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Survival and Infectivity of Entomopathogenic Nematodes Formulated in Sodium Alginate Beads

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Abstract

An alternative control method to the use of chemical insecticides against soil dwelling insect pests is the application of entomopathogenic nematodes formulated in alginate beads for enhanced shelf life. The aim was to compare the benefit on nematode survival and infectivity of: (i) pre-conditioning of juveniles, and (ii) coating of alginate beads. The nematodes *Steinernema glaseri*, *Steinernema carpocapsae,* and *Heterorhabditis bacteriophora* were reproduced in last instar larvae of the wax moth *Galleria mellonella* and the infective juveniles emerged were subjected to two capture treatments: white traps and plaster of Paris, the latter was utilized as a pre-conditioning treatment. A total of 1,000 infective juveniles were formulated in each sodium alginate bead with or without an alginate coating. The beads were stored at 23 ± 3°C and a bidistilled water suspension of nematodes was set as a control. The survivorship of these formulates and their infectivity on *Galleria mellonella* larvae were evaluated every 7 d post-formulation (dpf). In beads without pre-conditioning juveniles nor coating, *Steinernema carpocapsae* had the higher survival rate (58.8%), the longest survival time (28 dpf) and the higher infectivity (100%) as well. Pre-conditioning improved the survival and infectivity of *Steinernema glaseri* by 22.5% at 21 dpf and 70.0% at 14 dpf, respectively. Alginate coating increased survival (21.7%) and infectivity (95%) of *Heterorhabditis bacteriophora* for up to 28 dpf. The combination of pre-conditioning and coating slightly favored the survival (10% for up to 14 dpf) of *Steinernema glaseri* and infectivity (100% for up to 35 dpf) of *Steinernema carpocapsae*. Non pre-conditioned *Steinernema carpocapsae* formulated in uncoated beads was the combination with better performance in survivorship (58.8%) and infectivity (100%) at 28 dpf. It was concluded that non pre-conditioned *Steinernema carpocapsae* formulated in uncoated beads was the combination with better survivorship and infectivity.

Key words

Biological control, Coating, *Heterorhabditis*, Pre-conditioning, *Steinernema*.

Entomopathogenic nematodes (EPNs) (Nematoda: Heterorhabditidae and Steinernematidae) are effective biological control agents of insects pests due to their ease of adaptation, high lethality against major pests and safety (Grewal, 2002), are exempt from registration by the Environmental Protection Agency (EPA) in the U.S.A, are compatible with various chemical pesticides and are feasible for genetic selection (Kaya and Koppenhöfer, 2004). The applications of Steinernematidae have demonstrated efficacy for the control of insect pests from soil as white grubs (Subramanian and Muthulakshmi, 2016).

EPNs are commonly stored in an aqueous suspension and achieve a survival time of 6 to 9 mon

for Steinernematids and 3 to 4 mon for Heterorhabditids at 4°C to 15°C (Koppenhöfer, 2007; Stock and Goodrich-Blair, 2014). However, the application of EPN aqueous suspension on soil surface with conventional liquid spray technology, such as hand-operated sprinklers and hydraulic or pneumatic actuators, can affect the post-application effectiveness (Shapiro-Ilan et al., 2006; Hiltpold, 2015). Also, the cooling system required to maintain its storage stability during the transportation and spray technology required for the application increase the cost of use of EPNs (Chen and Glazer, 2005).

To promote the expansion of the use of EPNs as biological control agents, current research focuses on the development of low-cost formulations with: greater storage stability at room temperature, increased resistance during the transportation to the application site, where it dissolves for application in aqueous medium or it is deposited directly on moist soil (Burges, 1998; Grewal, 2000; Grzywacz et al., 2014), effective control of target insect pest and greater persistence in the field (Grewal, 2002; Shapiro-Ilan et al., 2012). The EPN formulation in gelling materials partially satisfies these requirements since it has been designed to provide a hydrophilic environment that prevents or delays the desiccation to increase its survival, especially on foliar surfaces (Navon et al., 2002). Since alginate biomaterials are used for tissue regeneration due to biocompatibility (Szekalska et al., 2016), its use in EPN formulation benefit its shelf life, is cheaper, and easier to elaborate and apply (Vemmer and Patel, 2013).

The success of EPN infection depends on the storage stability of the formulated product, which is also important for marketing; therefore, there is still scope for increasing that parameter (Navon et al., 2002; Sharma et al., 2011), but keeping it cheap and attending to its ease of use (Grzywacz et al., 2014). Some suggested strategies to improve the alginate formulation are: harden the surface to retain EPNs over an extended period of time (Hiltpold et al., 2012), slow down EPN metabolism before the formulation, probably forcing them into a partial anhidrobiosis through slow desiccation in glycerol, but maintaining the EPN infectiousness, and enhance it once the state dormancy breaks (Hiltpold, 2015; Kary et al., 2016). However, the survival of EPNs formulated in alginate beads (ABs) is no longer than 3 to 4 mon when stored at room temperature (22–25°C) (Kagimu et al., 2017).

The natural pre-conditionings of EPNs, before the formulation and coating of Abs, are cheaper strategies to improve the shelf life and their benefits and have been investigated marginally. The pre-conditioning of infective juveniles (IJs) in plaster of Paris prior to formulation in sodium ABs may have a positive effect on their survival time and infectivity since Cortés-Martínez et al. (2017) found that the survival of *Steinernema glaseri* mechanically pelletized in diatomaceous earth was improved if the IJs used were pre-conditioned on wet plaster of Paris after host emergence, compared to those from white trap.

The research questions we tried to answer was: can the functionality of the sodium ABs be improved to preserve the survival and infectivity of the EPNs by pre-conditioning the IJs prior to formulation and by slowing down the drying process through coating the ABs with an extra layer of sodium alginate? Therefore, the objective was to evaluate the survival and infectivity of IJs of three species of nematodes, with or without pre-conditioning in a modified trap of plaster of Paris, formulated in ABs with and without coating, under laboratory conditions at room temperature.

Materials and methods

Entomopathogenic nematodes

Infective juveniles of the nematodes *Steinernema carpocapsae* (*Sc*), *Heterorhabditis bacteriophora* (*Hb*), and *Steinernema glaseri* (*Sg*) collected within a week after their emergence from the host cadavers were used. The nematodes had been reared in last instar larvae of the greater wax moth, *Galleria mellonella*, at 23 ± 3°C and stored at 12°C.

Capture of entomopathogenic nematodes

Two capture methods of IJs were used, a modified White trap (WT) and a WT filled with a layer of plaster of Paris (PP) instead of the filter paper on small petri dish (Stock and Goodrich-Blair, 2014). The PP was used for the pre-conditioning of IJs upon emergence from infected hosts. A total of 30 *Galleria mellonella* larvae were inoculated with *Sc* or *Hb* and 60 with *Sg* IJs in petri dishes with a filter paper disc (90 mm diameter, Whatman No. 1). The inoculation rate was 100 IJs per insect. Three days after inoculation, the infected hosts were divided between the two IJs capture treatments and incubated at 23 ± 3°C. A few drops of bidistilled water were occasionally applied on the plaster to prevent desiccation of the host.

Infected cadavers placed in WT or PP were checked daily to inspect the emergence of IJs. Due to the faster movement of juveniles in PP, they were collected from the surrounding water three days after the onset of the massive emergence. The IJs from the water in the WTs were collected 7 d after the emer-

gence. Then, the collected juveniles were separately concentrated in bidistilled water suspension at the rate of 25,000 IJs/ml into partially closed 250 ml tissue culture flasks and stored at 12°C for a week.

Elaboration of the alginate beads

The ABs were made following the ionic gelation method (Vemmer and Patel, 2013). 20 ml of IJs aqueous suspension was deposited in 20 ml of a 1% solution of sodium alginate, 36% glycerol, and 0.05% green vegetable dye for 24 h at 12°C. Final composition was 0.5% of sodium alginate, 18% glycerol, and 0.025% green vegetable dye. Droplets of 83.33 µl of the alginate nematode suspension containing approximately 1,000 IJs were deposited in a 2% $CaCl_2$ solution using a 10 ml Mohr pipette with an exit hole of 3 mm in diameter, the drops remained in the solution for 20 min. A total of 470 ABs were recovered by treatment with a sieve, rinsed three times with bidistilled water to remove residues from the $CaCl_2$ solution and deposited in a 500 ml beaker which was covered with Parafilm® tape.

Coating of the alginate beads

Each AB was individually immersed for 2 s in a sodium alginate solution containing 0.5% of sodium alginate, 18% glycerol, and 0.025% green vegetable dye, to be deposited afterwards in the $CaCl_2$ solution for 20 min. The ABs were recovered with a sieve, rinsed three times with bidistilled water, and deposited in a 500 ml beaker which was covered with Parafilm® tape.

Storage conditions of the alginate beads

The beakers containing the ABs were stored at room temperature (23 ± 3°C) for 50 d. Monitoring of storage conditions was done with a temperature and relative humidity data logger (Control Company®, model 4040, accuracy of ±1°C and ±5% RH).

Bioassays of survival of entomopathogenic nematodes

Every two days, a dissected AB was placed in a petri dish (60 mm diameter) and rehydrated with 7 ml of bidistilled water for a period of 24 h to soften them and facilitate the release of IJs (Kaya and Nelsen, 1985). Then, a 1 ml sample containing approximately 143 IJs was taken, divided in ten 100 µl-subsamples and the alive and dead juveniles were counted using a stereoscopic microscope at 30×; the living ones had mobility by themselves or moved when stimulated with a dissecting needle, while dead IJs had shriveled surface or gas bubbles inside body (Peters, 2016). The survival rate (SR) was calculated as the proportion of alive juveniles over the total juveniles (alive and dead).

Bioassays of infectivity of entomopathogenic nematodes

Infectivity was evaluated every 7 d according to the mortality of *Galleria mellonella* (Ricci et al., 1996). A filter paper disc (15 mm diameter, Whatman no. 413) was placed in each cell of the 24-well plate, where 100 µl of bidistilled water and a dissected AB were placed afterwards. The plates were maintained in an incubator at 25 ± 2°C for 24 h for reactivation of the EPNs. Subsequently, the *Galleria mellonella* larvae were individually placed in each well plate, covered with Parafilm® tape, and returned to the incubator. Insect mortality was checked 72 h later. To verify the mortality of *Galleria mellonella* larvae by EPNs, each host with signs of infection was placed in a petri dish and incubated at 25 ± 2°C until the IJs emerged.

Statistical analysis of results

The experiment was performed by duplication and each bioassay was repeated five times. The results of the duplicate tests were combined for the final analysis. Survival analysis was performed using Kaplan-Meier plots and the Log-Rank test ($P \leq 0.001$). The analysis of variance (ANOVA on ranks) and the SNK test ($P < 0.05$) were performed between the species of EPNs, pre-conditioning, coating of Abs and % infectivity on *Galleria mellonella*. All analyzes were performed using SigmaPlot® 12 software (Systat Software, Inc., San Jose, CA, USA).

Results

Species of entomopathogenic nematodes

The survival rates per week of EPNs in ABs are presented in Table 1, which shows that *Sc* had the best viability, maintaining a SR value of 58.8% for 28 d, followed by *Hb* with a SR of 51.4% at 21 d and *Sg* with the shortest survival time with 7 d and 15% of SR. The differences between the survival curves by nematode species were highly significant ($\chi^2 = 2,507$, Log-Rank < 0.001).

Pre-conditioning nematodes

The SR of pre-conditioned EPNs in PP and formulated in ABs remained 39.6% and 53.3% in 7 d for *Sg* and *Sc*, respectively, while SR of *Hb* was above 9.8% in 21 d

Table 1. Survival over time of entomopathogenic nematodes (EPNs) formulated in sodium alginate beads (ABs) in relation to the pre-conditioning (Prec) of IJs or ABs coating (C) or both (Prec-C), stored at 23 ± 3°C. *Steinernema glaseri* (*Sg*), *Steinernema carpocapsae* (*Sc*), and *Heterorhabditis bacteriophora* (*Hb*).

		Survival rate after formulation (%)					
Factor	Treatment	7 d	14 d	21 d	28 d	35 d	MSt (days ± SE)
EPN	*Sg*	15	0	0	0	0	8.05 ± 0.079
	Sc	79	79	58.8	58.8	0	26.07 ± 0.389
	Hb	93.5	93.5	51.4	0	0	22.00 ± 0.209
Prec	*Sg*-Prec	39.6	39.6	22.5	0	0	13.21 ± 0.262
	Sc-Prec	53.3	23	9.8	0	0	13.02 ± 0.218
	Hb-Prec	69.3	69.3	7.6	0	0	14.69 ± 0.182
C	*Sg*-C	20	0	0	0	0	8.40 ± 0.088
	Sc-C	79	79	41.6	41.6	0	26.96 ± 0.355
	Hb-C	100	100	30.5	21.7	0	22.22 ± 0.262
Prec-C	*Sg*-Prec-C	40	0	0	0	0	9.80 ± 0.108
	Sc-Prec-C	74.4	67.8	31.5	31.5	10	22.06 ± 0.372
	Hb-Prec-C	92.4	0	0	0	0	13.46 ± 0.058

MSt, Mean survival time; d, days after formulation; SE, standard error.

(Table 1). The maximum extension of the survival time and the highest SR were, using *Sg*, 21 d and 22.5%, respectively. The differences between the survival curves of the treatments with pre-conditioning IJs were statistically significant (χ^2 = 3,207, Log-Rank < 0.001).

Coating of alginate beads

Amongst the three EPNs formulated in ABs coated with a layer of sodium alginate, *Sc* was maintained with an SR of 41.6% for 28 d, *Hb* with 21.7% for 28 d, and *Sg* with 20% for 7 d (Table 1). The differences between the survival curves of the EPNs in coated ABs were statistically significant (χ^2 = 5,086, Log-Rank < 0.001). The SR trends for *Sg* and *Sc* are similar to those observed in the corresponding treatments by EPN factor; therefore, no benefit on survival by coating of ABs was observed on EPNs.

Combined effect of the pre-conditioning of the EPN and the coating of the beads

The maximum extent of the survival time of *Sg* and *Hb* was just 7 d when pre-conditioned IJs were formulated in coated ABs. The SR of *Sg* decreased rapidly to 40% in the seventh day, whereas SR of *Hb* was maintained above 92.4% in the seventh day (Table 1). The SR of *Sc* remained above 67% in 14 d and its survival time was extended to 35 d, albeit with about 10% SR. The differences between the survival curves of the preconditioned EPNs in coated ABs were statistically significant (χ^2 = 4,288, Log-Rank < 0.001).

Infectivity of formulated EPNs

All three treatments within the four factors tested: nematode species (EPN), pre-conditioning (Prec), coating (C), and the Prec-C combination, were statistically significant different, reaching the lower *P*-values the first two (Table 2). For example, according to ANOVA, where the *F*-test applies to the means by factor, the infectivity (IN) of the EPNs in sodium ABs against *Galleria mellonella* was significantly different for each species applied (F = 6.289, P = 0.023) and the difference in the mean IN between *Sc* and *Sg* was greater (P < 0.05) than would be expected by chance after allowing for effects of differences in time

Table 2. Results of the statistical analysis over time of infectivity (IN) against *Galleria mellonella* by EPNs from sodium ABs, in relation to the pre-conditioning (Prec) of IJs or ABs coating (C) or both (Prec-C). *Steinernema glaseri* (*Sg*), *Steinernema carpocapsae* (*Sc*), and *Heterorhabditis bacteriophora* (*Hb*).

		Infectivity after formulation (%)[a]							
Factor	Treatment	7 d	14 d	21 d	28 d	35 d	Mean IN (% ± SE)	*F*	*P*
EPN	*Sg*	15b	0b	0b	0b	0b	3 ± 2.19bc	6.28	0.023
	Sc	100a	100a	100a	100a	0a	80 ± 9.18a		
	Hb	90a	100a	0b	0b	0a	38 ± 10.8ac		
Prec	*Sg*-Prec	75a	70a	0a	0a	0a	29 ± 9.5bc	5.79	0.006
	Sc-Prec	100a	100a	55a	0a	0a	51 ± 11.3a		
	Hb-Prec	100a	90a	0a	0a	0a	38 ± 10.8ac		
C	*Sg*-C	35b	0b	0b	0b	0a	7 ± 3.3b	219.59	<0.001
	Sc-C	100a	100a	100a	80a	0a	76 ± 9.58a		
	Hb-C	100a	100a	90a	95a	0a	77 ± 9a		
Prec-C	*Sg*-Prec-C	40b	10b	0b	00b	0b	10 ± 5.7b	160.54	<0.001
	Sc-Prec-C	100a	100a	90a	90a	100a	96 ± 2.7a		
	Hb-Prec-C	90a	10b	0b	0b	0b	20 ± 8.46b		

[a]Different letters in the same column within each factor and time period after formulation indicate statistically different values, SNK test ($\alpha = 0.05$).

after formulation. *Sc* was the most infectious, killing all larvae in a period of 28 d (100%), *Hb* killed almost all of them in a period of 14 d (~ 100%), but *Sg* killed a few larvae in the first 7 d (15%), which lowered its mean IN to 3.0% for a period of 35 d.

The IN of preconditioned EPNs formulated in sodium ABs against *Galleria mellonella* over time ranged from 55% to 100% (Table 2). The difference in the mean IN among the different EPN species is greater ($F = 5.795$, $P = 0.006$) than would be expected by chance after allowing for effects of differences in time after formulation, and the statistical difference was significant only between *Sg* and *Sc* nematodes ($P < 0.05$).

The IN of EPNs formulated in coated ABs on *Galleria mellonella* was between 35% and 100% and mean IN was significantly different between each specie applied ($P < 0.001$). *Sg* was the less infectious and therefore statistically significant different if compared with *Sc* or *Hb* ($P < 0.001$). As it can be seen in Table 2, this trend in statistical difference was maintained during all times after formulation.

The formulation of preconditioned EPNs in coated ABs results in statistically significant difference on mean IN for each species applied on *Galleria mellonella* larvae ($F = 160.548$, $P < 0.001$). The combined treatment Prec-C improves the mean IN of *Sc* much more than on *Sg* or *Hb* ($P < 0.05$). Table 2 shows that the mean IN of *Sc* had increased by 45% when compared with Prec and by 20% when compared with C and, the combination Prec-C produced negative results in *Sg* with respect to Prec only, while *Hb*-Prec-C had lower IN with respect to *Hb*-C only.

Mean survival time and mean infectivity of formulated EPNs

In Table 1, the mean survival time (MSt) of EPNs in ABs by each factor is shown for a time of 35 dpf. Non pre-conditioned *Sc* in uncoated ABs had the highest MSt (26.07 ± 0.389 d), but pre-conditioning of IJs before the formulation shows a negative effect on MSt (13.02 ± 0.218 d), and the same trend in MSt was observed using pre-conditioned *Hb*. The coating of ABs

did not show a significant difference on MSt of *Sg*, *Sc* or *Hb*, in relation to ABs uncoated. Only in the case of *Sc*, the coating of ABs reversed the negative effect of pre-conditioning of IJs, when both factors were combined, increasing MSt from 13.02 ± 0.218 d to 22.06 ± 0.372 d. *Sg* had the lowest performance formulated in Abs, but pre-conditioning alone increased the MSt significantly from 8.05 ± 0.079 d to 13.21 ± 0.262 d.

The mean infectivity (mean IN) increased significantly with the pre-conditioning of *Sg*, since it went from 3 ± 2.19% to 29 ± 9.5%, while for *Sc* a significant decrease occurred from 80 ± 9.18% to 51 ± 11.3% (Table 2). Coating of ABs had its greatest effect on the mean IN of *Hb*, with a value of 77 ± 9.0%, while the pre-conditioning combined with the ABs coating increase the mean IN of *Sc* to 96 ± 2.7%. Based on these mean results, it could be recommended doing more research to validate the use both pre-conditioning and coating treatments for the entire formulation process of *Sc* in ABs.

Discussion

The nematode *Sc* had the longest survival time (35 d), followed by *Hb* (28 d), and *Sg* (14 d); all of them were formulated in ABs stored at room temperature (23 ± 3°C). The differences in the maximum survival time were highly significant with the other EPN. The survival and infective fitness of EPNs are usually well maintained in sodium ABs, but the results of this study show marginal improvements, not enough to compare the values observed by other studies on formulations with the same species used.

A difference in the experiments reported is that the storage conditions were usually maintained at constant temperature. For example, after six months of storage in sodium ABs at 23°C and high relative humidity (100%), the SR of the *S. feltiae* IJs (IS-6) was almost 100% and its infectivity on *Galleria mellonella* was maintained similar to that of newly harvested IJs (Chen and Glazer, 2005). After three months of storage at 8°C and 25°C, the survival rate of 1,500 IJs glycerol dehydrated *Steinernema carpocapsae* (SH1) formulated in alginate granules was approximately 30% (Kary et al., 2016). Hiltpold et al. (2012) reported that *Hb* Poinar nematodes escape from alginate capsules stored at room temperature (25°C) and are able to kill *Galleria mellonella* larvae in 1 to 6 d. Recently, Kary et al. (2018) reported the survival of glycerol dehydrated *Heterorhabditis bacteriophora* (NIR1) formulated in sodium ABs stored at 25°C; in two months, their SR was 40% and minor to 10% in the third month. Non-published data on the formulation of *Steinernema glaseri* IJs in ABs to compare were found in the databases of Web of Science®.

The reported experiments of nematode formulation under variable storage conditions are limited. Only the survival of Egyptian strains of two EPNs contained in plastic bags with calcium alginate as carrier reported by Hussein and Abdel-Aty (2012) is similar to the survival of EPNs formulated in ABs, because they found that their nematodes show a decreasing trend in SR after 50 d of storage at 25 ± 2°C, resulting in an SR of approximately 25% and 50% for *Hb* (BA1) and *Sc* (BA2), respectively.

Although pre-conditioned *Sg* and *Hb* IJs survived in low percentages when are formulated in sodium ABs after 14 d, a high percentage of infectivity on *Galleria mellonella* larvae was registered at this time. The sodium alginate is a material that usually has good biocompatibility because it does not react to contact with the biological materials with which it interacts (Orive et al., 2002), so it has been acknowledged that this property has a positive effect on the maintenance of the infectivity of EPNs, as confirmed by our study.

The capture of IJs after the migration over plaster of Paris was a useful treatment for pre-conditioning *Sg* prior to formulation in ABs as it increased SU. Therefore, we confirmed that the formulation of pre-conditioned IJs of *Sg* (NJ-43) on PP significantly improved their survivorship as Perez et al. (2003) and Cortés-Martínez et al. (2017) suggested. However, we cannot offer a conclusion about the benefit of pre-conditioning IJs on infectivity of *Sg*, because the insect pests and results were different.

The coating of ABs produced a very high increase in the infectivity of *Sc* or *Hb* on *Galleria mellonella* with respect to *Sg*. As suggested by Ruiz-Vega et al. (2011), it is possible that the first two have a lower tolerance to desiccation, while *Sg* showed the highest drying tolerance, at the beginning of a selection process for tolerance to low A_w. As a result, the coating reduced the drying rate of ABs, giving additional moisture to the *Sc* and *Hb* nematodes which allowed them to remain viable for a longer time under the evaporative and osmotic conditions created by gelling materials such as sodium alginate (Block, 2002).

The combination of pre-conditioning and coating produced similar results to those observed in *Sc* or *Hb* when coating was applied alone; but on average, *Sc* significantly exceeded *Hb* as its infectivity had a significant increase associated with Prec. This behavior could also be associated with the higher response of *Sc* to Prec due to the greater tolerance of the genus *Steinernema* to low relative humidity. In the study by Ruiz-Vega et al. (2011), the nematode *Steinernema glaseri* showed the highest percentage of survival at low A_w, which agrees with the results obtained by Brown and Gaugler (1997), who found that the

emergence of nematodes of the genus *Steinernema* was less affected than that of *Heterorhabditis bacteriophora* under low relative humidity conditions.

Compared with the shelf life of available formulations as EPN infected insect cadavers or water dispersible granules (Kagimu et al., 2017), the proposed ABs are still not better because the two strategies tested marginally improve the survival and infectivity of nematodes, mainly depending on the fitness of each EPN species tested. Pre-conditioning benefits to *Sg*, coating of ABs benefits to *Hb* and Prec-C benefits slightly to *Sc*. However, the advantages of ease of IJs pre-conditioning and ABs preparation are maintained, which is important to develop low cost and simplest biopesticide technology for small farmers in less industrialized countries, where resources and equipment as liquid spray technology or air-conditioning are limited (Grzywacz et al., 2014).

Conclusions

Under a particular combination of formulation characteristics as EPN species, alginate coating and response of the EPNs to the pre-conditioning in plaster of Paris, the functionality of the sodium ABs to maintain viable and pathogenic IJs was favored. In relation to the pre-conditioning, only *Steinernema glaseri* IJs marginally favored their survival, as well as their infectivity on *Galleria mellonella*. The preconditioned IJs of *Steinernema glaseri* and *Heterorhabditis bacteriophora* showed a low percentage of survival to the formulation in sodium ABs; however, in the bioassays, a high percentage of infectivity was observed on 3rd instar larvae of *Galleria mellonella*. The coating of the sodium ABs resulted in the maintenance of a high infectivity in *Sc* and *Hb* up to 28 d, while the combination of pre-conditioning and coating slightly favored *Sc* followed by *Sg*.

The most promising combination to maintain nematode survival and infectivity at room temperature (23 ± 3°C) was using *Steinernema carpocapsae* IJs with or without pre-conditioning in coated or uncoated sodium ABs, which extended its survival time to 35 d with a minimal survival rate of 10% and preserved its infectivity (100%) against *Galleria mellonella* larvae.

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