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Evaluation of commercial products based on *Isaria fumosorosea* and *Verticillium lecanii* fungi as an alternative in the biocontrol of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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Evaluación de productos comerciales basados en los hongos *Isaria fumosorosea* y *Verticillium lecanii* como alternativa en el control biológico de *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae).

RESUMEN. En el noroeste de México, *Phenacoccus solenopsis* ha sido reconocida como una especie agresivamente invasiva en plantas de algodón. Por lo tanto, el objetivo del presente estudio fue evaluar la efectividad de dos bioinsecticidas fúngicos comerciales (*Verticillium lecanii* e *Isaria fumosorosea*) contra *P. solenopsis* en condiciones de laboratorio y semi-campo. Nuestros resultados mostraron una alta mortalidad por *V. lecanii* (100%) seguida de *I. fumosorosea* (40%) y control (0%) después de 96 horas de exposición. Después de treinta días de iniciar el experimento, se observó una reducción significativa del índice de severidad en plantas de algodón expuestas a *I . fumosorosea* y *V. lecanii* (50% y 48%) comparada con el control (76%). El uso de *V. lecanii* e *I . fumosorosea* no mostró cambios significativos en los parámetros fisiológicos de las plantas (valores de clorofila, contenido de polifenoles, antocianinas y eficiencia fotosintética) con respecto al control después de 30 días de exposición a los hongos entomopatógenos. Actualmente, la información disponible sobre el modo de acción de la formulación comercial empleando *I. fumosorosea* y *V. lecanii* para el control biológico de *P. solenopsis* es escasa en México en comparación con otros países. Por lo tanto, son necesarios estudios enfocados en su efectividad.

PALABRAS CLAVE. Algodón. Bio-insecticidas. Cochinilla harinosa. Control biológico. Entomopatógenos.

ABSTRACT. In the Northwest of Mexico, *Phenacoccus solenopsis* has been recognized as an aggressively invasive species on cotton plants. Therefore, the aim of the present study was to evaluate the effectiveness of two fungal commercial bioinsecticides (*Verticillium lecanii* and *Isaria fumosorosea*) against *P. solenopsis* under laboratory and semi-fields conditions. Our results showed a high mortality by *V. lecanii* (100%) followed by *I. fumosorosea* (40%) and control (0%) after 96 h of exposure. Thirty days after initiating the experiment, we observed a significant reduction of the disease index in cotton plants exposed to *I. fumosorosea* and *V. lecanii* (50% and 48%, respectively) compared with control (76%).

The use of *V. lecanii* and *I . fumosorosea* did not show significant changes in the physiological parameters of plants (chlorophyll, polyphenol contents, anthocyanins and potential photochemical yield values) compared with the control after 30 days of exposure to entomopathogenic fungi. Currently, the available information about mode of action of commercial formulations using *I. fumosorosea* and *V. lecanii* for biocontrol of *P. solenopsis* is scarce in Mexico compared with other countries. Therefore, studies focused on their effectiveness are necessary.

KEYWORDS. Bioinsecticides. Biological control. Cotton. Entomopathogens. Mealybug.

INTRODUCTION

Mexicali valley is located in the municipality of Mexicali, Baja California and has an arable land surface and irrigated area of 210,930 hectares (ha) of which 184,283 ha belong to Mexicali and 26,648 ha to San Luis Río Colorado. Agricultural production has a great economic relevance in the Mexicali valley; for example, over 15,000 people are employed in the local agricultural sector producing mainly cotton (González-Soto et al., 2017). Particularly, the cotton crop sown in Mexico has been genetically modified (GM) over twenty years (Rocha-Munive et al., 2018). GM cotton was authorized in Mexico since 1996 and the crop contains cry genes from *Bacillus thurigensis* (Bt) that confer resistance to larval stages of different lepidopteran pests (Terán-Vargas et al., 2005). The use of GM cotton has increased in Mexicali valley due to its high-yield production and management of pest control with reduced insecticide application (González-Soto et al., 2017).

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) have been reported as a severe pest of different crops in Mexico (García-Espinoza et al., 2017). This pest is a polyphagous insect with wide physiological and ecological adaptations in different localities around the world (Nagrare et al., 2009; Wu et al., 2014). The pest management strategies include the combination of chemical insecticides and the culture practices to degrade the complex layer of wax of *P. solenopsis* (Jhala et al., 2010; Ahmad et al., 2011). Although the insecticides provide high efficiency against *P. solenopsis* these may cause resistance (Noureen et al., 2016). In this sense, the use of entomopathogenic fungi in the agriculture represents a viable alternative to reduce the chemical pesticides. Currently, the employment of entomopathogenic fungi such as *Verticillium lecanii* and *Isaria fumosorosea* has a great relevance due to the fact that they are environmentally friendly and they do not use toxic chemicals in their formulation (Huang et al., 2012). In addition, entomopathogenic fungi as *Metarhizium anisopliae* and *Beauveria bassiana* have been previously tested against the mealybug *P. solenopsis* under laboratory conditions (Ram & Saini, 2010; Ujjan et al., 2015). However, studies about the use of entomopathogenic fungi for control of invasive GM cotton mealybug are scarce in Mexico. Therefore, this study was conducted to evaluate the effectiveness of two fungal commercial bioinsecticides (*V. lecanii* and *I. fumosorosea*) against *P. solenopsis* under laboratory and semi-fields conditions.

MATERIAL AND METHODS

Biological material

Phenacoccus solenopsis individuals were originally collected from infested GM cotton plants of Bollgard II variety of Deltapine 1044 B2RF during August 2018 in the Mexicali valley, Mexico. Branches of cotton plants infested with reproducing females of mealybugs were collected from cotton-fields located in Mexicali, Baja California, Mexico (Fig. 1). Identification of *P. solenopsis* in the samples was determined following the taxonomic keys of Hodgson et al. (2008). Insects were maintained on individual cotton leaves in a climate-controlled chamber (25 ± 3 °C, 60–70% RH, 12:12 photoperiod).

Laboratory bioassays

Two bioassays were conducted using commercial entomopathogenic formulae of *I. fumosorosea* (PAE-TRON®) and *V. lecanii* (VERTITRON®), respectively. For the first bioassays, stock suspensions of conidia were prepared according to the manufacturer's recommendations: PAE-TRON (5×10^6 conidia/mL) and VERTITRON (1×10^9 conidia/mL). Cellular concentration

Fig. 1. Cotton-field from Mexicali valley where *Phenacoccus solenopsis* was collected (A); Detail of damage in cotton plants by *P. solenopsis* (B).

was determined through serial dilutions (0.05% Tween 80 solution) using a Bio-Rad TC20 automated cell counter (Hercules, CA, USA). Stock suspensions were used for virulence bioassays with *P. solenopsis* and the percentage of viable conidia was determined prior to bioassay. Both commercial entomopathogenic fungi showed more than 90% of viable conidia using the trypan blue dye (Bio-Rad Laboratories, Hercules, CA, USA) and following the manufacturer´s protocol in a Bio-Rad TC20 automated cell counter. In the first bioassays, the insecticidal activity of both commercial entomopathogenic fungi was evaluated and compared with the control (only water). Thirty insects of adult females (15 days old) were selected and carefully transferred to Petri dishes containing a cotton leaf (Fig. 2). Then, 2 mL aqueous solution containing PAE-TRON (5×10^6 conidia/mL) was sprayed on insects using a small hand atomizer (treatment 1). In the second treatment, the insects were sprayed with 2 mL VERTITRON (1×10^9 conidia/mL); and the third treatment, 2 mL of water was only sprayed (control). All experiments were randomized using triplicates by treatment. The number of *P. solenopsis* survivors (mortality percentages) was counted after 96 h of initial application. Insect mortality in the first bioassays was calculated applying the following formula:

$$\textbf{Insect Mortality (\%)} = (\textbf{Dead insects/Total insects}) \times 100$$

which was corrected following Abbott (1925) if any mortality was observed in the control due to natural death.

Fig. 2. Selection and transferred of *Phenacoccus solenopsis* to Petri dishes

Semi-field bioassays

In the second bioassays, the efficacy of PAE-TRON® and VERTITRON® products against *P. solenopsis* population was evaluated under field conditions on September, 2018. This test was carried out on a plot of cotton plants untreated with pesticides and located at Mexicali valley (32º 33' 33.6'' N; 115º 17' 35.2'' W). Three replicates were performed *per* treatment using 11 m long rows, 0.80 m row spacing and 0.30 m plant spacing. Four grades of mealybug damage severity were considered in the cotton plants according to Prabhakar et al. (2013): grade 1: scattered appearance of few mealybugs on the plant (<25%); grade 2: severe infestation of mealybug on any one branch or on less than half of the plant (25–49%); grade 3: severe infestation of mealybug on more than one branch or half portion of the plant (50–75%) and grade 4: severe infestation of mealybug on the whole plant (>75%). The experiments were conducted in plants with grade 3 of mealybugs damage severity and these were classified as follows: PT) plants treated with PAE-TRON® (15 mL *per* plant containing 5×10^6 conidia/mL); VT) plants exposed to VERTITRON® (1×10^9 conidia/mL); C), plants treated with 15 mL of sterile water and 0.02% Tween 80 *per* plant (control). The plants were sprayed with suspensions of each treatment in the morning every other week for a month. The disease index (DX) and percentage of protection (PP) were calculated according to Zhu et al. (2013) based on the presence of mealybugs using one to four scales of mealybug infestation from fifty infested plants within each treatment and described as follows:

$$\textbf{Disease index (DX)} = [(0n_0+1n_1+2n_2+3n_3+4n_4)/4n] \times 100$$

$$\textbf{Protection (\%)} = [\textbf{DX (control)} - \textbf{DX (treatment)}]/\ \textbf{DX (control)}] \times 100$$

where n_0–n_4 were the numbers of plants with each of the corresponding grade of mealybug infestation, and n was the total number of plants tested.

On the other hand, entomopathogenic fungi are well known for their pest control potential. However, new roles have been reported especially in relation to their ability to improve the physiological status of plants (*e.g.*: chlorophyll) (Yong-Seong & Kim, 2019). Therefore, the determination of physiological parameters as leaf chlorophyll (Chl), polyphenol contents (EPhen), anthocyanins (Anth) and potential photochemical yield (Fv/Fm) from cotton leaves of each treatment were evaluated in this study. Chl, EPhen, Anth and Fv/Fm were analyzed using the Dualex® scientific sensor (FORCE-A, Orsay, France) and Chlorophyll Fluorometer (OS-30p, OPTI-SCIENCE, USA), according to Gonzalez-Mendoza et al. (2017). Determinations were taken at 30 days after exposure to PAE-TRON® and VERTITRON® products using fifty plants of each treatment.

Data analysis was conducted using the Statistics software package Version 9.0. Differences between thetreatment's means were compared using Tukey's test ($p \leq 0.05$), and significant differences were accepted if $p \leq 0.05$, for each bioassay (laboratory and field bioassays).

RESULTS

Laboratory bioassays

Figure 3 shows the changes in the mortality (%) of *P. solenopsis* after 96 h of the initial application of commercial product of *I. fumosorosea* (PAE-TRON®) and *V. lecanii* (VERTITRON®), respectively. The highest mortality (%) was recorded by *V. lecanii* (100%) followed by *I. fumosorosea* (40%) and the control (0%) after 96 h of exposure. The results showed significant toxicity of VERTITRON® against *P. solenopsis* after 96 h of exposure compared with the commercial product PAE-TRON® and the control.

Semi-field bioassays

On the other hand, after thirty days of the establishment of second experiment the lowest disease index of *P. solenopsis* was observed for the treatments compared with the control (Fig. 4). The results showed a significant reduction of insects exposed to *I. fumosorosea* and *V. lecanii* (50% and 48%, respectively) compared with the control (76%). In this sense, the use of commercial VT and PT products showed a significantly increase of percentage of protection of cotton plants after 30 days of aplication of products (Fig. 5).

The use of VERTITRON® (*V. lecanii*) and PAE-TRON® (*I. fumosorosea*) did not show significant changes on Chl, EPhen and Anth values compared to the control (Table I). Measurements of chlorophyll a fluorescence did not show a significant decrease on potential photochemical yield (Fv/Fm) value of cootton plants exposed to VERTITRON® and water (control) after 30 days of treatments (Table I).

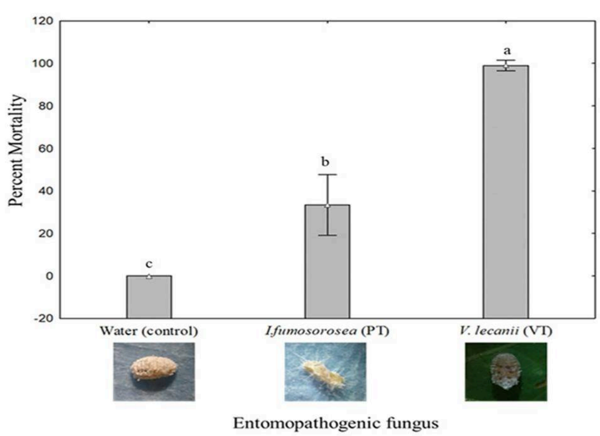

Fig. 3. Insect mortality of *Phenacoccus solenopsis* by application of commercial product of *Isaria fumosorosea* and *Verticillium lecanii* after 96 hours of treatment.

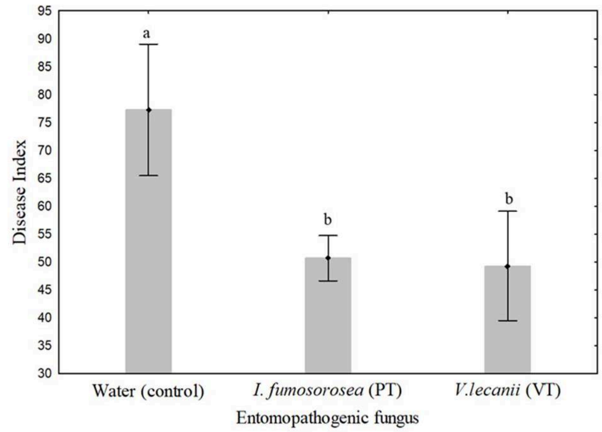

Fig. 4. Disease index in cotton plants exposed to *Isaria fumosorosea* and *Verticillium lecanii* after 30 days of treatment

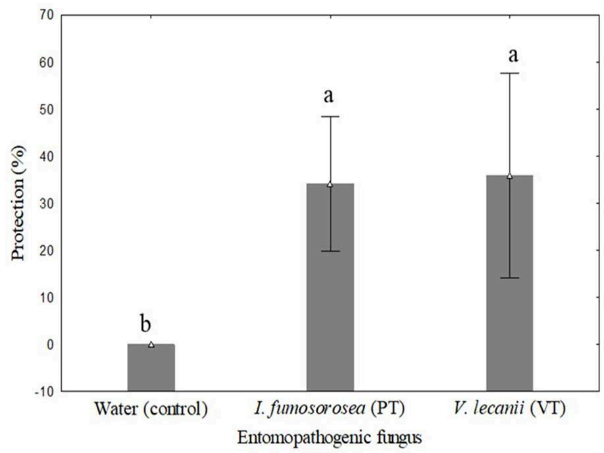

Fig. 5. Protection (%) in cotton plants exposed to *Isaria fumosorosea* and *Verticillium lecanii* after 30 days of treatment

DISCUSSION

In the present study, the commercial formulation of *V. lecanii* at 1 x 10^9 conidia/mL showed significant toxicity against *P. solenopsis* after 96 h of exposure compared with other commercial products of this entomopathogen. In this sense, Banu et al. (2010) reported that *V. lecanii* (2 x 10^8 CFU/g) showed less inhibitory effect against *P. solenopis* (42.22%) after 48 h of treatment. In addition, Kumar et al. (2012) reported a mortality maximum of 45.66% of this pest in the formulation containing *V. lecanii* (1 x 10^8 conidia/g) after five days of exposure. On the other hand, *I. fumosorosea* has also shown a great potential to control different insect pests. However, the efficacy of commercial formulation for the biocontrol of adults of mealybug was lower (40%) in the present study.

Treatments	Leaf chlorophyll Chl	Polyphenols EPhen	Anthocyanins Anth	Photochemical yield Fv/Fm
PAE-TRON® (PT)	24.16 ± 0.41ᵃ	1.89 ± 0.32ᵃ	0.15 ± 0.08ᵃ	0.60 ± 0.03ᵃ
VERTITRON® (VT)	24.12 ± 0.58ᵃ	1.93 ± 0.23ᵃ	0.15 ± 0.01ᵃ	0.57 ± 0.06ᵃ
Control (water)	24.32 ± 0.39ᵃ	1.95 ± 0.68ᵃ	0.16 ± 0.06ᵃ	0.58 ± 0.05ᵃ

Table I. Effects of commercial product based on *Isaria fumosorosea* (PT) and *Verticillium lecanii* (VT) on physiological parameters of cotton plants. Results are expressed as mean ± standard deviation of values from triplicate experiments. Values with the same letter within each line are equal according to the Tukey test at $p \leq 0.5$

Few data are available on the use of the commercial formulation of *I. fumosorosea* against mealybug in cotton plants in Mexico. However, recent studies indicated that *I. fumosorosea* has the potential to control the whitefly infestation of beans crops in nymphal stage, but not with the adults, which showed a very low susceptibility (Flores-Macas et al., 2013). Moreover, Huang et al. (2012) found that the chemical control caused an enhancement in the mortality of *P. solenopis* mainly on the first instars but it did not occur for adult females after 5 days under laboratory conditions. In this sense, the present study showed that both myco-insecticides had a significant impact against *P. solenopsis* in comparison with some commonly chemical insecticides used as flubendamide, chlorantraniliprole and imidacloprid (Nagrare et al., 2016).

On the other hand, the commercial formulations of *I. fumosorosea* (5 x 10^6 conidia/mL) and *V. lecanii* (1 x 10^9 conidia/mL) had a significant positive impact on the reduction of the disease index as well as the increase of protection of cotton plants after 30 days of aplication. Although the mechanism of reduction of the pest is not known yet, some studies suggest that the insects could cause a significant loss of plant nutrients through their feeding damage. In addition, the plants seem to have a mechanism to recover those nutrients, through the entomopathogenic fungi (Raya-Díaz et al., 2017).

Previous studies have revealed that some entomopathogenic fungi (*e.g.*: *Lacanicillium psalliotae*) increase the leaf chlorophyll content compared with untreated plants (Kumar et al., 2012). In contrast, our results showed that the commercial formulations of *I. fumosorosea* and *V. lecanii* did not change the leaf chlorophyll, polyphenol contents, anthocyanins and potential photochemical yield in cotton plants infested with *P. solenopsis*. These results could be explained by the length of infestation time, the effect of different environmental conditions on herbivore activity, the herbivore densities, or the interaction with biological agents of control (Huang et al., 2013).

Currently, the available information about mode of action of commercial formulations using *I. fumosorosea* and *V. lecanii* for biocontrol of *P. solenopsis* is scarce in Mexico compared with other countries. Therefore, studies focused on their effectiveness are necessary.

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