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Assessment of the entomopathogenic nematodes against maggots and pupae of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), under laboratory conditions

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Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the major insect pests which renders the fruit to become unfit for human consumption. In severe cases, losses may reach up to 100% in some fruit crops. The present study aimed to investigate the pathogenicity of entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. asiaticum* against *B. dorsalis* maggots and pupae under laboratory conditions. One milliliter of EPNs, having 50, 75, and 100 infective juveniles (IJs) against maggots and 100, 150, and 200 IJs against pupae, were poured into 9 cm Petri dishes with 20 g sterilized soil as supporting media. The highest maggots' mortality (70%) was obtained after 3 days of application of *H. bacteriophora* and *S. carpocapsae* and reached up to (96%) after 9 days. *S. asiaticum* and *H. indica* caused 91.16 and 85.87% mortality, respectively, after 9 days post treatment at the highest nematode concentration (100 IJs/ml). Whereas, against the fruit fly pupae, *H. bacteriophora* caused 69.08% mortality after 9 days at the highest concentration (200 IJs/ml). All nematode species showed high effectiveness against both stages of *B. dorsalis*. Their application can be further evaluated under field conditions to promote a good biological control of fruit flies for healthier fruit production.

Keywords: Fruit fly, *Bactrocera dorsalis*, EPN, IJs, Biological control

Background

Pakistan is ranked tenth among citrus producing countries in the world for citrus production (Mahmood and Sheikh 2006). Above 90% of the citrus fruits are produced in the Punjab province and are distributed through different value chains to domestic as well as international markets (GOP (Government of Pakistan) 2012). Citrus fruits are infested, from fruit setting to ripening and harvesting, by a number of insect pests and diseases but the fruit flies are the major destructive pests, which cause substantial yield losses. Fruit flies not only attack citrus but also attack a variety of fleshy fruits and vegetables in tropical and subtropical areas of the world (Roger et al. 2015). Among the fruit fly species, the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is an important insect pest of citrus fruits. Application of chemical insecticides has been the main tool of citrus growers to control this voracious insect pest, which not only leads towards the development of insecticidal resistance but also pose serious threats to the environment and human health. Application of entomopathogenic nematodes (EPNs) can be good alternatives to the conventional chemical insecticides as they have biological mode of action and resistance is unlikely to develop against these agents. The infective juveniles of the nematode enter the host body through body openings and release the associated bacterium, which

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multiply and deplete the host nutrients and ultimately kill it (Griffin et al. 2005).

EPNs have been used by different scientists in different countries for the management of various pests and have shown efficient results. In 2014, Nouh and Hussein used EPNs as bio-control agents against *Ceratitis capitata* (Wied.) and *Bactrocera zonata* (Saund.) in Egypt. Similarly, EPN, *Steinernema feltiae*, significantly decreased the larval population of *Leptinotarsa decemlineata* under field conditions (Laznik et al. 2010), while after the application of the same EPN, the damage to the leaves caused by *Frankliniella occidentalis* was reduced in greenhouse by lowering the pest population (Trdan et al. 2007).

The present study aimed to investigate the pathogenicity of *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. asiaticum* against *B. dorsalis* larvae and pupae under laboratory conditions.

Materials and methods

Sampling and rearing of *Bactrocera dorsalis*

B. dorsalis maggots-infested fruits, collected from the local fruit market, were brought to the Integrated Pest Management (IPM) Laboratory, College of Agriculture, Bahauddin Zakariya University (BZU), Bahadur Sub-Campus, Layyah, Pakistan, and were kept in plastic cages containing sterilized sand at 25 ± 1 °C and 65%RH. The full-grown maggots hopped from the fruits and pupated in the sand. Adult flies were fed with 20% honey solution in hanging inside cups containing fresh ripen fruits for egg-laying. After egg-laying, the fresh fruits were shifted to new rearing cages to get next progeny to be used in the experiments. The infested fruits were dissected to get the maggots for experiments.

Entomopathogenic nematodes

Infective juveniles (IJs) of *S. asiaticum*, *S. carpocapsae*, *H. bacteriophora*, and *H. indica* were obtained from Nematology Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. The EPNs were cultured on *Galleria mellonella* L. larvae (Lepidoptera: Pyralidae) following the procedures of Woodring and Kaya (1988).

Maggots' bioassay

To investigate the infectivity of EPNs, 3 different concentrations; 50, 75, and 100 IJs/ml^{-1} of all EPN species were prepared in glass jars. One milliliter of each EPN species was poured into 9 cm Petri dishes containing 20 g sterilized soil (40% sand, 40% silt, and 20% clay) as supporting media (Heve et al. 2017). Twelve maggots of *B. dorsalis* of uniform size and age (third instar) were released into each dish and wrapped with parafilm. Petri dishes, treated with distilled water only, served as control. The experimental conditions were maintained at 25 ± 2 °C, 75 ± 5% RH, and 12:12 (D:L) hour photoperiod in an incubator (Minas et al. 2016 and Raheel et al., 2017). Mortality data were recorded on third, sixth, and ninth day post exposure to EPNs. All the treatments were repeated five times.

Pupal bioassay

For pupal bioassay, the same procedure was used but the nematode concentrations were 100, 150, and 200 IJs/ml. Twelve, 1-day-old pupae were introduced in each Petri dish. The data for adult emergence was recorded on a daily basis for 10 days.

Statistical analysis

Mortality rates were corrected for control treatment mortality using Abbott's (1925) formula. The mortality and adult emergence data were subjected to analysis of variance (ANOVA), using Minitab 17 software (Minitab 17, 2010) and the means were separated by Tukey's Kramer test (HSD) (Sokal and Rohlf, 1995) at 5% significance level.

Table 1 Corrected percent mortality (mean ± SE) in *Bactrocera dorsalis* maggots (third instar) treated with *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. asiaticum* at different concentrations and time period

Time (days)	Concentrations (IJs/ml)	Percent mortality (mean ± SE) in *B. dorsalis* maggots			
		H. bacteriophora	*H. indica*	*S. carpocapsae*	*S. asiaticum*
3	50	20.17 ± 2.08f	13.37 ± 1.69f	21.87 ± 1.69f	21.87 ± 1.69f
	75	30.36 ± 3.17f	21.87 ± 1.69f	45.64 ± 2.08e	29.95 ± 2.10f
	100	69.42 ± 2.08cd	37.15 ± 2.08e	71.12 ± 2.08cd	47.34 ± 1.69d
6	50	45.64 ± 2.08e	38.85 ± 1.69e	62.62 ± 2.07d	47.34 ± 1.69d
	75	60.93 ± 2.07d	60.93 ± 2.07c	81.31 ± 1.69b	67.72 ± 1.69c
	100	81.31 ± 1.69b	72.82 ± 1.69b	93.20 ± 1.69a	84.71 ± 1.69ab
9	50	62.92 ± 3.30d	50.56 ± 2.16d	77.04 ± 2.16bc	50.56 ± 2.16d
	75	77.05 ± 2.16bc	75.28 ± 1.76b	92.93 ± 3.30a	77.04 ± 2.16bc
	100	96.13 ± 2.16a	85.87 ± 2.16a	96.46 ± 2.16a	91.16 ± 3.94a

*The numbers followed by different letters are significantly different from each other at $P < 0.01$. Data are the means of five replicates

Table 2 Lethal concentrations (LC_{50} and LC_{90}) of the tested EPNs against *Bactrocera dorsalis* maggots

Nematode spp.	LC_{50} (IJs/μL)	LC_{90} (IJs/μL)	Slope	Intercept	χ^2 (df = 1)	*P*
Heterorhabditis bacteriophora	41.88 (27.28–50.06)	87.40 (75.71–118.47)	1.74	− 3.74	2.97	< 0.01
H. indica	46.99 (31.74–55.58)	110.50 (91.12–178.47)	1.49	− 3.72	0.03	< 0.01
Steinernema asiaticum	48.08 (36.61–55.22)	96.39 (83.33–129.38)	1.84	− 4.38	0.06	< 0.01
S. carpocapsae	30.20 (9.34–41.16)	67.88 (56.87–87.72)	1.58	− 2.65	0.10	< 0.01

Results and discussion

Maggots' bioassay

B. dorsalis maggots were significantly susceptible to all the tested EPN species at all concentrations applied as compared to the control. *S. carpocapsae* caused the highest mean mortality rates at all concentrations (Table 1). At the concentration of 100 IJs/ml, *S. carpocapsae* caused 71.12, 93.20, and 96.46% mortality in maggots after 3, 6, and 9 days of application, respectively. The lowest mortality rate was achieved by the *H. indica*; 37.15, 72.82, and 85.87%, respectively, at the same concentration.

The LC_{50} for *S. carpocapsae* was 30.2 IJs/μl, while it was 41.88 IJs/μl for *H. bacteriophora*, 46.99 IJs/μl for *H. indica* and 48.08 IJs/μl for *S. asiaticum* (Table 2). Similarly, the LC_{90} of *S. carpocapsae* was also the lowest (67.88 IJs/μl) than all other tested EPNs, but the highest LC_{90} was recorded for *H. indica* (110.5 IJs/μl) (Table 2). Gazit et al. (2000) assessed 12 different species of EPNs against pre-pupae of *C. capitata*, from which *S. riobrave*, Texas isolate, was the most infective. The authors concluded that the activity of IJs was correlated with EPNs species as well as concentration rate. Lindegren and Vail (1986) recorded 92% mortality rate when they applied the concentration of 5000 IJs/insect and only 9% mortality rate after application with 50 IJs/insect. Similar results were obtained by other researchers against different insect species (Laborda et al. 2003; Almeida et al. 2007; Malan et al. 2011 and Rohde et al. 2012). Similarly, *S. carpocapsae* and *S. feltiae* were found to be the most efficacious species under laboratory, semi-field, and field conditions with larval mortality of 88, 78, and 88%, respectively, of European cherry fruit fly, while no mortality of pupae was observed (Köppler et al. 2003). Stark and Lacey (1999) reported that the highest infection rate was caused by *S. carpocapsae* (65%), *H. bacteriophora* (50%), *S. feltiae* (35%), and *H. marelatus* (15%) against *Rhagoletis indifferens* larvae. Karagoz et al. (2009) confirmed the effectiveness of five local EPN species against last-instar larvae of *C. capitata* under controlled conditions. Sirjani et al. (2009) stated that *S. feltiae* was highly virulent against third instar larvae of *Bactrocera oleae* (G.) compared to *S. carpocapsae*, *S. riobrave*, *S. glaseri*, *H. bacteriophora*, and *H. marelatus*.

Pupal bioassay

All the tested EPN species showed a high efficacy on the treated pupae at the highest concentrations, while at low concentrations caused the lowest mortality (Fig. 1). *H. bacteriophora* was superior and caused the highest mortality rates at all concentrations. The fruit fly pupae might be resistant to EPN penetration (Malan and Manrakhan, 2009). Many tested EPNs were found to

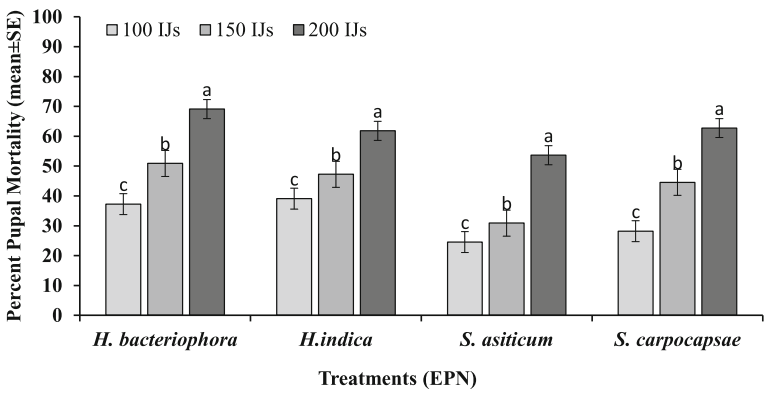

Fig. 1 Corrected percent mortality (mean ± SE) in *Bactrocera dorsalis* pupae after 10 days of treatment with *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. asiaticum* at different concentrations

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). Efficacy of *H. baujardi* LPP7 was evaluated against the fruit fly pupae by Minas et al. (2016) and recorded more than 80% mortality rate after application of 816 IJs/cm^2. Most fruit fly larvae come out from the fruit for pupation in the soil at depth of few centimeters. This is a good habitat for the IJs to search and locate the target hosts and initiate infection (Stark and Lacey, 1999 and Sirjani et al. 2009). Kepenekci and Susurluk (2006) evaluated two Turkish strains of *S. feltiae* (All and S3) on pupae of Medfly and detected a low death rate after application of both strains, All (26.6% and 33.3%) and S3 (30% and 40%) after application of 50 IJs/ and 100 IJs/ pupae, respectively. The low mortality rate of 1-day-old pupae could be due to cuticle hardness, which mainly reduces the penetration of the IJs. Minas et al. (2016) observed that mouth, spiracles, and anus, were quite open for penetration into pupae. However, the pupae of *Anastrepha fraterculus* were found to be vulnerable to infection by some EPN species, as it was highly susceptible to infection by *S. riobrave* and *H. bacteriophora* (Barbosa-Negrisoli et al. 2009). Similarly, Patterson Stark and Lacey (1999) gained high mortality rates (62.5 and 40%) of *Rhagoletis indifferens* pupae after application of *H. bacteriophora* and *S. riobrave*, respectively, but *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* effectively controlled all the life stages of *L. decemlineata*. The effectiveness of EPNs may vary depending upon the target species, life stage, and condition of the application (Trdan et al. 2007).

Conclusion

Results proved that EPNs are a promising tool to effectively reduce the populations of *B. dorsalis* larval stage. EPNs can be regularly used within an IPM plan to control the fruit fly pests under semi- and field conditions.

Abbreviations

ANOVA: Analysis of variance; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; IPM: Integrated pest management

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Authors' contributions

The authors carried out all the experiments, including the bioassay tests, analytical part, analysis of data, and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

This study does not contain any individual person's data.

Competing interests

The authors declare that they have no competing interests.

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