Indian Journal of Entomology 84(1): 29-33 (2022) DoI.: 10.55446/IJE.2021.30

SURVIVAL AND VIRULENCE OF NATIVE STRAINS OF *STEINERNEMA CARPOCAPSAE* AND *HETERORHABDITIS BACTERIOPHORA* IN FORMULATIONS

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ABSTRACT

In this study, entomopathogenic nematodes (EPN) isolated from cotton ecosystem viz., *Steinernema carpocapsae* (strain APKS2) and *Heterorhabditis bacteriophora* (strain KKMH1) were evaluated in formulations of alginate gel, talc, sponge and water concentrates. The survival or longevity of infective juveniles (IJs) in these formulations was evaluated under in vitro at storage temperatures of 5 and 25 °C. Simultaneously, virulence of these stored EPN infective juveniles (IJs) was evaluated against the spotted boll worm *Earias vitella* through in vitro bioassays. The results revealed that *S. carpocapsae* (APKS2) and *H. bacteriophora* (KKMH1) formulated in alginate gel survived 70- 100% up to 3 months with 55-100% infectivity at 5°C and survived 52- 100% with 42.5- 100% virulence for 2.5 months at 25°C. In talc formulation, these EPN remained alive to an extent of 55- 100% with 70-100% virulence at 5°C and stayed alive 45-100% with 52.5- 100% virulence at 25°C for 2 months. In sponge formulation, EPN strains survived 55-100% with 67.5-100% infectivity for 7 weeks at 5°C, but with 50-100% survivals and 62.5-100% virulence at 25°C. EPN strain *S. carpocapsae* (APKS2) can be stored in water at 5°C up to 8 weeks with viability of 50% and infectivity 60%. At 25°C, it can be stored for 5 weeks with survival of 62% and infectivity of 62.5- 100 %. It is inferred from the results that alginate and talc formulations at storage temperature of 5°C is better for long term storage of *S. carpocapsae* strain APKS2 and *H. bacteriophora* strain KKMH1. Thus, the results revealed that *S. carpocapsae* (strain APKS2) and *H. bacteriophora* (strain KKMH1) can be formulated in sponges for use within 2 months and can be kept as water concentrate if they would be utilized in 2-3 weeks.

Key words: *Earias vittella*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora,* formulations, alginate gel, talc, sponge and water concentrates, survival, virulence, storage temperature, viability, infectivity

Cotton (*Gossypium hirsutum*: Malvacea), popularly known as 'white gold', is an important cash crop of India. One of the prime challenges to attain high cotton production is damage caused by insect pests. The spotted boll worm *Earias vitella* (Lepidoptera: Noctuidae) is one of the most important pests affecting the cotton plants and it can cause yield loss of up to 50% (Dhaliwal et al., 2010). The habit of developing resistance to many insecticides including Bt transgenic cotton necessitate to find out an alternate strategy to manage *E. vitella*. In this situation, exploitation of naturally occurring entomopathogenic nematodes (EPN) from two families viz, Heterorhabditidae and Steinernematidae to develop biopesticide for the control of cotton bollworms is an ecologically sound approach. The infectivity of EPN species *Steinernema carpocapsae* (Weiser), *S. riobravus* Cabanillas and Poinar and *S. feltiae* (Weiser) on spotted boll worm *Earias insulana* was established from American continent (Glazer, 1997). Exploring indigenous EPN in cotton fields of Tamil Nadu in India, Seenivasan et al. (2012) recovered 27 strains belonging to 16 *S. carpocapsae*, 3 *Steinernema siamkayai* Stock, Somsook and Reid, 1 *Steinernema monticolum* Stock, Choo and Kaya and 7 *Heterorhabditis bacteriophora* Poinar from cotton ecosystem. Later, Seenivasan and Sivakumar (2012) established that strains of APKS2 (*S. carpocapsae)* and KKMH1 (*H. bacteriophora)* showed the advantages such as more virulence against *E. vittella* (Seenivasan and Sivakumar, 2014; Seenivasan, 2020). Recently, these EPN mass production under in vitro solid culture was standardized (Seenivasan, 2017). However, the successful use of these EPN strains as potential biopesticide against cotton bollworms is possible only after standardization of suitable formulation. EPN are widely formulated using either solid or semiliquid substrates immediately after they multiplied in vitro culture techniques. Formulation of infective juveniles (IJs) of EPN is very essential to enhance the storage or shelf life and for easy transport and handling. In a better formulation, the mortality of IJs is little and IJs are more virulent until they have to reach the end user or field. Hence, the present study was

carried out with the following objectives; i) to study the survival of *H. bacteriophora* strain KKMH1 and *S. carpocapsae* strain APKS2 formulated in alginate gel, talc and sponge in comparison to the IJs in water at refrigerated condition (5°C) and incubator at 25°C and ii) to study the virulence of the different formulations of *S. carpocapsae* APKS2 and *H. bacteriophora* KKMH1 against *E. vitella.*

MATERIALS AND METHODS

Two EPN strains namely APKS2 (*S. carpocapsae*) and KKMH1 (*H. bacteriophora)*, were taken from the Department of Nematology, Tamil Nadu Agricultural University (TNAU), Coimbatore. They were multiplied by in vitro solid culture technique using on modified Wouts medium as per Seenivasan (2017). Freshly harvested IJs were used for making different formulations. The test insect larvae *E. vittella* was collected from a standing cotton crop at TNAU farm and from the farmer's fields at Thondamuthur village in Coimbatore district of Tamil Nadu, India. They were sorted out and the fourth instar larvae of uniform size were used in the laboratory experiments. The alginate gel formulation of the IJs of *S. carpocapse* (APKS2) and *H. bacteriophora* (KKMH1) were prepared after the method given by Navon et al. (2002). Wettable powder and polyether-polyurethane sponge formulations was prepared after Divya et al. (2011). Freshly harvested lJs concentrated to 50,000 IJs/ ml was considered as water formulation. Each four formulations of *S. carpocapse* (APKS2) and *H. bacteriophora* (KKMH1) were stored at 5 and at 25°C. At weekly intervals, each formulation at two different temperatures were taken and used for survival and virulence test. For survival test, 1 g from alginate or talc and 1 ml from sponge or water were randomly taken and diluted in 10 ml distilled water. Three 1 ml subsample from each 10 ml suspension was used to count survival rate of IJs. The live and dead IJs were examined and counted under a stereozoom microscope (Kozo Zoom 645). The IJs were considered dead if they did not move on probing with a fine needle.

For virulence test, 1 g from alginate or talc and 1 ml from sponge or water were randomly taken, diluted in 2 ml distilled water and used as inoculum at 2 ml/ test unit. The test was conducted in 6-cm-diam petri dishes lined with moist filter paper disc. Five 4th instar larvae of *E. vitella* were put in petri dishes lined with filter paper. Then 2 ml of EPN suspension were applied to the petri dishes. Control plates received only 2 ml distilled water. The dishes were sealed with para-film, arranged in a completely randomized design (CRD) and incubated at room temperature. Each formulation at each temperature consisted of five replicates (One Petri dish = one replicate). After 4 days, larval mortality was recorded. The dead insects were dissected in Ringer's solution to confirm the death by EPN. Insect mortality was corrected according to the control treatment values using Abbott's formula (Abbott, 1925). Percentage data were arc sine transformed before analysis. Survival and virulence differences at weekly intervals from 1- 12 weeks for each formulation and at each temperature were detected through analysis of variance (ANOVA) and differences between time intervals were compared using Tukey's HSD (honestly significant difference) test. The software used for analysis was SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences among means in all experiments were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Alginate gel: In this formulation, *S. carpocapsae* (APKS2) and *H. bacteriophora* (KKMH1) survived longer up to 12 weeks with 70.2-76.1% survival at 5°C. At 25°C, APKS2 survived up to 62.0% until 12th week, but KKMH1 survived 38%. The results are similar to the findings of Grewal (2002) and Umamaheswari et al. (2006). EPN stored at low temperature (5°C) survived longer than at high temperature (25°C). Accumulation of more trehalose is attributed as a survival mechanism of IJs of EPN at low temperatures (Jagdale and Grewal, 2007). Infectivity was 100% up to 6-7 weeks when they stored at 5°C. The infectivity of both strains gradually decreased, but caused more than 50% mortality up to 12 weeks when they stored at 5°C. At 25°C, APKS2 has 52.5% infectivity when they stored for 12 weeks, but infectivity declined up to 25% for KKMH1. The reason for the less infectivity of KKMH1 at 12 weeks is attributed to its lowest survival rate (38%) recorded after 12 weeks (Table 1).

Talc formulation: In this, *S. carpocapsae* (APKS2) survived longer up to 10 weeks with 50% survival at 5°C, but at 25°C, 50% survival was found only for 8 weeks (Table 1). Hugar (2010) first tried the talc powder to formulate *H. indica.* This study showed that talc formulation retains survivability of *S. carpocapsae* and *H. bacteriophora* for >50% up to 2 months. Grewal (2000) reported that induced partial anhydrobiosis in *S. carpocapsae* by gradual desiccation. Hence, it is speculated that the wet talc formulations of the present EPN strains might have undergone partial anhydrobiosis during storage causing enhanced survival. Infectivity was 100% for APKS2 up to 5 weeks when they stored

Table 1. Survival and virulence of IJs of *S. carpocapsae* (APKS2) and *H. bacteriophora* (KKMH1)-formulated in alginate, talc, sponge and water

Period Weeks	Survival rate of IJs stored at 5°C (%)		Mortality of *Earias vitella* caused by IJs stored at 5°C (%)		Survival rate of IJs stored at 25°C (%)		Mortality of *Earias vitella* caused by IJs stored at 25°C (%)	
	APKS2	KKMH1	APKS2	KKMH1	APKS2	KKMH1	APKS2	KKMH1
I. Alginate gel								
1	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
2	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
3	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	95.3± 1.3 (81.1) b	92.7± 0.9 (77.8) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
4	92.5± 1.7 (77.6) b	90.3± 0.7 (75.2) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	95.0± 1.5 (80.7) b	90.0± 1.1 (74.9) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
5	92.0± 1.2 (77.0) b	90.0± 1.1 (74.9) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	90.0± 1.7 (74.9) cb	82.0± 1.7 (67.9) c	100± 0.0 (94.2) a	92.5± 0.8 (77.6) b
6	92.0± 1.9 (77.0) b	82.3± 2.4 (68.1) c	100± 0.0 (94.2) a	100± 0.0 (94.2) a	85.0± 2.2 (70.3) c	76.0± 2.3 (63.5) cd	97.5± 0.7 (84.7) b	90.0± 1.1 (74.9) b
7	90.3± 1.7 (75.2) b	80.7± 3.4 (66.9) e	100± 0.0 (94.2) a	92.5± 2.3 (77.6) b	82.0± 2.3 (67.9) c	75.0± 2.1 (62.8) d	92.5± 1.3 (77.6) c	87.5± 1.3 (72.5) cb
8	85.7± 2.4 (70.9) bc	80.3± 2.6 (66.6) c	92.5± 1.3 (77.6) b	90.0± 1.6 (74.9) b	80.0± 2.1 (66.4) c	70.0± 2.7 (59.4) d	87.5± 1.7 (72.5) cd	82.5± 1.6 (68.3) c
9	80.3± 2.7 (66.6) c	78.7± 2.3 (65.4) c	82.5± 2.1 (68.3) c	87.5± 2.7 (72.5) b	77.0± 3.7 (64.2) dc	62.0± 3.3 (54.3) e	75.0± 2.3 (62.8) e	70.0± 2.7 (59.4) d
10	80.0± 2.1 (66.4) c	77.3± 3.7 (64.4) c	70.5± 2.4 (59.7) d	75.0± 2.5 (62.8) c	70.0± 3.1 (59.4) de	52.0± 3.1 (48.3) f	62.5± 2.7 (54.7) f	42.5± 3.5 (42.6) e
11	77.3± 2.6 (64.3) c	74.3± 3.1 (62.3) cd	65.0± 3.8 (56.2) de	67.5± 3.1 (57.8) c	65.0± 3.3 (56.2) e	46.0± 3.3 (44.7) f	60.0± 3.8 (53.1) f	40.0± 3.2 (41.0) e
12	76.1± 3.2 (63.5) c	70.2± 2.6 (59.6) d	62.5± 3.2 (54.7) e	55.0± 3.4 (50.1) d	62.0± 3.9 (54.3) e	38.0± 3.9 (39.8) gf	52.5± 3.4 (48.6) gf	25.0± 3.7 (31.4) f
II. Talc powder								
1	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
2	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
3	100± 0.0 (94.2) a	95.0± 0.6 (80.7) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	97.0± 0.3 (83.8) b	96.0± 0.5 (82.1) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
4	95.0± 0.7 (80.7) b	95.0± 0.3 (80.7) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	95.0± 0.5 (80.7) b	95.0± 0.3 (80.7) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
5	90.0± 0.9 (74.9) c	85.0± 0.9 (70.3) c	100± 0.0 (94.2) a	97.5± 0.3 (84.7) b	87.0± 1.5 (72.1) c	82.0± 0.7 (67.9) c	95.0± 0.3 (80.7) b	92.5± 0.7 (77.6) b
6	85.0± 1.1 (70.3) cd	75.0± 1.6 (62.8) d	95.0± 0.6 (80.7) b	90.0± 0.5 (74.9) c	70.0± 1.7 (59.4) d	70.0± 2.3 (59.4) d	85.0± 0.8 (70.3) c	82.5± 0.9 (68.3) c
7	70.0± 2.3 (59.4) e	62.0± 1.7 (54.3) e	82.5± 0.9 (68.3) c	77.5± 1.3 (64.5) d	60.0± 2.9 (53.1) e	55.0± 3.1 (50.1) e	70.0± 1.7 (59.4) d	67.5± 2.4 (57.8) d
8	60.0± 2.7 (53.1) f	55.0± 2.1 (50.1) e	75.0± 1.1 (62.8) d	70.0± 1.1 (59.4) d	55.0± 2.7 (50.1) e	45.0± 1.7 (44.1) f	62.5± 3.4 (54.7) d	52.5± 3.3 (48.6) e
9	55.0± 2.5 (50.1) ef	40.0± 2.3 (41.0) f	67.5± 1.3 (57.8) e	57.5± 1.3 (51.6) e	45.0± 3.2 (44.1) f	30.0± 1.3 (34.7) g	57.5± 2.1 (51.6) ed	40.0± 1.5 (41.0) f
10	50.0± 3.4 (47.1) f	40.0± 2.1 (41.0) f	60.0± 2.6 (53.1) fe	50.0± 2.3 (47.1) fe	35.0± 1.7 (37.9) g	10.0± 0.7 (19.3) h	50.0± 1.6 (47.1) e	15.0± 0.7 (23.8) g
11	45.0± 3.1 (44.1) fg	30.0± 3.2 (34.7) g	52.5± 1.4 (48.6) g	42.5± 3.5 (42.6) g	20.0± 2.1 (27.8) h	0.0± 0.0 (0) i	22.5± 0.7 (29.6) f	0.0± 0.0 (0) h
12	40.0± 3.3 (41.0) g	25.0± 2.7 (31.4) g	50.0± 1.7 (47.1) g	35.0± 2.7 (37.9) hg	10.0± 1.6 (19.3) i	0.0± 0.0 (0) i	5.0± 0.3 (13.5) g	0.0± 0.0 (0) h

(contd...)

(contd. Table 1)

III. Polyurethane sponge								
1	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100 ± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
2	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
3	100± 0.0 (94.2) a	97.0± 0.0 (83.8) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	97.0± 0.3 (83.8) b	92.0± 0.5 (77.0) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
4	95.7± 0.4 (81.7) b	90.0± 0.7 (74.9) c	100± 0.0 (94.2) a	100± 0.0 (94.2) a	90.0± 0.7 (74.9) c	84.0± 1.3 (69.5) c	100± 0.0 (94.2) a	95.0± 0.7 (80.7) b
5	85.0± 0.9 (70.3) c	80.0± 1.1 (66.4) d	92.5± 0.8 (77.6) b	90.0± 0.6 (74.9) b	82.0± 1.1 (67.9) d	82.0± 1.5 (67.9) c	90.0± 1.1 (74.9) b	90.0± 0.9 (74.9) c
6	80.0± 1.3 (66.4) dc	65.0± 1.7 (56.2) e	90.0± 0.7 (74.9) b	77.5± 1.3 (64.5) c	70.0± 2.3 (59.4) e	65.0± 3.0 (56.2) d	80.0± 1.4 (66.4) c	77.5± 1.3 (64.5) d
7	60.0± 3.2 (53.1) e	55.0± 3.0 (50.1) fe	72.0± 1.4 (60.7) c	67.5± 2.1 (57.8) d	55.0± 1.9 (50.1) f	50.0± 1.6 (47.1) e	67.5± 2.7 (57.8) d	62.5± 2.1 (54.7) e
8	50.0± 2.7 (47.1) f	50.0± 2.1 (47.1) f	60.0± 2.5 (53.1) d	55.0± 1.7 (50.1) e	40.0± 3.0 (41.0) g	40.0± 0.8 (41.0) f	55.0± 3.2 (50.1) e	50.0± 2.5 (47.1) f
9	45.0± 1.3 (44.1) f	30.0± 1.1 (34.7) g	50.0± 3.1 (47.1) e	40.0± 1.1 (41.0) f	32.0± 1.1 (36.0) hg	45.0± 1.7 (44.1) ef	45.0± 1.6 (44.1) f	20.0± 0.8 (27.8) g
10	30.0± 0.7 (34.7) g	10.0± 0.7 (19.3) h	40.0± 2.3 (41.0) f	0.0± 0.0 (0) g	12.0± 0.7 (21.2) i	0.0± 0.0 (0) g	5.0± 0.3 (13.5) g	0.0± 0.0 (0) h
11	20.0± 0.7 (27.8) h	0.0± 0.0 (0) i	25.0± 1.1 (31.4) g	0.0± 0.0 (0) g	0.0± 0.0 (0) j	0.0± 0.0 (0) g	0.0± 0.0 (0) h	0.0± 0.0 (0) h
12	10.0± 0.5 (19.3) i	0.0± 0.0 (0) i	0.0± 0.0 (0) h	0.0± 0.0 (0) g	0.0± 0.0 (0) j	0.0± 0.0 (0) g	0.0± 0.0 (0) h	0.0± 0.0 (0) h
IV. Water								
1	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a
2	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	100± 0.0 (94.2) a	96.0± 0.3 (82.1) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a
3	92.0± 0.7 (77.0) b	85.0± 0.9 (70.3) b	100± 0.0 (94.2) a	100± 0.0 (94.2) a	90.0± 0.6 (74.9) b	80.0± 1.1 (66.4) c	100± 0.0 (94.2) a	92.5± 0.5 (77.6) b
4	90.0± 1.1 (74.9) b	70.0± 1.6 (59.4) c	100± 0.0 (94.2) a	85.0± 0.8 (70.3) b	80.0± 1.2 (66.4) c	72.0± 1.8 (60.7) d	90.0± 0.7 (74.9) b	80.0± 1.4 (66.4) c
5	80.0± 1.7 (66.4) c	65.0± 2.4 (56.2) c	90.0± 0.7 (74.9) b	77.5± 1.3 (64.5) c	62.0± 1.8 (54.3) d	60.0± 2.4 (53.1) e	77.5± 1.6 (64.5) c	70.0± 2.1 (59.4) d
6	65.0± 3.1 (56.2) d	50.0± 2.1 (47.1) d	70.0± 1.5 (59.4) c	60.0± 2.9 (53.1) d	45.0± 2.7 (44.1) e	45.0± 2.6 (44.1) f	65.0± 2.3 (56.2) d	65.0± 2.9 (56.2) d
7	55.0± 2.5 (50.1) e	43.0± 2.7 (42.9) e	65.0± 3.2 (56.2) c	55.0± 1.8 (50.1) d	32.0± 1.3 (36.0) f	25.0± 1.3 (31.4) g	50.0± 3.0 (47.1) e	50.0± 2.3 (47.1) e
8	50.0± 2.1 (47.1) fe	25.0± .0.9 (31.4) f	60.0± 2.6 (53.1) dc	37.5± 1.1 (39.5) e	30.0± 0.9 (34.7) f	10.0± 0.5 (19.3) h	45.0± 2.1 (44.1) ef	40.0± 1.4 (41.0) f
9	45.0± 2.4 (44.1) f	12.0± 0.5 (21.2) g	50.0± 2.1 (47.1) e	0.0± 0.0 (0) f	12.0± 0.5 (21.2) g	0.0± 0.0 (0) i	40.0± 1.7 (41.0) f	0.0± 0.0 (0) g
10	36.0± 1.3 (38.6) g	0.0± 0.0 (0) h	0.0± 0.0 (0) f	0.0± 0.0 (0) f	0.0± 0.0 (0) h	0.0± 0.0 (0) i	0.0± 0.0 (0) g	0.0± 0.0 (0) g
11	25.0± 1.5 (31.4) h	0.0± 0.0 (0) h	0.0± 0.0 (0) f	0.0± 0.0 (0) f	0.0± 0.0 (0) h	0.0± 0.0 (0) i	0.0± 0.0 (0) g	0.0± 0.0 (0) g
12	± 0.0 (0) i	± 0.0 (0) h	0.0± 0.0 (0) f	0.0± 0.0 (0) f	0.0± 0.0 (0) h	0.0± 0.0 (0) i	0.0± 0.0 (0) g	0.0± 0.0 (0) g

Means (± SD) followed by same letter in columns not significantly different at $p<0.05$ (Tukey's HSD test). Figures in parentheses arc sine transformed values.

at 5°C where as 4 weeks for KKMH1 (Table 1). The virulence of talc formulated *Steinernema seemae* against *Helicoverpa armigera* (up to 62% mortality) by Ali and Asif (2011) corroborate the present observations. In general, the survival and virulence were relatively lower in talc when compared to gel formulation.

Sponge formulation: In this formulation, both EPNs survived up to 8 weeks with 50-55% survival at 5°C, but at 25°C >50% survival found only up to 7 weeks. In sponge formulation EPN survival gradually decreased with increase of storage time and this effect was drastic at 25°C storage. The low survival at high

temperature may be due to rapid loss of water from sponge formulations. Infectivity was 100% up to 4 weeks when they stored at 5°C. At 25°C, these revealed 50-55% infectivity when stored for 8 weeks (Table 1). These results are in line with Hugar (2010) who observed 80% mortality of *H. armigera* with sponge formulated 60 days old *H. indica* stored at 10°C, but 36% mortality when stored at 28°C.

Water: In water, test EPNs survived 50% for 8 weeks at 5°C, but at 25°C, 62% survival found only for 5 weeks. The 100% survival was observed up to 2 weeks at both 5 and 25°C (Table 1). The results showed that the tested strains could be stored in plain water for short-term. Divya et al. (2011) also recorded observations close to the present ones such as 70% survival of *H. indica* up to 5 weeks. Infectivity was 100% for APKS2 up to 4 weeks when they stored at 5°C whereas 3 weeks when it was stored at 25 °C. APKS2 and KKMH1 showed 50% infectivity when stored for 7 weeks (Table 1). It indicates that virulence of the EPNs maintained better, though their survival rate decreases drastically. In addition, at low temperature (5°C) virulence was prolonged for long period up to 1 month with 100% virulence. The reason for this might be due to the reduced activity of IJs and more conservation of energy reserve at low temperature than at high temperature (Grewal, 2000). It is concluded that *S. carpocapsae* (APKS2) and *H. bacteriophora* (KKMH1) should be formulated either alginate gel or talc powder for long term storage up to 3 months. For usage in 1-2 months these can be formulated in sponges and stored at 5°C. The *S. carpocapsae* (APKS2) and *H. bacteriophora* (KKMH1) need not be formulated in any special media and can be kept as water concentrate for immediate use within 14-21 days.

ACKNOWLEDGEMENTS

The author thanks the Life Science Research Board, Defense Research and Development Organization, New Delhi for the financial support through a grant (No. DLS/81/48222/LSRB-136/FSB/2007).

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(Manuscript Received: November, 2020; Revised: March, 2021;
Accepted: March, 2021; Online Published: July, 2021)
Online published (Preview) in www.entosocindia.org Ref. No. e20424