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Research Article

Variability in foraging behaviour, thermal requirement and virulence of entomopathogenic nematodes against sod webworm, *Herpetogramma phaeopteralis* Gueneè (Lepidoptera: Crambidae)

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ABSTRACT: Variability in virulence among entomopathogenic nematodes, *Heterorhabditis indica*, *H. bacteriophora* and *Steinernema abbasi*, was studied for lethality, foraging behaviour in response to host volatiles, thermal requirements (degree-days), recyclability, persistence and field efficacy against Turfgrass Sod Webworm (TSW), *Herpetogramma phaeopteralis*. Comparatively, lethal concentration and time were lowest for *H. indica* against TSW. Recyclability of EPN ranged from 3.42×10^5 to 4.23×10^5 IJs g^1 of TSW. *H. bacteriophora* recorded highest movement rate on agar (0.38–0.78cm) and sand-agar (0.45–0.56cm), followed by *S. abbasi*, and *H. indica.* Responding to TSW volatiles, *S. abbasi* recorded maximum movement, *H. bacteriophora*, moderate, and *H. indica,* lowest. *Heterorhabditis indica* (with nictitation); *S. abbasi* and *H. bacteriophora* (without nictitation) were ambusher and cruiser, respectively. *S. abbasi* preferred warmer temperatures (30–33°C), *H. bacteriophora*, moderate (24–27°C), and *H. indica,* a wider range (24–30°C), for virulence based on thermal requirement. In field, EPNs were comparable to chlorpyriphos against TSW. We demonstrated the complementarity of thermal preferences of EPNs and insect pest was critical besides attributes like foraging behaviour, recyclability, persistence, and lethality values for their success in the field.

Keywords: Degree-days, foraging, *Herpetogramma phaeopteralis, Heterorhabditis indica, H. bacteriophora,* sod webworm, *Steinernema abbasi*, thermal requirement, turfgrass, virulence, variability.

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INTRODUCTION

Turfgrass (sod) production is an important industry covering more than 25 million ha annually in tropical and subtropical regions (Haydu *et al.,* 2006). Besides its recreational uses, turfgrass controls soil erosion, promotes and restores soil biological and physico-chemical properties, captures run-off water, and improves the aesthetics of urban landscapes. The production and maintenance are seriously affected by insect pests and diseases (Belair *et al*., 2010). Meagher *et al.,* (2007) reported that wide outbreaks of Tropical Sod Webworm (TSW), *Herpetogramma phaeopteralis* Gueneè (Lepidoptera: Pyralidae) have significant economic importance on turfgrasses in over 100 countries in tropical and subtropical areas of the world. Sod webworm larvae feed on a variety of turfgrasses viz. Bermudagrass, *Cynodon dactylon* (L.), Persoon, centipede grass, *Eremochloao phiuroides* (Munro) Hackel, seashore paspalum, *Paspalum vaginatum* Swartz, St. Augustine grass, *Stenotaphrum secundatum* (Walter) Kuntze), and zoysia-grass, *Zoysia japonica* (Steude), especially on newly established sod, lawns, athletic fields, and golf courses, resulting in yellowing, drying and patchy growth of foliage (Reinert *et al.,* 2009). Insecticides including Chlorpyrifos, phorate, carbaryl, pyrethroids, neonicoinoids, etc., are routinely and repeatedly administered for its control (Racke, 2000), and as a result, domestic animals and humans come in direct contact with these chemicals. Among the safer and effective alternatives to chemicals, biological control offers a potentially viable means of control on a long-term basis.

Use of Entomopathogenic nematodes (EPN) (Nematoda: Heterorhabditidae and Steinernematidae) could be a successful approach especially in the biological control of persistent insects in cryptic habitats (Lacey and Georgis, 2012) especially in lawn, turfgrass and landscape (Kaya, 1990; Grewal *et al*., 2005; Klein *et al.*, 2007). Entomopathogenic nematodes are natural enemies of soil insect pests, and naturally harbor monoxenic bacterial symbionts. Together they cause insect mortality in 24 to 72h depending on inset host, foraging strategies, exposure time and IJs concentration, soil type, etc., Tofangsazi *et al.,* (2014) demonstrated that

TBW could be controlled by using EPN formulations, with variability in virulence among species of EPN.

Considering the nature of occurrence of sod webworm in the top 5-7cm of turfgrass roots, we hypothesized that the successful suppression of sod webworm in turfgrass largely depends on the foraging ability, recyclability and persistence of EPNs in turfgrass rhizosphere. Sod grasses absorb heat and keep the surface temperatures lower than the uncovered. The heat absorbed by the lawn/turf has a direct effect on the biology and multiplication of the pest, i.e., sod webworm, and the behaviour of EPNs. With this in mind, we examined the thermal requirements of the sod webworm life-stages and infectivity of three species of EPNs with different foraging behaviour, viz. *Heterorhabditis indica*, *H. bacteriophora* and *Steinernema abbasi*, under laboratory and field conditions. Entomopathogenic nematodes are expected to move actively in soil, primarily for insect hosts and or for favourable moisture and temperature zones. Therefore, in this study we examined the movement and behavioural responses of Infective Juveniles (IJ) of these nematodes to insect and moisture stimuli in the lab and tried to relate their relative efficacy against the target pest. Further, the studies included recyclability (in *H. phaeopteralis* larvae) and persistence or recovery of EPNs in treated turfgrass in order to observe their long-term benefit in sod webworm control.

MATERIALS AND METHODS

Nematodes

Three species of EPNs from a culture collection maintained by the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, *viz. Steinernema abbasi* NBAIISa01 (NCBI Acc. No. HQ406728); *Heterorhabditis indica* NBAIIHi01 (NCBI Acc. No. HQ406730) and *H. bacteriophora* NBAIIHb05 (NCBI Acc. No. JN572120) were used for our laboratory and field studies. They were reared individually on late-instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), at 28 ± 2°C (Woodring and Kaya, 1988). Freshly emerged nematode juveniles, designated as IJ, were harvested (White, 1927), collected into tissue culture flasks, and used for experiments. Stocks were maintained in aqueous form at 10 ± 2°C.

Collection and maintenance of tropical sod webworm (TSW), *Herpetogramma phaeopteralis*

The larvae of *H. phaeopteralis* were collected from infested turfgrass at NBAIR experimental fields, Yelahanka, Bengaluru (12°58′34″ N, 77°36′11″ E; 914m) by the method described by Niemczyk (1981). The soil was predominantly red laterite with pH 6.47, organic carbon 0.88 and water holding capacity of 12–14%. Five litres of water was sprinkled over an area of 1m^2 of *H. phaeopteralis* infested turfgrass. Larvae that emerged to the surface were hand-picked and transferred to plastic trays (40 cm × 32 cm × 8 cm) containing pre-grown lawn of turfgrass. These trays were covered with black colour muslin cloth and maintained in a greenhouse at 28 ± 2°C. Approximately equal sized larvae of average body length 15 –16 mm with an average head capsule width of 1.374 mm (5th instar) were used for bioassays.

Behaviour and foraging of entomopathogenic nematodes (EPN)

Behaviour and foraging features of freshly harvested IJs of EPNs were investigated on agar media, with and without sand, as per the method detailed by Noosidum *et al*. (2010), and Glazer and Lewis (2000). A separate set of plates with respective media were prepared with a provision for TSW body volatiles to record the movement of IJs in response to insect host stimuli as per Glazer and Lewis (2000). The number of IJs that moved to different quadrants and circles of the media in Petri plates were counted, and the net movement rate for the three nematode species were calculated as:

$$\frac{(2A+3B+4C)}{N}100$$

where A, B, C are the numbers of IJs in 2nd, 3rd and 4th circles from centre of the Petri plate, respectively; 2, 3 and 4 are the distances in cm from the centre, and N is the total number of IJs in the opposite quadrants (Glazer and Lewis, 2000). Five replications were provided for each treatment.

In the case of host-stimuli-related movement assays of IJs (attraction assays), the protocols developed by Glazer and Lewis (2000) and Noosidum *et al.* (2010) were followed. The number of IJs in each arc in the quadrant containing the pipette tip with insect larva and the one opposite were counted every 5 min after introduction for 20 min. Movement was calculated as:

$$\frac{[(2A+3B+4C)-(2D+3E+4F)]}{N}100$$

where A, B, C are the numbers of IJs in 2nd, 3rd and 4th arcs from the centre with the pipette tip, respectively; D, E and F are the number of IJs in the 2nd, 3rd and 4th arcs from the centre in the opposite quadrant, respectively; each number was multiplied by the distance in centimeters from the centre for each arc, and N was the total number of IJs in the opposite quadrants (Glazer and Lewis, 2000). Five replications were provided for each treatment.

Nematode juveniles were also examined directly and on agar media in Petri plates, under a stereo zoom

microscope (light and phase contrast at 10–40×), to record the visual observations on nematode behaviour and foraging. Accordingly, the three nematode species were classified in to different foraging groups (Glazer and Lewis, 2000).

Another set of plain Petri plates was maintained with four cadavers per plate at 28 ± 2°C and near-saturated humidity. Cadavers were placed closely at the centre and a small wet cotton plug placed at the edge of the Petri plate. The cotton plug was maintained in wet to provide a moisture stimulus and observe the behaviour of IJs of three nematode species. Observations on aggregation, movement and body behaviour of IJs were observed visually under stereo zoom microscope.

In vitro assays for virulence of EPN against TSW

Nematode virulence was tested against 5th instar larvae of *H. phaeopteralis*. Larvae were exposed to 25, 50, 100, 200 and 400 IJs per Petri plate (90 × 12 mm) lined with filter paper. Petri plates were provided with small bits of turfgrass as food for TSW larvae. The plates were incubated at 28 ± 2°C in the dark. Five replicates of 25 larvae per treatment (five larvae per treatment) were maintained separately. Larvae treated with sterile distilled water served as control. Larval mortality was recorded at 12h interval for 3 days, and dead larvae were dissected to determine the presence of nematodes.

Another set of 5–6th instar larvae were used for determining the lethal time of exposure required to achieve 50 and 90 per cent mortality at 100 IJs per Petri plate. The methodology essentially remained the same as above, and the exposure time was extended until the death of larvae.

Influence of temperature on virulence of EPN (mortality period) against TSW

Influence of temperature on the mortality period of field-collected TSW larvae was studied under green house conditions. About 3000 IJs of *H. indica*, *S. abbasi* and *H. bacteriophora* were prepared from fresh batches and inoculated in to the poly trays (15 cm h × 15 cm w × 15 cm l) containing pre-grown turf grass. Ten 5th and 6th instar larvae were released per pot and kept at constant temperatures of 18, 23, 27, 30 and 33ºC, respectively. Observations were recorded on mortality days to understand the influence of temperature on the virulence of EPNs against TSW larvae.

Influence of temperature on survival and development of TSW

The effect of temperature on the developmental stages period of TSW (laboratory reared and field collected strains) was studied under laboratory conditions. Ten TSW females from the laboratory-reared and field (collected in batches from light traps in the vicinity of affected turfs and lawns) each were kept at different temperatures of 18, 23, 27, 30 and 33ºC for egg laying, larval growth, pupation and adult emergence. Observations were recorded on larval and pupal periods, and adult emergence to understand the influence of temperature on development of the two strains of TSW.

Recyclability of entomopathogenic nematodes on TSW

Recyclability of EPNs on TSW was estimated in terms of progeny production of the three nematodes on 5th instar sod webworm larvae. Larvae were individually exposed to each nematode species at a concentration of 100 IJs per Petri plate and incubated for 48–72h at 28 ± 1°C. Subsequently, cadavers were individually placed in Petri plates lined with moist filter paper and incubated at 28 ± 1°C for 4–5 days for the IJs to emerge from the cadavers. Emerged IJs were then collected into 150 ml plastic cup every day until the emergence of IJs completely ceased and were designated as stock. Further, a set of 5th instar *G. mellonella* larvae was also maintained as positive check for comparison. Progeny production of each nematode species on TSW and *G. mellonella* were determined by counting the IJs from stock through serial dilution under stereo zoom microscope 40×.

Field evaluation of entomopathogenic nematode strains against TSW

Field studies were conducted at two locations in the TSW-infested lawns at the NBAIR experimental fields, Yelahanka Campus, Bengaluru, during November 2013 and March 2014 with different, but fresh batches of nematodes. Soil type was red laterite with pH 6.74, organic carbon 0.88 and water holding capacity of 12–14 percent. Thirty-six microplots of 3 × 3m^2 size each were demarcated with a plastic sheet barrier (3 m × 3 m × 15 cm) placed for each plot to a depth of 15cm. The treatments included aqueous suspensions of *H. indica*, *S. abbasi* and *H. bacteriophora*each at 6.0 × 10^6 IJs per plot with Chlorpyrifos 2.5ml per litre water as chemical control and untreated control. The Latin Square Design model was used for conducting the experiments. The number of live larvae per m^2 was sampled at 10, 20 and 30 days after imposing the treatments in the same fashion as explained earlier (Niemczyk, 1981). Corrected mortality of larvae was calculated as per Abbot's formula and expressed in percentage. Mean values were arcsine transformed and subjected to analysis using LSD (least significant difference). Recommended agronomical practices were followed.

Persistence of nematodes in turf was determined at 10, 20 and 30 Days After Treatment (DAT). One hundred cc of soil samples (5-7cm depth) were drawn from EPNs-treated plots and used for insect-baiting technique. Ten 5th instar larvae of *G. mellonella* were placed in containers with soil samples, incubated at 30 ± 1°C for 96h and recorded larval

mortality due to EPN infection. Each treatment was replicated three times. Mean values were converted to percent larval mortality, and the standard error was calculated.

Data analysis

Differences in life-stage parameters between laboratory-reared and field-collected strains of TSW and, virulence of EPN at different temperatures were compared using *t*-test. The day-degrees were calculated using a modified formula given by Dhillon and Sharma (2009). Total day-degrees for treatments under constant temperatures = total development period (days) × temperature of exposure; while total degree-days for treatments under ambient conditions = total development period (days) × average of minimum and maximum temperatures across the development period.

Experiments on bioassay and progeny production (recyclability) of EPN were repeated twice using different, but fresh nematode batches, and the data on larval mortality were pooled. Larval mortality was corrected according to Abbott (1925) prior to statistical analysis. The data on larval mortality in relation to dose and exposure time to entomopathogenic nematodes were log-transformed. The values were subjected to Probit analysis (Probit model of Pearson Goodness of Fit Test at α=0.05% significance level) to determine lethal concentration (LC_{50} and LC_{90}) and lethal time (LT_{50} and LC_{90}) using SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

Behaviour and foraging of infective juveniles of EPN

In EPN movement rate studies, *Heterorhabditis bacteriophora* recorded the highest movement rate on agar and sand-agar in the range of 0.38–0.78cm and 0.45–0.56cm, respectively, across the time, followed by *Steinernema abbasi* with 0.29–0.60cm and 0.36–0.72cm, respectively (Table 1). *H. indica* recorded a movement rate of 0.10–0.35cm on agar and 0.15–0.48cm on sand-agar, in 45min. of observation. Although all three nematode species were attracted to TSW volatiles, foraging movement of *H. indica* was 0.18–0.46cm, while *H. bacteriophora* and *S. abbasi* recorded a movement of 0.42–0.85 and 0.31–0.68cm, respectively (Table 1). Visual observations under a stereo zoom microscope on the behaviour of IJs on agar and sand-agar showed that *H. indica* exhibited wavy and s-shaped movement, while IJs of *S. abbasi* and *H. bacteriophora* exhibited deep s-shaped active and vigorous movement. Infective juveniles of *H. indica* recorded nictitation, i.e. tails of IJs were attached to the substrate (sand particles), with body in upright position freely in space and body moving actively in wavy fashion (Table 2). Infective juveniles of *H. bacteriophora* and *S. abbasi* did not record this behaviour and they remained

Table 1. Movement rate of infective juveniles of *Heterorhabditis indica*, *H. bacteriphora* and *Steinernema abbasi* in the presence and absence of TSW volatiles

Nematode Species	Average movement rates (× ± SE) cm			Average movement rates (× ± SE) cm in the presence of TSW volatiles		
	15 min	30 min	45 min	15 min	30 min	45 min
H. indica	0.10 ± 0.02a(a)*/*/ 0.15 ± .03a(a)*/**	0.16 ± 0.02a(a)*/ 0.18 ± 0.02a(a)**	0.35 ± 0.02a(ab)*/ 0.48 ± 0.03a(ab)**	0.18 ± 0.02a(ab)	0.22 ± 0.03a(ab)	0.46 ± 0.03a(bc)
H. bacteriophora	0.38 ± 0.02c (bc)*/ 0.45 ± 0.04c (bc)**	0.62 ± 0.02c(cd)*/ 0.48 ± 0.07bc(bc)**	0.78 ± 0.0c(d)3*/ 0.56 ± 0.03bc(cd)**	0.42 ± 0.03c(bc)	0.69 ± 0.02c(cd)	0.85 ± 0.04c(d)
S. abbasi	0.29 ± 0.04bc (bc)*/ 0.36 ± 0.06bc (bc)**	0.45 ± 0.05bc(bc)*/ 0.52 ± 0.06bc(cd)**	0.60 ± 0.01c(cd)*/ 0.72 ± 0.08c(d)**	0.31 ± 0.02bc(b)	0.56 ± 0.06bc(c)	0.68 ± 0.06bc(cd)

Means were arcsine transformed and subjected to two-way ANOVA Means followed by the same letter are not significantly different by LSD at α = 0.05 between isolates. Means followed by same letter in parenthesis are not significantly different at α = 0.05 between time. There was no significance difference between *smooth agar plate; **sandy agar plate at α = 0.05

in crawling position. Infective juveniles of *H. indica* that emerged from cadavers on dry Petri plates characteristically aggregated and moved in radiating threads towards moisture droplets whereas IJs of *H. bacteriophora* were observed to aggregate, moved in small numbers, rarely in threads, while *S. abbasi* juveniles aggregated, actively moved individually or in small numbers, and thread formation was absent. None of the IJs were observed to show jumping behaviour movement, perhaps requiring a special set of experimental conditions for observation.

The behaviour of IJs, i.e. non-feeding and free-living stage of EPN, were reported to be highly variable and correlated to their host searching ability and host infectivity (Campbell and Gaugler, 1993, 1997). EPN have been categorized as ambushers, cruisers and intermediate foragers based on a suite of behavioural features (Lewis, 2002). Noosidum *et al.,* (2010) reported that all *Heterorhabditis* spp., characterized from Thailand, did not exhibit body-waving and standing behaviours, while the *Steinernema* isolates were more of intermediate foragers. Standing and jumping behaviour of IJs was attributed to cruiser forager (Campbell and Gaugler, 1993; 1997). In this study, we observed that IJs of *H. indica* not only moved relatively slower on agar and sand-agar than the IJs of *H. bacteriophora* and *S. abbasi,* but also exhibited nictitation and s-shaped movement; while the IJs of *H. bacteriophora* and *S. abbasi* did not show nictitation but exhibited active movement in deep s-shaped fashion. These observations suggest that *H. indica* was an ambusher than a cruiser, while *H. bacteriophora* and *S. abbasi* were cruiser type foragers as observed by Lewis and co-workers (Lewis *et al*., 1992; Lewis, 2002).

Attraction to host volatile cues was prominent in cruiser type foragers and not prominent in ambusher (Lewis *et al*., 1993). We observed that attraction of *H. bacteriophora* and *S. abbasi* to TSW volatile cues was significantly higher than that of *H. indica,* thus supporting the observations that these are cruiser type foragers. We also observed that besides host volatile cues, the nematodes characteristically were attracted and tactile to moisture. Infective juveniles of *Heterorhabditis indica* emerging from a *Galleria* cadaver on a dry Petri plate (with water droplets/moist cotton placed at a distance in the Petri plate) in response to moisture stimulus, first aggregated in to radiating threads or strands and then moved towards moisture, adhering to one another. This behaviour of aggregation and movement in strands was as well observed in *H. bacteriophora* but to a lesser extent, while IJs of *S. abbasi* preferred to actively move individually or in smaller number.

Infective juveniles are known to actively move in soil mainly in search of food, or for avoiding adverse abiotic stress. The common stimuli for their movement include host cues, moisture, temperature and pH. As observed in our study, relatively slow movement in simple s-shape manner and nictitation (standing and body-wave motion), in case of *H. indica*, were characteristic to an ambusher, while active and deep s-shape movement of IJs with no nictitation were characteristic to cruising type of foraging nematodes. Similar observations by Campbell and Gaugler (1993, 1997) and Lewis *et al.* (1993), supporting that *H. indica* strain under study can be categorized as an ambusher and *H. bacteriophora* and *S. abbasi* as cruisers. We believe that the behaviour of aggregation and movement in strands in response to moisture stimulus, as in case of *H. indica*, can be mistaken for cruising type of foraging.

Table 2. Behaviour of infective juveniles of *Heterorhabditis indica*, *H. bacteriphora* and *Steinernema abbasi* in the presence and absence of TSW volatiles and moisture

Nematode species	Visual observations under microscope (40-60×)		
	IJs on agar medium	IJs on sandy agar medium	IJs in dry conditions in a Petri plate
H. indica	S-shaped movement, jumping not prominently observed.	IJs nictitate on sand particles, jumping not prominently observed.	IJs aggregate, move in radiating threads towards moisture droplets.
H. bacteriophora	Deep s-shaped, active and vigorous movement, no jumping.	No nictitation, no jumping.	IJs aggregate, move in small numbers, rarely in threads.
S. abbasi	Deep s-shaped, active and vigorous movement, no jumping	No nictitation, no jumping.	IJs aggregate, actively move individually or in small numbers, thread formation is absent.

***In vitro* assays for virulence of EPN against TSW**

Heterorhabditis indica recorded the lowest LC_{50} and LC_{90} values (30 and 131 IJs, respectively), followed by *S. abbasi* and *H. bacteriophora,* which recorded 97 and 515, and 111 and 1252 IJs, respectively (Table 3). As the lowest slope value indicates the highest virulence, a lowest slope value of 1.5 was observed for *H. indica.* Similar trend was observed for *G. mellonella*, a positive check, with lowest LC values for *H. indica*, followed by *H. bacteriophora* and *S. abbasi*. In comparison, the LC values for *G. mellonella* were about 2.5 to 3.0 times lower than that of the LC values recorded for *H. phaeopteralis* for all the three nematodes. *Herpetogramma phaeopteralis* required higher inocula of EPN compared with *G. mellonella* to accomplish median and 90% larval mortality (LC_{50} and LC_{90}). These observations indicated two types of variability related to virulence of EPNs viz., variability among EPN species and variability due to insect host. Earlier Tofangsazi *et al.,* (2014) studied the effect of commercial formulations of five EPN species against TSW and reported variability in virulence among five EPN species against TSW.

Influence of temperature on virulence of EPN against TSW

The effect of temperature on virulence of EPN against 5th and 6th instar larvae of TSW (in terms of exposure period required for larval mortality) was studied under controlled conditions. Exposure period required for larval mortality at 18, 23, 27, 30 and 33°C were different for different nematode species (Fig. 1, 2; Table 7). At 18°C, the number of days

Table 3. Lethal concentration of *Steinernema abbasi*, *Heterorhabditis indica* and *H. bacteriphora* (IJs per larva) required to cause pathogenicity on the 5th instar larvae of *Herpetogramma phaeopteralis*

Nematode species	Slope ± SE	LC_{50} IJs	95% C.L.	LC_{90} IJs	95% C.L.	χ^2	*df*	*P*	Progeny production of IJs per g body weight of larva
S. abbasi	3.53 ± 0.63	97	69-36	515	309-1349	2.154	3	0.541	3.92×10^3
H.indica	1.53 ± 0.57	30	16-42	131	90-266	4.585	3	0.205	4.22×10^3
H. bacterio-phora	2.42 ± 0.76	111	69-186	1252	523-11546	0.310	3	0.958	3.44×10^3

In time–mortality response at 100 IJs, *H. indica* was observed to be the most virulent against *H. phaeopteralis* with significantly lower LT_{50} and LC_{90} values (21 and 72h, respectively) followed by *S. abbasi* (52 and 101, respectively) and *H. bacteriophora* (73 and 127, respectively) (Table 5). In case of *G. mellonella,* positive check, LT_{50} and 90 were significantly lower for all the three nematode species (Table 6) compared to *H. phaeopteralis,* and similar to the trend as observed with LC values. *G. mellonella* required shorted exposure time to nematodes in order achieve desired mortality compared to that of *H. phaeopteralis*.

Table 4. Lethal concentration of *Steinernema abbasi*, *Heterorhabditis indica* and *H. bacteriphora* (IJs per larva) required to cause pathogenicity on the 5th instar larvae of *Galleria mellonella*

Nematode species	Slope ± SE	LC_{50} IJs	95% C.L.	LC_{90} IJs	95% C.L.	χ^2	*df*	*P*	Progeny production of IJs per g body weight of larva
S. abbasi	3.04 ± 0.61	28	16-44	172	119-386	1.154	3	0.414	5.48×10^5
H.indica	1.13 ± 0.54	12	7-28	88	68-212	3.413	3	0.338	6.72×10^5
H. bacterio-phora	2.12 ± 0.76	24	14-40	149	102-346	2.312	3	0.578	5.13×10^5

Table 5. Exposure time of *Steinernema abbasi*, *Heterorhabditis indica* and *H. bacteriphora* required to cause mortality to the 5th instar larvae of *Herpetogramma phaeopteralis*

Nematode species	Slope ± SE	LT_{50} Hrs.	95% C.L.	LT_{90} Hrs.	95% C.L.	χ^2	*df*	*P*
S. abbasi	7.55 ± 1.36	52	45-60	101	81-157	5.239	4	0.264
H.indica	3.30 ± 0.69	21	15-27	72	54-122	4.891	4	0.299
H. bacteriophora	9.88 ± 2.32	73	63-99	127	95-285	4.699	4	0.320

Table 6. Exposure time of *Steinernema abbasi*, *Heterorhabditis indica* and *H. bacteriphora* required to cause mortality to the 5th instar larvae of *Galleria mellonella*

Nematodes	Slope ± SE	LT_{50} Hrs.	95% C.L.	LT_{90} Hrs.	95% C.L.	χ^2	*df*	*P*
S. abbasi	3.55 ± 1.12	41	30-56	54	41-72	4.139	4	0.410
H.indica	2.10 ± 0.29	17	12-26	36	28-48	3.112	4	0.599
H. bacteriophora	2.88 ± 1.22	33	28-43	49	35-67	3.729	4	0.452

Table 7. Mortality of TSW larvae exposed to *Heterorhabditis indica, H. bacteriphora* and *Steinernema abbasi* at different temperatures

Temperature (ºC)	Number of days required for TSW larval mortality exposed to different EPN species (mean ± SE)			*t*- Value *df*=7
	H. indica	*H. bacteriophora*	*S. abbasi*	
	Exposure period for 5th instar larval mortality (days)			
18	3.6 ± 0.22b(bc)	4.5 ± 0.24c(cd)	5.9 ± 0.11c(cd)	1.99
23	2.3 ± 0.34ab(ab)	3.6 ± 0.26b(bc)	4.6 ± 0.12c(cd)	1.81
27	1.8 ± 0.13a(a)	2.3 ± 0.32a(ab)	3.8 ± 0.22b(bc)	0.21
30	2.4 ± 0.12ab(ab)	2.8 ± 0.11a(ab)	2.2 ± 0.23a(ab)	0.39
33	3.3 ± 0.10b(bc)	3.8 ± 0.10b(bc)	3.3 ± 0.21b(bc)	1.26
	Exposure period for 6th instar larval mortality (days)			
18	5.2 ± 0.30cd(d)	5.8 ± 0.12d(d)	5.9 ± 0.14d(d)	1.96
23	3.5 ± 0.21bc(bc)	4.9 ± 0.26cd(d)	5.0 ± 0.12cd(d)	1.63
27	2.6 ± 0.36a(a)	3.1 ± 0.11ab(bc)	3.3 ± 0.23bc(bc)	0.42
30	2.8 ± 0.12ab(a)	3.3 ± 0.12bc(bc)	2.9 ± 0.22ab(a)	0.31
33	4.2 ± 0.22c(cd)	4.8 ± 0.71c(cd)	3.8 ± 0.16bc(c)	1.82

Note: Means were arcsine transformed and subjected to two-way ANOVA. Means followed by the same letter are not significantly different by LSD at α = 0.05 between temperature; Means followed by same letter in parenthesis are not significantly different at α = 0.05 between isolates

required for 5th instar larval mortality were 3.6 ± 0.22, 4.5 ± 0.24 and 5.9 ± 0.11 days, respectively for *H. indica, H. bacteriophora* and *S. abbasi,* and for 6th instar larval mortality, the number of days required were 5.2 ± 0.30, 5.8 ± 0.12 and 5.9 ± 0.10 days. At 33°C, exposure period required for 5th instar larval mortality were 3.3 ± 0.10, 3.8 ± 0.10 and 3.3 ± 0.21 days, respectively, for *H. indica, H. bacteriophora* and *S. abbasi* and 4.2 ± 0.22, 4.8 ± 0.70 and 3.8 ± 0.10 days,

Table 8. Survival and development of laboratory and field strains of TSW at different temperatures

Life table parameters/ temperature (ºC)	Laboratory strain (Mean ± SE)	Field strain (Mean ± SE)	*t*- Value *df*=7
	Larval period (days)		
18	50.8 ± 2.19c	53.5 ± 2.21c	-2.87**
23	48.6 ± 4.32bc	49.4 ± 3.10bc	-1.54
27	36.9 ± 1.16a	39.3 ± 2.12a	-0.32*
30	35.4 ± 2.22a	35.1 ± 1.38a	-0.97*
33	47.6 ± 3.17bc	50.8 ± 1.11c	-2.45
	Pupal period (days)		
18	7.4 ± 0.29bc	8.1 ± 0.20d	-1.57**
23	7.0 ± 0.15bc	7.8 ± 0.22cd	-0.98
27	5.6 ± 0.22a	6.3 ± 0.23ab	-0.21*
30	5.9 ± 0.10a	6.9 ± 0.15ab	-0.47*
33	6.8 ± 0.30ab	7.3 ± 0.18bc	-1.44
	Adult emergence (%)		
18	42.6 ± 0.93d	38.6 ± 0.21d	-4.57**
23	54.2 ± 0.67c	46.5 ± 0.18cd	-3.98
27	92.8 ± 0.39a	90.6 ± 0.53a	-1.21*
30	86.4 ± 0.49ab	93.2 ± 0.61a	-1.47*
33	67.6 ± 0.84bc	74.5 ± 0.82b	-3.44

Note: *, ** Values are significant at $P \leq 0.05$ and 0.01, respectively; the means were angular transformed and subjected to *t*-test using SPSS 16.0.

respectively, for 6th instar larval mortality. Significantly, temperatures that favoured minimum number of days for 5th and 6th instar larval mortality, for *H. indica* (1.80 ± 0.22 days, 2.60 ± 0.36 days), *H. bacteriophora* (2.30 ± 0.32 days, 3.10 ± 0.11 days) and *S. abbasi* (2.20 ± 0.23 days, 2.90 ± 0.22 days respectively) were 27, 27 and 30°C, respectively. Significantly, at 27ºC *H. indica* and *H. bacteriophora* recorded the lowest number of days for 5th and 6th instar larval mortality at 27°C, i.e. 1.8 ± 0.13 and 2.6 ± 0.36, and 2.3 ± 0.32 and 3.1 ± 0.11 days, respectively, and at 30ºC *S. abbasi* recorded the lowest number of days for 5th and 6th instar larval mortality (2.2 ± 0.23 and 2.9 ± 0.22 days, respectively) at 30°C, indicating variability in thermal preferences by the EPN for virulence.

The relationship between temperature and virulence of each nematode species against TSW larvae was further elaborated based on the degree-days at different temperatures. Number of degree-days for larval mortality were lowest for *H. indica* (94, 60 and 44 degree-days, respectively) at 18, 23 and 27°C compared with *H. bacteriophora* (118, 94 and 60 degree-days, respectively) and *S. abbasi* (153, 120 and 95degree-days, respectively). At higher temperatures of 30 and 33°C, the number of degree-days was lowest for *S. abbasi* (57 and 87 degree-days, respectively), followed by *H. indica* (61 and 87 degree-days, respectively) and *H. bacteriophora* (74 and 97 degree-days, respectively) for 5th instar larval mortality. For 6th instar larval mortality at 30°C, *H. indica* and *S. abbasi* were statistically on par (72 and 77 degree-days, respectively); and at 33°C, *S. abbasi* recorded lowest degree-days of 97 followed by *H. indica* (110 degree-days) and *H. bacteriophora* (123 degree-days).

Influence of temperature on development of soil stages (larva and pupa) of TSW

Data on effect of specific temperatures (18, 23, 27, 30 and 33°C) on developmental periods of TSW life-stages and adult emergence are given in Table 8. Larval periods for laboratory-reared and field strains of TSW ranged between 35.4 and 50.8, and 35.1 and 53.5 degree-days, respectively, and pupal periods between 5.6 and 7.4 degree-days and 6.3 and 8.1 degree-days, respectively, at different temperatures. Larval and pupal periods were longer at lower temperature of

18°C for both the laboratory-reared and field strains of TSW. With increase in temperature from 18 to 30°C, the number of days required for larval period decreased to 35.5 and 35.1 degree-days, respectively. At 33°C the larval period for laboratory-reared and field strain recorded an increase (47.6 and 50.8 degree-days). Pupal period too recorded an increase in number of degree-days at 33°C.

The association between temperature and longevity of TSW developmental stages were examined based on degree-days at different temperatures. Laboratory-reared TSW recorded a minimum of 917 degree-days at 30°C, and a maximum of 1310 degree-days at 18°C for complete development, while the field strain recorded a minimum of 876 and a maximum of 1325 degree-days at 30 and 18°C, respectively (Figure 3).

Thermal requirement of life-stages of TSW and virulence of EPN were examined in terms of degree-days to understand their mutual behaviour in different temperature regimes and identify mutually favourable temperature regimes. Between 18 and 27°C, the number of degree-days for TSW larval mortality was the lowest for *H. indica* compared with *H. bacteriophora* and *S. abbasi*. At higher temperatures (30 and 33°C) the number of degree-days was the lowest for *S. abbasi*, followed by *H. indica* and *H. bacteriophora* for TSW larval mortality. Number of degree-days for TSW larval stages was lowest at 27–30°C, indicating that the ideal overlapping temperatures for the insect pest (TSW) and virulence of the three nematodes. More specifically, *S. abbasi* preferred warmer temperatures of 30–33°C, while *H. bacteriophora*, milder temperatures of 24–27°C, and *H. indica,* a wider range of temperatures, 24–30°C, for virulence. Based on these observations we hypothesize that the thermal requirements of EPNs and a target insect pest contribute significantly to the success of EPN, thus and a specific nematode species can be employed for specific temperature regime in field.

Hill *et al.* (2015) reported acute thermal limits for survival and the thermal acclimation-related plasticity thereof for two key endemic South African EPN species, *S. yirgalemense* and *H. zealandica*. Shapiro *et al.* (2014) demonstrated that the *H. floridensis* strains possessed a wide niche breadth in temperature tolerance and had virulence and desiccation levels that were similar to a number of other EPNs. The strains may be useful for biocontrol purposes in environments where temperature extremes occur within short durations. These studies actually refer to the tolerance limits of the nematodes under controlled conditions, while our study demonstrated the actual thermal requirements of the EPNs for virulence and the relevance of matching thermal requirements of EPNs with that of pests.

Recyclability (progeny production) of EPN on TSW larvae

Progeny production of *H. indica, H. bacteriophora* and *S. abbasi* in the larvae of *H. phaeopteralis* and *G. mellonella* (a positive **control**) were estimated and expressed in terms of yield per gram of larval body weight for comparison. Progeny production of these nematode species on *H. phaeopteralis* ranged between 3.44×10^3 and 4.22×10^3 per g body weight, with a maximum yield of IJs of *H. indica* (4.22×10^3) followed by *S. abbasi* (3.92×10^3 IJs) and *H. bacteriophora* (3.44×10^3 IJs) (Table 10). Progeny production of the senematodes in *G. mellonella* was 1.2 to 1.6 times higher than that in *H. phaeopteralis.* Estimation of recyclability/productivity of EPN helps in possible survivability and persistence of these nematodes in treated fields/rhizosphere.

Field evaluation of EPN against TSW

Data recorded in two field trials, on the effect of EPNs on TSW populations at 10, 20 and 30 DAT, are presented in Table 9. In Trial-1, 10 DAT, *S. abbasi*, *H. bacteriophora* and *H. indica* recorded 22, 40 and 57% reduction in larval populations of TSW, respectively and Chlorpyrifos-treated plots recorded a reduction of 31%. At 20 and 30 DAT, *S. abbasi* recorded 31 and 47% reduction in TSW larval populations; *H. bacteriophora,* 52 and 68%, and *H. indica,* 69 and 83% reduction, respectively, while there was 48 and 37% reduction in Chlorpyrifos-treated plots. In Trial-2, similar trends were observed on the effect of EPN on TSW larval populations at all the three time intervals (Table 9). Compared to Trial-1, larval population reduction was significantly higher in *S. abbasi* treated plots, whereas, reduction in larval populations was at parin *H. indica* and *H. bacteriophora* treated plots in Trial-2. Among the three EPN species, *H. indica* recorded maximum reduction in TSW populations in both the trials. In Ttrial-1, *H. bacteriophora* recorded higher reduction of TSW populations compared to *S. abbasi*, while *S. abbasi* performed better than *H. bacteriophora* in Trial-2. Chlorpyrifos recorded comparatively lower reduction in TSW populations in Trial-2 than in Trial-1. In both the trials EPN treatments were superior to Chlorpyrifos treatment in reducing TSW populations.

All the three nematode species were recovered from respective treated plots at 15,30 and 60 DAT at two different trial sites (Table 10) indicating their persistence in turfgrass for 60 days. Kurtz *et al.* (2007) investigated the establishment and the short- and long-term persistence of *H. bacteriophora*, *H. megidis* and *S. feltiae* in three maize fields in southern Hungary, using the insect-baiting technique. All three EPN species equally established and persisted in maize fields. EPNs persisted for 2–5 months, i.e. they survived up to and throughout the crop period.

Table 9. Effect of EPN treatments on population density of *Herpetogramma phaeopteralis* in two trials

Treatments	Initial population per m^2	TRIAL -1 Reduction in population density per m^2 (%)			TRIAL -2 Reduction in population density per m^2 (%)		
		10 DAT	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
S. abbasi	91 ± 0.2	22 ± 0.2d	31 ± 1.4d	47 ± 1.7c	47 ± 0.8c	60 ± 1.4b	74 ± 1.6ab
.indica	95 ± 1.3	57 ± 0.6bc	69 ± 1.2b	83 ± 0.6a	58 ± 0.9bc	61 ± 1.0b	86 ± 0.3a
H. bacteriophora	98 ± 2.6	40 ± 0.5cd	52 ± 0.9bc	65 ± 0.9b	32 ± 1.3d	57 ± 1.5bc	61 ± 0.8b
Chlor-pyrifos	86 ± 2.4	31 ± 0.4d	48 ± 0.8c	37 ± 1.7d	27 ± 1.2d	30 ± 0.3cd	19 ± 1.4d
Untreated control*	93 ± 1.7	110 ± 0.8	116 ± 0.6	121 ± 0.4	101 ± 1.8	98 ± 1.9	119 ± 1.4

Note:*Untreated control values signify increase in population density recorded in untreated control over different DAT, while other values signify the percentage reduction in population density in all other treated plots over respective untreated controls. DAT denotes Days After Treatment. Means followed by the same letter are not significantly different by LSD at α = 0.05 between days.

Table 10. Recovery of *Steinernema abbasi, Heterorhabditis indica* and *H. bactriophora* from the treated turf grass micro plots and recyclability of these nematode species on TSW

Nematode species	Recovery of EPNs on *Galleria mellonella*						Progeny production of IJs per g body weight of larva	
	TRIAL -1			TRIAL -2			TSW	*G. mellonella*
	15 DAT	30 DAT	60 DAT	15 DAT	30 DAT	60 DAT		
S. abbasi	62 ± 0.3bc	48 ± 1.2b	22 ± 1.0bc	66 ± 1.2a	32 ± 1.4b	20 ± 1.4b	3.92×10^5	5.48×10^5
H. indica	86 ± 2.6a	89 ± 1.0a	85 ± 1.2a	61 ± 0.9a	82 ± 2.0a	70 ± 0.8a	4.22×10^5	6.72×10^5
H. bacteriophora	50 ± 1.9c	45 ± 0.8b	32 ± 0.3b	59 ± 1.3ba	27 ± 1.7cb	18 ± 0.8cb	3.44×10^5	5.13×10^5

Note: Means followed by the same letter are not significantly different by LSD at α = 0.05 between days.

Previously, Tofangsazi *et al.,* (2014) demonstrated that *S. carpocapsae* caused the highest mortality in comparison to *S. feltiae*, *H. bacteriophora*, *H. megidis* and *H. indica* against TSW, using their commercial formulations. Studies have also established the efficacy and applicability of EPNs against other insect pests of turfgrass. *Steinernema carpocapsae* was the best performing species than *S. feltiae* and *H. bacteriophora,* against black cutworm, *Agrotisipsilon*, on golf turf, indicating that there was inter-generic variation in efficacy (Ebssa and Koppenhofer, 2011). Similarly, Koppenhofer *et al.,* (2006) observed inter-generic variation in virulence among *H. bacteriophora, H.indica, H. zealandica*and *S. scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass.

CONCLUSION

Efficacy of all the three nematode species was comparable to that of Chlorpyrifos against TSW, as they possessed additional attributes like foraging and recyclability. Among the three EPNs studied, *Heterorhabditis indica* recorded lowest LC and LT values, better recyclability, field efficacy and persistence. We demonstrated that in addition to LC values, attributes like foraging behaviour, recyclability (progeny production in insect host) and persistence in treated locations, complementarity of thermal preferences of the target insect pest and the EPNs is critical feature for the success of EPNs in field on long-term basis. These attributes also help us in deciding the field dose, method and time of application of EPNs matching to the variability in EPNs and the insect pest.

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