Bio-efficacy of entomopathogenic fungi and vegetable oils against the pink pineapple mealybug: *Dysmicoccus brevipes* (Cockerell)

Bioeficacia de hongos entomopatógenos y aceites vegetales contra el piojo harinoso rosado de la piña: *Dysmicoccus brevipes* (Cockerell)

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Abstract

Dysmicoccus brevipes (Cockerell) (Hemiptera: Pseudococcidae) is an important insect pest of pineapple worldwide due to the direct damage it causes and because it is a vector of mealybug pineapple wilt. Entomopathogenic fungi are an alternative management tool for this pest. A preliminary experiment evaluated the lethal effects of two isolates of *Beauveria bassiana* (BbCT, BbCa) and one isolate of *Metarhizium anisopliae* (Ma) against adult female *D. brevipes*. Subsequently, the efficacy of the most virulent isolates and a commercial strain of *Paecilomyces fumosoroceus* (PAE-SIN) were evaluated under laboratory and greenhouse conditions, either alone or in combination with soybean oil or neem oil. Results showed variation amongst isolates and that *B. bassiana* was the most effective. Isolate BbCa at 1×10^7 mL^{-1} conidia, was the most effective against *D. brevipes* nymphs and adults at 26 ± 1°C, causing 66% ± 6% mortality 8 days after inoculation. BbCa was the most virulent with an LC_{50} of 3.45×10^7 mL^{-1} conidia and a LC_{95} of 2.29×10^8 mL^{-1} conidia, under controlled conditions. Efficacy of BbCa increased when combined with neem oil, causing 100% mortality 6 days after inoculation. In conclusion, a combination of *B. bassiana* isolate BbCa and neem oil achieved 100% mortality in *D. brevipes* under the experimental conditions reported in this study.

Keywords
entomopathogenic fungi • biological control • pineapple • *Dysmicoccus* • vegetable oils

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Resumen

Dismiccoccus brevipes (Cockerell) (Hemiptera: Pseudococcidae) es una de las plagas de la piña de mayor importancia a nivel mundial, no solo por los daños directos que ocasiona, sino por ser trasmisor del virus marchitez roja de la piña. Los hongos entomopatógenos son una alternativa para el manejo de este insecto. En un experimento preliminar se evaluó la patogenicidad y la virulencia de dos aislamientos de *Beauveria bassiana* (BbCT, BbCa) y uno de *Metarhizium anisopliae* (Ma), sobre hembras adultas de *D. brevipes.* Posteriormente, los aislamientos más virulentos y una cepa comercial de *Paecelomyces fumosoroceus* (PAE-SIN), fueron evaluados en otro experimento en condiciones de invernadero, solos o en combinación con aceite de soya y aceite de neem. Los aislamientos evaluados presentaron diferente grado de virulencia; sin embargo, *B. bassiana* resultó ser el más virulento. El aislamiento BbCa a una concentración inicial de 1×10^7 conidios mL^{-1} fue más efectivo contra adultos de *D. brevipes* comparado con el control, causando mortalidad del 66% ± 6% a los 8 días pos inoculación a 26 ± 1°C. BbCa presentó la mayor virulencia con una CL_{50}, de $3.45x10^7$ conidios mL^{-1} y una CL_{95} de $2.29x10^8$ conidios mL^{-1}, bajo condiciones controladas. Sin embargo, la eficacia se incrementó para BbCa, cuando se combinó con aceite de neem, al causar el 100 % de mortalidad a los 6 días pos inoculación. En conclusión, la combinación *B. bassiana* (BbCa) y aceite de neem fue el mejor tratamiento, con una mortalidad de 100% de *D. brevipes* bajo las condiciones experimentales reportadas en este estudio.

Palabras clave
entomopatógenos • control biológico • piña • *Dysmicoccus* • aceite vegetal

Introduction

Pineapple production generates significant economic resources worldwide. Mexico's main pineapple exports are destined for the United States market, with an annual value in 2020 of $30,602,000 USD (24). Unfortunately, the pineapple industry is affected by various phytosanitary problems. Since pineapple is grown intensively and in monoculture, pesticides are commonly applied for pest management, causing problems for human health, the environment, and agroecosystems. The mealybugs *Dysmicoccus brevipes* (Cockerell) (Hemiptera; Pseudococcidae) and *D. neobrevipes* Beardsley (Hemiptera: Pseudococcidae) are major pests of commercial pineapple cultivation (29) causing significant damage throughout the crop growth cycle; they are also vectors of Pineapple Mealybug Wilt associated Virus (PMWaV) which can cause up to 100% of export crop losses due to rejection of fruit (19). Recent management strategies for *D. brevipes* in pineapple are largely based on synthetic organophosphate insecticides. However, efficacy of chemical control is limited by the cryptic location and behavior of these insects on plants, and their waxy surface layer which is a barrier to the action of contact insecticides, even protecting eggs from residual effects. There is also increasing concern in general about the toxic risks of excessive pesticide use in agriculture. Therefore, exploration of economically viable and environmentally safe strategies is necessary. We hypothesize that commercial pineapple production could benefit from the use of botanical extracts and biological pest control agents, such as entomopathogenic fungi, within integrated pest management (33).

Entomopathogenic fungi can infect directly without the need for ingestion and so are effective against sucking pests such as aphids, mealybugs, whiteflies, and mosquitoes (4). Some entomopathogenic fungi have a combination of modes of action against arthropods: toxins; nutrient depletion; physiological disruption; and mechanical damage to internal tissues due to mycelium development (12). Efficacy of entomopathogenic fungi has been widely documented, particularly against mealybugs. For example, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales), *Lecanicillium lecanii* (Zimm.) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) infect and kill *Paracoccus marginatus* Williams & Granara de Willinks (2). Despite this, there are few laboratory and field studies on management of *D. brevipes* using entomopathogenic fungi in pineapple. One study by Miranda Vindas and Blanco Getzler (2013) has evaluated a range of options in the laboratory that included both entomopathogenic fungi and botanical oil extracts that are known for their repellent, anti-feeding, and growth inhibition properties; high degradability; and environmental safety (18). Specific evaluations included: *B. bassiana* (4.0 x10^{10} conidia/g); *M. anisopliae* (1.0 x10^{10} conidia/g); a mixture of both fungi (0.5 g + 0.5 g/L distilled water, 4.0 x10^{10} conidia/g + 1.0 x10^{10} conidia/g); potassium salts; fatty acids (7 mL/L); and botanical extracts (a mixture of hot chili, garlic, onion, mustard and jackass bitters) (7 mL/L). Results showed high efficiency of entomopathogenic fungi and that the botanical extract achieved the fastest mortality. In the same publication, the botanical extract was also evaluated in a commercial pineapple plantation in comparison with the typical chemical control options Diazinon® 60 EC (diazinon) (0.5 ml/L) and Sevin® 80 WP (carbaryl) (1 kg/ha); the lowest incidence of mealybugs was achieved in the botanical extract treatment (16).

These results suggest that combinations of entomopathogenic fungi and vegetable oil extracts have potential as control agents that may increase mortality of adult *D. brevipes* females. For this reason, the potential of two native fungal isolates was evaluated in comparison with a commercial product based on *Paecelomyces fumosoroseus*. Our specific objectives were to determine the pathogenicity and virulence of the entomopathogenic fungi, alone and in combination with neem oil or soybean oil, against the pineapple mealybug under laboratory and greenhouse conditions.

Material and Methods

Experiments were done at the Biological Control Laboratory, Postgraduate College, Tabasco Campus, Cárdenas, Tabasco, Mexico, between January and December 2021.

Collection and mass rearing of *D. brevipes*

Dysmicoccus brevipes adults were collected from two varieties of pineapple (MD2 and bighead) on commercial plantations in Huimanguillo, Tabasco. Insects were taken to the Biological Control laboratory of the Postgraduate College, Tabasco Campus, for laboratory breeding, following the methods of Pandey & Johnson (2006). For the breeding stock, 50 eight-month-old pineapple cloves (25 bighead and 25 MD2 variety) were transplanted from a commercial pineapple plantation in Huimanguillo, Tabasco, into plastic pots and kept in a greenhouse at 30-35°C. Twenty days after potting, they were infested with adult female *D. brevipes* (20 per plant) in the greenhouse.

Entomopathogenic fungal isolates

Pathogenicity and virulence evaluations were made on two *Beauveria bassiana* (BbCa, BbCT) isolates and one isolate of *Metarhizium anisopliae* (Ma); all were native entomopathogenic isolates from Tabasco, Mexico held in the collection of the Biological Control Laboratory of the Colegio de Postgraduados, Tabasco Campus (table 1). Subsequent bio-efficacy experiments included a commercial product based on *Paecilomyces fumosoroceus* (PAE-SIN®).

Mycelia from each isolate was grown on sterile Sabouraud Dextrose Agar (ADS, Bioxon, Mexico) in Petri dishes, 90 x 15 mm for 3 weeks at 26±1°C in darkness. Conidia were then scraped from the surface and suspended in 0.03% Tween 80. Conidial concentration was determined using a Neubauer chamber, following the method of Inglis *et al.* (2012).

Table 1. Reference of the fungi used in the evaluation of pathogenicity against *D. brevipes.*

Tabla 1. Referencia de los hongos usados en la evaluación de patogenicidad contra *D. brevipes.*

Species	Key	Host	Location
Beauveria bassiana	BbCT	*Hypsiphilla grandella*	Huimanguillo, Tab. Mexico
Beauveria bassiana	BbCa	*Hypothenemus* sp.	Huimanguillo, Tab. Mexico
Metarhizium anisopliae	Ma	*Aeneolamia* sp.	Cárdenas, Tab. Mexico

Pathogenicity and virulence of *D. brevipes*

Pathogenicity and virulence of *B. bassiana* (BbCa, BbCT) and *M. anisopliae* (Ma) against *D. brevipes* were determined experimentally using a completely randomized design with four replicates of each treatment and control; the entire experiment was repeated on three occasions. Groups of ten adult females were each placed on two basal pieces of MD2 pineapple leaf (8 x 8 cm) inside a plastic box (20 cm x 10 cm x 10 cm) with openings covered with organdy mesh for ventilation. Wet filter paper was placed in each box to provide moisture. Each group of adults was sprayed (from a spray bottle) with 1.5 ml of conidia (either 10^6, 10^7 or 10^8 mL^{-1}) suspended in 0.05% aqueous Tween 80; the control was sprayed with 0.05% aqueous Tween 80 only. Applications were made following the methodology of Ramírez-Sánchez *et al.* (2019). Boxes containing treated insects were incubated at 26 ± 1°C, 65 -70% RH and a 14:10 h light: dark regime). Mortality was assessed daily for 8 days. Dead insects were incubated to determine cause of death (mycosis), following the methodology of Butt and Goettel (2000). Abbott´s formula was used to correct data for control mortality (1).

Bio-efficacy of B. bassiana and *P. fumosoroseus*, either alone or in combination with vegetable oils, against *D. brevipes* under greenhouse conditions

An experiment was set up under greenhouse conditions based on the results of the aforementioned bioassays. Pineapple suckers (variety MD2 [40 cm in size]) were planted individually in replicate pots, each containing 2 kg of sandy soil collected from a commercial pineapple plantation in Huimanguillo, Tabasco, Mexico. To each pot twenty *D. brevipes* adults were inoculated at the base of the pineapple sucker and incubated for one month before experimental treatments were added.

A total of eight treatments were compared including the highly virulent *B. bassiana* isolate, BbCa, and a formulated strain of *P. fumosoroseus* (PAE-SIN®), either alone or in combination with soybean oil (CARRIER)® or neem extract oil (Nimicide 80®) (table 2). All treatments were applied as 20 ml solutions/ suspensions; all fungal treatments contained 1 x 10^7 conidia mL^{-1}. There were four replicates of each treatment arranged in a completely randomized design. After inoculation, insect mortality was assessed daily for 8 days.

Table 2. Treatments evaluated in the greenhouse assay.

Tabla 2. Tratamientos evaluados en el experimento de invernadero.

Treatments	Key	Conidia mL^{-1}
B. bassiana	BbCa	1 x 10^7
P. fumosoroceus	PAE-SIN	1 x 10^7
Soybean oil	SO (CARRIER®)	1.2 mL^{-1} L water
Neem oil	NO (NIMICIDE 80®)	1 mL^{-1} L water
BbCa + SO	BbCa + SO	1 x 10^7
BbCa + NO	BbCa + NO	1 x 10^7
PAE-SIN + SO	PAE-SIN + SO	1.0 x 10^7
PAE-SIN + NO	PAE-SIN + NO	1.0 x 10^7
Control	Tween 80	0.05 %

Statistical analysis

Probit analysis was used to estimate the LC_{50} and LC_{95} of each isolate with a 95% confidence limit. ANOVA and multiple comparisons of means for both isolates and their concentrations were also done with the Tukey test ($p \leq 0.05$) in SAS software (25). The probit regression model $\Phi^{-1}[\Pi(x)]=\alpha+\beta x$ and the formula LC (P) = (qnorm (P) - α) / β were used to estimate lethal concentrations. Virulence graphs were constructed using R software v. 1.0.143 (22).

Results

Pathogenicity and virulence

Both isolates of *B. bassiana* (BbCa and BbCT) caused higher levels of *D. brevipes* mortality at all conidia concentrations evaluated compared with *M. anisopliae* at the same concentrations. There were highly significant differences amongst treatments ($p < 0.0001$) in the mean daily mortality after 8 days at the $1x10^6$ conidia concentration. The highest daily mean % mortality was achieved by isolate BbCa (32.2%), followed by isolate BbCT (16.3%) and then Ma (15.8%); in the control mortality was 4.7% (figure 1 A1). However, cumulative mortality at day 8 after inoculation was 45, 40, 32.5, and 4.5% for isolates BbCa, BbCT, Ma and the control treatment, respectively (figure 1 B1).

Bars with different letters are significantly different to each other (Tukey p ≤ 0.05). Error bars represent ±1 standard error of the mean (SEM), n= 4.

Barras con diferentes letras son significativamente diferentes unas de otras (Tukey p ≤ 0,05). Barras de error representan ±1 el estándar error de la media (SEM), n= 4.

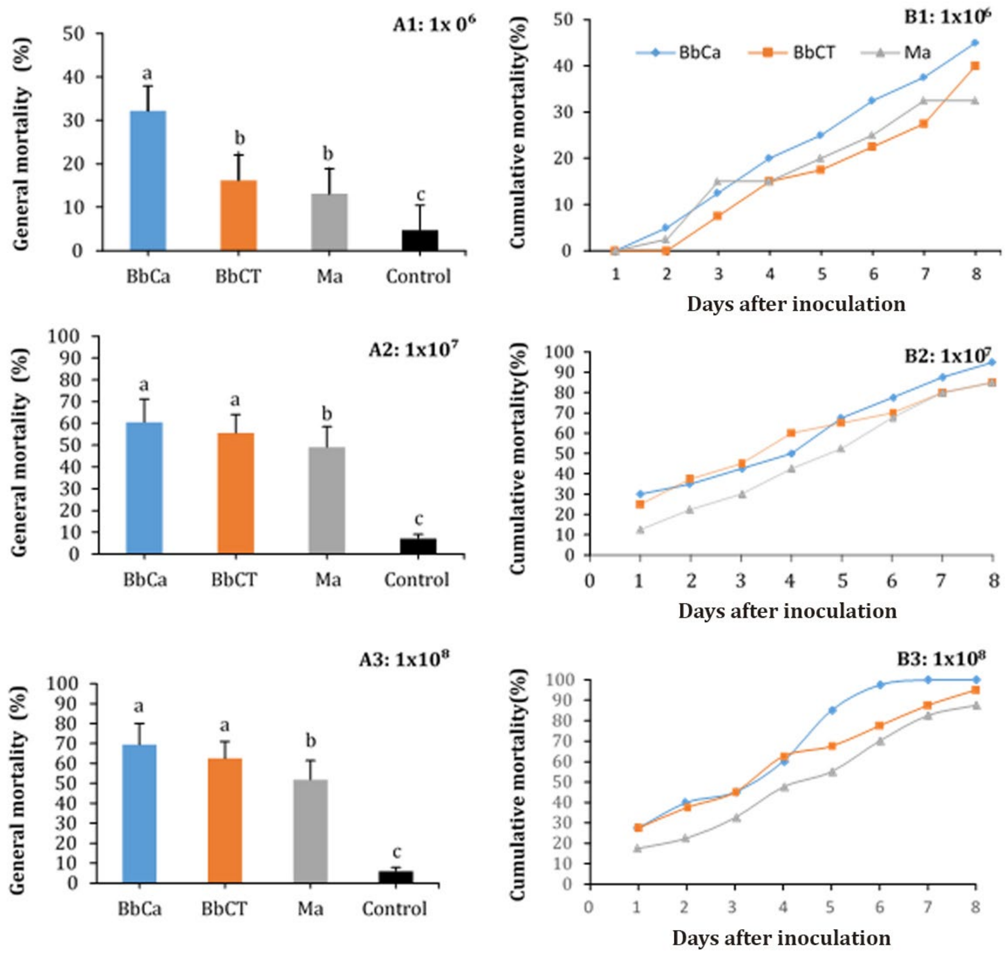

Figure 1. Dynamics of A) general mean mortality for the period 1 - 8 days post inoculation and B) daily cumulative mortality of adult female *Dysmicoccus brevipes* after treatment with 1×10^6 (A), 1×10^7 (B) or 1×10^8 (C) conidia mL^{-1}, of *B. bassiana* (BbCa, BbCT) and *M. anisopliae* (Ma).

Figura 1. Dinámica de A) mortalidad media general del periodo de 1 - 8 días post inoculación y B) mortalidad acumulada diaria de hembras adultas de *Dysmicoccus brevipes* después de los tratamientos con 1×10^6 (A), 1×10^7 (B) o 1×10^8 (C) conidios mL^{-1}, de *B. bassiana* (BbCa, BbCT) y *M. anisopliae* (Ma).

Mortality data for the 1 x 10^7 conidia mL^{-1} concentration showed an increase in % mortality compared with the 1 x 10^6 conidia mL^{-1} concentration. There were highly significant differences amongst treatments ($p < 0.0001$); the highest mean daily % mortality over 8 days was achieved by isolates BbCa and BbCT, with 60% and 58.43% mortality, respectively, followed by isolate Ma with 49.06% and the control with 7.18% (figure 1 A2, page 87). Cumulative mortality on day 8 was 95, 85, 82, and 7.18% for isolates BbCa, BbCT, Ma and the control, respectively (figure 1 B2, page 87).

Mortality data for the 1x10^8 conidia mL^{-1} concentration showed an even greater increase in % mortality compared with the 1 x 10^6 and 1 x 10^7 conidia mL^{-1}. Highly significant differences among treatments ($p < 0.0001$) were detected, with the highest mean daily % mortality achieved by isolates BbCa and BbCT being 69.4 and 62.5%, respectively, followed by Ma (51.9%), while in the control mortality was 5.9% (figure 1 A3, page 87). Cumulative % mortality on day 8 was 100, 95, 87.5 and 7.1% for isolates BbCa, BbCT, Ma and the control, respectively (figure 1 B3, page 87).

Concerning cumulative mortality, *B. bassiana* isolate BbCa was more effective from day 5 post-inoculation than isolate BbCT. However, both isolates of *B. bassiana*, at each concentration evaluated, showed high efficacy against pineapple mealybug with increasing cumulative mortality over time after inoculation (figure 1B, page 87).

Proliferation of mycelium and conidial structures was observed on cadavers of *D. brevipes* produced during the first 4 days after inoculation. Abundant sporulation was detected from day 8 after inoculation, particularly from cadavers killed by *B. bassiana* isolates (figure 2).

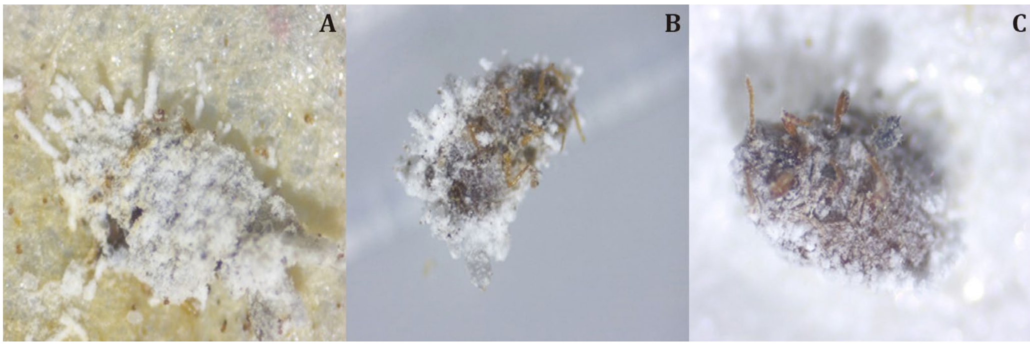

Figure 2. *Beauveria bassiana* isolates A) BbCa, B) BbCT and C) *Metarhizium anisopliae* (Ma) infecting adult female pineapple mealybug *Dysmicoccus brevipes* at 4 x magnification with an optical microscope.

Figura 2. Aislamientos de *Beauveria bassiana* A) BbCa, B) BbCT y C) *Metarhizium anisopliae* (Ma) infectando a hembras adultas del piojo harinoso de la piña *Dysmicoccus brevipes* a una magnificación de 4 x con un microscopio óptico.

Determination of virulence

LC_{50} values were 3.4 x 10^7, 5.2 x 10^7 and 8.5 x 10^7 mL^{-1} conidia for isolates BbCa, BbCT, and Ma, respectively. LC_{95} values were 2.29 x 10^8, 2.67 x 10^8 and 3.77 x 10^8 conidia mL^{-1} for isolates BbCa, BbCT and Ma, respectively (figure 3, page 89). Probit regression lines for *B. bassiana* were $Y = - 0.2864+8.401^{-09}$ (x) and $Y= - 0.3915+7.602^{-09}$(x), for BbCa, BbCT, respectively, whereas for *M. anisopliae*, it was $Y= - 0.4795+5.626^{-09}$(x), where 'Y' was the probit mortality and 'x' was the fungal concentration (figure 3, page 89). Data fitted well with the model and there was a positive correlation between conidial concentration evaluated and bioinsecticidal activity of both fungi.

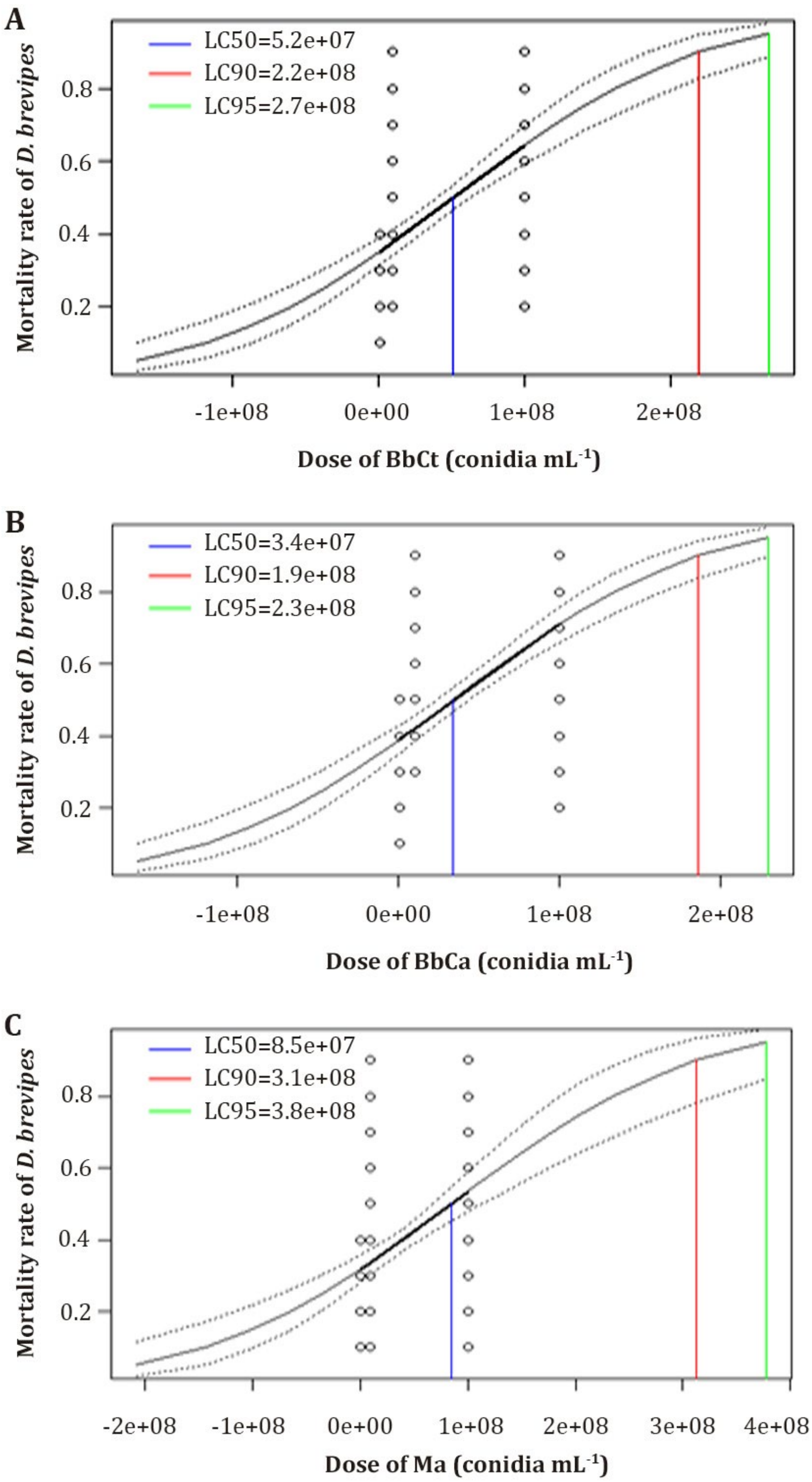

Figure 3. Corrected probit plot mortality of adult female *D. brevipes* treated with different conidia concentrations (log dose) of *B. bassiana* (BbCa [A], BbCT [B]) and *M. anisopliae* (Ma[C]) visualized using R software v. 1.0.143 (22).

Figura 3. Mortalidad probit corregida de hembras adultas de *D. brevipes* tratadas con diferentes concentraciones de conidios (log dosis) de *B. bassiana* (BbCa [A], BbCT [B]) y *M. anisopliae* (Ma[C]) visualizadas con el uso del programa R v. 1.0.143 (22).

Bio-efficacy of entomopathogenic fungi and vegetable oils against *D. brevipes*

Mortality of adult *D. brevipes* females following treatment with isolate BbCa (at 1 x 10^8 conidia mL^{-1}) or *P. fumosoroseus* alone or in combination with vegetable oils varied significantly amongst treatments ($p < 0.0001$). Mean mortality in the untreated control was 5.5%. The combination BbCa + neem oil achieved the highest mortality, 98.4%, ($p < 0.0001$) (figure 4). Neem oil treatment alone caused 81.4% mortality. The conidial concentrations of the commercial formulation of *P. fumosoroseus* used in this experiment, caused only 55.1% mortality. However, when *P. fumosoroseus* was combined with neem oil mortality was 66.1%.

Cumulative mortality data also showed that the combination of isolate BbCa and neem oil was sufficient to achieve high efficacy by day 6 after inoculation, which shortened the time to kill by 100% compared with neem oil alone.

Bars with different letters are significantly different to each other (Tukey p ≤ 0.05). Error bars represent ±1 standard error of the mean (SEM), n= 4.

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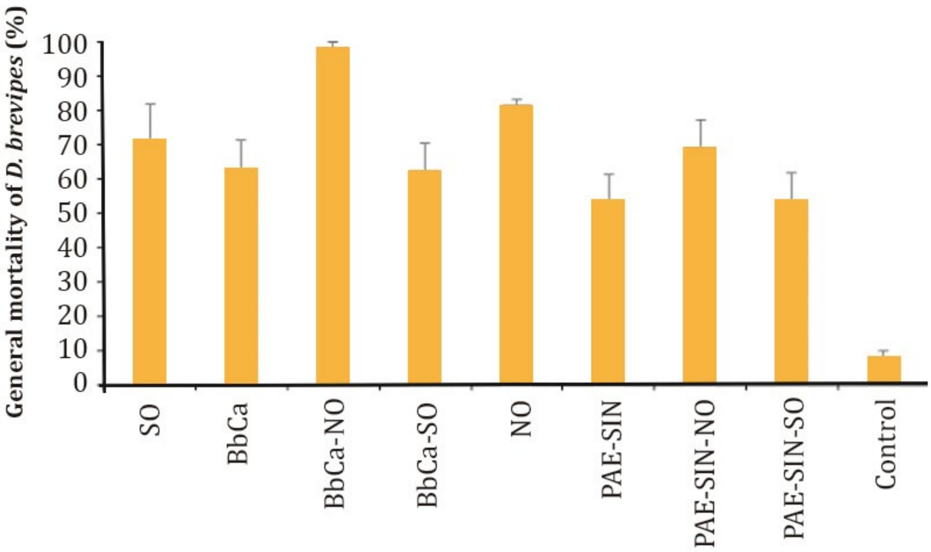

Figure 4. Overall mortality of *D. brevipes* after treatment with *B. bassiana* (isolate BbCa; 1 x 10^7 conidia mL^{-1}) or *P. fumosoroseus* at 1 x 10^7 conidia mL^{-1}(PAE-SIN®) either alone or in combination with soybean oil (SO) or neem oil (NO).

Figura 4. Mortalidad total de *D. brevipes* después de los tratamientos con *B. bassiana* (aislamiento BbCa; 1 x 10^7 conidios mL^{-1}) y *P. fumosoroseus* a 1 x 10^7 conidios mL^{-1} (PAE-SIN®) solos o en combinación con aceite de soya (SO) o aceite de neem (NO).

Discussion

In the present study isolates of both local native entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, were pathogenic. They caused mortality and variable mycosis in adult female *D. brevipes*. The BbCa isolate was significantly more virulent than the *M. anisopliae* isolate under laboratory conditions. *B. bassiana* isolates are widely used as biological control agents of a wide variety of insect pests, including sucking pests such as mealybugs (21, 26, 32). For example, previous research showed that two isolates of *B. bassiana* (FF and PPRC-56) caused 97% and 100% mortality in adults of the mealybug *Paraputo ensete* (Williams and Ferrero) (Hemiptera: Pseudococcidae) twenty days after inoculation (14). In another study, two isolates of *B. bassiana* (GAR 17 B3, GB AR 23133) caused 67.5% and 64% mortality in the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (5). Another isolate of *B. bassiana* caused 93% mortality in *Pseudococcus jackbeardsleyi* Gimpel & Miller (Hemiptera: Pseudococcidae) nymphs 5 days after treatment (10). The results of our research are in agreement with Mohamed (2016), who evaluated the virulence of *B. bassiana*, *M. anisopliae* and *Lecanicillium lecanii* isolates against adults of the mealybug, *Planococcus ficus* (Signoret) under laboratory conditions; they reported that virulence levels of *B. bassiana* were higher than those of *M. anisopliae* and *L. lecanii*, resulting in up to 98% mortality at a concentration of 5 x 10^8 mL^{-1} conidia. Moreover, Manjushree and Chellapan (2019) reported that isolates of *B. bassiana* caused higher mortality in *D. brevipes* (Cockerell) (Hemiptera: Pseudococcidae) at a concentration of 10^9 conidia mL^{-1} than isolates of *M. anisopliae* and *L. lecanii*. Like Surulivelu *et al.* (2012), Manjushree and Chellapan (2019) also reported that the same fungi were also effective against papaya mealybug *Paracoccus marginatus* (Williams) (Hemiptera: Pseudococcidae) under field conditions.

Concentration of inoculum (conidia) is a very important aspect of fungal pathogenicity and virulence. Results of our research suggest that isolate BbCa was the most virulent of the evaluated isolates with an LC_{50} of 3.4x10^7 conidia mL^{-1}, and that the higher the concentration of conidia the greater the mortality of *D. brevipes.*; overall 98% mortality was achieved 6 days after inoculation at 1 x10^7 conidia mL^{-1}. These results are consistent with other studies that report *B. bassiana* caused higher mortality of various mealybug species than *M. anisopliae* (11, 13). It has been reported that foliar applications of *V. lecanii* and *B. bassiana* (2×10^8 conidia mL^{-1}) in approximately 5 g/mL per L water is sufficient to reduce mealybug populations during months when the relative humidity is high (28). High effectiveness of *B. bassiana* and *P. fumosoroseus* against adult *D. brevipes* females was recorded from the 6^{th} day after inoculation. In a field-level study, Ugalde-Trejos (2010) found no differences in the efficacy of *B. bassiana*, *M. anisopliae*, *Trichoderma* spp., and *Bacillus thuringiensis* treatments against populations of *D. brevipes* infesting pineapple.

Results of the bio-efficacy assay showed that mortality of *D. brevipes* increased when *B. bassiana* (isolate BbCa) and neem oil were combined, making it possible to consider this treatment for future field trials. Fernández and Juncosa (2002) reported that the use of adjuvant oils improved effectiveness of entomopathogenic fungi. In the same way, Elósegui and Elizondo (2010) found that mixtures of entomopathogenic fungi and adjuvants increased efficacy and tolerance of the product to wider ranges of temperatures. Vásquez, (2000) evaluated *in vitro* effectiveness of *B. bassiana*, *M. anisopliae*, *Entomophthora*, soap, hydrated lime, garlic extract (*Allium sativum*) and neem extract for control of *D. brevipes* in an organic pineapple plantation, where the greatest efficacy was achieved with mixtures of entomopathogenic fungi and extracts of soap, garlic, neem, and hydrated lime. Results of the present study agree with Miranda Vindas & Blanco Getzler (2013), who found that botanical extracts were highly efficient in causing *D. brevipes* mortality under field conditions. Gopal *et al.* (2021), found that the maximum cumulative mortality of *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) was achieved when entomopathogenic fungi *B. bassiana* + *L. lecanii* (6 g/L + 6 g/L) were applied together rather than individually, resulting in 57.6% mortality, while neem and pongamia vegetable oils at 15 mL/L caused cumulative mortality of 81.4%, compared with the standard dose of neem oil (10 g/L) which caused 78.1% mortality. Our results showed that neem oil in combination with entomopathogenic fungi such as *B. bassiana* was the most efficient in killing adult female *D. brevipes* in greenhouse tests with up to 100% mortality by day 8 post-inoculation.

Conclusion

Both local isolates of *B. bassiana* and *M. anisopliae* were pathogenic to adult female *D. brevipes*. When *B. bassiana* and *P*. *fumosoroseus* were combined with neem oil under greenhouse conditions bio-efficiency increased by 20 % and 11%, respectively. *B. bassiana* (BbCa) combined with neem oil resulted in the highest mortality of *D. brevipes* reaching up to 100% by 8 days after inoculation.

We suggest that more research is needed to evaluate effectiveness of entomopathogenic fungi in combination with vegetable oils under field conditions. Design of biocontrol programs against pineapple mealybug is recommended in pineapple-growing regions of Mexico, as a strategy within integrated pest management programs. This option could reduce the use of toxic insecticides, which are harmful to the environment and human health.

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