



RESEARCH ARTICLE

EFFECT OF TEMPERATURE ON LONGEVITY OF ENTOMOPATHOGENIC NEMATODE,
HETERORHABDITIS INDICA POINAR

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ABSTRACT

Experiment was conducted to study the effect of temperature on longevity of *Heterorhabditis indica* under controlled laboratory conditions. The results showed the maximum survival rate of 77.29% at 20^oC after 90 days of incubation, followed by room temperature (25-28^oC) and 30^oC test temperature showing 61.30% and 54.60% survival rate after 90 days of incubation, respectively. At test temperatures 10^oC and 40^oC the survival rate was 0.0% and 0.33% after 20 days of incubation, indicating that the mortality of IJs of *H. indica* occurred due to lethal low and lethal high temperature effects, respectively. The results indicated the optimum temperature for storage of *H. indica* was 20^oC.

INTRODUCTION

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. EPNs have been described from 23 nematode families (Koppenhofer, 2007). Of all of the nematodes studied for biological control of insects, the Steinernematidae and Heterorhabditidae have received the most attention because they possess many of the attributes of effective biological control agents (Kaya and Gaugler, 1993; Grewal et al., 2005; Koppenhofer, 2007). The only free-living stage in a life cycle of entomopathogenic nematode is an infective juvenile (IJ₃) produced when host nutrients are depleted. The only function of the infective juveniles is to locate and parasitize new hosts (Poinar, 1990; Grewal et al., 1993). Infective juveniles may have mechanisms to survive under adverse environmental conditions. Persistence in soil, infectivity (Griffin and Downes, 1991; Kung and Gaugler, 1991), development, maturation and reproduction of entomopathogenic nematodes are influenced by temperature (Zervos and Webster, 1991; Grewal et al., 1993). Variation among species of entomopathogenic nematodes for temperature tolerance has been reported (Wright, 1992; Grewal et al., 1993). Temperature is an environmental factor of great biological significance.

As an environmental factor, temperature is variable both in space and time. EPNs isolated from diverse geographical regions and climate provides an opportunity to compare adaptations to a wide variety of thermal regimes. Keeping this in view, the Experiments were carried out under controlled laboratory conditions to study the effect of temperature on longevity of IJs of *Heterorhabditis indica*.

MATERIALS AND METHODS

The longevity of Entomopathogenic nematode cultures as affected by different controlled temperatures i.e. 10^oC, 20^oC, 30^oC, 40^oC and room temperature (25-28^oC) were assessed by placing the nematode suspension in BOD incubators adjusted to different test temperatures as mentioned above. The nematode suspension containing 1000 IJs/ml in sterile distilled water was prepared and 50 ml of suspension was stored in each conical flask of 100ml capacity. Survival of IJs was assessed at every 10 days interval till 3 months duration by drawing 1 ml nematode suspension from each conical flask. The percent live and dead IJs were counted under stereo-binocular microscope (10X magnification). Seven replications were maintained per each treatment. The data on dead and live IJs were collected and percent survival of IJs of nematodes were calculated by using following formula:

$$\text{Percent survival (\%)} = \frac{\text{Total number of IJs} - \text{Total number of dead IJs}}{\text{Total number of IJs}} \times 100$$

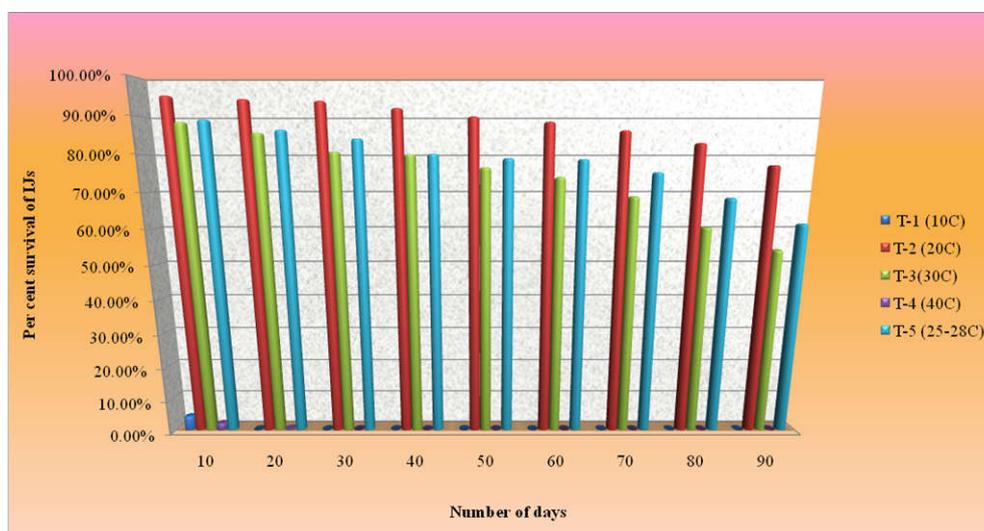
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Table 1. Effect of temperature on the survival rate and storage period of infective juveniles (IJs) of *Heterorhabditis indica*

Temperature (°C)	Survival percentage at 10 days interval									Mean	
	10	20	30	40	50	60	70	80	90		
10	4.24 (4.24)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.47 (0.47)
20	95.34 (126.43)	94.46 (123.63)	94.00 (122.26)	92.19 (117.29)	90.01 (111.99)	88.67 (109.02)	86.41 (104.34)	83.15 (98.18)	77.29 (88.34)	74.39 (85.32)	89.06 (111.28)
30	88.60 (108.86)	85.76 (103.06)	80.86 (94.18)	80.14 (92.96)	76.71 (87.43)	74.06 (83.39)	68.89 (75.99)	60.44 (64.90)	54.06 (57.12)	54.06 (57.12)	74.39 (85.32)
40	2.06 (2.06)	0.33 (0.33)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.27 (0.27)
25-28	89.24 (110.26)	86.58 (104.68)	84.31 (100.30)	80.31 (93.25)	79.14 (91.31)	78.89 (90.90)	75.38 (85.38)	68.46 (75.41)	61.30 (65.99)	54.06 (57.12)	78.18 (90.83)
Mean	55.90 (70.37)	53.43 (66.34)	51.83 (63.35)	50.53 (60.70)	49.17 (58.15)	48.32 (56.66)	46.14 (53.14)	42.41 (47.69)	38.53 (42.29)	38.53 (42.29)	

Note: Values in parentheses are arcsine transformed values.

	P- values	F	SEM±	CD
Temperature	9.45E-31	**	1.66	4.54
Storage period	0.000106	**	2.23	6.09
Temperature X Storage period	-	**	4.98	13.64

**Figure 1. Effect of temperature on longevity of infective juveniles (IJs) of *Heterorhabditis indica***

The data expressed in percentages was subjected to arc sine transformation. The survival data were subjected to analysis of variance (ANOVA). All the comparisons were tested at 5% level of significance.

RESULTS AND DISCUSSION

The results of effect of temperature on per cent survival of IJs of *H. indica* are presented in table 1 and fig 1. The results showed significantly maximum mean survival rate of IJs at 20°C, *ie.*, 89.06%, followed by control (room temperature 25-28°C) and 30°C, *viz.*, 78.18% and 74.39%, respectively. Whereas as, the lowest (10°C) and highest (40°C) temperature were found to have detrimental effect and recorded 0.47% and 0.27% survival rate, respectively. The effects of storage period on longevity of IJs of *H. indica* indicated that maximum mean survival of IJs was observed up to 30 days after incubation (70.37%) at different test temperatures. As the days of incubation increased the per cent longevity decreased, showing a negative linear relationship between temperature and longevity of IJs of *H. indica*. The mean per cent survival from 10 to 30 days (70.37% to 63.35%) was on par with each other and significantly greater than other storage periods of incubation.

The survival of *H. indica* observed at 40 to 60 days after incubation was found to be 50.53% to 48.32%, respectively and they were on par with each other. Whereas, the mean per cent survival of EPNs at 70 and 80 days (53.14% and 47.69%) were also on par with each other and the lowest mean survival rate was recorded at 90 days after incubation (42.29%).

The interaction effect of storage period at different temperatures revealed that, maximum survival of IJs was recorded at 20°C (95.34%). The percent survival at 20°C from 10th to 50th day was the maximum (95.34% to 90.01%) and on par with each other. The next best treatment was recorded at room temperature (25-28°C) of 10th to 30th day after incubation, where the survival rate of IJs ranged from 89.24% and 84.31% and was on par with each other. The percent survival at 30°C shown that, on 10th and 20th day after incubation the survival was high (88.60% and 85.76%), which was significantly greater when compared to per cent survival rate from 30th to 90th day (80.86% to 54.06%). The per cent survival rate of IJs at 10°C and 40°C were significantly lower, *ie.*, per cent mortality was observed at 20th day and 30th day after incubation, respectively.

The above results were in accordance with the results obtained by Mentiet *al.* (2000) who opined that the optimum temperature for survival, infection and development of *Steinernema feltiae* and *H. megidis* from Greece and the UK was 20- 23⁰C. Strouch and Ehlers (2000) indicated that the maximum survival of *H. indica* was achieved at 25⁰C and the highest mortality was observed at 5⁰C. Szcze-Panik (2000) showed that, rise in temperature from 10 to 30⁰C slightly increased the survival and infectivity of entomopathogenic nematodes. Hussainiet *al.* (2005) reported that temperature ranging from 25-32⁰C was found suitable for survival, infectivity and virulence of *S. carpocapsae*, *S. abbasi*, *S. tami* and *H. indica*. Studies carried out by Sunanda *et al.* (2012) indicated that highest number of IJs of *H. indica* survived at 30⁰C and the optimum temperature ranged from 20-30⁰C. Thus the results obtained from the studies of effect of temperature and storage period on survival of *H. indica* indicated that 20⁰C appears to be the best temperature for better storage of nematodes, as at this temperature they maintained highest survival rate of 94-95% up to one month period and even after 50 days of storage also they maintained 90% survival rate. The present findings are in accordance with the studies of Lalramliana and Yadav (2015) who stated that the IJs of EPN species can remain viable for at least 60 days at 25⁰C. The next best temperature was found to be the control where the nematodes exposed to 25-28⁰C room temperature survived better by recording 80% survival rate up to 40 days. Future studies on the identification of local EPN isolates which could tolerate high temperature would help in effective management of pests infecting at high temperature regimes.

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