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Effect of Storage Temperature on Survival and Infectivity of Entomopathogenic Nematodes

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The survival and infectivity of entomopathogenic nematodes at different storage temperatures were studied. The entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema glaseri* differed in their survival and ability to infect insects at different storage temperatures. The best storage temperatures for survival of *H. indica* were 20° and 25°C under BOD conditions for 90 days. Also, the infectivity was upto 90 days at different storage temperatures of 10°, 20° and 25°C when tested against *Corcyra cephalonica*. *S. glaseri* survived longer upto 120 days and 100 per cent survival were observed at the temperature regimes of 10°, 20° and 25°C and the infectivity was upto 60 days at 20°C when tested against *Corcyra cephalonica*.

Key words: Entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema glaseri, Storage temperature*.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabdidae with their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus,* respectively) are widely distributed in soils throughout the world. These nematode pathogens of insects kill them within 48 h with the aid of their associated bacterial symbionts and have great importance as biological control agents of many insect pests.

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 (Koppenhoffer and Kaya, 1999). The effects of temperature on survival and infectivity of nematode infective juveniles (IJs) have also been studied for some EPNs. In these studies, the different isolates of the same species have been found to exhibit differential responses to storage temperatures (Cagnolo and Campos, 2008).

After production, the entomopathogenic nematodes often need to be stored for several weeks. Regardless of how they are formulated, their quality declines with time. Maximum survival and stability of their infectivity is a goal for long term storage. General range of storage temperature for Steinernematids is 5-10°C and 10-15°C for Heterorhabditids. Steinernematids, especially *S. carpocapsae* can be stored up to 5 months at room temperature or up to 12 months under refrigeration (Georgis, 1990). Most Heterorhabditids, whether formulated or not, do not have a long shelf life and suffer high mortalities in storage (Kaya and Gaugler 1993). Nematode metabolism is temperature driven and warm temperature (20-30°C) increases metabolic activities and reduces viability.

Entomopathogenic nematodes are successfully used against numerous soil inhabiting pests and due to their poor survival nature at room temperature, storage prevents them from realizing their full potential as bio protectants. Infective juveniles of entomopathogenic nematodes do not feed and depend solely on stored reserves for their energy supply. Therefore, energy conservation is a vital factor in prolonging the survival of infective juveniles and extending the shelf life of entomopathogenic nematodes based bioinsecticides (Qiu *et al*., 2000). The infective juveniles of entomopathogenic nematodes can be stored for several months in water and refrigerated bubbled tank, but the high cost and difficulties in maintaining the quality prevent the practical use of this method. Several storage media have been developed to improve the survival of stored nematodes (Goud *et al*., 2010). 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 (Lalramliana, 2015).

Hence, in the present study, attempts were made to find out the influence of storage temperature on survival and infectivity of two species of entomopathogenic nematodes *viz*., *H. indica* and *S. glaseri*.

Materials and Methods

The nematodes *viz*., *H. indica* and *S. glaseri* were obtained from Sugarcane Breeding Institute, Coimbatore and mass cultured on *C. cephlonica*. The larvae were reared on broken cumbu grains sterilized at 100°C for 30 minutes (Kaya and Stock, 1997). The third stage infective juveniles (IJs) were harvested from water surrounding White's trap within 10 days of emergence from their hosts. A stock suspension of the IJs in distilled water was stored at 20°C for 2 weeks in BOD incubator before use.

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Effect of storage temperature on the survival of entomopathogenic nematodes

The effect of storage temperature on survival and infectivity of *H. indica* and *S. glaseri* was studied *in vivo*. The population densities of infective juveniles were adjusted to 1000 infective juveniles per ml in 20 ml of de-ionized water in 50 ml tissue culture flasks. The flasks were loosely closed and kept inside BOD incubator at three different temperatures *viz*., 10±2, 20±2 and 25±2°C for 120 days. Three replicates were maintained for each temperature. Before setting-up the experiment, the viability of nematode infective juveniles was checked by observing their movements under a stereozoom microscope. Accordingly, the nematode populations were assumed to be cent per cent viable on the first day of experiment. The survival of infective juveniles in the suspensions was monitored at 15, 30, 60, 90 and 120 days of storage periods for each test temperature. For this, about one ml of infective juvenile suspension was withdrawn from each conical flask and the viability of infective juveniles was determined under a stereozoom microscope by mobility criterion (Lalramliana, 2015).

Infectivity

Ten final instar larvae of *C. cephalonica* were released over two layers of Whatman No.1 filter paper on 9 cm dia. Petri dishes at different storage temperature and test nematodes were inoculated at 20 IJ/ larva. The Petri dishes were covered with lid and sealed with kling flim to conserve moisture. After 48 h of inoculation, the dead larvae were counted to check the infectivity of nematodes at different storage temperatures.

Results and Discussion

Effect of storage on survival of H. indica and S. glaseri

The survival of infective juveniles of *H. indica* and *S. glaseri* was tested at different storage temperatures of 10°, 20° and 25°C for 120 days. The infective juveniles stored at 10°, 20° and 25°C recorded a marked effect on the survival of *H. indica* upto 120 days. The storage temperatures of 10°, 20° and 25°C caused the highest survival of infective juveniles. Cent per cent survival was observed at 15 and 30 days for *H. indica* at different temperatures. At 60th day of storage, the survival of *H. indica* was 79.75, 92.35 and 85.07 per cent at 10°, 20° and 25°C, respectively.

At 90th day of storage period, survival of the infective juveniles was 50.92, 66.35 and 48.00 per cent, which reduced drastically at the temperatures of 10°, 20° and 25°C, respectively and no survival was observed at 120 days for *H. indica* at different storage temperatures.

Steinernema glaseri survived for longer periods (upto 120 days) and 100 per cent survival was observed upto 60 days at 10°, 20° and 25°C. After 60 days, the survival of *S. glaseri* was 95.42, 94.12 and 88.52 per cent; after 90 and 120 days, the survival was 85.82, 81.40 and 76.90 per cent, respectively at 10°, 20° and 20° and 25°C for *S. glaseri*.

Table 1. Effect of storage on survival of *H. indica*

Days	Survival (%)		
	Temperature (°C)		
	10	20	25
15	100 (89.17)	100 (89.17)	100 (89.17)
30	100 (89.17)	100 (89.17)	100 (89.17)
60	79.75 (63.27)	92.35 (73.97)	85.07 (67.56)
90	50.92 (45.53)	66.35 (54.55)	48.00 (43.84)
120	0 (0.28)	0 (0.28)	0 (0.28)
CD (p=0.05)	2.99	1.43	5.41

Figures in parentheses are arc sine transformed values

Effect of storage on infectivity of H. indica and S. glaseri

Infectivity of *H. indica* to *C. cephalonica* was maximum at 10° and 20°C for 15 and 30 days of storage period. The infective juveniles of *H. indica* showed the maximum infectivity of 97.50, 95.00 and 87.50 per cent; and 95.00, 92.50 and 75.00 per cent, respectively at 10°, 20° and 25°C for 15 and 30 days of storage period. Subsequently, the infectivity of infective juveniles was reduced to 72.50, 77.50 and 65.00 per cent; and 50.00, 55.00, 47.50 per cent at 10°, 20° and 25°C for 60 and 90 days of storage period for *C. cephalonica,* respectively.

Table 2. Effect of storage on survival of *S. glaseri*

Days	Survival (%)		
	Temperature (°C)		
	10	20	25
15	100 (89.17)	100 (89.17)	100 (89.17)
30	100 (89.17)	100 (89.17)	100 (89.17)
60	100 (89.17)	100 (89.17)	100 (89.17)
90	95.42 (78.29)	94.12 (77.64)	88.52 (70.63)
120	85.82 (68.08)	81.40 (64.33)	76.90 (61.39)
CD (p=0.05)	3.68	4.49	4.31

Figures in parentheses are arc sine transformed values

Infectivity of *S. glaseri* to *C. cephalonica* was 92.50 per cent at 10°C for 15 days of storage period. The per cent infectivity of infective juveniles reduced upon storage, which depends on storage temperature and nematode species. At temperatures of 10°, 20° and 25°C, the ability of *S. glaseri* to infect *C. cephalonica* larva was 72.50, 77.50 and 77.50 per cent; and 67.50,

55.00 and 55.00 per cent, respectively for 90 and 120 days of storage time.

The method of storage temperature of entomopathogenic nematodes should meet out the two major criteria a maximum survival of the infective juveniles and a maximum conservation of their infectivity.

Table 3. Infectivity of juveniles of *H. indica* on *C. cephalonica* under storage at different temperatures

Days	Infectivity (%)		
	Temperature (°C)		
	10	20	25
15	97.50 (85.17)	95.00 (80.64)	87.50 (69.53)
30	95.00 (80.64)	92.50 (76.10)	75.00 (60.11)
60	72.50 (58.45)	77.50 (61.77)	65.00 (55.28)
90	50.00 (45.00)	55.00 (47.88)	47.50 (43.55)
120	0 (0.28)	0 (0.28)	0 (0.28)
CD (p=0.05)	10.11	10.11	4.84

In the present study, survival of *H. indica* and *S. glaseri* decreased with increase in storage time up to 120 days for the temperatures of 10°, 20° and 25°C. The storage temperatures of 10°, 20° and 25°C caused the highest rate of survival of infective juveniles at 15 and 30 days for *H. indica*. It was in accordance with Hussaini *et al*. (2000), who reported that the survival of infective juveniles of *H. indica* was not drastically affected at the end of six weeks of storage at 8°C. Survival of *H. indica* was more at 20° and 25°C and lower survival was seen at 10°C. The increased mortality at low temperature was observed, which agrees with the findings of Cagnola and Campos (2008), who reported that the infective juveniles of *S. rarum* stored at 5±1°C showed less survival whereas, 23±2°C showed more than 95 per cent survival. Similar findings of Bedding (1981) also reported that 12°C is the ideal storage temperature for *Heterorhabditis* spp.

In the present study, *S. glaseri* survived longer period of up to 120 days and 100 per cent survival was observed upto 60 days at 10°C. However, at 20°C and 25°C 100 per cent survival was observed at 60 days. Poinar (1979) reported that the favourable temperature for the storage of *Steinernema* sp. varies between 5 and 9°C. In the present study, survival of infective juveniles of *H. indica* was drastically affected after 30 days of storage period at 10°C. The reason may be the poor storage stability of nematodes due to the presence of large quantity of unsaturated fatty acids in the freshly emerged juveniles or decrease in lipid content of IJ as reported by Selvan *et al*. (1993).

Table 4. Infectivity of infective juveniles of *S. glaseri* on *C. cephalonica* under storage at different temperatures

Days	Infectivity (%)		
	Temperature (°C)		
	10	20	25
15	92.50 (76.10)	87.50 (69.53)	87.50 (69.53)
30	85.00 (67.50)	77.50 (61.77)	77.50 (61.77)
60	82.50 (65.46)	75.00 (60.11)	75.00 (60.11)
90	72.50 (58.45)	77.50 (58.82)	77.50 (60.11)
120	67.50 (55.28)	55.00 (47.88)	55.00 (47.88)
CD (p=0.05)	8.00	5.41	5.56

At 90th day of storage period, the survival of infective juveniles was reduced drastically to temperatures of 10, 20 and 25°C, respectively and no survival of infective juveniles was observed at 120 days with *H. indica* at different temperatures. This result was similar to *S. glaseri,* which survived for 36 weeks (Selvan *et al*., 1993) and more than 450 days (Patel *et al*., 1997) at 8°C. The survival of *S. glaseri* was observed at different temperatures. Further more, 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* as indicated by Lalramliana (2015).

It is concluded that the storage potential of *H. indica* is inferior to *S. glaseri*. The results of this study indicated that the entomopathogenic nematode species show variation in their survival and infectivity with regard to storage periods. A temperature of 20° and 25°C appears to be comparatively better for the storage of *H. indica* and *S. glaseri;* at this temperature they can remain viable upto 3 months period.

In the present study, infective juveniles of *H. indica* showed maximum infectivity at 10°, 20° and 25°C for 15 and 30 days of storage period. After that the infectivity on *C. cephalonica* was reduced drastically at 10°, 20° and 25°C for 60 and 90 days of storage period. The present results are related for infective juveniles of *Heterorhabditis* sp. stored at 9°, 20° and 25°C. The maximum infectivity was observed at 9°C (Griffin, 1996), which is in line with Fitters and Griffin (2004) who found that the infectivity of *H. megidis* on *G. mellonella* was high in first two weeks and there after declined. The infectivity of *S. feltiae* and *Heterorhabditis* sp. were maintained at 5-6°C for 7-8 weeks during storage period (Schiroki and Hague, 1997; Klinger, 1990). Similarly, the infectivity of *H.*

indica reduced at different temperatures at different storage periods.

S. *glaseri* caused infectivity at 10°, 20° and 25°C for 30, 60 and 120 days of storage periods. The possible reason may be that the amount of trehalose accumulated by different nematodes varies with temperature and this may confer specific advantage to certain species as revealed in the present study for *Steinernema* sp. in which lipid reserves were preserved for longer periods at tested temperatures (Andalo *et al*., 2011). However from infectivity point of view at 25°C, *S. glaseri* can be best stored for about a month, as their infectivity lowers afterwards. It is confirmed that an extended period of storage and a reduction in total lipid content can lead to a decrease in the infectivity of infective juveniles (Fitters and Griffin 2004).

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