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IMPACT OF CERTAIN HOST PLANTS OF THE INSECT, *Spodoptera littoralis* ON NEMATODE PENETRATION AND REPRODUCTION OF *Heterorhabditis bacteriophora IN-VITRO.*

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ABSTRACT

The effect of eight host plants i.e. bean, castor, cotton, jaw's mallow, peanut , sesame , sweet potato and watermelon leaves of the cotton leaf worm, *Spodoptera littoralis* larvae on penetration and reproduction of entomopathogenic nematodes *Heterorhabditis bacteriophora* to *S. littoralis* larvae were studied under laboratory conditions. The last instar larvae of tested insect were reared on leaves of these host plants under study. Results indicated that the plant on which the host insects fed on significantly affected nematode penetration and reproduction in their larvae with various degrees. In general , the highest number of nematode penetration was recorded in the worms that fed on bean leaves with an averaged 8.33 , 21.33 and 47 IJs/larva at the concentrations of 25 ,50 and 100 IJs / larva, respectively, whereas, the lowest rate of nematode penetration was recorded from the larvae that reared on the jaw's mallow leaves. Meanwhile, the highest averages of nematode reproduction on *S. littoralis* larvae were produced in larvae that reared on the castor leaves (205400 IJs/cadaver), whereas, reproduction of IJs nematode from larvae which fed on peanut leaves showed the lower number than those of other tested host plant leaves.

Keywords: Entomopathogenic nematicides , *Heterorhabditis bacteriophora*, *Spodoptra littoralis*, reproduction, host plant , enetration .

INTRODUCTION

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus* , respectively , are safe antagonists as commercial bioinsecticide for many economically important insect pests in ornamentals ,vegetable , fruits and turf . Allthough results from laboratory tests with these nematodes have been promising , results from field evaluated have often been highly variable .Unreliable field efficacy has been an obstacle to expanding the commercial use of such entomopathogenic nematodes (Georgis and Gaugler,1991).

The success of natural enemies ,insect pathogens ,bacteria ,fungi and viruses as a biological control agent on insect pests was affected by different host plants (Bergman and Tingey,1979 ; Ramoska and Todd,1985 ;Duffey and Bloem,1986; Keating and Yendol,1987 and Reichelderfer,1991) .

The effect of host plant on entomopathogenic nematodes as a biological control agent against insect pests were studied by many authors. Jackson and Brooks, (1989) found that the soil inhabiting *Diabrotica* larvae cause direct yield loss by feeding on roots and other underground plant parts and indirect loss by providing entry for pathogenic and secondary microorganismso*tica* spp. are susceptible to infection by steinernematid and

heterorhabditid nematodes (Thurston and Yule, 1990)and are potential hosts for both naturally occurring and commercially produced nematodes. Barbercheck (1993) and Barbercheck *et al.*(1995) reported that the food plant of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, can affect mortality from entomopathogenic nematodes and IJ production from infected insects. Rootworms reared on corn produced the greatest numbers of IJ, whereas rootworms that fed on squash produced the lowest numbers of IJ. In addition, plant secondary compounds, cucurbitaceous, extracted from squash inhibited the in vitro growth of bacterial symbionts from some nematode isolates (Barbercheck and Wang, 1996). Plant influences on the internal "environment" of herbivorous insects that are potential hosts for nematodes may act as a selective pressure for nematodes that can tolerate the internal environment of the insect based on the insects food plant.

The results of experiments aim to determine the effect of eight host plants on the penetration of entomopathogenic nematodes *Heterorhabditis bacteriophora* in cotton leaf worm, *Spodoptera littoralis* and on nematode reproduction.

MATERIALS AND METHODS

Nematdes. Nematodes were routinely cultured in greater wax moth *Galleria mellonella* L. (Woodring and Kaya , 1988) entomopathogenic nematodes, *Heterorhabditis bacteriophora* was used in bioassays which collected at Behira Governorate (Anany,2005). Nematodes were stored at 15 °C. as aqueous suspensions in tissue culture flasks 250 ml, no longer than one week before use in bioassay.

Insects. The cotton leaf worm *Spodoptera littoralis* was used as a model to test the effect of host plant on entomopathogenic nematodes. The culture of the cotton leaf worm was reared under laboratory conditions of 26 C and 65 % R.H. The colony was reared for several generation befor using the larvae in the bioassay experiments . The egg mass were allowed to hach in clean jars provided with several leaves of tested host plants bean (*Vigna sinensis*) ,castor (*Ricinus communis*), Cotton(*Gossebium barbadence*) , jaw's mallow(*Corchorus olitorius*) , peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), sweet potato (*Ipomea batata*) and watermelon(*Citrullus lanatus*) . The larvae continued their development till pupation .The pupae were collected in separate jars until adult emergence . Moths were fed on 10 sugar solution.

Penetration assay. For each cotton leaf worm /host plant , one similarly sized of last instar larvae were exposed to entomopathogenic nematodes, *Heterorhabditis bacteriophora* in plastic cups (4.5x2.5 cm) filled with moist sterilized sand soil (60% v/v) . Each plastic cup was inoculated with nematode suspension at concentrations 25, 50 and 100 IJs / larva. Three replicates for each concentration were prepared to from a group for each one cotton leaf warm / host plant. After capping, the prepared cups were held at 25 °C. After 3 to 4 days of nematode exposure to the larvae, the dead larvae

transferred and dissected then counted number of the nematode penetrated to each insect larva and recorded.

Reproduction assay. This experiment was carried out as described in the penetration bioassay by using the nematode concentration 50 IJs/insect larva .After 48 h. inoculation at 25 °C , the infect cadavers were removed from the sand , rinsed , transferred to water traps and inoculated at 25 °C . All emerging IJs from a single larva were recovered over a period of 10 days and stored in culture flasks 250 ml. The content of each flask (nematode suspension from individual cadavers) was mixed thoroughly. Eight samples of 10 µ from each suspension were examined under a stereomicroscope and the total number of IJs nematode per cadaver was calculated (Susurluk, 2006).

Statistical analyses. Data were then analyzed according to Duncan's (1955).

RESULTS AND DISCUSSION

Penetration assay. The host plant leaves which cotton leaf worm *Spodoptera littoralis* larvae fed significantly affected number of IJs of entomopathogenic nematodes *Heterorhabditis bacteriophora* that found inside parasitized hosts after exposure of nematode (Fig. 1).The cotton leaf worm larvae which had fed on bean leaves recovered the highest number nematode penetration (8.33, 21.33 and 47 IJs/larva), followed by larvae which had fed on sweet potato leaves(10.33 , 18.66 and 37 IJs/larva), watermelon leaves (8 , 15.33 and 35 IJs/larva), castor leaves(6 , 19.66 and 29.66 IJs/larva), sesame leaves (9 , 13.66 and 28.66 IJs/larva), peanut leaves (5.66 , 10.33 and 26.66 IJs/larva) and cotton leaves (5.33 , 12.33 and 22.33 IJs/larva) at nematode concentration levels 25 ,50 and 100 IJs/larva , respectively (Table, 1). While the lowest number of IJs nematode penetration per insect larvae was found inside dead larvae which reared on jaw's mallow leaves (5, 11.66 and 19 IJs/larva) at the three tested concentrations , respectively.

These results agree with (Barbercheck ,1993 ; Barbercheck *et al.*1995 ; Eben and Barbercheck 1997).The observed effects of host plant on entomopathogenic nematode virulence may be due to metabolites in this plants that affect insect health as a host (Barbercheck *et al.*1995). Direct effects of protein in nematode media have been observed previously, Yang *et al.*, (1997) observed differences in *S. carpocapsae* dispersal and infectivity based on protein source. The fact that all lipid supplements increased host susceptibility except canola oil a situation which may indicate that source of lipid in such host diet is very important. Similarly, lipid source in invitro media has been shown to impact nematode quality (Yang *et al.*, 1997 ; Yoo *et al.*,2000 and Abu Hatab and Gaugler, 2001). Shapiro-Ilan *et al.* (2008) expect that varying diet components can alter the nutritional make-up of the host and thereby affect host susceptibility as well as nematode infectivity.

Table (1): Comparative invasion efficiency and reproduction of entomopathogenic nematode, *Heterorhabdits bacteriophora* on last instar of the cotton leaf worm, *Spodoptera littoralis* larvae which reared on eight host plants leaves.

Host plants	Means of nematode penetration/insect larvae at three concentration levels						ematode reproduction	
	25 ml		50 ml		100 ml			
Bean (*Vigna sinensis*)	8.33	b	21.33	a	47.00	a	144933	b
Castor (*Ricinus communis*)	6.00	c	19.66	ab	29.66	c	205400	a
Cotton (*Gossebium barbadence*)	5.33	c	12.33	de	22.33	d	13966	de
Jew's mallow(*Corchorus olitorius*)	5.00	c	11.66	de	19.00	e	68000	c
Peanut (*Arachis hypogaea*)	5.66	c	10.33	e	26.66	c	6611	e
Sesame (*Sesamum indicum*)	9.00	b	13.66	cd	28.66	c	37377	d
Sweet potato (*Ipomea batata*)	10.33	a	18.66	b	37.00	b	153200	b
Watermelon (*Citrullus lanatus*)	8.00	b	15.33	c	35.00	b	67166	c

Means followed by the same letters within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Reproduction assay. The different plant leaves which cotton leaf worm, *Spodoptera littoralis* larvae fed significantly affected nematode reproduction from larvae (cadavers) that infected by *Heterorhabditis bacteriophora* (Fig.2) reproduction from larvae which had fed on castor leaves was higher (at mean 205400 IJs/cadaver) than from those that larvae had fed on other host plants leaves, followed by cadavers which had fed on sweet potato , bean , jaw's , mallow, watermelon , sesame and cotton leaves at means 153200 , 144933 , 68000 ,67166 ,37377 and 13966 IJs/cadaver , respectively (Table 1). Thereupon, *S. littoralis* larvae which reared on peanut leaves produced lower number (at mean 6611 IJs/cadaver) than cadavers reared on other tested plants leaves.

These results agree with (Barbercheck ,1993 and Barbercheck *et al.*1995) . Epsky and Capinera (1994) observed greater nematode progeny production in black cutworm reared on artificial diet than from those reared on collard foliage. Morever, they attributed this difference to the greater lipid content in cutworm reared on artificial diet. Analyses of total protein, lipid, soluble sugars and polysaccharides in the plants and root worms of equal weight and age from the different host plants are probably not equal (quality) as hosts for nematode reproduction (Barbercheck *et al.*1995). Perhaps this difference in primary metabolite among insects because differences between host plants is related to difference in nematode progeny production from root worm.

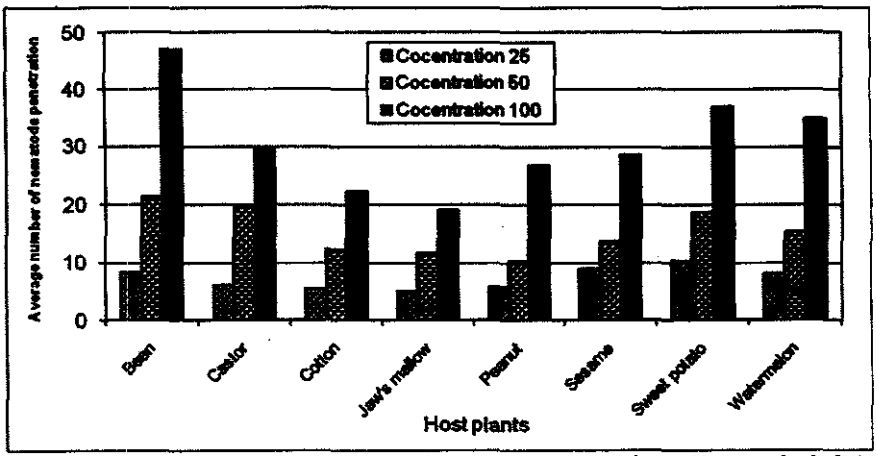

Fig.(1): Penetration of entomopathogenic nematodes, *Heterorhabditis bacteriophora* of last instar larvae of the cotton leaf worm, *Spodopetra littoralis* reared on eight host plant leaves.

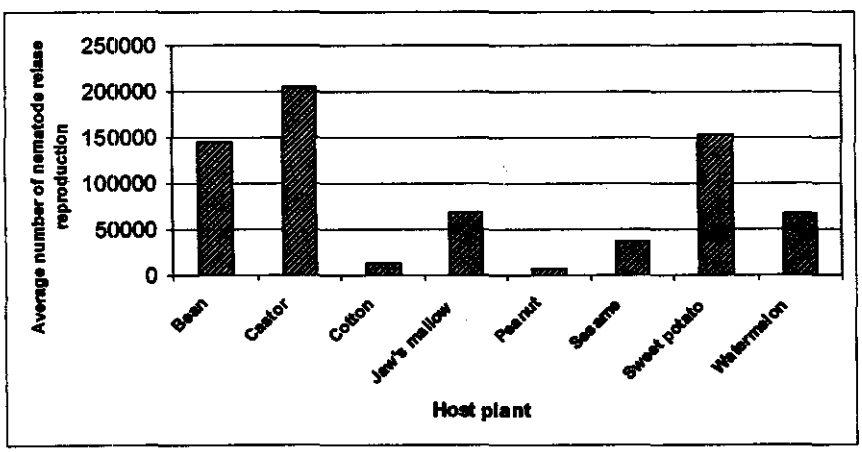

Fig.(2): Progeny production of entomopathogenic nematodes *H. bacteriophora* from cadavers of *Spodopetra littoralis* reared on eight host plan leaves.

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تأثير بعض العوائل النباتيه لحشرة دودة ورق القطن *Spodoptera littoralis* على اختراق وتكاثر النيماتودا الممرضه للحشرات *Heterorhabditis bacteriophora* تحت ظروف المعمل.

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تمت دراسة تأثير ثمانى عوائل نباتيه هم اللوبيا والخروع والقطن والملوخيه والفول السودانى والسمسم والبطاطا والبطيخ ليرقات حشرة دودة ورق القطن *Spodoptera littoralis* على قدرة النيماتودا الممرضه للحشرات *Heterorhabditis bacteriophora* على اختراق يرقات العمر الاخير لهذه الآفه وكذلك معدل تكاثر وانتاج النيماتودا للاطوار المعديه من جثث هذه الآفه . فى هذه الدراسة تم تربية يرقات دودة ورق القطن على أوراق العوائل النباتيه المختبره ولقد أوضحت النتائج ما يلي :

إن اختلاف العائل النباتى فى تربية يرقات دودة ورق القطن أدى الى فروق معنويه فى قدرة النيماتودا الممرضه للحشرات على اختراق العائل الحشرى وكذلك على معدل تكاثر وانتاج النيماتودا للاطوار اليرقيه المعديه من جثث هذه الآفه . وكانت اليرقات التى تم تربيتها على أوراق اللوبيا هى أكثر اليرقات تعرضا لاختراق النيماتودا للاطوار المعديه للنيماتودا بمتوسط (٨,٣٣ ، ٢١,٣٣ ، ٤٧ طور نيماتودى معدى / يرقة دودة ورق القطن)وذلك على ثلاثة تركيزات للعدوى ٢٥ ، ٥٠ ، ١٠٠ طور نيماتودى معدى / يرقة دودة ورق القطن على الترتيب . فى حين كانت اليرقات التى تم تربيتها على أوراق الملوخيه هى أقل اليرقات تعرضا لاختراق النيماتودا للاطوار المعديه للنيماتودا .

كما اوضحت النتائج أيضا أنه بالنسبه لتاثير اختلاف العائل النباتى على معدل تكاثر وانتاج النيماتودا للاطوار اليرقيه المعديه من جثث يرقات دودة ورق القطن كانت اليرقات التى تم تربيتها على أوراق الخروع هى أكثر اليرقات انتاجا للاطوار اليرقيه المعديه للنيماتودا وذلك بمتوسط (٢٠٥٤٠٠ طور نيماتودى معدى / جثه ليرقة دودة ورق القطن) بينما كانت اليرقات التى تم تربيتها على أوراق الفول السودانى هى الاقل انتاجا للاطوار اليرقيه المعديه للنيماتودا .