agronomy

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Laboratory Assessment of Plant Losses by *Sphenarium purpurascens* and Control with Entomopathogenic Fungi in Oil Emulsions

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Abstract: This study addresses the agricultural impact of the grasshopper *Sphenarium purpurascens* and evaluates the efficacy of entomopathogenic fungi (EPF), *Beauveria bassiana*, and *Metarhizium robertsii*, formulated in vegetable oil emulsions as sustainable pest control agents. The losses caused by *S. purpurascens* at different developmental stages (N4, N5, and adult) were assessed in five economically significant crops (*Medicago sativa*, *Zea mays*, *Helianthus* sp., *Cynodon dactylon*, and *Cucurbita pepo*), revealing a marked preference for *Helianthus* sp. and *C. pepo*, with consumption rates reaching 0.92 g/48 h during N4 and N5 stages, while adults showed preference for *M. sativa* (1.18 g/48 h) and *Z. mays* (1.15 g/48 h). The viability of EPF in oil emulsions (20% and 40% concentrations) was evaluated, demonstrating that formulations with *Azadirachta indica* and *Moringa oleifera* maintained over 99% fungal viability compared to the control absolute with distilled water (DW). The effectiveness of EPF against *S. purpurascens* adults was tested, with EPF on *M. robertsii* combined with *Persea americana* achieving 100% mortality within 72 h. Finally, the pathogenicity and dispersion of EPF in oil emulsions were evaluated, demonstrating that, at 240 h, the *B. bassiana* + *A. indica* strain (with three inoculated insects) achieved 100% mortality. It was observed that the number of inoculated adults directly influenced the mortality of *S. purpurascens*. These findings highlight the potential of EPF as a sustainable pest management strategy, emphasizing the need for further field trials to optimize its application and mitigate agricultural losses caused by *S. purpurascens*.

Keywords: *Beauveria bassiana*; crops; grasshopper; pest; *Metarhizium robertsii*

Received: 5 February 2025
Revised: 8 March 2025
Accepted: 9 March 2025
Published: 13 March 2025

Citation: Cruz-García, K.; Aquino-Bolaños, T.; Ortiz-Hernández, Y.D.; Aquino-López, T. Laboratory Assessment of Plant Losses by *Sphenarium purpurascens* and Control with Entomopathogenic Fungi in Oil Emulsions. *Agronomy* **2025**, *15*, 690. https://doi.org/10.3390/agronomy15030690

1. Introduction

Alfalfa (*Medicago sativa* L., 1753) is one of the main forage crops in Mexico, covering an area of 583,561 ha, which represents 57.1% of the total forage crop area. It is considered the most important forage legume in livestock farming, while corn (*Zea mays* L., 1753), oats (*Avena sativa* L., 1753), and sorghum (*Sorghum bicolor* (L.) Moench, 1794) together account for the remaining 42.9% of cultivated forage [1–4]. *Z. mays* is another key agricultural crop in Mexico, with significant cultural, economic, social and ecological importance due to its widespread distribution and essential role in people's diets [4,5]. However, various factors, particularly biotic ones, have negatively affected crop growth, development, and productivity, with insect pests causing losses of up to 30% in agricultural production [5,6].

Orthoptera of the Pyrgomorphidae family, commonly known as locusts and grasshoppers, are among the insect pests responsible for such damage. These insects have adapted to various terrestrial habitats, with 12 species known to cause severe losses by feeding on the foliage of crops such as common beans (*Phaseolus vulgaris* L., 1753), *M. sativa*, pumpkin (*Cucurbita pepo* L., 1753) and *Z. mays* [7–9]. The genus *Sphenarium* is a group of grasshoppers of both economic and cultural importance, not only as a food source but also as an agricultural pest [10,11]. The grasshopper (*Sphenarium purpurascens* Charpentier, 1842) (Orthoptera: Pyrgomorphidae) is a wingless insect known for its distinctive body coloration during the adult stage, which varies from dark brown to bright olive green with black spots [12]. It has a univoltine life cycle, with egg hatching occurring between May and June following the rainy season in central and northern Mexico [13]. Its feeding activity intensifies as it matures, a behavior associated with the development of its mandibular structures. In recent years, this grasshopper has become one of the most significant agricultural pests due to its high reproductive capacity and broad range of host plants [7,14,15]. While pest control is commonly achieved using chemical pesticides, their excessive use has led to pesticide resistance and poses risks to human health and the environment [16,17]. Therefore, alternative biological control methods using beneficial organisms such as entomopathogenic fungi (EPF) are being explored [18,19]. These fungi can penetrate the insect cuticle, causing immobility, loss of coordination, feeding cessation and eventually, death [20,21]. Several EPF species with these unique insecticidal properties and specificity include *Beauveria bassiana* (Bals-Criv) Vuill., 1912, *Metarhizium anisopliae* (Metschn.) Sorokín (1883), *Metarhizium acridum* (Driver & Milner, 1998) and *Entomophaga grylli* (Fresen., 1964) A. Batko, all of which are natural pathogens of grasshoppers [8,14,21–23]. The genus *B. bassiana* is a cosmopolitan EPF that reproduces asexually and is associated with a wide range of hosts, infecting up to 707 insect species across the orders Orthoptera, Coleoptera, Lepidoptera, and Diptera [24]. Additionally, it can persist in the soil as a saprotrophic organism after killing the host, which exhibits a characteristic white-to-yellowish coloration [25,26]. Meanwhile, *Metarhizium robertsii* (Metschn., 1883) Sorokín) previously known as *M. anisopliae*, can infect over 200 species of insect pests [27]. Infected insects initially display white mycelial growth, which later develops into a green coloration as the fungus sporulates [26,28]. Furthermore, ecological alternatives such as plant extracts, aqueous solutions, and essential oils have demonstrated insecticidal activity. One study reported successful control of *S. purpurascens* in amaranth (*Amaranthus hypochondriacus* L., 1753) crops using a mixture of castor oil plant extract and chili, combined with *B. bassiana* and alternated with soap [29]. Additionally, the combination of *B. bassiana* with *Persea americana* (Mill, 1768) and *Prunus dulcis* (Mill, 1967) D.A. Webb., achieved 100% effectiveness against *Strategus aloeus* (L., 1758) within 48 h [30], while *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, 2011, resulted in over 80% mortality in *Leptopharsa gibbicarina* (Froeschner, 1976) [31]. In *Conotrachelus dimidiatus* (Champion, 1904), mixtures of *B. bassiana* and plant extracts achieved 90% mortality, demonstrating the potential of EPF and botanical extracts for pest management [32].

This research was undertaken to address the growing need for biological alternatives to chemical insecticides in agricultural pest control, with a particular focus on *S. purpurascens*. Therefore, the objective of this study was to determine, under laboratory conditions, the losses in economically important crops that were caused by *S. purpurascens* and to evaluate control methods based on EPF, specifically *B. bassiana* and *M. robertsii*, formulated in vegetable oil emulsions of *Azadirachta indica* (A. Juss), *Moringa oleifera* (Lam, 1783), *P. americana* and *P. dulcis*.

2. Materials and Methods

This study was conducted in September to November 2024 at the Biological Control Laboratory at CIIDIR- Oaxaca located at 1564 m above sea level. Under laboratory conditions, the relative humidity (RH) levels varied from 56% ± 8.36% and dropped to 45% ± 8.68%. Meanwhile, the temperature varied from 23 °C ± 2.16 °C to 21 °C ± 2 °C.

The grasshoppers used in the experiment were obtained through direct collection methods using entomological nets in Santa Cruz Xoxocotlán (17°00′11.4″ N, 96°45′02.2″ W). For evaluation, only the required nymphal and adult stages of *S. purpurascens* were considered. Subsequently, the collected individuals were confined in four-liter plastic containers with modified lids, featuring a circular cut covered with a 1 mm diameter mesh to allow gas exchange, thereby facilitating their transport under optimal conditions to the laboratory. Once in the laboratory, grasshoppers were quarantined for 120 h in containers to allow for acclimatization and observation of possible infections. They were then separated into groups and fed with the evaluated crop, which had been previously disinfected with a 5% sodium hypochlorite solution for 20 min and rinsed with distilled water. Excreta were removed daily to maintain hygienic conditions.

The strains of EPF used in the experiment were obtained from the strain collection of the Biological Control Laboratory at CIIDIR-Oaxaca, and had been previously characterized.

2.1. Losses Caused by S. purpurascens in the N4, N5 Stages and Adult Crops of M. sativa, Z. mays, Helianthus sp., C. dactylon and C. pepo

Experiments were conducted from 13 September to 4 October 2024, to determine the losses caused by *S. purpurascens* at different developmental stages (N4 and N5 nymphs and adults). The specimens were selected based on their morphological characteristics [33,34]. Experiments were conducted using transparent plastic containers with a capacity of 300 mL. Each container houses one insect, which was fed 3 g of the evaluated crops: *M. sativa*, *Z. mays*, *Helianthus* sp. (L.), *Cynodon dactylon* (L.) Pers., 1805 and *C. pepo*. The weight of the foliage was recorded using a digital analytical balance PA2202C (OHAUS, Parsippany, Morris County, NJ, USA), with an accuracy of 200 g × 0.1 g.

Foliage weight was recorded and replaced every 48 h to document the weight loss per crop (g/48 h) and by the *S. purpurascens* stage of development. Since measurements were taken every 48 h, three measurements were collected each week (days 2, 4, and 6). The last measurement of each week (day 6) was averaged with the measurement taken at the start of the next week to maintain consistency in the calculations. The same insects were used throughout the experiment, allowing the change in their consumption to be assessed as their development progressed. A total of 15 treatments, with 12 repetitions per treatment over a period of 21 days, were approved.

2.2. Entomopathogenic Fungi and Their Culturing

The identification of EPF (*M. robertsii* and *B. bassiana*) was carried out using taxonomic keys and specific morphological traits of each species during September 2024. The EPF were propagated on a solid Potato Dextrose Agar (PDA) substrate in Petri dishes (80 × 10 mm) and kept at a regulated temperature of 25 ± 2 °C for 10 days. Conidia were extracted manually using a microbiological inoculation loop, then suspended in sterile distilled water (DW) at a concentration of 1×10^9 conidia/mL and preserved in a beaker before being formulated in vegetable + oil emulsions.

2.3. Preparation of Oil Emulsion-Based Conidia Suspensions

During September 2024, *M. robertsii* and *B. bassiana* fungi were suspended in oil emulsions prepared using the following vegetable oils: *A. indica* (Herbofilia, Tlaxcala, Mexico), *M. oleifera* (Plant Guro Inc., Plainfield, NJ, USA), *P. americana* (Monte Persea

Walmart, Oaxaca, Mexico) and *P. dulcis* (Aromatica Fresly, Oaxaca, Mexico). Tween 80 was used as a dispersing agent at 0.1% to facilitate the separation and dispersion of mycelia in water. Subsequently, serial dilutions were carried out using distilled water with 0.1% of Tween until a concentration of 1×10^9 conidia/mL was achieved. Vegetable oil emulsion concentrations of 20% and 40% (by volume) were added to each glass Petri dish (55 mm diameter) using a 10–100 µL micropipette, (Pipet-Lite XLS, Rainin, Oakland, CA, USA). Finally, a volume of 0.5 mL of the aqueous suspension containing 1×10^9 conidia was added to each glass Petri dish, and mixed for three minutes using a magnetic stirrer. The obtained conidia were counted using a Neubauer chamber at a concentration of 1×10^9 conidia/mL.

2.4. Viability of Entomopathogenic Fungi in Vegetable Oil Emulsions

An oil emulsion with conidia in suspension (1 mL) was prepared and maintained at 26 ± 1 °C to evaluate conidial viability. Germination was analyzed for 96 h after preparation (from October 18 to 22, 2024) [35]. For microscopic observation, 1 µL of the suspension was placed on a glass slide and covered with a coverslip. A total of 100 conidia were counted per slide, and those whose germ tube was equal to or greater than their diameter were considered viable. A total of 18 treatments were evaluated, including an absolute control with DW each with 12 replicates. The formulations were prepared at two concentrations: 20% (0.2 mL of vegetable oil + 0.8 mL DW) and 40% (0.4 mL of vegetable oil + 0.6 mL DW). In both cases, the emulsions contained a conidial suspension with a concentration of 1×10^9 conidia/mL.

2.5. Effectiveness of Entomopathogenic Fungi in Oil Emulsions on S. purpurascens Under Laboratory Conditions

Effectiveness was determined from 28 October to 1 November 2024, and was defined as the mortality of *S. purpurascens* insects after inoculation with *B. bassiana* and *M. robertsii* fungi. These fungi were applied at a concentration of 1×10^9 conidia/insect in 2 mL of oil emulsions (prepared from *A. indica*, *M. oleifera*, *P. americana* and *P. dulcis*) at a 40% concentration. For emulsion preparation, a 100 µL micropipette was used with the following proportions: 0.40 µL of vegetable oil + 0.60 µL EPF suspension. *S. purpurascens* adults were placed individually in 300 mL cylindrical plastic containers, each containing *M. sativa* (2 g) as food. The *M. sativa* was previously disinfected with 5% sodium hypochlorite for 20 min and rinsed thoroughly with distilled water to ensure the removal of any contaminants. A 1 mL dose of the oil emulsion was administered to both the insects and their food. Mortality was recorded every 24 h until 96 h with deceased individuals added to the cumulative count. A total of 13 treatments were tested, including an absolute control with distilled water, each treatment replicated 12 times. Insects exhibiting symptoms of EPF infection were positioned in humid chambers at >90% RH and observed with a stereoscopic microscope at 200× magnification to determine the presence or absence of mycelial growth on the cadavers.

2.6. Pathogenicity and Dispersion of B. bassiana and M. robertssi in Oil Emulsions in S. purpurascens Adults

The dispersion and pathogenicity of adult *S. purpurascens* were determined by inoculating them with *B. bassiana* + *A. indica* or *M. oleifera*, and *M. robertsii* + *P. americana* or *P. dulcis*, between November 5 and November 15, 2024. Inoculation was carried out at a concentration of 1×10^9 conidia/insect using oils at 40% purity.

In the experiment, ten adult *S. purpurascens* were placed in 4 L containers and fed *M. sativa* foliage. The insects were acclimated for 48 h before inoculation. To evaluate the ability of the fungi to infect healthy grasshoppers, inoculated insects were introduced into containers containing ten healthy grasshoppers. The number of inoculated insects varied according to

the treatment, with 5, 3, or 1 inoculated insect per container. This experimental design allowed the evaluation of the fungi's dispersal capacity and its effect on the mortality of healthy insects. Twelve treatments and absolute control were evaluated, with each treatment repeated 12 times to ensure the reliability and reproducibility of the results.

Observations were made every 48 h over a period of 240 h with deceased individuals added to the cumulative count. Dead insects were placed in humid chambers to induce mycelial growth, allowing for the determination of mortality induced by the fungi. Simultaneously, Koch's postulates were applied to confirm the causal relationship between the fungi and insect mortality [36].

2.7. Statistical Analysis

The viability, efficacy, and pathogenicity percentages of the fungi were calculated using the following formula:

$$\%\text{Effectiveness} = \frac{Pi - Pf}{Pi} \times 100 \quad (1)$$

where *Pi* = initial population, *Pf* = Final population. The obtained data were normalized using an arcsine transformation and analyzed through an analysis of variance (ANOVA) to establish Tukey's means at an alpha level of 0.05%, SAS® Statistical software, version 9.1 [37].

3. Results

3.1. Losses Caused by the Developmental Stages of S. purpurascens in Five Crops

Average losses over a 21-day period per insect were calculated, with a preference observed in two crops at the N4 development stage: T3-*Helianthus* sp., with 0.91 g/48 h and T5-*C. pepo* with 0.93 g/48 h. In the N5 stage, the same preference was observed in T8-*Heliantus* sp., with 0.90 g/48 h, and T10-*C. pepo* with 0.95 g/48 h. These treatments in the N4 and N5 stages were statistically different from T1, T2, T4, T6, T7, and T9 treatments. The highest consumption was observed in the adult stage across all evaluated crops, with the most significant consumption recorded in T11-*M. sativa* with 1.18 g/48 h and T12-*Z. mays* with 1.15 g/48 h. Notably, a difference of more than 0.20 g/48 h was observed in the consumption of *C. dactylon*, *Helianthus* sp., and *C. pepo* (Table 1).

Table 1. Average consumption of *S. purpurascens* in five cultures in three weeks (g/48 h).

Treatment	$\bar{X}$/Week 1	$\bar{X}$/Week 2	$\bar{X}$/Week 3	$\bar{X}$ Total
T1 N4 + *M. sativa*	0.56 ± 0.10 cd	0.64 ± 0.12 cd	0.65 ± 0.12 cd	0.61 ± 0.13 cd
T2 N4 + *Z. mays*	0.52 ± 0.09 cd	0.63 ± 0.11 cd	0.66 ± 0.16 cd	0.60 ± 0.09 cd
T3 N4 + *Helianthus* sp.	0.87 ± 0.15 ab	0.89 ± 0.17 ab	0.98 ± 0.10 ab	0.91 ± 0.16 ab
T4 N4 + *C. dactylon*	0.67 ± 0.20 cd	0.69 ± 0.13 cd	0.72 ± 0.09 cd	0.69 ± 0.34 cd
T5 N4 + *C. pepo*	0.87 ± 0.17 ab	0.94 ± 0.09 ab	0.99 ± 0.11 ab	0.93 ± 0.15 ab
T6 N5 + *M. sativa*	0.66 ± 0.25 cd	0.78 ± 0.15 cd	0.95 ± 0.16 ab	0.79 ± 0.15 cd
T7 N5 + *Z. mays*	0.65 ± 0.07 cd	0.70 ± 0.09 cd	0.94 ± 0.11 ab	0.76 ± 0.15 cd
T8 N5 + *Helianthus* sp.	0.85 ± 0.26 ab	0.88 ± 0.24 bc	0.98 ± 0.27 ab	0.90 ± 0.11 ab
T9 N5 + *C. dactylon*	0.75 ± 0.16 bc	0.76 ± 0.15 cd	0.80 ± 0.13 cd	0.77 ± 0.18 cd
T10 N5 + *C. pepo*	0.91 ± 0.15 ab	0.95 ± 0.15 ab	0.99 ± 0.11 ab	0.95 ± 0.16 ab
T11 Adult + *M. sativa*	1.06 ± 0.11 a	1.15 ± 0.21 a	1.33 ± 0.11 a	1.18 ± 0.14 a
T12 Adult + *Z. mays*	1.01 ± 0.03 a	1.10 ± 0.22 a	1.34 ± 0.14 a	1.15 ± 0.23 a
T13 Adult + *Helianthus* sp.	0.90 ± 0.13 ab	0.93 ± 0.17 bc	0.99 ± 0.21 ab	0.94 ± 0.11 ab
T14 Adult + *C. dactylon*	0.78 ± 0.15 bc	0.80 ± 0.14 bc	0.90 ± 0.18 ab	0.82 ± 0.15 bc
T15 Adult + *C. pepo*	0.91 ± 0.16 ab	0.95 ± 0.16 ab	1.10 ± 0.14 a	0.98 ± 0.15 ab

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

An increase in consumption was observed from week 1 to week 3, possibly due to the higher energy demands of *S. purpurascens* at more advanced developmental stages. Additionally, some crops may have experienced changes in their nutritional content or texture over time, influencing the consumption rate.

3.2. Evaluation of Entomopathogenic Fungi Viability in Vegetable Oil Emulsions

After 96 h, it was found that the oils of *A. indica* and *M. oleifera*, at concentrations of 20% and 40%, had no effect on the viability of *B. bassiana* and *M. robertsii*, registering percentages greater than 99% in all evaluated treatments (T1, T2, T5, T6, T9, T10, T13, and T14). In contrast, the absolute controls (T17 and T18), treated with distilled water, showed 0% survival, indicating complete fungal dehydration (Table 2).

Table 2. Viability percentage for *B. bassiana* and *M. robertsii* to two concentrations.

Treatment	EPF	Oil	Concentration (%)	Viability (96 h)
T1	*B. bassiana*	*A. indica*	20	100 ± 0.0 a
T2	*B. bassiana*	*M. oleifera*	20	100 ± 0.0 a
T3	*B. bassiana*	*P. americana*	20	92.4 ± 4.4 ab
T4	*B. bassiana*	*P. dulcis*	20	90.5 ± 6.8 ab
T5	*B. bassiana*	*A. indica*	40	100 ± 0.0 a
T6	*B. bassiana*	*M. oleifera*	40	100 ± 0.0 a
T7	*B. bassiana*	*P. americana*	40	92.71 ± 6.8 ab
T8	*B. bassiana*	*P. dulcis*	40	94 ± 1.14 ab
T9	*M. robertsii*	*A. indica*	20	99.0 ± 1.14 a
T10	*M. robertsii*	*M. oleifera*	20	100 ± 0.0 a
T11	*M. robertsii*	*P. americana*	20	91.1 ± 1.14 ab
T12	*M. robertsii*	*P. dulcis*	20	97.2 ± 1.14 ab
T13	*M. robertsii*	*A. indica*	40	99.4 ± 6.8 a
T14	*M. robertsii*	*M. oleifera*	40	99.2 ± 3.9 a
T15	*M. robertsii*	*P. americana*	40	97.4 ± 6.8 ab
T16	*M. robertsii*	*P. dulcis*	40	96.57 ± 4.6 ab
T17	*B. bassiana*	DW (absolute control)	0	0 d
T18	*M. robertsii*	DW (absolute control)	0	0 d

Mean ± standard deviation. Values with different letters indicate a significant difference ($p \leq 0.05$), according to Tukey's test. DW: distilled water. Tukey's mean. Values with different letters indicate a significant difference ($p \leq 0.05$).

Both *B. bassiana* and *M. robertsii* responded similarly in terms of viability when formulated with *A. indica* and *M. oleifera*. Overall, the oil concentration (20% vs. 40%) did not have a negative impact on EPF viability. However, the results highlight the importance of oil selection in formulations, with *A. indica* and *M. oleifera* standing out as the most promising options for ensuring fungal stability.

3.3. Effectiveness of Entomopathogenic Fungi in Vegetables Oil Emulsions on S. purpurascens Adults Under Laboratory Conditions

The effectiveness began to be observed 24 h after inoculation in treatments T10 and T11, with a 57.14% mortality rate in *S. purpurascens*. Applying the effectiveness formula, it was determined that after 72 h, T11 (*M. robertsii* + *P. americana*) achieved 100% mortality, which was statistically different from the rest of the treatments. This suggests better compatibility between this fungus and the oil in controlling *S. purpurascens*. Meanwhile, four treatments (T4, T7, T8, T10) achieved mortality rates of 85.7%. At 96 h, only two treatments (T2, T3) showed mortality rates below 60%, which were statistically different from

the other treatments. No mortality was recorded throughout the experiment's evaluations in the case of the absolute control (T13) (Table 3).

Table 3. Efficacy of *B. bassiana* and *M. robertsii* in 40% oil emulsions against *S. purpurascens* adults.

Treatment	Concentration (%)	Time (h)			
		24	48	72	96
T1 *A. indica*	40	28.5 ± 0.4 c	42.8 ± 0.5 c	57.1 ± 0.5 d	85.7 ± 0.3 b
T2 *M. oleifera*	40	28.5 ± 0.4 c	28.5 ± 0.4 d	42.8 ± 0.5 e	57.1 ± 0.5 c
T3 *P. americana*	40	28.5 ± 0.4 c	28.5 ± 0.4 d	57.1 ± 0.5 d	57.1 ± 0.5 c
T4 *P. dulcis*	40	42.8 ± 0.5 b	57.1 ± 0.5 b	85.7 ± 0.3 b	100 ± 0.0 a
T5 *B. bassiana* + *A. indica*	40	14.2 ± 0.3 d	28.5 ± 0.4 d	57.1 ± 0.5 d	100 ± 0.0 a
T6 *B. bassiana* + *M. oleifera*	40	28.5 ± 0.4 c	42.8 ± 0.5 c	71.4 ± 0.4 c	100 ± 0.0 a
T7 *B. bassiana* + *P. americana*	40	0 ± 0.0 e	42.8 ± 0.5 c	85.7 ± 0.3 b	100 ± 0.0 a
T8 *B. bassiana* + *P. dulcis*	40	14.2 ± 0.3 d	71.4 ± 7.3 a	85.7 ± 0.3 b	85.7 ± 0.3 b
T9 *M. robertsii* + *A. indica*	40	14.2 ± 0.3 d	42.8 ± 0.5 c	57.1 ± 0.5 d	85.7 ± 0.3 b
T10 *M. robertsii* + *M. oleifera*	40	57.1 ± 0.53 a	71.4 ± 0.4 a	85.7 ± 0.3 b	85.7 ± 0.3 b
T11 *M. robertsii* + *P. americana*	40	57.1 ± 0.53 a	57.1 ± 0.5 b	100 ± 0.0 a	100 ± 0.0 a
T12 *M. robertsii* + *P. dulcis*	40	42.8 ± 0.0 b	71.4 ± 0.4 a	71.4 ± 0.4 c	100 ± 0.0 a
T13 DW (absolute control)	100	0 ± 0.0 e	0 ± 0.0 e	0 ± 0.0 f	0 ± 0.0 d

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

The treatments without EPF (T1, T2, T3, and T4) showed lower effectiveness compared to those containing EPF, with a slower effect and a lower mortality rate by the end of the experiment. However, *P. dulcis* oil (T4) exhibited a certain insecticidal effect on its own, with 100% mortality achieved at 96 h.

The use of *B. bassiana* and *M. robertsii* in emulsions significantly improved both mortality and the speed of action against *S. purpurascens.* These results highlight the higher effectiveness of *B. bassiana* compared to *M. robertsii* in infecting *S. purpurascens.*

3.4. Pathogenicity and Dispersion of B. bassiana and M. robertsii in Oil Emulsions on S. purpurascens Adults

Insect mortality progressively increased over time in all treatments, reaching its peak at 240 h. The treatment T2 *B. bassiana* + *A. indica* (three inoculated insects) showed the highest effectiveness, reaching 100% mortality at the end of the experiment. Other highly effective treatments were T4 *B. bassiana* + *M. oleifera* (five inoculated insects) and T7 *M. robertsii* + *P. americana* (five inoculated insects), both reaching 80% mortality at 240 h (Table 4).

It was also observed that the number of inoculated insects influenced mortality, as it was higher when five infected insects were introduced into the experimental containers (Figure 1a). In contrast, treatments T8, T9, and T12 with *M. robertsii* + *P. dulcis* and *P. americana* showed lower effectiveness, with a maximum mortality of 46.1% at 240 h. The absolute control (T13) showed 0% pathogenicity at all time intervals, confirming that the observed effects were due to the combinations of fungi and oils.

Our results suggest that oil emulsion formulations can enhance the dispersion and pathogenicity of these EPF (Figure 1b,c).

Table 4. Percentage of pathogenicity of *B. bassiana* and *M. robertsii* in *S. purpurascens* oil emulsions.

Treatments	Inoculated Insects	Time (h)			
		48	96	192	240
T1 *B. bassiana* + *A. indica*	5	20 ± 0.3 e	33.3 ± 0.4 e	53.3 ± 0.4 ef	66.6 ± 0.7 c
T2 *B. bassiana* + *A. indica*	3	23 ± 0.4 d	38.4 ± 0.5 c	84.6 ± 0.5 a	100 a
T3 *B. bassiana* + *A. indica*	1	27.2 ± 0.3 b	36.3 ± 0.4 d	54.5 ± 0.6 de	63.6 ± 0.7 c
T4 *B. bassiana* + *M. oleifera*	5	26.6 ± 0.4 c	26.6 ± 0.4 g	73.3 ± 0.4 b	80 ± 0.1 b
T5 *B. bassiana* + *M. oleifera*	3	15.3 ± 0.4 f	15.3 ± 0.4 i	46.1 ± 0.2 fg	61.5 ± 0.6 c
T6 *B. bassiana* + *M. oleifera*	1	0 ± 0 h	9 ± 0.1 j	36.3 ± 0.4 h	45.4 ± 0.5 d
T7 *M. robertsii* + *P. americana*	5	33.3 ± 0.4 a	66.6 ± 0.7 a	74 ± 0.1 b	80 ± 0.1 b
T8 *M. robertsii* + *P. americana*	3	15.3 ± 0.2 f	30.7 ± 0.8 f	38.4 ± 0.5 d	46.1 ± 0.2 d
T9 *M. robertsii* + *P. americana*	1	0 ± 0 h	18.1 ± 0.2 i	36.3 ± 0.4 d	36.3 ± 0.4 e
T10 *M. robertsii* + *P. dulcis*	5	33.3 ± 0.4 a	46.6 ± 0.7 b	66.6 ± 0.7 bc	66.6 ± 0.7 c
T11 *M. robertsii* + *P. dulcis*	3	23 ± 0.1 d	38.4 ± 0.5 c	61.5 ± 0.6 bc	61.5 ± 0.6 c
T12 *M. robertsii* + *P. dulcis*	1	9 ± 0.1 g	9 ± 0.1 j	18.1 ± 0.2 i	36.3 ± 0.4 e
T13 DW (absolute control)	0	0 ± 0 h	0 ± 0 h	0 ± 0 h	0 ± 0 h

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

(**a**)

(**b**)

(**c**)

Figure 1. (**a**) Pathogenicity process in *S. purpurascens*; (**b**) ineffectiveness of *M. robertsii* and (**c**) *B. bassiana*.

4. Discussion

This study, performed under laboratory conditions, has provided valuable information on the feeding behavior of *S. purpurascens* at its N4, N5, and adult developmental stages. This research specifically focuses on the insect's preference for certain crops and its potential impact on agricultural productivity. Previous study reported an incidence of 25 insects/m^2, considered a severe infestation threshold requiring control measures due to the damage and losses caused to producers [8,38]. In contrast, our findings indicate that feeding behavior is influenced by crop characteristics. Similar trends have been observed in other species: *Dociostaurus maroccanus* (Thunberg, 1815) and *Tettigonia viridissima* (Linnaeus, 1758) showed a preference for potato leaves over other plants, while *Calliptamus barbarus barbarus* (Costa, 1836) did not exhibit a significant preference [39]. In our study, *C. dactylon* exhibited lower consumption rates across all stages (N4, N5, and adults), averaging 0.76 g/48 h. Despite being replaced every 48 h, its rapid dehydration reduced its water content, decreasing its palatability. The ability of *S. purpurascens* to select food sources that support its growth and development is crucial for understanding its ecology and potential agricultural impact.

Currently, there are no reports or references on losses caused by *S. purpurascens* at its different development stages (N4, N5 and adults) with regard to the crops evaluated. However, the results of this study suggest a clear preference for specific crops at each of the insect's developmental stages. A study on *Scotussa lemniscata* (Stal, 1861) revealed significant variations in consumption rates by developmental stage and sex, with pre-reproductive adults consuming the most. Notably, adult females consumed 118.81 ± 9.41 mg/day, significantly more than males and fifth-instar females [40]. This trend aligns with our findings, in which *S. purpurascens* adults exhibited the highest intake, particularly in *M. sativa* and *Z. mays*.

In addition to the developmental stage, food selection plays a crucial role in feeding dynamics. Previous research on *Dichroplus maculipennis* (Blanchard, 1851) demonstrated significant variations in consumption rates when they were fed *Glycine max* (Willd, 1802), *Z. mays*, *Triticum aestivum* (L., 1753), and *A. sativa* [41], highlighting the influence of nutritional composition and structural characteristics on dietary preferences. Our findings suggest that crops with higher moisture content are more attractive to *S. purpurascens*, likely due to their role in maintaining hydration and facilitating digestion.

Field studies on *D. maculipennis* reported an average intake of 236.0 ± 0.02 mg/individual/day in *Festuca arundinacea* (Schreb) pastures [42], reinforcing the idea that body size and metabolic demands drive feeding rates. The increased consumption observed in adult *S. purpurascens* supports this hypothesis, as larger individuals require more energy to sustain their physiological processes.

Our results highlight the importance of considering developmental stage and crop susceptibility when designing integrated pest management (IPM) strategies. The significant consumption of *M. sativa* and *Z. mays* by adult *S. purpurascens* suggests that these crops are particularly vulnerable in later instars, making biological control approaches, such as EPF in oil emulsions, a promising alternative.

The high viability observed in *B. bassiana* and *M. robertsii* exposed to *A. indica* and *M. oleifera* oils suggests that these oils represent an ideal option for the development of fungus-based biopesticides. Their compatibility with EPF ensures that these fungi maintain their survival capacity and efficacy when used under field conditions. The use of the highest concentration (40%) did not significantly reduce viability, which is particularly noteworthy. This indicates that these oils can be applied at higher doses without compromising fungal efficacy, a key advantage for field applications where higher concentrations may be required to enhance persistence or coverage. This emphasizes the importance of developing formulations compatible with the strains used to ensure their effective application against the target pest.

For example, the use of Tween 80 at 0.05% as a surfactant enhances the dispersion and viability of *B. bassiana* [43], facilitating spore adhesion to the insect cuticle [44] and protecting them from external factors, thereby increasing conidia viability. The moderate (yet still high) viability observed with *P. americana* and *P. dulcis* oils suggests that these are also suitable, though slightly less effective compared to *A. indica* and *M. oleifera*. These findings align with previous research highlighting the importance of selecting compatible adjuvants for EPF formulations. For instance, a biopreparation based on Zeolite + Diatomite + *B. bassiana* was evaluated, reporting a conidial viability of 89.1% [23]. It was suggested that selecting preparations with higher viability is more effective for field applications, as these are more resistant to biotic and abiotic factors. Finally, it was noted that spore growth and germination are directly associated with the virulence of EPF [45].

Regarding the effectiveness of EPF in the field, previous studies have shown that combining extracts from *Argemone mexicana* (Linnaeus, 1753) and *Capsicum frutescens* (L., 1753) (chili) with *B. bassiana* reduced the incidence of *S. purpurascens* in *A. hypochondriacus* crops from 48% to 18%. Furthermore, *B. bassiana* strains at concentrations of 1×10^8 and 1×10^9 spores/mL caused 100% mortality in second- and fourth-stage nymphs after 8 days and 76–81% mortality in adults 6 days after application [28]. Similar results were obtained in our study, where vegetable oil emulsions with *M. robertsii* and *P. americana* caused 100% mortality in *S. purpurascens* adults after 72 h. These differences in effectiveness may be attributed to factors beyond concentration, such as the formulation type, application method, or environmental conditions. Although higher concentrations generally enhance strain efficacy, other studies have primarily focused on strain variability rather than the effects of concentration [45–47]. This finding highlights the importance of considering the developmental stage of the insect when designing biological control strategies. Reports of two *M. anisopliae* var. anisopliae isolates, Driver and Milner (Alberta 11S-1, Alberta 6W-2, and Green Guard), for *Melanoplus bivittatus* (Say) control at a 5×10^7 conidia concentration in sunflower oil caused 50% mortality within 5 to 6 days and more than 90% by day 7 [48].

This bioassay helped us to understand the importance of ineffective processes, where EPF associate with healthy organisms, in relation to the ecology of the insects (habits). Grasshoppers act as spore dispersal vectors when they come into contact with the fungus, promoting its spread during movement. During mating, the transfer of spores between males and females can affect their reproductive viability. This is relevant, as a female lays between six and eight oothecas, each containing 20 to 40 eggs [49].

On the other hand, studies have demonstrated that combining canola oil with wheat bran and *B. bassiana* (1×10^8 conidia) resulted in undesirable effects for third-stage nymphs of *D. maculipennis* in both laboratory and field cage settings. Mortality was observed between 10 and 15 days after inoculation [35]. In contrast, the combination of *B. bassiana* + Tween 80 at 0.05% + DW + *A. indica* showed 100% mortality 10 days after application under laboratory conditions. These results highlight the variability in efficacy depending on the formulation and target species. Furthermore, oil emulsions containing *B. bassiana* and *M. robertsii* significantly increased pathogenicity against *S. purpurascens*. This enhanced efficacy may be attributed to the oils' ability to improve spore adhesion and distribution on the insect cuticle, a critical step in the fungal infection process. Moreover, fungal spores can persist in the environment for several months or even years. Additionally, by reproducing from dead insects, they maintain their role as infectious agents, enabling them to continue their life cycle and propagate [50,51]. This persistence and reproductive capability make EPF a sustainable and effective option for biological control. Similar results confirmed the potential of FPf for the control of *Schistocerca gregaria* (Forskal, 1775), highlighting *B. bassiana* and *M. anisopliae* var. *acridum* as effective alternatives to chemical insecticides. Green Muscle (*M. anisopliae* var. *acridum*) demonstrated the highest efficacy in the field,

achieving up to 81.7% mortality in greenhouse trials in 14 days. However, *B. bassiana* isolates 341, 231, and 334, formulated in liquid paraffin, exhibited superior pathogenicity under laboratory conditions, exceeding 99% mortality in adult locusts in the same period [52,53].

5. Conclusions

In conclusion, this study demonstrates the potential of oil emulsions combined with *B. bassiana* and *M. robertsii* as effective biological control agents against *S. purpurascens* under laboratory conditions. The results emphasize the need for field trials to validate these findings across diverse crops and environments.

Furthermore, this study highlights the significant impact of *S. purpurascens* at different developmental stages on agricultural productivity, underscoring the importance of timely interventions by farmers.

Author Contributions: Conceptualization, T.A.-B.; data curation, Y.D.O.-H. and T.A.-B.; formal. analysis, K.C.-G., T.A.-B. and T.A.-L.; writing—original draft, Y.D.O.-H. and T.A.-B.; writing—review and editing, K.C.-G., Y.D.O.-H., T.A.-B. and T.A.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Instituto Politécnico Nacional through project number 20231509.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The first author thanks the Secretaría de Ciencias, Humanidades, Tecnología e Innovación (SECIHTI) for awarding a grant to the first author to undertake their post-graduate studies in Conservation and Use of Natural Resources-Biological Control Laboratory of IPN-CIIDIR Oaxaca for its support, and Sistema Nacional de Investigadoras e Investigadores (SNII-SECIHTI).

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

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