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ORIGINAL ARTICLE

Effects of storage temperature on survival and infectivity of three indigenous entomopathogenic nematodes strains (Steinernematidae and Heterorhabditidae) from Meghalaya, India

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Abstract Three locally isolated strains of entomopathogenic nematodes (EPNs), viz. *Heterorhabditis indica*, *Steinernema thermophilum* and *Steinernema glaseri*, from Meghalaya, India were characterized in terms of storage temperature and survival and infectivity of their infective juveniles (IJs). The survival and infectivity of nematode IJs was studied at, 5 ± 2 and 25 ± 2 °C, for a period of 120 days, using deionized water as storage medium. The viability of nematode IJs was checked by mobility criterion at different storage periods, while the infectivity of nematode IJs was ascertained on the basis of establishment of IJs, using *Galleria mellonella* larva mortality tests in petridishes. The results of this study revealed that storage temperature markedly affects the survival as well as the establishment of nematode IJs of the three EPN species. At 5 °C, comparatively higher rate of IJ's survival (i.e. 74–86 %) was observed for 15 days of storage, but the same reduced drastically to 28–32 % after 30 days of storage for *H. indica* and *S. thermophilum*. On the other hand, at 25 °C, the survival of nematode IJs was observed till 120 days for all the three studied EPNs. In case of *S. thermophilum* and *S. glaseri*, higher rate of IJs survival (>75 %) was observed respectively at 15 and 30 days of observation. The study also showed that the establishment of IJs of the three EPN species declines with increase in storage periods, at both the test temperatures. In general, the nematodes stored at 25 °C showed comparatively better establishment than those stored at 5 °C. Among the three EPN studied, the establishment of *S. glaseri* was comparatively better than the rest of the species at both the temperatures and for different storage durations. In conclusion, our study adds further valuable information about the effect of storage temperature on survival and infectivity of three indigenous EPN species of Meghalaya, India which appears to be promising biocontrol agents of local insect pests.

Keywords Entomopathogenic nematodes · *Heterorhabditis indica* · *Steinernema thermophilum* · *Steinernema glaseri* · *Galleria mellonella* · Biological control

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Introduction

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are obligate parasites of insects and are frequently used as biological control agents of many soil-inhabiting insect pests (Georgis et al. 2006). At present, there are about a dozen of EPN species that are available commercially as biocontrol agents for various insect pests. By and large, most of these commercial EPNs target the high-value crop pests, either in North America or European countries, and a few such products are also being used in Korea (Kaya et al. 2006). Many studies have demonstrated that the native species/isolates of EPNs, that are adapted to local environmental conditions, are especially good biocontrol agents for local insect pests (Koppenhöfer and Kaya 1999). Several

previous studies have also reported that the isolates of EPNs from different geographical regions exhibit considerable variations in terms of their host range, infectivity potentials, reproduction, tolerance of various environmental factors, etc (Bedding 1990). Therefore, to a great extent, the biocontrol potentials of different EPN species/strains are greatly influenced by a number of specific abiotic and biotic factors (Kaya 1990; Koppenhöfer et al. 1995).

In India, prior to any scientific report about the occurrence of indigenous EPNs, initially attempts were made to utilize a few exotic species/strains of EPNs, like *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, etc that were imported by the researchers from other countries, as biocontrol agents (Rao et al. 1971; Hussaini 2003). However, in none of these attempts the exotic EPN isolates were able to establish successfully in the fields due to their poor adaptability to the prevailing agro-climatic conditions of the country. As a result, in the later years, attention was diverted to search the native strains of EPNs, assuming that perhaps they may adapt well to the local climatic conditions of the country (Nayak et al. 1977; Singh 1977). The surveys made in different regions of India have resulted into isolation of a number of indigenous species/isolates of EPNs (Rahman et al. 2000; Hussaini 2003). Ever since, efforts are being made to understand the basic biology and ecology of the native EPN isolates, so as to explore their possible use against the local insect pests of the country (Ganguly and Singh 2001; Ganguly and Gavas 2004a, b). However, at present, none of the indigenous EPN species/strain is available as commercial product for possible use against any insect pest in India.

In a recent study, three indigenous isolates of EPNs, viz. *Heterorhabditis indica*—Poinar, Karunakar and David, *Steinernema thermophilum*—Ganguly and Singh and *Steinernema glaseri* (Steiner) were isolated from the forest soils in Meghalaya, India (Lalramliana 2007). In laboratory trials, the isolates of these nematodes showed promising control against two important local insect pests, viz. the mustard sawfly, *Athalia lugens proxima* (Klug) and against a chrysomelid beetle, *Aplosonyx chalybaeus* (Hope), a pest of taro (Yadav and Lalramliana 2012a, b). It is widely recognized now that the selection of a suitable EPN species as a biocontrol agent in a given geographical area requires adequate knowledge about the biology and ecology of the nematode. Information relating to the above three EPN isolates, such as the effects of soil moisture on the establishment and the effects of relative humidity on the emergence and reproduction were studied by Lalramliana and Yadav (2009) and Yadav and Lalramliana (2012c). Among other abiotic factors, the temperature is also considered as an important factor in determining the suitability of EPN as an appropriate biocontrol agent. For example, the temperature may affect the mobility, development, persistence, survival, reproduction, etc of nematodes in several ways (Griffin 1993; Grewal et al. 1994; Koppenhöfer and Kaya 1999; Ganguly and Singh 2001; Cagnolo and Campos 2008). The effects of temperature on survival and infectivity of nematodes infective juveniles (IJs) have also been studied for some EPNs. In these studies, the different isolates of the same species have also been found to exhibit differential responses to storage temperatures (Cagnolo and Campos 2008). In view of this, it is necessary to characterize the indigenous nematodes to be used for the biocontrol of local insect pests in terms of temperature requirements as well. Hence, in the present study, attempts have been made to find out the influence of storage temperature on survival and infectivity of isolates of three indigenous EPNs, i.e. *H. indica*, *S. thermophilum* and *S. glaseri*.

Materials and methods

The isolates of nematodes used in this study, viz. *H. indica, S. glaseri and S. thermophilum*, were collected using *Galleria mellonella* traps from the forest soils of Ri-Bhoi District (91′40′16″ latitude and 25′40′–26′20′N longitude) in Meghalaya, India. These EPNs have been duly identified using light and scanning electron microscope and also by morphometric criteria (Lalramliana 2007). For the present study, the nematodes were reared in the laboratory on late instar larvae of *G. mellonella* at 25 °C, as described by Woodring and Kaya (1988). The freshly emerged IJs (3–5 days after first day of emergence) of each nematode species were then collected from wax moth larval cadavers, using modified white traps (Woodring and Kaya 1988), and used for various experiments.

To determine the effect of storage temperature on survival and infectivity of EPNs, the population densities of IJs were adjusted to 1,000 IJs/ml in 20 ml of de-ionized water in 50 ml conical flasks. The flasks were then plugged with non-absorbent cotton and kept inside BOD incubators at two different temperatures, i.e. 5 ± 2 and 25 ± 2 °C, for 120 days. The selection of test temperatures was done in accordance with the prevailing average minimum and maximum monthly temperatures of the area from where the nematode isolates were collected. Before setting-up the experiment, the viabilities of nematode IJs were checked by observing their movements under a stereomicroscope. Accordingly, the nematode populations were assumed to be 100 % viable on the first day of experiment. The survival of IJs in the suspensions was monitored at 15, 30, 60, 90 and 120 days of storage periods for each test temperature. For this, about 1 ml of IJs suspension was withdrawn from each conical flask and the viability of IJs was determined

under a stereomicroscope by mobility criterion. To ascertain the mobility status of IJs, the immobile IJs were first located in the suspension and later they were touched with a fine-wire probe, those that did not react by touching were considered as dead IJs. To determine the establishment percentage of nematodes, the infectivity tests of IJs were done by petridish assay, using eight numbers of *G. mellonella* larvae at the concentration of 100 IJs/larva and the number of IJs established/larva were recorded (Lalramliana 2007). For control group, the establishments of IJs were also checked on the day the experiments were set-up (shown as day 0), wherein the same numbers of moth larvae were exposed to 100 IJs from the original stock suspension of IJs to record the number of IJs established/larva. There was three replicates for each test temperature and IJs observation for survival and infectivity tests.

Statistical analysis

The data were analyzed statistically and are represented as mean ± standard error of the mean (SEM). The significance of the difference was determined by the one-way analysis of variance (ANOVA) and student's *t* test. *P* values <0.05 were accepted as statistically significant. Correlation between the parameters was determined by regression analysis.

Results

The data regarding the survival and establishment of EPN IJs after storage at 5 °C for 120 days are shown in Fig. 1. The storage temperature showed a marked effect on both the survival and establishment of the IJs of three EPN species. At 5 °C, the survival of IJs was observed only till 60 days for *H. indica* and *S. thermophilum*. At this storage condition, comparatively higher rates of IJ's survival (i.e. 74–86 %) were observed for 15 days of storage for both these EPN species. The IJs survival, however, reduced drastically to 28–32 % after 30 days of storage and no survival of nematode IJs was observed beyond 60 days storage for both these EPNs. In contrast to *H. indica* and *S. thermophilum*, the IJs of *S. glaseri* survived till 120 days and the highest rate of survival (99.37 %) was observed for 15 days storage period. At the same temperature, the infectivity of EPN IJs (as measured by rate of IJs establishment in *G. mellonella*) was also significantly ($p < 0.05$) affected by storage durations. The infectivity of *H. indica* and *S. thermophilum* IJs was maximum at 15 days storage period. However, the IJs of *S. glaseri* showed the maximum infectivity rate at 30 days storage time (Fig. 1).

The data pertaining to the survival and establishment of EPN IJs after storage at 25 °C are presented in Fig. 2. At

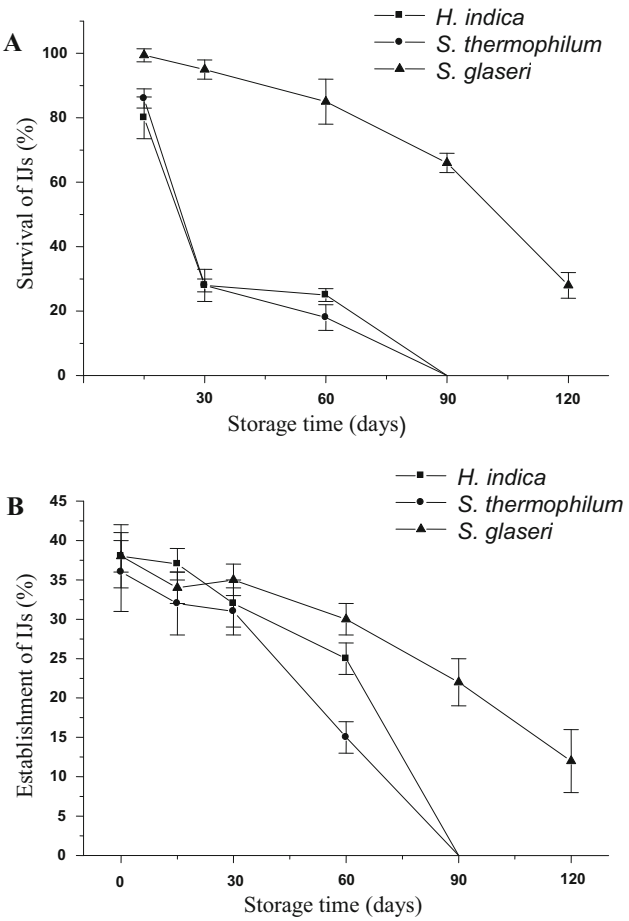

Fig. 1 Percentage survival and establishment of infective juveniles (IJs) of entomopathogenic nematode species at 5 °C. **a** Survival of IJs and **b** establishment of IJs

this temperature, the survival of nematode IJs was observed till 120 days for all the three test EPN species. The rate of IJ survival was moderately high till 90 days observation time, but reduced significantly thereafter. As far as the infectivity of IJs is concerned, significant differences ($p < 0.05$) were observed in the infectivity of IJs of *H. indica* and *S. thermophilum* from 90 days to 120 days storage periods with the IJs from control. However, no such differences were observed in the infectivity of IJs of *S. glaseri* when compared to the infectivity of IJs from control at 120 days storage period (Fig. 2).

Discussion

One of the important requirements in utilizing EPNs as biocontrol agent for insect pests is the proper storage of EPN IJs, in a given population and also at optimum temperature, so that maximum IJs can survive for long periods before they are used for field applications. The aim of this study was to

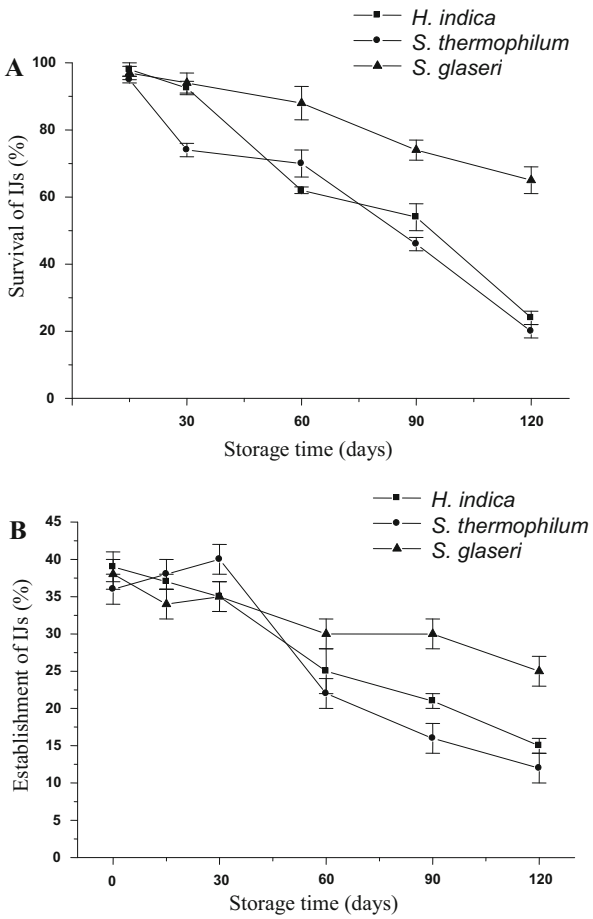

Fig. 2 Percentage survival and establishment of infective juveniles of (IJs) of entomopathogenic nematode species at 25 °C. **a** Survival of IJs and **b** establishment of IJs

characterize the three locally isolated strains of EPNs from Meghalaya (*H. indica*, *S. thermophilum* and *S. glaseri*) in terms of storage temperature on survival and infectivity of IJs. It was observed in this study that, at the lower temperature (5 °C), the survival rate of *H. indica* and *S. thermophilum* IJs drastically reduced beyond 60 days and no survival of IJs was observed after 90 days storage. On the other hand, at the same temperature, the survival of IJs was observed even up to 120 days for *S. glaseri*. At the higher temperature (25 °C), survival of nematode IJs was, however, observed till 120 days for all the three studied nematode species. Nevertheless, at this temperature, the survival rate of *H. indica* and *S. thermophilum* was comparatively low (<25 %) as compared to *S. glaseri* which revealed >60 % survival. It is thus obvious from these results that the IJs of these EPN species can remain viable for at least 60 days. Poinar (1979) reported the favorable temperature for the storage of *Steinernema* sp. varies between 5 and 9 °C. Likewise, Bedding (1981) also reported that 12 °C is the best storage temperature for *Heterorhabditis* spp. Our present observation on EPN IJs survivals at 25 °C is in disagreement with the findings of Poinar (1979) and Bedding (1981). A possible reason behind these observed differences may be that in the present study the isolates of both *H. indica* and *S. thermophilum* species were isolated from the areas where the mean monthly temperature varies between 20 and 25 °C and this may perhaps be a possible reason as to why at lower temperature of 5 °C the nematode IJs showed relatively less survival beyond 30 days storage periods. In a related study, Katti et al. (2006) reported 100 % survival of *S. thermophilum* after storage up to 50 days at 28 °C, but thereafter the survival declined to 10 % after 150 days of storage. The differences in survival of two *S. thermophilum* isolates could be due to differences in their natural origins. While our isolates were collected from forest soils in Meghalaya, the EPNs in Katti et al. studies were collected from soils in Hyderabad. In another study, Strauch et al. (2000) reported that a maximum survival of *H. indica* can be achieved at 15 °C and the highest mortality of IJs occurs at 5 °C. The increased mortality at low temperature observed in this study agrees with the findings of Cagnolo and Campos (2008) where the IJs of *Steinernema rarum* stored at 5 ± 1 °C showed less survival whereas those stored at 23 ± 2 °C showed above 95 % survival rate. The observed survival of IJs of the three EPN species at 25 °C for 120 days duration also reflects the longevity of IJs without the host at the prevailing temperature, from where these isolates were collected. Furthermore, a comparatively high rate of survival of *S. glaseri* at 25 °C may be due to the slower utilization of lipid reserves in this species as compared to *H. indica* and *S. thermophilum*. It is reported that large nematode species, like *S. glaseri,* survive starvation longer than small species with the same percentage lipid content (Atkinson 1980). Grewal (2000) also opined that the differences in longevity of IJs between species of *Steinernema* correlates very well with the initial lipid content and with the rate of lipid utilization by nematode.

Apart from these results, in the present study, though the IJs establishment showed a decreasing trend following the storage period, the insect mortality was observed to be still high wherever IJs established. In a related study, Cagnolo and Campos (2008), while comparing the effect of storage temperature (5 ± 1 and 23 ± 2 °C) on survival and infectivity of *S. rarum*, reported that both infectivity and survival of IJs would be higher when stored at 23 ± 2 °C. Furthermore, Fan and Hominick (1991) also reported that IJs of *Steinernema* sp. and *S. feltiae*, after being stored in sand at 5 °C, lose their ability to parasitize a host, however, effects of storage temperature on infectivity were not reflected in the data for mortality of the insects, which generally remained high in all the tests. In contrast, O'Leary et al. (1998) found that the host finding ability of IJs of *H. megidis* improved with increased duration of storage.

Taken together, the results of our study indicate that the three EPN species show variations in their survival and infectivity with regard to storage periods. A storage temperature of 25 °C appears comparatively better for the storage of all the three EPNs, as at this temperature they can remain viable up to 2 months period. However, from infectivity point of view, at 25 °C these EPNs isolates can be best stored for about a month, as their infectivity lowers afterwards. It is expected that these ecological data on effects of storage temperature on isolates of indigenous strains of EPNs from Meghalaya may be useful in exploring the role of these species as the biocontrol agents of local insect pest species.

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