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Assessment of Entomopathogenic Nematodes in Oil Emulsions to Control *Scyphophorus acupunctatus* in Agave under Laboratory Conditions

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Abstract: The weevil *Scyphophorus acupunctatus* Gyllenhal causes damage and losses in agave crops and has traditionally been controlled using contact and systemic agrochemicals. Implementing microbial control strategies is proposed as an alternative to mitigate the environmental impact associated with agrochemicals. The objective of this study was to determine the survival of entomopathogenic nematodes in oil emulsions for the control of adult *S. acupunctatus*. Three species of entomopathogenic nematodes were evaluated: *Steinernema carpocapsae*, *S. glaseri*, and *Heterorhabditis bacteriophora*. We used two concentrations (50 ± 5 and 100 ± 10 infectious juvenile nematodes), and oil emulsions derived from *Salvia hispanica*, *Triticum vulgare*, and *Olea europea* with oil purity of 20% and 40%. The effectiveness of these treatments was assessed by determining the mortality rate of *S. acupunctatus*. The results indicate that the combination of *S. glaseri* and *H. bacteriophora*, at concentrations of 50 ± 5 and 100 ± 10 nematodes, respectively, with *T. vulgare* and *O. europea* oils, achieved a mortality rate of 85.76% in *S. acupunctatus* adults at 24 h. At 120 h, a mortality rate of 100% was achieved with specific formulations, such as *S. glaseri* with 100 ± 10 nematodes + *O. europea*, and *H. bacteriophora* with 100 ± 10 nematodes + *O. europea*. Consequently, we conclude that oil formulations combined with nematodes show potential as an effective and environmentally friendly alternative for the control and management of *S. acupunctatus*.

Keywords: pest control methods; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae*; *S. glaseri*

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1. Introduction

In the state of Oaxaca, Mexico, more than 10,000 hectares of *Agave* spp. are cultivated, and 200,000 tons of agave are allocated to the mezcal industry, which is registered to generate a total of USD 1389 million at national and international sales [1]. The production of mezcal is limited by technological and socio-economic factors, but principally due to the incidence of pests and diseases. Because the leaves and "piñas" of mature plants (heart of the agave plant where carbohydrates are stored) have a high sugar content, they are cooked, fermented, and distilled to produce mezcal. However, also due to their high sugar content, they are affected principally by *Scyphophorus acupunctatus* larvae and adults [2] (Figure 1), which damage mature plants and cause production losses of 20–40%, causing plant death [3,4]. Cultural, chemical, biological, and etiological control methods have not been successful because the life cycle of the insect takes place in the internal part of the plant (leaves and stem), making it difficult for the chemical pesticides to come into contact with the agave weevil.

Figure 1. (**A**,**B**) Agave plant and "piña" damaged by weevils. (**C**) Heart of the agave plant with adult *Scyphophorus acupunctatus*. (**D**,**E**) Healthy agave plant and "piña".

Agriculture demands less harmful alternatives for the environment and for farmers. Among the fundamental aspects for the integrated management of this insect, which has had contributions from biology and ecology, biological control by means of parasitoids or predators has been a viable alternative [5–7]. Entomopathogenic nematodes species (EPNs) have been successful in dealing with agricultural plagues [6,8,9]. However, in the field, their successful use is highly unlikely because biotic and abiotic factors reduce their useful life, and results are not consistent [10].

In Mexico, there are few documented experiences of the use of nematodes as biological control agents for insect pests. The published studies have focused on two different aspects: one involving the search for new isolation methods and another on the management and control of pest insects living in the soil. Nematodes of the genera *Heterorabditidae* and *Steinermatidae* are considered to have high potential as an alternative for the control of insects that live their entire lives in the soil or protected in plant parts [5,6]. Under laboratory conditions, they have been shown to be general pathogens due to their capacity to kill several species of insects [8,9]. However, in the field, this is very unlikely because of biotic and abiotic factors that reduce their useful life, generating mixed results. Entomopathogenic nematodes are compatible with chemical pesticides and adherents, and commercial production of these nematodes is easy using fermentation tanks or insect larvae [10].

Dehydration is the constraining factor in EPNs' mode of action [11]. For this reason, they are used with additives, processing aids, or formulations to improve their pathogenicity and survival in the field [10,12]. The use of vegetable oils together with EPNs improves and generates more efficient formulations against insects [13,14]. Some positive results have been found in the survival and anti-desiccation of the *Heterhabditis bacteriophora* and *Sterneinerma feltiae* nematodes using Tween and glycerol at 1% in the formulations [15]. Local integrated pest management programs (Programas Locales de Manejo Integrado de Plagas—PIHLP) promote the local production of EPNs [6,16]. In this context, it is necessary to develop new systems to guarantee high survival and virulence of the infective juveniles (IJs) after medium-term storage, without increasing costs and with relatively accessible products. Here, we studied three vegetable oils in a possible coadjuvant model.

The misuse of contact insecticides such as malathion and endosulfan contaminates the environment, and their residues remain in the soil and in the agave plants. For this reason, it is necessary to assess the compatibility of oils and biological control agents (entomopathogens nematodes) to determine whether there is any nematicidal activity and to find the exact combinations of entomopathogenic nematodes and oil, either as coadjuvants or additives, to increase the biological activity.

Based on the above information and under the hypothesis that the oils are compatible, to identify synergic or antagonistic effects in the use of nematodes, the objective of this research was to determine the survival of entomopathogenic nematodes (*H. bacteriophora*, *S. glaseri*, and *S. carpocapsae*) in vegetable oil emulsions at two concentrations and the mortality of adult *Scyphophorus acupunctatus* caused by these treatments under laboratory conditions.

2. Materials and Methods

This research was conducted in June–October 2022 at Santa Cruz Xoxocotlán, Oaxaca, México (Latitude 17°01'31"N, 96°43'11"W, and 1500 m above sea level). Nematode survival and mortality experiments were conducted in the Biological Control Laboratory at CIIDIR Oaxaca, at maximum and minimum relative humidities of 56 ± 8.36% and 45 ± 8.68%, respectively, and maximum and minimum temperatures of 33 ± 2.16 °C and 23 ± 2.2 °C, respectively.

Salvia hispanica, *Tritricum vulgare*, and *Olea hispanica* oils were used. These oils have been used in formulations with entomopathogenic fungi [3], and can be found on the market at 100% purity (Laboratorios Hersol, S.A. de C.V., Edo. México, México).

The entomopathogenic nematodes *S. carpocapsae*, *S. glaseri*, and *H. bacteriophora* in their infective juveniles (IJ) stage were reproduced in the last instars of the wax moth *Galleria mellonella* under laboratory conditions.

2.1. Survival of Entomopathogenic Nematodes in Oil Emulsions

The survival of three entomopathogenic nematodes (*S. carpocapsae*, *S. glaseri*, and *H. bacteriophora*) was evaluated; the nematodes were obtained from the strain repository at CIIDIR Unidad Oaxaca. The nematodes (50 ± 5 EPNs) were mixed in three vegetable oil emulsions, chia seed (*Salvia hispanica*), wheat (*Triticum vulgadere*), and olive (*Olea europea*), at two concentrations (20 and 40% purity). The concentrations of 20/40 Volume % (Vol.-%) of the emulsions were fixed on a glass plate measuring 55 mm in diameter using a Science MED 100–1000 µL capacity micropipette for the oil concentration of 20% (200 µL oil + 800 µL of nematodes in DW) and the oil at 40% (400 µL oil + 600 µL nematodes in DW). A 1 mL for both concentrations was used, and they were mixed using a magnetic stirrer for about 3 min. The mixtures were set in 55 mm wide × 15 mm high glass Petri dishes. Survival observations were carried out every 24 h for 168 h after applying the oil to the solution of nematodes. Twenty-one treatments, including the control (distilled water), were evaluated (Table 1), and each treatment was replicated 20 times.

Table 1. EPN survival in oil emulsions.

Treatment	Concentration (%)	Nematode	Treatment	Concentration (%)	Nematode
T1 DW (absolute control)	0	*H. bacteriophora*	T12 *T. vulgare*	20	*S. glaseri*
T2 DW (absolute control)	0	*S. carpocapsae*	T13 *T. vulgare*	40	*H. bacteriophora*
T3 DW (absolute control)	0	*S. glaseri*	T14 *T. vulgare*	40	*S. carpocapsae*
T4 *S. hispanica*	20	*H. bacteriophora*	T15 *T. vulgare*	40	*S. glaseri*
T5 *S. hispanica*	20	*S. carpocapsae*	T16 *O. europea*	20	*H. bacteriophora*
T6 *S. hispanica*	20	*S. glaseri*	T17 *O. europea*	20	*S. carpocapsae*
T7 *S. hispanica*	40	*H. bacteriophora*	T18 *O. europea*	20	*S. glaseri*
T8 *S. hispanica*	40	*S. carpocapsae*	T19 *O. europea*	40	*H. bacteriophora*
T9 *S. hispanica*	40	*S. glaseri*	T20 *O. europea*	40	*S. carpocapsae*
T10 *T. vulgare*	20	*H. bacteriophora*	T21 *O. europea*	40	*S. glaseri*
T11 *T. vulgare*	20	*S. carpocapsae*			

DW: distilled water.

2.2. Mortality of Scyphophorus acupunctatus Caused by Nematodes in Oil Emulsions

After the evaluation of entomopathogenic nematodes' (EPNs') survival in oil emulsions, the mortality of adult of *S. acupunctatus* was determined (Figure 2). Two species of nematodes (*S. glaseri* and *H. bacteriophora*) were tested at two concentrations (50 ± 5 and 100 ± 5 nematodes/*S. acupunctatus*) in the *T. vulgare* and *O. europea* oil emulsions at a concentration of 40%. The *S. acupunctatus* adults were placed individually in cylindrical 300 mL capacity plastic containers with a small piece of agave as food.

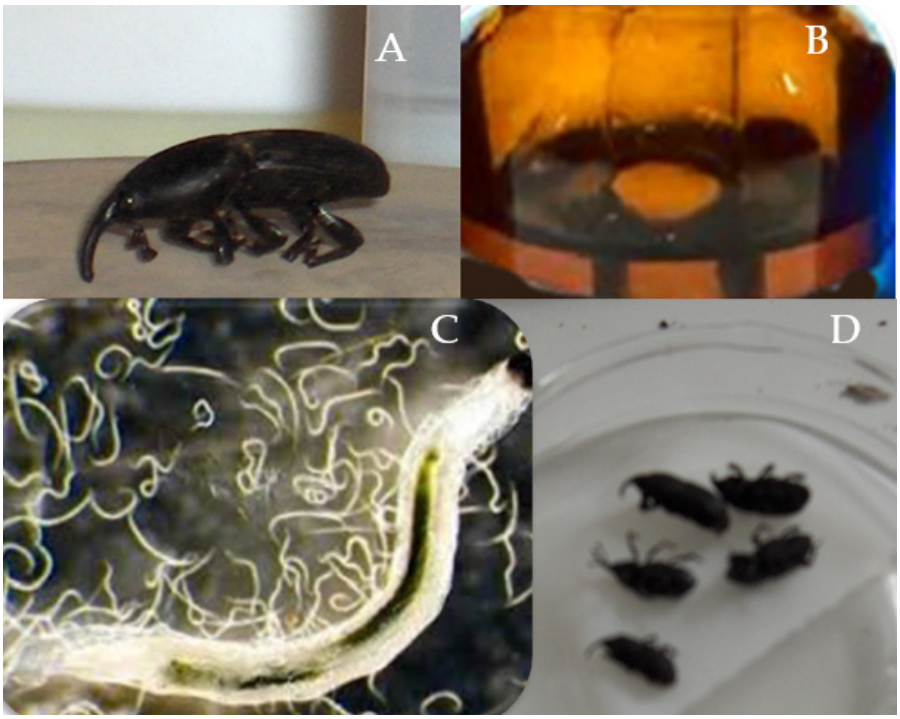

Figure 2. (**A**) *Sciphophorus acupunctatus* adult. (**B**) Vegetable oil. (**C**) Emergence of entomopathogenic nematodes. (**D**) Infected weevil cadavers after receiving oil emulsions with entomopathogenic nematodes.

After 48 h with the insects still alive, 1 mL of the each of the nematode concentrations of oil emulsion were applied over the insects and the food. Eleven treatments with 20 repetitions per treatment were evaluated with one insect per repetition. A technique was selected from *Insect Nematology* [17] and the insects were observed every 24 h until 100% mortality of the adult *S. acupunctatus* was obtained. The weevil cadavers were placed in white wet chambers and checked to determine whether there were any EPNs.

Likewise, the emergence of nematodes in *Galleria mellonella* larvae was determined via the Koch postulates. To confirm the formation of adults, 10 larvae were dissected and another 10 larvae were kept at 23 ± 2 °C to record the accumulated production of infective juveniles, counting the daily emergence until total exhaustion of the *G. mellonella* larvae.

2.3. Statistical Analysis

The percentages obtained for survival and efficiency were normalized via arcsine transformation $\sqrt{(x/100)}$ and processed through ANOVA. The statistical differences among average mortalities of each treatment were established via a Tukey test ($\alpha = 0.05$). All analyses were performed using the statistical software SAS/ETS® 9.1 [18].

3. Results and Discussion

3.1. Survival of Entomopathogenic Nematodes Formulated in Oils

After 24 and 48 h, it was found that the three evaluated nematodes (*S. glaseri*, *S. carpocapsae*, and *H. bacteriophora*) had the highest survival in *O. europea* oil at concentrations of 20 and 40%, with a mean of survival of 94.1–84.2%, followed by *T. vulgare* oil with 83.9–72.9%. The lowest survival of nematodes was found in *S. hispanica* with 71.8–56.4%. The three nematodes had 100% survival in the control. At the time of evaluation (24 and 48 h), the nematode that presented the highest survival (95.6–85.9%) was *S. glaseri* with *O. europea,* followed by *S. hispanica* (88.6–79.1%), and finally *T. vulgare* oil (78.6–68.6%). After 72 h, the survival of the evaluated nematodes (*H. bacteriophora, S. carpocapsae,* and *S. glaseari*) was 0% in the control (DW). This treatment was statistically different from the other treatments. Finally, it was found that after 120 h, all the evaluated nematodes remained viable. It was found that the *S. carpocapsae* nematode in *O. europea* oil at 40% purity had a survival of 62.2%, which was statistically different from the other treatments (Table 2).

Table 2. Survival of entomopathogenic nematodes in oil emulsions.

Treatment	Concentration (%)	Nematode	Time (h)				
			24	48	72	96	120
T1 DW (absolute control)	0	*H. bacteriophora*	100.0 ± 0.0 a	71.2 ± 2.0 bc	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
T2 DW (absolute control)	0	*S. carpocapsae*	100.0 ± 0.0 a	81.6 ± 1.7 b	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
T3 DW (absolute control)	0	*S. glaseri*	100.0 ± 0.0 a	70.8 ± 2.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
T4 *S. hispanica*	20	*H. bacteriophora*	56.4 ± 1.5 c	26.8 ± 2.8 e	12.6 ± 1.0 e	9.6 ±0.9 e	3.2 ± 0.2 d
T5 *S. hispanica*	20	*S. carpocapsae*	47.6 ± 2.0 e	17.6 ± 1.3 e	14 ± 1.2 e	12.8 ± 1.0 e	0.0 ± 0.0 d
T6 *S. hispanica*	20	*S. glaseri*	88.4 ± 3.3 b	78.6 ± 2.3 b	47.8 ± 3.7 c	46.2 ±3.3 b	41.2 ± 3.0 bc
T7 *S. hispanica*	40	*H. bacteriophora*	67.6 ± 2.8 c	57.4 ± 1.5 c	44.4 ± 3.4 c	32 ± 3.2 bc	14 ± 1.2 d
T8 *S. hispanica*	40	*S. carpocapsae*	82.4 ± 1.7 b	78.8 ± 2.3 b	58.8 ± 1.5 b	42.4 ± 3.2 b	32.4 ±3.1 c
T9 *S. hispanica*	40	*S. glaseri*	88.8 ± 3.3 b	79.6 ± 2.4 b	49.6 ± 3.9 c	35.6 ± 3.6 bc	30 ± 3.1 c
T10 *T. vulgare*	20	*H. bacteriophora*	84.8 ± 1.8 b	74.6 ± 2.2 b	46.2 ± 3.6 c	29.4 ± 1.8 bc	15.4 ± 1.2 d
T11 *T. vulgare*	20	*S. carpocapsae*	88.4 ± 3.3 b	73.2 ± 2.2 b	63.4 ± 2.5 ab	55 ± 1.5 ab	38 ± 3.7 c
T12 *T. vulgare*	20	*S. glaseri*	74.4 ± 2.2 bc	64.6 ± 2.5 bc	31.8 ± 3.2 cd	20.8 ± 2.0 c	10 ± 0.9 e
T13 *T. vulgare*	40	*H. bacteriophora*	90.6 ± 2.6 a	80.2 ± 1.6 b	73.8 ± 2.2 b	50.8 ± 1.7 ab	35.2 ± 3.6 d
T14 *T. vulgare*	40	*S. carpocapsae*	82.4 ± 1.7 b	72.4 ± 2.1 bc	54 ± 1.5 b	42 ± 3.2 b	30.8 ± 3.1 c
T15 *T. vulgare*	40	*S. glaseri*	82.8 ± 1.7 b	72.8 ± 2.1 b	66.8 ± 2.8 ab	54 ±1.4 ab	41.2 ± 3.0 bc
T16 *O. europea*	20	*H. bacteriophora*	96.2 ± 2.6 a	86.2 ± 1.9 ab	44.8 ± 3.2 c	20.4 ± 2.0 c	12.8 ± 1.0 e
T17 *O. europea*	20	*S. carpocapsae*	90.8 ± 2.6 a	80.8 ± 1.6 ab	35.6 ± 3.6 cd	33.2 ± 3.2 e	30 ± 3.1 c
T18 *O. europea*	20	*S. glaseri*	96 ± 2.0 a	86 ± 1.8 ab	63.6 ± 2.5 ab	50.8 ± 2.1 ab	42.4 ± 3.1 bc
T19 *O. europea*	40	*H. bacteriophora*	90.4 ± 2.6 a	80 ± 1.6 ab	42 ± 3.0 c	29.6 ± 2.6 c	17.2 ± 1.3 d
T20 *O. europea*	40	*S. carpocapsae*	96 ± 2.0 a	86.4 ± 1.9 ab	76.4 ± 2.3 a	68.4 ±2.8 a	62.2 ± 2.4 a
T21 *O. europea*	40	*S. glaseri*	95.2 ± 1.9 a	85.8 ± 1.8 ab	40.8 ± 3.0 c	20.8 ± 2.0 c	12.7 ± 1.0 c

Mean ± standard deviation. Means with different letters in each column are statistically different (Tukey, $p \leq 0.05$). DW: distilled water.

It is notable that the species of nematode and the concentrations of 20 and 40% in the three evaluated oils have a positive effect on nematode survival time and hydration. An evaluation of *Azadirachta* oil [19] reported that, at 100% purity after 120 h, the survival of the nematode *S. filtae* was 100%. Other studies [15] indicate that using glycerin and Tween at a concentration of 1% results in survival of the *H. bacteriophora* and *S. feltiae* nematodes after 168 h.

Survival rates [20] were linked to EPN metabolism, in which temperature and humidity are determining factors. The survival time found in our study on EPNs indicates important advances in the formulations of the emulsions with useful oils for managing insect pests in the field.

3.2. Mortality of Scyphophorus acupunctatus and Emergence of Nematodes Adhered to Oils

After 48 h, it was found that *O. europea* oil at a concentration of 40% purity with *S. glaseri* and *H. bacteriophora* at concentrations of 50 ± 5 and 100 ± 10 nematodes caused 50% mortality in *S. acupunctatus* adults (T3, T4, T7 and T8). These treatments are statistically different from the rest of the treatments. After 96 h, two treatments caused 90% mortality in *S. acupunctatus* adults: T4 (*S. glaseri* 100 ± 10 nematodes + *O. europea*) and T8 (*H. bacteriophora* 100 ± 10 nematodes + *O. europea*). After 120 h, these same treatments

caused 100% mortality in *S. acupunctatus* adult insects. An influential factor in the mortality of *S. acupunctatus* adults is the concentration of the nematodes *S. glaseri* and *H. bacteriophora* applied of 100 nematodes/insect had the highest control percentages. After 48–168 h, the control with distilled water and the oils *T. vulgare* and *O. europea* at 40% purity did not produce any mortality in *S. acupunctatus* adults These same treatments were statistically different from all the established treatments in the mentioned periods (Table 3).

Table 3. Percentage of *Scyphophorus acupunctatus* mortality caused by nematodes bonded with oils (hours).

Treatment (40%)	Time (h)					
	48	72	96	120	144	168
T1 *S. glaseri* 50 ± 5 nematodes + *T. vulgare*	20 ± 2.4 b	30 ± 3.8 bc	50 ± 1.6 b	80 ± 1.5 ab	80 ± 1.5 b	90 ± 2.7 a
T2 *S. glaseri* 100 ± 10 nematodes + *T. vulgare*	30 ± 3.8 ab	50 ± 1.6 b	70 ± 2.4 bc	70 ± 2.4 b	90 ± 2.7 a	90 ± 2.7 a
T3 *S. glaseri* 50 ± 5 nematodes + *O. europea*	50 ± 1.6 a	70 ± 2.4 ab	80 ± 1.5 ab	80 ± 1.5 ab	90 ± 2.7 a	100.0 ± 0.0 a
T4 *S. glaseri* 100 ± 10 nematodes + *O. europea*	50 ± 1.6 a	90 ± 2.7 a	90 ± 2.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
T5 *H. bacteriophora* 50 ± 5 nematodes + *T. vulgare*	20 ± 2.4 b	50 ± 1.6 b	70 ± 2.4 ab	80 ± 1.5 a	90 ± 2.7 a	90 ± 2.7 a
T6 *H. bacteriophora* 100 ± 10 nematodes + *T. vulgare*	20 ± 2.4 b	40 ± 3.9 bc	70 ± 2.4 bc	70 ± 2.4 b	70 ± 2.4 bc	80 ± 1.5 b
T7 *H. bacteriophora* 50 ± 5 nematodes + *O. europea*	50 ± 1.6 a	70 ± 2.4 ab	70 ± 2.4 bc	90 ± 2.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a
T8 *H. bacteriophora* 100 ± 10 nematodes + *O. europea*	50 ± 1.6 a	70 ± 2.4 ab	90 ± 2.7 a	100 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
T9 DW (absolute control)	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
T10 Triticum vulgare (positive control)	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
T11 Olea europea (positive control)	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c

Mean ± standard deviation. Means with different letters in each column are statistically different (Tukey, $p \leq 0.05$). DW: distilled water.

Currently, there is little information on oil emulsions with EPNs. Nematodes applied with water might kill the larvae in a period between 24 and 48 h with a mortality rate of 50% before becoming dehydrated owing to environmental factors such as temperature [21]. The lethal time to kill in 95% of larvae was between 3 and 5 days with *Steinernema* spp. and *H. bacteriophora*, respectively, in *Phyllophaga vetula* [22]. In *Collaria scenica* nymphs inoculated with *S. glaseri*, 24 h after infection, 75% mortality was found, and at 48 h, mortality was 82% with the nematode *Heterorhabditis* sp. [23].

The entomopathogenic nematodes *S. feltiae* and *S. carpocapsae* at a concentration of 9000 JIs applied in water presented 60% mortality 44 days after inoculation in *S. acupuncatatus* adults [22]. With *Lippia sidoides* oil associated with entomopathogenic nematodes, 100% mortality was observed in the control of *Rhipicephalus microplus* (Acari: Ixodae) [19,24]. It is important to continue searching for chemical materials and their combinations either as coadjuvants or additives to increase the mortality of biological controllers [25]. This suggests that the mixture of oils with EPN may generate synergy to favor nematode activity. Although formulations for nematodes are based on the formulations of traditional pesticides, it is important to reduce the effects of extreme temperatures, improve the release and distribution of JI in water, improve and maintain infection capacity, and improve their survival after implementation in the field [26].

Likewise, nematode virulence (larval mortality and the necessary time to kill the insect larvae) considerably decreased after 14 days. From days 1 to 7, apart from the used coadjuvant, more than 80% larval mortality was recorded at most of the temperatures. This high larval mortality could be because only the survival of nematodes is necessary to kill an insect. Similar values for the EPN species [14] *S. wesbteri* and *H. bacteriophora* formulated with *C. citratus* and *J. virginiana* were found, while *S. carpocapsae* formulated with *C. citratus* produced 60% larval mortality. In this study, less than 50% of larva mortality was obtained for the IJ stored for 7 days at 24 °C. This result contrasts with the 100% mortality found for the oily coadjuvants of plant origin in similar conditions (5 days at 24 °C). There are no registers referring to the activity of oil emulsions with entomopathogenic nematodes for the control of *S. acupunctatus* in the field. For this reason, it is necessary to continue evaluating more oils or coadjuvants to find the biological formulations that could be used in the field.

3.3. Emergence of Nematodes in Galleria Mellonella Larvae

With mean relative humidity of 56 ± 8.36% and an average temperature of 23 ± 2.6 °C, it was found that a concentration of 50 nematodes/*G. mellonella* larvae led to the emergence and reproduction of *S. glaseri* in 19–21 days and of *H. bacteriophora* in 23–25 days. With both *S. glaseri* and *H. bateriophora*, the communities that started from 50 ± 5 parent nematodes generated about 10,000 IJs per 10 larvae of *G. mellonella*, from the fourth instar, where the EPNs were obtained in three reproductive cycles under laboratory conditions.

EPNs are considered an alternative for the biological control of insects due to their killing and infecting capacity. This is possible due to their symbiotic association with bacteria, which are in the inner part of the nematode. This association [27–29] kills the host after few hours of being infected (24 to 48 h), and both organisms benefit [30,31] from the emergence and reproduction of EPNs.

The dissection of the infected larvae of *G. mellonella* at different moments after infection showed that the development of the nematode and the final production of the infectious juveniles of individual insects were very similar. The oils evaluated in this study did not affect the nematodes' reproductive capacity or their viability; they were effective in killing *S. acupunctatus* adults. It is also important to note that the variations observed in nematode emergence may be the result of several factors in combination, such as the temperature, relative humidity, ventilation [32,33], size of the host used [34], nematode species [11,35], time of emergence, and length of the cycle in the host [36].

4. Conclusions

Under laboratory conditions, the nematodes *S. glaseri* and *H. bacteriophora* in *Olea europea* oil emulsions were pathogenic for *S. acupunctatus*. The mortality of *S. acupunctatus* exposed to EPN depended on the concentration of the formulations. A mortality rate of 100% was achieved at 120 h with the combination of *S. glaseri* + *O. europea* oil. Formulations with nematodes could be a viable and environmentally friendly alternative for the control and management of *S. acupunctatus*, thereby helping to reduce the use of chemical pesticides. However, further studies are needed to manage the agave weevil using other oils with EPN.

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