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Temperature dependent virulence of the entomopathogenic nematodes against immatures of the oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae)

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Abstract

Fruit flies have a great influence on fruit and vegetable industry of Pakistan. *Bactrocera dorsalis* Hendel (Tephritidae) is a polyphagous pest in large number of fruit and vegetable crops worldwide. Virulence of 4 entomopathogenic nematodes (EPNs) species, *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, and *S. asiaticum*, was evaluated at different temperature degrees (15, 20, 25, 30 and 35 °C) against the immature stages of fruit fly species, *B. dorsalis*. The tested EPNs species showed a temperature dependent virulence against the fruit fly immatures. All EPNs showed a poor infectivity at the lowest temperature (15 °C) and a high infectivity at the highest temperature (35 °C). In overall, *H. bacteriophora* performed the best against the fruit fly larvae and pupae at all temperature degrees. At 35 °C, all the EPNs caused more than 95% mortality in fruit fly maggots, but *H. bacteriophora* and *S. carpocapsae* performed better than the others. The EPNs infectivity increased with increasing the temperature and exposure time. Similar results were recorded in case of pupae. *H. bacteriophora* and *S. carpocapsae* caused more than 70% pupal mortality rates at 35 °C.

Keywords: *Bactrocera dorsalis*, Entomopathogenic nematodes, Virulence, Temperature

Background

Fruit flies are economically important insect pests responsible for attacking a wide range of fruits and fleshy vegetables in the tropical and subtropical zones of the world (Roger et al. 2015). The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a highly polyphagous species with wide dispersals. It is the most damaging pest of more than 170 commercial fruits and vegetable varieties in tropical and sub-tropical zones of the world (Ye and Liu 2005).

Fruit flies control options mainly occupied by using synthetic chemicals and pheromone traps. Insecticide resistance is becoming a big issue due to their frequent utilization. Therefore, fruit fly control is difficult (Pereira et al. 2009). Biopesticides, comprised of certain living entities (natural enemies) or their products (phyto- and microbial chemicals), are used to manage the injurious insect pests. Entomopathogens like fungi, bacteria, viruses and nematodes are commercially used to develop many biopesticides (Hussain et al. 2019). Several biological control options which have shown potential results for the effective biological control of fruit flies were evaluated. Few entomopathogenic fungi (EPF) such as *Metarhizium* spp. and *Beauveria bassiana* and their

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lethal metabolites are also helpful to reduce the fruit fly damages in different fruit crops (Yousef et al. 2013). EPNs have a great potential for the control of large numbers of different insect pests of economically important crops. An efficient and workable method for the management of *B. dorsalis* could be the use of EPNs, when applied on soil-borne phases of the *B. dorsalis* life cycle. The 3rd instar larvae of *B. dorsalis* usually escape from the infested fruits and tends to hide in the top 4 cm layer of soil ahead of pupating (Hou et al. 2006). Numerous studies have reported the association of EPNs and environment as they are well adapted to the prevailing environmental conditions and consequently regarded as one of the best biological control agents of insect pests (Zadji et al. 2014).

Among climatic factors, temperature is the most vital factor regulating the growth and development of EPNs. Heat stress can impose enough impact on the pathogenicity and reproductive capacity of EPNs (Chen et al. 2003). It directly impacts host finding ability (Susurluk 2008), infectivity (Pervez et al. 2008), and persistence of EPNs (Ali et al. 2010). Temperature can affect the adhesion, invasion, infectivity, and reproducibility of several isolates of infective juveniles (IJs) of EPNs to greater extent (Ali et al. 2010). Influence of temperature on EPN fluctuates with the species and isolates because they can acclimatize with the prevailing environmental conditions (Pervez et al. 2008). Extreme temperature degrees, 0 and 40 °C, could be fatal to EPNs, as temperature below 10 °C can reduce their agility, while above 40 °C induce diapause. However, some isolates and species exhibit improved adaptability to temperature variation, which can help them to survive and infect the hosts (Berry et al. 1997).

Therefore, the present study aimed to evaluate different EPN strains at different temperature degrees to find out the favorable one for their application as a control measure against fruit flies under laboratory conditions.

Materials and methods

Nematodes cultures

EPN strains, obtained from the Nematology Lab (Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan), *Steinernema asiaticum*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, and *H. indica* were reared using last instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 25 °C as described by Aatif et al. (2019). *G. mellonella* larvae, treated by EPNs, were placed on a white trap, and the infective juveniles (IJs) emerged from the cadavers were collected and stored at 10 °C in tissue culture flasks. IJs were used within 2 weeks for the experiments.

Insect cultures

Adults of *B. dorsalis* were collected from guava orchard at College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah, Pakistan (latitude of 30.9, longitude of 70.9, and altitude of 143 m above sea level) and reared in aerial plastic rearing cages (60 × 60 × 60 cm) at 25 ± 1 °C, 70 ± 5% RH, and 12 h photoperiod provided with sterilized sand according to Aatif et al. (2019) for maggots and pupae provision for further studies.

Maggots bioassay

To evaluate the impact of temperature on EPNs' infectivity, the EPNs (100 IJs/μl) were tested against the fruit fly maggots at 15, 20, 25, 30, and 35 °C. Twenty grams of sterilized and air-dried sandy soil (60 sand, 20 clay, and 20% silt) were added to Petri dishes. The moisture level in soil was maintained at 10% (v/w) by addition of distilled water. The Petri dishes were placed for overnight at the required temperature ranges to have uniform temperature before the addition of nematodes. In each Petri dish, 12 individuals of the 3rd instar fruit fly maggots were placed, and 0.5 ml of a prepared concentration of all tested EPNs were added. Petri dishes with no EPNs served as control. All treatments were maintained at environmental chambers under controlled temperature degrees of 15, 20, 25, 30, and 35 °C, 70 ± 5% RH, and 12 h photoperiod for 10 days.

Pupal bioassay

Effect of temperature on pathogenicity of EPNs against the pupal stage of *B. dorsalis* under the same tested conditions was studied, using the concentration of 150 IJs/μl. Twelve-day-old fully sclerotized pupae were placed in Petri dishes, and 0.5 ml of prepared EPNs' concentrations were added. The died maggots and pupae, recovered from soils, were placed on a sieve (710 μm) and gently sprayed with distilled water to eliminate the soil particles and nematodes on their cuticle. Afterwards, they were placed on White traps for microscopic examination of emerging IJs (Heve et al. 2018).

Data analyses

Treatments were replicated 4 times. Maggot mortality was noted at 3rd, 6th, and 9th days post-exposure to EPNs. The pupal mortality rate was recorded daily for 10 days. The corrected percent mortalities, obtained using Abbott's formula (Abbott 1925), were subjected to factorial ANOVA, and means were compared with Tukey's HSD test at 5% significance level. The data analyses were performed using the standard statistical software (Minitab 17 2010).

Results and discussion

Infectivity of the 4 EPN species significantly varied against the fruit fly maggots at different temperature degrees. The highest mortalities were reported at 35 °C after 3rd, 6th, and 9th days of exposure to all the EPN strains. The highest mortality rate was recorded by *H. bacteriophora* at all temperature degrees. *H. bacteriophora* caused 72, 87, and 97% mortality rates after 3, 6, and 9 days of EPN treatments, respectively. *S. asiaticum*, *H. indica*, and *S. carpocapsae* also recorded similar mortalities at 35 °C. It was noted that the EPN infectivity was increased by increasing temperature and time of exposure. At the highest degrees of temperature (35 °C), the difference between the EPN infectivity was non-significant but at the lowest ones (15, 20, and 25 °C); EPNs showed significant variations in their infectivity after 3, 6, and 9 days of exposures. Temperature can cause significant variations in EPN infectivity (Trdan et al. 2009). The exposure time was also an important factor of causing maggot mortality. *H. bacteriophora* and *S. asiaticum* caused the fastest casualties, at the highest temperature, in maggots after 3 days of exposure, which was 72% in both cases. The fruit fly maggot mortality rate attained 87% post 6 days after exposure to all EPNs, while (more than 95%) was recorded after 9 days of exposure at all EPNs at 35 °C (Table 1).

Similarly, at different temperature degrees, the efficacy of the 4 EPN species significantly varied against the pupae of *B. dorsalis*. At the lowest temperature of 15 °C, all EPN species caused the least pupal mortality (less than 20%), with the highest mortality rate (21.58%) shown by *S. carpocapsae*. As the temperature increased, infectivity of all the EPN strains was improved leading towards high pupal mortalities. *H. bacteriophora* caused the highest mortality rates (39, 53, 67, and 72%) at 20, 25, 30, and 35 °C, respectively, which were higher than all other EPN given mortalities. *S. carpocapsae* was the second effective candidate to cause pupal deaths in *B. dorsalis* (50, 60, and 71% pupal mortality at 25, 30, and 35 °C). *H. indica* and *S. asiaticum* resulted moderate pupal mortalities at all the temperature degrees. Overall, temperature was positively linked with pupal mortality because EPN infectivity was increased at high temperature degrees (Fig. 1). Similarly, the adult emergence was around 95% in control treatments, while it was reciprocal to their mortality values in treated pupae. These data were used to calculate the corrected percent pupal mortality.

To identify the optimum climatic factors required for a high efficacy of EPNs is critical in biological control. EPNs achieve varying levels of insect pest control under variable temperature degrees. *S. feltiae* and *H. bacteriophora* showed different mortality rates in *Bothynoderes punctiventris* for different soil depths at different temperature levels (Susurluk 2008). Langford et al. (2013) also noted the addition of *S. feltiae* significantly improved the insect mortality after exposure to EPN at 20 and 25 °C than at 15 °C. Significantly, the highest infectivity effects of *H. bacteriophora* were observed above 30 °C, which led to significantly high mortality rates in

Table 1 Corrected percent mortality (mean ± SE) in *Bactrocera dorsalis* maggots (3rd instar) exposed to different EPNs at different temperature degrees and time periods (DF = 24, $F = 6.20$, $P = 0.00$)

Time (days)	Temperature (°C)	Percent mortality (mean ± SE) in *B. dorsalis* maggots			
		Heterorhabditis bacteriophora	*H. indica*	*Steinernema carpocapsae*	*S. asiaticum*
3	15	17.18 ± 1.62 tu	12.95 ± 1.66 u	17.19 ± 1.60 tu	19.32 ± 1.65 tu
	20	44.91 ± 2.05 m–q	38.58 ± 1.98 o–r	33.18 ± 1.87 qrs	29.91 ± 1.90 rst
	25	55.41 ± 2.15 i–n	46.92 ± 1.99 l–q	38.18 ± 2.06 o–r	44.41 ± 1.87 m–q
	30	63.90 ± 2.25 f–j	59.65 ± 2.07 g–l	44.81 ± 2.07 m–q	59.65 ± 2.06 g–l
	35	72.92 ± 2.32 d–g	68.35 ± 2.25 e–i	55.41 ± 2.32 i–n	72.25 ± 2.15 d–g
6	15	42.74 ± 2.11 n–r	34.18 ± 1.90 qrs	38.34 ± 1.90 o–r	21.92 ± 1.70 stu
	20	68.15 ± 2.25 e–i	59.25 ± 2.07 g–l	51.18 ± 2.25 j–o	63.08 ± 1.99 f–j
	25	85.15 ± 2.29 a–d	61.78 ± 2.19 g–k	63.83 ± 2.30 f–j	70.58 ± 2.25 e–h
	30	79.16 ± 2.33 cde	85.14 ± 2.36 a–d	76.64 ± 2.33 c–f	76.34 ± 2.33 c–f
	35	87.49 ± 2.37 abc	87.49 ± 2.40 abc	87.49 ± 2.39 abc	87.49 ± 2.39 abc
9	15	60.15 ± 2.19 g–k	53.65 ± 2.15 j–n	35.98 ± 1.99 pqr	49.18 ± 1.98 k–p
	20	75.24 ± 2.33 c–f	60.49 ± 2.19 g–k	58.02 ± 2.32 h–m	71.31 ± 2.07 e–h
	25	77.93 ± 2.33 cde	69.01 ± 2.30 e–h	77.25 ± 2.41 cde	80.14 ± 2.33 b–e
	30	93.38 ± 2.90 ab	93.37 ± 2.90 ab	86.75 ± 2.39 abc	86.75 ± 2.39 abc
	35	97.79 ± 3.05 a	95.58 ± 3.01 a	97.83 ± 3.01 a	95.58 ± 2.15 a

*The numbers followed by different letters are significantly different from each other (Tukey's HSD test, $\alpha = 0.05$)

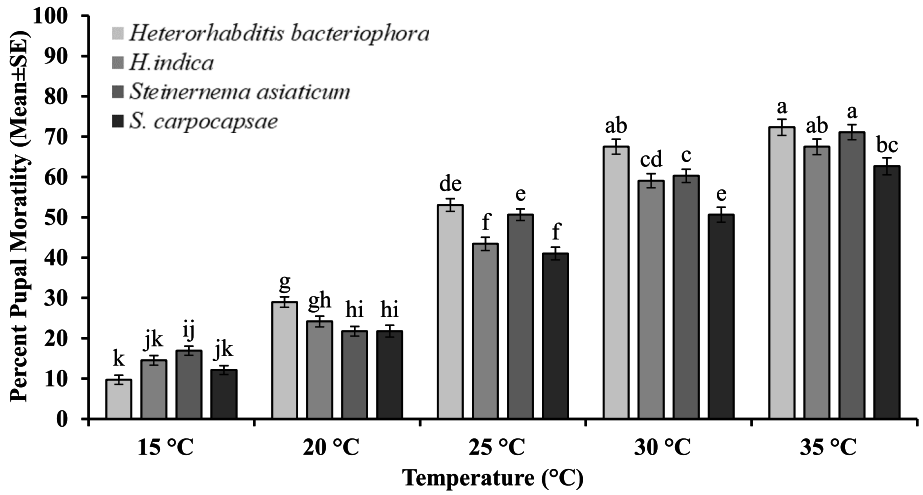

Fig. 1 Pupal corrected percent mortality (mean ± SE) in *Bactrocera dorsalis*, 10 days post exposure to EPNs at different temperature degrees (DF = 12, $F = 9.20$, $P = 0.00$; Tukey's HSD test, $\alpha = 0.05$)

maggots and pupae of *B. dorsalis*. Similarly, the study of Kepenekci et al. (2015) confirmed that the pathogenicity of locally isolated EPN species was highly boosted by temperature and EPN concentration against *Rhagoletis cerasi* fruit fly maggots, where *S. feltiae* was the most virulent EPN species at various tested temperatures and IJs concentrations. In contrast, *Heterorhabditis* species are known for their adaptability to warmer temperature levels (Grewal et al. 1994) and can give considerable insect infections above 30 °C (El Khoury et al. 2018). The used EPN strains gave a promising efficacy at moderate temperature degrees, while their infectivity was greatly lowered below 20 °C.

Heterorhabditis is different than *Steinernema* in their infectivity levels due to their searching capacity. The majority of *Heterorhabditis* species are fast moving, while *S. carpocapsae* is ambusher (Foelkel et al. 2016). Temperature can be an additional possible reasoning factor, since *Steinernema* species are perceived to be highly effective at 25 °C, whereas at 30 °C *Heterorhabditis* indicated greater efficacy (Rohde et al. 2010). Furthermore, *Steinernema* species are appearing to be more adjustable to various soil textures than *Heterorhabditis* species (Kaya and Stock 1997). Accordingly, Grewal et al. (2002) found that it is common that EPNs from an insect species are found to be less infectious than those isolated from other insect or geographical origin. Additionally, specificity is an important factor for virulence of EPNs than their adaptation to the environment (Foelkel et al. 2016). All the studies dealing with the EPN applications on tephritid flies have concluded that 3rd instar maggots are highly susceptible to EPNs infection (Barbosa-Negrisoli et al. 2009; Langford et al. 2013; and Shaurub et al. 2015).

Pathogenicity of EPNs was shown for the first time against the *Bactrocera oleae* pupae (Torrini et al. 2017). In the majority of orchards, fruit fly maggots move into the soil after infesting the fruits during late autumn or in winter; then, temperature degrees are relatively low for EPNs application. The significant results achieved in this study, regarding the EPNs capability to kill the pupae of *B. dorsalis* at various temperature degrees, signified their utilization in controlling the fruit flies in different environments. EPNs can be applied under the canopy fruit trees during early spring, when adult flies emerge from the soil with moderate temperature degrees (Shaurub et al. 2015). However, the pupal mortality was less than attained mortality in maggots due to pupal resistance against EPNs penetration. These results are in agreement with the findings of some researches with limited ability of IJs to permeate into pupal cuticle or spiracles. The virulence of various EPN strains might be unable to cause high infections against pupal stage of different species of fruit flies. Their effectiveness can be enhanced by increasing their concentrations or using them at optimum temperature degrees (Toledo et al. 2005; Aatif et al. 2019), but this fact needs to be further investigated because performance of EPNs and other biological control agents can be affected be the temperature variations (Laznik and Trdan 2015).

Conclusion

EPN species proved effective to manage *B. dorsalis* populations at 30 to 35 °C. However, further researches are required for assessment of the mechanism for EPNs penetration into the pupae and the possibility of using them to reduce fruit flies population in orchards.

Abbreviations
ANOVA: Analysis of Variance; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles

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Authors' contributions
The authors carried out all the experiments including the bioassay tests, analytical part, and 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 the study data have been presented in the manuscript, and high-quality analytical 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 have no competing interests.

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