Susceptibility of Ceratitis Capitata (Wied) Larvae and Pupae to Entomopathogenic Nematodes, Steinernema Carpocapsae, and Heterorhabditis Bacteriophora

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Abstract

The pathogenicity of the entomopathogenic nematodes, Steinernema carpocapsae and Heterorhabditis bacteriophora to larvae, and pupae of the Mediterranean fruit fly, Ceratitis capitata (Wied.) was investigated. The activity of IJs was correlated with EPNs species as well as concentration rate. S. carpocapsae caused the highest mortality rates of larvae at all concentrations in filter paper and soil assays. In filter paper assay; S. carpocapsae at the concentrations of 50,100 and 200 IJs/cm2, caused mortality rates of 68.7, 79.3, and 85.2% respectively, while the H. bacteriophora caused 53.6, 61.8, and 72.3%, respectively, at the same concentrations. The LC50 values were 21.77 IJs/cm2 for S. carpocapsae, and 42.96 IJs/cm2 for H. bacteriophora,. In soil assay; the differences between the two species were not significant, with the persistence of the probability of the S. carpocapsae, which was confirmed by the LC50 value (25.58 IJs/ cm2for S. carpocapsae and 81.42 IJs/ cm2for H. bacteriophora). EPN species were less efficient in infecting pupae,. In filter paper assay; H. bacteriophora caused the higher mortality rates at all concentrations without significant differences (48.37, 52.3, and 58.6%) respectively, while they were 34.6, 38.3, and 53.27 % for S. carpocapsae . The LC50 for S. carpocapsae was 184.8 IJs/ cm2, while it was 55.57 IJs/ cm2 for H. bacteriophora. Treating of the pupae in soil gave 31.37, 42.3, and 45.6% mortality for H. bacteriophora and 24.6, 28.27, and 36.27% for S. carpocapsae at the mentioned concentrations respectively.

Keywords: Steinernema carpocapsae and Heterorhabditis bacteriophora , Ceratitis capitata, Pathogenicity.

1. Introduction

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) is one of the most important fruit fly species because it is the most cosmopolitan and invasive species, occurring in all biogeographic regions of the world and, consequently, causing the most damages to fruit production (Sticker et al., 2019). The Medfly is a polyphagous pest attacking more than 400 host plant species (Suzuki et al., 2002). Chemical pesticides are still a widely used strategy in fruit fly management as they give fast and effective results but it contributes to an imbalance in the ecosystem, affecting natural enemies and non-target organisms. In addition, pesticides are expensive and can leave toxic residues on fruits, which are prejudicial to the fresh fruit marketing and consumption (Muriithi et al., 2016). Recently, researchers are seeking for alternatives pest control tools like biological control agents. Entomopathogenic nematodes (EPNs) are obligate insect pathogens found in soil that are used as biological agents. They have a wide host range, able to kill their hosts with the help of their symbiotic bacteria within 24–48 h, easy to mass produce, able to actively search and find their hosts, can stay alive for a long time in the absence of hosts, and do not harm the environment and humans. Studies of isolating EPNs and investigating their virulence have increased, and are of great importance for biological control studies (Hazır et al. 2003). Entomopathogenic nematodes (EPNs) from the families; Steinernematidae and Heterorhabditidae are widely regarded as promising biological control agents for a broad range of insect pests in soil and cryptic habitat (Kung and Kaya, 1990). Third stage infective juveniles (IJs) leave the host cadaver in the soil, seek a new host, penetrate its body and release their symbiotic bacteria which multiply and kill the insect host by septicaemia. Larvae of the insect pests are the common target of EPNs especially those inhabit and/or pupate in soil. Thus, the objectives of this study were to evaluate the efficiency of applications with different concentrations of the nematodes; Steinernema carpocapsae and Heterorhabditis bacteriophora in controlling larvae and pupae of C. capitata in laboratory and greenhouse assays.

2. Materials and methods

Rearing of the Medfly

The adult colony originated from infested fallen fruits of

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citrus collected from different orchards in Madain. Upon emergence, adults were transferred to screened plastic cages (30 cm × 30 cm × 30 cm). Adult flies were provided with water and an adult diet comprised of sugar and yeast (3:1 ratio) (Sookar et al. 2014). A plastic bottle (500 mL) containing citrus juice was covered with a lid that had small holes (1 mm in diam.) to collect fruit fly eggs (Kena et al. 2015). The collected eggs were transferred to an artificial diet (Quesada-Moraga et al. 2006). Larvae were reared on a diet of water (50.5%), sucrose (16.2%), bran (24.2%), torula yeast (8.0%), citric acid (0.6%), and benzoic acid (0.5%). The environmental conditions were maintained at 25 ± 1 ◦C and 60–70% relative humidity (RH) (Usman et al. 2021).

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

Rearing of entomopathogenic nematode species

Nematodes (Steinernema carpocapsae and Heterorhabditis bacteriophora) were reared in last-instar Galleria mellonella L. (Lepidoptera: Pyralidae) at 25 °C as described by (Kaya and Stock (1997). Infected G. mellonella larvae were kept on a White's trap (White 1927), and the emerging infective juveniles (IJs) were collected over 7 days and stored in sterile distilled water at 10°C for no longer than 3 weeks before being used in the experiments.

Bioassay

Filter paper assays

Bioassays were carried out in a randomized design with three replications. The plots consisted of Petri dishes with 10 individuals. The larva trial was carried out using standardized individuals at the end of the third instar; and the pupa bioassay was tested on young (2-day-old). The individuals were transferred to Petri dishes (9 cm diameter) with two sheets of filter paper, where 1 mL of the nematode suspension (Steinernema carpocapsae and Heterorhabditis bacteriophora) was added at concentrations of 0 (control treatment), 50, 100, and 200, IJs (infective juveniles) cm2; 1 mL of distilled water was used for the control treatment. These Petri dishes were kept in an incubator (27±1 °C, relative humidity of 70±10%, and photoperiod of 12 hours). The insects were evaluated at 7 days after application, and the dead ones were analyzed for typical symptoms of death by Steinernema sp. (dark brown color) or Heterorhabditis sp. (red color), the number of pupae formed was recorded and then maintained in the treatments for adult emergence. In addition, the dead ones were dissected and the presence of nematodes confirmed their death by nematodes.

Soil assay

A quantity of 20 g of autoclaved sandy soil was added to a petri dish (diameter 9 cm). The top of the soil was levelled; Ten third-instar larvae were placed on moistened soil and inoculated with the nematode suspension (Steinernema carpocapsae and Heterorhabditis bacteriophora) at concentrations of 0 (control treatment), 50, 100, and 200, IJs (infective juveniles) cm-2 . The final soil moisture was adjusted to ca 10 %. For controls, distilled water was applied. Three replicates for each EPN treatment were made and then incubated at 27 ◦C. For treatment to pupae in soils, ten pupae were buried in grooves (depths 2–3 mm, one for each pupa), and incubated in the same way as the larvae. Emerging adult flies from inoculated larvae and pupae were recorded between 2 and 3 weeks following the date of inoculation.

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 in dose response

3. Results and discussion

The activity of IJs was correlated with EPNs species as well as concentration rate. C.capitata larvae were susceptible to the tested EPN species at all concentrations applied. S. carpocapsae caused the highest mean mortality rates of larvae at all concentrations in filter paper and soil assays (Tables 1 and 2). In filter paper assay; at the concentrations of 50,100 and 200 IJs/cm2, S. carpocapsae caused mortality rates of 68.7, 79.3, and 85.2% respectively, with significant differences between the concentrations of 100 IJs/ cm2 and 200 IJs/ cm2 from the concentration 50 IJs/ cm2. The correlation coefficient value between concentrations and the mortality rate was 0.69. The mortality rate achieved by the H. bacteriophora was 53.6, 61.8, and 72.3%, respectively, at the same concentrations. There were Significant differences between the both species of nematodes at concentrations of 50, and 200 IJs/cm2. This was confirmed by the LC50 value which was 21.77 IJs/cm2 for S. carpocapsae, and 42.96 IJs/cm2 for H. bacteriophora,. In soil assay; the differences between the two species were not significant, with the persistence of the probability of the S. carpocapsae, which was confirmed by the LC50 value (25.58 IJs/ cm2for S. carpocapsae and 81.42 IJs/ cm2for H. bacteriophora) .

The fruit fly pupae might be resistant to EPN penetration, EPN species was less efficient in infecting pupae compared with its effectiveness against larvae, (table 3). In filter paper assay; H. bacteriophora caused the higher mortality rates at all concentrations without significant differences (48.37, 52.3, and 58.6%) respectively, while they were 34.6, 38.3, and 53.27 % for S. carpocapsae . The LC50 for S. carpocapsae was 184.8 IJs/ cm2, while it was

55.57 IJs/ cm2 for H. bacteriophora. The correlation coefficient between concentrations and mortality was 0.85 for S. carpocapsae , and 0.62 for H. bacteriophora . Treating of the pupae in soil gave 31.37, 42.3, and 45.6% mortality at concentrations of 50,100 and 200 IJs/ cm2 respectively for H. bacteriophora and 24.6, 28.27, and 36.27% for S. carpocapsae at the mentioned concentrations respectively. The LC50 value for S. carpocapsae was 1040 IJs/ cm2, while it was 255 IJs/ cm2 for H. bacteriophora. The correlation coefficient between concentrations and mortality were 0.81 and 0.69 for H. bacteriophora and S. carpocapsae respectively (table 4).

Several studies have been carried out to evaluate the efficiency of steinernematid and heterorhabditid nematodes against C. capitata, In (Morocco, Mokrini et al. 2020) evaluated the efficiency of EPN strains against C. capitata. In laboratory assays, S. feltiae-SFMOR9, S. feltiae-SF-MOR10 and H. bacteriophora-HBMOR7 strains showed significantly higher infectivity. (Gazit& Gavish 2000) assessed 12 different species of EPNs against pre-pupae of C. capitata, from which S. riobrave, Texas isolate, was the most infective. It was emphasized by (Yee & Lacey (2003) that the higher susceptibility of larvae to EPNs may be related with 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 infect more easily. 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 a main reason of pupal resistance (Grewal et al., 2005). It was also confirmed by (Chergui et al. (2019), who used a Turkish S. feltiae isolate and observed that the final instar larvae and newly formed pupae of C. capitata were more susceptible to EPNs than old pupae under laboratory conditions. Many tested EPNs were found to have no ability to cause infection to pupal stage of different species of fruit flies (Lindegren and Vail, 1986; Yee and Lacey, 2003; Soliman, 2007 and Karagoz et al. 2009).

Griffin et al., 2005 explained the superior of H. bacteriophora to cause higher pupal mortality than S. carpocapsae by dorsal tooth of Heterorhabditis species used to penetrate the host cuticle more easily. Increasing of larvae and pupae mortality caused by all 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. The result of this study need to be further evaluated by testing the most effective isolates under field conditions. 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 (Laforgue& Mokrini,2020; Kapranas et al.,2023).

Table 1 Effect of different concentrations (50, 100, and 200, IJs/cm2) of Heterorhabditis bacteriophora and Steinernema carpocapsae on ceratitis capitata larvae in filter paper

Nematode species	50 IJs/ml	100IJs/ml	200 IJs/ml	R value	LC50
Heterorhabditis bacteriophora	53.6A	61.8B	72.3C	0.95	42.96 IJs/ ml
Steinernema carpocapsae	68.7A	79.3AB	85.2B	0.69	21.77 IJs/ml
P value	0.041	0.076	0.033		
*Means followed by same letter in the same raw are not significantly different					

Table 2 Effect of different concentrations (50, 100, and 200, IJs/cm2) of Heterorhabditis bacteriophora and Steinernema carpocapsae on Ceratitis capitata larvae in soil

Nematode species	50IJs/cm2	100IJs/cm2	200IJs/cm2	R value	LC50
Heterorhabditis bacteriophora	43.5A	48.7A	61.7B	0.85	81.42 IJs/cm2
Steinernema carpocapsae	57.7A	64.3A	69.6A	0.74	25.58 IJs/cm2
P value	0.075	0.085	0.094		
*Means followed by same letter in the same raw are not significantly different					

Table 3 Effect of different concentrations (50, 100, and 200, IJs/cm^2) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on *Ceratitis capitata* pupae in filter paper

Nematode species	50IJs/ cm2	100IJs/ cm2	200IJs/ cm2	R value	LC50
Heterorhabditis bacteriophora	48.37A	52.3A	58.6A	0.62	55.57 **IJs**/ cm^2
Steinernema carpocapsae	34.6A	38.3A	53.27B	0.84	184.8 **IJs**/ cm^2
P value	0.075	0.085	0.094		

Means followed by same letter in the same raw are not significantly different

Table 4 Effect of different concentrations (50, 100, and 200, IJs/cm^2) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on *Ceratitis capitata* pupae in soil

Nematode species	50IJs/ cm2	100IJs/ cm2	200IJs/ cm2	R value	LC50
Heterorhabditis bacteriophora	31.37A	42.3B	45.6B	0.81	255.27 IJs/ cm2
Steinernema carpocapsae	24.6A	28.3A	36.27A	0.69	1040.38 IJs/ cm2
P value	0.17	0.085	0.1		

Mean followed by same letter in the same raw are not significantly different

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