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ORIGINAL ARTICLE

A laboratory study on survival and infectivity of entomopathogenic nematodes formulated in gum katira-based biogel compositions

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Abstract

Insufficient analysis of carriers and their interaction with biocontrol agents can lead to ineffective formulations. Therefore, it is important to thoroughly study the compatibility and interactions between carriers and biocontrol agents for high-quality biological formulations. The present study aimed to evaluate the effect of four different formulation compositions containing gum katira biogel, vermiculite, and diatomaceous earth on the survival and infectivity of infective juveniles (IJs) of two entomopathogenic nematodes (EPNs) species namely, *Steinernema abbasi* and *Heterorhabditis indica* at 25 and 35 °C. The physicochemical analysis of the prepared formulations revealed their slightly acidic nature with pH ranging from 5.65 to 6.10. Rheological studies validated the solid-like behavior of the developed formulations. The highest survival of *S. abbasi* IJs was observed in the case of gum katira biogel-vermiculite blended composition at both 25 °C (94.2%) and 35 °C (88.4%) after 90 days of storage. Interestingly, gum katira biogel alone sustained maximum survival percentage (63.5%) of *H. indica* as compared to control (41.5%) after 90 days at 25 °C. The formulation compositions failed to retain alive *H. indica* IJs at 35 °C even after seven days of storage. Moreover, the gum katira singly or in combination with vermiculite resulted in superior infectivity against *Galleria mellonella* (4th instar larvae) as compared to the other treatments in case of both the nematode species. The presence of diatomaceous earth in all the compositions irrespective of moisture and EPN species showed a negative impact on the survival and infectivity of IJs after 90 days of storage. Gum katira biogel alone or in combination with vermiculite was favorable to sustaining the survivability and infectivity of test EPN IJs at ambient storage temperatures. These formulants can further be used to develop biocontrol EPN formulations for organic farming and integrated pest management programs.

Keywords *Steinernema abbasi* · *Heterorhabditis indica* · Gel · Vermiculite · Diatomaceous earth · EPN

Introduction

Pesticides are indispensable agro-inputs in achieving food security, both in terms of quantity and quality (Anani et al. 2020). However, crop growers' indiscriminate and injudicious use of synthetic pesticides, as well as violations of prescribed safety norms and application techniques, have caused major health concerns to the environment and living creatures (Damalas and Koutroubas 2018). Biopesticides are safe and preferable alternatives in this situation, which include naturally occurring bioactive compounds from microbial, animal, and plant origin, as well as living organisms (Samada and Tambunan 2020). When compared to synthetic pesticides, biopesticides have several intrinsic advantages (Kaya and Lacey 2007; Kaya and Vega 2012). Biopesticides have no residue problems, are less or non-toxic to non-target organisms and the environment, have high target pest specificity, and, have the additional benefit of reproducing in their target hosts and causing long-term pest population suppression through horizontal and vertical transmission even if the application is not repeated (Sporleder and Lacey 2013). Therefore, microbial biopesticides, particularly as a component of IPM strategies, are

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gaining popularity internationally. Microbial biopesticides are also considered as an integral part of integrated pest management and integrated crop management strategies, which employ a variety of complementary techniques to reduce pest populations below the level of economic injury while minimizing effects on other agro-ecosystem elements (Ortiz and Sansinenea 2022). They make up the greatest range of broad-spectrum biopesticides that are pest specific and eco-friendly of all the biopesticides utilized today. Entomopathogenic nematodes (EPNs) are another type of potential biocontrol agent for the development of sustainable pest management strategies (Singh et al. 2019).

Weevils, gnats, white grubs, and other Sesiidae species that live in cryptic habitats can be controlled by EPNs (Koul 2012). Commonly, the infective juveniles (IJs) of the nematode genera *Steinernema* and *Heterorhabditis* are utilized for pest control all over the world (Kaya and Gaugler 1993). EPNs of the Steinernematidae and Heterorhabditidae families can detect, infect, and kill a large variety of both above- and below-ground insect pests. The IJs of Steinernematids and Heterorhabditids have symbiotic bacteria namely, *Xenorhabdus* and *Photorhabdus*, respectively in their guts. Though these nematodes and their symbiotic bacteria are highly effective insect pathogens, they are not harmful to humans, plants, or other non-target creatures (Manochaya et al. 2022). Several carriers such as peat, activated charcoal, sponge, cadaver, etc. have been employed to date to develop EPN-based bioinsecticidal formulations (Mukhopadhyay et al. 2019, 2020). Because of the convenience of handling and application, storing IJs in talc formulation is a favored technique. Hussaini et al. (2003) found that for *Steinernema* spp., the survival of IJs was highest in water, followed by talc and alginate, and that the combination of talc + china clay and alginate formulations was superior in retaining the maximum viable IJs of *H. indica* during a storage period of 90 days. Divya et al. (2011) compared the survival as well as virulence *H. indica* IJs in different carriers namely, talc, sawdust, coir dust, water dispersible hydrogel, and sponge, and found the best nematode survival in the hydrogel for a period of 11 weeks at 27 °C storage temperature, while other carriers, including aqueous suspensions, reported poor performance. Guo et al. (2017) developed vermiculite and humus-based formulations of different *Steinernema* and *Heterorhabditis* sp. and reported showing better survival of *S. longicaudum*, *S. feltiae* and *S. carpocapsae* at 5 °C in the vermiculite formulation, while H. indica and *H. bacteriophora* survived better in aerated water at a storage temperature of 15 °C. The improper carrier-EPN compatibility had resulted in poor survival of EPN IJs at ambient conditions in most of the developed products to date. The poor shelf life of EPNs, particularly as a result of their sensitivity to UV rays and desiccation as well as their low tolerance to high temperatures, is also a significant barrier to the application of their biocontrol potential (Nxitywa and Malan 2021). Due to the moisture-retaining, anti-desiccation, and ease of bioagent immobilization features, gelling biopolymers provide a flexible option to immobilize EPN IJs. The reason behind the selection of biopolymer as one of the matrix components in the present study was not only to avoid the negative impacts of synthetic or semi-synthetic polymers but also to provide an eco-friendly carrier suitable for the bioinsecticidal formulation, which is in line with the United Nation's "sustainable development goal" of "responsible production and consumption patterns" (SDG 12) (United Nations 2015). The biopolymer selected in the present study is gum katira, a hetero-polysaccharide of plant origin. It is an exudate from the softwood tree *Cochlospermum religiosum*, which belongs to the Cochlospermaceae family (Jain and Babbar 2002). L-rhamnose, D-galactose, and D-galacturonic acid make up the chemical structure of gum katira in the molar ratios of 3:2:1, respectively, with traces of ketohexose (Ojha et al. 2008). This water insoluble biopolymer is used in curing of cough, diarrhea, dysentery, pharyngitis, gonorrhea, syphilis, and trachoma. It has also been effectively employed as a gelling agent in tissue culture media and is widely used in the cigar, paste, and ice cream industries (Ahuja and Bhatt 2015). In the present study, the hypothesis behind the selection of the carriers for EPNs was that the use of hydrophilic as well as porous nature of the biopolymer singly or in combination with vermiculite and/or diatomaceous earth having porous characteristics would sustain the immobilized EPN IJs providing sufficient aeration and moisture during storage, which are two prerequisite parameters for the survival of EPNs. Therefore, in this piece of work, gum katira-based different biogel admixtures were prepared employing gum katira along with vermiculite and/or diatomaceous earth as carriers for EPNs, and their influence on survival and infectivity of *S. abbasi* and *H. indica* at two different storage temperatures (25 and 35 ºC) was assessed. As *S abbasi* and *H indica* are the most prevalent species of EPNs in North Indian Indo Gangetic plains and well adapted to local conditions, we have selected these two EPN species for development of formulations.

Materials and methods

Biocontrol agents' suspension

The IJs of *Steinernema abbasi* and *Heterorhabditis indica* were reared in vivo in the laboratory on the 4th stage instar larvae of *Galleria mellonella* as host (Wang and Bedding 1996). From one fourth-instar *Galleria*, up to 2,50,000 IJs were harvested. Live active IJs were collected in aqueous

suspension and stored in tissue culture flasks by maintaining nematode density of 3000 IJs/ mL at 15 °C in a BOD incubator. The *Steinernema abbasi* IARI strain (Ganguly et al. 2010) and the *H. indica* IARI-EPN-Hms1 strain (Kumar et al. 2015) were used for the present study and for preparation of formulation compositions, freshly hatched IJs (7 days old) were used.

Formulants

The biogels are gel-forming hydrophilic polymers of natural origin. The biogel used in this study was gum katira (*Cochlospermum religiosum*) (lumps, water absorption capacity 50–70 g g^{-1}). The gum katira was procured from the local market, powdered (120–240 mesh), sieved, and autoclaved before use. Powdered vermiculite (240 mesh) was purchased from the local market and directly used; whereas, powdered diatomaceous earth (food grade) was purchased from Casa de Amor (Madhya Pradesh, India) and directly used.

Toxicity study of prepared compositions with test EPN IJs

To select appropriate formulation recipes suitable for the immobilization of EPN IJs, four different compositions (Table 1) were prepared by immobilizing *S. abbasi* and *H. indica* IJs, separately. For this, an appropriate amount of formulants were added to aqueous nematode suspension of volume 100 mL having 5000 nematode IJs per mL. An aqueous nematode suspension of 100 mL volume containing 5000 IJs per mL was taken as no treatment control (NTC). The prepared compositions were placed in sealed tissue culture flasks and placed in a BOD incubator at a storage temperature of 25 ºC. Survival of IJs was evaluated after seven days to check the effect of compositions on nematode survival. The experiment was carried out to assess the feasibility of the prepared compositions in sustaining the test EPN IJs. Three biological replicates were taken with three technical replicates each time.

Table 1 Compositions used for immobilization of EPN IJs and their pH

Treatment	Composition	pH
Gk	5 g gum katira	5.65
Gk + DE	3.5 g gum katira + 1.5 g Diatomaceous earth	6.10
Gk + V	3.5 g gum katira + 1.5 g powdered vermiculite	5.75
Gk + DE + V	3.5 g gum katira + 0.75 g powdered vermiculite + 0.75 g Diatomaceous earth	5.70

Physico-chemical properties of prepared compositions

The pH of the prepared compositions was measured using a digital pH meter. Rheological analyses of the prepared compositions were carried out using a rheometer (MCR 102, Anton Paar, Germany) fitted with a parallel plate measurement system (25 mm diameter) with a gap of 1 mm between the plates. The viscoelastic properties of the prepared compositions were determined by an amplitude sweep test followed by a frequency sweep test. All analyses were carried out at 25 ºC temperature. Firstly, by performing amplitude sweep tests within a range of 0.1–100% of shear strain (γ) at a constant frequency of 10 Hz, the linear viscoelastic region (LVER) was identified. Then, frequency sweep tests (frequency: 0.1–10 Hz) in logarithmic progression were carried out in controlled strain mode at a constant shear strain (γ) of 0.1%, which was well within the LVER for all of the test compositions (Supplementary information Tab S1).

Effect of different formulation compositions on survival of EPN IJs

Based on the findings of the toxicity study, separate sets of four different compositions as mentioned in Table 1 were prepared by immobilizing *S. abbasi* and *H. indica* IJs to assess the effect of different formulation compositions on survival of test EPN IJs as a function of both storage time and temperature. For this, a specific weight of dry pure gum katira powder or gum katira-vermiculite and/or diatomaceous earth dry mix powder was added to aqueous nematode suspension (Volume: 100 mL, nematode concentration: 5000 IJs per mL) and mixed thoroughly to prepare the test compositions. Prepared formulation compositions were placed in sealed tissue culture flasks and stored at temperatures 25 and 35 ºC in BOD incubator for the study of survival of EPN IJs immobilized in prepared formulations. Proper aseptic conditions were maintained within the BOD incubator throughout the experimental period. A total of three different sets of each nematode species comprising different formulation compositions were prepared to evaluate the effect on survival of EPN IJs periodically up to 90 days.

Study on survival of immobilized EPN IJs in prepared formulations as a function of temperature and time

To evaluate the survival of nematodes, approximately 500 mg of the sample was first mixed with 5 mL of water and placed on a magnetic stirrer for around 5–10 min for the release of nematodes (Ganguly et al. 2008). Then, 100 µL aliquot containing the released nematodes were then observed under a stereo microscope (Model: Leica MZ6) to assess the number of alive and dead nematodes by touching

them with a fine needle. The nematodes which showed movement on touching were considered alive and those which showed no movement on touching were considered dead. The initial population of IJs in the prepared compositions was around 5 IJs/mg of composition. Therefore, the percent survival of nematode IJs in each treatment was computed using the following formula:

$$\text{Percent survival} = \frac{\text{Alive nematode IJs per mg of composition}}{\text{Initial count of nematode IJs per mg of composition}} \times 100$$

The mean percent survival for each treatment was determined by averaging the three technical replicates' percent survival values. Three biological replicates with three technical replicates each time were kept for each experiment.

Infectivity evaluation against Galleria mellonella

The formulation compositions having at least survival period for 30 days, i.e., *S. abbasi*-based compositions stored at 25 and 35 °C and *H. indica*-based compositions stored at 25 °C were assessed for their infectivity potential using a Petri dish bioassay method (Guo et al. 2017). Infectivity potential of the test compositions was periodically assessed for 90 d, in terms of insect mortality under laboratory conditions. To accomplish this, around 20 mg of sample containing 100 immobilized IJs was periodically drawn from each test formulation separately. The drawn sample was diluted in 1 mL water. Then, the 1 mL suspension was placed in a covered 9 cm Petri dish containing five larvae of *Galleria mellonella* and left as such for seventy two hours to check their mortality. Three biological replicates were taken with three technical replicates each time.

Statistical analysis

All experiments were analyzed using a Completely Randomized Design (CRD) setup. The analysis was carried out using open-source software, Web Agristat Package 2.0 (WASP 2.0) (https://ccari.icar.gov.in/wasp2.0/index.php). For comparison of treatment means, the least significant difference (LSD) test was used at a 5% level of significance.

Results

Toxicity study of prepared compositions with test EPN IJs

After 7 days of incubation, no mortality of the EPN IJs (both *S. abbasi* and *H. indica*) in aqueous suspension and prepared compositions was observed, suggesting no negative impact of any of the test compositions on EPN survival (100% survival in all the test compositions). Therefore, all the prepared compositions were further used to evaluate their relative performance on survival of test EPN IJs as a function of storage time and temperature.

Physico-chemical properties of prepared compositions

pH

The prepared formulations were found to be slightly acidic in nature (Table 1), with pH values ranging from 5.65 (Gk) to 6.10 (Gk + DE).

Viscoelastic properties

Variation in storage modulus (G') or loss modulus (G'') with frequency provides a material's "mechanical spectrum". The mechanical spectrum of the prepared compositions is shown in Fig. 1. In all the treatments storage modulus predominated over loss modulus, suggesting solid-like behavior of the prepared compositions. The treatment comprising only biogel (Gk) revealed higher mechanical strength than all the other compositions.

The complex viscosity of the prepared compositions at all the frequency points has been shown in Fig. 2. At all test data points, the highest complex viscosity was observed in the treatment comprising only biogel (Gk). The addition of diatomaceous earth or vermiculite or both to the gum katira biogel has resulted in a lowering of the complex viscosity of the matrix. The complex viscosity trends in all test data points followed the trend: Gk > Gk + DE + V > Gk + DE > Gk + V.

Effect of different formulation compositions on survival of *S. abbasi* IJs at 25 and 35 °C storage temperature

The survival of *S. abbasi* IJs in different formulation compositions at 25 ºC storage temperature has been shown in Fig. 3a. On the 30th day, the highest survival was observed in gum katira biogel-vermiculite blend (97.6%), followed by gum katira biogel (96.7%), both of which were higher than the control (91.8%). Both the gum katira biogel and gum katira biogel-vermiculite blend retained their superior performance on the 60th day, where > 94.00% survival of

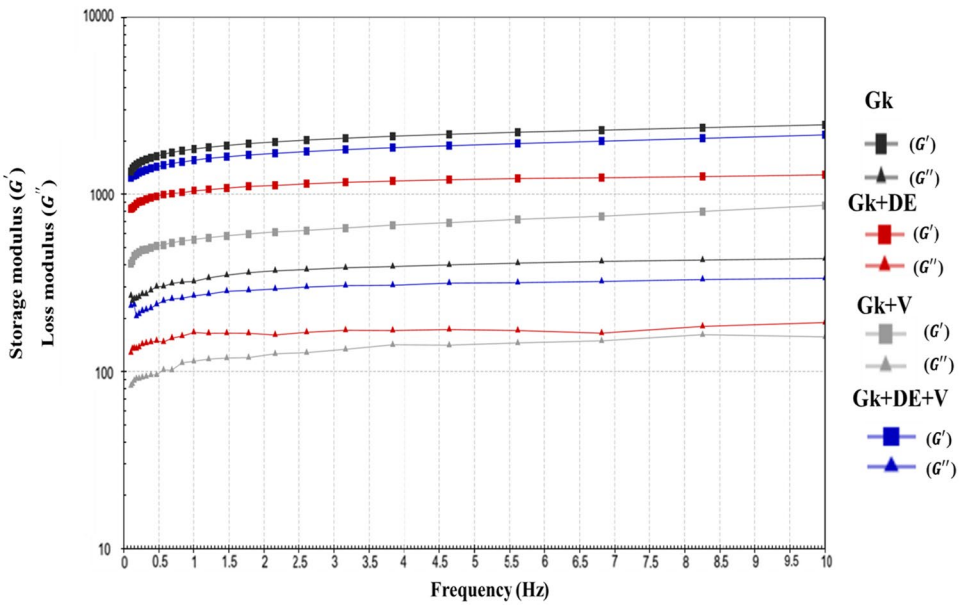

Fig. 1 Storage modulus and loss modulus *vs* frequency of prepared compositions. (Gk = Gum katira, DE = Diatomaceous Earth, V = Vermiculite)

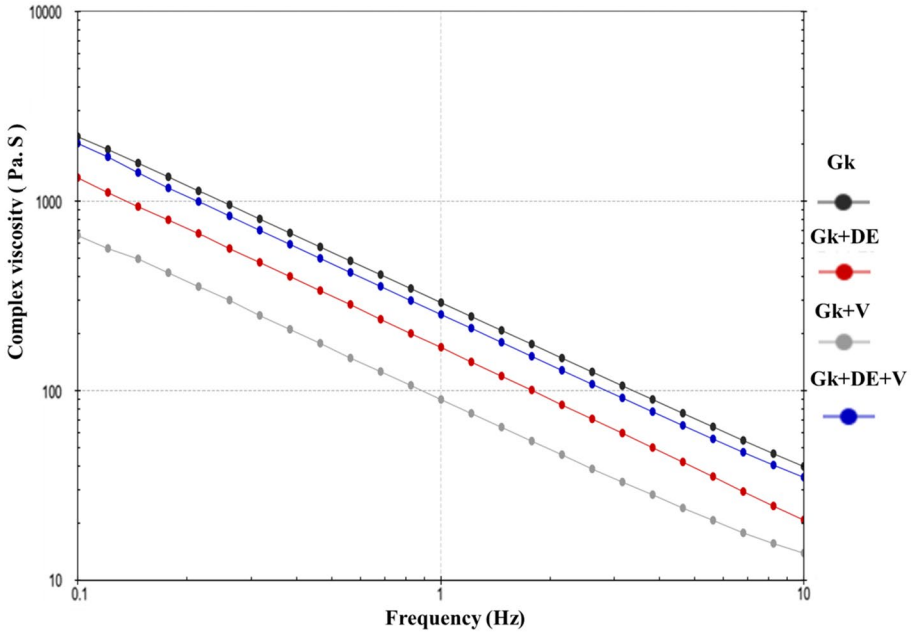

Fig. 2 Complex viscosity of prepared compositions at different frequencies (Gk = Gum katira, DE = Diatomaceous Earth, V = Vermiculite)

nematode IJs was observed in those two treatments, which were significantly higher than the other compositions. On the 90th day, gum katira biogel-vermiculite blended admix exhibited the highest survival of 94.2% which was significantly superior to other treatments including the control (88.0%). The presence of diatomaceous earth in combination with gum katira biogel (Gk + DE) and gum katira biogel-vermiculite blend (Gk + DE + V) failed to sustain even as much live IJs as compared to control.

The survival of *S. abbasi* IJs in different compositions at 35 ºC storage temperature has been depicted in Fig. 3b. On the 30th day, the highest survival was observed in gum katira biogel-vermiculite blend (97.5%), followed by gum katira biogel (95.2%), both of which were significantly higher than the control (92.5%). A similar trend was observed on the 60th day, where gum katira biogel-vermiculite blend retained its superior performance with 93.2% nematode survival. At 35 °C storage temperature, after 90 days, the highest survival in gum katira biogel-vermiculite blend composition (88.4%) followed by gum katira biogel (82.2%) as compared to 40% in control was observed. The incorporation of diatomaceous earth in the test compositions resulted in a drastic reduction in the survival rate of IJs. Gum katira biogel-vermiculite blend maintained the viability of IJs to

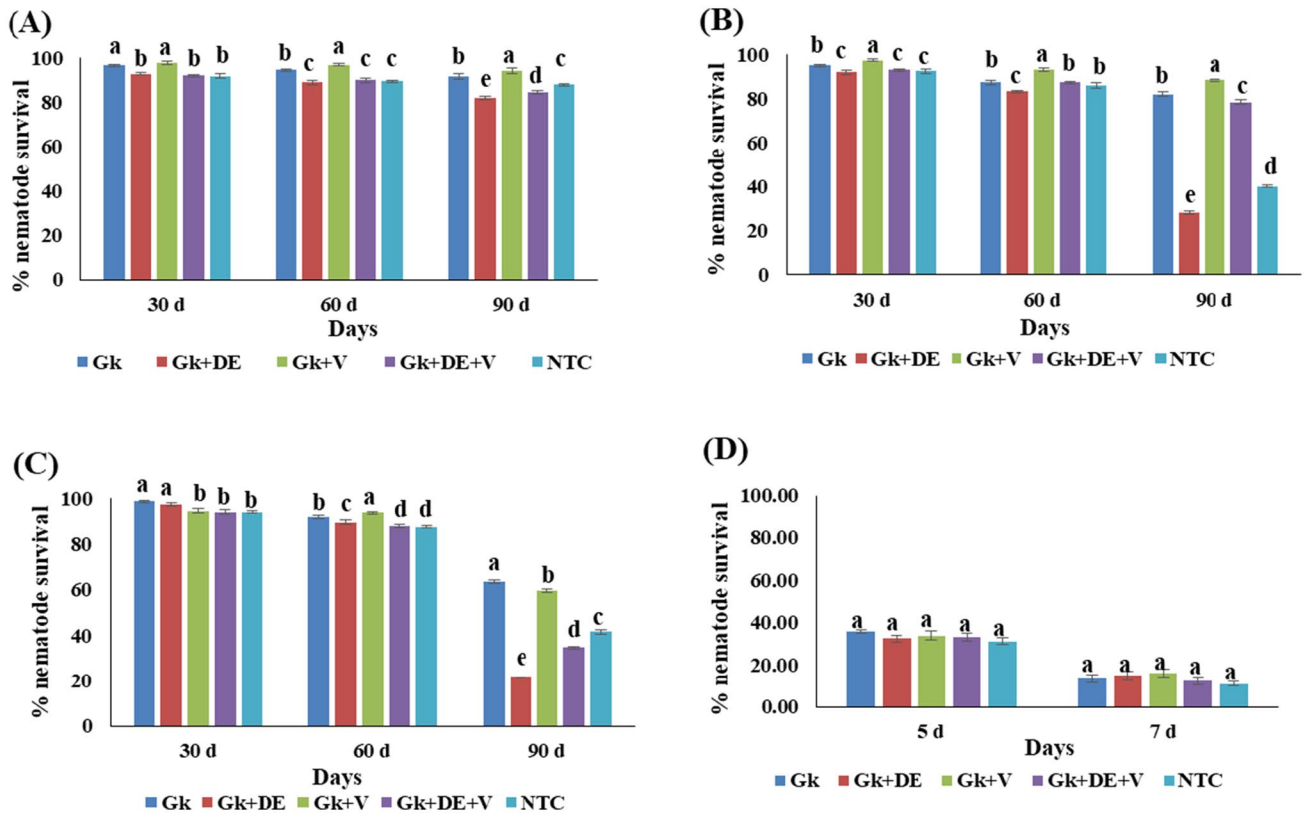

Fig. 3 Relative survival of *S. abbasi* IJs at 25 °C (**a**), and 35 °C (**b**) and *H. indica* IJs at 25 °C (**c**), and 35 °C (**d**) in prepared compositions (Gk = Gum katira, DE = Diatomaceous Earth, V = Vermiculite, NTC = Aqueous Nematode Suspension) (Treatments containing at least a single common letter in each time point are not statistically significant at LSD with $p = 0.05$ level. Error bars are standard deviations.)

97.5% after 30 days and though it decreased to 88.4% after 90 days of storage, significantly higher percentage survival of IJs than the other treatments was observed.

Effect of different formulation compositions on survival of *H. indica* IJs at 25 and 35 °C storage temperature

The survival of *H. indica* IJs in different prepared compositions at 25 ℃ storage temperature has been shown in Fig. 3c. The relatively superior performance of gum katira biogel and gum katira biogel-vermiculite blend than the aqueous suspension (control) could be seen till 90 days of storage period. On the 30th day, the highest percentage survival was observed in the gum katira biogel treatment (98.5%). On the 60th day, gum katira biogel-vermiculite blend revealed the highest survival (93.6%), which was significantly higher than the other treatments. On the 90th day, the highest survival was observed in gum katira biogel (63.5%), followed by gum katira biogel-vermiculite blend (59.6%), as compared to only 41.5% survival in aqueous suspension. For *H. indica*, both gum katira biogel (63.5%) and gum katira biogel-vermiculite blend (59.6%) showed statistically significant superior performance as compared to other treatments in terms of survival of IJs at 25 °C after 90 days of storage. The presence of diatomaceous earth along with the biogel (Gk + DE), and biogel-vermiculite (Gk + DE + V) had resulted in significantly lower nematode survival as compared to the other treatments.

In the present study, *H. indica* IJs were found to be highly sensitive to the temperature of the surrounding environment (Fig. 3d). At 35 °C storage temperature, IJs in both the aqueous suspension and test admixes showed around 31–35% survival on 5th day and around 11–16% on 7th day. Beyond 7th day, no IJ was survived in any of the compositions.

Infectivity evaluation against *Galleria mellonella*

In the present study, effect of prepared formulation compositions stored at 25 and 35 °C on infectivity of *S. abbasi* IJs against *Galleria mellonella* has been shown in Fig. 4a and b. The test insect mortality was statistically non-significant ($p < 0.05$) in all treatments irrespective of the storage period at 25 °C. At 35 °C, all the treatments showed at par performance

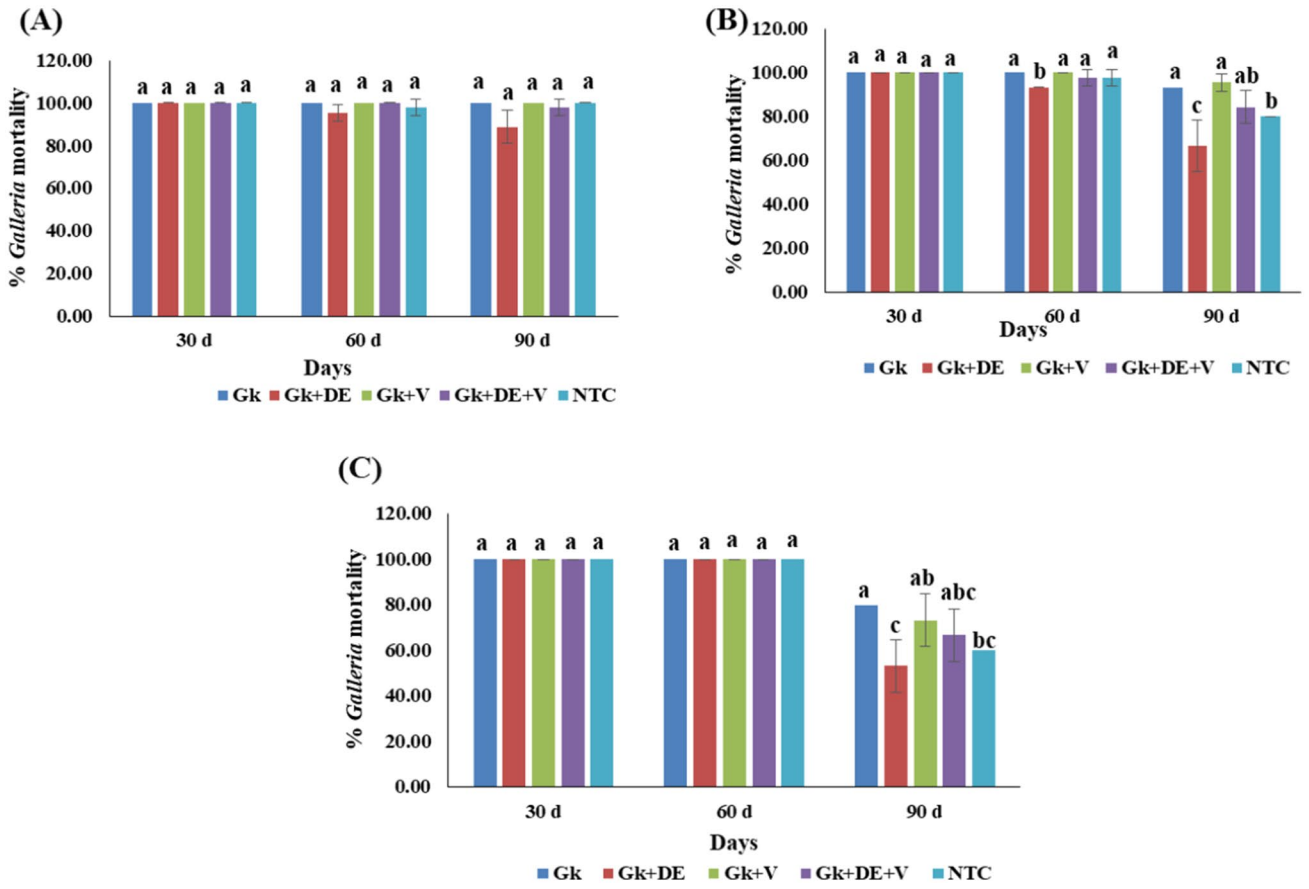

Fig. 4 Mean percent mortality of *G. mellonella* infected with *S. abbasi* IJs stored at 25 °C (**a**), and 35 °C (**b**) and with *H. indica* IJs stored at 25 °C (**c**) in prepared compositions (Gk = Gum katira, DE = Diatomaceous Earth, V = Vermiculite, NTC = Aqueous Nematode Suspension) (Treatments containing at least a single common letter in each time point are not statistically significant at LSD with $p = 0.05$ level. Error bars are standard deviations.)

resulting in 100% mortality of the test insect on 30th day. On 60th day, all compositions except gum katira-diatomaceous earth blend (93.33%) showed > 97% mortality of the test insect. On 90th day, the gum katira-vermiculite blend showed highest mortality (95.55%) of the test insect, followed by only gum katira biogel-based treatment (93.33%), both of which are significantly higher than the other treatments. The effect of *H. indica* IJs-based compositions stored at 25 °C on insect mortality has been depicted in Fig. 4c. All the compositions resulted in 100% mortality of the test insect on 30th and 60th days of storage. However, on 90th day, the gum katira biogel-based treatment showed highest insect mortality (80%) among all the test treatments, which was significantly higher than the control (53.33%) and the gum katira-diatomaceous earth based treatment (60%).

Discussion

The current research report highlights the efficacy of gum katira biogel-vermiculite blend in promoting the survival and infectivity of *S. abbasi* IJs at both 25 and 35 °C. Moreover, the study revealed that both gum katira biogel and gum katira biogel-vermiculite blend were the most suitable carriers for *H. indica* IJs at 25 °C. The results of the study suggest that the gum katira biogel-vermiculite blend could be considered as a reliable carrier for the effective application of *S. abbasi* IJs in soil, while for *H. indica* IJs, both gum katira biogel and gum katira biogel-vermiculite blend could be used as potential carriers at 25 °C.

The results obtained in this study align with the findings of Matadamas-Ortiz et al. (2014) and Cortés-Martínez et al. (2016), indicating that moisture loss is a significant factor affecting the survival percentage of IJs. Considering this, hydrophilic carriers, such as gum katira biopolymer and vermiculite clay, were employed in the present study to formulate compositions of EPN that can help mitigate severe moisture loss during storage. The use of such carriers has the potential to significantly enhance the shelf life of EPN formulations, thereby improving their efficacy in biological control applications.

Gel-based formulations have a semisolid appearance and are frequently provided with the benefit of longer shelf lives than solid formulations. The consistency or viscosity of the gel carrier, as well as structural stability, pH, moisture content, and pathogen resistance, are all important factors in the formulation's effectiveness (Chandrika et al. 2016). There have been multiple reports of EPN IJs being encapsulated in alginate gel beads or capsules. However, the gel beads showed several problems too. For instance, the aggregate composition prepared by Bedding and Butler (1994) and Bedding et al. (2000) using polyacrylamide to partially desiccate the EPNs, but at room temperature, the nematode survival period was reported to be very short and the formulation was also found to be difficult to get dissolved in water for release of the entrapped nematodes. This poor survival problem in alginate-based biogels has been tried to resolve in the present study using gum katira-based biogel compositions, having sustained survival and infectivity of *S. abbasi* IJs in biogel-vermiculite blended composition at both 25 and 35 °C. However, all the test compositions failed to retain the test *H. indica* IJs alive at 35 °C after seven days of storage. At 25 °C storage temperature, the Gk-based treatment being the best composition among the test treatments in terms of nematode percentage survival registered around 63.5% survival of the immobilized *H. indica* IJs after 90 days of storage. At elevated temperature, the poor storage potential of Heterorhabditis nematodes, such as *H. indica*, may be attributed to their low tolerance of high storage temperatures. In contrast to Steinernematids, including *S. carpocapsae*, *S. feltiae*, and *S. riobrave*, *H. indica* has demonstrated inferior storage potential, as reviewed by Kagimu et al. (2017). The current results are consistent with the research of Strauch et al. (2000), which indicated that the highest survival rate of *H. indica* IJs was achieved at a storage temperature of 15 °C. The percentage of nematode survival decreased as the storage temperature increased. The prepared compositions of the present work except for gum katira biogel-diatomaceous earth blend showed good compatibility with *S. abbasi* IJs. The gum katira biogel-vermiculite admixture registered > 90% survival and infectivity of *S. abbasi* IJs at both 25 and 35 °C storage temperatures after 90 days of storage. The composition may have provided better insulation and aeration to the encased *S. abbasi* IJs at both storage temperatures due to the porosity of the matrix. The present study also reports poor storage potential of *H. indica* as compared to *S. abbasi* in the prepared compositions. *H. indica* exhibits a lower tolerance for high storage temperatures than other species of nematodes, such as the Steinernematids like *S. carpocapsae*. It is worth noting that Heterorhabditis nematodes, including *H. indica*, have been found to have poorer storage potential in comparison with Steinernematids (Kagimu et al. 2017; Strauch et al. 2000). Temperature is very crucial on the viability of EPN IJs in storage as EPNs are sensitive to cold and heat temperature extremes (like 25 and 35 °C in the present study). This effect varies in EPN species and strains (Kagimu et al. 2017). This may have implications for the use of Heterorhabditids in various applications and should be taken into consideration when selecting nematodes for storage and use. Moreover, moisture retaining properties of both the matrix component, i.e., gum katira biogel and vermiculite may be helpful in attaining higher survival of *S. abbasi* IJs even at 35 °C storage temperature. This finding is of the utmost importance to the present research work since vermiculite's per-unit cheap cost and large volume qualities, along with biogel's humectant, antidesiccant, porous, and binding properties, complement each other that favor the design of EPN bioformulations. A similar kind of gel-clay compatibility though for a lesser time as compared to the present investigation was observed for the development of EPN-based formulations in the case of a synthetic gel-based formulation developed by Leite et al. (2018), where a combination of vermiculite and double polyacrylamide gel showed viability of encased *S. feltiae* IJs around 77% after 42 days at 35 °C storage temperature. However, the bigger granule size of vermiculites was a limitation of the research work. The present investigation also explored the virulence of *S. abbasi* and *H. indica* IJs against *G. mellonella*, focusing on different formulation compositions and storage conditions. *S. abbasi* formulations showed stability at 25 °C, with consistent infectivity. At 35 °C on the 90th day, all formulations were effective, except for the gum katira-diatomaceous earth blend, highlighting *S. abbasi*'s robustness at elevated temperature. Lalitha et al. (2023) found similar results, indicating that extended storage in formulations did not adversely affect the infectivity of EPNs against Culex quinquefasciatus larvae. Moreover, *H. indica* formulations of the present study at 25 °C exhibited rapid and consistent insect mortality. On the 90th day, the gum katira biogel-based treatment showed the highest mortality (80%), possibly due to improved bioagent stability in the formulation matrix. This is in line with the findings of Gayathri and Nisha (2023) who observed over 50.00% mortality of epilachna beetle (*Henosepilachna. septima* Poinar) when *H. indica* juveniles were stored in alginate gel and talc-based formulation matrices for up to 8 weeks. Wu et al. (2023) also pointed out the protective role of formulation matrices by demonstrating that a liquid starch-based EPN formulation provided optimal UV protection for immobilized EPNs, preserving both infective juvenile viability and virulence.

In the current study, the analysis of the physico-chemical properties revealed the acidic nature of the prepared compositions, which may be due to the acidic pH of gum katira employed as the major component of the matrices. The slightly acidic pH of the matrix is favorable for *S. abbasi* IJs (Mukhopadhyay et al. 2019). However, the highest pH of the matrix was observed in Gk + DE composition (6.10), where the increase in pH might be due to the presence of diatomaceous earth. The pH of diatomaceous earth was 8.5–9.0, whereas that of vermiculite was 7.5–8.0. This difference in pH of the individual carriers might have affected the survival of test *S. abbasi* EPN IJs when they were in a combined form with the biogel. This observation is in line with the previous report by Guo et al. (2017), where the diatomite-based formulations of *Steinernema* spp. registered the least survival among the selected carriers. In future, the test EPN species of the present study should be formulated in biogel compositions containing gum katira singly or in combination with vermiculite. DE with pH in the alkaline range may be avoided in these two species for formulation development purposes. Also, in future, DE of different grades having varying physicochemical properties may be used to study the compatibility with different EPN species. The viscosity of the compositions had not been found to have any direct effect on the survival of test EPN IJs. So, in brief, the present work demonstrates the successful application of gum katira, a pure biopolymer of plant origin, for the development of bio gel-based bioinsecticidal formulations of *S. abbasi* with improved survival at ambient and high storage temperatures, mitigating nematode survival constraints of tropical and sub-tropical countries. However, more information on the compatibility of *H. indica* IJs and formulation auxiliaries based on their physiological behavior should also be generated in the future for the successful preparation of bioformulations based on these nematode species. Furthermore, biopolymers are reported to be prone to microbial attacks (Pathak 2017). Therefore, in the present study, the selected biopolymer gum katira was used after autoclaving and no microbial contamination was observed throughout the experimental period. Additional studies may further be conducted to find suitable antimicrobial agents for biopolymer-based EPN formulations for their large-scale applications and adaptability. The formulation prototypes reported in this present study may be used in the future to develop EPN-based bioformulations for different pest management programs, as a step forward toward attaining agricultural as well as environmental sustainability.

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Authors' contributions AM, JA, AK and DK conducted the laboratory-based experiments. AS, VSS, and SMN provided resources. AM performed the statistical analysis. AS conceptualized the study, and supervised the experiment. AS, VSS, AD and AM prepared the original draft. All authors contributed to the article and approved the submitted version.

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Data availability statement The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests The authors declare that they have no competing interests.

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