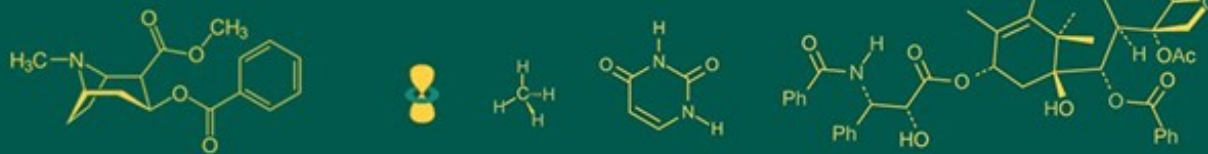


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Survival rate of the entomopathogenic nematode *Heterorhabditis indica* in different carrier materials

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ABSTRACT

Entomopathogenic nematodes (EPNs) are effective biological control agents against soil-dwelling insect pests such as white grubs, but their field efficacy depends on maintaining the viability of infective juveniles (IJs) during storage and transport. A laboratory study was conducted to evaluate the survival of *Heterorhabditis indica* in six carrier materials – talc, vermicompost, cocopeat, moss, lignite and fine sand – at 7, 14, 21 and 28 days after inoculation (DAI). Survival declined progressively with storage duration in all carriers, but the rate of decline differed markedly among them. Cocopeat consistently recorded the highest survival at every interval, with a mean survival of 94.63 per cent over the observation period, followed by vermicompost (92.58%) and talc (91.14%). Moss, lignite and sand recorded comparatively lower mean survival of 88.98, 85.16 and 81.68 per cent, respectively, with sand showing the sharpest decline (76.45% at 28 DAI). The favorable moisture-retention and physical properties of cocopeat appear to provide a suitable microenvironment for maintaining IJ viability. These findings identify cocopeat as a promising, locally available carrier for the development of a practical, storable and transportable formulation of *H. indica* for field application against white grubs.

Keywords: Entomopathogenic nematode, *Heterorhabditis indica*, Carrier material, Cocopeat, Infective juveniles, Survival rate, Formulation

1. Introduction

Entomopathogenic nematodes (EPNs), particularly members of the genera *Steinernema* and *Heterorhabditis*, are important biological control agents used against insect pests. Their infective juveniles (IJs) carry symbiotic bacteria *Xenorhabdus spp.* in *Steinernema* and *Photorhabdus spp.* in *Heterorhabditis* that contribute to rapid mortality of susceptible insects. Under suitable conditions, EPNs and their bacterial symbionts can kill target insects within 24–72 h, making them attractive components of biologically based pest management programs. The efficacy of an EPN product, however, depends not only on nematode virulence but also on maintaining IJ viability and infectivity during storage, transport and application. For *Heterorhabditis*, successful pest suppression requires that nematode material reaches the user in adequate physiological condition. Consequently, formulation and storage systems must provide an environment that supports survival and preserves infectivity.

A variety of materials including sponge, vermiculite, clays, gels, alginate and other substrates—have been evaluated for EPN storage and delivery. Previous studies have shown that formulation substrate and storage conditions can markedly influence survival and persistence, and that performance may vary with nematode species and storage environment. Cocopeat and other locally available materials may therefore have practical value, but their suitability needs to be assessed experimentally for the particular EPN isolate or species being formulated. The present study was undertaken to compare the survival of *Heterorhabditis indica* in six carrier materials, namely talc, vermicompost, cocopeat, moss, lignite and fine sand, over a 28-day period. The objective was to identify a carrier that could better maintain *H. indica* viability and thereby serve as a basis for further development of a practical, storable and transportable formulation.

2. Materials and Methodology**Preparation of *Heterorhabditis indica* culture**

Fresh culture of *Heterorhabditis indica* was used for preparation of carrier-based formulations. The nematodes were harvested in sterilized tissue culture flasks, and a stock suspension was maintained in a 250 ml capacity flask for preparation of formulations.

Preparation and sterilization of carrier materials

The carrier materials selected for the study were talc, vermicompost, cocopeat, moss, lignite and fine sand. The selected carrier materials were sterilized before use; carrier material with distilled water was autoclaved at 15 psi pressure and 121 °C for 20 minutes.

Preparation of carrier-based formulations

Approximately 100 g of each sterilized carrier material was placed in a polythene bag and 20 ml of distilled water was added to the carrier material. Three bags were maintained as replications for each carrier treatment. Each carrier formulation was inoculated with 20 ml of *H. indica* suspension containing 1,000 infective juveniles (IJs) per ml. The inoculated formulations were maintained at room temperature and survival was assessed at weekly intervals for 28 days.

Assessment of survival of infective juveniles

For estimation of nematode survival, 1 g of each carrier-based formulation was transferred to a 50 ml capacity conical flask containing 9 ml of distilled water. The material was mixed thoroughly for uniform distribution of nematodes and 1 ml of the suspension was transferred to a nematode counting dish. The number of live and dead infective juveniles was counted under a stereo zoom binocular microscope. Survival (%) was calculated as (number of live IJs / total number of IJs counted) × 100. The survival percentage was recorded at 7, 14, 21 and 28 days after inoculation (DAI). Nematodes that were immobile and showed no observable movement were considered non-viable, following the criterion specified in the experimental protocol.

Carrier materials evaluated

Talc; Vermicompost; Cocopeat; Moss; Lignite; Fine sand

Statistical Analysis

The laboratory experiment was analyzed using a Completely Randomized Design (CRD) with three replications. Percentage data were transformed using the arcsine transformation prior to statistical analysis.

3. Result and Discussion

Survival rate of *Heterorhabditis indica* in different carriers

The survival rate of *Heterorhabditis indica* in different carriers at 7, 14, 21 and 28 days after inoculation (DAI) is presented in Table 1. The results revealed variation in the survival of *H. indica* among the carriers throughout the observation period. In general, the survival rate declined gradually with an increase in the period after inoculation; however, the extent of decline varied among the carriers.

At 7 DAI, the highest survival rate of *H. indica* was recorded in cocopeat (96.32%), followed by vermicompost (94.66%) and talc (93.28%). Moss and lignite recorded 90.65% and 90.16 per cent survival, respectively, whereas the lowest survival was observed in sand (87.03%). At 14 DAI, cocopeat continued to record the highest survival (95.14%), followed by vermicompost (93.30%) and talc (92.41%); survival in moss, lignite and sand was 90.00, 86.31 and 82.69 per cent, respectively.

At 21 DAI, the survival rate in cocopeat was 94.64 per cent, followed by vermicompost (91.61%) and talc (90.30%); moss, lignite and sand recorded survival rates of 88.26, 83.70 and 80.57 per cent, respectively. At 28 DAI, cocopeat maintained the highest survival rate (92.44%), followed by vermicompost (90.76%) and talc (88.58%); the corresponding survival rates in moss, lignite and sand were 87.04, 80.50 and 76.45 per cent, respectively.

The mean survival rate over the four observation intervals was highest in cocopeat (94.63%), followed by vermicompost (92.58%) and talc (91.14%). Moss, lignite and sand recorded mean survival rates of 88.98, 85.16 and 81.68 per cent, respectively. Thus, cocopeat maintained the highest survival of *H. indica* throughout the observation period, whereas sand recorded the lowest survival. The reduction in survival from 7 to 28 DAI was relatively small in cocopeat compared with lignite and sand, indicating better maintenance of nematode viability in cocopeat during the experimental period.

Table 1. Survival rate (%) of *Heterorhabditis indica* in different carrier

T No.	Carriers	Per cent survival rate at different DAI				Mean
		7	14	21	28	
T ₁	Talc	93.28 (75.04)*	92.41 (74.11)	90.30 (72.09)	88.58 (70.29)	91.14 (72.72)
T ₂	Vermicompost	94.66 (76.66)	93.30 (75.05)	91.61 (73.28)	90.76 (72.34)	92.58 (74.23)
T ₃	Cocopeat	96.32 (79.02)	95.14 (77.40)	94.64 (76.64)	92.44 (74.12)	94.63 (76.65)
T ₄	Moss	90.65 (72.21)	90.00 (71.65)	88.26 (69.96)	87.04 (68.38)	88.98 (70.63)
T ₅	Lignite	90.16 (71.90)	86.31 (68.72)	83.70 (66.33)	80.50 (63.93)	85.16 (67.38)
T ₆	Sand	87.03 (69.21)	82.69 (65.48)	80.57 (63.96)	76.45 (61.08)	81.68 (64.69)
	SE ±	1.58	1.81	1.68	1.55	0.82
	CD 1%	6.82	7.82	7.27	6.71	3.54
	CV (%)	3.70	4.35	4.14	3.93	1.99

*Figures in parentheses are arcsine transformed values; DAI – Days after inoculation.

(continued on next page)

The present findings are in conformity with earlier studies which demonstrated that the type of carrier or formulation has a considerable influence on the survival and persistence of entomopathogenic nematodes. Divya *et al.* (2011) [1] evaluated different carrier materials, including sawdust, hydrogel, coir dust, talc, water and sponge, for maintaining *Heterorhabditis indica* and observed differences in nematode survival among the formulations, with survival gradually declining with increasing storage duration; their findings indicated that suitable carrier materials could maintain comparatively higher nematode viability for a longer period. Similarly, Strauch *et al.* (2000) [2] reported that formulation and storage conditions significantly influenced the survival and infectivity of *H. indica* and *H. bacteriophora*, highlighting the importance of selecting appropriate carrier materials for maintaining nematode viability. Gayathri *et al.* (2023) [3] also recorded differences in the survival of *H. indica* in sponge, talc and alginate-based formulations and reported that suitable formulations maintained higher nematode survival over an extended period. The present observation of comparatively higher survival of *H. indica* in cocopeat, followed by vermicompost and talc, is therefore broadly consistent with these earlier findings. The higher survival observed in cocopeat is associated with its favourable physical characteristics and moisture-retention capacity, which could provide a suitable microenvironment for maintaining infective juveniles.

4. Conclusion

Among the six carrier materials tested, cocopeat provided the most favorable conditions for maintaining the survival of *Heterorhabditis indica* during the 28 day laboratory observation period. Mean survival was highest in cocopeat (94.63%), followed by vermicompost (92.58%) and talc (91.14%), while fine sand recorded the lowest mean survival (81.68%). Cocopeat retained 92.44% survival at 28 DAI, compared with 76.45% in fine sand. The results support the selection of cocopeat for further development and optimization of *H. indica* formulations. Future experiments should include longer storage periods, controlled temperature and humidity treatments, physico-chemical characterization of carriers, infectivity assays against target white-grub species and field validation.

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