# EFFECT OF STORAGE PERIODS ON SURVIVAL AND INFECTIVITY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES OF INSECT PESTS OF RICE

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**Summary.** Laboratory investigations were carried out to study the survival and infectivity of two indigenous species of entomopathogenic nematodes (EPN), *Rhabditis* (*Oscheius*) sp. and *Steinernema thermophilum*, stored at room temperature for varying periods (5-150 days), on two insect hosts, greater wax moth, *Galleria mellonella* and rice moth, *Corcyra cephalonica*. Observations were made on per cent survival, time taken for mortality of host larvae as well as recovery of infective juveniles (IJs) of the two EPN species from the host cadavers. There was 100% survival of both species of EPN on the two hosts after storage for up to 50 days, but thereafter the survival of *Rhabditis* (*Oscheius*) sp. declined to zero and that of *S. thermophilum* to 10% after 150 days of storage. Both EPN species took more time to kill *G. mellonella* than *C. cephalonica*. Inoculation with *Rhabditis* (*Oscheius*) sp. stored for five days resulted in maximum recovery from *G. mellonella*, while there were no significant differences in recoveries of IJs from *C. cephalonica*. The IJs of *S. thermophilum* stored for 100 days took 20.8 h to kill *G. mellonella*, while those stored for 50 and 100 days took 19.6 to 25.6 h to kill *C. cephalonica.* The recovery of IJs of *S. thermophilum* was greater from *G. mellonella* (36033 to 49298/larva) than from *C. cephalonica* (24161 to 28154/larva). There were no significant differences in recoveries of IJs after storage at different periods from these two hosts.

**Key words:** *Corcyra cephalonica*, *Galleria mellonella*, *Rhabditis* (*Oscheius*) sp., *Steinernema thermophilum.*

Rice (*Oryza sativa* L.) is attacked by several insect pests that can cause severe economic losses throughout its growing cycle**.** The major insect pests of rice include stem borers, leaf folders and plant hoppers. Chemical control is widely adopted for the management of these pests (Matteson, 2000). However, in the last decade, the role of entomopathogenic nematodes (EPNs) as a safe alternative to the use of insecticides in IPM of different crops, including rice, has gained worldwide attention (Georgis, 1987; Kaya 1985, 1990; Klein, 1990; Mason and Wright, 1997; Rahman *et al.*, 2000). In India, efforts were made during the 1970s to study the effectiveness of an exotic entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin *et* Bedding (DD-136) against insect pests of rice (Rao and Manjunath, 1966; Israel *et al.*, 1969; Yadava and Rao, 1970; Rao *et al.*, 1971). However, the nematode was not able to become established in field trials and, therefore, attention was paid to indigenous strains in the hope that they could adapt to local conditions better than imported strains (CRRI, 1975, 1977; Nayak *et al.*, 1977). Studies undertaken at the Directorate of Rice Research (DRR), Hyderabad, demonstrated the efficacy of two indigenous species of entomopathogenic nematodes, *viz.*, *Rhabditis* (*Oscheius*) sp. and *Steinernema thermophilum* Ganguly *et* Singh, against yellow stem borer and leaf folder in rice (Gururaj Katti *et al.*, 2003; Prasad *et al.*, 2003). A study on the life cycle of *Rhabditis* (*Oscheius*) sp. on rice moth, *Corcyra cephalonica* Stainton, revealed that up to 1300 infective juveniles (IJs) emerged from each female nematode on the fifth day after inoculation and *endotokia matricida* was a common feature (J.S. Prasad, personal communication). Mohandas and Rajamma (2005) described *Rhabditis* (*Oscheius*) sp. as an effective EPN against arecanut spindle bug and red ant. A symbiotic bacterium, identified as *Xenorhabdus* sp., was isolated from dead larvae of *C. cephalonica*, arecanut spindle bug and a red ant (Mohandas and Rajamma, 2005; Mohandas *et al.*, 2005). However, mass multiplication of these EPNs in the laboratory is a pre-requisite for their deployment in the field. Storage period studies are also important to ascertain the viability and infectivity of EPN. Hence, as a part of the ongoing investigations, studies were undertaken on the effect of storage duration on survival and infectivity of these two indigenous species of EPNs.

## MATERIALS AND METHODS

*Rhabditis* (*Oscheius*) sp. was isolated from soils (Gururaj Katti *et al.*, 2003) and *S. thermophilum* was isolated from greater wax moth, *Galleria mellonella* L., collected from deserted honey comb by placing the infected larvae onto White traps (White, 1927), at Hyder-

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**Table I.** Effect of storage periods on pathogenicity and multiplication of *Rhabditis* (*Oscheius*) sp. on *Galleria mellonella* and *Corcyra cephalonica*.

| Treatment | | Time for host larval mortality (hrs) | No. of EPNs recovered from host larva | MR |
|---|---|---|---|---|
| Host species | Storage period (days) | | | |
| *G. mellonella* | 110 | 57.6 b | 39955 b | 399.6 |
| *G. mellonella* | 50 | 65.6 b | 48464 b | 484.6 |
| *G. mellonella* | 5 | 61.6 b | 54482 a | 544.8 |
| *C. cephalonica* | 110 | 24.4 a | 16566 c | 165.7 |
| *C. cephalonica* | 50 | 26.4 a | 14646 c | 146.5 |
| *C. cephalonica* | 5 | 65.2 b | 19019 c | 190.2 |

MR - Multiplication rate.
Figures within a column followed by different letters are significantly different at P = 0.05, following DMRT.

abad, India. Both *Rhabditis* (*Oscheius*) sp. and *S. thermophilum* were maintained *in vivo* on two insect hosts *viz*., *G. mellonella*, reared on artificial diet as described by Singh (1994) and *C. cephalonica*, reared on coarse *Sorghum bicolor* (Ganguly, 2000) at the Directorate of Rice Research (DRR), Rajendranagar, Hyderabad. The infective juveniles of each EPN were harvested separately using a modified White trap method (White, 1927), surface sterilized with 0.1% formalin and stored in water (2000 IJs/ml) at room temperature (27-30 $^0$C).

*Effect of storage on survival of EPNs at room temperature.* Freshly harvested IJs of *Rhabditis* (*Oscheius*) sp. and *S. thermophilum* were washed three times in tap water and stored in 100 ml distilled water in 250 ml conical flasks at a concentration of 2000 IJs/ml. Formalin (0.1%) was added to avoid contamination and the flasks were plugged with non-absorbent cotton. Treatments were storage periods for 50, 80, 100, 135 and 150 days at 28 ± 2 $^0$C, arranged according to a completely randomized block design, with five replicates per EPN species and storage period. The survival of IJs was monitored by counting active nematodes at the end of each storage period (Krishna Prasad and Rao, 1980). The per cent survival of EPNs was then calculated.

*Effect of storage on the infectivity of EPNs.* After storage at room temperature for varying periods, the two species of EPNs were evaluated for their infectivity, pathogenicity and multiplication rate on final instar larvae of *G. mellonella* and *C. cephalonica*.

Nematode samples were maintained at 28 ± 2 $^0$C in five batches for storage periods of 5, 50 and 110 days for *Rhabditis* (*Oscheius*) sp. and of 5, 50, 100 and 150 days for *S. thermophilum*. Suspensions of infective juveniles (100 IJs/ml) were pipetted onto moist filter paper placed in 2.5-cm-diameter Petri-plates. A single larva of the host was then placed in each Petri-plate for exposure to each EPN for 12 h. After exposure, the larvae were washed with formalin (0.1%) and transferred to fresh Petri-plates containing food. Partially ground sorghum grains were provided to *C. cephalonica* (25 g/larva) and solid artificial diet was provided to *G. mellonella* (25 g/larva). Observations on infectivity were made at six-hour intervals until 100% larval mortality was observed in all the treatments. Only the time taken for 100% mortality was considered in analysis of the data. The dead insect larvae were carefully placed on White traps and the IJs that emerged (White, 1927) were counted by dilution count under a stereozoom microscope. The data were analysed by ANOVA and means were compared by Duncan's Multiple Range Test (DMRT).

## RESULTS

*Effect of storage on survival of EPNs at room temperature.* The survival of IJs of EPNs under storage varied between the two species. There was 100% survival of both isolates up to 50 days of storage at room temperature. Thereafter, survival of *Rhabditis* (*Oscheius*) sp. decreased to 75%, 50%, 30% and zero after 80, 100, 135 and 150 days of storage, respectively. Survival of *S. thermophilum* was 80%, 75%, 50% and 10% after 80, 100, 135 and 150 days of storage, respectively (Fig. 1).

*Effect of storage on the infectivity of EPNs.* The time taken by *Rhabditis* (*Oscheius*) sp. to kill the insects ranged from 57.6 to 65.6 h for *G. mellonella* and from 24.4 to 65.2 h for *C. cephalonica*, after periods of storage varying from 5 to 110 days (Table I). Significantly more *Rhabditis* (*Oscheius*) sp. IJs were recovered from *G. mellonella* (39956 to 54482/larva) than from *C. cephalonica* (14646 to 19019/larva).

*Steinernema thermophilum* also took more time to kill *G. mellonella* (20.8 to 74.4 h) than C. *cephalonica* (19.6 to 57.2 h), after periods of storage varying from 5

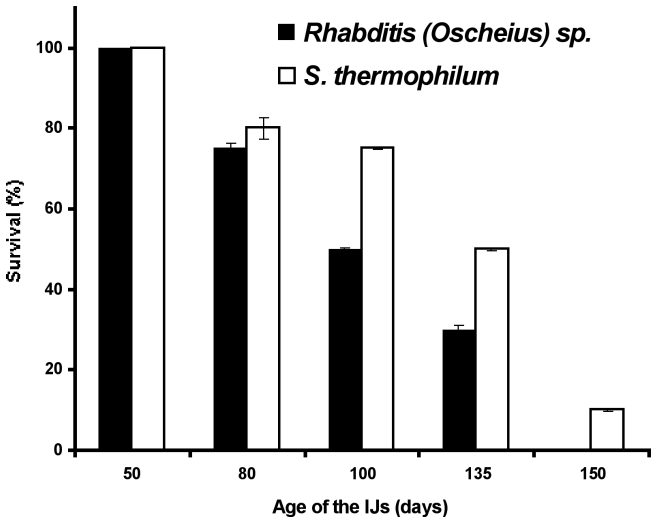


**Fig. 1.** Survival of the EPNs under different storage periods in the laboratory. Bars represent standard errors.

to 150 days (Table II). The IJs stored for 100 days took least time (20.8 h) to kill *G. mellonella*, while storage of IJs for periods as long as 150 days or as short as 50 or 5 days required significantly more time (36.8 to 74.4 h). The IJs stored for 50 or 100 days required the least time (19.6 to 25.6 h) to kill *C. cephalonica* followed by IJs stored for 150 days (50.4 h) and 5 days (57.2 h). The recovery of *S. thermophilum* was greater from *G. mellonella* (36033 to 49298/larva) than from *C. cephalonica* (24161 to 28154/larva).

The multiplication rate of both nematode species was similar on *G. mellonella* except for *Rhabditis* (*Oscheius*) sp. stored for 110 days. When compared to *Rhabditis* (*Oscheius*) sp., *S. thermophilum* had a slightly higher multiplication rate on *C. cephalonica*.

## DISCUSSION

*Steinernema thermophilum* survived better than *Rhabditis* (*Oscheius*) sp. at room temperatures. Selvan *et al.* (1993), studying the shelf life of *Heterorhabditis bacteriophora* Poinar and *S. glaseri* (Steiner) Wouts, Mracek, Gerdin *et* Bedding, which survived for 7 and 36 weeks, respectively, deduced that poor storage stability in entomopathogenic nematodes could be due to an increase with storage of unsaturated fatty acids in the freshly emerged infective juveniles. The differences in survival and infectivity of the two EPNs could also be due to difference in activity of the IJs under storage (Hussaini *et al.*, 2005).

There was no significant effect of the storage period of IJs on time taken by *Rhabditis* (*Oscheius*) sp. to kill *G. mellonella*, while the time necessary to kill *C. cephalonica* decreased with increase in storage time. The IJs of *S. thermophilum* stored for 50 and 100 days were significantly more effective than those stored for 5 and 150 days in killing both hosts. Jung (1996) reported low infectivity of EPN isolates during the first week of emergence. Lewis *et al.* (1997) also observed that IJs of *S. carpocapsae* became more mobile with age, while O'Leary *et al.* (1998) found that the host finding ability of IJs of *H. megidis* improved with increased duration of storage.

*Galleria mellonella* larvae yielded significantly more IJs of *S. thermophilum* than the larvae of *C. cephalonica*, but storage periods did not affect the recovery of IJs. Rajkumar *et al.* (2003) also observed greater recovery of IJs of *Heterorhabditis* sp. from *G. mellonella* in comparison to *Steinernema* sp., while Zaki *et al.* (2000) observed no differences in recovery of IJs of *Steinernema* sp. and *Heterorhabditis* sp. from *Bombyx mori*.

The survival of *Rhabditis* (*Oscheius*) sp. and *S. thermophilum* declined after 50 days of storage. Although

**Table II.** Effect of storage periods on pathogenicity and multiplication of *Steinernema thermophilum* on *Galleria mellonella* and *Corcyra cephalonica*.

| Treatment | | Time for host larval mortality (hrs) | No. of EPNs recovered from host larva | MR |
|---|---|---|---|---|
| Host species | Storage period (days) | | | |
| *G. mellonella* | 150 | 74.4 c | 48977 a | 489. 8 |
| *G. mellonella* | 100 | 20.8 a | 49298 a | 493.0 |
| *G. mellonella* | 50 | 36.8 b | 36033 a | 360.3 |
| *G. mellonella* | 5 | 60.8 c | 41575 a | 415.7 |
| *C. cephalonica* | 150 | 50.4 b | 24161 b | 2416.1 |
| *C. cephalonica* | 100 | 25.6 a | 24744 b | 247.4 |
| *C. cephalonica* | 50 | 19.6 a | 27432 b | 274.3 |
| *C. cephalonica* | 5 | 57.2 c | 28154 b | 281.5 |

MR - Multiplication rate.
Figures within a column followed by different letters are significantly different at P = 0.05, following DMRT.

the infectivity of *Rhabditis* (*Oscheius*) sp. increased after longer storage, in *C. cephalonica* there was no significant effect of storage period on the recovery of nematode. In *S. thermophilum*, the IJs took less time to kill when stored for 50-100 days, irrespective of the host. The recovery of IJs was more in *G. mellonella* than *C. cephalonica*. However, within each host the storage period had no significant effect on the recoveries of *S. thermophilum*. As both EPNs are amenable to quick and easy multiplication, studies to further improve their shelf life could contribute to the development of appropriate delivery systems for field management of rice pests.

Awareness of hazards caused by the usage of chemicals in agriculture, is increasing the demand for pesticide-free produce in India, particularly in northern areas where quality rices and basmati varieties are grown mainly for export purposes. In these areas, stem borer and leaf folder are the major insect pest problems and the farmers are being encouraged to resort to non-pesticidal and organic inputs for their management. With the identified potential of the EPNs and the added advantage of organic rice attracting premium prices, development of EPNs as a component in rice pest management will be economically beneficial to the farmers.

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