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VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO *Ceratitis capitata* (WIED.) (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The virulence of entomopathogenic nematode species Steinernema carpocapsae (All) and Heterorhabditis bacteriophora (HP88) was determined against the 3rd instar larvae of Ceratitis capitata (Wied.) throughout three tested media under laboratory conditions. Analysis of data revealed that filter paper media has significant effect than another two media. Such effect descendingly arranged follow: filter paper followed by soil and soil with manure, mortality rates were 40.74, 29.63 and 25.37 respectively. S. carpocapsae (All) was the most virulent than H. bacteriophora (HP88) at all tested media and concentrations. Mortality rates were 78.14, 65.93, 53.33, 39.26 and 21.86% respectively for S. Capocapsae (All) while they were 60.74, 52.22, 37.40, 28.52 and 12.52% respectively, for H. bacteriophora (HP88). Lc_{50} at 72 hours were obtained for the two entomopathogenic nematode species at the three tested media. Temperature influences nematode virulence, the optimum temperature for nematode pathogenicity was (25^{o}C) then mortality decreased at the lowest (20^{o}C) and highest (30^{o}C) in two tested entomopathogenic nematode species.

***Results showed that** S. carpocapsae (All) and H.bacteriophora (HP88) caused higher mortality in loamy soil than clay or sandy soil. Mortality rates of medfly larvae were reduced in all soil types mixed with cow manure compared with soil without manure.*

Key words: Virulence, entomopathogenic nematodes, *Ceratitis capitata*, diptera : tephritidae .

INTRODUCTION

Medfly, *Ceratitis capitata* (Wiedemann) (Diptera : Tephritidae) is considered as one of the world's most destructive fruit pests because of its high capability to damage the production, it's global distribution and it's wide range of hosts.

It causes significant fruit losses worldwide due to damages resulting from their oviposition into fruits and from pulp consumption by their larvae.

Damage occurs because females oviposite in ripening fruit which opens a door for microorganisms, resulting in fruit contamination and decay. It is a major pest capable of infesting many different species of fruits, vegetables and nuts.

Until the end of the 1980's, fruit fly control was based exclusively on chemical control. However due to indiscriminate use of chemical products, problems arisen such as the resurgence of pests, resistant populations, environmental contamination, and residues in the final sales product, which required the adoption of other control practices to fight the pest. This insect is difficult to control with insecticide because larvae develop inside the fruit and move to the soil for pupation. Entomopathogenic nematodes belonging to families steinernematidae and heterorhabditidae are available commercially in many parts of the world to control a number of different soil insect pests (Kaya and Caugler , 1993).

One method of biological control that could be used is the application of entomopathogenic nematodes against alive stages in the soil. (Dolinski and Samuels, 2002). *H. bacteriophora* was pathogenic against *S. littoralis* and *A. ipsilon* larvae as reported by Hammad (1996, 2001).Aggarwal *et al*. (2014) determined the efficacy and interaction between entomopathogenic nematodes and *Bacillus thuringiensis* Var. Kurstaki against 2^{nd} and 3^{rd} larval instars of diamondback moth (DBM) *Plutellaxylostella* under laboratory conditions. Insect larvae were susceptible to nematode infestation. There was no imporovement in the efficacy of the combination treatment over that of nematodes or bacterium when each was used alone. Entomopathogenic nematodes have shown potential as bio-control agents of pests especially those with a soil phase (Georgis and Gaugler, 1991; Alekseev *et al*., 2006).

Ceratitis capitata spends parts of it's life cycle in the soil, where entomopathogenic nematodes are found (Lindegren *et al*., 1989 and Gazity *et al*., 2000). Steinernematid and Heterorhabditid are promising for the control of *C. capitata*, as the pest's behavior of leaving the fruit and penetrating the soil for pupal development allows nematode action.

Studies have indicated that tephritid larvae are susceptible to these nematodes; although pupae are more resistant (Beavers & Calkins, 1984) *S. carpocapsae* (Mexican isolate) has caused 87% mortality in *C. capitata* larvae at doses of up to 500 infective juvenils/cm^2 (Grewal *et al*., 2001). The pathogenicity of the nematode *Heterorhabditis* spp. (isolate IBCBn05) has been evaluated against the pre-pupal stages of medfly, and was found to be

effective at the concentration of 200 infective juveniles/medfly (Almeida *et al*., 2007).

Therefore, the objective of this study was to examine the virulence of *S. carpocapsae* (All) and *H. bacteriophora* (HP88) against the 3rd instar larvae of the medfly *C. capitata* under laboratory conditions. Also, investigating the factors (nematode dose, experimental temperatures, soil types and adding manure with soil) that may affect the susceptibility of the larvae to infection with nematodes.

MATERIALS AND METHODS

Insect:

Mediterranean fruit fly, *Ceratitiscapitata* (Wiedemann) was provided by horticulture insects department, plant protection research institute ARC, Ministry of Agriculture. Insect have been rearing in our laboratory at the department of plant protection, Faculty of Agriculture, Al-Azhar University. The colony was stated with pupae and was maintained under controlled conditions (25±2°C, 70±5% RH). After the emergence of pupae flies were provided with water (through apiece of sponge) and food (sugar and enzymatic yeast hydrolysate in ratio 3:1, respectively) (El-Sayed, 1979). The larval diet ingredients (Tanaka *et al*., 1969) consists of wheat bran 23.5% Molasses 8.5%, yeast 5.8%, sodium benzoate 0.1%HCl 0.1% and tap water 58.5%. In the present work, late third instar larvae were collected within two hours after they had exited from the diet to pupate.

Entomopathogenic nematodes:

Entomopathogenic nematode strains used in the present investigation were *H. bacteriophora* HP88 and *S. carpocapsae.* All obtained from Biological Control Laboratory, Plant Protection Department, National Research Center. The nematodes were reared in *Galleria mellonella* (L.) (Pyralidae: Lepidoptera) larvae according to procedures in (Wooding and Kaya, 1988). Larvae were starved for several hours before infection. Every ten healthy mature larvae were placed on a filter paper in a Petridish where 1000 of nematode Ijs in 1.5 ml sterilized distilled water was added. The dish was completely dosed. Dishes were kept at 25°C±2 in an incubator. Two days after inoculation, dead larvae were removed and cadavers were placed on white traps for extraction according to the technique of (White, 1927). The infective juveniles were harvested and stored in sterilized water at 10°C for 7-12 days before use (Woodring and Kaya, 1988). Viability of nematodes was determined based on movement as seen under microscopebefore use.

***Pathogenicity of nematodes on 3rd larval instar of C. capitata at different temperatures*:**

Before the laboratory studies a third medfly larvae were starved for 7 hours prior to experiment. The two nematode species were screened for their virulence against the 3rd instar medfly larvae at three constant temperatures: 20, 25 and 30°C and 70±5% RH. Tests were conducted in conical plastic cups (6.5cm diameter x 4.5 cm lower surface x 3cm height) with perforated covers lined with moistened filter paper.

For each cup, 1ml of a suspension containing 750, 1500, 3000, 5000 and 7000 nematodes were applied to the filter paper. Ten larvae were placed in each cup and replicated three times for each concentration. Cups with distilled water free of nematodes used as check ones. Larvae were observed, larval mortality were recorded at 72 hours post treatment.

Influence of soil type and manure on nematode species infectivity to C. capitata larvae:

The two nematode species were laboratory screened for their virulence against the 3rd instarmedfly larvae at 25°Cand 70±5% RH using two methods of application, three different soil types (Clay, Loamy and Sandy). Different types of soil used were obtained from Soils & Water Resources Department, Faculty of Agriculture, Al-Azhar University. Tests were conducted in conical plastic cups (6.5 cm diameter x 4.5cm lower surface x 3cm height) 40 gm soil was added for each cup. Soil was moistened by atomizer adequate distilled water before inoculation with nematodes. For each cup, 1 ml of a suspension containing 750, 1500, 3000, 5000 and 7000 nematodes was applied to soil. Ten medfly larvae were placed in each cup and replicated three times for each concentration. In another assay, the soil types were substituted by 5 gm of cow manure mixed with the same three soil types. Cups with distilled water or containing clean soil free of nematode were used as check ones. Larvae were observed and larval mortality was recorded at 72 hours post treatment.

Statistical analysis:

Based on the data obtained, following statistical analysis has been carried out probit analysis (Finney, 1971) and analysis of variance (Three Way ANOVA) (SAS Institute, 2002).

RESULTS

Under controlled laboratory conditions, the present study demonstrated that the medfly larvae *C. capitata* were susceptible to *H.bacteriophora* (HP88) and *S. carpocapsae* (All) as a biological control

agent. The pathogenicity of these entomopathogenic nematodes were investigated.

Media:

There was no mortality in the control. Mortality percentage of the third instar larvae of the medfly to these nematodes at five concentrations under three different media are shown in Table (1) in the filter paper assay (irrespective to soil or soil with manure media), analysis of data generally, revealed that the highest mortality in *C. capitata* third larval instar. Amean of 40.74 dead larvae treated with *H. bacteriophora* (HP88) were recorded in filter paper it was followed discendingly by soil (29.63).Significantly efficiency than soil and soil with manure. Descending order filter paper > soil > soil with manure. Showing mortality rate 40.74, 29.63 and 25.37, respectively.

Steinernema carpocapsae also showed that the same trend but significantly effect filter paper and soil than soil with manure, the percentage mortality rate were 46.30, 48.33 and 34.63, respectively. On the other hand, *S. carpocapsae* (All) was more efficient than *H. bacteriophora* (HP88) in all three treatments (filter paper), soil and soil with manure respectively, as shown in Table 1. Mortality percentages were 46.30 and 40.74, 48.33 and 29.63, 34.63 and 25.37 irrespective to any another factors.

Temperature:

Temperature pathogenicity relationship showed that there is significant difference in the pathogenicity of the tested entomopathogenic nematode species to the 3rd larval instar of *C. capitata*at the tested temperature as shown in Table 1. There is difference between mean mortality values caused by *H. bacteriophora* (HP88) due to the difference of temperatures. The higher efficiency was at 25^{o}C while lower at 20^{o}C and the lowest was at 30^{o}C. There is a significant deference between the pathogenicity at 25^{o}C and 30^{o}C. Mortality rate were 37.22, 33.33 and 25.19, respectively. There was no mortality in the control. The pathogenicity of *S. carpocapsa* (All) was the highest at 25^{o}C than 30^{o}C and 20^{o}C, mortality percentages were 51.67, 39.44 and 38.15 respectively. Significant efficiency between the virulence at 25^{o}C and both 30^{o}C and 20^{o}C. On the other hand, both *H.bacteriophora* and *S.carpocapsae* were more pathogenic at 25^{o}C but, *S. carpocapsae* (All) also more virulent than *H. bacteriophora* (HP88) at all tested temperatures, mortality rate were 51.67, 37.22 and 39.44, 25.18 and 38.15, 33.33 at 25^{o}C, 30^{o}C and 20^{o}C, respectively.

Concentrations:

The data obtained indicated that percentage of larval mortalities of *C. capitata* increased by increasing the concentrations of the tested nematodes

(Table 1). The highest concentration 7000 Ijs/ml caused 78.15% larval mortality but the lowest concentration 750 Ijs/ml. Cause 21.85 mortality percentage of *S. carpocapsae* (All) on the 3rd larval instar of *C. capitata* treated with the same trend was obtained in case of *H. bacteriophora*, mortality rates ranged between 60.74 and 12.52 at the highest and the lowest concentrations. On the other hand, *S. carpocapsae* (All) was the most virulent than *H. bacteriophora* in comparison at all concentrations. Mortality rates were 78.14, 65.93, 53.33, 39.26 and 21.86%, respectively at *S. carpocapsae* (All), while they were 60.74, 52.22, 37.40, 28.52 and 12.52%, respectively, at *H. bacteriophora* (HP88).

Two entomopathogenic nematode species were selected to determine the median concentrations (LC_{50}) under controlled laboratory conditions. The results in Table 2, Figures (1 and 2) presented the LC_{50} and LC_{90} for *S. carpocapsae* (All) and *H. bacteriophora* under three tested constant temperatures 20°C, 25°C and 30°C and laboratory conditions. These results indicated that the LC_{50} of *H. bacteriophora* (HP88) at 25°C was the lowest compare with 20°C and 30°C (1795.6, 1903.2 and 5656.6), respectively. So that the pathogenicity was the highest at 25°C.

Table 1: Factorial analysis of different aspects of *H.bacteriophora* (HP88) and *S.carpocapsae* (All) on the 3rd medfly larval mortality percentage.

Factor	Level	*H.bacteriophora*	*S.carpocapsae*
Media	Filter paper	40.74 a	46.30 a
	Soil	29.63 b	48.33 a
	Soil + Manure	25.37 b	34.63 b
	P	0.0001	0.0001
Temperature	20°C	33.33 a	38.15 b
	25°C	37.22 a	51.67 a
	30°C	25.18 b	39.44 b
	P	**0.0001**	**0.0001**
Concentrations	Control	0.00	0.00
	750 Ijs/ml	12.52	21.85
	1500 Ijs/ml	28.52	39.26
	3000 Ijs/ml	37.41	53.33
	5000 Ijs/ml	52.22	65.93
	7000 Ijs/ml	60.74	78.15
	P	**0.0001**	**0.0001**

Means with the same letter are not significantly different.

Table 2: Relative efficiency of *H. bacteriophora* (HP88) and *S.carpocapsae* (All) at different temperatures using filter paper as media.

Nematodes	Temperature	LC_{50}	LC_{90}	Slope	Index
***H.bacteriophora* (HP88)**	20°C	1903.2	11494	1.64	94.34
	25°C	1795.6	12874	1.50	100.0
	30°C	5656.6	25898	1.94	31.74
***S.carpocapsae* (All)**	20°C	3040.0	22812	1.46	39.71
	25°C	1207.1	4899	2.11	100.0
	30°C	2827.4	24183	1.38	42.69

Moreover, *S. carpocapsae* (All) at 25°C was more virulent than 30°C and 20°C based on LC_{50} (1207.1, 2827.4 and 3040.0), respectively. Moreover, the virulence of *S. carpocapsae* (All) was more pathogenic than *H. bacteriophora*. LC_{50} of them were 1207.1 and 1795.6 at 25°C, respectively.

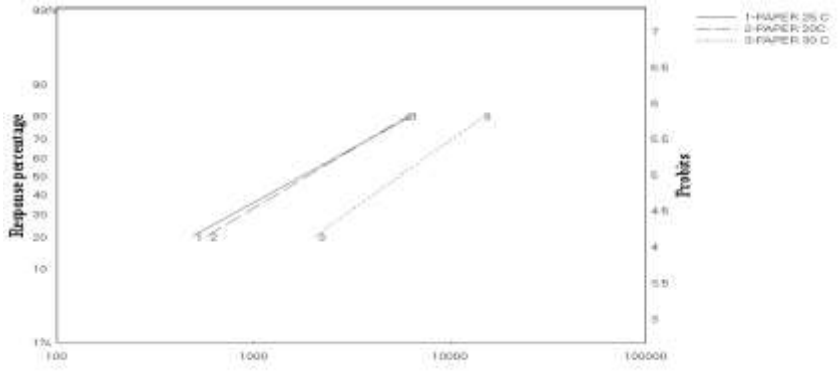

Figure 1: Ld-p line for the mortality of *C. capitata*larvae at different temperatures using filter paper as media affected by *H. bacterophora*.

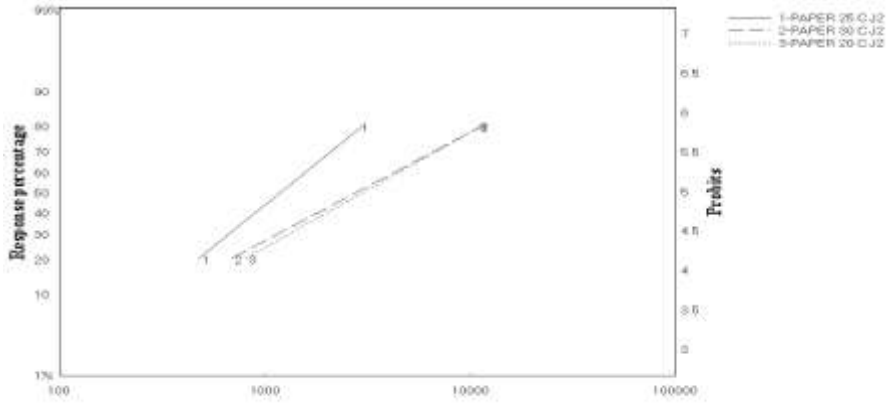

Figure 2: Ld-p line for the mortality of *C. capitata*larvae at different temperatures using filter paper as media affected by *S. carpocapsae*.

Results obtained in Table (3), Figures (3 and 4) present the LC_{50} and LC_{90} for the two tested entomopathogenic nematode species under controlled laboratory conditions. It is clear that the 3rd larval instar of *C. capitata* was susceptible to applied nematodes at the tested soil types; loamy, clay and sandy. LC_{50} values indicate that the percentage of mortality was the greatest at loamy soil. LC_{50} values were 4303.1, 5318.3 and 7250.3 at loamy, clay and sandy soil at *H. bacteriophora* (HP88). The same trend was also shown in *S. carpocapsae* (All). The LC_{50} values are (1505.4, 1884.0 and 2381.3) at loamy, clay and sand soil respectively. So that the loamy soil was the most susceptible for the highest infectivity of *S. carpocapsae* (All) on *C. capitata* larvae.

Table 3: Relative efficiency of *H.bacteriophora* (HP88) and *S. carpocapsae* (All) at different soil types.

Nematodes	Soil	LC_{50}	LC_{90}	Slope	Index
***H. bacteriophora* (HP88)**	Loamy	4303.1	42267	1.29	100.0
	Clay	5318.3	53243	1.28	80.91
	Sandy	7250.3	77034	1.25	59.35
***S.carpocapsae* (All)**	Loamy	1505.4	7807	1.79	100.0
	Clay	1884.0	13978	1.47	79.91
	Sandy	2381.3	20543	1.37	63.22

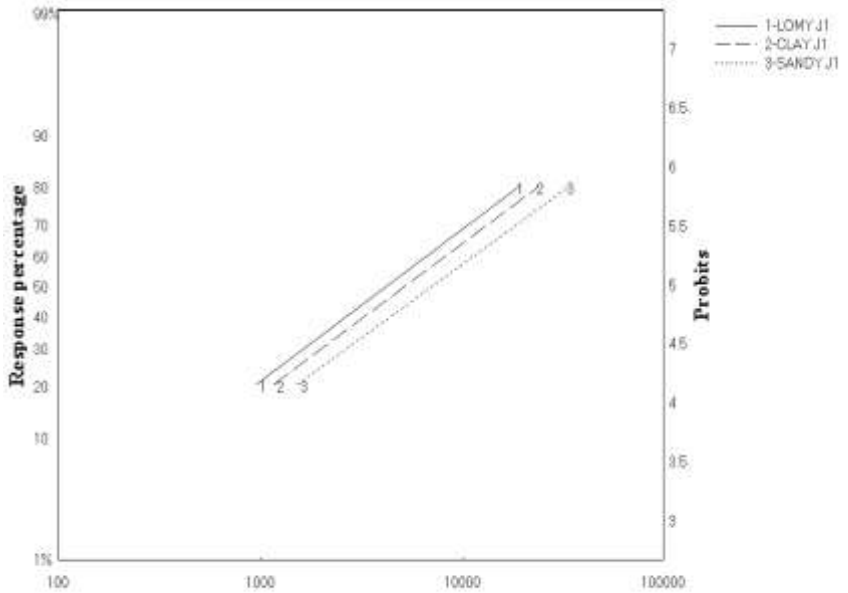

Figure 3: Ld-p line for the mortality of *C. capitata*larvae at different soil types affected by *H. bacterophora*.

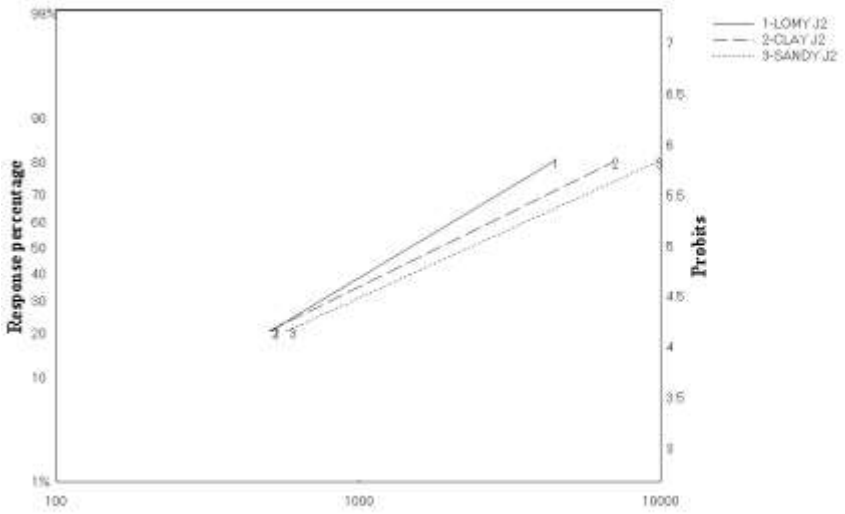

Figure 4: Ld-p line for the mortality of *C. capitata* larvae at different soil types affected by *S. carpocapsae*.

The results in Table 4, Figures (5 and 6) present the LC_{50} and LC_{90} for the two tested entomopathogenic nematode species on 3rd larval instar of *C. capitata* in three soil types (loamy, sandy, clay) mixed with cow manure under laboratory conditions. It is clear that the loamy soil was the most suitable soil for the pathogenicity of *H. bacteriophora* (HP88) or *S. carpocapsae* (All) to the *C. capitata* larvae. In case of *H. bacteriophora* (HP88) LC_{50} values were 5442.5, 6968.6 and 7177.6 at loamy soil with manure, sandy soil with manure and clay soil with manure, respectively. Data also, provided that *S. carpocapsae* (All) was most greatest efficiency than *H. bacteriophora* (HP88) at all soil types mixed with cow manure. Values of LC_{50} were 2519, 3794.6 and 4943.7 at loamy, sandy and clay mixed with manure, respectively.

Table 4: Relative efficiency of *H.bacteriophora* (HP88) and *S.carpocapsae* (All) at different soil types with Manure.

Nematodes	Soil with manure	LC_{50}	LC_{90}	Slope	Index
***H.bacteriophora* (HP88)**	Loamy	5442.5	52361	1.30	100.0
	Sandy	6968.6	59584	1.38	78.10
	Clay	7177.6	38954	1.75	75.83
***S.carpocapsae* (All)**	Loamy	2519.0	24580	1.30	100.0
	Sandy	3794.6	20371	1.76	66.38
	Clay	4943.7	22734	1.93	50.95

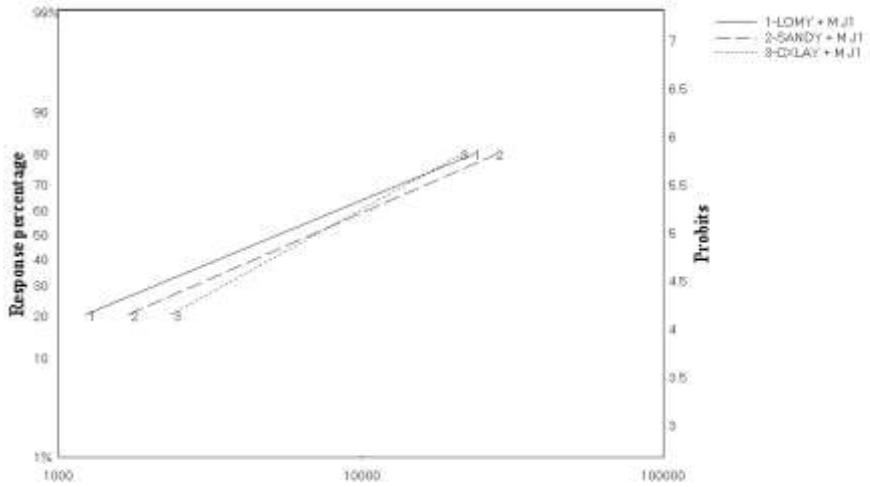

Figure 5: Ld-p line for the mortality of *C. capitata*larvae at different soil types with manure affected by *H. bacterophora*.

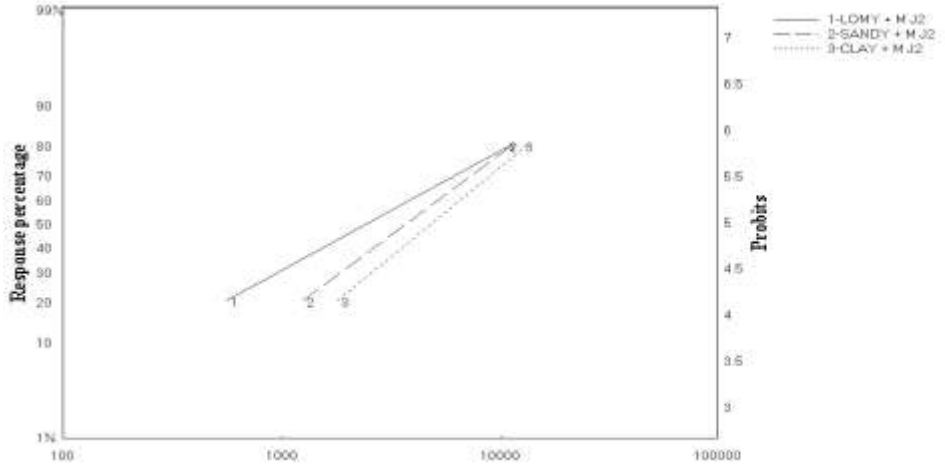

Figure 6: Ld-p line for the mortality of *C. capitata* larvae at different soil types with manure affected by *S. carpocapsae*.

DISCUSSION

Laboratory studies of entomopathogenic nematode species in Petridish assays at different temperatures, soil types and soil with cow manure generally, showed that *S. carpocapsae* (All) tested were more virulent to medfly larvae than *H. bacteriophora* (HP88). Irrespective to other studying factors Petridish assays with these species supported this result and indicate

that both *S. carpocapsae* (All) and *H. bacteriophora* (HP88) were pathogenic and killing the target insect. The greatest larval mortality was obtained for nematode concentration of 7000 Ijs/ml. These result demonstrated the ability of this nematode to find and kill medfly larvae at three different media, petridish assays at different temperatures, soil types and soil with cow manure. Overview, higher mortality rates and LC_{50} were obtained with filter paper than other media.

Firstly, temperature has been reported as an important environmental factor in survival, mortality, infection, development and reproduction of entomopathogenic nematodes (Poinar, 1979).In the present investigation, mortality rates of *C. capitata* larvae were directly correlated with the exposure temperatures, the optimum temperatures for nematode pathogenicity was 25^{o}C then mortality decreased at the lowest (20^{o}C) and highest temperature (30^{o}C) (Grewal *et al*., 1993) reported that maximum penetration of *Galleria mellonella* L. larvae by *S. carpocapsae* was at 24^{o}C, with reduced penetration occurring at 30 and 35^{o}C. Hammad and Abdel-Hamid (2007) reported that housefly larvae were susceptible to the entomopathogenic nematodes at 25^{o}C. Temperature is the most influential environmental factor, which has great biological significance. Temperature influences nematode mobility, reproduction and development (Mason & Hominik, 1995). In another study, Shapiro-Ilan *et al*., (2002) observed that temperature limits the virulence of steinernematids by its influence on nematode activity, bacterial symbiont or both. Mahar *et al*., (2005) found that the maximum number of *S.carpocapsae* was produced in the vine weevil larvae *Otiorhynchussulcatus* F. at 25^{o}C. The present study demonstrated that *S. carpocapsae* (All) was more efficient than*H. bacteriophora* with 78.15% and 60.74% mortality, respectively. The higher efficiency of *S. carpocapsae* at higher temperatures has already been reported (Grewal*et al*., 1994). Several studies have demonstrated the influence of temperature on the infectivity of entomopathogenic nematodes (El-Sadawy, 2001; Hazir *et al*., 2001; Ebssa *et al*., 2003 and Subramanian & Senthamizh, 2004). Hussaini*et al*., (2005) also observed a direct relationship between lethal time and temperature. These results indicate that thisentomopathogenic nematodes can be used to control insect pests that occur in tropical climate regions.

The second part of our experiment was carried out to determine the effects of soil type on the pathogenicity of entomopathogenic nematodes against medfly larvae. Both *S. carpocapsae* and *H. bacteriophora* caused different levels of mortality on larvae of *C. capitata*in all tested soil types. The reason for the efficacy of nematodes in all soils might be due to the

small size of test containers, and consequently the shorter distance which nematodes much move to reach the host insect. The results showed that *S. carpocapsae* (All) and *H. bacteriophora* (HP88) in loamy soil caused higher mortality than clay or sandy soils. This observation is consistent with early reports that showed infectivity of *S. carpocapsae* and *H. bacteriophora* were higher in lighter soils (Kung*et al.*, 1990; Choo and Kaya, 1991). Mortality rates of medfly larvae were reduced in all soil types with cow manure compared with soil without manure. The decrease of the entomopathogenic nematode pathogenicity may be due to that the increase of organic matter not suitable for the activity of nematodes. Our results appear to differ from those of Bednarek and Gaugler (1997) who found that *S. Feltiae* populations actually increased with the addition of manure to soils. However, studies of Shapiro *et al.*, (1996) supported our result; they found that manure reduced nematode virulence when added to soil as a fertilizer. The high efficiency of *S. carpocapsae* (All) on *C. capitata* larvae could be related to the relatively small size of their infective juveniles, ranging between 438-650 µm. (Adams & Naguyen, 2002) thus facilitating the penetration mode of the steinernematids, which take place through the natural openings of the host (Spiracles, mouth and anus). Most *C. capitata* larvae exposed to the nematode died during the pupal stage. This fact was also observed by Rohde *et al*., (2010) who studying the susceptibility of *C. capitata*, larvae to the entomopathogen, and Yee & Lacy (2003) obtained results showed that both *S. carpocapsae* (All) and *H. bacteriophora* (HP88) caused mortality on *C. capitata* larvae at all applied concentrations, except in the control treatment. Data also indicate that the higher nematode inoculums levels. However, caused higher mortality than the lower levels.

In brief, our results showed that entomopathogenic nematodes were pathogenic against 3rd larval instar of *C. capitata* the virulence of *S. carpocapsae* (All) was more than *H. bacteriophora* (HP88). Results also proved that nematode species, concentration, temperature, soil type or soil with manure were significantly affected mortality of medfly larvae under experimental conditions. Further studies are still required to define application strategies for these entomopathogenic nematodes in order to control this insect pest.

CONCLUSION

The tested entomopathogenic nematode species *S. capocapsae* (All) and *H. bacteriophora* (HP88) were pathogenic to 3rd instar larvae of *C. capitata.* At all three tested media and concentrations, *S . capocapsae* (All) more virulent than *H. bacteriophora* (HP88). The optimum temperature for

nematode pathogenicity was 25°C. Loamy soil was suitable for the pathogenicity than clay or sandy soil. Mortality rates of medfly larvae were reduced in all tested soil types with manure compared with soil without manure.

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تأثير النيماتودا الممرضة للحشرات على ذبابة الفاكهة

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تم تقدير فاعلية إثنتين من أنواع الديدان الخيطية الممرضة للحشرات *شتينرنيما كاربوكابساى و هيتيرورابديتيس باكتيروفورا* كعناصر للمكافحة الحيوية ضد يرقات العمر الثالث من ذبابة فاكهة البحر المتوسط وذلك تحت ظروف المختبر فى (ثلاث بيئات مختلفة) وبتحليل البيانات تبين أن بيئة أطباق بترى تؤثر على النسبة المئوية لموت اليرقات معنوياً مقارنة بكل من بيئتى التربة بمفردها والتربة بعد إضافة السماد حيث كانت نسبة الوفيات ٤٠.٧٤% ، ٢٩.٦٣% ، ٢٥.٣٧% على التوالى. وقد كان النوع *شتينرنيما كاربوكابساى* أكثر فاعلية عن *هيتيرورابديتيس باكتيروفورا* وذلك خلال التركيزات المختبرة حيث كانت النسبة المئوية للوفيات ٧٨.١٤% ، ٦٥.٩٣% ، ٥٣.٣٣%، ٣٩.٢٦% ، ٢١.٨٦% على التوالى. بينما سجل النوع *هيتيرورابديتيس باكتيروفورا* نسب الوفيات الآتية (٦٠.٧٤%، ٥٢.٢٢%، ٣٧.٤٠%، ٢٨.٥٢%، ١٢.٥٢%) على التوالى وقد تم أيضاً تقدير التركيز المميت لـ ٥٠% من اليرقات بعد تعريضها لمدة ٧٢ ساعة وذلك لكلا النوعين *شتينرنيما كاربوكابساى و هيتيرورابديتيس باكتيروفورا* فى البيئات الثلاثة المختبرة. كما أثرت درجات الحرارة المختبرة على كفاءة النيماتودا الممرضة للحشرات حيث كانت أعلى نسبة لوفيات اليرقات عموماً عند درجة ٢٥°م بينما تناقصت هذه النسبة عند درجتى الحرارة (٢٠°م) و (٣٠°م).

التوصية: أوضحت النتائج أن التربة الصفراء الخفيفة أفضل من التربة الطينية أو الرملية من حيث كفاءة النيماتودا الممرضة للحشرات على يرقات ذبابة الفاكهة كما أن نسبة الوفيات قد تناقصت عند إضافة السماد العضوى إلى أنواع التربة الثلاثة المختبرة مقارنة بالتربة غير المسمدة.