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Standardization of Formulation and Application Approaches for Field Implementation of EPNs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Entomopathogenic nematodes (EPNs) live parasitically inside the infected insect host, and so they are termed as endoparasitic. They infect different types of insect living in the soil like the larval forms of moths, butterflies and beetles as well as adult forms of beetles, grasshoppers and crickets. The present experiment was conducted under both laboratory and field conditions at CCS Haryana Agriculture University, Hisar. Two most virulent strains of EPNs, *Metarhabditis amsactae* strain HAR-St-II and HAR-Ht-III were taken and their formulations were standardized using six different media *i.e.* cadaver based formulation, water, alginate gel, foam chips, clay chips and water dispersible granules. Among these formulations, cadaver based formulation was found to be best. Maximum numbers of active IJs after 90 days were obtained from this formulation were 3370 and

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2728 /Petri plate in strain HAR-St-II and HAR- Ht-III of *M. amsactae*, respectively. Further this, experiment was conducted at field level for EPNs suspension was standardized using different size of nozzle opening i.e. 25 µm, 50 µm, 75 µm and 100 µm on knapsack sprayer. *Spodoptera litura* was selected as a test insect in okra crop for foliar application of EPNs and results revealed that 100 µm nozzle opening size gave maximum larval mortality of *S. litura*. Per cent mortality of *S. litura* was increased with increase in size of nozzle and period of observation

Keywords: Entomopathogenic nematodes; Spodoptera litura; formulation; nozzle size; mortality.

1. INTRODUCTION

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae have been applied with great success as a biocide against a wide range of insect pests [1]. Both families have similar traits and life cycles, despite not being closely related with the bacterial symbiont of *Steinernema* species belonging to the genus *Xenorhabdus,* whereas *Heterorhabditis* is associated with *Photorhabdus* [2]. Steinernematids and heterorhabditids have a free-living stage, called the infective juveniles (IJs). This stage occurs freely in the soil, where the IJs can actively seek out and find a suitable host insect. The success of EPNs formulated products for soil application, as well as their foliar application in the greenhouse production of crops [3,4,5], has rekindled an interest in their commercial field application against above-ground insect pests [6]. However, as soil-adapted organisms, EPNs are poorly suited to above-ground environments, which often feature low relative humidity, extremes of temperature, and exposure to ultraviolet radiation. The above-mentioned factors result in rapid desiccation and death, negatively impacting on EPNs efficacy as biocontrol agents.

The main factor appears to be humidity, with nematode survival being prolonged in humid environments. Various formulations have been developed in order to preserve the intergrity of stored EPNs, to facilitate their storage and application. These formulations include activated charcoal, alginate and polyacrylamide gels, baits, clay, paste, peat, polyurethane sponge, vermiculite and water-dispersible granules. Successful storage under refrigeration conditions ranges from one to seven months depending on the EPN species [7]. EPNs formulated and applied as insect cadavers showed higher efficacy for the pest control than the EPNs applied in an aqueous solution. Additives used in the formulation have been found to increase the survival and maintain the virulence of the EPNs. Aqueous suspension is commonly used formulation that is mainly used for storage, transportation and applications [8]. Live insects pre-infected with EPNs (living insect bombs) are the latest formulation and application approach to improve the efficacy of EPNs [9]. Tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is a polyphagous, notorious leaf feeding insect globally important serious crop pest distributed widely from tropical to temperate regions, throughout the Middle East, East Asia, Pacific Islands and Oceania (Bhati, 2020; Chandi et al. 2022). Variations in the plant host species have been recorded by various authors and this insect attacking more than 120 different host plants including cotton, okra, tobacco, maize, cabbage, cauliflower, tomato, greengram, groundnut, tea etc., causing significant economic loss in vegetable and field crop production around the Asia- Pacific region [10,11]. Larvae of *S. litura* feed on various parts of a plant comprising leaves, flowers and fruits, causing leaf skeletalization in the early growth stage of the crop and severe defoliation in later stages overall leading to a reduction in the photosynthetic capacity of plants (Bhati, 2020).

2. MATERIALS AND METHODOLOGY

2.1 Standardization of Formulation of Native Strain of EPNs

The experiment was conducted under laboratory conditions in the Department of Nematology. Two most virulent strains of EPNs, *Metarhabditis amsactae* strain HAR-St-II and HAR-Ht-III were taken and their formulations were standardized using six different media. These media used as treatments were water, alginate gel, dead host cadaver/ cadaver based formulations, water dispersible granules, clay chips and foam chips with inoculum level of 100 IJs/ treatment. The experiment was laid out with completely randomized design with four replications. The number of active IJs per formulation was counted thrice under a stereoscopic microscope and

mean value was worked out at 15 days intervals up to 3 month.

2.2 Efficacy of IJs Survival After 90 Days in Different Formulations

Three formulation viz., water, dead cadavers and alginate gel consisting EPNs were inoculated on *Galleria mellonella* at inoculum level of 10 IJs/larva with five replications under laboratory conditions. Observation on mortality of *G. mellonella* larvae was recorded on 1, 2, 3 and 4 days after inoculation.

2.3 Standardization of Application Technology of EPNs

Two most virulent strains HAR-St-II and HAR-Ht-III of *M. amsactae* were selected for their application against test insect, *S. litura* in okra field. The experiment was done in research area of Department of Nematology. Suspension of EPNs was standardized using different size of nozzle opening *i.e.* 25 µm, 50 µm, 75 µm and 100 µm on knapsack sprayer. Okra crop was grown in plots of 2x2 m with spacing of 60 x 45 cm. Five plants per plot were selected and each treatment was replicated five times. Observations on the number of dead insect per plant were recorded 1, 2, 3 and 4 days after spray. Number of dead insects on aerial parts of the plants was counted on 1, 2, 3 and 4 days after spray.

2.4 Statistical Analysis

The statistical analysis of data obtained in experiments was done based on CRD/ RBD as per experiment requirement using OPSTAT software available online at CCS HAU website (www.hau.ernet.in). Comparison of treatments was made at 5 % level of significance. Necessary transformation of data was done as per requirement.

3. RESULTS

3.1 Standardization of Formulation of Native Strains of EPNs

Survival of strains HAR-St-II and HAR-Ht-III was evaluated in five formulations to standardize the formulations.

Data presented in Tables 1 revealed that cadaver based formulation was found best among all the formulations tested and it had significantly highest number of active IJs (3801.4/ Petri plate). In rest of the formulations, alginate gel showed maximum survival (58.5/ Petri plate) though it was significantly less than survival in water. As the observation time increased, there was decrease in number of active IJs irrespective of formulations. Maximum mean survival of IJs was 749.3 IJs/Petri plate after 15 days which declined to 578.2 IJs/ Petri plate after 90 days. In clay chips, there was no survival after first observation. In water dispersible granules and foam chips, survival declined rapidly upto 60 days and there was no survival after this period. After one month, in alginate gel and foam chips, survival was 80 per cent which was less than survival in water (100 %). Recovery of active IJs in cadaver based formulation was maximum (nearly 4154/ Petri plate) on 15th day which slowly declined upto 90 days.

The results obtained on formulations for *M. amsactae* strain HAR-Ht-III were also similar to strain HAR-St-II. Data in Table 2 show that maximum mean survival of IJs was recorded in cadaver based formulation followed by water as control. In all other formulations, except cadaver based formulation, survival of IJs was less than water. Considering mean survival, irrespective of formulation, the number of active IJs significantly declined at each observation time. The number of active IJs recovered in cadaver based formulation was maximum (3176 IJs/ Petri plate) on 15th day which showed significant decline at each observation finally reaching to (2728 IJs/ Petri plate) after 90 days. In clay chips survival was less than 10 per cent and it was negligible after 30 days onwards. In water dispersible granules, after 30 days survival was less than 30 per cent which was declined to less than 10 per cent after 60 days. In foam chips and alginate gel about 50 per cent survival was recorded on 45th and 60th day, respectively but the survival in these formulations was also less than water.

3.2 Efficacy of IJs Survived After 90 Days in Different Formulations on *G. mellonella*

The IJs surviving in water, cadaver based formulation and alginate gel after 90 days were tested for their efficacy on *G. mellonella*. Results of both the strains of HAR-St-II and HAR-Ht-III were presented in Table 3. Since, there was no survival in clay chips, water dispersible granules and foam chips after 90 days, these formulations were not included in this efficacy test.

Table 1. Survival of IJs of *Metarhabditis amsactae,* strain HAR-St-II in different formulations

Formulations	Number of active IJs at different time periods						
	15th day	30th day	45th day	60th day	75th day	90th day	Mean
Water	100.0 (10.1)	100.0 (10.1)	97.3(9.9)	92.8(9.7)	87.8 (9.5)	81.0(9.1)	93.0 (9.7)
Alginate gel	91.5(9.7)	80.0(9.1)	70.0(8.5)	59.3 (7.8)	31.5(5.8)	18.8(4.6)	58.5(7.6)
Cadaver based Formulation	4153.8 (64.5)	4039.0 (63.6)	3919.8 (62.6)	3776.5 (61.5)	3550.3 (59.6)	3369.3 (58.0)	3801.4 (61.6)
Water dispersible Granules	51.0 (7.3)	23.8 (5.1)	13.0 (3.9)	6.5 (2.9)	0.0 (1.4)	0.0 (1.4)	15.7 (3.7)
Clay chips	6.3 (2.9)	1.0 (1.4)	0.0 (1.4)	0.0 (1.4)	0.0 (1.4)	0.0 (1.4)	1.2 (1.7)
Foam chips	93.0 (9.7)	80.3 (9.1)	31.3 (5.8)	11.5 (3.6)	0.0 (1.4)	0.0 (1.4)	36.0 (5.2)
Mean	749.3 (17.4)	720.5 (16.4)	688.5 (15.4)	657.8 (14.5)	611.6 (13.2)	578.2 (12.7)	
CD at 5 %	formulation: (0.26), time: (0.26), time x formulation: (0.65)						

Values in parentheses are n+ 1 square root transformations, initial inoculum- 100 IJs/ Petri plate

Table 2. Survival of IJs of *Metarhabditis amsactae* strain HAR-Ht-III in different formulations

Formulations	Number of active IJs at different time periods						
	15th day	30th day	45th day	60th day	75th day	90th day	Mean
Water	98.5(10.0)	95.0(9.8)	92.5(9.7)	89.3(9.6)	86.0(9.4)	82.3(9.2)	90.6 (9.6)
Alginate gel	89.5(9.6)	78.0(8.9)	67.3(8.3)	49.8(7.2)	24.0(5.1)	12.5(3.8)	53.5 (7.2)
Cadaver based formulation	3176.0 (56.4)	3094.3 (55.6)	2989.8 (54.7)	2897.8 (53.8)	2800.0 (52.9)	2728.0 (52.2)	2947.6 (54.3)
Water dispersible granules	57.5(7.7)	29.5(5.6)	18.5(4.5)	9.3(3.3)	0.8(1.6)	0.0(1.4)	19.3 (4.0)
Clay chips	9.3 (3.3)	1.8(1.9)	0.0(1.4)	0.0(1.4)	0.0(1.4)	0.0(1.4)	1.8(1.8)
Foam chips	94.5(9.8)	85.5(9.4)	49.5(7.2)	24.3(5.1)	5.8(2.8)	0.8(1.6)	43.4 (6.0)
Mean	587.5 (16.1)	564.0(15.2)	536.3 (14.3)	511.7 (13.4)	486.1 (12.2)	470.6(11.6)	
CD at 5 %	formulation: (0.29) time (0.29), time x formulation: (0.71)						

Values in parentheses are n+ 1 square root transformations, initial inoculum- 100 IJs/ Petri plate

Table 3. Efficacy of IJs of HAR-St-II and HAR-Ht-III recovered from different formulations on *G. mellonella*

Treatments	Per cent mortality of *G. mellonella* larvae using strain HAR-St-II					Per cent mortality of *G. mellonella* larvae using strain HAR-Ht-III				
	24 h	**48 h**	**72 h**	**96 h**	**Mean**	**24 h**	**48 h**	**72 h**	**96 h**	**Mean**
Water	10.1(16.4)	28.0(31.7)	62.0(52.2)	89.9(76.3)	47.5(44.1)	8.1(13.8)	22 .0(27.6)	56 .0(48.5)	81.9(66.8)	42.1(39.2)
Cadaver based Formulations	28.0(31.7)	74.0(59.8)	91.9(76.1)	99.8(87.1)	73.4(63.7)	14 .0(21.7)	42 .0(40.3)	76 .0(60.8)	93.8(79.2)	56.4(50.5)
Alginate gel	4.2(9.1)	22.0(27.2)	54.0(47.3)	80.0(65.5)	40.0(37.3)	0(2.9)	14.1(19.9)	36 .0(36.6)	71.9(61.0)	30.5(30.1)
Untreated check	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)	0(2.9)
Mean	10.6(15.0)	31.1(30.4)	52.0(44.6)	67.5(57.9)	-	5.6(10.3)	19.5(22.7)	42. 1(37.2)	62.0(52.5)	-
CD at 5 %	formulation: (4.8), time: (4.8), formulation x time: (9.8)					formulations: (4.9), time: (4.9), formulations x time: (9.9)				

Values in parentheses are angular transformations, inoculum level: 10 IJs/ larva

Data showed in Table 3, revealed that mean per cent mortality using strain HAR-St-II, was highest in cadaver based formulation *i.e.* 73.4 per cent followed by IJs survived in water (47.5 %). In alginate gel, mortality of *G. mellonella* larvae was significantly less (40.0 %) than IJs survived in water. Mean mortality of *Galleria* larvae increased with period of observations. It was maximum (67.5 %) after 96 h of inoculation and minimum (10.6 %) after 24 h. After 24 h, 10.1 per cent mortality was recorded in water which was statistically at par in alginate gel but lower than cadaver based formulation. Similarily, 28.0 per cent mortality was observed in water after 48 h which was statistically at par in alginate gel but significantly less than cadaver based formulation. After 72 h, 62.0 per cent mortality was statistically different in water and alginate gel. After 96 h, 90.0 per cent mortality was observed in water which significantly higher than alginate gel but lower than cadaver based formulation. In strain HAR-Ht-III, data showed in Table 3 that mean mortality in all the treatments was significantly different maximum being in cadaver based formulation and minimum in alginate gel. Irrespective of formulations, mean mortality of *Galleria* larvae at period of observation differed significantly. It was highest after 96 h followed by 72, 48 and 24 h. After 24 h, mortality of *Galleria* larvae was statistically similar in water and cadaver based formulation whereas no mortality was observed in alginate gel and control. After 72 h, maximum mortality (76 %) of *G. mellonella* larvae was achieved in cadaver based formulation followed by water (56.0 %) and alginate gel (36.0 %) and all these differed significantly.

3.3 Standardization of Application Technology of EPNs

The application of *M. amsactae* strains HAR-St-II and HAR-Ht-III was standardized using four size of nozzles i.e. 25 µm, 50 µm, 75 µm and 100 µm on knapsack sprayer. Eighty ml nematode suspension containing 10,000 IJs/ml was used in each treatment. Observations were recorded daily on dead larvae of *S. litura,* upto four days. Data in Table 4 indicate that maximum mean mortality (61.7 %) was observed at 100 µm nozzle which differed significantly from all other treatments. Mortality of *S. litura* increased with increase in period of observations.

Irrespective of nozzle size, mean mortality on 1st day was minimum (18.1 %), which increased to 62.2 per cent on 4th day. On 1st day, there was no mortality in spray with 25 µm nozzle but 15.6, 25.6 and 31.1 per cent mortality was observed in 50, 75 and 100 µm nozzle, respectively. On 2nd day, significantly higher (56.1 %) mortality was observed in 100 µm nozzle than all other nozzle sizes. On 3rd day, mortality of *S. litura* increased to 70.0 per cent which was significantly higher than the mortality in other treatments. On 4th day of spray highest per cent mortality (89.4 %) was observed in 100 µm nozzle followed by 77.2 per cent in 75 µm nozzle. Data in Table 5 show that as the mean per cent mortality of *S. litura* caused by *M. amsactae* strain HAR-Ht-III significantly increased with increase in size of nozzle and it was maximum (62.6 %) in 100 µm nozzle and minimum (11.2 %) in 25 µm nozzle.

As the period of observations increased, there was significantly increase in mean per cent mortality of *S. litura* which was maximum (62.2 %) on 4th day and minimum (18.0 %) on 1st day. Mortality of *S. litura* was similar in 100 µm nozzle on 3rd day (75.0 %) to mortality in 75 µm nozzle on 4th day (75.6 %). Similarily, mortality on 3rd day in 75 µm nozzle and on 4th day in 50 µm nozzle were statically different. More than 50.0 per cent mortality was observed on 3rd day in 50 µm nozzle whereas it was observed on 2nd day in 100 µm nozzle.

Table 4. Effect of spray of *Metarhabditis amsactae* strain HAR-St-II by different size of nozzles on mortality of *Spodoptera litura*

Treatments (size of nozzle)	Per cent moratlity of *Spodoptera litura* at different time intervals				
	1st day	2nd day	3rd day	4th day	Mean
25 µm	0.3 (3.1)	7.8 (16.1)	16.7 (24.0)	26.7 (31.1)	12.8 (18.6)
50 µm	15.6 (23.1)	34.4 (35.9)	45.0 (42.1)	57.2 (49.1)	38.1 (37.6)
75 µm	25.6 (30.3)	44.4 (41.8)	58.3 (49.8)	77.2 (61.5)	51.4 (45.8)
100 µm	31.1 (33.9)	56.1 (48.5)	70.0 (56.8)	89.4 (71.1)	61.7 (50.2)
Mean	18.1 (22.6)	35.7 (35.6)	47.5 (43.2)	62.2 (53.2)	-
C.D. at 5 %	treatment: (1.1), time: (1.1), time x treatment: (2.3)				

Values in parentheses show angular transformations

Table 5. Effect of spray of *Metarhabditis amsactae* strain HAR-Ht-III by different size of nozzles on mortality of *Spodoptera litura*

Treatments (size of nozzle)	Per cent moratlity of *Spodoptera litura* at different time intervals				
	1st day	2nd day	3rd day	4th day	Mean
25 µm	0.3 (3.1)	6.7 (16.5)	15.0 (32.1)	22.8 (36.2)	11.2 (22.0)
50 µm	8.3 (14.9)	37.2 (37.6)	51.1 (40.2)	65.0 (53.3)	40.4 (34.7)
75 µm	28.3 (22.7)	41.7 (45.6)	65.6 (54.1)	75.6 (60.0)	52.8 (45.6)
100 µm	35.0 (28.5)	52.2 (53.7)	75.0 (60.4)	88.3 (70.2)	62.6 (53.2)
Mean	18.0 (17.3)	34.4 (38.4)	51.7 (46.7)	62.2 (53.2)	-
C.D. at 5 %	treatment: (1.3), time: (1.3), treatment x time: (2.6)				

Values in parentheses show angular transformations

Data on the mortality of *S. litura* caused by strains HAR-St-II and HAR-Ht-III and its presence on aerial part are presented in Table 6. In strain HAR-St-II, dead larvae of *S. litura* available on plant significantly increased with increase in size of nozzle. Maximum (57.8 %) insects available on aerial parts were recorded in 100 µm size of nozzle and minimum (15.6 %) in 25 µm nozzle. Similarly, in strain HAR-Ht-III, dead larvae of *S. litura* available on plant significantly increased with increase in size of nozzle. Maximum (61.7 %) insects available on aerial parts were recorded in 100 µm size of nozzle followed by 75 µm nozzle (52.2 %) and minimum (20.6 %) in 25 µm nozzle.

3.4 Isolation of EPNs from Dead Insects

Isolation of IJs of *M. amsactae* strain HAR-St-II and strain HAR-Ht-III from cadaver of *S. litura* by spray of EPNs using four size of nozzles, results in Fig. 1 (left) clearly indicated that highest recovery (1521 IJs/cadaver) was obtained in 100 µm nozzle size followed by 75, 50 and 25 µm nozzle size, with yield of IJs 1241, 1022 and 607 IJs/cadaver of *S. litura* at inoculum level of 80 ml suspension per plot containing 10,000 IJs/ ml suspension. Maximum recovery (1880 IJs/cadaver) of *M. amsactae* strain HAR-Ht-III (Fig. 1, right) was obtained in 100 µm nozzle size. Least multiplication (877 IJs/cadaver) was recorded in 25 µm nozzle size, emerged from the cadavers of the *S. litura* with spray of 10,000 IJs/ml suspension.

4. DISCUSSION

In present study, six formulations i.e. cadaver based formulation, water, alginate gel, foam chips, clay chips and water dispersible granules were used to see which cadaver based formulation was found best. In cadaver based formulation, maximum recovery of active IJs was found on 15th day i.e. 3170 IJs in HAR-Ht-III and 4150 in HAR-St-II strains. This number declined gradually upto 90th day. On 90th day, number of active IJs recovered in this formulation was 27 times more in HAR-Ht-III and 33 times more in HAR-St-II of initial number of IJs. This highest number of IJs recovered from cadaver based formulation was due to multiplication of IJs in insect larvae. Cadaver based formulation can be used for the management of insect-pests on small-scale as suggested by Del Valle et al. [12] and Monteiro et al. [13]. Use of this formulation may not be cost effective due to high cost of diet for culturing of *G. mellonella* larvae and is also labour-intensive. If some cheap and easy technique for culturing of *G. mellonella* or some other suitable insects that can be used as host of EPNs for their large scale multiplication is developed then it can be possible to use this formulation for pest management. Advantage of this type of mass multiplication of EPNs is that no specialized equipment or application skill is required. Monteiro et al. [13] suggested that for the control of soil dwelling insect- pests, cadavers containing EPNs can be applied in field or glasshouse simply by broadcast. In present studies, survival of IJs in alginate

Table 6. Number of dead insects on aerial parts of plant

Treatments (size of nozzle)	Per cent mortality of *Spodoptera litura* on aerial parts	
	Strain HAR-St-II	Strain HAR-Ht-III
25 µm	15.6 (23.1)	20.6 (26.8)
50 µm	41.7 (40.1)	47.2 (43.3)
75 µm	49.4 (44.6)	52.2 (46.2)
100 µm	57.8 (49.5)	61.7 (51.8)
C.D. at 5 %	(2.9)	(2.2)

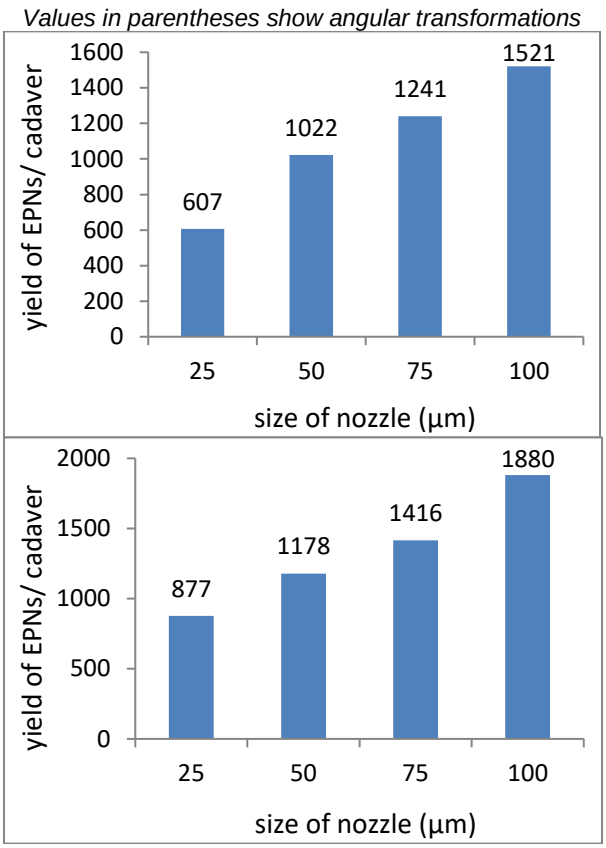

Fig. 1. Isolation of *Metarhabditis amsactae,* strain HAR-St-II (left) and HAR-Ht-III (right) from dead larvae of *Spodoptera litura*

gel and foam chips, was 80 per cent, upto one month, which was less than survival in water (100 %). After 90 days, survival in water was more than 80.0 per cent and no survival was found in foam chips. So, alginate gel and foam chips formulation did not prove better than water. Less survival of IJs in alginate gel and foam chips as compared to water may be due to dessication. Studies of Jangid et al. [14] suggested that maximum (84 %) recovery of IJs of *S. masoodi* was from calcium gel alginate capsules followed by water dispersible granules (80 %) and vermiculate formulations (76 %), respectively at 25 ^{o}C after one month and after second and third month, survival of IJs in all the three formulations declined. Grewal et al. [15] reported that in calcium gel alginate capsule formulation, energy burn rate of EPNs is reduced, which extends the shelf life of *S. carpocapsae* upto 5 to 6th month at room temp. According to Georgis et al. [16], in sponge formulations, EPNs are simply squeezed from the sponge while alginate gel does not directly dissolve in water and sodium citrate can be added for dissolving it. Early morning application of EPNs is recommended as it prevents the dessication and that can negatively affect their efficacy [17]. In present study, survival declined rapidly upto 60 days in water dispersible granules and foam chips and there was no survival after this period. In clay chips, there was no survival after first observation (15 days). Same results were obtained by Maru et al. [18] who found that no survival of *S. carpocapsae* was reported in talc powder formulation after one month. In water dispersible granules and sponge bits, they found 7.73 and 18.81 per cent survival, respectively, after two months, at room temperature. Grewal [19] found that the shelf life and infectivity of *S. carpocapsae* was more in wettable granule formulation than liquid storage at 25°C. The survival in wettable granule formulation lasted up to seven months, as compared to four months in EPNs stored in liquid and the infectivity on *G. mellonella* did not differ significantly in both formulations, after storing IJs upto three months. An effective formulation should be able to withstand adverse conditions like extreme temperature and dessication, and easy in transportation and handling. Therefore, further attempts are required to develop such formulations of EPNs for field applications.

When both the strains of *M. amsactae* were sprayed using 25, 50, 75 and 100 µm size of nozzles, larval mortality of *S. litura* increased with increase in size of nozzle and observation period. Mean mortality of *S. litura* was 62.2 per cent on 4th day in both strains. Maximum mortality of *S. litura* larvae was observed 89.4 per cent in strain HAR-St-II and 88.3 per cent in strain HAR- Ht-III. Among all nozzles, 100 µm nozzle was found the best. This may be due to the reason that delivery of viable EPNs was more in 100 µm nozzle size than other nozzles. Laczynski et al. [20] found that there was no difference in the effect of viability of EPNs using four size i.e. 11008 VK, 11006 VK, 11004 VK and 11002 VK of TeeJet flan fan nozzles. Brusselman et al. (2012) while evaluating the spray of EPNs by flat fan, air induction flat fan, TwinJet spray nozzle, air support system and row application system, found that nozzle type has a minor effect on number of EPNs delivered. Use of air support system and row application system improved nematode delivery on cabbage plant.

5. CONCLUSION

The cadaver-based formulation outperformed other formulations tested, yielding the highest number of active IJs after 90 days, with 3370 and 2728 per Petri plate in strains HAR-St-II and HAR-Ht-III, respectively. These IJs, surviving in water, cadaver-based formulation, and alginate gel, displayed efficacy in killing *G. mellonella* larvae after the 90-day period. Additionally, when both strains of *M. amsactae* were sprayed using nozzles of varying sizes (25, 50, 75, and 100 µm), larval mortality of *S. litura* increased proportionally, with the 100 µm nozzle proving most effective. Strain HAR-Ht-III exhibited higher IJ recovery from *S. litura* cadavers compared to strain HAR-St-II across all nozzles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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