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VIRULENCE AND EFFICACY OF CERTAIN STEINERNEMATID AND HETERORHABDITID NEMATODES AGAINST TWO STRAINS OF MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WIED.) (DIPTERA: TEPHRITIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT: Several laboratory assays were carried out to determine the efficiency of different entomopathogenic nematodes against Spanish and Egyptian strains of Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wied.), (Diptera: Tephritidae) (Larval stage). Five species of Heterorhabditid and Steinernematid nematodes (*Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser) (All and German strains), *S. riobravis* Cabanillas (Texas strain), *S. glaseri* (Steiner) and *Heterorhabditis bacteriophora* Poinar (HP88 strain)) have been evaluated. Results showed that the efficacy of the entomopathogenic nematode differed among the different strains of the fruit flies, where *S. carpocapsae* (Weiser) (All strain) was the highest in Egyptian strain of MFF infestation, the percentage of mortality due to 50 IJs/ cm^2 was 92% ±1. In adverse, *Heterorhabditis bacteriophora* Poinar (HP88 strain) was the highest virulent Entomopathogenic nematode against the Spanish strain of MFF, where 50 IJs/ cm^2 produced 74.7% ±2.5 mortality.

INTRODUCTION

Fruit flies, members of Family Tephritidae, are serious pests of fruits and vegetables in many tropical and subtropical areas of the world. The introduction of any of the Tephritid species into an area where they are not established has required extensive and costly eradication procedures (Hagen *et al.*, 1981). The Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae) is one of the most important pest of fruits, vegetables and nuts, where its host range includes more than 350 plant hosts round the world (Liquido *et al.*, 1991).

Different methods of control have been used to reduce the fruit flies population, Most of them are concentrated to control the pest in the adult stage and few of them control the pest in the immature stages. Nematodes of the genera *Heterrhabditids* Poinar and *Steinernema* Travasses, in

conjunction with their symbiotic bacteria *Photorhabdus* spp. and *Zenorhabdus* spp., are pathogenic to many insect pests (Poinar, 1979) and are effective biological control agents for soil associated insects (Klein, 1990). Host infestation is accomplished as third-stage (dauer) nematodes enter natural body opening or intact cuticle in certain cases and introduce the symbiotic bacteria that usually kill the host insect within 24-48 h (Poinar, 1979 and Kaya, 1985). These nematodes and associated bacteria are not pathogenic to vertebrates (Poinar *et al.*, 1982 and Obendorf *et al.*, 1983) and recent advances in artificial culture (Bedding, 1984) have made them more attractive as control agents for insect pests. The susceptibility of various species of fruit flies to heterorhabditid and steinernematid nematodes under laboratory and natural condition (Poinar *et al.*, 1977, Poinar & Hislop, 1981, Beavers & Calkins, 1984, Lindegren & Vail, 1986 and Stark & Lawrence, 1999) suggests that they have potential as biological control agents for tephritid flies. Laborda *et al.*, (2003) studied the susceptibility of MFF to mixture of *Steinernema* spp "Biorend C" and the initial laboratory tests showed high susceptibility of larvae to infestation by these nematodes with >90% mortality and under the field condition, the mortality decreased to 70%. Gazit *et al.*, (2000) examined 12 EPN strains against the larvae of the MFF (Palestine strain) and found that the highest mortality was due to *S. riobrave* (Texas strain) infestation. In Egypt, Shams Eldean *et al.*, (1994) examined the direct contamination of hetrorhabditid nematode isolate EGB1 against MFF larvae and other 11 different plant insect pests and they found that the isolate killed all the larvae under investigation.

This study was conducted to determine the susceptibility of MFF mature third instar larvae to five species of entomopathogenic nematode (EPN): *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser) (All and German strains), *S. riobrave* Cabanillas (Texas strain), *S. glaseri* (Steiner) and *Heterorhabditis bacteriophora* Poinar (HP88 strain), and compare between the Spanish and Egyptian strains of the MFF to be attacked with the EPN.

MATERIALS AND METHODS

Nematode and fly cultures:

There were two experiments, the first used the Spanish strain of MFF and second used Egyptian strain. The Spanish strain was delivered by Dr Elisa Vinuele, Unidad de Protection de Cultivous, Universided

Politecninca de Madrid, Madrid, Spain and the experiment has been conducted in the Institute for Plant Protection in Fruit Crops, Dossenheim, Germany. The Egyptian experiment used the Egyptian strain supplied from stock culture that have been presented in Plant Protection Research Institute (PPRI), ARC, Dokki, Giza, Egypt. Spanish strain larvae were reared on the wheat bran diet of Boller (1984), while the Egyptian strain was reared according to Mohamed (2003). Both strains were reared at 25 °C and 65% RH.

Nematodes were reared using late-instar greater wax moth, *Galleria mellonella* (L.), (Lepidoptera: Pyralidae) as described by Dutky *et al.*, (1964). Nematodes were harvested with White traps (White, 1927), quantified by counting the number of nematodes in 100 µL for five times samples, using the average to dilute the solutions to the needed concentration and stored in tissue culture flasks at 4 °C except the *Heterorhabditis* spp. which was kept in 10 °C. 24 hours before the experiment, the EPN mixtures were moved to the room temperature for acclimation.

Susceptibility of maggot to EPN:

The virulence of *Heterorhabditis* spp. and *Steinernema* spp. toward the MFF larvae was tested using fine sand soil application. The late third instar larvae that had exited from the diet to pupate within 2 hours time interval was received into water. The arena is a plastic cup, 7.5 cm diameter with surface area of 44.2 cm^2 and lined with 100 ml of dry fine sand soil. The nematodes were counted and poured to the surface according to the desired number and left for 2 hours before placing the MFF larvae. The treatments were replicated five times. Each test consisted of four treatments for each EPN with doses of 0, 10, 25 and 50 IJs/cm^2. According to the amount of water was added with the EPN, the moisture content was adjusted to be 10% of sand volume. Each arena received 5 homogenous last instar MFF larvae by placing the larvae on the soil surface allowing them to burrow naturally into it. After the addition of the larvae, the cups were covered with a plastic perforated lid and incubated at 25 °C and 65% RH. After ten days, the sand was sifted for dead larvae, pupae and emerged adult flies. The larvae and pupae were individually dissected to determine pupal mortality and verify nematode parasitism.

Data analysis:

LC_{50} and LC_{90} were calculated using the probit regression analysis of SPSS computer program version 16 (Green & Salkind, 2003). A One-way analysis of variance (ANOVA) with a complete block design was used to analyze larval mortality. Mortality data are presented as percentage mortality after correction in comparison to the control of the experiment. Least Significant Difference (LSD) was used for multiple mean comparisons between each treatment and the other (Green & Salkind, 2003).

RESULTS AND DISCUSSION

This study showed that last larval instar (prepupae) of Mediterranean fruit fly, (MFF), *C. capitata* (Wiedemann) is susceptible to the infective stages of the entomopathogenic nematodes and this coincides with Shams Eldean *et al.*, (1994), Gazit *et al.*, (2000) and Laborda *et al.*, (2003). During this study, five species of EPN have been evaluated, which were *Steinernema carpocapsae* (Weiser) (All and German strains), *S. riobrave* Cabanillas (Texas strain), *S. feltiae* (Filipjev), *S. glaseri* (Steiner) and *Heterorhabditis bacteriophora* Poinar (HP88 strain). Comparing among the results and using the probit regression analysis of the SPSS program, The LC_{50} and LC_{90} of different EPN species against Spanish and Egyptian strains of the MFF have been calculated (Table 1).

Table (1). Dose effect of certain entomopathogenic nematode species/strains on different mature larvae of two strains of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.).

Nematode species	strain	Mediterranean fruit fly strains			
		Spanish strain		Egyptian strain	
		LC_{50} [z]	LC_{90}	LC_{50}	LC_{90}
Steinernema carpocapsae (Weiser)	All	-[@]	-[@]	18.61	40.4
S. carpocapsae (Weiser)	German	37	72.12	21.75	45.72
S. riobrave Cabanillas	Texas	-[@]	-[@]	21.81	49.02
S. feltiae (Filipjev)		41.44	95.66	32.66	70.23
S. glaseri (Steiner)		-[@]	-[@]	46.96	103.89
Heterorhabditis bacteriophora Poinar	HP88	24.32	58.23	37.11	91.61

[z]: LC_{50} and LC_{90} expressed as number of infective juveniles per cm^2 surface area, Probit analysis,

[@] : could not be estimated

The LC_{50} for the *S. carpocapsae* (Weiser) (German strain) was higher in case of Spanish strain of MFF (37 IJs/cm^2) than the Egyptian strain (21.8 IJs/cm^2). The same result was concluded with *S. feltiae* (Filipjev) where the LC_{50} for Spanish strain was 41.4 IJs/cm^2 and for the Egyptian strain was 32.7 IJs/cm^2. The case was changed with *H. bacteriophora* Poinar (HP88 strain), where the Egyptian strain was more resistant for infestation. The LC_{50} was 37.1 IJs/cm^2 and for the Spanish strain was 24.3 IJs/cm^2. The other three EPN, *S. carpocapsae* (Weiser) (All strain), *S. riobrave* Cabanillas (Texas strain) and *S. glaseri* (Steiner) were not evaluated against the Spanish strain but for the Egyptian strain, the LC_{50} values were 18.6, 21.8 and 47 IJs/cm^2, respectively.

Larvae of the Spanish strain were sensitive to the three EPN used (Table 2), and there was a complete direct correlation between the EPN concentrations and percentages of corrected mortality.

Table (2): Influence of different concentrations of entomopathogenic nematode on mature larvae of Mediterranean fruit fly (Spanish strain), *Ceratitis capitata* (Wied.) (Diptera: Tephritidae).

Nematode Species	Strain	Concentration (IJs / cm^2)	% corrected mortality	± SE‡
Steinernema carpocapsae (Weiser)	German	10	20	1.78
		25	48	0.98 a
		50	58	2.56 a
S feltiae (Filipjev)		10	30.6	1.28
		25	38	1.6 b
		50	52	2.04 b
Heterorhabditis bacteriophora Poinar	HP88	10	48	1.6 c
		25	58.7	1.96 c
		50	74.7	2.54 c

‡ Means followed by the same letter are not significantly different ($P<0.05$, Least Significant Difference (LSD) test, using SPSS computer program Ver.16).

The highest percentage of corrected mortality produced from *H. bacteriophora* Poinar (HP88), where the percentages were 48% ± 1.6, 58.7 % ± 2 and 74.7 % ±2.5 for the EPN concentrations 10, 25 and 50 IJs/cm^2, respectively. *S. carpocapsae* (Weiser) (German strain) was the second in rank, where the percentages of corrected mortality were 20% ±1.8, 48% ± 1 and 58% ±2.6, respectively and the last one was *S. feltiae* (Filipjev) which recorded 30.6% ±1.3, 38% ±1.6 and 52% ±2, respectively. Results indicated that there is a significance difference

among treatments and between concentrations except between concentration 25 and 50 IJs/cm^2 of *S. carpocapsae* (Weiser) (German strain), between concentration 25 and 50 IJs/cm^2 of *S. feltiae* (Filipjev) and between 10, 25 and 50 IJs/cm^2 of *H. bacteriophora* Poinar (HP88 strain). Previously, Laborda *et al.*, (2003) studied the susceptibility of MFF (Spanish strain) to EPN *Steinernema* spp. "Biorend C" under laboratory conditions and found that 50 and 25 IJs/cm^2 produced percentage of 88%±5 and 99% ±1 mortality, respectively in case of sandy loam soil and 52% ±17 and 57%±21 respectively in case of clay soil.

The Egyptian strain of MFF was also sensitive to the EPN infestation but in a different rank. The highest percentage of mortality (Table 3) was represented in *S. carpocapsae* (Weiser) (All strain), where the percentage of corrected mortality was 42.3% ±1.7, 72% ±1.6 and 92% ±1 for the concentrations 10, 25 and 50 IJs/cm^2, respectively. There was a

Table (3): Influence of different concentrations of entomopathogenic nematode on mature larvae of Mediterranean fruit fly (Egyptian strain), *Ceratitis capitata* (Wied.) (Diptera: Tephritidae).

Nematode Species	Strain	Concentration (IJs / cm^2)	% corrected mortality	± SE‡
Steinernema carpocapsae (Weiser)	All	10	42.3	1.66
		25	72	1.6
		50	92	0.98
S. carpocapsae (Weiser)	Germany	10	19.3	0.84
		25	79	1.54 a
		50	80	1.48 a
S. riobrave Cabanillas	Texas	10	32	2.34
		25	65.3	1.56 b
		50	80	1.5 b
S feltiae (Filipjev)		10	24.7	1.6 c
		25	48.7	2.72 cd
		50	62.7	1.74 d
S. glaseri (Steiner)		10	26	2.18 e
		25	32	1.5 e
		50	45.72	2.84 e
Heterorhabditis bacteriophora Poinar	HP88	10	38.7	2.22 f
		25	44.7	2.98 f
		50	52.1	2.64 f

‡ Means followed by the same letter are not significantly different (P<0.05, Least Significant Difference (LSD) test, using SPSS computer program Ver.16).

significant difference between concentrations at P<0.05 and also between treatments. Gazit *et al.*, (2000) studied the same EPN using the Palestinian strain of MFF and they found the percentage of mortality for

the concentration 100 IJs/ cm^2 was <10%. The second and third EPN were *S. carpocapsae* (Weiser) (German strain) and *S. riobrave* Cabanillas (Texas strain), where the concentration 50 IJs/cm^2 produced 80% ±1.5 for both and there was a significant difference between treatments, except between concentration 25 and 50 IJs/cm^2. Gazit *et al.*, (2000) found that *S. riobrave* (Texas strain) infest the MFF larvae and the mortality percentage was 82.5% ±2.84 at concentration of 100 IJs/ cm^2. The fourth EPN was *S. feltiae* (Filipjev) and the percentages of corrected mortality were 24.7% ±1.6, 48.7% ±2.7 and 62.7% ±1.7 for the concentrations 10, 25 and 50 IJs/cm^2, respectively. There was no significant difference between concentration 10 and 25 IJs/cm^2 and also between concentration 25 and 50 IJs/cm^2. Poinar & Hislop (1981) found that adult MFF was susceptible to *S. feltiae* (Mexican strain) and *H. heliothidis* in laboratory tests, but due to a limited amount of host materials no tests for larval or pupal susceptibility were made. Also, Lindegren & Vail (1986) found that 3rd-instar larvae of MFF, Melon fly (*Dacus cucurbitae* Cuquillett) and Oriental fruit fly (*D. dorsalis* Hendel), were also susceptible to *S. feltiae* (Mexican strain). The fifth EPN was *Heterorhabditis bacteriophora* Poinar (HP88 strain), where the percentage of corrected mortality for 10, 25 and 50 IJs/cm^2 concentrations were 38.7% ±2.2, 44.7% ±3 and 52.1% ± 2.6 respectively. The lowest EPN in infestation of MFF was *S. glaseri* (Steiner) where the percentages of corrected mortality were 26% ±2.2, 32% ±1.5 and 45.7% ±2.8 respectively. For both *H. bacteriophora* Poinar and *S. glaseri* (Steiner), there was significant difference between the control and the other treatments but there was no significant difference between treatments. Previously, Shams Eldean *et al.*, (1994) examined direct contamination of *Heterorhabditis* nematode isolate EGB1 against the MFF larvae and found that the isolate killed all the larvae and pupae.

In comparison between the two strains of the MFF in susceptibility to the EPN infestation at concentration 50 IJs/cm^2, it was found that there is a difference in the infestation, where the percentages of mortality of the Egyptian strain was higher when infected with *S. carpocapsae* (Weiser) (German strain) (80 % ± 1.5) followed by *S. feltiae* (Filipjev) (62.7 % ± 1.7) and the lowest was *H. bacteriophora* Poinar (HP88 strain) (52.1 % ±2.6). While in case of the Spanish strain, the highest susceptibility was related to *H. bacteriophora* Poinar (HP88 strain) (74.7 % ±2.5) followed by *S. carpocapsae* (Weiser) (German strain) (58 % ±2.6) and the lowest was *S. feltiae* (Filipjev) (52 % ± 2).

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قدرة وفاعلية بعض نيماتودا الإشتينرنيماتيد، والهيتيرورهابدتيد ضد سلالتين من ذبابة فاكهة البحر المتوسط، سيراتيتيس كابياتاتا (ثنائيات الأجنحة : تيفرتيدى) تحت الظروف المعملية

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أجريت دراسات معملية للتعرف على تأثير عدة أنواع من النيماتودا الممرضة على العمر اليرقى الثالث للسلالة الأسبانية والمصرية من ذبابة فاكهة البحر المتوسط (ثنائيات الأجنحة : تيفرتيدى) (العمر اليرقى)، حيث تم تقويم خمسة أنواع من النيماتودا فى تسلسل من التركيزات. أثبتت النتائج أن تأثير النيماتودا الممرضة يتغير حسب سلالة ذباب الفاكهة حيث كانت النيماتودا شتينرنيما كاربوكابسا هى الأعلى فى مهاجمة السلالة المصرية من ذبابة فاكهة البحر المتوسط. وكانت نسبة موت اليرقات عند تعرضها لتركيز ٥٠ يرقة/سم^{٢} هى ٩٢% ±١. وفى المقابل كانت النيماتودا هيتيرورهابديتيس باكتيريوفورا هى الأقوى فى الأنواع المستعملة ضد السلالة الأسبانية من ذبابة فاكهة البحر المتوسط حيث قضت نسبة ٥٠ يرقة/سم^{٢} من النيماتودا على ٧٤,٧% ±٢,٥ من يرقات الذبابة.