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RESEARCH ARTICLE

Applying a saponin complex of Pseudalbizzia inundata (Mart.) Koenen & Duno as surfactants of Beauveria bassiana (Balsamo) Vuillemin conidia to control Bemisia tabaci Gennadius

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ABSTRACT

Biosurfactants secondary plant metabolites, such as saponins, could be an efficient green alternative dispersant for entomopathogenic fungi propagules. This work aimed to evaluate the potential of a saponins' complex extracted from Pseudalbizzia inundata (Mart.) Koenen & Duno (PSC) to disperse conidia of Beauveria bassiana IBCB66 (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) as also its' insecticidal activity against Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae). Despite a negligible effect on fungal germination and mycelial growth at the highest concentration of PSC (1,000 mg L-1), conidia production was significantly inhibited, resulting in a biological index of 52.03 at the higher dose, which characterises PSC as moderately toxic to B. bassiana. However, no effect was detected on the viability of conidia produced in media added with PSC. When applied directly on cowpea leaves, the saponins did not show an insecticidal effect on B. tabaci eggs and nymphs. PSC solution produced an adequate dispersion of B. bassiana IBCB66 conidia, causing nymph mortality higher than 90%, similar to Triton X-100, and adult mortality of 60% with a median survival time (ST50) of 8 days. A probit analysis of dose-effect of IBCB on both surfactants showed no difference between PSC solutions and Triton according to the parallelism test, with LC50 of 2.35 and 1.44 × 107 conidia mL-1 for PSC and Triton, respectively. These findings underscore the potential of PSC as a safe and effective substitute for synthetic surfactants to disperse the conidia of entomopathogenic fungi.

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Green additive; plant-based surfactant; entomopathogen

Introduction

Biological control of plant arthropod pests is increasingly popular worldwide, with Brazil standing out as a notable example (Fischer et al., 2023; Sabbahi et al., 2022). Among these methods, entomopathogenic fungi (EPF) are the most commonly used biocontrol agents

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(Fischer et al., 2023). The widespread use of EPF has been accompanied by technological advances such as formulations and adjuvants for tank mix preparation to improve wettability, surface adhesion, photoprotection, and prevent dehydration (Burges, 1998; Holka & Kowalska, 2023). Surfactants act as wetting agents, enhancing the dispersion of hydrophobic conidia and ensuring uniformity and adhesion to the target organism (Arnosti et al., 2019). The impact of surfactants on control efficacy can vary based on their composition and characteristics (Mwamburi et al., 2015). However, given the hazardous effects of chemical pesticides and adjuvants on human health and the environment, there is an urgent need for a better strategy to manage agricultural pests based on biopesticides and biological or green adjuvants (Dunlap et al., 2014; Gayathiri et al., 2022). Therefore, using plant-based surfactants is an eco-friendly alternative to synthetic surfactants due to their biodegradability and low risk of bioaccumulation (Dunlap et al., 2014; Tmáková et al., 2015).

Plants can produce bioactive secondary metabolites exhibiting surfactant activity, such as saponins and rhamnolipids (Khursheed et al., 2022; Tmáková et al., 2015). Saponins are natural surface-active glycosides with detergent properties, consisting of a steroid or triterpenoid aglycone linked to an oligosaccharide moiety (Rai et al., 2021). They can reduce water surface tension, making them a natural alternative for dispersing hydrophobic propagules of fungal-based biopesticides. Some saponins have shown insecticidal activity, attributed to their impact on the epidermis's waxy layer and integument and their ability to disrupt cell membranes (Cui et al., 2019; Rai et al., 2021). Their disruptive effect on the insect integument potentially facilitates fungal infection (Qasim et al., 2020).

Bemisia tabaci Gennadius, also known as whitefly, is a significant threat to various plant crops due to its short life cycle, fertility, and ability to spread rapidly. It can thrive on multiple host plants, maintaining a high population between crop seasons (Bevilaqua et al., 2023). *Bemisia* whitefly (BWF) causes severe crop damage by stunting growth, chlorosis, and transmitting several plant viruses, consequently reducing productivity (Abubakar et al., 2022). Although BWF infestations are typically controlled with synthetic insecticides such as neonicotinoids (Bevilaqua et al., 2023), the excessive use of these chemicals has led to the selection of resistant populations (Abubakar et al., 2022; Bevilaqua et al., 2023). Additionally, neonicotinoids have raised concerns about their impact on bees, resulting in their ban in some countries (Athanasios, 2018). Therefore, an efficient integrated management of BSF should include the application of entomopathogenic fungi (Li et al., 2024).

A study by Mascarin et al. (2013) showed that *B. bassiana* CG1229 and *Cordyceps* (*Isaria*) *fumosorosea* CG1228 resulted in mortality of eggs (30-60%), nymphs (71-86%), and adults (50-93%) of *B. tabaci*. Further research showed that the isolates were compatible with silicon-based surfactants, enhancing biocontrol activity (Mascarin et al., 2014). Recently, Boaventura et al. (2021) also showed that *Cordyceps* sp. strains were virulent to all developmental stages of *B. tabaci*. Additionally, do Nascimento Silva et al. (2019) showed that a rhamnolipid extracted from *Pseudomonas aeruginosa* PA1 significantly increased the survival of conidia of *B. bassiana* and *C. javanica* compared to a standard laboratory surfactant, resulting in significant mortality of *B. tabaci*.

Pseudalbizzia inundata (Mart.) Koenen & Duno is a Fabaceae with a pantropical distribution in South American countries, primarily found in seasonally dry biomes (Peraza et al., 2022). This species produces a complex of saponins, including oleanane-type

triterpene, acacic acid, echinocystic acid, oleanolic acid, and acacic acid-lactone saponins, with various bioactivities (Bahgat, 2015; Zhang et al., 2011). This study aimed to assess the surfactant potential of the saponins complex extracted and partially purified from P. *inundata* for dispersing conidia of *B. bassiana* IBCB66, which is known to be virulent to *B. tabaci* (Mascarin et al., 2013). Additionally, the study investigated the insecticidal potential of the saponins complex against eggs, nymphs, and adults of *B. tabaci*.

Material and methods

Saponins extraction and preparation

A saponin complex was extracted from *P. inundata* seeds using the method described by Zhang et al. (2011). Briefly, dried seeds were ground into powder in liquid nitrogen. The powder was extracted three times using CH_2Cl_2-MeOH (50:50), followed by evaporation of the solvents. After drying, the extract was stored at 10°C. The Pseud*albizzia* saponin complex (PSC) was dissolved in sterilised distilled water (ADE), obtaining a stock solution of 10,000 mg L^{-1}. The solution was centrifuged at 10,000 rpm for 10 minutes to remove particles and sterilised by filtration in a 0.22 µm sterile filter.

Bemisia tabaci rearing and manutention

B. tabaci whitefly (BWF) used in the experiments was obtained from a colony maintained by the Laboratory of Entomology of Embrapa Semiarid. The initial insect cohorts were collected from an area cultivated with yellow melons in the experimental field of Embrapa Semiarid. The insects were reared in cages kept in a greenhouse using cabbage (*Brassica oleracea* L. var. *acephala* DC) plants as hosts. The cabbage plants were grown in 5.0 L plastic pots filled with a substrate of soil, sand, and manure in equal proportions. The plants were kept in cages built with anti-aphid screens (200 × 120 × 80 cm). Periodic replacements of damaged plants were carried out to maintain optimal rearing conditions.

The cowpea plants *(Vigna unguiculata* (L.) Walp.) used in the bioassays were planted in 0.5 L pots containing the same substrate mentioned above. They were kept in a greenhouse with a temperature of 26.4 ± 4.56°C and an average relative humidity of 70.0 ± 12.3%. For the bioassays, leaflets were used and supported by plastic straws with petioles inserted in microtubes filled with distilled water.

Insecticide effect of *P. inundata* saponins against *B. tabaci*

In an experiment to test the toxicity of saponin against BWF (Black-eyed Wilt) eggs, cowpea plants with mature leaves were placed in rearing cages with adult insects to allow for egg-laying. After 24 hours, the number of eggs on the lower side of each leaflet was counted using a stereoscopic microscope. Each leaflet had between 15 and 35 eggs, resulting in approximately 145 per treatment. A similar process was used to collect insect nymphs. After egg-laying, the cowpea plants were moved to cages built with anti-aphid screens (60.0 × 40.0 × 80.0 cm), resulting in approximately 130 s-stage nymphs per treatment.

The cowpea leaflets with eggs or nymphs were removed from the plants and then sprayed on the underside with 0.50 mL of PSC solution with 200, 400, 800, 1,600, and 3,200 mg L^{-1} using a Potter's Tower with a pressure of 5.0 bar cm^{-2}. A control group was sprayed with sterilised distilled water. The leaf petioles were placed in assay tubes (4 mL) containing distilled water (Baldin et al., 2015). The treated leaflets were kept in growth chambers at 25.0 ± 1.0°C, 60.0 ± 10.0% relative air humidity, and a 12-hour photophase.

The number of BWF nymphs hatched between the fifth and twelfth day after the treatments were recorded. Nymph mortality was assessed daily between three and seven days after the treatments were applied. Nymphs that changed colour or dried out were considered dead (Boaventura et al., 2021). The experiment was conducted using a completely randomised design and repeated twice using different insect cohorts and freshly prepared saponin solutions.

Conidial dispersion in PSC solution

B. bassiana IBCB66 was cultivated in Sabouraud-dextrose agar with added yeast extract (SDY) (consisting of dextrose 5.0 g, meat peptone 2.0 g, yeast extract 2.0 g, agar 20.0 g, and water 1.0 L; pH 6.0). After fifteen days of inoculation, conidial suspensions in Triton X-100 0.01% (v/v) were harvested from the Petri dishes and then inoculated into parboiled rice with 40% water (v/w) content. The mixture was then incubated at 27.0 ± 2.0 °C, 60 ± 10% RH, and a 12-hour photophase. After maximal growth was achieved (14 days), the rice, along with fungal biomass, was partially dehydrated in a forced air chamber at 30 °C, and conidia were extracted using a Mycoharvester M5 (ACIS R&D, Devon, UK).

Subsequently, 10 milligrams of conidia were added to 50 mL Falcon conical tubes containing saponin solutions with concentrations of 0.0, 0.1, 1.0, 10.0, 100.0, and 1,000.0 mg L^{-1}, with a Triton X-100 0.01% (v/v) solution as a standard treatment. The tubes were vortexed for two minutes and left to rest. Samples of 10 µL were extracted from a 10.0 mm depth at 0, 15, 30, and 60 minutes after shaking. Conidia and conidia clusters were counted in a Neubauer chamber under an optical microscope (400× magnification). The experiment was conducted three times, with three replicates for each treatment and freshly prepared saponin solutions.

In vitro compatibility of PSC and *B. bassiana* IBCB66

A group of experiments was conducted to assess the impact of PSC on conidial germination, mycelial growth, conidial production, and germination of *B. bassiana* IBCB66. In the conidial germination experiment, a fresh conidial suspension was transferred to SDY media added with increasing concentrations (0.0, 0.1, 1.0, 10.0, 100.0, and 1,000 mg L^{-1}) of PSC (12 days incubation at 27.0 ± 2.0 °C and 12 h photophase) was standardised to 10^8 conidia mL^{-1} after counting in a Neubauer chamber. The conidial suspension was transferred to SDY, obtaining a final concentration of 10^5 conidia mL^{-1}.

The conidial germination assays were carried out in 96-well plates containing 100 µL of SDY mixed with the different PSC solutions and incubated at 27.0 ± 1.0 °C for 17 hours. Subsequently, ten microliters of a solution of methylene blue (20.0 g L^{-1} in

lactic acid 1.0 M) were added to stop the germination process. Conidia were considered germinated when the germination tube reached twice its diameter. The total and germinated conidia were counted using an optical microscope (400× magnification), with at least 300 conidia counted per treatment.

Mycelial growth and conidial production and viability

Sabouraud agar medium added with yeast extract (SDY) (dextrose 40 g L^{-1}, soybean peptone 10 g L^{-1}, yeast extract 10 g $L^{-1,}$ agar 20 g L^{-1}, pH 6,5) was mixed with the same concentrations of saponin as described above. Then, 10 µL of IBCB66 conidial suspension (10^8 conidia mL^1) was added to the media surface. The mycelial growth was assessed daily by measuring the colony diameter in perpendicular segments marked on the plates until the control treatment (SDY without saponin) reached the edge of the plates. After 12 days, a 0.5 cm Ø disk was taken from the centre of each plate and transferred to microtubes with 1.0 mL of 0.01% (v/v) Triton X-100, then vortexed for 60 s. Conidia in the suspension were counted in a Neubauer chamber using an optical microscope (at 400× magnification). Ten microliters of these suspensions were placed in SDY-A medium, incubated for 17 hours, and added with methylene blue plus lactic acid to evaluate conidia germination.

Toxicity of *B. bassiana* IBCB66 and *P. inundata* against *B. tabaci* eggs and nymphs

Two experiments were conducted to assess the impact of PSC, a surfactant of *B. bassiana* IBCB66, on the mortality of eggs, nymphs, and adults of *B.* tabaci in cowpea (*V. unguiculata*) plants. In the experiments, cowpea leaflets containing eggs or nymphs were placed in a polystyrene Petri dish with filter paper moistened with sterilised distilled water to keep the leaflets hydrated. A commercial wettable powder (WP) formulation of IBCB66 (Granada, Lalemand Brasil SA) was dispersed in either a saponin solution (1,000 mg L^{-1}) or Triton X-100 (0.01%) to achieve 10^4, 10^5, 10^6, 10^7, and 10^8 conidia mL^{-1}. The suspensions were sprayed onto the leaflets at 5 bar cm^{-2} pressure using a Potter tower (Burkard Scientific). The control group consisted of leaflets sprayed only with saponin solution (1,000 mg L-1), Triton X-100 (0.01%), or distilled water. After air-drying, the plates were transferred to an incubation chamber at 25 ± 1° C for further evaluation. Nymph eclosions were evaluated 5–8 days after spraying in the first experiment to assess the ovicidal effect of the treatments. In the second experiment evaluating the insecticidal effect of PSC on nymphs, they were observed for discolouration, dehydration symptoms, and fungal growth.

A similar experiment evaluated the mortality caused by IBCB66 in BWF adult insects using either saponin or Triton as surfactants. Cowpea leaves were sprayed with the conidial suspensions using a Potter tower and then placed in laboratory cages with 30 adult couples that were 24 hours old. Leaves in the control group were sprayed with distilled water. The cages were kept in a growth chamber at 25.0 ± 1.0°C, 70.0 ± 10.0% relative humidity, and a 12.0-hour photophase. Insect mortality was observed from 2 to 10 days after the treatment. The dead insects were collected and monitored for conidiogenesis to confirm mortality. The experiments were repeated twice using distinct insect

cohorts in a completely randomised design. Each cage was considered a replicate, resulting in six replicates per treatment.

Data analysis

Mycelial growth, conidia production, and germination data were converted to the proportion of the control treatment, arcsin transformed and submitted to one-way analysis of variance (ANOVA). Fungal compatibility with saponins was estimated using the biological index (BI) described in Rossi-Zalaf et al. (2008):

$$BI = [47VG + 43SP + 10G]/100$$

VG is the percentage of vegetative or mycelial growth, *G* is the percentage of conidial germination, and *SP* is the percentage of conidia production in a medium enriched with saponin as a proportion of the control treatment. Toxicity was classified using the scale: BI 0–41 – toxic; BI 42–66 – moderately toxic; BI > 66 – compatible.

All data sets were evaluated for normality and homogeneity of variance using Kolmogorov-Smirnof and Levene tests, respectively. Proportion and percentage data were transformed using $X' = arcsin(X/100)$. Mortality data from saponins dose-effect experiments using nymph and adult insects were corrected for natural mortality using the Schneider-Orelli formula (Püntener, 1992) for ANOVA ($p < 0{,}05$). Once determined the significance of the treatments by the F test, mortality data was applied in Probit analysis and control treatment data used to estimate natural mortality using SPSS v. 20 software (SPSS Inc., Chicago, IL, USA).

Data from the experiments evaluating the ovicidal potential of saponins and IBCB66 were analyzed using a generalised linear model (GLM) with binomial distribution. When a significant difference was detected, multiple comparisons (Tukey test; $p < 0.05$) were carried out using the function *glht multicomp* package, with adjustment of the *p* values (Cedney et al., 2021). GLM ANOVA, Tukey test, and Kaplan-Meier analysis were performed using R for Windows v. 4.3.1 (R Core Team, 2013) with the R-Studio interface (R Studio Team, 2019).

Data from the nymph and adult mortality experiments were corrected for natural mortality using the Schneider-Orelli formula (Püntener, 1992), and ANOVA ($p < 0{,}05$) was performed using SPSS v. 20 software (SPSS Inc., Chicago, IL, USA). Once determined the significance of the treatments by the F test, mortality data over time were applied to build a mortality curve, which was tested through success-failure analysis using the Kaplan-Meier procedure (Andersen & Vaeth, 2014) and compared through the log-rank and Mantel–Cox (M–C) test ($p < 0.05$) using GraphPad Prism v. 8.0.0 for Windows (GraphPad Software, San Diego, California, EUA).

Results

PSC insecticide activity

The application of saponin spray did not significantly affect the final egg-hatching percentage of BWF, as evidenced by the consistent number of nymphs ($p = 0.0975$) and the overall hatching rates over time ($p = 0.6898$). Despite the treatment, egg hatching

exceeded 90% over the 12-day evaluation period. Additionally, there was no observable impact on the survival of BWF nymphs, even when subjected to a concentration of 3.2 g L^{-1} of saponin ($p = 0.0509$). The survival rate remained above 80% seven days after being sprayed.

Saponin effect on mycelial growth and conidial production and germination

Increasing PSC concentrations in solid culture media showed a dose-dependent effect on IBCB66 conidial germination (F = 4.075; $p = 0.026$; df = 4; 12). However, only the 1.0 g L^{-1} concentration reduced conidial germination, reaching 86.89% (Figure 1). Similar dose-dependent results were observed in vegetative growth (F = 3.461; $p = 0.02$; df = 4; 20), with only the highest concentration reduced mycelial growth.

Adding saponin to the SDY medium with PSC significantly decreased conidia production (F = 7.451; $p < 0.01$; df = 4; 20). Even at the lower dose (1.0 mg L^{-1}), IBCB66 conidia production was 57.7% lower than in the control treatment (Figure 1). However, there was no significant impact on conidia germination extracted from colonies grown in the saponin-amended SDY medium (F = 0.508; $p = 0.767$; df = 4; 20). As a result, PSC concentrations higher than 100 mg L^{-1} exhibited a BI of 65.63, and the highest concentration (1.0 g L^{-1}) resulted in a biological index of 52.03, classifying it as moderately toxic to *B. bassiana* IBCB66.

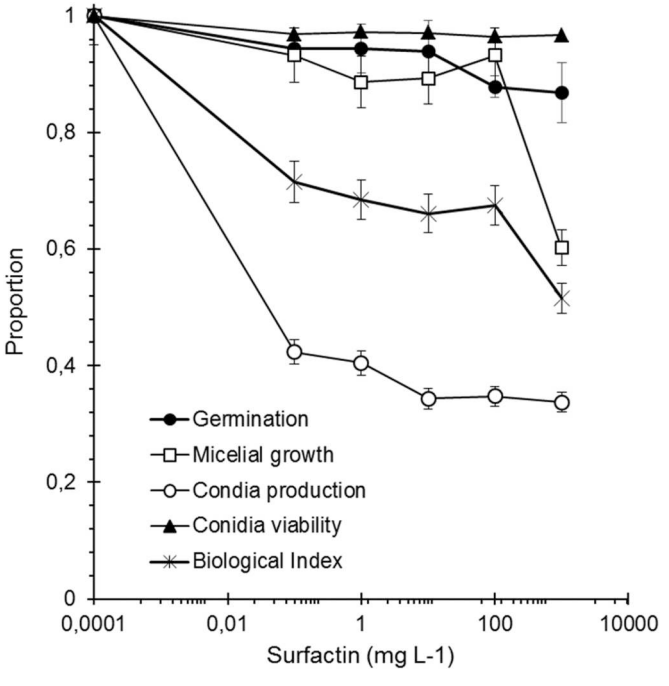

Figure 1. Conidial germination, mycelial growth, and conidial production in an SDY culture medium added with increasing concentrations of *P. inundata* saponin complex. Conidia viability (▲) refers to the germination of IBCB66 conidia produced in saponin-amended culture media. The biological index was represented as a proportion (BI/100). Results are presented as a proportion of the values obtained in the control treatment without saponin, and the results are the average (± SD) of two experiments.

Effect of PSC on conidial dispersion

The results from the experiment on conidial dispersion in different solutions are shown in Figure 2A. Figure 2B illustrates the ratio (proportional dispersion) of conidia counts in saponin and Triton X-100 at increasing concentrations of both surfactants. The saponin concentration significantly affected conidial dispersion over time ($F = 2.9735$; $p = 0.043$; $df = 12; 40$). The data was fitted to a multiple quadratic regression model (multiple $R^2 = 0.8665$; $F = 103.863$; $p < 0.001$; $df = 2; 69$), which showed that the highest dispersion occurred at the highest concentration in the experiment, with a slight sedimentation observed over time for all treatments. The results were comparable to those obtained using Triton X-100 as the standard treatment, as indicated by the flat plane obtained in linear multiple regression (multiple $R_2 = 0.7452$; $F_{2;\ 69} = 433.104$; $p < 0.001$) (Figure 2B). Based on these findings, the ratio between the conidial dispersion in PSC and the optimal concentration of Triton X-100 varied between 85% and 100%. Additionally, the ratio of aggregates observed in both treatments also conformed to a linear multiple regression model (multiple $R_2 = 0.5798$; $F_{2;\ 69} = 17.471$; $p < 0.001$), indicating that the lower number of aggregates was observed at the higher concentration of surfactants, and this number did not change over time (Figure 2C).

Insecticide efficiency of IBCB66 using PSC as surfactant

Egg and nymph mortality

Two experiments were conducted to assess the insecticidal activity of increasing concentrations of IBCB66 conidia using PSC and Triton X-100 at the same concentration (1.0 g L^{-1}) against eggs and second-stage nymphs of BWF. IBCB66 had a significant impact on egg hatching, which was dependent on the conidial doses ($F = 2.610$; $p = 0.028$; $df = 5$; 126), while the surfactants did not significantly affect the ovicidal activity of IBCB66 ($F = 1.017$; $p = 0.411$; $df = 5$; 126). The ovicidal activity for 10^8 conidia mL^{-1} of IBCB66

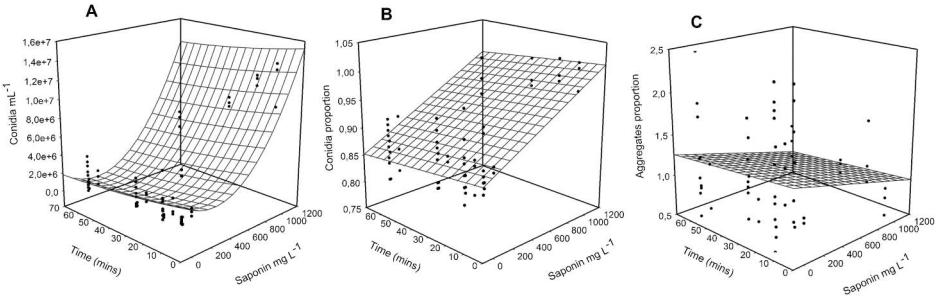

Figure 2. Dispersion of *B. bassiana* IBCB66 conidia in solution with different concentrations of *P. inundata* saponins complex over time (A), the proportion of conidial dispersion (B), and the proportion of conidia aggregates (C) as a ratio of the counts obtained in an equivalent concentration of Triton X-100. In the regression equation, D = conidial dispersion; D_p = ratio between conidial dispersion in saponin and Triton-X-100; A_p = ratio between aggregates number in saponin and Triton-X-100; T = time (minutes); Spn = saponins complex concentration. $D = 2{,}53 \times 10^6 - 2.77 \times 10^3 \times Spn - 1.81 \times 10^3 \times T + 13{,}86 \times Spn^2 - 30{,}97 \times Spn \times T + 214{,}5 \times T^2$ $D_P = 0.885 + 0.733Spn - 0.13T$ $A_P = 1.614 + 0.575Spn - 0.076T$.

dispersed in saponin was 37.28%, while it reached 36.58% when dispersed in Triton X-100.

There was a significant interaction between conidia doses and different surfactant concentrations on nymph mortality (F = 2,8771, $p = 0.021$, df = 5, 60), with mortality exceeding 90% at the highest concentration for both surfactants (Figure 3). The nymph mortality data fit the probit model based on the Pearson χ^2 test ($\chi^2 = 15.259$, $p = 0.99$, df = 69). The parallelism test in probit analysis showed no significant difference in the slope of nymph mortality between the surfactants in the dose–response experiment ($\chi^2 = 11.837$, $p = 0.175$, df = 1). The LC_{50} estimated for IBCB66 was 2.35 and 1.44×10^7 conidia mL^{-1}, and the LC_{90} was 6.99 and 6.08×10^7 conidia mL^{-1} for saponins and Triton X-100, respectively.

Effect of surfactants and IBCB6 on BWF adult mortality

The insecticidal activity of IBCB66 using different surfactants against BWF (Bean Weevil) adults was tested by spraying conidia dispersed in Triton or saponin solutions at the same concentration (0.10% w/v) on cowpea leaves. Anova of accumulated mortality data showed a significant effect of IBCB66 on insect survival (F = 13.4936; $p = 0.007$; df = 1; 43) but no effect of the surfactant or the interaction of these factors ($p > 0.05$). The Kaplan-Meier method showed a significant effect of the treatments on the insect mortality curve over time ($\chi^2 = 151.8$; df = 3; $p < 0.0001$). Treatments with only the surfactants did not significantly affect insect mortality (M–C test, $p < 0.05$), resulting in a survival rate of 86.0% and 83.0% for PSC and Triton X-100, respectively. There was a noticeable difference between the mortality curves of the control treatments and those

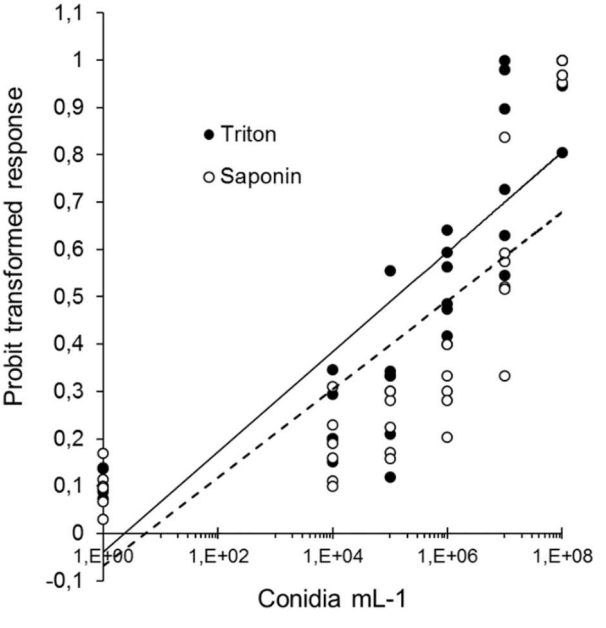

Figure 3. Mortality and probit transformed response of *Bemisia* whitefly nymphs treated with increasing concentrations of *B. bassiana* IBCB66 conidia using Triton X-100 or *A. inundata* saponin complex solutions as dispersant at the same concentration (1.0 g L^{-1}).

obtained when the biocontrol agent was applied with either surfactant (Figure 4). There was no significant difference among BWF mortality curves when Triton or saponin 0.01% were used as a dispersant for *B. bassiana* IBCB66 conidia according to the M–C test ($\chi^2 = 1.24$; df = 1; $p = 0.265$). The adult median survival time to IBCB66 (ST_{50}) was eight days when exposed to both PSC or Triton X-100 preparations.

Discussion

The study investigated the surfactant properties of a saponin complex extracted from P. *inundata* on *B. bassiana* IBCB66 conidia, compared to Triton X-100, a surfactant usually applied in laboratory conditions. The PSC solutions were applied to different developmental stages of BWF, and its compatibility with IBCB66 and potential insecticidal effects were also tested. The amphipathic nature of saponins allowed for the efficient extraction of a partially purified PSC using a moderately hydrophobic solvent mixture as the first step. The carbohydrate radical facilitated their solubilisation in water during the second extraction step, and a partially purified saponin complex from *P. inundata* seeds was extracted and standardised to a 10.0 g L^{-1} solution.

Although saponins have already shown insecticide activity when applied topically against the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* (Bagavan et al., 2008) and the Lepidoptera *Ectropis obliqua* Prout (Cui et al., 2019), the application of PSC solutions caused negligible mortality of *B. tabaci* in this work. Even the highest doses of PSC had no significant effect on egg hatching, nymph survival, or adult mortality.

Saponins are commonly classified as triterpenoids with an amphiphilic molecule structure with one hydrophilic and one hydrophobic fragment, typically a sugar

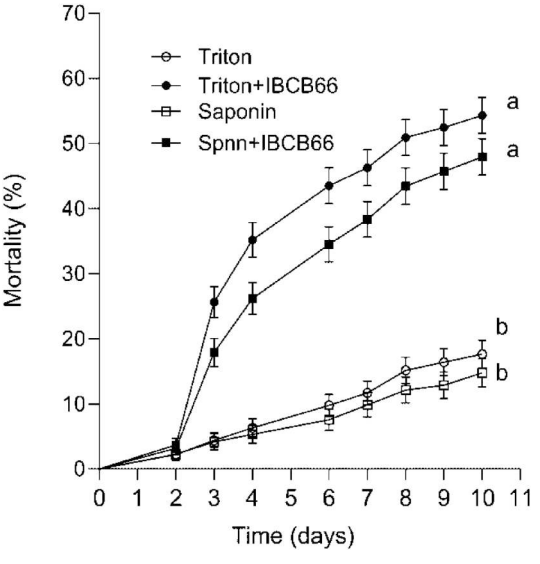

Figure 4. Mortality curve of *Bemisia* whitely adults treated with 10^8 conidia mL^{-1} of *B. bassiana* IBCB66 conidia using Triton X-100 or *A. inundata* saponin complex solutions as dispersant at the same concentration (1.0 g L^{-1}). Mortality of treatments showing the same letter at the end of the experiment did not differ by Tukey test ($p < 0.05$).

compound (Rai et al., 2021; Tmáková et al., 2015). As a result, saponins can act as a surfactant for hydrophobic conidia of EPF. They can efficiently disperse hydrophobic conidia by producing micelles by attaching the hydrophobic moieties on the conidial surface, while the hydrophilic sugar radical allows them to mix with water (Rai et al., 2021). Although PSC requires a higher concentration than Triton X-100, it reduced conidial wetting time and decreased the proportion of conidia aggregates after an initial stirring, resulting in the highest number of individual conidia in the suspensions. However, both surfactants showed slight conidia sedimentation after 60 minutes. Dunlap et al. (2014) did not report conidial sedimentation when they evaluated saponins from *Yucca schidigera* Roezl as surfactants for *Metarhizium brunneum* and *Cordyceps fumosorosea*. The observed sedimentation over time may have been caused by increased conidia individual weight due to the imbibition process, which is the first step for conidial germination (Mishra et al., 2015).

Only the highest concentrations of PSC significantly reduced the germination and growth of IBCB66 conidia. However, even at the lowest concentration, PSC reduced conidia production to around 40% of those in the control treatment. Nevertheless, it did not affect the viability of conidia produced in the medium with doses as high as 1,000 mg L^{-1} compared to the saponin-free medium. Previous reports have mentioned the harmful effects of saponins on conidial germination. Saponins extracted from tea (*Camellia sinensis* Kuntze) caused abnormalities in the germ tube production and cell wall thickening of the plant pathogen *Pestalotiopsis thea* (Speg.) Zhao & Li (Yang & Zhang, 2012). Similarly, a commercial surfactant formulation containing *Yucca schidigera* saponins reduced the germination of *B. bassiana* conidia by 34% (Dunlap et al., 2014).

Based on the biological index, PSC was categorised as moderately toxic to *B. bassiana* IBCB66. This classification was mainly due to the significant emphasis on conidia production in the formula used to calculate the biological index. However, it is crucial to consider the impact on conidial survival and germination when evaluating an additive for tank mix, given that germination is the initial stage of the host infection process. Additionally, mycelial growth occurs on the insect integument when it is still susceptible to a bioactive compound in the tank mix or during insect body colonisation when it is almost completely protected from external influences (Chandler, 2017). On the contrary, a detrimental effect on the production of conidia after exteriorisation would have a lesser impact on the pathosystem, as it is necessary for conidia dispersion and the establishment of natural epizooties or enzooties (Holka & Kowalska, 2023). Therefore, an index that places greater weight on the conidia germination and mycelial growth processes could enhance the assertion of the Biological Index regarding the impact of additives on formulations or tank mixes, as opposed to the efficiency of biocontrol agents.

The effectiveness of IBCB66 in controlling the different life stages of the black vine weevil (BWF) was examined using various surfactants. It was found that the ovicidal activity of IBCB66 was low when using PSC or Triton X-100 as surfactants (37.28% and 36.58%, respectively). However, nymph mortality was higher than 90% for both surfactants, with similar LC90 values. Additionally, adult mortality using 10^8 conidia mL^{-1} showed similar results for both surfactants, with an average survival time of 6–7 days. These findings suggest a significant potential for applying IBCB66 in the integrated management of BWF, as it can effectively target all life stages of the insect population.

Notably, a strain of entomopathogenic fungi (EPF) that is virulent against all insect life stages can potentially improve control efficiency in the field(Akutse et al., 2019; Samuels et al., 2002). Al Khoury et al. (2021) demonstrated low virulence of *B. bassiana* strains against eggs of the mite *Sarcoptes scabiei* (DeGeer), and only a few *M. anisopliae* isolates were found to be virulent to eggs of *Spodoptera frugiperda* (Smith) (Akutse et al., 2019). However, the authors noted that the larval stage of *S. frugiperda* was highly susceptible to EPF infection. The varying levels of susceptibility of different insect life stages to EPF infection suggest the existence of age specificity in the interaction between EPF and the host insect (da Silva et al., 2015).

The effective dispersal of conidia and the minimal impact on fungal development and IBCB66 virulence against BWF suggest that PSC could be an eco-friendly alternative to surfactants when applying conidia-based formulations of EPF. Although it demonstrates varying virulence against different life stages of BWF, *B. bassiana* IBCB66 has proven virulent to *B. tabaci*, as initially reported by Mascarin et al. (2013). Consequently, combining the reduction of egg hatching with the high mortality of nymphs would constrain the growth of the adult population, thereby eliminating the risk of its spread and virus transmission. Therefore, *B. bassiana* IBCB66 could become a crucial tool for the integrated management of BWF.

Disclosure statement

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References

Abubakar, M., Koul, B., Chandrashekar, K., Raut, A., & Yadav, D. (2022). Whitefly (Bemisia tabaci) management (WFM) strategies for sustainable agriculture: A review. *Agriculture (Switzerland)*, *12*(9), 1317.

Akutse, K. S., Kimemia, J. W., Ekesi, S., Khamis, F. M., Ombura, O. L., & Subramanian, S. (2019). Ovicidal effects of entomopathogenic fungal isolates on the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Applied Entomology*, *143*(6), 626–634. https://doi.org/10.1111/jen.12634

Al Khoury, C., Nemer, N., Bernigaud, C., Fischer, K., & Guillot, J. (2021). First evidence of the activity of an entomopathogenic fungus against the eggs of *Sarcoptes scabiei*. *Veterinary Parasitology*, *298*, 109553. https://doi.org/10.1016/j.vetpar.2021.109553

Andersen, P. K., & Vaeth, M. (2014). Survival analysis. In S. Kotz, C. B. Read, N. Balakrishnan, B. Vidakovic, & N. L. Johnson (Eds.), *Encyclopedia of statistical sciences* (2nd ed, pp. 1–14). John Wiley and Sons Inc.

Arnosti, A., Junior, I. D., Conceschi, M. R., D'Alessandro, C. P., Travaglini, R. V., & Camargo-Mathias, M. I. (2019). Interactions of adjuvants on adhesion and germination of *Isaria*

fumosorosea on adults of *Diaphorina citri*. *Scientia Agricola*, *76*(6), 487–493. https://doi.org/10.1590/1678-992x-2017-0240

Athanasios, V. (2018). Neonicotinoid insecticides. Banned by the European Union in 2018 after scientific studies concluded that harm honey bees. *Scientific Reviews*, *June*, 1–37.

Bagavan, A., Rahuman, A. A., Kamaraj, C., & Geetha, K. (2008). Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, *103*(1), 223–229. doi:10.1007/s00436-008-0962-z

Bahgat, D. S. A. (2015). Saponins from Genus Albizia: Phytochemical and biological review. *Medicinal & Aromatic Plants*, *s3*. https://doi.org/10.4172/2167-0412.s3-001.

Baldin, E. L. L., Aguiar, G. P., Fanela, T. L. M., Soares, M. C. E., Groppo, M., & Crotti, A. E. M. (2015). Bioactivity of Pelargonium graveolens essential oil and related monoterpenoids against sweet potato whitefly, Pelargonium graveolens biotype B. *Pelargonium Graveolens*, *88*(1), 191–199. https://doi.org/10.1007/s10340-014-0580-8.

Bevilaqua, J. G., Padilha, G., Pozebon, H., Marques, R. P., Cargnelutti Filho, A., Ramon, P. C., Boeni, L., Castilhos, L. B., da Luz, G. R., Brum, A. L. S., de, S., Biruel, N., Wojahn, B., Leão, J. D. J., & Arnemann, J. A. (2023). A sustainable approach to control whitefly on soybean: Integrating entomopathogenic fungi with insecticides. *Crop Protection*, *164*, 106145. https://doi.org/10.1016/J.CROPRO.2022.106145

Boaventura, H. A., Quintela, E. D., Santos, E. N., Silva, J. F. A., & Humber, R. A. (2021). Susceptibility of all nymphal stages of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) to three Brazilian isolates of *Cordyceps* sp. (Hypocreales: Cordycipitaceae) in a screenhouse under variable temperature and moisture conditions. *Neotropical Entomology*, *50*(1), 100–113. https://doi.org/10.1007/s13744-020-00823-5

Burges, H. D. (1998). *Formulation of microbial biopesticides: Beneficial microorganisms, nematodes and seed treatments*. Springer Netherlands.

Cedney, J.-B. M., de Brida, A. L., Bernardi, D., da Costa Dias, S., de Bastos Pazini, J., Leite, L. G., Wilcken, S. R. S., & Garcia, F. R. M. (2021). Effectiveness of entomopathogenic nematodes against Ceratitis capitata (Diptera: Tephritidae) pupae and nematode compatibility with chemical insecticides. *Journal of Economic Entomology*, *114*(1), 248–256. https://doi.org/10.1093/jee/toaa301

Chandler, D. (2017). Basic and applied research on entomopathogenic fungi. In L. A. Lacey (Ed.), *Microbial control of insect and mite pests: From theory to practice* (pp. 69–89). Academic Press.

Cui, C., Yang, Y., Zhao, T., Zou, K., Peng, C., Cai, H., Wan, X., & Hou, R. (2019). Insecticidal activity and insecticidal mechanism of total saponins from *Camellia oleifera*. *Molecules*, *24*(24), 4518. https://doi.org/10.3390/molecules24244518

da Silva, R. A., Quintela, E. D., Mascarin, G. M., Pedrini, N., Lião, L. M., & Ferri, P. H. (2015). Unveiling chemical defense in the rice stalk stink bug against the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, *127*, 93–100. https://doi.org/10.1016/J.JIP.2015.03.009

do Nascimento Silva, J., Mascarin, G. M., de Paula Vieira de Castro, R., Castilho, L. R., & Freire, D. M. (2019). Novel combination of a biosurfactant with entomopathogenic fungi enhances efficacy against *Bemisia* whitefly. *Pest Management Science*, *75*(11), 2882–2891. https://doi.org/10.1002/ps.5458

Dunlap, C. A., Behle, R. W., & Jackson, M. A. (2014). Compatibility of OMRI-certified surfactants with three entomopathogenic fungi. *Biocontrol Science and Technology*, *24*(4), 436–447. https://doi.org/10.1080/09583157.2013.870532

Fischer, H., Marcondes de Almeida, J. E., Limberger, C., Harakava, R., Sato, M. E., Costa, V. A., Schoeninger, K., Leite, L. G., Chacon-Orozco, J. G., & Baldo, F. B. (2023). The rise of bioinputs in the Brazilian agri-industry: Trends, cases and hurdles. *Outlooks on Pest Management*, *34*(3), 106–118. https://doi.org/10.1564/V34_JUN_04

Gayathiri, E., Prakash, P., Karmegam, N., Varjani, S., Awasthi, M. K., & Ravindran, B. (2022). Biosurfactants: Potential and eco-friendly material for sustainable agriculture and rnvironmental safety – a review. *Agronomy*, *12*(3), 662. https://doi.org/10.3390/agronomy12030662

Holka, M., & Kowalska, J. (2023). The potential of adjuvants used with microbiological control of insect pests with emphasis on organic farming. *Agriculture*, *13*(9), 1659. https://doi.org/10.3390/AGRICULTURE13091659

Khursheed, A., Rather, M. A., Jain, V., Wani, A. R., Rasool, S., Nazir, R., Malik, N. A., & Majid, S. A. (2022). Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. *Microbial Pathogenesis*, *173*, 105854. https://doi.org/10.1016/j.micpath.2022.105854.

Li, Y., Mbata, G. N., Simmons, A. M., Shapiro-Ilan, D. I., & Wu, S. (2024). Management of *Bemisia tabaci* on vegetable crops using entomopathogens. *Crop Protection*, *180*, 106638. https://doi.org/10.1016/j.cropro.2024.106638

Mascarin, G. M., Kobori, N. N., Quintela, E. D., Arthurs, S. P., & Júnior, ÍD. (2014). Toxicity of non-ionic surfactants and interactions with fungal entomopathogens toward *Bemisia tabaci* biotype B. *BioControl*, *59*(1), 111–123. https://doi.org/10.1007/s10526-013-9543-x

Mascarin, G. M., Kobori, N. N., Quintela, E. D., & Delalibera, I. (2013). The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. *Biological Control*, *66*(3), 209–218. https://doi.org/10.1016/j.biocontrol.2013.05.001

Mishra, S., Kumar, P., & Malik, A. (2015). Effect of temperature and humidity on pathogenicity of native *Beauveria bassiana* isolate against *Musca domestica* L. *Journal of Parasitic Diseases*, *39*(4), 697–704. https://doi.org/10.1007/s12639-013-0408-0

Mwamburi, L. A., Laing, M. D., Miller, R. M., Mwamburi, L. A., Laing, M. D., & Miller, R. M. (2015). Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*. *Brazilian Journal of Microbiology*, *46*(1), 67–74. https://doi.org/10.1590/S1517-838246120131077

Peraza, G. A., Koenen, E. J. M., Riina, R., Hughes, C. E., Ringelberg, J. J., Carnevali Fernández-Concha, G., Mercedes, I., Morillo, R., Can Itza, L. L., Tamayo-Cen, I., Humberto, J., Prado, R., Cornejo, X., Mattapha, S., Duno De Stefano, R., Duno, R., & Stefano, D. (2022). Re-establishment of the genus *Pseudalbizzia* (Leguminosae, Caesalpinioideae, Mimosoid clade): The New World species formerly placed in *Albizia*. *Phytokeys*, *205*, 317–400. https://doi.org/10.3897/phytokeys.205.76821

Püntener, W. (1992). *Manual for field trials in plant protection*. Ciba-Geigy.

Qasim, M., Islam, W., Ashraf, H. J., Ali, I, & Wang, L. (2020). Saponins in insect pest control. In J.-M. Mérillon & K. G. Ramawat (Eds.), *Co-evolution of secondary metabolites - Reference series in phytochemistry* (pp. 898–924). Springer. https://doi.org/10.1007/978-3-319-76887-8_39-1.

Rai, S., Acharya-Siwakoti, E., Kafle, A., Devkota, H. P., & Bhattarai, A. (2021). Plant-derived saponins: A review of their surfactant properties and applications. *Sci*, *3*(4), 44. https://doi.org/10.3390/sci3040044

R Core Team. (2013). *R: A language and environment for statistical computing*. https://www.r-project.org/

Rossi-Zalaf, L. S., Alves, S. B., Lopes, R. B., Silveira Neto, S., & Tanzini, M. R. (2008). Interação de microorganismo com outros agentes de controle de pragas e doenças = Microorganism interaction with other pest and disease control agents. In S. B. Alves, & R. B. Lopes (Eds.), *Controle microbiano de pragas na América Latina: avanços e desafios = Microbial control of pest in Latin America: Advances and challenges* (pp. 279302). Fealq.

R Studio Team. (2019). *RStudio: Integrated development environment for R*. http://www.rstudio.com/

Sabbahi, R., Hock, V., Azzaoui, K., Saoiabi, S., & Hammouti, B. (2022). A global perspective of entomopathogens as microbial biocontrol agents of insect pests. *Journal of Agriculture and Food Research*, *10*, 100376. https://doi.org/10.1016/j.jafr.2022.100376

Samuels, R. I., Coracini, D. L. A., dos Santos, C. M., & Gava, C. A. T. (2002). Infection of *Blissus antillus* (Hemiptera: Lygaeidae) eggs by the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. *Biological Control*, *23*(3), 269–273. https://doi.org/10.1006/bcon.2001.1009

Tmáková, L., Sekretár, S., & Schmidt, Š. (2015). Plant-derived surfactants as an alternative to synthetic surfactants: Surface and antioxidant activities. *Chemical Papers*, *70*(2), 188–196. https://doi.org/10.1515/chempap-2015-0200

Yang, X., & Zhang, H. (2012). Synergistic interaction of tea saponin with mancozeb against *Pestalotiopsis theae*. *Crop Protection*, *40*, 126–131. https://doi.org/10.1016/J.CROPRO.2012.04.013

Zhang, H., Samadi, A. K., Rao, K. V., Cohen, M. S., & Timmermann, B. N. (2011). Cytotoxic oleanane-type saponins from *Albizia inundata*. *Journal of Natural Products*, *74*(3), 477–482. https://doi.org/10.1021/np100702p