agronomy

MDPI

Article

Viability of Entomopathogenic Fungi in Oil Suspensions and Their Effectiveness against the Agave Pest *Scyphophorus acupunctatus* under Laboratory Conditions

Teodulfo Aquino-Bolaños, Yolanda Donají Ortiz-Hernández *, Angélica Bautista-Cruz and Marco Aurelio Acevedo-Ortiz

Instituto Politécnico Nacional, CIIDIR-Oaxaca, Hornos 1003, Santa Cruz Xoxocotlán 71230, Mexico
* Correspondence: yortiz@ipn.mx; Tel.: +52-9515170610 (ext. 82723)

Abstract: Oaxaca, Mexico, is home to over 30 species of the genus *Agave*, and its cultivation is of great economic and social importance for the mezcal industry, which depends on its production. The incidence of the pest *Scyphophorus acupunctatus* causes severe losses and damage. Agrochemicals are used for its control, but a viable alternative is microbial control. The objectives of this study were to determine the natural occurrence of the entomopathogenic fungi (EPF) *Beauveria bassiana* and *Metarhizium anisopliae*, isolated from *S. acupunctatus* in agave crops, and to evaluate the effect of vegetable oil in water emulsions containing conidia from the native fungi against adults of *S. acupunctatus* under laboratory conditions. Viability of the fungal isolates was determined at a concentration of $\times 10^8$ conidia/mL in avocado (*Persea americana*), mamey (*Mammea americana*), chia (*Salvia hispanica*), or olive (*Olea europaea*) oil in water emulsions at two concentrations, 20% and 40%. The most effective oil emulsion on *S. acupunctatus* adults was determined under laboratory conditions. Naturally occurring fungi in 900 field collected insects was 2.21%. Ninety-six hours after preparing a 20% emulsion in *P. americana* oil, *B. bassiana* and *M. anisopliae* had a viability of 75% and 66.5%, respectively, while the control conidia suspended in distilled water remained viable for only 48 h. Twenty-four hours after applying *M. anisopliae* conidia in a 40% *P. americana* oil emulsion, effectiveness was 100% on *S. acupunctatus*, followed by *M. anisopliae* in 20% *P. americana* oil emulsion with 75% effectiveness. At 72 h post-application, all fungus in oil emulsions achieved an accumulated insect mortality of 100%, while the control showed no effect on adult *S. acupunctatus*. The most promising combination was 40% *P. americana* oil emulsion, which achieved 50% viable *B. bassiana* or *M. anisopliae* conidia up to 96 h after preparation, and its accumulated effectiveness on *S. acupunctatus* adults was 87.5% after 24 h.

Keywords: weevil; conidia; natural occurrence; oil in water formulation

Citation: Aquino-Bolaños, T.; Ortiz-Hernández, Y.D.; Bautista-Cruz, A.; Acevedo-Ortiz, M.A. Viability of Entomopathogenic Fungi in Oil Suspensions and Their Effectiveness against the Agave Pest *Scyphophorus acupunctatus* under Laboratory Conditions. *Agronomy* **2023**, *13*, 1468. https://doi.org/10.3390/agronomy13061468

Academic Editors: Adrian Wiater, Kamila Wlizło, Artur Nowak and Christos Athanassiou

Received: 12 April 2023
Revised: 23 May 2023
Accepted: 23 May 2023
Published: 25 May 2023

1. Introduction

Mexico is home to over 75% of the world's agave species, distributed in arid and semi-arid regions, and extensively cultivated in Oaxaca, Sonora, Morelos, Puebla, Guerrero, San Luis Potosí, Zacatecas, Durango, Michoacán, Tamaulipas, and Guanajuato. These perennial plants are part of agroforestry systems of great cultural, agronomic, ecological, and social importance [1,2]. They are used to obtain syrup, fiber, construction materials, and bioplastics, and to control soil erosion, among other uses [3,4]. The agave agroindustry generates jobs for 85,000 workers and 17,000 producers who cultivate more than 111,420 hectares to produce "tequila" and "mezcal", alcoholic beverages with national and international demand; sales were over a 1 billion USD in 2017 [5–7].

In the Central Valleys and Sierra Sur of the state of Oaxaca, Mexico, more than 10,000 hectares of *Agave* spp. are cultivated [8], and 200,000 tons of agave are destined for the mezcal industry [9,10]. *Agave angustifolia*, known as "maguey espadín", is the most

widely produced species in the area [5]. However, as with other agaves, yield of the plants for mezcal production is affected by environmental problems and by incidence of aggressive pathogens, such as the bacterium *Pectobacterium carotovora*, for which *Scyphophorus acupunctatus* is a vector [11,12].

S. acupunctatus (Coleoptera: Curculionidae), the agave weevil, is a year-round pest, that causes severe damage to agave plants and affects their growth [13]. The larvae bore into the plant's stem, causing weakening, and larvae and adults feed on the leaves and stems, causing plant death [14]. Furthermore, the weevil transmits the bacteria *P. carotovorum*, which causes putrescence. The loss of plant tissue due to damage caused by *S. acupunctatus* and *P. carotovorum* is 10% in *A. angustifolia* and up to 90% in *A. tequilana* [15].

S. acupunctatus is controlled with contact pesticides such as malathion and endosulfan, which pollute the environment, and their residues remain in both the soil and agave plants. Misuse of these products increases resistance of insect pest populations and causes intoxication to farmworkers [16–18]. Biological control with entomopathogenic agents is an alternative for management of this coleopteran because *S. acupunctatus* larvae and adults are susceptible to infection caused by entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) [19–21].

Application of EPF in the field using spray technology requires support adjuvants, such as sunscreens and mineral or vegetable oils in suspensions of conidia to protect them against adverse abiotic factors such as high temperature [22], rapid desiccation [23], and UV radiation [24]. Vegetable oil provides some protection from the physical damage to the propagules caused during preparation in suspensions with distilled water, which diminishes their effectiveness in field applications [25]. It also increases adhesion of conidia on the insect cuticle and plant surface, thus increasing contact time to facilitate germination and penetration [26]. In addition, because of their viscosity, the evaporation of oils is slower than water and they contribute to maintaining the viability of EPF conidia [17].

Prepared emulsions of soybean oil [27] and water with *Beauveria bassiana* (strain ESALQ-PL63) or *Metarhizium anisopliae* (strain ESALQ-1037), which were originally isolated from ants, were exposed to a temperature of 36 °C. After 6 h, viability decreased to less than 7% for both species, while in water suspension they showed decreasing viability from 60 to 7% [27]. When *M. anisopliae* (IP 46) conidia formulated in pure canola vegetable oil was heated (45 ± 0.2 °C), almost 50% remained viable after 48 h [22]. Indeed, the biocompatibility of some vegetable oils with EPF has been reported [28], and in some cases, the mixtures have had a repellent effect against Acari, such as ticks [29] or a toxic effect on insects.

Isolated native EPF are effective agents for controlling local insect pests, and oil adjuvants are not aggressive for conidial viability in aqueous suspension. Oil emulsions might extend the viability of conidia and increase the mortality of *S. acupunctatus* adults isolated from *Agave* spp. The objectives of this study were (1) to determine the natural occurrence of EPF in *S. acupunctatus* populations collected from agave crops and (2) to evaluate the effect of conidia from the native fungi *B. bassiana* or *M. anisopliae* in vegetable oil emulsions on conidia viability and the effectiveness of the fungi against *S. acupunctatus* adults under laboratory conditions.

2. Materials and Methods

2.1. Presence of Entomopathogenic Fungi (EPF) in Scyphophorus Acupunctatus Adults

S. acupunctatus adults were collected from mature agave plants on plantations located in Ocotlán, Oaxaca, Mexico [16°47′29″ north latitude, 96°40′29″ west longitude, 1500 m altitude, 600–800 mm annual precipitation, average annual temperature of 26.9 °C, subhumid warm climate]. The agave plants exhibited a degree of damage of 3 (more than six lesions on the leaf) or 4 (necrotic lesions on the plant's base and healthy core of the plant "piña"), according to the classification scale reported [11]. From June 2021 to May 2022, 12 samplings were conducted. Every 30 days, 75 live adult insects were collected, and inmediately divided into two populations, each of which was placed in plastic containers.

A total of 900 adult specimens were collected and kept in a 500 mL cylindrical plastic container without a lid; the aperture was covered with a fine mesh fixed with an elastic band. The insects were fed with small pieces of ripe agave leaves and maintained under controlled conditions in the entomology laboratory of the Interdisciplinary Research Center for Integral Regional Development (IPN-CIIDIR Oaxaca), at a temperature of 21 ± 2 °C to 23 ± 2.16 °C, with a relative humidity of 45 ± 8.68 to 56 ± 8.36% and 10 h light.

Positive samples (insects with signs of infection by EPF) were separately placed in humid chambers [30] and incubated at 24 ± 2 °C and 50–85% relative humidity for 10 days to promote mycelium growth on the insects. The insect cadavers were then observed with a stereoscopic microscope at 200× magnification to determine mycelium growth (without distinguishing the EPF genera) and the percentage of natural EPF occurrence on the insect sample was calculated with Equation (1).

$$\text{Natural occurrence of EPF} = (\text{ISI} + \text{IMG}/\text{TIS}) \times 100 \quad (1)$$

where, ISI = Individuals with Signs of Infection, IMG = Individuals with Mycelium Growth, and TIS = the Total of Individuals Sampled.

To confirm Koch's postulates [31] and the actual death of *S. acupunctatus* adults, insects identified as infected by EPF were individually deposited in humid chambers and observed for mycelial growth. Once isolated, the EPF were seeded on potato dextrose agar Sabouraud (PDA) culture medium. The conidia were tested in bioassays against adult insects.

2.2. Culture of the Entomopathogenic Fungi

In the Biological Control Laboratory of CIIDIR, two isolated EPF were identified with the support of taxonomic keys and of the morphological characteristics of the species described as *M. anisopliae* and *B. bassiana* [32]. EPF were cultured on a solid Sabouraud potato dextrose agar medium in Petri dishes (80 × 10 mm) and incubated at 26 ± 1 °C for 15 days. The conidia were harvested manually with a microbiological inoculation loop, concentrated in sterile distilled water (DW) at a ratio of 1×10^8 conidia/mL, and placed in a beaker before preparation in vegetable oil emulsions.

2.3. Preparation of Conidia Suspension in Oil Emulsions

The native fungi *M. anisopliae* or *B. bassiana* (isolated from *S. acupunctatus*) were suspended in oil emulsions made with one of the following vegetable oils: avocado (*Persea americana* Miller) Laboratorios Hersol®, olive (*Olea europaea* Linnaeus) Español®, chia (*Salvia hispanica* Linnaeus), and Ines® or mamey (*Mammea americana* Linnaeus). A dispersant 0.1% Tween 80 was used to separate and disperse the mycelia in water, and then serial dilutions were made in DW plus 0.1% Tween until a concentration of 1×10^8 conidia/1 mL was achieved.

Using a 10–100 µL micropipette (Pipet-Lite XLS), 20/40 vol-% concentrations of vegetable oil emulsions were each placed in a glass Petri dish (55 mm diameter). A volume of 0.5 mL of the aqueous suspension containing 1×10^8 conidia was then added to the glass Petri dishes and both liquids were mixed with a magnetic stirrer for 3 min. The conidia obtained were counted in a Neubauer chamber, and we worked with a concentration of 1×10^8 conidia/1 mL.

2.4. Bioassay of the Viability of M. anisopliae and B. bassiana Suspended in Vegetable Oil Emulsions

One mL of oil emulsion with the suspended conidia was incubated at 26 ± 1 °C for germination, and their viability was determined every 24 h post-preparation for four days. The suspension was placed on slides and a coverslip was used to facilitate observation of the spores; 100 conidia were counted per slide. The conidia in oil emulsion were observed under a light microscope at 200× magnification and were considered viable when they had germ tubes that were longer than their diameters. Viability was also determined by

germination of conidia on insects and their growth in a potato dextrose agar (PDA) culture medium.

The treatments with *M. anisopliae* or *B. bassiana* were applied. Two controls were included: *M. anisopliae* (T1) or *B. bassiana* (T2) in 1 mL sterile DW without vegetable oil (Table 1). Sixteen treatments used vegetable oil (*P. americana*, *O. europaea*, *S. hispanica* and *M. americana*) at two oil concentrations (20% or 40%) with *B. bassiana* or *M. anisopliae*. For each treatment, there were twelve replications, and one insect was used per repetition. The conidia in oil emulsions at two oil concentrations were prepared as follows: (1) 20% (0.2 mL vegetable oil + 0.8 mL DW) with suspension of 1×10^8 conidia, and (2) 40%, (0.4 mL vegetable oil + 0.6 mL DW), with suspension of 1×10^8 conidia.

Table 1. Viability percentage for *B. bassiana* and *M. anisopliae* (concentration of 1×10^8 conidia/mL) suspended in emulsions of four vegetable oils at concentrations of 20% or 40%, at four different times.

Treatment	EPF (1×10^8 Conidia)	Oil Concentration (%)	Time after Formulation (h)			
			24	48	72	96
			None			
T1	*B. bassiana*	0	50 ± 0.67	37.5 ± 0.67	0	0
T2	*M. anisopliae*	0	62.5 ± 0.67	12.5 ± 0.67	0	0
			P. americana oil emulsion			
T3	*B. bassiana*	20	87.5 ± 0.52	87.5 ± 0.52	75 ± 0.52	75 ± 0.62
T4		40	62.5 ± 0.67	37.5 ± 0.67	37.5 ± 0.67	0
T5	*M. anisopliae*	20	50 ± 0.67	62.5 ± 0.67	62.5 ± 0.67	62.5 ± 0.67
T6		40	75 ± 0.62	37.5 ± 0.62	37.5 ± 0.62	0
			O. europaea oil emulsion			
T7	*B. bassiana*	20	37.5 ± 0.67	37.5 ± 0.67	37.5 ± 0.67	0
T8		40	100	50 ± 0.52	50 ± 0.52	0
T9	*M. anisopliae*	20	12.5 ± 0.67	62.5 ± 0.67	62.5 ± 0.67	0
T10		40	50 ± 0.67	37.5 ± 0.67	37.5 ± 0.67	0
			S. hispanica oil emulsion			
T11	*B. bassiana*	20	12.5 ± 0.67	12.5 ± 0.67	12.5 ± 0.67	0
T12		40	25 ± 0.67	0	0	0
T13	*M. anisopliae*	20	37.5 ± 0.67	25 ± 0.67	25 ± 0.67	0
T14		40	50 ± 0.67	0	0	0
			M. americana oil emulsion			
T15	*B. bassiana*	20	62.5 ± 0.67	0	0	0
T16		40	50 ± 0.67	25 ± 0.67	25 ± 0.67	0
T17	*M. anisopliae*	20	87.5 ± 0.52	50 ± 0.52	37.5 ± 0.52	0
T18		40	0	0	0	0

2.5. Bioassay of the Effectiveness of M. anisopliae or B. bassiana Conidia Suspended in Oil Emulsions on S. acupunctatus Adults under Laboratory Conditions

Effectiveness was defined as the proportion of *S. acupunctatus* adults that died after exposure to the native fungi *B. bassiana* or *M. anisopliae*, which were applied at a concentration of 1×10^8 conidia/insect in 2 mL of a *P. americana* oil emulsion. Effectiveness was measured every 24 h. The viability of conidia proved to be the highest in *P. americana* oil emulsion and, thus, this emulsion was later used to test its effectiveness against pest insects. Two oil concentrations were prepared: (1) 20% *P. americana* oil emulsion consisting of 0.4 mL oil + 1.6 mL of a suspension of conidia in DW and (2) 40% *P. americana* oil emulsion consisting of 0.8 mL of oil + 1.2 mL of a suspension of conidia in DW. Eight treatments were tested with twelve replications each (Table 2). The treatments comprising four *P. americana* oil emulsions at 20% or 40% with *B. bassiana* (T1 and T2) or *M. anisopliae* (T3 and T4), two *P. americana* oil emulsions at 20% (T5) or 40% (T6) without conidia, an absolute control (T7) of DW with no conidia or vegetable oil, and a treatment of only vegetable oil (T8).

Table 2. Cumulative effectiveness of *B. bassiana* and *M. anisopliae* (1×10^8 conidia/insect) applied in emulsions of *P. americana* oil at concentrations of 20% or 40% on *S. acupunctatus* adults.

Treatment	Composition (%)	Time after Application (h)		
		24	48	72
T1	*B. bassiana* + *P. americana* 20	14.2 ^{b}	57.1 ^{b}	100 ^{a}
T2	*B. bassiana* + *P. americana* 40	71.4 ^{ab}	87.5 ^{a}	100 ^{a}
T3	*M. anisopliae* + *P. americana* 20	87.5 ^{a}	87.5 ^{a}	100 ^{a}
T4	*M. anisopliae* + *P. americana* 40	100 ^{a}	100 ^{a}	100 ^{a}
T5	80 DW + *P. americana* 20	42.8 ^{ab}	100 ^{a}	100 ^{a}
T6	60 DW + *P. americana* 40	87.5 ^{a}	100 ^{a}	100 ^{a}
T7	100 DW (absolute control)	0 ^{c}	0 ^{c}	0 ^{c}
T8	100 *P. americana* (positive control)	100 ^{a}	100 ^{a}	100 ^{a}

For statistical analysis, the original dates were transformed into arcsine $\sqrt{(x/100)}$. Means with different letters in each column are statistically different (Tukey, $p \leq 0.05$). DW: distilled water.

Adult *S. acupunctatus* were placed individually in 300 mL cylindrical plastic jars with a piece of agave as food, and 1 mL of oil emulsion was then applied on the insects and food. Twelve replications per treatment were conducted, and one insect was used per repetition. Insect mortality was checked every 24 h post-application for 7 days. The insects showing signs of infection by EPF were placed in humid chambers and the reason of the death was confirmed by the appearance of mycelial growth on the cadaver, seen through a light microscope at a 200× magnification.

2.6. Statistical Analysis

Cumulative viability of the EPF conidia in the oil emulsions for each treatment was expressed as a percentage, based on the number of replications that had mycelium germination divided by the total number of replicas. Mortality was quantified every 24 h for 96 h. Data of the accumulated percentage of insect mortality every 24 h during 72 h were normalized by arcsine transformation $\sqrt{(x/100)}$ and processed through an analysis of variance (ANOVA). Statistical differences between the mean mortality of each treatment were established by a Tukey test ($\alpha = 0.05$). All analyses were performed with the statistical software SAS® [33].

3. Results and Discussion

3.1. Natural Occurrence of Entomopathogenic Fungi on S. acupunctatus

The natural occurrence of EPF in the sample of 900 insects was 2.21%. The native EPF isolated from positive adult *S. acupunctatus* insects collected from Agave spp. plants in Oaxaca, Mexico, were identified as *M. anisopliae* and *B. bassiana*. This percentage is low, compared to the 18.1% of two entomopathogenic fungi (1% for *M. anisopliae* and 17.1% for *B. bassiana*) found in insects in agricultural soils in chaparral habitat associated with *Agave lechuguilla* in Saltillo, Coahuila, Mexico [34]. The isolation of *B. bassiana* had been previously reported in mummified cadavers of *S. acupunctatus* adults collected from *Agave cocui* plants in Estado Falcón, Venezuela [35]. Our study is of great importance since there are no previous reports of any species of *Metarhizium* spp. isolated from insects associated with *Agave* spp.

3.2. Viability of B. bassiana and M. anisopliae Suspended in Oil Emulsions

The maximum mean viability of conidia suspended in vegetable oil emulsion 96 h after preparation can be seen in (Table 1). Twenty-four hours after preparing emulsions of vegetable oil with suspended conidia, three treatments exceeded 80% viability, the highest of which was T8 (*B. bassiana* + DW + 40% *O. europaea*) with 100%, and T3 and T17 with 85%, while T2 (Control) maintained 62.5%. After 48 h, conidia suspended in DW were completely dehydrated, and the treatments that showed viability higher than 60% were T3 (*B. bassiana* + DW +20% *P. americana*) with 87.5% and T5 and T9 both with 62.5%. At 72 and

96 h, mycelial growth was observed in treatment T3 (*B. bassiana* + DW + 20% *P. americana*) with 75% viability and T5 (*M. anisopliae* + DW + 20% *P. americana*) with 67%. These results suggest that *P. americana* oil contributed more than the other vegetable oils to preventing dehydration of EPF conidia and maintained viability at 23 ± 4 °C for up to 96 h. Hence, the experimental results support the proposal of using EPF conidia in oil–water emulsion (mainly with *P. americana* oil) at a concentration of 20%, which maintains viability for up to 96 h. Comparing avocado oil and olive oil, the omega 3/omega 6 ratio is higher in avocado oil than in olive oil [36], which may make it more biocompatible with EPF. Some of the benefits could be protection against physical damage to cells and repair of conidia damage from adverse environmental factors, such as UV radiation, heating, or aging, among others [37].

3.3. Effectiveness of B. bassiana and M. anisopliae Suspended in P. americana Oil Emulsions on S. acupunctatus Adults under Laboratory Conditions

Accumulated effectiveness of the EPF in oil–water emulsions on *S. acupunctatus* adults at 24–72 h after application is presented in (Table 2). Twenty-four hours post-application, effectiveness of treatments T4 (*M. anisopliae* + 60% DW + 40% *P. americana*) and T8 (40% *P. americana*) were 100% and 87.5% for treatments T6 (60% DW + 40% *P. americana*) and T3 (*M. anisopliae* + DW + 20% *P. americana*). Effectiveness of these four treatments was statistically similar, but different from the other treatments. After 48 h, the accumulated effectiveness of treatments T5 and T6 increased to 100%. It was not until after 72 h that treatments T1, T2, and T3 accumulated 100% effectiveness on *S. acupunctatus*. The absolute control T7 (DW without EPF) did not cause mortality in adult insects and was statistically different from all the other treatments. However, at 48 h, two treatments were found to be 100% toxic/lethal to adults of *S. acupunctatus* T8 (oil *P. americana*) and T4 (*M. anisopliae* + *P. americana* 40%).

The effectiveness of *B. bassiana* suspended in 20% *P. americana* oil-in-water emulsion on *S. acupunctatus* adults (100%) is similar to another report [38], in which *B. bassiana* applied in sunflower oil to control the coffee berry borer *Hyphotenemus hampei* resulted in 100% mortality of adult insects after 120 h. Mortality of *Leptinotarsa decemlineata* larvae after application of a dispersion of mineral oil formulation with *B. bassiana* (GHA) at a concentration of 254 conidia/mm^2 was 58.6% [39].

The effectiveness of *M. anisopliae* applied in 40% *P. americana* oil-in-water emulsion (T4) on *S. acupunctatus* was even higher in less time (100% in 24 h) compared with other reported results [40], who found a synergistic effect between the combination of *M. anisopliae* (ATCC-Nr. 90448) and some vegetable oils. They found that effectiveness on white flies (*Trialeurodes vaporariorum* and *Bemisia tobaci*) increased up to three times when combined. With sunflower oil, effectiveness was 100%, but 8 days post-application. *B. bassiana* against the coffee berry borer caused 100% mortality within 6 days [38]. Hence, based on these reports, mortality of insects caused by EPF conidia can be a good alternative as a control method against insect pests. The high mortality (100%) of *S. acupunctatus* by application of only *P. americana* oil (T8) is in accord with the toxic effect of avocado oil on insects, which has been reported in previous studies, but this effect is not usually evident so quickly on the first day post-application. For example, application of avocado idioblast cell oil against *Spodoptera exigua* (Hübner) caused 100% mortality after seven days [41]. Against *Drosophila suzukii* (Matsumura), application of *P. americana* essential oil had a pesticidal effect [42].

Against the coleoptera *Conotrachelus psidii*, the effectiveness of the fungi *M. anisopliae* and *B. bassiana* at 1 × 10^8 conidia/mL applied with sunflower oil + Tween 80 was 57.3 to 84.3% and 77.3 to 95.3%, respectively. However, this mortality is a value accumulated over 20 days post-application [43]. A rate of 5 × 10^6 conidia/mL of *M. anisopliae* was formulated into a water-in-oil emulsion, whose composition was coconut oil (19%), soybean oil (28.5%), Tween 20 (2.5%), sterile DW (45.25%), glycerin (4%), and water-soluble wax (0.75%). The formulation was stored at 20 ± 1 °C, maintaining 50% conidia viability for 4.6 months, and the effectiveness against *Bemisia tabaci* and *Tetranychus cinnabarinus* was 58 to 100% within

a period of 3 to 4 days after application [17]. The viability and effectiveness results of our study were below reported values. However, it is important to mention that the difference in storage temperature was a distinctive factor in the execution of our experiments with respect to others [44].

4. Conclusions

Conidia from naturally occurring fungi *B. bassiana* and *M. anisopliae* isolated from *S. acupunctatus* adults collected from agave plantations and suspended in *P. americana* oil emulsion proved to be pathogenic to adult insects and highly effective for their control under laboratory conditions, probably due to the contribution of the toxicity of avocado oil. This is the first report of the pesticidal effect of avocado oil (*P. americana*) on *S. acupunctatus* adults collected from *Agave* spp. Fungal formulation will serve as a more environmentally friendly alternative to control the agave weevil, *S. acupunctatus*.

Author Contributions: Conceptualization, T.A.-B.; Data curation, T.A.-B. and Y.D.O.-H.; Formal analysis, T.A.-B. and Y.D.O.-H.; Validation, T.A.-B.; Writing—original draft, T.A.-B. and Y.D.O.-H.; Writing—review and editing, T.A.-B., Y.D.O.-H., A.B.-C. and M.A.A.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Instituto Politécnico Nacional through project SIP-IPN.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Instituto Politécnico Nacional (IPN) of Mexico and Entomology Laboratory of IPN-CIIDIR Oaxaca (Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Oaxaca IPN). Thanks to the anonymous reviewers for improving the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. García-Mendoza, A.J. Agavaceae. In *Flora del Valle de Tehuacán-Cuicatlán*; Instituto de Biología, Universidad Nacional Autónoma de Mexico: Mexico City, Mexico, 2011; Volume 88, pp. 1–95, ISBN 968-36-3108-8/978-607-02-2566-6.
2. Torres-García, I.; Rendón-Sandoval, F.J.; Blancas, J.; Casas, A.; Moreno-Calles, A.I. The genus Agave in agroforestry systems of Mexico. *Bot. Sci.* **2019**, *97*, 263–290. [CrossRef]
3. Martínez-Herrera, R.E.; Rutiaga-Quiñones, O.M.; Alemán-Huerta, M.E. Integration of agave plants into the polyhydroxybutyrate (PHB) production: A gift of the ancient aztecs to the current bioworld. *Ind. Crops Prod.* **2021**, *174*, 114188. [CrossRef]
4. Ozuna, C.; Franco-Robles, E. Agave syrup: An Alternative to conventional sweeteners? A review of its current technological applications and health effects. *LWT* **2022**, *162*, 113434. [CrossRef]
5. SAGARPA. *SAGARPA Planeación Agrícola Nacional 2017–2030*, 1st ed.; Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA): Mexico City, Mexico, 2017; pp. 1–20. Available online: https://www.gob.mx/cms/uploads/attachment/file/257066/Potencial-Agave_Tequilero_y_Mezcalero.pdf (accessed on 11 April 2023).
6. Consejo Regulador del Tequila. *Mexico Exportó 213 Millones de Litros de Tequila en 2017*; LIDERES Mexicanos: Mexico City, Mexico, 2018. Available online: https://lideresmexicanos.com/noticias/mexico-exporto-213-millones-de-litros-de-tequila-en-2017/ (accessed on 11 April 2023).
7. Consejo Mexicano Regulador de la Calidad del Mezcal (COMERCAM). *Información Estadística General del Sector Mezcalero*; Consejo Mexicano Regulador de la Calidad del Mezcal (COMERCAM): Oaxaca, Mexico, 2022. Available online: https://comercam-dom.org.mx/wp-content/uploads/2022/06/INFORME-2022-_II_-SINTESIS.pdf (accessed on 11 April 2023).
8. Servicio de Información Agroalimentario y Pesquera (SIAP). Anuario Estadístico de la Producción Agrícola de Agave en Oaxaca. Available online: https://nube.siap.gob.mx/cierreagricola/ (accessed on 11 April 2023).
9. Martinez-Gutiérrez, G.A.; Íñiguez-Covarrubias, G.; Ortiz-Hernández, Y.D.; López-Cruz, J.Y.; Bautista-Cruz, M.A. Tiempos de apilado del bagazo del maguey mezcalero y su efecto en las propiedades del compost para sustrato de tomate. *Rev. Int. De Contam. Ambient.* **2013**, *29*, 209–216. Available online: https://www.revistascca.unam.mx/rica/index.php/rica/article/view/34948 (accessed on 12 March 2023).
10. Ortiz-Hernández, Y.D.; Gutiérrez-Hernández, G.F.; Corzo-Ríos, L.J.; García-Ramírez, E.; Martínez-Tomás, S.H. Varietal and germinative characterization of *Agave potatorum* (Asparagaceae) seeds with different origins. *Bot. Sci.* **2018**, *96*, 628–639. [CrossRef]
11. Aquino-Bolaños, T.; Ortiz-Hernández, Y.D.; Martínez-Gutiérrez, G.A. Relationship between *Scyphophorus acupunctatus* Gyllenhal damage and nutrient and sugar content of *Agave angustifolia* Haw. *Southwest. Entomol.* **2013**, *38*, 477–486. [CrossRef]
12. Aquino-Bolaños, T.; Sánchez-García, J.A.; Ortíz-Hernández, Y.D.; Hernández-Cruz, J.; Cortés-Martínez, C.I. Carrier and vector of *Pectobacterium Carotovorum* Subsp. *Carotovorum* and its handling through a base of entomopathogenic fungi in *Agave* sp. *Fla. Entomol.* **2020**, *103*, 243–246. [CrossRef]

13. Romo, A.; Morrone, J.J. Especies mexicanas de *Curculionidae* (Insecta: Coleoptera) asociadas con *Agaves* (Asparagaceae: Agavoideae). *Rev. Mex. Biodivers.* **2012**, *83*, 1025–1035. [CrossRef]
14. González Hernández, H.; Solís Aguilar, J.F.; Pacheco Sánchez, C.; Flores Mendoza, F.J.; Rubio Cortés, R.; Rojas, J.C. Insectos Barrenadores del Agave Tequilero. In *Manejo de Plagas del Agave Tequilero*; González-Hernández, H., del Real-Laborde, J.I., Solis-Aguilar, J.F., Eds.; Colegio de Postgraduados: Montecillo, Mexico, 2007; pp. 39–67, ISBN 968-839-527-7.
15. Aquino-Bolaños, T.; Parraguirre-Cruz, M.A.; Ruiz-Vega, J. *Scyphophorus acupunctatus* (=*interstitialis*) Gyllenhal (Coleoptera: Curculionidae). Pest of agave mezcalero: Losses and damage in Oaxaca, Mexico. *Rev. Científica UDO Agrícola* **2007**, *7*, 175–180. Available online: http://www.bioline.org.br/pdf?cg07018 (accessed on 14 January 2023).
16. Terán-Vargas, A.P.; Azuara-Domínguez, A.; Vega-Aquino, P.; Zambrano-Gutiérrez, J.; Blanco-Montero, C. Biological effectivity of insecticides to control the agave weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), in Mexico. *Southwest. Entomol.* **2012**, *37*, 47–53. [CrossRef]
17. Batta, Y.A. Invert Emulsion: Method of preparation and application as proper formulation of entomopathogenic fungi. *MethodsX* **2016**, *3*, 119–127. [CrossRef] [PubMed]
18. Cuervo-Parra, J.A.; Pérez-España, V.H.; Pérez, P.A.L.; Morales-Ovando, M.A.; Arce-Cervantes, O.; Aparicio-Burgos, J.E.; Romero-Cortes, T. *Scyphophorus acupunctatus* (Coleoptera: Dryophthoridae): A weevil threatening the production of agave in Mexico. *Fla. Entomol.* **2019**, *102*, 1–9. [CrossRef]
19. Aquino-Bolaños, T.; Ruíz-Vega, J.; Iparraguirre-Cruz, M. Biological control of the black weevil (*Scyphophorus interstitialis* Gyllenhal) with entomophatogenic nematodes and fungi in agave in Oaxaca, Mexico. *Rev. Científica UDO Agrícola* **2006**, *6*, 92–101. Available online: https://tspace.library.utoronto.ca/bitstream/1807/45371/1/cg06012.pdf (accessed on 16 January 2023).
20. Delgado-Gamboa, J.R.; Ruíz-Vega, J.; Ibarra-Rendón, J.E.; Aquino-Bolaños, T.; Giron-Pablo, S. Isolation and identification of native entomopathogenic nematodes (Nematoda: Rhabditidae) and potential for controlling *Scyphophorus acupunctatus* in a laboratory. *Southwest. Entomol.* **2015**, *40*, 731–739. [CrossRef]
21. Gkounti, V.T.; Markoyiannaki, D.; Kontodimas, D.C. Pathogenicity of indigenous strains of three entomopathogenic fungi to the sisal weevil, *Scyphophorus Acupunctatus* (Gyllenhal) (Coleoptera: Curculionidae). *Hell. Plant Prot. J.* **2015**, *8*, 46–54. [CrossRef]
22. Paixão, F.R.S.; Muniz, E.R.; Barreto, L.P.; Bernardo, C.C.; Mascarin, G.M.; Luz, C.; Fernandes, K.K. Increased heat tolerance afforded by oil-based conidial formulations of *Metarhizium anisopliae* and *Metarhizium robertsii*. *Biocontrol Sci. Technol.* **2017**, *27*, 324–337. [CrossRef]
23. Prior, C.; Jollands, P.; le Patourel, G. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). *J. Invertebr. Pathol.* **1988**, *52*, 66–72. [CrossRef]
24. Behle, R.W.; Compton, D.L.; Laszlo, J.A.; Shapiro-Ilan, D.I. Evaluation of soyscreen in an oil-based formulation for UV protection of *Beauveria bassiana* conidia. *J. Econ. Entomol.* **2009**, *102*, 1759–1766. [CrossRef]
25. Faria, M.; Hajek, A.E.; Wraight, S.P. Imbibitional damage in conidia of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium acridum*, and *Metarhizium anisopliae*. *Biol. Control* **2009**, *51*, 346–354. [CrossRef]
26. Ortiz-Urquiza, A.; Luo, Z.; Keyhani, N.O. Improving mycoinsecticides for insect biological control. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1057–1068. [CrossRef]
27. de Oliveira, D.G.P.; Lopes, R.B.; Rezende, J.M.; Delalibera, I. Increased tolerance of *Beauveria bassiana* and *Metarhizium anisopliae* conidia to high temperature provided by oil-based formulations. *J. Invertebr. Pathol.* **2018**, *151*, 151–157. [CrossRef]
28. Mohan, M.C.; Reddy, N.P.; Devi, U.K.; Kongara, R.; Sharma, H.C. Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. *Biocontrol Sci. Technol.* **2007**, *17*, 1059–1069. [CrossRef]
29. Benelli, G.; Pavela, R. Repellence of essential oils and selected compounds against ticks—A systematic review. *Acta Trop.* **2018**, *179*, 47–54. [CrossRef] [PubMed]
30. Kaya, H.K. Entomogenous Nematodes for Insect Control in IPM Systems. In *Biological Control in Agricultural IPM Systems*; Hoy, M.A., Herzog, D.C., Eds.; Academic Press: New York, NY, USA, 1985; pp. 283–302, ISBN 978-0-12-357030-7.
31. Sharma, L.; Oliveira, I.; Torres, L.; Marques, G. Entomopathogenic fungi in portuguese vineyards soils: Suggesting a '*Galleria-Tenebrio*-bait method' as bait-insects *Galleria* and *Tenebrio* significantly underestimate the respective recoveries of *Metarhizium* (*robertsii*) and *Beauveria* (*bassiana*). *MycoKeys* **2018**, *38*, 1–23. [CrossRef]
32. Humber, R.A. Preservation of Entomopathogenic Fungal Cultures. In *Manual of Techniques in Invertebrate Pathology*; Lacey, L.A., Ed.; Academic Press: London, UK, 2012; pp. 317–328, ISBN 9780123869005.
33. SAS Institute Inc. *SAS/ETS 9.1 User's Guide*, 1st ed.; SAS Institute Inc.: Cary, NC, USA, 2004; ISBN 1590472446.
34. Sánchez-Peña, S.R.; Lara, J.S.-J.; Medina, R.F. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, Mexico, and their virulence towards thrips and whiteflies. *J. Insect. Sci.* **2011**, *11*, 1. [CrossRef] [PubMed]
35. Velázquez, J.; Joly, L.J.; García, J.L.; Romero, Y.; González, M.; Medina, M. Enemigos naturales del "picudo del Agave" *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) en el Estado Falcón, Venezuela. *Entomotropica* **2006**, *21*, 185–193.
36. Berasategi, I.; Barriuso, B.; Ansorena, D.; Astiasarán, I. Stability of avocado oil during heating: Comparative study to olive oil. *Food Chem.* **2012**, *132*, 439–446. [CrossRef]
37. Lin, T.-K.; Zhong, L.; Santiago, J.L. Anti-Inflammatory and Skin Barrier Repair Effects of Topical Application of Some Plant Oils. *Int. J. Mol. Sci.* **2018**, *19*, 70. [CrossRef]

38. Morales-De León, A.D.; Jarquín-Gálvez, R.; Gómez-Ruiz, J.; Díaz-Gómez, O.; Marín-Sánchez, J. Evaluación de un formulado de aceite vegetal de *Beauveria bassiana* en condiciones de laboratorio para el control de la broca del café. *Fitosanidad* **2014**, *18*, 5–14. Available online: https://www.redalyc.org/pdf/2091/209131412001.pdf (accessed on 14 January 2022).
39. Wraight, S.P.; Ramos, M.E. Effects of inoculation method on efficacy of wettable powder and oil dispersion formulations of *Beauveria bassiana* against colorado potato beetle larvae under low-humidity conditions. *Biocontrol Sci. Technol.* **2017**, *27*, 348–363. [CrossRef]
40. Malsam, O.; Kilian, M.; Oerke, E.-C.; Dehne, H.-W. Oils for increased efficacy of *Metarhizium anisopliae* to control whiteflies. *Biocontrol Sci. Technol.* **2002**, *12*, 337–348. [CrossRef]
41. Rodriguez-Saona, C.; Millar, J.G.; Maynard, D.F.; Trumble, J.T. Novel antifeedant and insecticidal compounds from avocado idioblast Cell Oil. *J. Chem. Ecol.* **1998**, *24*, 867–889. [CrossRef]
42. Erland, L.A.E.; Rheault, M.R.; Mahmoud, S.S. Insecticidal and oviposition deterrent effects of essential oils and their constituents against the invasive pest *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). *Crop Prot.* **2015**, *78*, 20–26. [CrossRef]
43. Brito, E.S.; De Paula, A.R.; Vieira, L.P.; Dolinski, C.; Samuels, R.I. Combining vegetable oil and sub-lethal concentrations of imidacloprid with *Beauveria bassiana* and *Metarhizium anisopliae* against adult guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae). *Biocontrol Sci. Technol.* **2008**, *18*, 665–673. [CrossRef]
44. Batta, Y.A. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes). *Crop Prot.* **2003**, *22*, 415–422. [CrossRef]