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Susceptibility of *Ceratitis capitate* (Wiedemann) To Native and Imported Entomopathogenic Nematodes and Compatibility with Abamectin and Fenamiphos

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ABSTRACT

Infectivity of two native Egyptian isolates belonging to *Heterorhabditis* spp. was evaluated against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), compared with imported *Steinernema* and *Heterorhabditis* species by two laboratory assays and compatible responses with recommended application concentration (RC) and 0.5 RC of abamectin and fenamiphos. The efficacy of EPNs is directly associated with an increase in concentrations, and percentages of mortalities were higher in the third larvae stage than in the pupal stage. Moreover, native *H.bacteriophora* (Ar-4 strain) caused the high mortality rates of imported *H.bacteriophora* (HP88 strain).At concentration 200 IJs/ larvae or pupa, *S. feltiae* (Filipjev) showed the shortest median lethal time (LT_{50}) for the killing of medfly with LT_{50} 1.19 days in third-instar larvae and 1.26 days with pupae of *C. capitata* followed by *S. glaseri* (NC strain) and *S. carpocapase* (All strain). On the other hand, native *H. bacteriophora* (Ht strain) caused the least larvae and pupae mortalities compared to other Egyptian isolates, *H. bacteriophora* Ar-4 strain with LT_{50} 2.25 days and 2.38 days in larvae and pupal stage with mentioned concentration, respectively. Regarding the compatibility of abamectin and fenamiphos with EPN species, incompatible responses were revealed with the RC of abamectin. In contrast, an additive effect was exhibited by combining EPN species and 0.5 RC of the tested nematicides. Also, larvae of medfly showed a compatible interaction (additive) when compared with the pupal stage, and *H.bacteriophora* (Ar-4 strain) was more compatible with two nematicides compared to *H.bacteriophora* (Ht strain). Current results indicate the feasibility of the integrated management of EPNs with a low dose of chemical pesticides in crop protection.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae), is recognized as the most disturbing and damaging fruit fly pest worldwide that reduced the production and quality of more than 360 different hosts ranging from citrus to soft and stone fruits and vegetables (Liquido *et al*., 1991; Papadopoulos *et al*., 1998; Satar *et al*., 2016). *C. capitata* can tolerate climatic conditions better than most other fruit flies, negatively impacting global fruit production. A successful integrated pest management (IPM) strategy uses a combination of natural enemies of pest arthropods and other alternative

measures that play significant roles in crop protection. Also, it minimized pesticides' negative environmental impacts and other deleterious effects while providing a more sustainable approach to pest control.

Several microbial control agents, such as viruses, bacteria, fungi, and nematodes, offer effective control that can be combined with other tactics. Moreover, they are safe for the environment, beneficial insects, applicators, and the food supply, and they can be applied just before field fruit harvest (Kaya & Lacey, 2007).

Laboratory and field studies assessed numbers of natural enemies, including parasitoids that can use their ovipositor to locate and parasitize medfly eggs and larvae within the fruit but are protected from entomopathogens and only entomopathogens including entomopathogenic nematodes (EPNs) (Bazman *et al.*, 2008; Gazit *et al*., 2000; Lindegren and Vail, 1986; Lindegren *et al.* 1990) or fungi (Ekesi *et al.*, 2007) could infect larvae during leave the fruit and enter the soil. To date, 16 out of 117 currently described EPN species (12 *Steinernema* and 4 *Heterorhabditis*) (Shapiro-Ilan *et al*., 2018), besides additional isolates and species, remain undescribed.

C. capitata is one of numerous soil insect pests successfully candidates for biological control by ENPs belonging to families of steinernematidae and heterorhabditidae, which are associated with mutualistic bacteria belonging to the genus *Xenorhabdus* in the genus *Steinernema* and *Photorhabdu*s in the genus *Photorhabdu*s respectively. The bacterial cells are carried as symbionts in the intestinal tract of the only free-living stage of the nematode, the infective juvenile (IJ) (Kaya *et al.*, 2006). Most applied EPN imported abroad and with several species isolated from Egyptian soils (El-Ashry *et al.*, 2018; Alexandros Dritsoulas *et al.*,2022).

Laboratory control studies have been conducted on medflies (Gazit *et al.*,2000; Karagoz *et al*.,2009; Minas *et al*.,2016; Mokrini *et al*.,2020; Kapranas *et al*.,2021). So, it has been proposed that applying EPNs in the soil beneath the tree canopy can kill a significant number of the soil-dwelling stages of medflies.

In recent studies, different authors elucidated the efficacy of steinernematid and heterorhabditid species against larvae and pupae of *C. capitata* in reducing population densities in laboratory assays or under field conditions (Mokrini *et al*.,2020; Kapranas *et al*.,2021).

In vitro assay helps improve our knowledge about combinations of virulent EPN species (native and imported species) in subsequent field management tactics combination between biocontrol agents used against fruit flies on crops (Hooper *et al*.,2005; Neumann & Shields, 2008; Finke & Snyder, 2010). However, *in vitro* assays of previous studies by El-Ashry *et al*., 2020; Aioub *et al.*, 2021 compatible studies showed that the combining effect with various pesticides could be antagonistic, synergistic, or additive with different EPN species.

Therefore, the current study focused on the efficacy of native and imported EPNs as implemented biological control agents with two pesticides used in the control of larvae and pupae of *C. capitata* in soil under laboratory conditions to improve our knowledge that will increase our understanding of inclusive EPNs as a biocontrol agent in fruit fly IPM programs.

MATERIALS AND METHODS

Rearing of Mediterranean Fruit Fly, *Ceratitis capitata:*

According to (Vargas, 1989), the laboratory culture of *C. capitata* colony was previously supplied as pupae by Plant Protection Institute in Dokki, Giza, Egypt., and then reared on an artificial diet. Adults of medfly were provided with water and a standard adult

diet consisting of a mixture of Brewer's yeast, sugar, and water at a 4:1:5 ratio and reared in wire-screened wooden holding cages (40×40×65 cm) (Pašková, 2007). Eggs were collected from modified oviposition domes and provided with water and (*Citrus aurantium* L.; Rutaceae) bitter orange juice to stimulate oviposition. Larvae were reared on an artificial diet prepared by mixing 330 g wheat bran, 82.5 g sugar, 82.5 g yeast extract, 4 g citric acid, 4 g sodium benzoate, and 500 ml water (Leftwich *et al.,* 2017). Daily collected eggs from the adult cages were transferred to the artificial diet and reared until the third instar in the laboratory at 25±2ºC and 60±5% RH. Mature larvae or pupae were collected from the artificial diet with a 2 mm diameter sieve for the bioassays

Pesticides Used:

Two registered commercial formulations of pesticides available in the market and used for controlling insect and nematode pests in Egypt were obtained from the Central Laboratory of Pesticides, Dokki, Giza. The tested pesticides were used in the current study at recommended application rate (RC) and 0.5 RC. One of them was a biopesticide, abamectin (Tervigo 2% SC) was used at the recommended concentration (RC), 3 L/feddan, and 0.5 RC (1.5 L/feddan). In contrast, the other one was organophosphate; fenamiphos (Dento 40%EC) was used at RC 6 L/feddan, and 0.5 RC (3 L/feddan).

Nematode Cultures:

Steinernema carpocapsae (All strain), *S. feltiae* (Filipjev), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain) used in these experiments included two native species, *H. bacteriophora* (Ar-4strain) and *H. bacteriophora* (Ht strain) isolated from EL-Arish and Belbis district, Egypt, according to El-Ashry *et al*., 2018 and used in all laboratory bioassays.

EPN species reared in last-instar greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), at approximately 24 ±2°C according to procedures described in Kaya and Stock (1997). Infective juveniles (IJs) that emerged from insect cadavers in White traps (White, 1927) were stored in shallow water in transfer flasks at 15°C for up to 7 days before use.

Bioassays Studies:

Two laboratory techniques were conducted to bioassay the native and imported EPNs against larvae and pupae of *C. capitata*.

A. Plastic Cups Method:

Plastic cups (60 mm diameter × 110 mm high) filled with 180 g of sterilized sand were used. The sand of cups was kept at 10 % moisture and provided 20 individuals of medfly (3rd larval instars or pupae). To evaluate the efficacy of native EPNs species [*H.bacteriophora* (Ar-4strain) and *H.bacteriophora* (Ht strain)] or imported EPNs [*Steinernema carpocapsae* (All strain), *S.feltiae* (Filipjev), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain)], EPNs doses of 100, 150 and 200 IJs/larva or pupa were used in this study. The administered cups were capped by a lid, punctured for aeration, and kept at room temperature (26 ±2ºC). Mortality was recorded daily for seven days after EPNs inoculation; to approve the infection, the dead larvae and pupae that showed typical infection signs were placed in White traps in moisten chamber to approve infection (White, 1927). Emerged adults were counted, and mortality was calculated by subtracting the emerged adults from the initial number of larvae or pupae. The mortality of larvae and pupae and the efficiency of EPNs were also determined according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae or pupae}}{(\text{Total number of dead larvae or pupae})} x100$$

The control treatment of *C. capitate* received the same volume of distilled water used in treatments of *C. capitata* larvae and pupae and the bioassay was performed twice.

B. Petri Dishes Assay:

Native and imported EPNs were used for the Petri dish assay. Each Petri dish of 9 cm filled with 25 g of dry, autoclaved sand (0.3 to 0.5 mm particle size). About 100, 150, and 200 IJs/Petri dish was used in 1 ml, and each dish introduced 20 healthy medfly larvae or pupae. Control Petri dishes received only distilled water. The medfly mortalities (in larvae or pupae) were recorded daily for seven days. Where mortality percentages were calculated according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae or pupae}}{(\text{Total number of dead larvae or pupae})} x100$$

If mortality in the controls is between 5% and 20%, cumulative mortality counts obtained from experiments were corrected for natural mortality using Abbott's formula:

$$\text{Corrected Mortality (\%)} = \frac{\text{X - Y}}{\text{100 - Y}} \times 100$$

X = percentage mortality in the treated sample, and Y = percentage mortality in the control. As well as, the median lethal Dose (LD_{50}) and lethal Time (LT_{50}) of nematodes were estimated. Dead larvae and pupae were dissected after mortality.

The Combined Effect of Entomopathogenic Nematodes Abamectin and Fenamiphos Using Plastic Cups Technique:

The biopesticide abamectin and fenamiphos were used to test the combined effect with EPN species. Both nematicides were tested for their effectiveness against larvae and pupae of *C.capitata* as concomitant treatments (EPNs + RC or 0.5 RC) (El-Ashry *et al*.,2020). Previous plastic cups (60 mm diameter × 110 mm high) filled with 180 g of sterilized sand were used to conduct the bioassay. Each plastic cup with 20 individuals of 3rd larval stage or pupae treated with 2 ml of tested native or imported EPNs contained 200 IJs and was sprayed immediately with 10 ml of recommended application rate (RC) or 0.5 RC of the tested nematicides. Plastic cups of_Medfly stages (larvae or pupae) in control treatments were provided with the same number of larvae or pupae sprayed with 2 ml of distilled water only free of EPNs juveniles or tested concentrations of nematicides.

Medfly mortalities in larvae or pupae were observed and recorded daily for seven days

Analysis of the Chemo/Bio-Interaction:

Interaction data for mixtures were estimated using Limpel's formula reported by Richer (1987) as follows:

$$\mathbf{E} = \frac{(\text{X} + \text{Y}) - (\text{XY})}{100}$$

Where:

E: The expected additive effect of the mixture.

X: The effect is due to component A alone.

Y: The effect is due to component B alone.

The expected effect was compared with the actual effect obtained experimentally from the mixture to determine the additive, synergistic or antagonistic effects, according to the equation given by Mansour *et al.* (1966) as follows:

$$\text{Co- toxicity factor} = \frac{\text{The observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100$$

This factor was used to classify results into three categories: ≥ +20 is considered potentiation, ≤ -20, and -20: +20 indicates only additive effect.

Statistical Analysis:

The experiments were carried out in a completely randomized design. Each treatment was replicated five times. Data were subjected to analysis of variance (ANOVA) using MSTAT VERSION 4 (1987). Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS

1-Pathogenicity of Multiple Entomopathogenic Nematode (EPN) Species On *C. Capitata* Larvae And Pupae:

1. a: Pathogenicity of *Steinernema* and *Heterorhabditis* species/ isolates on *C. capitata* larvae and pupae:

Imported three *Steinernema* species [*S. carpocapse* (All strain), *S. fetltiae* (Filipjev) and *S. glaseri* (NC strain)] and two imported *Heterorhabditis* species, *H. bacteriophora* (HP88 strain) besides two Egyptian *Heterorhabditis* isolates (Ar-4 strain and Ht strain) were screened by plastic cups methods against medfly, *C. capitata* larvae (3rd instar) and pupae to identify their virulence under laboratory condition. In this assay, three doses of 100, 150, and 200 IJs/larvae or pupa were tested to assess the LD_{50} (lethal dose concentration able to kill 50% of tested larvae or pupae).

From current results, LD_{50} decreased gradually by increasing doses of EPN species and varied according to EPN species/isolates and medfly stages.

LD_{50} resulted from the pupa of medfly treated with 100, 150 and 200 IJs were 4.99, 4.75, 3.6874 IJs/pupa, whereas LD_{50} of 3rd instar larva was 4.78, 3.51 and 3.16 IJs/larva in treatment of *S. carpocapse* (All strain), respectively. The parallel values with pupa and larva of medfly treated with *S fetltiae* (Filipjev) were 4. 4.61, 4.32, 3.88 & 4.11, 3.70, 3.42, respectively. Also, with *S. glaseri* (NC strain), LD_{50} with pupae and larvae recorded 4.9861, 3.98, 3.74 & 4.81, 3.89, and 3.4012, respectively. Results in Table (1) exhibited that *Steinernema* species were more virulence against larvae than pupae of medfly, and *S. fetltiae* (Filipjev) was more virulent, followed by *S. carpocapse* (All strain) and *S. glaseri* (NC strain), respectively.

Table 1: Comparative infectivity of imported nematodes and native isolates of *H. bacteriophora* against medfly, *C. capitata* 3rd larval instars and pupae in plastic cup method by using the median lethal dose.

Nematode species (Strains)	Dose (No. of IJs/ cup)	Pupae LD_{50} (No. of IJs/pupa)	Larvae (3rd instar) LD_{50} (No. of IJs/pupa)
S. carpocapsae (All strain)	100	5.00	4.79
	150	4.76	3.52
	200	3.69	3.17
S. feltiae (Filipjev)	100	4.62	4.11
	150	4.33	3.71
	200	3.89	3.42
S. glaseri (NC strain)	100	4.99	4.82
	150	3.99	3.90
	200	3.75	3.40
H. bacteriophora (HP88 strain)	100	4.46	4.25
	150	3.79	3.55
	200	3.33	2.86
H. bacteriophora (Ar-4 strain)	100	5.14	4.83
	150	4.76	4.44
	200	3.83	3.34
H. bacteriophora (Ht strain)	100	5.81	5.61
	150	5.25	4.90
	200	3.90	3.93

*Each value is a mean of five replicates with 20 medfly larvae or pupae.

**Tested medfly larvae /pupae were observed for seven days.

Concerning imported and native *Heterorhabditis* species/ isolates, the virulence of imported *H. bacteriophora* (HP88 strain) overwhelmed the two native isolates, *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain). For instance, LD_{50} with papa and larvae recorded 5.81, 5.25, 3.90& 5.61, 4.90, 3.93 with *H. bacteriophora* (Ht strain) whereas the parallel values with native *H. bacteriophora* (Ar-4 strain) were 5.14, 4.75, 3.82 & 4.82, 4.44, 3.34, respectively.

The results revealed that native species were less virulent against larvae, and pupae of medfly, *C. capitata*, and LD_{50} decreased steadily as the increase in concentration dose tested against larvae of pupae under laboratory conditions assay.

In plastic cup analysis, results displayed that the species of *Steinernema* belonging to EPNs were more virulent than *Heterorhabditis* species and showed the least LT_{50} (lethal time required for killing of 50% larvae or pupae treated with 200IJs), particularly when compared with two native *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain). For instance, LT_{50} (After four days) with *S. carpocapse* (All strain), *S. fetltiae* (Filipjev), and *S. glaseri* (NC strain) were 1.87, 1.19, and 1,50 days with larvae and 2.05,1.26 and 1.65 days with medfly pupae. LT_{50} with imported *H. bacteriophora* (HP88 strain) was 1.50 and 1.65 with 3ed instar and medfly pupae, respectively. On the other hand, native *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain) were less virulent against medfly, *C. capitata* larvae, and pupae. For instance, LT_{50} were 2.25 & 2.71 with larvae and 2.38 &2.70 with pupae of medfly treated with *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain), respectively. As Fig. (1) illustrates, larvae were more sensitive to EPNs, and *Steinernema* species were more virulent against medfly larvae and pupae than *Heterorhabditis* species. Moreover, native *Heterorhabditis* species were less aggressive than imported *H. bacteriophora* (HP88 strain).

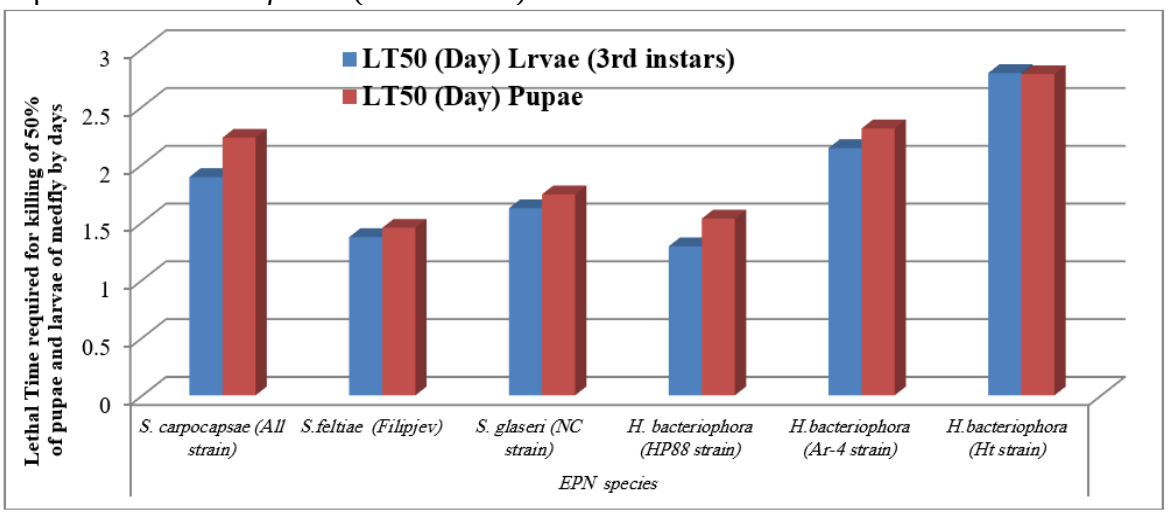

Fig. 1 . Median lethal time (LT_{50}) of *C. capitata* larvae (3^{rd} instars) and pupae, after four days post utilization of imported nematodes and native isolates of *H. bacteriophora* in plastic cup method.

2. Mortality Percentages (%) Of EPN Species at Different Concentrations Against Larvae and Pupae of *Ceratitis capitata* in Plastic Cups*:*

In all treatments (larvae/pupae) tested concentrations (100,150, and 200 IJs) against medfly, *C. capitata* were more effective than controls. Moreover, as the concentration of tested EPNs increased in steinernematid and heterorhabditid species, the mortality rate of the larvae and pupae individuals increased in direct proportion ($p < 0.05$).

The highest mortality rate detected at a concentration of 200 IJs/larvae or pupae against medfly and *S. feltiae* (Filipjev) caused the highest mortality (96.2& 99.9 and 88.6 &

90.8% with larvae and with pupae) after 48 and 96 hr followed by *S. glaseri* (NC strain) with 95.7&99.2% and 89.9& 94.6% with larvae and pupae after 48 and 96 hr, respectively. In contrast, the mortality percentages with *S. carpocapsae* (All strain) were 94.9&99.4 % and 88.4 & 91.9%, respectively.

On the other hand, the lowest percentage of mortalities was assessed with native heterorhabditid species, *H. bacteriophora* (Ht strain). Moreover, *H. bacteriophora* (Ar-4strain) scored 95.8& 99.1 % and 89.0 & 91.2 % with larvae and pupae after 48 and 96 hrs post-treatment, respectively (Table 2). In general, all steinernematid species and imported *H. bacteriophora* (HP88 strain) were more effective against larvae and pupae of *C. capitata* at all tested concentrations (100, 150 and 200 IJs) than the two native heterorhabditid species, *H. bacteriophora* (Ar-4strain) and *H. bacteriophora* (Ht strain).

Table 2: Percentages of mortality (%) in larvae and pupae of medfly, *C. capitata* in plastic cup method after 48 and 96 hours of exposure to nematode species.

Nematode species (Strain)	Concentrations (IJs/ Plastic cup)	% Mortality in medfly, *C. capitata*			
		Larvae (3rd instars)		Pupae	
		48 h	96 h	48 h	96 h
S. carpocapsae (All strains)	100	29.9^{i}	56.8^{f}	22.9^{g}	49.2^{hi}
	150	64.6^{ef}	89.8^{b}	58.6^{d}	82.2^{d}
	200	94.9^{a}	99.4^{a}	88.4^{a}	91.9^{a}
S. feltiae (Filipjev)	100	37.8^{h}	44.8^{h}	30.0f^{g}	40.4^{j}
	150	68.2^{de}	77.9^{d}	53.6^{d}	68.6^{f}
	200	96.2^{a}	99.9^{a}	88.6^{a}	90.8^{abc}
S. glaseri (NC strain)	100	47.8^{g}	56.4^{f}	32.6^{f}	46.8^{i}
	150	72.9^{d}	86.8^{c}	58.8^{d}	77.5^{e}
	200	95.7^{a}	99.2^{a}	89.9^{a}	94.6^{a}
H. bacteriophora (HP88 strain)	100	48.6^{g}	56.9^{f}	41.9^{e}	51.4^{h}
	150	82.9^{c}	90.1^{b}	68.8^{c}	81.6^{d}
	200	98.8^{a}	99.8^{a}	84.4^{ab}	89.2b^{c}
H. bacteriophora (Ar-4strain)	100	35.9^{h}	52.6^{g}	31.2^{fg}	46.7^{i}
	150	78.4^{c}	80.6^{d}	54.8^{d}	70.8^{f}
	200	95.8^{a}	99.1^{a}	89.0^{a}	91.2^{ab}
H. bacteriophora (Ht strain)	100	34.4^{h}	44.2^{h}	31.8^{f}	39.6^{j}
	150	58.9^{f}	71.5^{e}	44.5^{e}	58.4^{g}
	200	80.7^{b}	93.8^{b}	80.6^{b}	86.9^{c}

[1] Reported number represent the means of five replicate counts.

[2]Different letters in the same column represent significant differences ($P < 0.05$) among different nematode strains according to Duncan's multiple range test.

General percentage mortalities in larvae and pupae of medfly in plastic cup method after 48 and 96 hr illustrated in (Fig.2a &b). Among *Heterorhabditis* species /isolates, one native (*H. bacteriophora* Ar-4 strain) and three imported *Steinernema* species (*S. carpocapsae* All strain, *S. feltiae* (Filipjev) and *S. glaseri* NC strain had excellent performances against 3rd instar larvae of medfly, and pupae achieved more efficacy against larvae than pupae. Moreover, *the H. bacteriophora* HP88 strain, followed by the native *H. bacteriophora* Ar-4 strain, exhibited good performances with insignificant differences ($p < 0.05$) with the imported *H. bacteriophora* HP 88 strain. At the same time, the native *H. bacteriophora* Ht strain was the least efficient isolate.

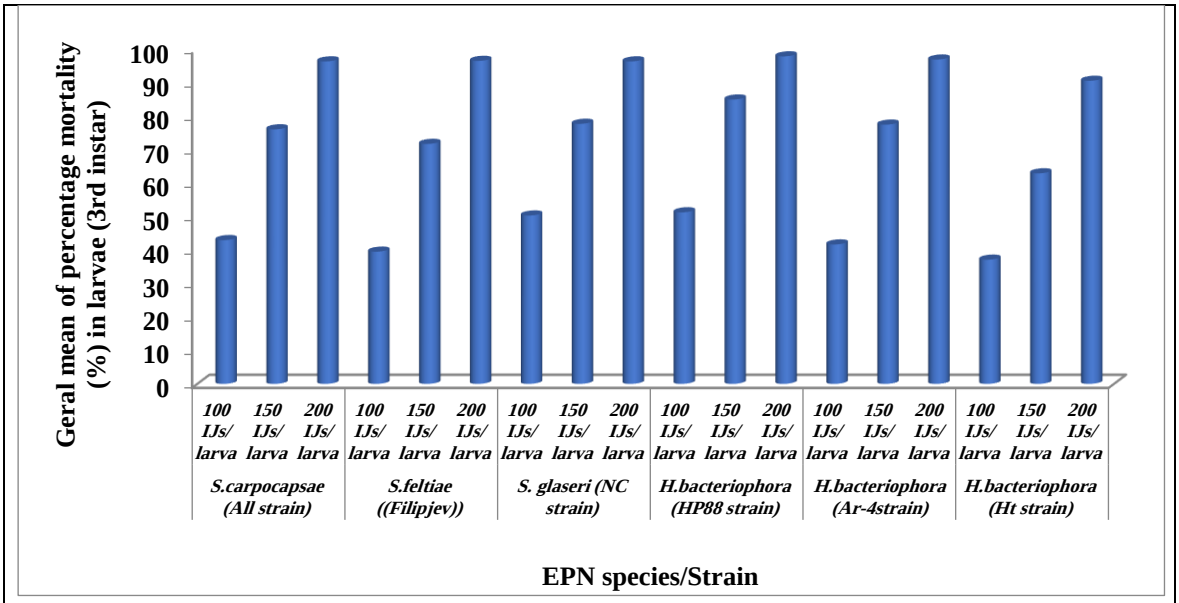

Fig. 2a: General means percentages mortality in 3rd instars larvae of *C. capitata* in plastic cup method resulted from 48 and 96 hours of exposure to EPNs species/strains.

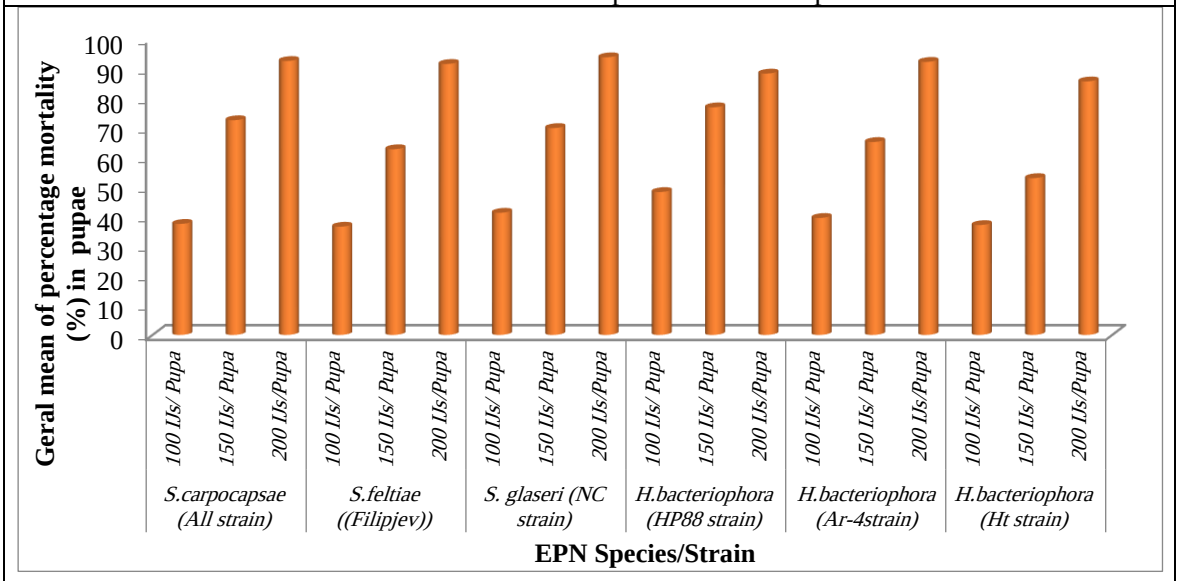

Fig. 2b: General means percentages mortality in pupae of *C. capitata* in plastic cup method resulted from 48 and 96 hours of exposure to EPNs species/strains.

3. Mortality Percentages (%) of EPN Species at Different Concentrations Against Larvae and Pupae of *C. capitata* in Petri Dishes*:*

The percentage of mortalities obtained from the Petri dishes assay was less than those of plastic cups with larvae and pupae of medfly at the three tested concentrations. As mentioned before with the plastic cups assay, as the concentration of tested EPNs increased in steinernematid and heterorhabditid species, the mortality rate of the larvae and pupae individuals increased in direct proportion ($p < 0.05$).

The concentration of 200 IJs/larvae or pupae exhibited the highest mortality rate against medfly, and *S. feltiae* (Filipjev) caused the highest mortality (86.8 & 91.9% and 81.0 & 84.3% with larvae and pupae) after 48 and 96 hr followed *S. glaseri* (NC strain) with 85.4

& 88.4 % and 80.1& 84.1% with larvae and pupae after 48 and 96 hr, respectively. The mortality percentages resulting from *S. carpocapsae* (All strain) were 85.3 & 88.7% and 71.2 & 82.5%, respectively.

On the other hand, the lowest percentage of mortalities was evaluated with native heterorhabditid species, *H. bacteriophora* (Ht strain). However, *H. bacteriophora* (Ar-4strain) scored 77.3& 80.8 % and 68.8 & 72.1% with larvae and pupae after 48 and 96 hr post-treatment, respectively (Table 3). Generally, steinernematid species and imported *H. bacteriophora* (HP88 strain) were more effective against larvae and pupae of medfly at concentrations of 100, 150 and 200 IJs than the two native heterorhabditid species, *H. bacteriophora* (Ar-4strain) and *H.bacteriophora* (Ht strain).

Table 3: Percentages of mortality in larvae and pupae of *C. capitata* in the Petri dish method after 48 and 96 hours of exposure to nematode species.

Nematode species	Strains	Concentrations (IJs/Petri dish or Plastic cup)	Larvae (3rd instars)		Pupae	
			48 h	96 h	48 h	96 h
S. carpocapsae	(All strain)	100	24.9^{h}	48.7^{fg}	17.9^{hi}	38.3^{h}
		150	57.9^{d}	78.6^{c}	50.5^{d}	72.4^{c}
		200	85.3^{a}	88.7^{b}	71.2^{b}	82.5^{ab}
S. feltiae	(Filipjev)	100	28.5^{h}	34.9^{i}	20.3^{gh}	30.7^{j}
		150	58.7^{d}	68.8^{d}	44.5^{e}	58.8^{f}
		200	86.8^{a}	91.9^{a}	81.0^{a}	84.3^{a}
S. glaseri	(NC strain)	100	38.6^{fg}	46.5^{gh}	24.4^{g}	36.0^{hi}
		150	66.3^{c}	76.7^{c}	50.4^{d}	67.2^{e}
		200	85.4^{a}	88.4^{ab}	80.1^{a}	84.1^{a}
H. bacteriophora	(HP88 strain)	100	35.7^{g}	47.8^{fgh}	29.9^{f}	31.6^{ij}
		150	72.9^{b}	81.7^{c}	54.5c	72.1^{cd}
		200	88.9^{a}	90.3^{ab}	74.2^{a}	78.2^{b}
H. bacteriophora	(Ar-4strain)	100	17.8^{i}	26.7^{j}	13.8^{i}	21.5^{k}
		150	51.7^{e}	60.5^{e}	30.4^{f}	50.4^{g}
		200	77.3^{b}	80.8^{c}	68.8^{b}	72.1^{c}
H. bacteriophora	(Ht strain)	100	35.6^{g}	43.6^{h}	14.2^{hi}	20.2^{k}
		150	41.8^{f}	51.4^{f}	21.9^{g}	37.8^{h}
		200	73.7^{b}	77.9^{c}	45.8^{e}	67.5^{de}

[1]Reported numbers represent the means of five replicate counts.

[2]Different letters in the same column represent significant differences ($P < 0.05$) among different nematode strains according to Duncan's multiple range test.

Interactions Response Between EPNs and Nematicides:
Abamectin and EPN Species/Strains with Pupae:

Unfortunately, combinations between RC of abamectin are completely incompatible with *S.carpocapase* (All strain), *S.feltiae* (Filipjev), *S.glaseri* (NC strain), *H.bacteriophora* (HP88 strain), *H.bacteriophora* (Ar-4 strain) and *H.bacteriophora* (Ht strain) against medfly, *C. capitata* (Wiedemann) pupae in plastic cups method after three days of application (Table 4). High incompatible results from the combination between native heterorhabditid nematodes, *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain), followed by *S.glaseri* (NC strain), *S.feltiae* (Filipjev) and *S.carpocapase* (All strain).

Whereas, combined application between EPN species and 0.5 RC of abamectin and EPNs in plastic cups assay against pupae of medfly after three days post-treatment exhibited incompatible reaction (antagonism) with EPN species except with *S.carpocapase* (All strain) and *S.feltiae* (Filipjev) which showed additive effect (Table 4).

Table 4: Interaction responses between recommended application rate (RC) of abamectin and different EPNs strains on mortality of *C. capitata* pupae in plastic cups method after three days of application.

Application rate	Treatments	Mortality (%) (EPN +Nematicide)		Co-toxicity factor (CF)	Interaction type
		Observed	Expected		
1RC	Abamectin + Sc	78.5	91.12	-13.86	Antagonism
	Abamectin + Sf	67.5	90.70	-25.58	Antagonism
	Abamectin + Sg	51.7	91.03	-43.21	Antagonism
	Abamectin + HP 88	55.6	90.23	-38.38	Antagonism
	Abamectin + Hb Ar-4	48.9	93.95	-47.95	Antagonism
	Abamectin + Hb Ht	45.5	91.96	-50.52	Antagonism
0.5 RC	Abamectin + Sc	76.8	84.10	-8.68	Additive
	Abamectin + Sf	75.6	86.26	-12.36	Additive
	Abamectin + Sg	63.5	84.67	-25.01	Antagonism
	Abamectin + HP 88	63.5	84.83	-25.15	Antagonism
	Abamectin + Hb Ar-4	55.6	83.30	-33.26	Antagonism
	Abamectin + Hb Ht	65.8	83.05	-20.77	Antagonism

[1] Reported numbers represent the means of five replicate counts.

[2]Different letters in the same column represent significant differences ($P < 0.05$) among different nematode strains according to Duncan's multiple range test.

Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).

**RC = Recommended application rate and 1/2 RC = Half recommended application rate.

Abamectin and EPN Species/Strains with Larvae:

The compatible response appeared with all EPN species and RC treatments or 0.5 RC of abamectin treatments in plastic cups. Utilization of 0.5 RC of abamectin was more compatible than RC application against medfly third instar larvae. The high additive response appeared with *H. bacteriophora* (HP88 strain) followed by *H. bacteriophora* (Ar-4 strain), *S.carpocapase* (All strain), and *S.feltiae* (Filipjev), respectively, with 0.5 RC application of abamectin. Although additive response exhibited with RC of abamectin, native heterorhabditid species, *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain) were less compatible with CF of -18.34 and -7.79 when compared with *Steinernema* species, *S.carpocapase* (All strain) and *S.feltiae* (Filipjev), with CF -8.68 and -9.44, respectively (Table5).

Table 5. Interaction responses between half recommended application rate of abamectin and different EPNs strains on mortality of *C. capitata* larvae (3rd instars) in the plastic cups method after three days of application.

Application rate	Treatments	Mortality (%) (Nematode +Nematicide)		Co-toxicity factor (CF)	Response
		Observed	Expected		
1RC	Abamectin + Sc	71.8	77.86	-8.68	Additive
	Abamectin + Sf	70.5	77.20	-9.44	Additive
	Abamectin + Sg	69.8	77.08	-12.49	Additive
	Abamectin + HP 88	70.2	80.22	-14.24	Additive
	Abamectin + Hb Ar-4	71.1	82.90	-18.34	Additive
	Abamectin + Hb Ht	62.2	76.17	-7.79	Additive
0.5 RC	Abamectin + Sc	86.2	80.38	7.24	Additive
	Abamectin + Sf	84.1	79.80	5.39	Additive
	Abamectin + Sg	80.1	79.69	0.52	Additive
	Abamectin + HP 88	89.7	82.47	8.77	Additive
	Abamectin + Hb Ar-4	88.9	84.85	4.78	Additive
	Abamectin + Hb Ht	78.8	78.88	-0.10	Additive

*Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).

**RC = Recommended application rate and 1/2 RC = Half recommended application rate.

Tervigo and EPN Species/Strains:
Various Interactions Between Larvae of Medfly and Tervigo with Larvae:

Antagonism effect exhibited with RC of fenamiphos and S. *feltiae* (Filipjev), *S.glaseri* (NC strain) against larvae of medfly, *C. capitata* whereas additive effect appeared in treatment of combination between RC of fenamiphos and *S.carpocapase* (All strain). Also, native heterorhabditid species, *H.bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ht strain) showed incompatible reactions with RC of fenamiphos. Only *H. bacteriophora* (HP88 strain) showed additive effects when combined with RC of fenamiphos three days post-treatment.

On the other hand, the application of 0.5 RC of fenamiphos in the treatment of heterorhabditid species, *H.bacteriophora* (HP88 strain), *H. bacteriophora* (Ar-4 strain), and *H .bacteriophora* (Ht strain) revealed additive effect as well as steinernematid species, *S. carpocapase* (All strain) only (Table 6).

Table 6. Interactions response between recommended and half recommended application rate of Tervigo and different EPNs strains on mortality of medfly, *Ceratitis capitata* (Wiedemann) larvae (3rd instars) in the plastic cups method after three days of application.

Application rate	Treatments	Mortality (%) (Nematode +Nematicide)		Co-toxicity factor (CF)	Response
		Observed	Expected		
1RC	Fenamiphos + Sc	69.85	76.78	-9.03	Additive
	Fenamiphos + Sf	43.45	74.44	-41.64	Antagonism
	Fenamiphos + Sg	45.05	76.53	-41.14	Antagonism
	Fenamiphos + HP 88	63.02	75.66	-16.71	Additive
	Fenamiphos + Hb Ar-4	41.86	78.97	-46.99	Antagonism
	Fenamiphos + Hb Ht	37.99	74.05	-48.70	Antagonism
0.5 RC	Fenamiphos + Sc	75.54	75.42	0.15	Additive
	Fenamiphos + Sf	49.48	72.95	-32.18	Antagonism
	Fenamiphos + Sg	58.47	75.16	-22.21	Antagonism
	Fenamiphos + HP 88	73.60	74.24	-0.86	Additive
	Fenamiphos + Hb Ar-4	72.24	77.74	-7.08	Additive
	Fenamiphos + Hb Ht	60.63	72.54	-16.42	Additive

*Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).
**RC = Recommended application rate and 1/2 RC = Half recommended application rate.

With the recommended dose of fenamiphos, compatibility and incompatibility effects were assessed with EPNs against medfly pupae three days post-treatment. For instance, *S. carpocapase* (All strain), *H. bacteriophora* (HP88 strain) and *H. bacteriophora* (Ar-4 strain) showed additive effect (compatibility) with RC of fenamiphos whereas *S.feltiae* (Filipjev), *S.glaseri* (NC strain) and *H. bacteriophora* (Ht strain) exhibited antagonism (incompatible) response (Table 7). On the other hand, using 0.5 RC of fenamiphos with EPN species revealed an additive response with all EPN species except with *S. feltiae* (Filipjev).

Table 7. Interactions response between recommended and 0.5 application rate of fenamiphos and different EPNs strains on mortality of *C. capitata* pupae (3rd instars) in the plastic cups method after three days of application.

Application rate	Treatments	Mortality (%) (Nematode +Nematicide)		Co-toxicity factor (CF)	Response
		Observed	Expected		
1RC	Fenamiphos + Sc	86.75	82.1	-5.36	Additive
	Fenamiphos + Sf	86.23	58.7	-31.93	Antagonism
	Fenamiphos + Sg	86.01	56.8	-33.97	Antagonism
	Fenamiphos + HP 88	85.57	74.8	-12.60	Additive
	Fenamiphos + Hb Ar-4	85.50	68.7	-19.66	Additive
	Fenamiphos + Hb Ht	83.38	60.3	-27.68	Antagonism
0.5 RC	Fenamiphos + Sc	92.28	85.3	-7.57	Additive
	Fenamiphos + Sf	91.99	59.9	-34.89	Antagonism
	Fenamiphos + Sg	91.86	78.3	-14.76	Additive
	Fenamiphos + HP 88	91.60	89.4	-2.41	Additive
	Fenamiphos + Hb Ar-4	91.56	85.2	-6.95	Additive
	Fenamiphos + Hb Ht	62.2	76.16	-18.34	Additive

*Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).
**RC = Recommended application rate and 1/2 RC = Half recommended application rate

DISCUSSION

EPN species varied in their efficacy against larvae and pupae of *C. capitata*. Among evaluated the six species/isolates, only the native *Heterorhabditis* species, *H. bacteriophora* (Ht strain), caused the least larvae and pupae mortalities and 5 EPN species had varied mortality rates between moderate and higher mortalities than 60% after four days, namely *S. carpocapase* (All strain), *S. feltiae* (Filipjev), *S. glaseri* (NC strain), *H. bacteriophora* (HP88 strain) and *H. bacteriophora* (Ar-4 strain).

The specificity of EPN species against larvae or pupae of medfly cannot be clarified based on a single trait since a diversity of factors act upon that relationship. Specificity is directly associated with EPN's efficiency in locating, infecting, developing, and reproducing without being recognized by the host's immune system (Rohde *et al*.,2012). Moreover, most *C. capitata* larvae exposed to the tested EPNs died during the pupal stage, and no essential differences between the genera studied against *C. capitata* larvae or pupae as well, as native *Heterorhabditis* isolates also caused the lowest mortalities against the pupal stage.

Moreover, only native isolate, *H. bacteriophora* (Ar-4 strain), caused the highest mortality rates belonged to *Heterorhabditis*, and nearby all *Steinernema* species, *S. carpocapase* (All strain), *S. feltiae* (Filipjev) and *S. glaseri* (NC strain) showed high efficacy with insignificant differences between each other.

Based on LT_{50}, *S. feltiae* (Filipjev) showed the least L T_{50} (1.19 days) with third-instar larvae and 1.26 days with pupae of *C. capitata*, followed by *S. glaseri* (NC strain) and *S. carpocapase* (All strain) and also, higher susceptibility of the third-instar larvae of *C. capitata* was obtained at infection with *Heterorhabtitis* species at least time with imported species *H. bacteriophora* HP88 strain (1.38 with larvae and 1.50 with pupal stage) while LT_{50} scored with the native isolate, *H. bacteriophora* Ar-4 strain were 2.25 and 2.38 days with larvae and pupal stage, respectively.So, the mortality of *C. capitata* larvae/pupae in the

soil increased proportionally to the nematode dose (LD_{50}) with the least time to kill 50% (LT_{50}) of larvae and pupal stage of medfly, *C. capitata*.

Results revealed that *S. carpocapase* (All strain), *S. feltiae* (Filipjev), and *S. glaseri* (NC strain) produced mortalities higher than *Heterorhabditis* species under laboratory and field conditions, particularly with native isolates, and these findings are in agreement with Lindegren, 1990 and Rohde *et al*.,2012.

In laboratory assays, plastic cups exhibited the greatest control against larvae and pupae of medfly than Petri dishes assay. Many factors significantly affect EPNs efficacy against medflies, like soil humidity and optimum temperature. Kapranas *et al*.,2021 mentioned that higher virulence of EPN species against medfly stages due to moderate temperatures (~ 20°C), which could provide control over four weeks and better adapted to lower temperatures species such as *S. carpocapsae* and *S. feltiae* is suitable for reproduction (Grewal *et al*.,1994; Hazir *et al*.,2001).

Variations in virulence of the same EPNs against the *C. capitata* stage were noticed in the current assays as observed with *S. carpocapsae* All strain that exhibited highly virulent *C. capitata* larvae was probably little effective against the pupal stage because it is an ambusher strategist, making encounters more difficult between pupae (sedentary) and infective juveniles (Rohde *et al*.,2012).

Many authors as Lewis *et al*.,2006 mentioned to critical that cruiser strategists of EPN species (i.e., those that actively search for the host) may have a greater probability of finding hosts with cryptic or sedentary habits, while those of ambusher strategists (i.e., those that sit and wait for the hosts in order to attack) are more effective in finding high-mobility hosts. This finding could be attributed to variation in genetics (Gaugler *et al.*, 1989), infectivity (Grifn & Downes,1991), physiology (Fitters *et al.*,1999), climatic adaptation (Solomon *et al*.,1999), and morphology (Stock *et al*., 2000) of different nematode strains of the same species.

The higher susceptibility of larvae over that of pupae to EPNs may be due to their locomotor activity and higher release of CO^2, which attracts nematodes (Shapiro-Ilan *et al*., 2017). Moreover, larvae have less sclerotized integuments and large natural openings that facilitate the insect's infection by EPNs (Minas *et al*., 2016; Rohde *et al*.,2020). These factors could separately or synergistically increase the susceptibility of larvae to EPNs. Although pupae were less susceptible to EPNs, Chergui *et al.,* 2019 revealed that young pupae of medfly were more susceptible than older pupae due to fewer integuments sclerotized in young pupae than those of older pupae. EPN species can enter young pupae more efficiently, making them more susceptible to infection than older pupae (Godjo *et al.*, 2018).

Compatible pesticides with EPN species could be improved the control strategies against various pests under field conditions after conducting laboratory assays. So, each candidate product used in the IPM system should be tested individually with the specific EPN species or isolates (Krishnayya & Grewal, 2002).

Various combination responses between EPNs and RC or 0.5 RC of abamectin were observed with larvae and pupae of medfly. The current results showed an incomputable reaction between the RC of abamectin and medfly pupae in plastic cups assay. At the same time, RC or 0.5 RC of abamectin showed additive response with the third instar of medfly. These results agree with Mostafa *et al*.,2022 when examining RC or 0.5 RC of abamectin against two termite species using imported EPN species and two native *Heterorhabditis* species, *H.bacteriophora* (Ar-4 strain) and *H.bacteriophora* (Ht strain). Moreover, the incompatibility effect with *Heterorhabditis* species and *Steinernema* species is principally due to the ingredients of abamectin having a well-known nematicide effect (Mahfooz *et*

al.,2008). Moreover, when reduced the dose used (0.5 RC) with some EPN species, i.e., *H. bacteriophora* (HP88 strain and *H. bacteriophora* (Ar-4 strain), improved percentage mortalities in larvae of medfly and exhibited an additive effect (Lazinik and Trdan, 2014).

The same trend was observed with RC or 0.5 RC of Tervigo against larvae and pupae of medfly. In joint action between Tervigo and *Helicoverpa armigera* (Hübner), the highest larval mortality was observed in descending order for combinations between *H.bacteriophora* (HP88 strain) with fenamiphos (Tervigo) (El-Ashry *et al*.,2020). Moreover, results indicate the feasibility of the integrated management of EPNs and a low dose of chemical pesticides in crop protection.

Conclusion:

All species and strains of EPNs tested were pathogenic to larval and pupal stages of *C. capitata*. The larval stage of *C. capitata* was more susceptible to EPNs than pupae. The results found in this study indicate the great potential of the *Steinenema* species than *Heterorhabditis* species against two stages of Mediterranean fruit fly. Moreover, the incompatible response of two native isolates of *H. bacteriophora* with RC of abamectin and Tervigo in plastic cups and Petri dishes assays. However, field studies are needed to confirm the laboratory assays. Also, additional studies are still required to find new isolates of EPNs compatible with pesticides and could tolerate temperate conditions effectively under field conditions.

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ARABIC SUMMARY

إصابة ذبابة البحر الأبيض المتوسط *Ceratitis capitate* (Wiedemann) بالأنواع المحلية والمستوردة للنيماتودا المتطفلة على الحشرات وتوافقها مع الأبامكتين والفينامیفوس

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تم تقييم القدرة على الاصابة لعزلتين مصريتين محليتين تابعتين لنيماتودا هيتيرورابديتس ضد ذبابة فاكهة البحر الأبيض المتوسط (Wiedemann) *Ceratitis capitata* ومقارنتها مع أنواع مستوردة تابعة لجنسى *Steinernema* و *Heterorhabditis* من خلال نوعين من التحليل المعملية واستجاباتها التوافقية مع معدلات التطبيق الموصى بها (RC) ونصف الموصى بها (0.5 RC) لكل من abamectin وfenamiphos .

كشفت النتائج عن وجود اختلافات في فعالية النيماتودا الممرضة للحشرات (EPNs) ضد يرقات وعذارى البحر الأبيض المتوسط *C. capitata*. و ترتبط فاعلية النيماتودا الممرضة للحشرات EPNs ارتباطا مباشرا بزيادة التركيزات المستخدمة ، وكانت النسب المئوية للموت أعلى في يرقات العمر الثالث عن طور العذراء. علاوة على ذلك ،أحدثت العزلة المحلية *H.bacteriophora* (Ar-4 strain) معدلات موت تقترب من تلك الناتجة من النيماتودا المستوردة *H.bacteriophora* (HP88 strain) .

واستنادا إلى قيم LT_{50} ، أظهرت نيماتودا *S.feltiae* (Filipjev) أقل وقت لقتل ذبابة البحر الأبيض المتوسط حيث كانت قيمة LT_{50} هى 1.19 يوم فى يرقات العمر الثالث و 1.26 يوم فى عذارى *C. capitata* تليها نيماتودا *S.glaseri* (NC strain) ونيماتودا *S.carpocapase* (All strain) .

ومن ناحية أخرى، كانت أقل نسب موت فى كل من يرقات وعذارى ذبابة البحر الأبيض المتوسط ناتجة من السلالة المحلية *H.bacteriophora* (Ht strain) مقارنة مع العزلة المصرية الأخرى *H. bacteriophora* Ar-4 وبقيم LT_{50} مقدارها 2.25 يوم و 2.38 يوما مع كل من اليرقات والعذارى على التوالى.

فيما يتعلق بتوافق كل من abamectin وfenamiphos مع أنواع النيماتودا الممرضة للحشرات (EPNs) ، أسفرت النتائج عن عدم توافق النيماتودا مع التركيز الموصى به RC من Abamectin وفى المقابل ، ظهرت استجابات التوافق (الإضافة) من خلال الجمع بين أنواع EPN ونصف التركيز الموصى به 0.5 RC من المبيدات النيماتودية. أيضا أظهرت يرقات البحر الأبيض المتوسط استجابة توافق (تفاعل الإضافة) بدرجة أكبر عند مقارنتها مع طور العذراء ، وكانت النيماتودا المحلية *H.bacteriophora* (Ar-4 strain) أكثر توافقا مع المبيدين النيماتودين عند مقارنتها مع *H.bacteriophora* (Ht strain) .

وتشير النتائج الحالية إلى جدوى الإدارة المتكاملة للافات باستخدام جرعات منخفضة من المبيدات الكيماوية مع النيماتودا الممرضة للحشرات فى حماية المحاصيل.