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# Survival and infectivity of entomopathogenic nematode, *Heterorhabditis bacteriophora* in different formulations

**Jisna George, Gitanjali Devi and Badal Bhattacharyya**

**Abstract**
Entomopathogenic nematode, *Heterorhabditis bacteriophora* was formulated in six different formulations such as talc, sawdust, alginate gel, water dispersible granule and compost: charcoal powder mixture. All formulations tested *in vitro* at two temperatures (5 ˚C and 30 ˚C) to evaluate their survival and infectivity against wax moth, *Galleria mellonella*. Alginate gel and Sawdust formulations enhanced highest survival of infective juveniles of *H. bacteriophora*. Mean percent survival of *H. bacteriophora* infective juveniles (IJs) storage at 5 ˚C in alginate gel was 99.40% and in sawdust 98.76%, whereas mean percent survival was less (98.90% and 98.36% respectively) at 30 ˚C. Mean larval mortality of *G. mellonella* by *H. bacteriophora* stored at 5 ˚C in alginate gel was 66.66% and 63.33% in sawdust formulation whereas larval mortality stored at 30 ˚C in alginate gel was 59.33% and 56.00% in sawdust formulation. However from economic point of view sawdust formulation seems to be more efficient formulation.

**Keywords:** Entomopathogenic nematodes (EPNs), survival, infectivity, formulation, storage temperature

## Introduction
Entomopathogenic nematodes (EPNs), *Steinernema* and *Heterorhaditis* in the family Steinernematidae and Heterorhabditidae, order Rhabditida are important biocontrol agents of insect pests. They are considered as one of the most significant non-chemical alternatives for pest management and thus using in integrated pest management systems (Georgis *et al*., 2006). Sometimes EPNs do not show encouraging results under field condition. This is due to the poor stability of the product during storage prior to application, amount of active infective juvenile actually reaching the target, and rapid degradation of the active material on the target. The infective stage nematodes need to be mass produced and formulated into solid or semi liquid substrates which warranty survival for a period necessary to transport and field application. In order to maintain viability and storage stability, formulation depends on oxygen, temperature, moisture requirements of nematodes, microbial contamination. Selection of an appropriate formulation can improve product stability, enhance and extend activity, and may reduce inconsistency of field performance of the EPNs. The products should have improved balance between efficiency and cost. Nematode formulation is the most important aspect in the commercialization of nematode as biocontrol agent. Various formulations of entomopathogenic nematodes with increasing shelf-life have been developed for soil as well as foliar application, a number of these are currently in use (Georgis, 1990; Grewal, 1998; Navon *et al.*, 1998; Gokte Narkhedkar *et al.*, 2003) [6, 9, 10, 14]. Keeping this view, the present investigations were undertaken to standardize formulation of entomopathogenic nematodes, *Heterorhabditis bacteriophora*.

## Materials and Methods
### Rearing of *Galleria mellonella*
Mother culture of *G. mellonella* was obtained from honey comb and maintained on a semi-synthetic diet using cereals, yeast and glycerol in the laboratory (David and Kurup, 1988) [3].

**Nematode culture**

Entomopathogenic nematode *Heterorhabditis bacteriophora* was isolated from rhizosphere of brinjal from ICR Farm, AAU, Jorhat (26˚72′7.393′′N; 94˚19′70.01′′E) and identified by morphological and morphometric methods during 2018-2019. The isolate was mass cultured on final instar larvae of *Galleria mellonella*. Emerged infective juveniles (IJs) were harvested through modified White trap method (Glazer and Novon, 1990) [8] and stored them in tissue culture flasks (250 ml) for 2 days at 25±2 ˚C in the laboratory before use in the experiments.

**Preparation of nematode formulation**

**1. Talc formulation**

Talc powder (250 g) was added to 25 ml of distilled water in a 500 ml beaker and mixed thoroughly. Fifty ml of freshly harvested IJs of *Heterorhabditis bacteriophora* (2000 IJs/ml) were added in the above moisten talc and then the contents were thoroughly mixed till the nematode suspension spread over evenly into the talc.

**2. Sawdust formulation**

The sawdust material was grinded separately to get fine dust with the help of mixer and grinder and sieved with fine mesh and then sterilized under sunlight for 1 hr. Two hundred grams of sawdust were moistened by adding 50 ml of distilled water separately. IJs suspension of 50 ml (2000 IJs/ml) were added evenly and mixed them gently till nematodes spread over into the saw dust.

**3. Alginate gel**

A solution of gel matrix was prepared by dissolving 2 g of sodium alginate in 100 ml of water and blended for 4-5 minutes. Drops of this solution when placed into a 100 mM solution of $CaCl_2.2H_2O$ (The complexing solution) formed discrete capsule of calcium alginate. Fifty milliliter of nematode suspension (2000 IJs/ml) were placed into the solution of Sodium alginate which is water-insoluble, gelatinous, cream coloured substance and then dripped into the complexing solution which was continuously stirred. Capsules were allowed to complex for 20-30 minutes and then separated from the complexing solution by sieving, rinsed in deionised water and stored at a temperature of 50C and 300C for further survival and infectivity observations. The actual number of nematodes was determined by dissolving five capsules in 9.5 ml of 0.5M sodium citrate containing 0.1% Triton X-100. The capsules were stirred with magnetic spin bar until dissolution (about 30 minutes), and the nematode in 1 ml of suspension were counted using a Hawksley counting dish.

**4. Water dispersible granule (WDG)**

Clay, aloe gel and starch were mixed at the ratio of 1:1:1. IJs suspension of 50 ml (2000 IJs/ml) were added evenly and mixed them gently till nematodes spread over into the above mixture. Granules of 10-20 mm diameter were prepared and packed in polythene envelope.

**5. Compost and charcoal powder mixture**

The formulation in which vermicompost and charcoal powder were mixed at the ratio of 1:1. IJs suspension of 50 ml (2000 IJs/ml) were added evenly and mixed them gently till nematodes spread over into the above mixture.

**6. Control (Water)**

Freshly harvested infective juveniles were washed twice in distilled water and 50 ml (2000 IJs/ml) of suspension was stored in 250 ml conical flask. Flasks were closed with non-absorbent cotton.

The prepared formulations were packed in polythene envelope and stored at a temperature of 5 ˚C and 30 ˚C for further survival and infectivity observation.

**Bioassay study**

**1. Survival of infective juveniles of *Heterorhabditis bacteriophora***

Survival of infective juveniles in different formulations were evaluated by weekly interval up to 6 weeks at 5 ˚C and 30 ˚C temperature, by diluting 1.00 g of formulated IJs in 5 ml distilled water from each and the per cent IJs survival was counted and the percent mean data of survived IJs was recorded. Five replicates for each treatment were done. Data obtained in a per cent survival of IJs were transformed to arcsine for statistical analysis. Data were statistically analyzed using two factorial completely randomized block design.

**2. Infectivity of *Heterorhabditis bacteriophora* against *Galleria mellonella***

**Soil bioassay**

The experiment was conducted in 250 ml capacity beaker. Two fifty grams of sterilized soil were kept in each beaker and 15% moisture was maintained. Five grams of *Heterorhabditis bacteriophora* formulations that were stored at 5 ˚C and 30 ˚C each with 5 replications were tested against 10 larvae of the greater wax moth, *G. mellonella*. Observations on mortality were done at 24 h intervals for three days. The data from percent larval mortality induced by *Heterorhabditis bacteriophora* were transformed to arcsine for statistical analysis. Data were statistically analyzed using two factorial Completely Randomized Block Design.

**Results and Discussion**

The data on effect of different formulations at different storage period on survival of *Heterorhabditis bacteriophora* at 5 ˚C are presented in table1. Irrespective of storage time, the formulations (T) of Alginate gel (99.40%) were found to be significantly effective followed by the formulation of Sawdust (98.76%) in survival of *H. bacteriophora* when compared with control (water). Similarly, irrespective of formulation (T), the storage period (t) was also significantly effective for survival of *H. bacteriophora.* Survival was more (99.76%) in 1st week of storage followed by 98.83% in 2nd week, 96.73% in 3rd week, 94.13% in 4th week, 91.30%, in 5th week and 87.70% in 6th week of storage. Following the significant interaction of formulation and storage time (T x t), up to 4th week of storage at 5 ˚C, Alginate gel and sawdust formulation, *H. bacteriophora* survival was 100% and it was at par with control (water). During 6th week of storage, survival % of *H. bacteriophora* at 5 ˚C was significantly higher in Alginate gel formulation as compared to sawdust (95.40%) and control (88.40%).

**Table 1:** Percent survival of infective juveniles of *Heterorhabditis bacteriophora* in different formulations stored at 5 ˚C (Mean of five replications).

| Formulations | Survival (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | |
| T1: Talc | 100 (89.78) | 100 (89.78) | 100 (89.78) | 92.00 (73.61) | 90.40 (71.95) | 84.80 (67.06) | 94.53 (80.33) |
| T2: Sawdust | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100.00 (89.78) | 97.20 (81.44) | 95.40 (77.78) | 98.76 (86.39) |
| T3: Alginate gel | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 96.40 (79.27) | 99.40 (88.03) |
| T4: Water dispersible granule (WDG) | 98.60 (85.66) | 93.00 (76.27) | 85.20 (67.49) | 82.80 (65.56) | 78.80 (62.59) | 75.00 (60.67) | 85.80 (69.60) |
| T5: Compost : Charcoal powder mixture (1:1) | 100 (89.78) | 100 (89.78) | 95.20 (77.55) | 90.00 (71.59) | 89.00 (70.64) | 86.20 (68.22) | 93.40 (77.93) |
| T6: Control (Water) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 92.40 (74.16) | 88.40 (69.88) | 96.80 (83.86) |
| Mean | 99.76 (89.09) | 98.83 (87.53) | 96.73 (84.03) | 94.13 (80.02) | 91.30 (75.10) | 87.70 (70.37) | |
| CD (P=0.05) Formulation (T): (1.11) Storage Time (t): (1.11) Formulation (T) x Storage Time (t): (2.71) | | | | | | | |

Figures in parentheses are arc sin transformed values.

The data on effect of different formulation at different storage period on survival of *Heterorhabditis bacteriophora* at 30 ˚C are presented in table 2. Irrespective of storage time, the formulation (T) of Alginate gel (98.90%) was found to be significantly effective followed by sawdust in survival of *H. bacteriophora* at 30 ˚C when compared with control. Similarly, irrespective of formulation (T), the storage period (t) was also significantly effective for survival of *H. bacteriophora* was more (99.40%) in 1st week of storage followed by 97.13% in 2nd week, 95.53% in 3rd week, 93.26% in 4th week, 88.83% in 5th week and 85.40% in 6th week of storage. The results have confirmed observations by Jung (1996) [13] and Georgis and Kaya (1998) [7] that energy reserves usually exhausted much faster as storage time increase. Following the significant interaction of formulation and storage time (T x t), up to 4th week of storage at 30 ˚C, Alginate gel and sawdust formulation, *H. bacteriophora* survival was 100% and it was at par with control (water). Fan and Hominick (1991) [5] observed that temperature is the important factor affecting nematode survival in formulations, they recorded a positive influence of cold storage on the survival of nematode IJs. *S. carpocapsae* formulated in alginate gel could be stored up to 6-12 months at refrigerated conditions and 1-3 months at room temperature (Georgis, 1990) [6]. *S. feltiae* with alginate capsules with 99.8% survival has been reached up to 6 months at 23 ˚C (Chen & Glazer 2005) [2]. (Umamaheswari *et al*., 2006) [16] observed 100 percent survival of *H. indica*, *S. siamkayai* and *S. glaseri* at 5 ˚C up to 4 weeks in alginate gel formulation. Survival of *S. glaseri* was more (7.5%) up to 24 weeks followed by *S. siamkayai* (5.0%) up to 22 weeks and *H. indica* (12.5%) upto 18 weeks at 5 ˚C. At the storage temperature 25 ˚C, 100 percent survival of these EPNs was observed up to 2 weeks. Divya *et al*. (2011) [4] developed five different formulations i.e., sawdust, hydrogel, coirdust, talc and sponge of *H. indica*, and were evaluated its survival at 27 ± 2 ˚C. Sawdust (95%) and hydrogel (85%) formulations were enhanced highest survival followed by coirdust (80%), talc (75%) and sponge (65%) till 5th week period. A maximum shelf-life of more than 11 week periods achieved in hydrogel formulation with 65% of survival than sawdust formulation. Hussein and Abdel-Aty (2012) [12] observed that among the three formulation *viz*., hydrogel, kaolinite and calcium alginate, storage potential of *S. carpocapsae* juveniles was more (more than 50% in 40 days) than that of *H. bacteriophora* in case of formulation with calcium alginate at room temperature (25±2 ˚C). Trehalose accumulation at low temperature appears to be more among EPNs and may be a survival strategy during environmental stress (Grewal, 2002) [11].

The data on effect of different formulation at different exposure period stored at 5 ˚C on larval mortality of *Galleria mellonella* by *Heterorhabditis bacteriophora* in soil bioassay are presented in table 3. Among all the formulations (T) of *H. bacteriophora*, talc, sawdust, alginate gel and compost charcoal mixture showed higher mortality of *Galleria* larva as compared to control (water), irrespective of exposure time. Alginate gel formulation was found to be most effective on larval mortality of *Galleria* (66.66%) than sawdust (63.33). Similarly irrespective of formulation treatment (T), the exposure time (t) showed significant effect on *Galleria* larval mortality. During 24h of exposure time there was no larval mortality of *Galleria* by *H. bacteriophora*. Larval mortality by *H. bacteriophora was* 67.33% and 80.66% at 48 h and 72 h respectively. Hundred percent mortality of *Galleria* was recorded during 48 h of exposure time in case of Alginate gel formulation followed by sawdust formulation (90%), compost charcoal mixture (60%) and talc formulation (56%). Hundred percent mortality of *Galleria* was recorded during 72 h of exposure time in case of Alginate gel and sawdust formulation followed by compost charcoal mixture (90%) and talc formulation (76%). Alginate formulation showed larval mortality which is 85.18% and 66.66% increased over control during 48 h and 72 h exposure time respectively.

**Table 2:** Percent survival of infective juveniles of *Heterorhabditis bacteriophora* in different formulations stored at 30 ˚C. (Mean of five replications).

| Formulations | Survival (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | |
| T1: Talc | 100 (89.78) | 100 (89.78) | 100 (89.78) | 91.60 (73.28) | 87.00 (68.93) | 82.00 (64.93) | 93.43 (79.56) |
| T2: Sawdust | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 96.40 (79.37) | 93.80 (75.64) | 98.36 (85.69) |
| T3: Alginate gel | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 98.00 (82.77) | 95.40 (78.11) | 98.90 (86.67) |
| T4: Water dispersible granule (WDG) | 96.40 (79.18) | 90.80 (72.65) | 84.80 (67.08) | 80.60 (63.95) | 77.80 (62.00) | 74.80 (59.88) | 84.20 (67.50) |
| T5: Compost : Charcoal powder mixture (1:1) | 100 (89.78) | 92.00 (75.35) | 88.40 (70.40) | 87.40 (69.45) | 84.20 (66.67) | 79.00 (62.76) | 88.50 (72.40) |
| T6: Control (Water) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 100 (89.78) | 89.60 (71.29) | 87.40 (69.23) | 96.16 (83.27) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mean | 99.40 (88.07) | 97.13 (84.52) | 95.53 (82.77) | 93.26 (79.34) | 88.83 (71.98) | 85.40 (68.43) | |
| CD (P=0.05) Formulation (T): (1.24) Storage Time (t): (1.24) Formulation (T) x Storage Time (t): (3.04) | | | | | | | |

Figures in parentheses are arc sin transformed values.

**Table 3:** Percent larval mortality of *Galleria mellonella* by *Heterorhabditis bacteriophora* in different formulations stored at 5 ˚C in soil bioassay. (Mean of five replications).

| Formulations | Larval mortality (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | % increase/ decrease over control | 72 hrs | % increase/ decrease over control | |
| $T_1$: Talc | 0.00 (0.30) | 56.00 (48.46) | 3.70 (+ve) | 76.00 (60.77) | 26.66 (+ve) | 44.00 (36.51) |
| $T_2$: Sawdust | 0.00 (0.30) | 90.00 (73.56) | 66.66 (+ve) | 100 (89.69) | 66.66 (+ve) | 63.33 (54.52) |
| $T_3$: Alginate gel | 0.00 (0.30) | 100 (89.78) | 85.18 (+ve) | 100 (89.69) | 66.66 (+ve) | 66.66 (59.89) |
| $T_4$: Water dispersible granule (WDG) | 0.00 (0.30) | 44.00 (41.53) | 14.21 (-ve) | 58.00 (49.66) | 3.33 (-ve) | 34.00 (30.50) |
| $T_5$: Compost: Charcoal powder mixture (1:1) | 0.00 (0.30) | 60.00 (50.81) | 11.11 (+ve) | 90.00 (75.56) | 50.00 (+ve) | 50.00 (42.22) |
| $T_6$: Control (Water) | 0.00 (0.30) | 54.00 (47.30) | | 60.00 (50.81) | | 38.33 (32.80) |
| Mean | 0.00 (0.30) | 67.33 (58.56) | | 80.66 (69.37) | | |
| CD (P=0.05) Formulation (T): (3.30) Exposure period (t): (2.33) Formulation (T) x Exposure period (t): (5.72) | | | | | | |

Figures in parentheses are arc sin transformed values.

The data on effect of different formulation at different exposure period on larval mortality of *Galleria mellonella* by *Heterorhabditis bacteriophora* stored at 30 ˚C in soil bioassay are presented in table 4. Among all the formulations (T) of *H. bacteriophora*, Alginate gel formulation showed higher mortality of *Galleria* larva as compared to control (water). Similarly irrespective of formulation treatment (T), the exposure time (t) showed significant effect on *Galleria* larval mortality. During 24h of exposure time there was no larval mortality of *Galleria* by *H. bacteriophora*. Larval mortality by *H. bacteriophora was* 55.66% and 75.00% at 48 h and 72 h respectively. Hundred percent mortality of *Galleria* was recorded during 72 h of exposure time in case of Alginate gel and sawdust formulation followed by compost charcoal mixture (86%). The result of the present study are in agreement with those of Umamaheswari *et al*. (2006) [16] who revealed *H. indica* infectivity (55%) up to 12 weeks of storage at 5 ˚C where as 50% infectivity was observed up to 8 weeks of storage at 25 ˚C in alginate gel formulation. Infectivity was more 52.5% in both *S. siamkayai* and *S. glaseri* at 5 ˚C up to 12 weeks of storage. Survival and infectivity is conserved at lower temperatures due to the tendency of IJs to be less active (Grewal, 2002) [11]. Similarly, *Heterorhabditis indica* infectivity under formulation was lead to cause 85% and 70% pathogenicity on *Helicoverpa armigera* larvae in hydrogel and sawdust respectively, exposed for 48 h treated 100 infective juvenile/larva (Divya *et al*. 2011) [4]. Hussein and Abdel-Aty (2012) [12] observed the infectivity in the three formulations, Hydrogel, Kaolinite and Calcium Alginate against *Galleria mellonella*. It was concluded that *S.carpocapsae* was more virulent than *H.bacteriophora* to *G. mellonella* in all tested formulations at room temperature. Navon *et al.* (1998, 2002) [14, 15] encapsulated *S. carpocapsae* in an edible-to-insects gel to control *Helicoverpa armigera* and *S. littoralis*, at a concentration of 1000 *S. carpocapsae* IJs/g, which caused 95% mortality in *H. armigera* and 100% in *S. littoralis* larvae. Andalo *et al.* (2010) [1] who achieved 89.3 and 57.5% survival in sponge formulation (3000 IJs/ml) after 90 and 180 days storage, respectively, at 16 ˚C for *Steinernema carpocapsae*. In their study, foam maintained a higher percentage of live IJs than other substrates tested, namely, fine sand, course sand, agar, soil, starch, expanded clay and phenolic foam.

**Table 4:** Percent larval mortality of *Galleria mellonella* by *Heterorhabditis bacteriophora* in different formulations stored at 30 ˚C in soil bioassay. (Mean of five replications).

| Formulations | Larval mortality (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 24hrs | 48hrs | % increase/ decrease over control | 72hrs | % increase/ decrease over control | |
| $T_1$: Talc | 0.00 (0.30) | 42.00 (40.38) | 16.00 (-ve) | 54.00 (47.30) | 1.81 (-ve) | 32.00 (29.33) |
| $T_2$: Sawdust | 0.00 (0.30) | 68.00 (55.71) | 36.00 (+ve) | 100 (89.78) | 81.81 (+ve) | 56.00 (48.57) |
| $T_3$: Alginate gel | 0.00 (0.30) | 78.00 (62.40) | 56.00 (+ve) | 100 (89.78) | 81.81 (+ve) | 59.33 (50.80) |
| $T_4$: Water dispersible granule (WDG) | 0.00 (0.30) | 36.00 (36.64) | 28.00 (-ve) | 54.00 (48.51) | 1.81 (-ve) | 30.66 (28.48) |
| $T_5$: Compost : Charcoal powder mixture (1:1) | 0.00 (0.30) | 60.00 (50.86) | 20.00 (+ve) | 86.00 (72.61) | 56.36 (+ve) | 48.66 (41.26) |
| $T_6$: Control (Water) | 0.00 (0.30) | 50.00 (45.00) | | 56.00 (48.46) | | 35.33 (31.24) |
| Mean | 0.00 (0.30) | 55.66 (48.50) | | 75.00 (66.04) | | |
| CD (P=0.05) Formulation (T): (3.75) Exposure period (t): (2.65) Formulation (T) x Exposure period (t): (6.49) | | | | | | |

Figures in parentheses are arc sin transformed values.

**Table 5:** Ingredient composition and cost per Kg of formulation.

| Formulation | Ingredients | Amount | Cost |
|---|---|---|---|
| $T_1$: Talc | Talc Fine powder | 250 g | Rs. 45.00 |
| $T_2$: Sawdust | Sawdust | 250 g | Rs. 10.00 |
| $T_3$: Alginate gel | Sodium alginate | 2 g/50 ml suspension | Rs. 20.00 |

| | | | |
|---|---|---|---|
| | Calcium chloride | 1.1 g/100 ml | Rs. 0.30 |
| $T_4$: Water dispersible granule (WDG) | Clay | 84 g | Rs. 10.00 |
| | Aloe gel | 84 g | Rs. 56.00 |
| | Starch | 84 g | Rs. 218.40 |
| $T_5$: Compost : Charcoal powder mixture (1:1) | Vermicompost | 125 g | Rs. 1.25 |
| | Charcoal powder | 125 g | Rs. 387.50 |
| $T_6$: Control (Water) | Water | - | - |

## Conclusion

In alginate gel formulation 100 per cent survival of *H. bacteriophora*, was observed at 5 °C up to 4 weeks. In alginate gel formulation 100 per cent larval mortality of *G. mellonella* was observed at 5 °C up to 3 weeks in soil bioassay. The present study confirms the scope for formulating *Heterorhabditis bacteriophora* in alginate gel and sawdust. From the economic point of view, sawdust formulation is the best for *Heterorhabditis bacteriophora* (Table 5). Improved understanding of the nematode behaviour and physiology could lead to the development of better formulations.

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## References

1. Andalo V, Cavalcanti RS, Molina JP, Moino Jr A. Substrates for storing entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Sci. Agric. 2010;67:342-347.
2. Chen S, Glazer I. A novel method for long-term storage of the entomopathogenic nematode *Steinernema feltiae* at room temperature. Biol. Control. 2005;32:104-110.
3. David H, Kurup NK. Techniques for mass production of *Sturmiopsis inferens* Tns. In: Biocontrol Technology for Sugarcane Pest Management. Sugarcane Breeding Institute, Coimbatore, India, 1988, 87-92.
4. Divya K, Sankar M, Marulasiddesha KN, Rao S, Krupanidhi K. Formuiation technotogy of entomopathogenic nematode for the control of the cotton bollworm, *Helicoverpa armigera*. Biosci. Discov. 2011;02(2):174-180.
5. Fan X, Hominick WM. Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). Rev. Nematol. 1991;14:407-412.
6. Georgis R. Formulation and application technology. In: Entomopathogenic Nematodes in Biological Control. R. Gaugler, and H. K. Kaya, eds. Boca Raton, FL: CRC Press, 1990, 173-194.
7. Georgis R, Kaya HK. Formulation of entomopathogenic nematodes. In: Formulation of Microbial Biopesticides. Burges HD (ed). Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998, 289-308.
8. Glazer I, Navon A. Activity and persistence of entomopathogenic nematodes tested against *Heliothidis armigera*. J Econ Entomol. 1990;83:1795-1800.
9. Gokte-Narkhedkar N, Banerjee SK, Lavhe NV, Mayee CD. Utilization of entomopathogenic nematodes for control of cotton pests and progress of work at Central Institute for Cotton Research, Nagpur. In: Current Status of Research on Entomopathogenic Nematodes in India. Hussaini, S.S., Rabindra, R.J. and Nagesh, M. (eds) PDBC, ICAR, Bangalore, India, 2003, 175-178.
10. Grewal PS. Formulations of entomopathogenic nematodes for storage and application. JPN J Nematol. 1998;28:68-74.
11. Grewal PS. Formulation and application technology. In: Entomopathogenic nematology. Gaugler R (edi) USA: CABI Publications, 2002, 265-287.
12. Hussein MA, Abdel-Aty MA. Formulation of two native entomopathogenic nematodes at room temperature. J Biopest. 2012;5(Supplementary):23-27.
13. Jung K. Storage of entomopathogenic nematodes of the genus Heterorhabditis at two temperatures. Effect on infectivity, energy reserve and number of bacteria. IOBC/WPRS Bulletin. 1996;19:103-106.
14. Navon A, Keren S, Salame L, Glazer I. An edible-to-insects calcium alginate gel as a carrier for entomopathogenic nematodes. Biocontrol Sci Technol. 1998;8:429-437.
15. Navon A, Nagalakshmi VK, Levski S, Salame L, Glazer I. Effectiveness of entomopathogenic nematodes in an alginate gel formulation against lepidopterous pests. Biocontrol Sci Technol. 2002;12:737-746.
16. Umamaheswari R, Sivakumar M, Subramanian S. Biocontrol efficacy of entomopathogenic nematodes on *Spodoptera litura* (Lepidoptera: Noctuidae) in blackgram. Indian J Nematol. 2006;36:19-22.