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Novel bioinsecticidal gel formulation with improved shelf life and infectivity

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ABSTRACT

In this study, the infective juveniles (IJ) of *Steinernema thermophilum* were immobilized in biopolymer based green gels with lipid metabolism arrestant compound (OA) as adjuvant. Four test bioformulations were prepared using binary biopolymeric crosslinked composite (GkCBC) and the biopolymer (Gk) and stored at 25° C. The survival (%) of IJs infectivity potential of stored bioformulations was assessed under *in vitro* conditions up to 4 months. Shelf life evaluation at 25ºC, showed 75-90 % survival of IJs in gel and composite formulations after 4th month than 68 % in aqueous suspension. Addition of OA as adjuvant enhanced the nematode survival than without OA. Formulation GkCBC with OA at 75-100 % moisture content proved best for nematode survival and showing the allelopathic effects against test insect. The IJs immobilized formulations containing OA, irrespective of moisture content were more virulent against *Galleria mellonella* (4th instar larvae), than other compositions. New findings from this work will be validated to develop the biocontrol EPN technology for organic farming.

Keywords: Allelochemicals, allelopathic effects, biocontrol, bio-formulation, entomopathogenic nematode (EPN), *Galleria mellonella*, gel, *in-vitro*, lipid metabolism, *Steinernema thermophilum*.

INTRODUCTION

Indiscriminate and injudicious use of pesticides by formers has posed serious health risks to environment and living beings (5). Hence, the current focus is to develop alternatives to chemical pesticides, either as complete replacement or as component of Integrated Pest Management Programmes. In this context, biopesticides are safe and preferred option. It is expected that between late 2040s and the early 2050s, biopesticides will come at par with synthetic pesticides (14).

Of all present biopesticides, microbial biopesticides are the largest group of broad-spectrum biopesticides, which are pest specific and environmentally friendly. There are > 1500 naturally occurring insect-specific microorganisms, out of which around 100 are insecticidal (13). Bioinsecticides include formulations using the pest antagonistic bacteria, fungi, viruses, nematodes and protozoa. Allelochemicals mediated plant parasitic nematode control by plant based products is known (10). The secondary metabolites produced by plants, bacteria or fungi disrupt the metabolic pathway in insects but not in mammals, could pave way for innovative biocontrol strategies. Allelochemicals causes biopesticidal action not only in plants but also in biocontrol agents [bacteria, fungus, entomopathogenic nematodes (2,6,9). Ahmed *et al.* (1). reported that next to oxamyl (synthetic nematicide), entomopathogenic nematodes decreased the number of galls and egg

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masses in *M. incognita* due to superior performance of EPNs over plant based essential oils due to allelopathic interactions, both repellent and killing in nature.

EPNs controls the weevils, gnats, white grubs and various species of Sesiidae family inhabiting cryptic habitats (13). Infective juveniles of nematodes of *Steinernema* and *Heterorhabditis*, genera used for pest control carry symbiotic bacteria in their guts (11). The insecticidal action is primarily due to septicaemia of insect body caused by the allelochemicals released from multiplication of bacteria in the insect gut. These versatile biocontrol agents controls both below (15), and above ground pests (16). The allelopathic potential of secondary metabolites and toxins produced by the symbiotic bacteria present in the guts of EPNs is function of viability of the nematodes, which is limited due to their high mobile nature and sensitivity to external environment, leading to fast death. The major problem in use of allelopathic potential of EPNs is poor shelf life, necessitating strong formulation strategies. Globally, efforts have been made to develop formulations to overcome these limitations (8). However till now, no bioinsecticidal formulation has been developed with prolonged shelf-life.

Various carriers (clay, sponge, activated charcoal, vermiculite, peat, hydrogel etc.) have been used to formulate EPNs. However, the non-reproducibility and non-uniformity of their performance is serious constraint to maintain the quality of formulations. Biopolymers and biopolymer based hydrogels offer versatile option to immobilize EPN IJs due to their moisture enrichment and anti-desiccation properties and ease of microbe immobilization. We earlier developed a semisynthetic hydrogel-based bio-pesticidal formulation, 'Pusa Nemagel' based on heat tolerant EPN *Steinernema thermophilum* (7). However, major constraints in the product were pH of hydrogel carrier, moderate shelf life, cost and bulkiness leading to handling issues. This study aimed to develop a novel biogel based EPN formulation with lipid metabolism arrestant (OA) to enhance the shelf life and infectivity of immobilized EPNs.

MATERIALS AND METHODS

Study site and period

All experiments were DONE under laboratory conditions in ICAR- Indian Agricultural Research Institute (Latitude 28°38'23"N, Longitude: 77°09'27"E., Altitude: 228.61m above), Pusa, New Delhi during October, 2017 to May, 2018.

Biogels

Katira **(***Cochlospermum religiosum***)** gum (lumps, moisture 80 % w/w at 25°C and pH in deionised water 6.0), was purchased from local market, powdered and sieved (120-240 mesh). We developed Cross linked Katira composite (GkCBC) with optimized composition and powdered it (120-240 mesh) before use.

Auxiliary for bioformulations

Organic acid (benzoic acid derivative) (OA) was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India and was used as such without further purification. Stock solution of 100 ppm concentration of test compound was prepared in 100 mL distilled water. From the stock solution, solution of 10 ppm concentration was prepared with distilled water and kept at 25 °C for further use.

Biocontrol agent suspension

Infective juveniles of EPN, *Steinernema thermophilum* were selected for the study.

Maintenance of nematode culture

Steinernema thermophilum was reared *in-vivo* in the lab using *Galleria mellonella* as host. The *Galleria* larvae can easily be reared, act as model host for multiplication of nematodes. Fourth stage instar larvae are suitable for this purpose and from one fourth-instar *Galleria*, up to 2, 50,000 IJs can be harvested.

The details of *in vivo* rearing process of *Steinernema thermophilum* are given below:

Infecting *Galleria*

One mL of IJs suspension containing ~ 200 nematodes/mL was used to infect *Galleria* larvae. 1 mL suspension was distributed evenly on 5.5 cm Whatman # 1 filter paper in 5.5 cm dia Petri dish and 10 conditioned *Galleria* larvae were placed on it. The aim was to have about 20 nematodes per larva. Petri dish containing *Galleria* and nematodes was labelled and stored at 28 °C. Infected larvae were placed into modified White traps, 4-5 days after infection for harvesting of IJs (12).

Harvesting

Modified White traps were prepared by placing in small Petri dishes (5.5 cm dia) lined with Whatman #1 filter paper in large glass Petri dish (15 cms dia) touching its inner wall surface (Figure 1). Infected larvae were placed on the moist filter paper in small petri dish. Ringer's solution was added to the large Petri dish to activate the infective juveniles to migrate into the ringer's solution. IJs start to exit 7-10 days after infection. Once nematodes started to appear, they were harvested daily until the production decrease (4-5 days). Live active IJs were collected in Ringer's solution and stored in tissue culture flasks (nematode density of 5000 IJs/mL) at 15 °C in BOD incubator.

Figure 1. Harvesting of *S. thermophilum* infective juveniles by using modified white trap

Screening of formulation recipes to immobilize EPN IJs

Sixteen different recipes enlisted in (Table 1) were prepared in laboratory to determine the most suitable composition/s for immobilization of *S. thermophilum*. The prepared compositions containing IJs of *S. thermophilum* were kept at 25 °C in BOD incubator.

Table 1. Compositions and performances of EPN bioformulations (storage temperature 25 °C)

No.	Composition	Grading*	Observations
1.	Granules, Gk+china clay powder (1:2), moisture 75-100% (w/w)	-	Rapid drying leading to death of nematodes
2.	Granules, Gk +rice husk powder (1:2), moisture 75-100% (w/w)	-	Fungal attack, all nematodes died in 7 days
3.	Granules, Gk+corn cob powder (1:2), moisture 75-100% (w/w)	-	Fungal attack; all nematodes died in 7 days
4.	Wettable powder, Gk +China clay	-	Rapid drying leading to death of nematodes in 10 days
5.	Wettable powder, Gk +China clay +Corn cob powder	-	Fungal attack; all nematodes died in 7 days
6.	Wettable powder, Gk +China clay +rice husk	-	Fungal attack; all nematodes died in 7 days
7.	Wettable powder, Gk + silica gel powder	-	Rapid drying leading to death of nematodes
8.	Granules, GkCBC composite+ china clay powder (1:2), moisture 75-100% (w/w)	-	Rapid drying leading to death of nematodes
9.	Granules, GkCBC composite+ rice husk powder (1:2), moisture 75-100% (w/w)	-	Fungal attack all nematodes died in 7 days
10.	Granules, GkCBC composite+ corn cob powder (1:2), moisture 75-100% (w/w)	-	Fungal attack all nematodes died in 7 days
11.	Wettable powder, GkCBC composite +China clay	-	Rapid drying leading to death of nematodes
12.	Wettable powder, GkCBC composite +China clay +Corn cob powder	-	Fungal attack all nematodes died in 7 days
13.	Wettable powder, GkCBC composite +China clay +rice husk	-	Fungal attack all nematodes died in 7 days
14.	Wettable powder, GkCBC composite + silica gel powder	-	Rapid drying leading to death of nematodes
15.	Gel formulation, Gk gelling carrier moisture, 75-100% (w/w)	+	No negative effect on nematode survival
16.	Gel formulation, GkCBC composite gelling carrier, moisture 75-100% (w/w)	+	No negative effect on nematode survival

- : Inhibitory effects on nematode survival, + : No inhibitory effects on nematode survival

(i). Preparation of recipes 1-3, 8-10:

Specific amount of dry solid powder (gel/composite with the filler) was added to 100 mL of aqueous nematode suspension containing 5000 IJs/mL and blended by spatula carefully to prepare the dough. The dough was then placed in a syringe and pressed slowly. The thin noodle like material was then placed over a stainless steel mesh sieve (mesh size

60-120 mesh) prefilled with filler. The sieve was then shaken slowly to get the granules. The prepared granules were then collected and stored in glass vials sealed with perforated aluminium foils at 25 °C in BOD incubator.

(ii). Preparation of recipes 4-7, 11-14:

Specific amount of dry powdered gel/composite was added to a 200 mL glass beaker containing 100 mL of aqueous nematode suspension containing 5000 IJs/mL and left for 10 minutes for entrapment of IJs within the gel/composite. Thereafter, filler was added to the gel mass with continuous hand shaking until desired powder like consistency was not achieved. The prepared wettable powders were then stored in glass vials sealed with perforated aluminium foils at 25°C in BOD incubator.

(iii). Preparation of recipes 15-16:

A specific weight of dry powdered pure katira gum biopolymer or optimized Gk-CA-B-Na CMC composite (GkCBC) was added to a particular volume of aqueous nematode suspension containing 5000 IJs/mL. The gels were then stored in sealed plastic boxes at 25°C in BOD incubator.

Criteria chosen for selection of composition were:

- Negligible or no mortality of entrapped infective juveniles
- Resistance of test composition to fungal attack

All the compositions were graded at (+) and (-) scale according to their performance against chosen criteria.

Determination of optimum moisture content to prepare the bioinsecticidal EPN gel formulations

To determine the optimum moisture content in gel based EPN formulations, moisture per centage of formulations (in both gel and composites) were adjusted to 75-100 % and 25-50 % and were maintained gravimetrically during the investigation period of 4 months. Description of test compositions are given Table 2. The pH of the compositions were adjusted to 6.0-6.5 by addition of optimized amount of Gk alone (gel based formulations) or in combination with GkCBC composite (composite based formulations). Sampling was done periodically at intervals of 0 d, 1, 2, 3 and 4 months to check per cent survival of *Steinernema thermophilum* IJs. Two biological replicates were taken for this experiment with three technical replicates each time.

Table 2. Treatments to determine optimum moisture content in gel based EPN formulations

S. No.	Code	Treatments
1.	Gk100	Nematodes entrapped in Gk with 75-100% moisture(w/w)
2.	Gk50	Nematodes entrapped in Gk with 25-50% moisture (w/w)
3.	GkCBC100	Nematodes entrapped in GkCBC with 75-100% moisture (w/w)
4.	GkCBC50	Nematodes entrapped in GkCBC with 25-50% moisture(w/w)
5.	PN	Pusa Nemagel (reference standard)
6.	NTC	Control (aqueous nematode suspension)

Preparation of OA fortified bioinsecticidal EPN gel formulations

Based on optimum moisture content for gel based EPN bioformulations, novel compositions of gel based formulations of *Steinernema thermophilum* were developed in the laboratory. A generalized procedure followed was as follows: a specific weight of dry pure *C. religiosum* powder or GkCBC composite powder was added to 100 mL of aqueous

nematode suspension (with or without OA) containing 5000 IJs/mL. Moisture per centage of the formulations were maintained at 75-100% (w/w). The pH of the compositions were adjusted to 6.0-6.5 as mentioned above. Prepared compositions were stored in sealed plastic boxes at temperature 25 ℃ in BOD incubator. Description of test compositions are given Table 3.

Table 3. Bioformulations for shelf-life and infectivity assessment

S. No.	Code	Treatments
1.	T1	Nematodes entrapped in Gk with OA
2.	T2	Nematodes entrapped in Gk without OA
3.	T3	Nematodes entrapped in GkCBC with OA
4.	T4	Nematodes entrapped in GkCBC without OA
5.	PN	Pusa Nemagel (Reference standard)
6.	NTC	Control (Aqueous nematode suspension)

Shelf-life evaluation to assess per cent IJ survival as a function of time

All test compositions were kept at 25°C in B.O.D. incubators. Sampling was done periodically at intervals of 0 d, 1, 2, 3 and 4 months. 500 mg sample was dissolved in 60 mL water and placed on a magnetic stirrer for 4-5 minutes for release of nematodes and the released nematodes were observed under stereo microscope to check dead and live nematodes. The dead nematodes were confirmed as dead by touching with a fine needle. The dead nematodes did not move when touched whereas inactive but alive nematodes showed motility of varying degree. Percent survival of infective juveniles in each treatment was computed by-

Per cent survival = [(Total IJs per count – Dead IJs per count)/Total IJs per count]*100

The mean per cent survival value of three technical replicates was considered as average percent survival of each treatment. Two biological replicates with three technical replicates each time were taken for the experiment *in vitro*.

Infectivity evaluation against *Galleria mellonella in-vitro*.

Infectivity potential of the prepared bioformulations stored at 25°C was periodically assessed for four months, in terms of insect mortality under *in vitro* conditions. Sampling was done periodically at intervals of 0 d, 1, 2, 3 and 4 months. 500 mg sample containing 200-250 immobilized IJs was periodically drawn from each test formulation. The drawn sample was diluted in 2 mL water and the semisolid mass so obtained was placed in a covered Petri plate containing five *Galleria mellonella* fourth instar larvae and left as such for seventy two hours. Two biological replicates were taken with two technical replicates each time.

Per cent mortality of the test insect was checked according to the following formulae

Per cent mortality = (Number of dead insects /Number of total insects) * 100 (16)

Statistical analysis

All experiments were analysed using Completely Randomized Design (CRD) set up. The analysis was carried out using PROC GLM procedure of SAS 9.3 (SAS Institute, Cary, North Carolina, USA). Analysis by ANOVA showed that treatments are significantly different from each other, hence for further multiple pairwise comparison, Tukey's honest significant difference test was used at 1% level of significance.

RESULTS AND DISCUSSION

Screening of formulation recipes to immobilize EPN IJs

Different test compositions containing and the respective outcomes at 25 °C storage temperature is shown in Table 1. All except the neat Katira gum (Gk) and Gk-CA-B-Na CMC (GkCBC) composites qualified as carriers and were chosen for further formulation experiments. In present study, a total of sixteen formulation recipes were prepared to choose suitable one for EPN formulations. Most of the formulation, particularly those containing agri-residue developed fungal infection leading to fast death of EPN IJs within three days of storage at 25 °C. The study registered only two recipes formulations employing Gk biopolymer and cross-linked hydrogel composites as a suitable option for developing EPN bioformulations. Regarding Gk biopolymer, the finding is as expected. Higher acidic character is reported to favour fungal infestation (2). Slightly acidic pH (6.0-6.5) of both Gk and GkCBC based compositions helped in resisting infection. The optimal moisture rich environment and the network properties of composite also favoured the survival of IJs. Both the biopolymer and composite based granules and wettable powder formulations using corn cobs or rice husks as an attribute were severely attacked by fungus. Similar findings were observed in granular "Pesta" formulations where using wheat flour as an attribute led to severe fungal attack to developed granules (4).

Use of China clay or dry silica powder in the formulations resulted in rapid drying of the matrix leading to death of the nematodes, as moisture is an essential and unavoidable requirement for EPN formulations. Our study does not support the finding on anhydrobiosis to a satisfactory extent. Gel formulations, when subjected to slow desiccation led to fast death of nematodes. There is a need to develop robust strategy of desiccating the nematodes at an optimized moisture supported by simulation studies. Based on screening study, the two carriers namely Gk and GkCBC were used to prepare four compositions for determination of optimum moisture content for preparation of bioinsecticidal EPN gel formulations.

Determination of optimum moisture content to prepare the bioinsecticidal EPN gel formulations

The effect of moisture content of formulation on per cent survival of nematodes has been shown in Figure 2. After 1st month, all the gel and composite treatments irrespective of moisture content showed significantly higher nematode survival as compared to control. At 75-100% moisture, gel (Gk100) and composite (GkCBC100) resulted nearly 6% higher nematode survival than Pusa Nemagel. At 25-50% moisture, gel (Gk50) and composite (GkCBC50) showed at par performance with Pusa Nemagel. Both gel and composite treatments with 75-100% moisture showed significantly higher nematode survival than the treatments with 25-50% moisture.

After 2nd month, all the gel and composite treatments irrespective of moisture content showed significantly higher nematode survival as compared to control. At 75-100% moisture, gel (Gk100) and composite (GkCBC100) showed 5% and 7% higher nematode survival respectively than Pusa Nemagel. At 25-50% moisture, gel (Gk50) and composite (GkCBC50) showed at par performance with Pusa Nemagel. Both gel and composite treatments with 75-100% moisture showed higher nematode survival than the treatments with 25-50% moisture though they were not statistically significant.

After 3rd month, similar findings were observed in case of all the gel and composite treatments irrespective of moisture content which maintained their higher nematode survival per centage as compared to control. At 75-100% moisture, gel (Gk100) and composite (GkCBC100) showed nearly 4% and 6% higher nematode survival respectively than Pusa Nemagel. At 25-50% moisture, gel (Gk50) showed significantly lower nematode survival than Pusa Nemagel and composite (GkCBC50) showed at par performance with Pusa Nemagel. Among the gel based formulations, compositions with 75-100% moisture content showed higher nematode survival per centage as compared to the compositions with 25-50% moisture content.

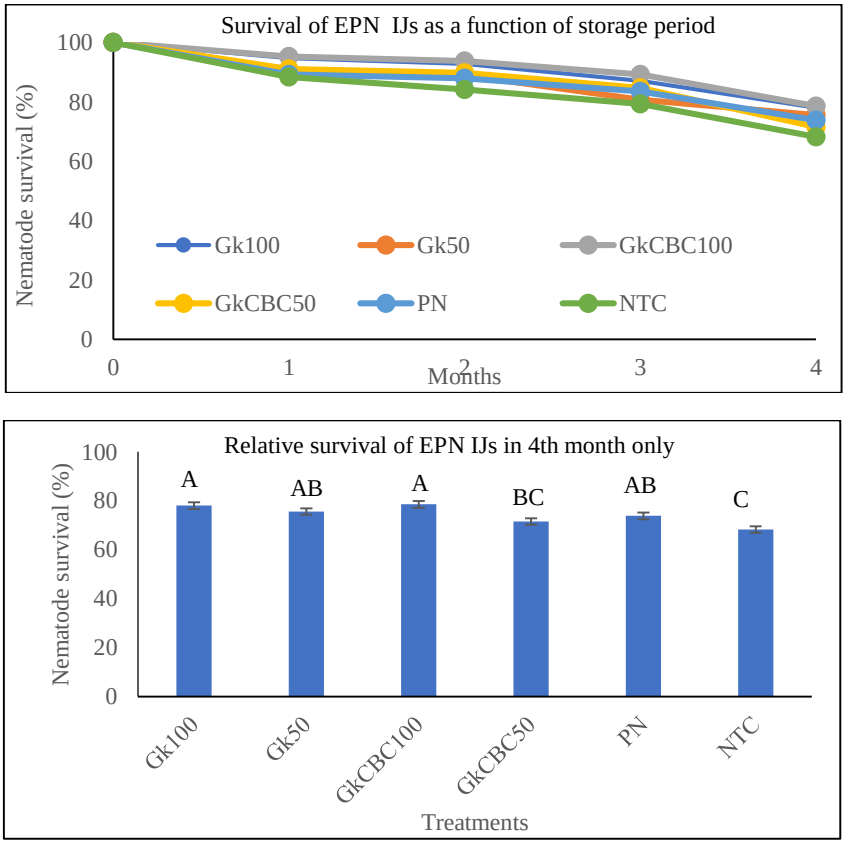

Figure 2. Variation in survival of *Steinernema thermophilum* IJs as a function of moisture content of gel compositions. Bars are standard errors of means. Different letters on the bars indicate significant differences at P< 0.01 within the treatments. Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Difference. For each treatment, n= 2 biological replicates, where 1biological replicate= mean of three technical replicates.
[Gk100: Nematodes entrapped in Gk with 75-100% moisture (w/w), Gk50: Nematodes entrapped in Gk with 25-50% moisture (w/w), GkCBC100: Nematodes entrapped in GkCBC with 75-100% moisture (w/w), GkCBC50: Nematodes entrapped in GkCBC with 25-50% moisture (w/w), PN: Pusa Nemagel (reference standard) and NTC: Control (aqueous nematode suspension)]

After 4th month, all the gel and composite treatments irrespective of moisture content showed higher nematode survival per centage as compared to control. At 75-100% moisture, both gel (Gk100) and composite (GkCBC100) showed nearly 5% higher nematode survival than Pusa Nemagel. Between the composite treatments, GkCBC100 showed significantly higher nematode survival than GkCBC50 and between the gel treatments, Gk100 showed nearly 3-5% higher nematode survival than Gk50 though they are not statistically significant. Based on these findings, final compositions were prepared by adjusting moisture content of the formulations at 75-100% (w/w). Treatments comprising OA (10 ppm) was imposed on the prepared compositions.

Gel based OA fortified EPN bioformulations

(i). Shelf life evaluation of test formulations stored at 25 °C

The effect of finalized recipes on per cent survival of the IJs of test nematode, *Steinernema thermophilum* at 25°C has been shown in Figure 3. After 1st month, all the treatments were significantly superior as compared to control. Very interestingly, in gel and composite treatments, addition of OA significantly enhanced percent IJ survival than Pusa Nemagel.

After 2nd month, all the treatments showed significantly higher nematode survival as compared to control. In gel treatment (T1) and composite treatment (T3) OA addition resulted in 4% and 6% increase in nematode survival respectively as compared to the corresponding treatments without OA treatments.

After 3rd month, all the treatments were significantly superior as compared to control. At 75-100% moisture without OA, gel treatment (T2) and composite treatment (T4) showed 4% and 6% higher percent survival respectively as compared to Pusa Nemagel whereas addition of OA enhanced the survival to 10% and 13% over Pusa Nemagel in gel (T1) and composites (T3) respectively. In gel treatment (T1) and composite treatment (T3), OA addition led to about 6% and 7% higher nematode survival respectively as compared to the corresponding treatments without OA.

After 4th month, all the gel and composite treatments continued their superior performance as compared to control. At 75-100% moisture without OA, both the gel treatment (T2) and composite treatment (T4) showed nearly 3-4% higher nematode survival as compared to Pusa Nemagel whereas addition of OA resulted in 8% and 15% higher survival than Pusa Nemagel in gel (T1) and composites (T3) at 100% moisture respectively. In gel treatment (T1) and composite treatment (T3), OA addition led to about 5% and 11% higher nematode survival respectively as compared to the corresponding treatments without OA.

(ii). Infectivity evaluation against *Galleria mellonella in-vitro*

It is well established that the symbiotic EPN and bacteria complex produces protein toxins and several secondary metabolites, which also act as effective allelochemicals. The EPNs also release their symbiont bacteria into the insect haemocoel, and kills the insects by causing septicaemia and production of toxins, bacteriocins and other secondary metabolites. In addition, several EPN and bacterial metabolites may work as effective allelochemicals against rhizosphere micro fauna (9), therefore, appropriate formulations may preserve these allelopathic properties for prolonged duration. Formulation quality is thus an important aspect.

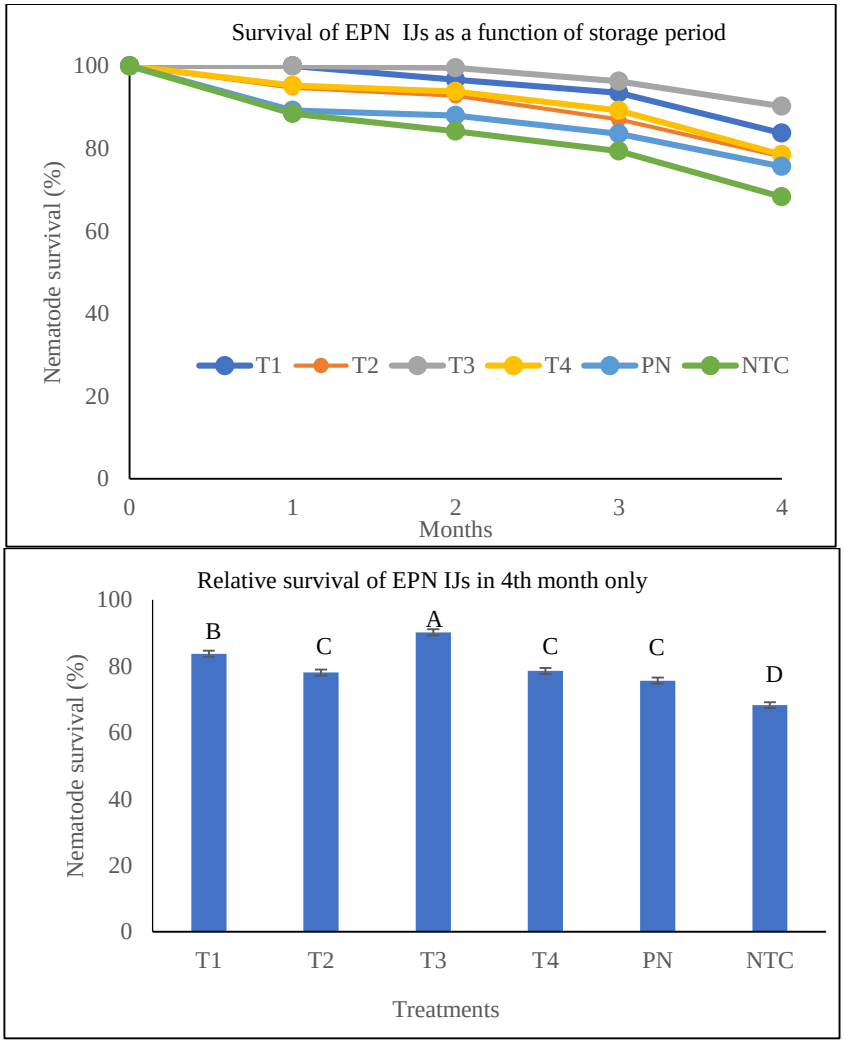

Figure 3. Relative shelf lives of final test formulations at 25°C. Bars are standard errors of means. Different letters on the bars indicate significant differences at P< 0.01 within the treatments. Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Difference. For each treatment, n= 2 biological replicates, where 1biological replicate= mean of three technical replicates.
[T1: Nematodes entrapped in Gk with OA, T2: Nematodes entrapped in Gk without OA, T3: Nematodes entrapped in GkCBC with OA, T4: Nematodes entrapped in GkCBC without OA, PN: Pusa Nemagel (reference standard) and NTC: Control (aqueous nematode suspension)]

In the present study, effect of prepared formulations stored at 25°C on infectivity of *S. thermophilum* IJs against *Galleria mellonella* is presented in Figure 4. After 1st month, all the gel and composite treatments showed performance at par with the Pusa Nemagel and

control resulting in 100% mortality of the test insects. After 2nd month, all the gel and composite treatments resulted in 100% mortality of the test insects which was at par with Pusa Nemagel but superior over control. After 3rd month, again all the composite treatments and the gel treatments with or without OA resulted in 100% mortality of the test insects which was 10% and 25% higher than Pusa Nemagel and control respectively. After 4th month, all the gel and composite treatments retained their superior performance to the control. Addition of OA to the gel (T1) and composite (T3) at 75-100% moisture resulted in 10% and 15% higher mortality of the test insect respectively as compared to Pusa Nemagel. Among all the gel and composite treatments, T3 resulted in highest mortality of the test insect.

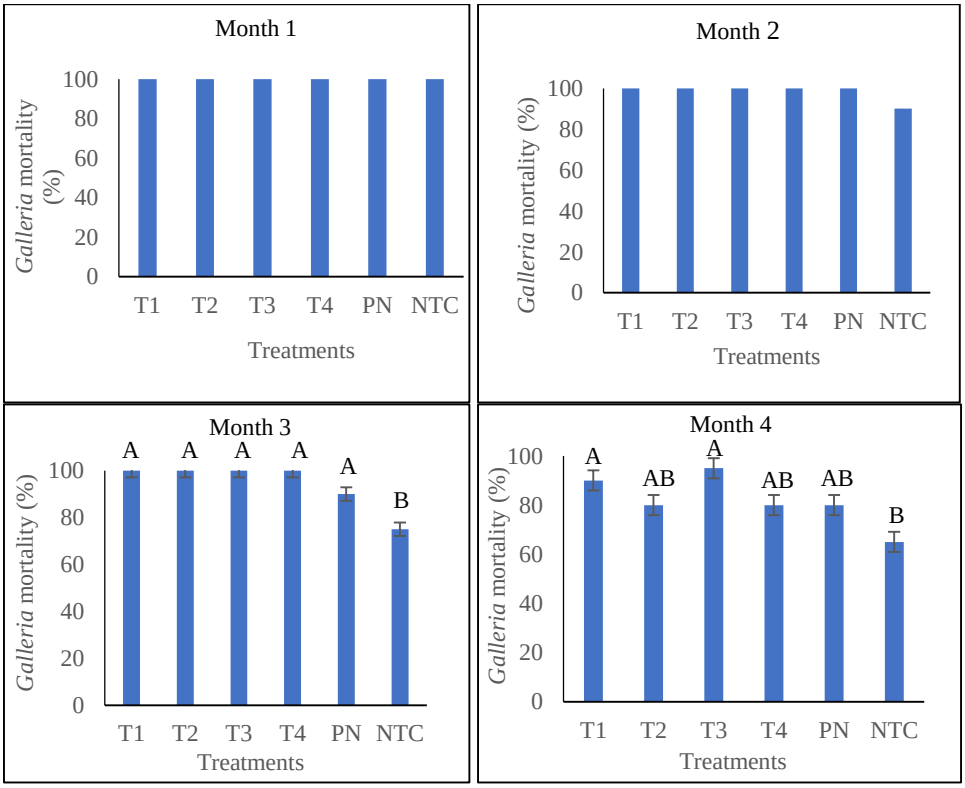

Figure 4. Relative virulence of IJs in test bioformulations stored at 25°C. Bars are standard errors of means. Different letters on the bars indicate significant differences at P< 0.01 within the treatments. Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Difference. For each treatment, n= 2 biological replicates, where 1biological replicate= mean of two technical replicates.

[T1: Nematodes entrapped in Gk with OA, T2: Nematodes entrapped in Gk without OA, T3: Nematodes entrapped in GkCBC with OA, T4: Nematodes entrapped in GkCBC without OA, PN: Pusa Nemagel (reference standard) and NTC: Control (aqueous nematode suspension)]

As evident from the results, the developed compositions have distinct elements of novelty and superiority over gel formulations reported so far in literature including our own reports on Pusa Nemagel, bioinsecticidal formulation. In the sodium alginate gel formulations, after 2 months all the test nematodes were found to be dead at room temperature (7). *S. siamkayai* in alginate gel formulation survived at 25°C only up to 14 weeks (17). Hence, the formulation carriers used in our study based on scientific reasoning based selection qualify for green technology, are easy to form and use and have immediate upscalation potential.

CONCLUSIONS

To prepare EPN formulation with longer shelf life and improved nematode infectivity, we tested new formulations and additives. All the gel and composite formulations, irrespective of organic acid (OA) addition, showed significantly higher nematode survival and *Galleria mellonella* infectivity than control. The gel and composite treatments stored for 4-months at 25 °C, killed 75-95 % test nematodes and the composite with OA at 75-100 % moisture content showed the highest EPN infectivity (95 %) against *Galleria*. By enhancing the survival through preservation of lipid reserves and immobilization, the formulation has retained the allopathic traits of the EPNs, a finding that will be further verified in field experiments and expressions at molecular level.

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