Evaluation of Entomopathogenic Nematodes *Steinernema Carpocapsae*, And *Heterorhabditis Bacteriophora* Against the Peach Fruit Fly, *Bactrocera Zonata* (Saunders) (Diptera: Tephritidae)

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Abstract
The Peach fruit fly, Bactrocera zonata (Saunders) (Diptera: Tephritidae) is a destructive polyphagous pest infests most of the horticultural fruits in addition to some vegetables. The use of entomopathogenic nematodes (EPNs) represents an important alternative tool for insecticides. This study was designed to estimate the efficacy of entomopathogenic nematode, Steinernema carpocapsae and Heterohabditis bacteriophora against B.zonata larvae, and pupae as a safe control tool to reduce chemical environmental hazards. The virulence of different concentrations (100, 200, and 400 IJs/ml) of both nematodes was evaluated. The mortality rate of larvae increased with increasing concentration (high positive correlation). The superiority with significant difference was recorded to S. carpocapsae species that gave mortality rates of 51.7, 65.3, and 90.63 % at concentrations 100, 200, and 400 IJs/ml respectively, While the second species achieved mortality rates 39.5, 53.7, and 86.7% respectively. The LC50 values were 146 IJs/ml and 104 IJs/ml for H. bacteriophora and S. carpocapsae respectively. In case of pupae treatment, the superiority was to the H. bacteriophora species without a significant difference from the other one, the mortality rates were dependent on the concentrations. The LC50 values were 145 IJs/ml H. bacteriophora and 184 IJs/ml for S. carpocapsae.
Keywords: Bactrocera zonata · Entomopathogenic nematode · Biological control

1. Introduction

There is increasing concern about reducing pesticide inputs because of the risk they pose to humans and the environment as well as increased resistance in pest populations. Management of insect pests by biological control is an alternative approach that results in no risk to the environment. Among the different agents of biological control, entomopathogenic nematodes (EPNs) are one of them. The EPNs of the families Steinernematidae and Heterorhabditidae are obligate insect parasites that have been successfully used against a wide range of insect pests (Georgis et al.2006). These nematodes have evolved a mutualistic association with bacteria in the genera Xenorhabdus and Photorhabdus. Nematode locates their potential host by following insect cues (Lewis et al.2006).

After IJs locate the insect, they infect the host through the anus, mouth, spiracles, or by penetrating the cuticle. Once IJs enter the host, they shed their outer cuticle and begin ingesting hemolymph, which triggers the release of symbionts by defecation or regurgitation. The nematode-bacteria complex kills the host within 24 to 72 hrs. Fruit flies (Diptera: Tephritidae) are considered to be the most damaging pests of fruit crops worldwide as they deposit eggs under fruit pulp and cause blemishes which ultimately reduce the fruit quality. Among fruit fly species, the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a destructive fruit pest of peach, guava, mango, apricot, and citrus in tropical countries (Kapoor 1993; Shehata et al. 2006). The difficulty of controlling this pest comes from its larvae hiding inside the fruits and its pupae pupate in the soil. Nematodes can reach larvae and newly formed pupae in the soil as well as adults during their emergence from pupae. The present study aimed to evaluate theefficacy of entomopathogenic nematode isolate *S.carpocapsae and H.bacterophora* against stages of the peach fruit fly *B. zonata.*

2. Materials and methods

Rearing of Galleria mellonella larvae

Galleria mellonella's larvae were reared on a artificial diet, contained 890 g flour, 222 g dry baker's yeast, 500 g glycerin, 500 g honey, 445 g milk powder and 125 g bee wax in an incubator with 16/8 h lighting

doi.org/10.31838/hiv23.03.250 Received: 10.11.22, Revised: 25.12.22, Accepted: 27.01.23.

set at 24–25 °C (Mohamed and Coppel 1983).

Rearing of entomopathogenic nematode species

The IJs of EPNs were cultured in vivo, using Galleria larvae on modified White's traps approximately 2 weeks prior to screening (Kaya and Stock, 1997). Twenty insect larvae were placed in a Petri dish (90 mm diam.), lined with filter paper and inoculated with 800 µL of the EPN suspension. After 2 days in a growth chamber maintained at 25 ± 2°C, the dead larvae were transferred to clean Petri dishes. After 7 to 10 days, the cadavers were placed on modified White's traps, so as to allow for IJ emergence (White, 1927). Emerged IJs were harvested, with the suspension of IJs and distilled water transferred to vented culture flasks daily within a period of 7 to 14 days, and then stored at 12.8°C, before being used for the screening tests within 4 weeks.

Bioassay

Nematode hosts exposure were carried out in Petri dishes (10cm) containing 30 gm of sand. Different concentrations of EPN were prepared at: 100, 200 and 400 IJs/ml of both nematodes species and added to Petri dishes. The final soil moisture was adjusted to *ca* 10 %. Twenty of full grown larvae (third larval instar) and 20 of newly formed pupae were introduced to nematodes. Each concentration was replicated three times. Control test (untreated) was carried out at the same time in parallel to the nematodes tests. These Petri dishes were kept in an incubator (27±1 °C, relative humidity of 70±10%, and photoperiod of 12 hours). Insect mortality was recorded after adult emergence, and was corrected against natural mortality that was obtained from check treatment using Abbott's formula (Abbott, 1925). All individuals failed to emerge successfully considered dead. Dead insects were kept in a Petri-dish having moist filter paper, under sterilized conditions, at 25°C. To confirm the existence of IJs, the dead larvae and pupae were dissected under the microscope (Poinar, 1976)

3. Statistical analysis

All statistical analyses were conducted using SPSS20. Mortality (each stage) for the treated group was corrected for control mortality by using the Abbott formula [Abbott, 1925] and then the data were subjected to analysis of variance (ANOVA). Whenever appropriate, treatment means were separated with Duncan test with a significance level of 5%. Probit analysis was used to determine the LC50.

4. Results and discussion

The results of the study showed the efficacy of both nematode species H. bacteriophora and S. carpocapsae against the larvae of the peach fruit fly (table1), the mortality rate increased with increasing concentration (high positive correlation). The relative superiority with a significant difference was recorded to S.carpocapsae species that gave mortality rates of 51.7, 65.3, and 90.63 % at concentrations 100, 200, and 400 IJs/ml respectively, While the second species achieved mortality rates of 39.5, 53.7, and 86.7% respectively. The LC50 values were 146 IJs/ml and 104 IJs/ml for H. bacteriophora and S. carpocapsae respectively.

In the case of pupae treatment (table2), the superiority of the H. bacteriophora species appeared without a significant difference from the other one, the mortality rates were dependent on the concentrations. The mortality rate of pupae by S. carpocapsae were 34.6, 40.3, and 82.27% at concentrations of 100, 200, and 400 IJs/ml respectively, while they were 41.7, 51.3, and 85.6 % for H. bacteriophora at mentioned concentrations respectively. The LC50 values were 145 IJs/ml H. bacteriophora and 184 IJs/ml for S. carpocapsae.

Increasing of larvae and pupae mortality caused by nematode isolates as the dose increased, reported in Studies of Nouh & Hussein (2014) and Minas et al. (2016) that gave similar results, higher mortality with higher IJs doses. Kepenekçi & Susurluk (2006) used two Turkish isolates against C. capitata pupae and obtained higher mortality with 100 IJs/insect compared to 50 IJs/insect. Similar trends in the efficacy of the EPN isolates were observed in the bioassays performed under different conditions.

EPN species were less efficient in infecting pupae compared with its effectiveness against larvae; many tested EPNs were found to have no ability to cause infection to the pupal stage of different species of fruit flies (Yee and Lacey, 2003; Soliman, 2007 and Karagoz et al. 2009). It was emphasized by Yee & Lacey (2003) that the higher susceptibility of larvae to EPNs may be related to the higher release of CO2 at that stage, attracting the nematodes. Also, large natural openings and the poorly sclerotized integument of the larva enable EPNs to infect more easily. Toledo et al. (2005) mentioned that younger larvae were more susceptible to infection, possibly because the period of exposure, from inoculation to pupation, was longer in young larvae. In contrast, pupae are totally refractory to penetration by nematodes, probably due to the toughness of the puparium and the limited possibility to penetrate pupal spiracles. In contrast, the lower susceptibility of pupae could be due to the small spiracle opening size for nematode penetration (Toledo et al., 2005). The closure of all-natural openings owing largely to sclerotization and thickening of the cuticle into puparial cells is the main reason for pupal resistance (Grewal et al., 2005). Griffin et al., 2005 explained the superiority of H. bacteriophora to cause higher pupal mortality than S. carpocapsae by the dorsal tooth of Heterorhabditis species used to penetrate the host cuticle more easily.

In conclusion, H. bacteriophora and S. carpocapsae showed high virulence toward third instar larvae of B.zonata

Table (1) Effect of different concentrations (100, 200, and 400 IJs/ml) of *Heterorhabditis bacteriophora* and *Steinernema* carpocapsae on *Bactrocera zonata* larvae

Nematode species	100IJs/ml	200IJs/ml	400IJs/ml	R value	LC50
Heterorhabditis bacteriophora	39.5A	53.7B	86.7C	0.99	146 IJs/ml
Steinernema carpocapsae	51.7A	65.3B	90.6C	0.99	104 IJs/ml
P value	0.002	0.03	0.4		
*Means followed by same letter in the same raw are not significantly different					

Table (2) Effect of different concentrations (100, 200, and 400 IJs/ml) of Heterorhabditis bacteriophora and Steinernema carpocapsae on Bactrocera zonata pupae

Nematode species	100IJs/ ml	200IJs/ ml	400IJs/ ml	R value	LC50
Heterorhabditis bacteriophora	41.7A	51.3A	85.6B	0.98	145 IJs/ ml
Steinernema carpocapsae	34.6A	40.3A	82.27B	0.96	184 IJs/ ml
P value	0.12	0.34	0.37		
*Mean followed by same letter in the same raw are not significantly different					

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