or popped larval instar) and 100 of newly formed pupae were introduced to nematodes. Each concentration was replicated three times for each concentration for each insect species. Control test (untreated) was carried out at the same time in parallel to the nematodes tests.

Also, 100 adult flies (7 days old) were used for each concentration of the EPN in sugary solution put in small glassy vials (10ml) containing cotton wick piece, as a source of food, drink and nematode infection. Adult flies were put in large glassy pots (250ml), covered with piece of fabric and then tied by elastic threads. Control treatment had only sugary solution. Also, each concentration was replicated three times for each insect species. Control test (untreated) was carried out at the same time in parallel to the nematodes tests according to Fetoh and El-Gendi (2006).

All tests were observed daily to detect the mortality of exposed larvae, pupae and adults were recorded till 7days.

Data analysis

The mortality resulted from the effect of EPN was calculated and corrected according to Abbott's formula (Abbott, 1925). Log- probity lines and relative toxicity for different concentrations were obtained by Finney (1971) using a detected software program. Duncan's multiple range test (Duncan, 1955) was used to differentiate between the means of mortalities.

RESULTS AND DISCUSSION

Efficiency of the entomopathogenic nematodes (EPN) on different stages of PFF and CFF

1. On the full grown larvae (Leaping larvae or 3 rd instar larvae)

The obtained data in Table (1) indicate that the both used EPN were effective and virulent on the full grown larvae of PFF and CFF, while Sc nematode was lower virulence than Hb nematode on the full grown larvae of PFF and CFF.

The means mortality percent were increased in parallel manner with the increasing in EPN concentration (50, 100, 150, 200, 250 IJs/cm^2), means mortality percent

were ranged from 9.3 to37.7%, from 12.7 to 52.3% , for the full grown larvae of PFF and CFF, respectively. The means mortality percent were : 9.3, 14.7, 25.0, 37.7 and 42.7 % , respectively for PFF larvae treated with Sc nematode . The means mortality percent were: 12.7, 20.0, 26.7, 37.7and 52.3 %, respectively, for CFF larvae treated with Sc nematode.

Hb nematode showed the same effect and the means mortality percent were ranged from 67.3 to 100.0% for PFF larvae and from 46.3 to 100.0% for CFF larvae. The means mortality percent were: 67.3, 79.7, 86.3, 94.7 and 100.0% for PFF larvae treated by Hb nematode. The means mortality percent were: 46.3, 72.7, 86.7, 98.0 and 100.0% for CFF larvae treated by Hb nematode, respectively. Moreover, Hb nematode was highly virulent than Sc nematode on the full grown larvae of both tested flies (PFF and CFF). The larvae of CFF were more susceptible to Sc and Hb nematodes than the larvae of PFF.

The calculated LC_{50} was 325.3and 286.2 Ijs/cm^2 for Sc nematode on the larvae of PFF and CFF, respectively, LC_{50} was 28.8 and 56.7 Ijs/cm^2 for Hb nematode on the larvae of PFF and CFF, respectively, LC_{90} was 1718.6 and 1650 Ijs/cm^2 for Sc nematode on the larvae of PFF and CFF, respectively, and LC_{90} was 167.2 and 156.1 Ijs/cm^2 for Hb nematode on the larvae of PFF and CFF, respectively, (Table 4 and Figs 1&2).

2. On the newly formed pupae:

The results in Table (2) revealed that the both used EPN were effective and virulent on the newly formed pupae of PFF and CFF, while Sc nematode was lower virulence than Hb nematode on the pupae of PFF and CFF.

The means mortality percent were increased in ascending manner with the increasing in EPN concentration (50, 100, 150, 200, 250 IJs/cm^2), means mortality percent were ranged from 2.7 to 32.7%, from 1.7 to 23.3%, for the newly formed pupae of PFF and CFF, respectively. The means mortality percent were: 2.7, 9.0, 12.0, 21.7 and 32.7 % , respectively for PFF pupae treated with Sc nematode . The means mortality percent were: 1.7, 4.0, 9.0, 17.1 and 23.3 %, respectively, for CFF pupae treated with Sc nematode.

Hb nematode showed the same effect and the means mortality percent were ranged from 12.7 to 51.7% for PFF pupae and from 6.3 to 39.3% for CFF pupae. The means mortality percent were: 12.7, 20.3, 35.7, 47.7 and 51.7 % for PFF pupae treated by Hb nematode. The means mortality percent were: 6.3, 8.7, 14.7, 25.0 and 39.3% for CFF pupae treated by Hb nematode, respectively. Moreover, Hb nematode was highly virulent than Sc nematode on the pupae of both tested

flies (PFF and CFF). Pupae of PFF were more susceptible to Sc and Hb nematodes than the pupae of CFF.

The calculated LC_{50} was 540.2 and 447.4 Ijs/cm^2 for Sc nematode on the pupae PFF and CFF, respectively, LC_{50} was 235.0 and 420.8 Ijs/cm^2 for Hb nematode on PFF and CFF, respectively, LC_{90} was 1785.4 and 2009.8Ijs/cm^2 for Sc nematode on PFF and CFF, respectively, and LC_{90} was 1167.0 and 1914.5 Ijs/cm^2 for Hb nematode on the pupae of PFF and CFF, respectively, (Table 5and Figs 3&4).

3. On the adults

The obtained data in Table (3) showed that the both used EPN were effective and virulent on the adults of PFF and CFF, while Sc nematode was lower virulence than Hb nematode on the adults of PFF and CFF.

The means mortality percent were increased in parallel manner with the increasing in EPN concentration (50, 100, 150, 200, 250 IJs/cm^2), means mortality percent were ranged from 35.0 to 78.7% and from7.7 to 50.3% , for the adults of PFF and CFF, respectively. The means mortality percent were: 35.0, 39.7, 48.0, 62.3 and 78.7 % , respectively for PFF adults treated with Sc nematode . The means mortality percent were: 7.7, 13.7, 22.7, and 28.3 and 50.3 %, respectively, for CFF adults treated with Sc nematode.

Hb nematode gave the same effect and the means mortality percent were ranged from 41.7 to 90.3% for PFF adults and from 17.0 to 67.7% for CFFadults. The means mortality percent were: 41.7, 51.7, 66.7, 73.3and 90.3% for PFF adults treated by Hb nematode. The means mortality percent were: 17.0, 25.3, 37.7, 43.7 and 67.7% for CFF adults treated by Hb nematode, respectively. Moreover, Hb nematode was highly virulent than Sc nematode on the adults of both tested flies (PFF and CFF). The adults of PFF were more susceptible to Sc and Hb nematodes than the adults of CFF.

The calculated LC_{50} was 116.8 and 261.6 IJs/cm^2 for Sc nematode on the adults of PFF and CFF, respectively, LC_{50} was 77.3 and 196.7 IJs/cm^2 for Hb nematode on the adults of PFF and CFF, respectively, LC_{90} was 319.3 and 375.0 IJs/cm^2 for Sc nematode on the adults of PFF and CFF, respectively, and LC_{90} was 290.8 and 235.7 IJs/cm^2 for Hb nematode on the adults of PFF and CFF, respectively, (Table 6 and Figs 5&6).

The obtained results emphasized that the entomopathogenic nematodes (EPN) could be used successfully in controlling both of PFF and CFF. Both of Sc and Hb nematodes were effective and virulent on the larvae and adults of PFF and CFF , while the pupae were less susceptible .This is in the same trend with Gaugler and Kaya (1990) who mentioned that EPN of genera *Heterorhabditis* and *Steinernema*

(Nemartoda: Rhabditidae) have emerged as excellent insect biocontrol agents. Also it agree with other attempts concerning the infectivity of EPN on the peach fruit fly, *Bactrocera zonata* (Attala *et al*., 2002), the cucurbit fly, *Dacus ciliatus* (Fetoh and El-Gendi, 2006) , peach fruit fly, *Bactrocera zonata* and the med fly *Ceratitis capitata* (Soliman, 2007 a&b).

The non-feeding, third-stage infective juvenile of nematodes (IJs) is the only stage that survives outside of the host. The IJ carries cells of symbiotic bacteria in its intestine. When the IJ finds a suitable host, it invades and enters into the host's heamocel through the natural openings and releases the bacteria that kill the host within 48 hours. These bacteria produce antibodies that prevent other micro-organisms from colonizing the cadavers of host. Furthermore, serving as a food source for nematodes, the bacteria digest the host tissues, thereby providing suitable nutrients for nematodes growth and development (Ehlers, 2001).

Table 1. Effects of selected concentrations of the entomopathogenic nematodes on Bactrocera zonata and Dacus ciliatus larvae

Concentration	*Bactrocera zonata*		*Dacus ciliatus*	
IJs/cm^2	Sc	Hb	Sc	Hb
50	9.3(9-10)e	67.3(65-69)E	12.7(10-15)e	46.3(45-48)E
100	14.7(13-16)d	79.7(78-81)D	20.2(19-21)d	72.7(70-75)D
150	26.0(25-27)c	86.3(85-88)C	26.7(25-29)c	86.7(85-88)C
200	37.7(36-39)b	94.7(93-96)B	37.7(35-40)b	98.0(96-99)B
250	42.7(40-45)a	100(100-100)A	52.3(50-55)a	100(100-100)A

The same letter in the same column is non-significant.

Table 2. Effects of selected concentrations of the entomopathogenic nematodes on Bactrocera zonata and Dacus ciliatus pupae

Concentration	*Bactrocera zonata*		*Dacus ciliatus*	
IJs/ cm^2	Sc	Hb	Sc	Hb
50	2.7(2-3)e	12.7(11-15)E	1.7(1-2)c	6.3(5-8)E
100	9.0(8-10)d	20.3(18-23)D	4.0(3-5)d	8.7(7-10)D
150	12.0(11-13)c	35.7(33-38)C	9.0(8-10)c	14.7(13-16)C
200	21.7(20-23)b	47.7(54-50)B	17.1(15-20)b	25.0(22-28)B
250	32.7(30-35)a	51.7(50-53)A	23.3(22-25)a	39.3(36-42)A

The same letter in the same column is non-significant.

Table 3. Effects of selected concentrations of the entomopathogenic nematodes on Bactrocera zonata and Dacus ciliatus adults.

Concentration IJs/ cm^2	*Bactrocera zonata*		*Dacus ciliatus*	
	Sc	Hb	Sc	Hb
50	35.0(33-37)e	41.7(40-43)E	7.7(6-9)e	17.0(16-18)E
100	39.7(38-41)d	51.7(50-53)D	13.7(12-15)d	25.3(23-28)D
150	48.0(46-50)c	66.7(65-68)C	22.7(20-25)c	37.7(35-40)C
200	62.3(60-65)b	73.3(72-75)B	28.3(27-30)b	43.7(42-45)B
250	78.7(77-80)a	90.3(89-92)A	50.3(48-53)a	67.7(65-70)A

The same letter in the same column is non-significant.

Table 4. The calculated LC50, LC90 and relative toxicological potency of the two species of nematodes on Bactrocera zonata and Dacus ciliatus larvae

Treatment	*Bactrocera zonata*				*Dacus ciliatus*			
	LC_{50}	LC_{90}	Relative potency	Slope	LC50	LC90	Relative Potency	Slope
Sc nematode (IJs/cm^2)	325.3	1718.6	1.1	1.7	286.2	1650.0	1	1.7
Hb nematode (IJs/cm^2)	28.8	167.2	1	1.6	56.7	156.1	1.9	2.9

Table 5. The calculated LC50, LC90 and relative toxicological potency of the two species of nematodes on Bactrocera zonata and Dacus ciliatus pupae

Treatment	*Bactrocera zonata*				*Dacus ciliatus*			
	LC_{50}	LC_{90}	Relative potency	Slope	LC_{50}	LC_{90}	Relative potency	Slope
Sc nematode (IJs/cm^2)	540.2	1785.4	1	2.1	447.4	2009.8	1.2	2.3
Hb nematode (IJs/cm^2)	235.0	1167.0	1	1.8	420.8	1941.5	1.8	1.9

Table 6. The calculated LC50, LC90 and relative toxicological potency of the two species of nematodes on Bactrocera zonata and Dacus ciliatus adults

Treatment	*Bactrocera zonata*				*Dacus ciliatus*			
	LC_{50}	LC_{90}	Relative potency	Slope	LC_{50}	LC_{90}	Relative potency	Slope
Sc nematode (IJs/cm^2)	116.8	319.3	1	1.5	261.6	375.0	2.7	2.0
Hb nematode (IJs/cm^2)	77.3	290.8	1	1.8	196.7	253.7	2.5	1.9

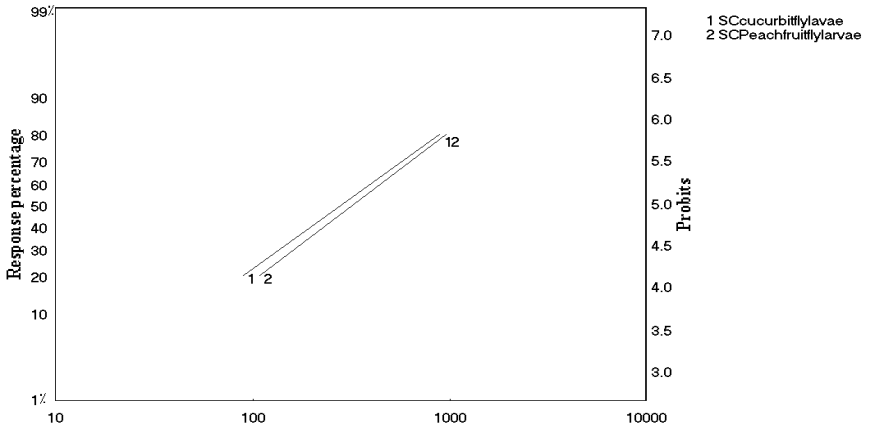

Fig. 1. Log-probity curve of percent of Sc entomopathogenic nematode on the peach fruit fly and cucurbit fly larvae

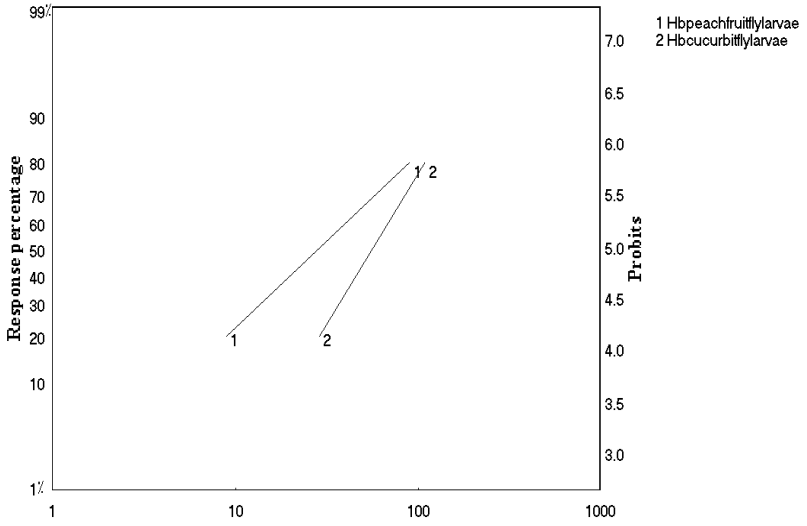

Fig. 2. Log-probity curve of percent of Hb entomopathogenic nematode on the peach fruit fly and cucurbit fly larvae

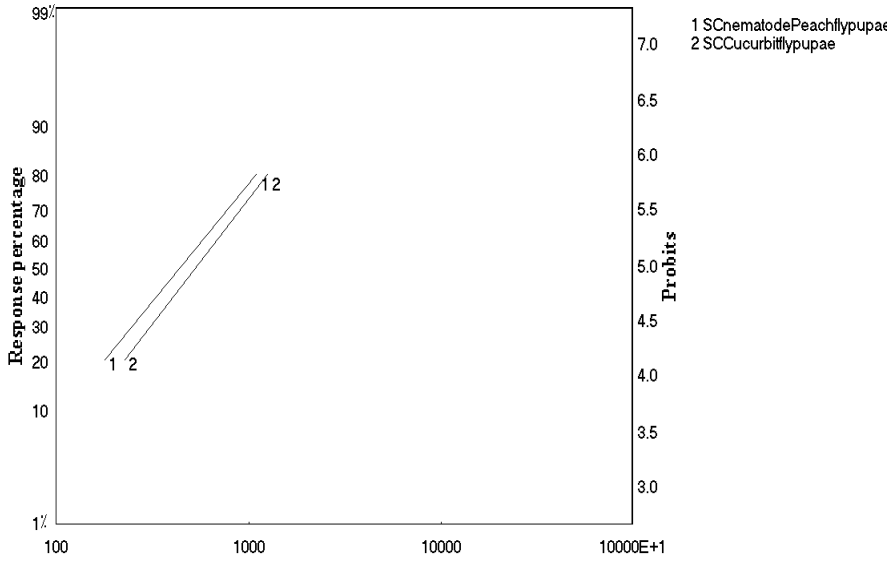

Fig. 3. Log-probity curve of percent of Sc entomopathogenic nematode on the peach fruit fly and cucurbit fly pupae

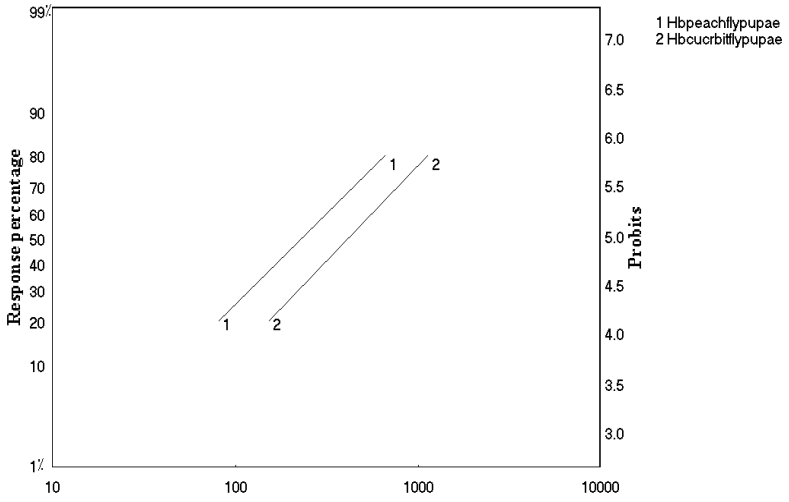

Fig. 4. Log-probity curve of percent of Hb entomopathogenic nematode on the peach fruit fly and cucurbit fly pupae

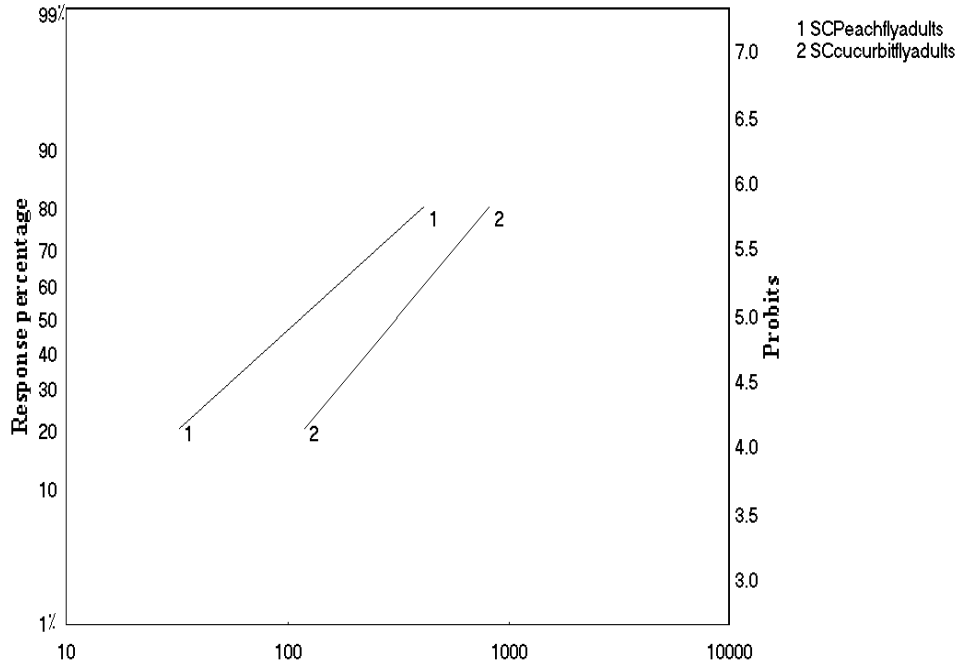

Fig. 5. Log-probity curve of percent of Sc entomopathogenic nematode on the peach fruit fly and cucurbit fly adults

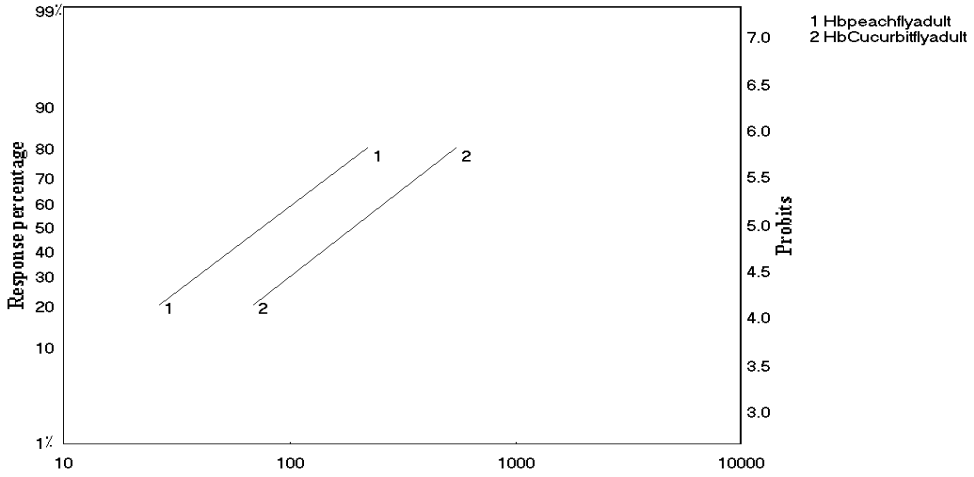

Fig. 6. Log-probity curve of percent of Hb entomopathogenic nematode on the peach fruit fly and cucurbit fly adults

The previous studies indicated that larvae of the fruit flies were highly susceptible to EPN , but pupae were generally less susceptible to EPN (Gazit *et al.*, 2000).

Fetoh and El-Gendi (2006) and Soliman (2007a) reported that EPN were effective on the newly formed pupae than aged pupae, and used EPN to control the adults of PFF and CFF. Soliman (2007a) used sugar solutions 1% and 4% mango, guava, orange juices, agar solution and a nematode- water suspensions to control both of the peach fruit fly and the med fly.

Wallace(1958**)** and Soliman (2007a) mentioned that carbon dioxide gas plays a role in EPN attraction to its host. Full grown larvae showed high activity after existing from their plant hosts searching for a suitable site to pupate. It was acceptable that this stage produces carbon dioxide gas therefore, high infection occurred by nematodes. Although the pupae were apparently less susceptible to nematodes infection than the full grown larvae, Hb infective juveniles were able to cause moderate pupal mortality rates at early pupal stages than Sc infective juveniles. This might be due to presence of the terminal tooth which was a discriminative feature of heterorhabditids that enable them to penetrate their host bodies through cuticle.

The obtained data in this offered work showed that Hb nematode was more effective than Sc nematode. It could be concluded that Hb nematode was the best candidate for control fruit flies as it caused high mortality to target pests. The high virulence of Hb nematode native isolates to the tested insects was unclear but may be able to be attributed to the rapid penetration in or rapid bacterial growth inside host's body and this in the agreement with Soliman (2007b). Toledo *et al.*, (2006) used Hb nematode on *Anastrepha ludens* (Diptera: Tephritidae) and mentioned that Hb nematode caused high pathogenicity. Glazer (1992) stated that *Steinernema carpocapsae* All strains was less effective than *Heterorhabditis bacteriophora* HP88 when applied to different lepidopteron pests according to LD50 and LT 50 values.

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التأثيرات الممرضة والمميتة للنيماتودا الممرضة للحشرات علي كل من ذبابة ثمار الخوخ وذبابة القرعيات

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أجريت تجارب معملية لتقدير التأثيرات الممرضة والمميتة لنوعين من النيماتودا الممرضة للحشرات من جنسي هيتيرورابيدتيس وشتينرنيما علي يرقات كاملة النمو و عذارى حديثة التكون وأفراد كاملة عمرها سبعة أيام ، وتراوحت نسب الموت من 9.3 إلي 42.7% و من 12.7 إلي 52.3% ليرقات كلا من ذبابة ثمار الخوخ وذبابة القرعيات المعاملة بالنيماتودا من جنس شتينرنيما ، ومن 67.3 إلي 100% و من 46.3 إلي 100% ليرقات كلا من ذبابة ثمار الخوخ وذبابة القرعيات المعاملة بالنيماتودا من جنس هيتيرورابيدتيس ، بينما كانت نسب الموت لعذارى ذبابة ثمار الخوخ المعاملة بالنيماتودا من جنس شتينرنيما بين 2.7 و 32.3% ، وبين 1.7 و 23.3% لعذارى ذبابة القرعيات المعاملة بالنيماتودا من جنس شتينرنيما ، وبين 12.7 و 51.7% لعذارى ذبابة ثمار الخوخ المعاملة بالنيماتودا من جنس هيتيرورابيدتيس، و بين 6.3 و 39.3% لعذارى ذبابة القرعيات المعاملة بالنيماتودا من جنس هيتيرورابيدتيس ، وتنوعت نسب الموت في الأفراد الكاملة من 35.0 إلي 78.7% ومن 7.7 إلي 50.3% لذبابة ثمار الخوخ وذبابة القرعيات المعاملة بالنيماتودا من جنس شتينرنيما ، ومن 41.7 إلي 90.3% و من 17.0 إلي 67.7% للأفراد الكاملة لذبابة ثمار الخوخ وذبابة القرعيات المعاملة بالنيماتودا من جنس هيتيرورابيدتيس علي التوالي، كما تم حساب قيم LC_{90} و LC_{50} ، وعموما كلا النوعين المستخدمين من النيماتودا المختبرة كانت فعالة ومؤثرة علي كل من ذبابة ثمار الخوخ وذبابة القرعيات وان كانت ذبابة ثمار الخوخ أكثر تأثرا بالنيماتودا الممرضة للحشرات عن ذبابة القرعيات ، وكانت اليرقات والأفراد الكاملة أكثر حساسية للنيماتودا الممرضة للحشرات عن العذارى للذبابتين.