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REVIEW

Susceptibility of Economic Dipteran Fruit Flies to Entomopathogenic Nematodes

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ABSTRACT

The present review article demonstrates laboratory and field evaluations of entomopathogenic nematodes (EPNs) against different developmental stages of fruit flies. The virulence of the EPNs differed clearly even on the same insect species and / or by the same nematode species. Such differences might be attributed to some reasons such as the method of treatment as well as the concentrations of the tested nematodes. Fruit flies are among the most important insect pests infesting vegetables and fruits causing considerable losses in the yields worldwide. In laboratory studies, the tested nematodes proved to be highly virulent to larvae as percentage of mortality may reach 100 %. As for treated pupae, at different ages, the results are variable and controversially; some studies revealed their moderate or high susceptibility to nematode infection and others indicated low susceptibility or resistance to infection .Treated adults, or those emerged from treated larvae or pupae, are also susceptible to infection. In semi-field and field trials, EPNs proved to be successful for reducing the populations of some fruit flies with up to 85 % at concentrations not less than 100 infective juveniles (IJs) / cm^2 of soil. However, the field applications of commercial EPNs have been recommended to be 2.5 - 5 x 10^9 IJs / ha (25-50 IJs/cm^2 of soil).

1. Introduction

The free-living, non-feeding 3rd stage infective juveniles (IJs) of the entomopathogenic nematodes (EPNs) (Families Steinernematidae and Hetrorhabditidae) possess attributes of both insect parasitoids or predators and microbial pathogens. Like parasitoids and predators, they have chemoreceptors and are motile, in soil, looking for suitable host. Like pathogens they are highly virulent , killing the host quickly and can be cultured easily in vivo and in vitro [1]. The members of both families are associated with mutualistic bacteria of the genera *Xenorhabdus* (forSteinernematidae) and *Photorhabdus* (for Heterorhabditidae) [2]. IJs can locate the host by detecting the insect excretory products, carbon dioxide levels, temperature gradients and movement of the host. The IJs then penetrate the host through natural openings; mouth, anus or spiracles, and, in addition, IJs in heterorhabditids possess a tooth that enable them to penetrate the host through the cuticle of certain insects. Once they enter the hemocoel they release the bacteria which multiply and kill the host by cepticaemia [3]. EPNs have positive characters including their broad host range, safety to vertebrates, plants and non- target organisms [4]. exempting from registration in many countries,

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easily applied using a standard spray equipment [5], compatible with many chemical and bio-pesticides and amenable to genetic selection [6]. In field application, commercially, a dose of 2.5 - 5 x 10^9 IJs / ha was recommended to give effective control comparable to chemical insecticides [7]. The ENPs have a great potential to be used in integrated pest management programmes. They are more specific, proven to be safe and effective alternatives to chemical pesticides. The susceptibility of insect pests varies depending on the selectivity and applied rates of EPN species. Location of the host by IJs within the soil is one of the most important factors where their movements are affected by temperature and oxygen levels. Temperature, moisture, aeration and soil type, the species of EPN, age of target insects and soil fauna are important factors affecting the activity of EPNs [6].

Dipteran insects are among the most important insect pests infesting vegetables, fruits and field crops causing considerable losses in the yields worldwide. Besides, human, animals and chickens suffer from the attack by a number of dipteran insects that may transmit fatal diseases [8].

The present article reviews some laboratory and field evaluations of the efficacy of the entomopathogenic nematodes against different developmental stages of some economic dipteran insect pests belonging to different families.

2. Susceptibility of Fruit Flies (Fam. Tephritidae) to EPNs

2.1 Laboratory Experiments

2.1.1 The Mediterranean Fruit Fly, *Ceratitiscapitata* (Wied)

The susceptibility of full-grown larvae and one day old pupae of *C.capitata* to three species of EPNs was studied at a concentration of 200 IJs/larva or pupa [9]. The results showed that % mortality by *H.bacteriophora* averaged (80, 0, and 35 %) for full-grown larvae, pupae and adults, respectively. The respective values were (90, 0, and 45 %) by *H.zealand* and (60, 0, and 75%) by *S.khoisanae*. Infectivity of *H. noenieputensis* and *S. yirgalemense* to 3rd instar larvae of *C. capitata* was evaluated in containers (250 ml) containing sterile sand sprayed with 2000 IJs .The containers were kept at 25° C for 14 days and the dead larvae, pupae and adults were then dissected to ensure infection. The results showed that percent mortality was 99% in larvae and 0.0 % in pupae and adults by *H. noenieputensis*. As for *S. yirgalemense*, % mortality was 0.0 % in pupae, 2% in larvae and 53% in adults [10].

The virulence of 10 species and isolates of *Heterorhabditis* and 5 species of *Steinernema* to full grown larvae and pupae (5-8 days old) of *C.capitata* was studied in Petri-dishes (10 larvae or pupae/dish) at a concentration of 200 IJs/larva or pupa [11]. % mortality in larvae ranged 40 - 87.5 % by *Heterorhabditis* spp. and isolates and 32.5 - 86.3 % by *Steinernema* spp. The respective values for pupae were 3.8 - 43.8 % and 17.5 - 43.8 %.

Similarly, 8 species and strains of *Heterorhabditis* were tested against 3rd instar larvae and one day old pupae of *C.capitata* [12]. The highest averages of mortality were obtained by *Heterorhabditis* sp. (LPP17), *Heterorhabditis* sp. (LPP14) and H.baujardi being 98.5, 95.5 and 90 %, respectively. % mortality in one day old pupae above 80 % was at a concentration of 816 IJs / cm^2 of soil. Also, the virulence of 15 strains of *S.feltiae* and 2 strains of *S.carpocapsae* against one day old pupae of *C.capitata* at a concentration of 250 IJs/pupa was investigated [13]. Percent mortality by *S.feltiae* strains ranged 23 - 45 % while it ranged 12 - 28% by the two strains of *S.carpocapsae*. 12 EPNs strains and species were evaluated against 3rd larval instar of *C. capitata* [14]. *S. riobrave* (Sr TX strain) and *Heterorhabditis* sp. (H IS-5 strain) caused more than 80 % mortality. However, 6 strains caused more than 30% and 4 strains caused less than 20% mortality.

The pathogenicity of *H.bacteriophora* and S.riobrave to one day old pupae of *C.capitata* was tested at concentrations of 250, 500 and 1000 IJs/cm^2 of sand in cups (one pupa/cup) [15]. Mortality % ranged 48-72 % by *H.bacteriophora* and 36-52 % by S.riobrave. Also, the pathogenicity of *S.carpocapsae* and *H.bacteriophora* against full grown larvae and pupae (8-day old) of *C.capitata* was investigated in cups (8 larvae or pupae/cup) [16]. Six concentrations of the nematodes were used (500-16,000 IJs/cup). *H.bacteriophora* caused 24-98 % mortality in full-grown larvae and 22-92 % in pupae at the 6 tested concentrations. In contrast, *S.carpocapsae* and *H.bacteriophora* did not cause mortality in *C.capitata* pupae (1,3,and 6-day old) at concentrations of 12.5, 25 and 50 IJs / cm^2 of sand in cups [17]. However, the adults emerged from treated 6-day old pupae at the same concentrations were highly susceptible to infection as % mortality at 12.5, 25 and 50 IJs/cm^2 ranged 73.3 - 100 % by *S.carpocapsae* and 60 - 86.7% by *H.bacteriophora*.

The pathogenicity of *S.riobrave* and *H.bacteriophora* against 5 ages of *C.capitata* pupae (8, 24, 48 and 72 hrs old as well as 9-day old ones) was tested in plastic cups, with sand, at a concentration of 3x10^3 IJs / cup / 10 pupae. % mortality averaged 24.5, 26.2, 4.4, 2.2 and 29.0% in 8, 24, 48, 72 hrs old pupae and 9-day old ones, respectively, by *S.riobrave*. The respective values by *H.bacteriophora* were 63.0, 29.8, 19.1, 17.0 and 24.4 % [18]. In a similar study, the efficacy of S. bacteriophora against pupae and

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adults of *C.capitata* was evaluated in cages (25x20x15 cm) containing sand at a concentration of $1x10^5$ IJs/ cage/100 pupae [19]. Three ages of the pupae were used; 12 and 24 hrs. as well as 8-day old ones. As for adults, 9-day old pupae were treated in similar cages (100 pupae/ cage) at the same concentration. The results showed no mortality in the treated pupae. % mortality in adults by *S. carpocapsae* was 42.7 % while it was 72% by *H.bacteriophora*. Similarly, when adults of *C.capitata* were exposed to *S.carpocapsae* in Petri-dishes for longer periods the nematode caused 45 - 100 % mortality in such adults [20]. Infection of pre-pupae and pupae of *C.capitata* by *S.carpocapsae* in cups at 5 concentrations from 5000 to 50,000 IJs/cup revealed that % mortality in pre-pupae ranged 9 - 92 % while the pupae were resistant to infection [21].

2.1.2 The Natal Fruit Fly, *Ceratitis rosa* (Cert.)

The susceptibility of full-grown larvae and one day old pupae of *C.rosa* to three species of EPNs was investigated at a concentration of 200 IJs/larva or pupa [9]. The results showed that % mortality averaged 30, 0, 40 % in larvae, pupae and adults, respectively, by *H.bacteriophora*. The respective values were 65, 0, 60 % by *H.zealand* and 15, 0, 80 % by *S.khoisanae*.

2.1.3 The Peach Fruit Fly, *Bactrocera zonata* (Saund.)

The efficacy of *S.carpocapsae* and *H. bacteriophora* against pupae and adults of *B.zonata* was tested in cages (25x20x15 cm) containing sand at a concentration of $1x10^5$ IJs/cage/100 pupae [19]. Three ages of the pupae were used; 12 and 24 hrs as well as 8-day old ones. As for adults, 9-day old pupae were treated in similar cages (100 pupae/cage) at the same concentration. The results showed that no mortality was obtained in the treated pupae. % mortality in adults was 29.4% by *S.carpocapsae* and 60% by *H.bacteriophora*. However, at 6 concentrations of IJs (500 - 16,000 IJs/cup) *H.bacteriophora* caused 20-94 % mortality in full-grown larvae and 16-88 % in pupae (8-day old) of *B.zonata* [14]. *S.riobravis* did not cause mortality to 0 - 4-hr as well as 5-day old pupae of *B.zonata* when treated in cups ,with sterile sand, (10 pupae/cup) at concentrations of 25, 50 and 100 IJs/cm^2 of sand [22]. Also, pathogenicity of *S.carpocapsae* and *H.bacteriophora* to pupae (1,3,and 6-day old) and adults (emerged from treated 6-day old pupae) of *B.zonata* was carried out in cups with sand at concentrations of 12.5, 25 and 50 IJs / cm^2 of sand (3 pupae/cup) [17]. The results (depending on dissection of dead insects) indicated that the treated pupae were resistant to infection (no infection was noticed). Dissection of treated pupae was carried out because the high humidity, not the nematodes, in the cups was found to kill the pupae in the control cups. However, the adults emerged from treated 6-day old pupae were highly susceptible to infection as % mortality at 12.5, 25 and 50 IJs/cm^2 ranged 80 -86% by *S.carpocapsae* and 53 - 60% by *H.bacteriophora*.

The pathogenicity of *S.riobrave* and *H.bacteriophora* against 5 ages of *B.zonata* pupae (8, 24, 48 and 72 hrs old as well as 9-day old ones) was tested in plastic cups, with sand, at a concentration of $3x10^3$ IJs / cup / 10 pupae. % mortality averaged 25, 4.3, 7.1, 2.1 and 17.0% in 8, 24, 48, 72 hrs old pupae and 9-day old ones, respectively, by *S.riobrave*. The respective values by *H.bacteriophora* were 29.0, 20.0, 11.1, 4.4 and 21.3 % (18). Two experiments using plastic cups half-filled with sterilized soil were carried out to estimate the virulence of *S.feltiae* to *B. zonata*. In the 1stexperiment, *S.feltiae* (25 IJs/ cm^2 of soil) was sprayed in the cups then guava fruits (infested with larvae of *B.zonata*) were placed on the treated soil. In the 2ndone, the nematode was sprayed at the same concentration on the soil that already contained the pupae of the insect (more than one day old). After 20 days, the results of the 1st experiment showed that mortality percentages were 12.6, 13.3 and 35.6 % in larvae, pupae and adults, respectively. The results of the 2nd one showed that % mortality was 0.0% in pupae and 81.1 % in adults [23].

The pathogenicity of *S.carpocapsae*, *S.riobrave* and *H.bacteriophora* against full-grown larvae and pupae (their age was not given) of *B.zonata* was studied at 5 concentrations (100 - 1600) IJs/ml in Petri-dishes (1ml/ dish/18 larvae or pupae) [24]. The results showed that % mortality in larvae ranged 40.9 - 88%, 65.8 - 96% and 70.8 -93% by *H.bacteriophora*, *S.carpocapsae* and *S.riobrave*, respectively. In pupae, the respective % mortality ranged 33-55%, 6 - 43.7% and 16.6 - 49.7% .Similarly, the virulence of *S.carpocapsae*, *S.riobrave* and *H. bacteriophora* was tested against pre-pupae and pupae (their age was not given) of *B.zonata* at 4 concentrations (250, 500, 1000 and 2000 IJs / ml) in cups with sand (25 individuals/cup).The results showed that % mortality in pre-pupae ranged 34 - 93%, 27 - 92% and 26 - 81% at the tested concentrations for *S.riobrave*, *S.carpocapsae* and *H. bacteriophora*, respectively. As for pupae, the respective % mortality ranged 32-79%, 29 - 74% and 39-91% % [25].

Also, at concentrations of 50 - 800 IJs / Petri-dish, *S.feltiae* caused 4 - 56 % and 0 - 20 % mortalities in one and 6-day old pupae of *B.zonata*, respectively. Mortality percent in 3rd instar larvae at the same concentrations ranged 32 - 88 % [26]. The newly formed and 5-day old pupae of *B.zonata* were treated with nematodes at concentrations of 25, 50 and 100 IJs / cm^2 of sand in cups (5 pupae/

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cup). *Steinernema* sp. and H.indicus caused 86.7 - 100 % and 93.3 - 97.8 % mortalities, respectively, in newly formed pupae. No mortality was noticed in 5-day old pupae at all tested concentrations of both nematode species [27]. The virulence of *H. marelatus* and *H.bacteriophora* was evaluated against B. zonata pupae (7-day old) at 5 and 15 IJs/cm^2 in plastic containers, 100 cm^2 surface area, filled with sandy soil and containing 25 pupae each. Seven days post treatment, the adult cadavers were dissected for nematode infection. The mortality percentages in adults at the concentration of 5 IJs/cm^2 of soil were 45 and 35% by *H.marelatus* and *H.bacteriophora*, respectively. The respective values for 15 IJs/cm^2 were 77.5 and 75% [28].

2.1.4 The Olive Fruit Fly, *Bactrocera oleae* (Rossi.)

The virulence of 6 EPN species against the 3rd larval instar of olive fruit fly, B.oleae was tested in cups with sand at a concentration of 25 IJs/cm^2. *S.feltiae* caused the highest mortality (67.9%) while *H.marelatus* caused the least one (19%) (29). *S.carpocapsae* and *H.bacteriophora* were evaluated against B.oleae pupae (their age was not given) in 24-well plates filled with sand (one pupa/well/100 IJs). They caused 62.5 and 40.6% mortality, respectively, in the treated pupae. *S.carpocapsae* caused 21.9% mortality in emerged adult flies [30].

2.1.5 The Queensland Fruit Fly, *Bactrocera tryoni* (Frog)

The virulence of *S.feltiae*, *S.carpocapsae* and *H.bacteriophora* was tested against 3rd instar larvae of B.tryoni at 5 concentrations (50 - 1000 IJs/cm^2 of sand) in 24-well bioassay plates (one larva/well). *S.feltiae* caused the highest mortality rates at the 5 concentrations (65-90%), *S.carpocapsae* caused 37-90% while *H.bacteriophora* caused 20-65% mortality. However, the 3 species did not cause mortality in the one day old pupae when treated at the concentration of 1000 IJs/cm^2 [31].

2.1.6 The Mango Fruit Fly, *Bactrocera* (*Dacus*) *dorsalis* (Hend)

Treatment of pre-pupae and pupae of *Bactrocera (Dacus) dorsalis* and the melon fly, *Bactrocera (Dacus) cucurbitae* (Coquillett) by *S.carpocapsae* in cups at 5 concentrations from 5000 to 50,000 IJs/cup revealed that % mortality in pre-pupae ranged 9 - 85 % for *B.dorsalis* and 0.0 - 86 % for *B.cucurbitae*, 6 days post treatment. Pupae of the 2 species were not susceptible to infection [21]. Pathogenicity of 12 EPN species and strains to *B. dorsalis* at a concentration of 100 IJs /one 3rd instar larva in containers was investigated [32]. After14 days insect mortality ranged 7 - 96 % by the 12 tested nematode species and strains (infected pupae were considered larvae).The same technique was used to estimate susceptibility of *B.dorsalis* larvae and pupae to *H.taysearae* and *Steinernema* sp. The results showed that % mortality in 3rd instar larvae was 94% by *H.taysearae* and 99% by *Steinernema* sp. As for pupae, % mortality averaged 5.5% and 1% in 3-day old pupae by *H.taysearae* and *Steinernema* sp., respectively [32].

2.1.7 The Cucurbit Fly, *Dacus ciliates* (Loew.)

Susceptibility of 2nd instar larvae and pupae (the age was not given) of *Dacus ciliates* to 5 species of nematodes was investigated in Petri-dishes (5 larvae or pupae/dish) [33]. Two concentrations of the IJs were used for larvae (500 and 1000 / 5 larvae / dish) and four concentrations for pupae (500 - 4000 IJs / dish). The results showed that % mortality in the larvae was 100 % at the two concentrations by all tested nematodes. The LC_{50} values for *S.feltiae* were 254 IJs / larva and 587 IJs / pupa whereas they were 269 and 1787, respectively, for *H.bacteriophora*. The bio-control potential of *H.bacteriophora* and *S.carpocapsae* against 3rd instar larvae, pupae (one day old) and adults of *D.ciliatus* was tested [34]. The LC_{50} for larvae was 27.8 and 325.7 IJs/larva, respectively. % mortality in pupae averaged 12.5 % by *S.carpocapsae* and 8.9 % by *H.bacteriophora*. As for adults, the respective % mortality averaged 55.6 and 44.6 %.

2.1.8 The European Cherry Fruit Fly, *Rhagoletis cerasi* (Linn.)

Four native EPNs from Turkey were evaluated against the 1st instar larvae of *Ragoletis cerasi* in soil at 3 concentrations; 100, 500 and 1000 IJs/larva [35]. The nematodes were *S.carpocapsae*, *S. feltiae*, *H. bacteriophora*, and *H.marelatus*. *S. feltiae* was the most virulent species at all concentrations and at 1000 IJs/larva it caused 95% mortality, followed by *H. marelatus* (82%) and *H. bacteriophora* (76%).

2.1.9 The Western Cherry Fruit Fly, *Rhagoletis indifferens* (Rhag.)

The susceptibility of full-grown larvae of *R.indifferens* to 5 species of EPNs was investigated at a concentration of 10^6 IJs/20 larvae/ Petri-dish [36]. 7 days post treatment, % mortality was estimated in the formed pupae as the larvae pupated within 24 hrs. % mortality averaged 65, 50, 35, 17.5 and 15 % by *S.carpocapsae*, *H. bacteriophora*, *S.feltiae*, *S.riobravis*, and *H. marelatus*, respectively. The infectivity of *S.carpocapsae* and *S.feltiae* was evaluated against *R.indifferens* larvae, pupae and adults in cups with

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soil [37]. % mortality of larvae at a concentration of 50 IJs/ cm^2 averaged 78.6 and 77.5 %, respectively. The pupae were not infected but the emerged adult flies were infected (0.0 - 53%) by the 2 nematode species at a concentration of 100 IJs/cm^2 .

2.1.10 The South American Fruit Fly, *Anastrepha fraterculus* (Wied)

Eight nematode isolates were tested against late 3rd instar larvae of *A.fraterculus* in plastic containers (50 ml) (12 larvae/container) at a concentration of 100 IJs/larva. 20 days later individuals not giving rise to adults were dissected to check for nematode -infection (79% of larvae developed to pupae during the 1st week of treatment). The highest mortality was caused by *S.carpocapsae* (84%) followed by *H.amazonensis* (54%) while *Heterorhabditis* sp. caused 28% mortality [38].

2.1.11 The Caribbian Fruit Fly, *Anastrepha suspense* (Loew)

Larvae (4-5 days old), pupae (4-8 days old) and adults (2-5 days old) of *A.suspense* were treated with nematodes in Petri-dishes (1000 IJs/25 individuals/dish). The nematodes were *S.feltiae*, S.glaseri, *H.bacteriophora* and *H.heliothidis*. % mortality in adults were 91.7, 58.1, 75.6 and 86.7 %, respectively. The respective values in larvae were 90.7, 15.7, 78.7 and 86.6 %. The pupae showed very little infection (0.0 - 1.1%) by the 4 species [39]. Also, the pathogenicity of 12 species / isolates of EPNs to 3rd larval instar and pupae (their age is not given) of *A.suspense* was evaluated in Petri-dishes lined with filter paper or soil (10 larvae or pupae / dish / 2000 IJs). The results showed that larval mortality by the 12 species / isolates ranged 15 - 95% and 10 - 60% in filter paper and soil assays, respectively. The respective values for pupae were 0.0 - 30 and 1.0 - 30 % [40].

2.1.12 The Mexican Fruit Fly, *Anastrepha ludens* (Anst.)

The LC_{50} for 3rd instar larvae of *A.ludens* treated with *H.bacteriophora* in plastic cups lined with soil was found to be 15 IJs/ $cm^2$of soil [41].

2.1.13 The Wheat Fruit Fly, *Oscinella frit* (L.)

The efficacy of *S.carpocapsae*, *S.feltiae* and *H.bacteriophora* against 3rd instar larvae of *Oscinella frit* (Family Chloropidae) was evaluated in Petri-dishes (5 larvae/dish) at concentrations of 250, 500 and 1000 IJs/ml. % mortality by *S.feltiae* averaged 80, 90 and 96% at the 3 concentrations, respectively, whereas it averaged 74, 90 and 100% by *S.carpocapsae* and 90, 94 and 100% by *H.bacteriophora* [42].

2.2 Semi-field and Field Experiments

S.carpocapsae was applied in the field against pre-pupae and pupae of *Bactrocera (Dacus) dorsalis* and the melon fly, *Bactrocera (Dacus) cucurbitae* at a concentration of 500 IJs/ cm^2 of the soil [21]. The emerging adult populations of both insects were reduced by 89% and 94%, respectively, than those in the control. Also, application of *H.bacteriophora* and *S.feltiae* in a greenhouse at a concentration of 30,000 IJs / plant reduced, significantly, the number of the larvae of cabbage root fly, *Delia radicum* (Linn.) that developed to pupae as well as the root damage caused by such larvae [43]. Soil surface application by *S.feltiae*, in the field, at concentrations of 100,000 and 200,000 IJs / plant was more effective than sub-surface application in preventing damage by the insect larvae [43]. *S.carpocapsae* and *S.feltiae* were equally effective against larvae of *Rhagoletis indifference* (59-85% mortality) when applied to soil under cherry trees at 50-100 IJs/cm^2 of soil. In addition, mortality rates in the emerged adults ranged 0.0 - 53 % [37].

Application of *H.bacteriophora* against *Anastrepha ludens* (Anst.) at concentrations of 115 and 345 IJs / cm^2 of soil in experimental plots in a mango orchard resulted in 46.7 and 76.7 % infection in the insect larvae, respectively [41]. Similarly, *H.bacteriophora* was applied against *A.ludens* at a concentration of 125 IJs / cm^2 of soil after releasing 50 - 500 third instar larvae of the insect in 0.25 m^2 plot [44]. It was noticed that the application did not significantly influence the prevalence of infection by the nematode. However, in subsequent experiments, % infection of the insect pupae were positively correlated with the IJs concentrations and reached 74% at the concentration of 250 IJs / cm^2 of soil. Double applications, at 4-day intervals, did not greatly improve the prevalence of infection than a single application.

The efficacy of indigenous EPNs from Brazil against *A.fraterculus* in peach orchards was tested and it was found that the LC_{50s} of *H.bacteriophora* and *S.riobrave* against 3rd larval instar of the insect were 229 and 347 IJs/cm^2 of soil, respectively [45]. A semi-field experiment was carried out using EPNs against *C.capitata* in a guava orchard. It was found that at a concentration of 10 IJs/cm^2 of soil *H.indica* caused 66 - 93% mortality in pre-pupae and one day old pupae of the insect [46]. In a wheat field experiment, application of *S.carpocapsae*, *S.feltiae* and *H.bacteriophora* twice at a concentration of $2.5x10^9$ IJs/ha reduced the infestation level by the wheat fruit fly, *O. frit* and increased the yield of the crop [42].

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Table 1. Susceptibility or resistance of fruit flies pupae (at different ages) to EPNs infection (The letter X indicates susceptibility to infection)

Insect	Age of pupa	Nematode species	Method of Treatment	Concentration of Nematode	Mortality %	Reference
Ceratitis capitata and *C.rosa*	One day	*H.bacteriophora* *H.zealand* *S.khoisanae*	24- well plates	200 IJs / pupa	0.0 0.0 0.0	Malan and Manrachan (2009)
C.capitata X	5-8 day	Ten *H.*spp. and Five *S.*spp.	Petri-dishes	200 IJs / 10 pupae	3.8-43.8 17.5-43.8	Rhode etal (2012)
C.capitata X	One day	H.baujardi	Tubes with sand	155 IJ , l3 pupae	80	Minas et al . (2016)
C.capitata X	One day	15 strains of *S . feltiae* 2 strains of *S. carpocab-sae*	24 - well plates	250 IJ s / pupa	23-45 12 - 28	Campos - Herrera and gutierres (2009)
C.capitata X	One day	*H.bacteriophora* S. riobrave	Cups with sand	250 - 1000 IJs / cm^2/ pupa	48 - 72 36 - 52	Nouh (2001)
C.capitata X	One day	*H.indica*	Semi - field experi-ment	10 IJs / cm^2 of soil	66 - 93	Silva et al. (2010)
C.capitata *Bacterocera zonata*	1,3,6 days 1,3,6 days	S.carpocapsae. *H.bacteriophora* *S.carpocapsae* *H.bacteriophora*	Cups with sand	12.5,25,50 IJs / cm^2 of sand / 8 pupae	0.0 0.0 0.0 0.0	Abbas et al. (2016)
C.capitata *X*	6,24,72 hr and 9 days old	S. riobrave H.bacteriophora	Plastic cups with sand //	3000 IJs / 10 pupae //	33 , 16 , 2 and 29%re-spectively 74 , 30 , 17 and 24 % respectively	Ibrahim et al. (2014)
C.capitata and *B.zonata*	8 , 12 , 24 hr and 8 days	*S. riobrave* *H.bacteriophora*	Plastic cups with sand	10^5 IJs / cup / 100 pupae	0.0 0.0 0.0 0.0	Soliman (2007)
C.capitata X B.zonata	8 -day //	*H.bacteriophora* //	Cups with sand //	500 - 16000 IJ s / 8 pupae //	22 - 92% 16 - 88%	Nouh and Hussein (2014)
C.capitata *Dacas dorsales* *D.cacurbita*	Not given	*S. carpocapsae*	Cups with sand	500 - 50.000 IJs / cup /100 pupae	0.0 0.0 0.0	Lindegren (1990)
B.zonata	More than one day	*S.feltiae*	Plastic cups with sand	25 IJs / cm^2 of sand	0.0	Mahmoud et al (2016)
B.zonata X	One day	S.carpocapsae S.riobrave *H.bacteriophora*	Cups with sand	1600IJs/18 pupae	43.7 49.7 55.4	Rashad et al . (2015)
B.zonata X	Not given	*S.carpocapsae* S.riobrave *H.bacteriophora*	Cups with sand	250 IJs / 25 pupae	29 32 39	Atallah and Eweise (2002)
B.zonata X	One day 6 days	*S. feltiae*	Petri - dishes	50 - 800 IJs/ pupa	4-56 0.0 - 20	Mahmoud and Osman (2006)
B.zonata	4 hr 5 days	*S. riobrave*	Soil in cups	25,50,100 IJs / cm^2 / 10 pupae	0.0 0.0	Abbas and Mahmoud (2009)
B.zonata	5 days	*Steinernema sp. H.indi-ca.*	Sand in cups	25 ,50,100 IJs/cm^2	0.0 0.0	Abdel-Samad &Abul-Fadl (2009)
B.dorsales X	One day 5 days	*Steinernema sp.* *H.taysearae* *Steinernema sp. H.bacte-riophora.*	24 - well plates	100 IJs / pupa	4.5 % 22.8 % 1.0% 5.5%	Godjo et al (2017)
B. tryoni	One day	*S.carpocapsae.* *S.feltiae* H.bacteriophora	24 - well plates	1000 IJs / cm^2 / one pupa	0.0 0.0 0.0	Langford et al (2014)
B.oleae X	Unknown	*S.carpocapsae.* H.bacteriophora	Petri - dishes	100 IJs / pupa	62.5 40.6	Torrini et al (2017)
Ragoletis indifferens	Not given	*S.carpocapsae.* *S.feltiae* S.intermedium	Soil in cups	100 IJs / cm^2 / pupa	0.0 0.0 0.0	Yee & lacey (2003)
Anastrepha suspensa	4-8 days	*S.feltiae* *S.glaseri* *H.bacteriophora* *H.heliothidis*	Petri - dishes	1000 IJs / 25 pupae	0.0 - 1.1 by the 4 species	Beavers & Calkins (1984)
A.suspense X	Not given	12 specis and / or isolates	Petri - dishes	2000 IJs / dish / 10 pupae	0.0-30%	Heve et al (2017)
Dacus ciliates X	Not given	*S.feltiae* *H.bacteriophora*	Petri - dishes	1000,2000,4000 IJs / 5 pupae	LC_{50} 828 IJs / pupa LC_{50} 676 IJs / pupa	Hussein et al (2006)
D.ciliatus X	One day	*S.carpocapsae.* H.bacteriophora	Containers with soil	50,100,150 IJs / 10 pupae	12.5% 8.9%	Kamali et al (2013)

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3. Discussion

The present article proved the high virulence of EPNs to larvae and adults of fruit flies under laboratory conditions as mortality may reach 100 % in both stages. As for the pupae, however, some studies revealed the susceptibility of different ages of the pupae to nematode-infection while others indicated low susceptibility and / or resistance to infection especially the late-aged pupae (Table 1). It can be suggested that the low susceptibility and / or resistance of dipteran pupae to nematode infection (as observed in considerable number of references may be attributed to different reasons: (1) The completion of puparium and the closer of the anal and oral apertures [47], (2) The toughness of the puparium and the limited ability of IJs to penetrate through pupal spiracles [41], and (3) The small size of spiracle-openings that makes penetration of IJs difficult [11]. What supports such reasons is that the IJs of *S. carpocapsae* and *H. bacteriophora* were found to adhere to treated one day old pupae of *D. ciliates* at the natural openings but no evidence of entry via these openings was noticed [34]. However, newly formed pupae (less than one hr old) were found to be susceptible to nematode-infection as the IJs entered the host via the anus and possibly through the mouth as evidenced by video [47]. In contrast, the relatively moderate or high susceptibility of more than one day old pupae to EPNs, as reported by some authors, can be attributed, partially, to: (1) Injuries in pupae from handling or pupae with incomplete integument that facilitates penetration of the IJs [48]. (2) Very high humidity in the procedure of treatment was found to kill the nematode-treated pupae as well as the untreated ones [17]. So, it is necessary to prove the nematode-infection of treated insects by dissection of the dead insects or by using White-traps for migration of infective juveniles (IJs).

In semi-field and field studies, EPNs were successful for reducing the populations of fruit flies (up to 85 %) at concentrations not less than 100 IJs / cm^2 of soil. It is to be noted that the field commercial application of EPNs has been recommended to be 2.5 - 5 x 10^9 IJs / ha (25-50 IJs/cm^2).

List of Abbreviations

EPNs (Entomopathogenic Nematodes) ; ha (hectare) ; hr (hour) : IJs (Infective juveniles).

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References

[1] Gauglar,R.and Kaya,H.K. Entomopathogenic nematodes in biological control. Boca Raton,Florida,USA: CRC Press Inc., 1990: 365.

[2] Poinar,G.O. Jr. Taxonomy and biology of *Steinernema*tidae and Heterorhabditidae. In: Gauglar,R. and Kaya, H.K.(eds) Entomopathogenic Nematodes in Biological Control. CRC Press. Boca Raton, Florida, USA, 1990: 23 - 61.

[3] Georgis,R. Present and future prospects for entomopathogenic nematode products. Biocontrol Science and Technology, 1992, 2: 83-99.

[4] Akhurst, R.J. Safety to non-target invertebrates of nematodes of economically important pests, in: Safety of Microbial Insecticides (Laird,L.A.; Lacey, E.W. and Davidson E.W., Eds) CRC Press, BacoRaton, FL, USA, 1990: 233-240 .

[5] Georgis, R. Commercialization of *Steinernema*tid and heterorhabditid entomopathgenic nematodes. Brighton Crop Protection Conference - Pests and Diseases, 1990, 1: 275-280.

[6] Kaya, H.K., Gauglar, R. Entomopathogenic nematodes. Ann.Rev.Entomol., 1993, 38: 181 - 206.

[7] Georgis, R., Hague,N.G.M. Nematodes as biological insecticides. Pesticide Outlook, 1991, 2: 29-32.

[8] Axtell,R.C., Arends, J.J. Ecology and management of arthropod pests of poultry. Annual. Rev. Entomol., 1990, 35: 101-126.

[9] Malan, A.P., Manrakhan, A. Susceptibility of Med. fruit fly, Ceratitis capitata and the natal fruit fly, *C.rosa* to EPNs. J.Invert. Pathol., 2009, 100(1): 47-49

[10] James, M., Malan, A.P., Addison, P. Surveying and screening South African EPNsfor the control of Ceratitis capitata Wied. Crop Protection, 2017, 115: 41-48.
http://doi.org/10.1016/j.cropro.2017

[11] Rhode, C., Moino, A., Carvalho, F., da Silva, P. Selection of entomopathogenic nematodes for the control of the fruit fly, Ceratitis capitata. Agraria Ravista Brasileria de Celincias Agrilas. 2012. ISSN online:1981-0997.

[12] Minas, R. S., Sauza, R. M., Dolinski, C., Carvallo, R.S. Potential of EPNs to control Mediterranean fruit fly, Ceratitis capitata soil stages. Nematoda. 2016, 3.
http://dx.doi.org/10.4322

[13] Campos-Herrera, R., Gutierrez, C. Screening Spanish isolates of *Steinernema*tid nematodes for use as biological control agents through laboratory and green-

  DOI: https://doi.org/10.30564/jzr.v2i1.2040

house studies. J.Inverteb.Pathol., 2009, 100: 100-105.

[14] Gazit, Y., Rossler, Y., Glazer, I. Evaluation of entomopathogenic nematodes for the control of the Mediterranean fruit fly. Biocontrol Science and Technology, 2000, 10: 157-164.

[15] Nouh, G.M. EPNs as biological control agents against Ceratitis capitata. M.Sc. Thesis, Fac.Agric., Zagazig Univ. 2001: 122.

[16] Nouh,G.M., Hussein, M.A. The role of entomopathogenic nematodes as biocontrol agents against some tephritid flies. Advances in Biological Research, 2014, 8(6): 301-306.

[17] Abbas, M.S.T., Nouh, G.M., Abdel-Samad, S.S.M., Negm, A.A. Infectivity of EPNs as bio-control agents to Spodoptera littoralis, Ceratitis capitata and Bactrocera zonata. Egypt.J.Biol.Pest Control, 2016, 26(3): 609-613.

[18] Ibrahim, A,A., Solimann N.A., Shams El-din,M.M., Ramadan, N.F., Farag, S.R. Entomopathogenic nematodes and fungi as bio-control agents for Bacterocera zonata and Ceratitis capitata. Egypt. J.Biol.Pest Control, 2016, 24(2): 497-502.

[19] Soliman, N.A. Efficacy of *Steinernema*riobravis and *Heterorhabditis* bacteriophora against Bactrocera zonata and Ceratitis capitata. Egypt. J.Biol.Pest Control, 2007, 17(2): 77-82.

[20] Ghalley, S.E. Pathogenecity of *Steinernema* feltiae in relation to different insect hosts. J.Egypt. Society of Parasitology, 1989, 18: 297-304.

[21] Lindegren, J.E. Field suppression of three fruit fly species with *Steinernema* carpocapsae. 5th Intern. Conf. Inverteb. Pathol. and Microbial Control, Adelaide, Australia, 1990: 20-24 .

[22] Abbas, M.S.T., Mahmoud, B.A. On the pathogenicity of EPN, *Steinernema* riobravis to some economic insect pests. Egypt. J.Biol. Pest Control, 2009, 19(1): 49-53.

[23] Mahmoud, Y.A., Ebadah, I.A., Metwally, H.M.S., Saleh, M.E. Controlling of larvae, pupae and adults of Bactrocera zonata with EPN, *Steinernema* feltiae. Egypt. J. Biol. Pest Control, 2016, 26(3): 615-617 .

[24] Rashad, M.M., El-Heneidy, A.H., Djelouah. K., Hassan, N., Shaira, S.A. On the pathogenicity of entomopathogens to the peach fruit fly, Bactrocera zonata. Egypt. J. Biol. Pest Control, 2015, 25(3): 649-654.

[25] Atallah, F.A., Ewais, M.A. Preliminary investigation on the utilization of EPNs as biological control agents against the peach fruit fly, Bactrocera zonata. Egypt, J. Agric. Res., 2002, 80(3): 1045-1053.

[26] Mahmoud, F.M., Osman, M.A.M. Biological control of the peach fruit fly, Bactrocera zonata by using EPN, *Steinernema* feltiae crosses N 33. 9th Arab Conference of Plant Protection, Damascus, Syria, 2006: 19-23.

[27] Abdel-Samad, S.S.M., Aboul-Fadl, H.A.A. EPNs as bio-control agents for Spodoptera littoralis and Bactrocera zonata. J. Egypt. Ger. Soc. Zool. (58 E), Entomology, 2009: 71-81.

[28] Saleh,M.M.E., Metwally, H.M.S., Mahmoud,Y.A. Potential of entomopathogenic nematode, *Heterorhabditis* marelatus in controlling the peach fruit fly, Bactrocera zonata. Egypt. J. Biol. Pest Control, 2018, 28: 22.

[29] Sirjani, F.O., Lewis, E.E., Kaya. H.K. Evaluation of EPNs against the olive fruit fly, Bactrocera oleae. Biological Control, 2009, 48: 274-280.

[30] Torrini,G., Mazza,G., Benvenuti,C., Roversi, P.F. Susceptibility of olive fruit fly, Bactrocera oleae to entomopathogenic nematodes. J. Plant Prot. Res., 2017, 57(3): 318-320.

[31] Langford, E.A., Nielsen, U.N., Johnson, S.N., Riegler, M. Susceptibility of fruit fly Bacterocera tryoni to EPNs. Biol.Control, 2014, 69: 34-39 .

[32] Godjo, A., Zadje, L., Decraemer, W., Afouda, L. Pathogenecity of indigenous EPNs from Benin against mango fruit fly, Bactrocera dorsalis under laboratory conditions. Biological Control, 2017. http://doi.org/10.1016/j.biocontrol.2017.

[33] Hussein, M.A., El-Wakeil, N., El-Sebai, T. Susceptibility of melon fruit fly, Dacus ciliatus to EPNs and to insecticides. Intern. J. Nematology, 2006, 16(1): 13-18.

[34] Kamali, S., Karimi, J., Hossini, M., Herrera,C., Duncan, L.W.. Biocontrol potential of entomopathogenic nematodes, *Heterorhabditis* bacteriophora and *Steinernema* carpocapsae on cucurbit fly, Dacus ciliatus (Dipt.:Tephritidae). Biocontrol Science and Technology, 2013, 23(11): 1307-1323.

[35] Kepenikci, I., Hazir, S., Ozdem, A. Evaluation of native entomopathogenic nematodes for the control of the European cherry fruit fly Rhagoletis cerasi L. (Diptera: Tephritidae) larvae in soil. Turk. J. Agric. 2015, 39: 74-79.

[36] Patterson-Stark, J.E., Lacey, L.A. Susceptibility of cherry fruit fly, Rhagoletis indifferens to five species of EPNs in laboratory studies. J. Inverteb. Pathol., 1999, 74: 206-208.

[37] Yee,W.L., Lacey, L.A. Stage-specific mortality of *Rhagoletis indifference* exposed to three species of *Steinernema* nematodes. Biological Control, 2003, 27: 349-356.

[38] Foelkel, E., Monteiro, L.B., Voss, M. Virulence of nematodes against larvae of South-American fruit fly in laboratory using soil. CienciaRural,Santa Maria,

 DOI: https://doi.org/10.30564/jzr.v2i1.2040

2016, 46(3): 405-410.

[39] Beavers, J.B., Calkins, C.O. Susceptibility of Anastrepha suspense (Dipt.:Tephretidae) to *Steinernema*-tid and heterorhabditid nematodes. Environ.Entomol. 1984, 13: 137-139.

[40] Heve, W.K., El-Borai, F.E., Carrillo, D., Duncan, L.W. Biological control potential of EPNs for management of Caribbean fruit fly, Anastrepha suspense. Pest Management Science, 2017, 73(6): 1220-1228.

[41] Toledo, E.M., Ibarra, J.E., Liedoi, P., Mezi,A.G., Williams, T. Infection of *Anastrepha ludens* (Dipt.:Tephretidae) larvae by *Heterorhabditis* bacteriophora under laboratory and field conditions. Bio-control Science and Technology, 2005, 15: 627-634 .

[42] El-Wakeil, N., Volkmar,C. Efficacy of entomopathogenic nematodes against the fruit fly, *Oscinella frit* (Dipt.:Chloropidae) on spring wheat. J. Kulturpflanzen, 2013, 65(1): 9-18.

[43] Schroeder, P.C., Ferguson, C.S., Shelton, A.M., Wilsey, W.T., Hoffman, M.P., Petzoldt, C. Greenhouse and field evaluations of cabbage maggot on cabbage .J.Econ.Entomol. 1996, 89(5): 1109-1115 .

[44] Toledo, J., Williams, T. Infection of *Anastrepha ludens* following soil applications of *Heterorhabditis* bacteriophora in a mango orchard. Entomologia Experimentalis et Applicata, 2006, 119(2): 155-162.

[45] Barbosa-Negrosoli, C.R.C., Garcia, M.S., Dolinski, C., Nigrisoli, A.S., Nava, D.E. Efficacy of indigenous EPNs from Rio Grande do Sul Brazil against Anastrepha fraterculus in peach orchards. J.Enverteb. Pathol., 2009, 102: 6-13 .

[46] Silva, A.C., Patista, F.A., Leite, L.G., Tavares, F.M., Scmidt, F.S. Effect of EPNs on the mortality of Ceratitis capitata and guava weevil, Conotrachelus psidii. Nematol. Bras, 2010, 34: 31-40 .

[47] Lebeck, L.M., Gauglar, R., Kaya, H.K., Hara, A.H., Johnson, M.W. Host stage suitability of the leafminer, Liriomyza trifolii to the entomopathogenic nematode, *Steinernema* carpocapsae. J.Invert. Pathol. 1993, 62: 58-63 .

[48] Henneberry, J., Lindegren, E., Forlow, J., Burke, R.A. Pink bollworm, cabbage looper and beet armyworm pupal susceptibility to *Steinernema*tid nematodes. J. Econ. Entomol. 1995, 88(2): 835-839.

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