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Influence of storage temperature, population density and duration of storage on the survival of three species of entomopathogenic nematodes

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ABSTRACT: Among the five different temperatures tested for storage of infective juveniles (IJs), survival of *Steinernema feltiae* Filipjev and *S. glaseri* Steiner was higher at 7.5° C, while the survival of *Heterorhabditis indica* (Poinar, Karunakar and David) was higher at 10° C. All the three species of nematodes showed higher percentage of survival when stored at 250 IJs per ml compared to 500 and 1000 IJs per ml. A negative correlation was observed between duration of storage and per cent survival in *S. feltiae* (r = - 0.9831 **), *S. glaseri* (r = - 0.9847 **) and *H. indica* (r = - 0.9437 **). Based on the results of the study, the best combination of temperature, population density and duration of storage for the entomopathogenic nematodes was 7.5° C, 250 IJs per ml and 120 days, respectively for *S. feltiae*, 7.5° C, 250 IJs per ml and 90 days for *S. glaseri* and 10° C, 250 IJs per ml and 90 days for *H. indica*.

KEY WORDS: *Heterorhabditis indica*, population density, *Steinernema feltiae*, *S. glaseri*, storage

Two steinernematids, *Steinernema glaseri* Steiner and *S. feltiae* Filipjev and a heterorhabditid, *Heterorhabditis indica* (Poinar, Karunakar and David) are being considered for their possible use in the management of white grub, *Holotrichia serrata* F. a serious pest of sugarcane in certain parts of India (David and Ananthanarayana, 1986). One of the important steps in the utilization of entomopathogenic nematodes for biocontrol programme is the storage of the infective juveniles (IJs) in a given population density at optimum temperature, so that maximum IJs can survive for long periods before they are utilized for field application.

The storage temperature, duration of storage and number of IJs to be stored per unit volume may vary between the entomopathogenic nematode species. The results of the studies carried out on these aspects for *S. feltiae*, *S. glaseri* and *H. indica* at Sugarcane Breeding Institute, Coimbatore, are discused in this paper.

METERIALS AND METHODS

Nucleus stock of *Steinernema glaseri* Steiner obtained from Dr. Bedding, Canberra Scientific and Industrial Research Organization, Tasmanian Research Laboratory, Tasmania, Australia; *S.*

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feltiae Filipjev (DD-136 strain) obtained from C. A. B. Institute of Biological Control, Bangalore, India and *Heterorhabditis indica* (Poinar, Karunakar and David) collected in the vicinity of Coimbatore (Poinar *et al.*, 1992) were used in this study. Fifth instar larvae of greater wax moth, *Galleria mellonella* (L.), which were reared on artificial diet (David and Kurup, 1988) and field collected larvae of sugarcane top borer, *Scirpophaga excerptalis* Walker were used as laboratory host for the multiplication of *S. glaseri* and *H. indica*, while *S. feltiae* was multiplied on larvae of rice meal moth, *Corcyra cephalonica* (Stainton). The basic *in vivo* production method outlined by Woodring and Kaya (1988) was followed for the multiplication of the entomopathogenic nematodes.

Freshly emerged IJs of *S. feltiae, S. glaseri* and *H. indica* collected from White traps in formalin (0.1%) were ascertained for 100 per cent activity. Thereafter, these IJs were allowed to settle at the bottom of the container, supernatant aspirated or formalin (0.1%) added and the population density adjusted to 1000 IJs per ml. Dilutions of 500 and 250 IJs per ml were made from this concentration. Twenty ml of each concentration was pipetted out in Petri-dishes (9 x 1.5 cm), which were covered with larger Petri-dishes (10 x 1.25cm). Each population level was replicated thrice. The Petri-dishes were stored in refrigerators at 5±1° C and 7.5±1°C and in BOD incubators at 10±1°C and 15±1°C and one set kept in an air conditioned room at 24±1°C. The activity of the nematodes was checked at 10 days interval for 4 months by pipetting out one ml of the sample from each concentration into a counting chamber. The activity of the nematodes was determined based on the movement / no movement of randomly selected IJs. The experiments were conducted in factorial randomized block design and the survival data on 10, 30, 60, 90 and 120 days of storage alone given in the tables.

RESULTS AND DISCUSSION

Influence of storage temperature on survival

Among the five different temperatures tested, the mean per cent survival of *S. feltiae* and *S. glaseri* was significantly higher at 7.5°C (99.1 and 95.9 %) followed by 10°C (97.8 and 95.1%) and 15°C (94.3 and 92.5%) (Table 1 and 2). But the mean per cent survival of *H. indica* was significantly higher at 10°C (96.0 %), followed by 15, 24 and 7.5°C (Table 3). Five and 20°C were not found suitable for storing *S. feltiae* and *S. glaseri* where as for *H. indica* it was 5°C

Influence of population density on survival

Among the three population densities tested, the least population density of 250 IJs per ml resulted in significantly higher mean survival of *S. feltiae* (93.5%), *S. glaseri* (91.1%) and *H. indica* (63.7 %). It is evident that as the population density during storage increased, the survival of IJs decreased in all the three nematode species.

Influence of duration of storage on survival

Significantly higher mean survival of 100 per cent was observed in *S. feltiae* up to 20 days from the date of storage. This was on par with the survival rate on 30th day (99.2 %). In *S. glaseri*, the mean survival rate was significantly higher up to 20 days (100 %) but in *H. indica* it was only up to 10 days (93.2 %). It was evident from this study that the mean survival percentage decreased as the duration of storage increased. A negative correlation was observed between the duration of storage and per cent survival in *S. feltiae* (r = - 0.9831 **), *S. glaseri* (r = - 0.9847**) and *H. indica* (r = - .9437**). The mean survival percentage observed at the end of four months was 80.1, 72.8 and 42.8 in *S. feltiae, S. glaseri* and *H. indica*, respectively.

Table 1. Influence of storage temperature and population density on the survival of *S. feltiae*

Temperature (° C)	Population of IJ s / ml.	Per cent survival of IJ s on the day of					
		10	30	60	90	120	Mean
5	250	100.0(90.0)	100.0 (90.0)	93.3 (75.90)	73.7 (59.50)	61.7(52.00)	85.4(72.08)
	500	100.0(90.0)	98.7(86.40)	90.3 (72.42)	71.3(57.95)	56.0(48.74)	82.6(69.69)
	1000	100.0(90.0)	96.7 (80.47)	87.3(69.66)	66.7 (55.05)	53.0(47.01)	80.3(67.47)
	Mean	100.0(90.0)	98.5 (85.63)	90.3 (72.66)	70.6(57.50)	56.9(49.26)	
7.5	250	100.0(90.0)	100.0 (90.0)	100.0 (90.0)	99.3(87.30)	98.0(83.27)	99.5(88.20)
	500	100.0(90.0)	100.0(90.0)	99.3 (87.30)	98.7 (84.86)	98.0(83.27)	99.1(86.77)
	1000	100.0(90.0)	100.0(90.0)	99.3 (87.66)	98.0 (83.27)	96.0(80.05)	98.6(85.83)
	Mean	100.0(90.0)	100.0 (90.0)	99.6(88.32)	98.7(85.18)	97.3(82.20)	
10	250	100.0(90.0)	100.0 (90.0)	99.7 (88.65)	98.7(84.96)	98.0(82.97)	99.3(87.53)
	500	100.0(90.0)	100.0 (90.0)	98.7(85.31)	96.0(79.54)	95.0(77.80)	97.9(83.88)
	1000	100.0(90.0)	100.0 (90.0)	96.7(80.47)	94.3 (76.96)	91.0(73.07)	96.2(81.36)
	Mean	100.0(90.0)	100.0 (90.0)	98.3(84.81)	96.3 (80.40)	94.7(77.95)	
15	250	100.0(90.0)	100.0(90.0)	98.7(83.96)	94.0(76.54)	90.0(72.06)	96.6 (82.17)
	500	100.0(90.0)	100.0(90.0)	96.7 (80.34)	92.7(74.90)	86.0(68.45)	95.0(80.21)
	1000	100.0(90.0)	100.0(90.0)	93.3(75.70)	85.7 (68.18)	80.0(63.81)	91.2 (75.78)
	Mean	100.0(90.0)	100.0(90.0)	96.2 (80.0)	90.8(73.20)	85.3(68.11)	
24	250	100.0(90.0)	100.0 (90.0)	93.3(75.7)	73.3(59.33)	72.0(58.40)	86.7 (72.71)
	500	100.0(90.0)	96.7 (80.47)	87.7(79.09)	69.7 (56.89)	66.0(54.65)	82.3 (68.50)
	1000	100.0(90.0)	96.0 (79.54)	87.3 (69.63)	66.7(55.06)	61.0(51.65)	80.4(67.17)
	Mean	100.0(90.0)	97.6 (83.34)	89.4 (71.81)	69.9(57.09)	66.3(54.90)	

Figures in parentheses are arcsine percentage transferred values.

	SEM±	CD (=0.05)
Temperature		
Population	0.22**	0.61
Duration	0.17**	0.47
Temperature x Population	0.34**	0.94
Temperature x Duration	0.38**	1.05
Population x Duration	0.76**	2.11
	0.59**	1.63

Table 2. Influence of storage temperature and population density on the survival of *S. glaseri*

Temperature (° C)	Population of IJ s / ml	Per cent survival of IJ s on the day of 10	30	60	90	120	Mean
5	250	100.0(90.0)	99.0(86.31)	90.3(72.89)	70.7(57.70)	57.7(49.92)	83.0 (70.30)
	500	100.0(90.0)	97.7(82.58)	87.3 (69.70)	62.3(52.44)	52.7(46.82)	79.2(66.92)
	1000	100.0(90.0)	94.7(77.59)	80.3 (64.06)	58.3 (50.11)	50.0 (45.29)	75.6(63.92)
	Mean	100.0(90.0)	97.1(82.16)	86.0(68.89)	63.8(53.42)	53.5(47.34)	
7.5	250	100.0(90.0)	100.0(90.0)	100.0(90.0)	96.7(80.54)	93.3 (75.75)	97.8(84.51)
	500	100.0(90.0)	100.0 (90.0)	96.7(80.50)	93.3(75.75)	90.7(73.08)	96.0(81.26)
	1000	100.0(90.0)	100.0(90.0)	96.7(80.54)	90.0 (72.15)	86.0(68.52)	93.8(78.95)
	Mean	100.0(90.0)	100.0 (90.0)	97.8(83.68)	93.3(76.15)	90.0 (72.45)	
10	250	100.0(90.0)	100.0(90.0)	97.0(81.23)	93.3 (75.75)	90.0 (72.06)	96.5(82.41)
	500	100.0(90.0)	100.0 (90.0)	94.7(77.52)	93.0 (75.26)	87.0 (69.37)	95.1(80.27)
	1000	100.0(90.0)	100.0 (90.0)	93.3(75.70)	90.0 (72.05)	85.0(67.69)	93.8 (78.77)
	Mean	100.0(90.0)	100.0(90.0)	95.0 (78.15)	92.1(74.35)	87.3 (69.71)	
15	250	100.0(90.0)	100.0(90.0)	96.7 (80.54)	89.7(72.04)	85.3(67.94)	94.7(80.16)
	500	100.0 (90.0)	100.0(90.0)	96.7(80.34)	88.0(70.22)	80.0(63.79)	92.8(78.25)
	1000	100.0 (90.0)	100.0(90.0)	92.0(74.18)	85.3(67.89)	75.3(60.55)	89.9(75.16)
	Mean	100.0(90.0)	100.0 (90.0)	95.1(78.35)	87.7(70.05)	80.2 (64.10)	
24	250	100.0(90.0)	99.3(87.65)	93.3(75.66)	70.0(58.11)	66.0 (54.63)	83.4(70.13)
	500	100.0(90.0)	96.7 (81.83)	86.7(69.10)	63.0(52.88)	56.3(48.93)	79.0(66.59)
	1000	100.0(90.0)	95.0 (77.96)	77.0 (61.70)	57.7(49.71)	37.0(37.75)	73.6 (62.88)
	Mean	100.0(90.0)	97.0(82.48)	85.7(68.82)	63.6(53.57)	53.1(47.10)	

Figures in parentheses are arcsine percentage transferred values.

	SEM±	CD (=0.05)
Temperature	0.21**	0.59
Population	0.17**	0.46
Duration	0.33**	0.92
Temperature x Population	0.37**	1.03
Temperature x Duration	0.74**	2.06
Population x Duration	0.57**	1.59

Table 3. Influence of storage temperature and population density on the survival of *H. indica*

Temperature (°C)	Population of IJ s / ml.	Per cent survival of IJ s on the day of 10	30	60	90	120	Mean
5	250	71.3 (57.97)	19.0(26.15)	0.0(4.05)	0.0(4.05)	0.0(4.05)	11.5(13.71)
	500	68.3(56.08)	15.3(23.28)	0.0(4.05)	0.0(4.05)	0.0(4.05)	10.6(13.14)
	1000	62.3(52.45)	12.0(20.66)	0.0(4.05)	0.0(4.05)	0.0(4.05)	9.4(12.37)
	Mean	67.3(55.50)	15.4(23.36)	0.0(4.05)	0.0(4.05)	0.0(4.05)	
7.5	250	99.7(88.65)	90.0((72.05)	0.0(4.05)	0.0(4.05)	0.0(4.05)	28.2(27.52)
	500	98.7(86.41)	89.0(71.18)	0.0(4.05)	0.0(4.05)	0.0(4.05)	
	1000	98.0(84.06)	86.0(68.67)	0.0(4.05)	0.0(4.05)	0.0(4.05)	26.1(25.08)
	Mean	98.8 (86.37)	88.3 (70.63)	0.0(4.05)	0.0(4.05)	0.0(4.05)	
10	250	100.0(90.0)	99.7(88.65)	99.0(86.31)	96.7(80.47)	90.7(72.74)	97.3(83.43)
	500	100.0(90.0)	99.3(87.66)	98.3(85.06)	95.3(78.29)	85.7(68.21)	95.9(81.99)
	1000	100.0(90.0)	98.7(85.31)	97.3(81.72)	94.7(77.84)	80.3(64.04)	94.6(79.53)
	Mean	100.0(90.0)	99.2 (87.21)	98.2(84.36)	95.6(78.87)	85.6(68.33)	
15	250	100.0 (90.0)	100.0 90.0)	99.0(86.31)	95.0 (77.8)	85.0(67.7)	96.3(82.2)
	500	100.0(90.0)	99.3(87.30)	96.7(80.47)	93.7(76.07)	80.0(63.81)	94.6 (79.3)
	1000	100.0 (90.0)	98.3 (84.62)	93.3(75.7)	83.3(66.31)	61.7(52.04)	88.8(73.93)
	Mean	100.0(90.0)	99.2 (87.31)	97.0(80.82)	90.7(73.39)	75.6(61.17)	
24	250	100.0(90.0)	99.3(87.3)	90.0(72.06)	73.3(59.24)	62.0(52.25)	85.1(71.11)
	500	100.0(90.0)	98.0 (84.06)	89.0(71.11)	70.3(57.36)	56.0(48.74)	82.2 (69.01)
	1000	100.0 (90.0)	96.7(80.47)	86.7(69.1)	61.7(52.05)	40.0(39.51)	77.8(65.85)
	Mean	100.0 (90.0)	98.0 (83.94)	88.6(70.76)	68.4 (56.22)	52.7(46.83)	

Figures in parentheses are arcsine percentage transferred values.

	SEM±	CD (=0.05)
Temperature		
Population	0.24**	0.67
Duration	0.19**	0.52
Temperature x Population	0.37**	1.04
Temperature x Duration	0.42**	1.16
Population x Duration	0.83**	2.32
	0.65**	1.80

The reduction in the survival during the prolonged period of storage was maximum in *H. indica* (57.2 %) followed by *S. feltiae* (27.2 %) and *S. glaseri* (19.9 %).

Based on the studies, the best combination of temperature, population density and duration of storage was 7.5 °C, 250 IJs per ml and 120 days; for *S. feltiae;* 7.5 °C, 250 IJs per ml and 90 days for *S. glaseri*; and 10° C, 250 IJs per ml and 90 days for *H. indica,* respectively. Poinar (1979) reported that the favourable temperature range for prolonged period of storage of *S. feltiae* was 5 to 9°C. In general, steinernematids can be stored at 4 to 10°C for 6 to 12 months without much loss of infectivity. *Heterorhabditis* spp. do not store so well and 2 to 4 months of storage at 4 to 10°C is considered good (Woodring and Kaya, 1988). Bedding (1981) reported that 12°C was the best for storage of *Heterorhabditis* sp. in culture flasks. These results are in agreement with the present study. It is evident that steinernematids can be stored at lower temperature (7.5°C) than *H. indica* (10°C). The reason may be that *H. indica* was isolated from tropical region (Coimbatore) and hence it may be acclimatized for higher temperatures of tropical climate.

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