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ORIGINAL

Evaluation of the effectiveness of an entomopathogenic consortium for the control of mealybug (Planococcus citri) located on the María Auxiliadora campus of the Salesian Polytechnic University

Evaluación de la eficacia de un consorcio entomopatógeno para el control de cochinilla harinosa (Planococcus citri) ubicada en el campus María Auxiliadora de la Universidad Politécnica Salesiana

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ABSTRACT

Introduction: in the city of Guayaquil, the mealybug pest poses a threat to various plant species. Biological control strategies, such as the use of one of its natural predators, ladybugs, are also possible, as are other solutions such as the use of entomopathogenic fungi.
Objective: to evaluate the efficacy of an entomopathogenic consortium consisting of the species *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, and *Purpureocillium lilacinum* against the mealybug species Planococcus citri in lemon plants.
Method: the experimental strategy was carried out on the grounds of the Salesian Polytechnic University, María Auxiliadora campus. Lemon trees were infected with *Planococcus citri* for 4 to 6 months, two weeks before the application of the treatments. The 7 treatments, except for the application control, consisted of 3 different concentrations (200 %, 100 %, and 50 %) of the positive control (chemical detergent) and the entomopathogenic consortium.
Results: the mortality rate of scale insects and the efficacy of the consortium were analyzed. Treatment 3 (100 %) produced the highest mortality rate; while treatment 2, the positive control, and treatment 3, the entomopathogenic consortium, showed no significant differences in efficacy. In addition, chlorophyll was analyzed before and after treatment; treatment 3 showed significant differences compared to the positive control treatments.
Conclusions: the entomopathogenic fungal consortium proved to be an effective alternative to chemical insecticides for controlling Planococcus citri, as higher mortality of the pest and a significant population reduction were observed with the use of this treatment compared to the negative and positive controls.

Keywords: Entomopathogenic Consortium; Planoccocus Citri; Biological Control; Invertebrates; Bioinsecticide.

RESUMEN

Introducción: en la ciudad de Guayaquil, la plaga de la cochinilla supone una amenaza para diferentes especies de plantas. Estrategias de tipo control biológico como el uso de uno de sus depredadores naturales, las mariquitas, es posible utilizar otras soluciones como el uso de hongos entomopatógenos.
Objetivo: evaluar la eficacia de un consorcio entomopatógenos conformado por las especies *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* y *Purpureocillium lilacinum* contra la especie de cochinillas *Planococcus citri* en plantas de limón.

Método: la estrategia experimental fue llevada a cabo en los predios de la Universidad Politécnica Salesiana, campus María Auxiliadora. Se infectaron *Planococcus citri* en los limoneros de entre 4 a 6 a meses, dos semanas antes de la aplicación de los tratamientos. Los 7 tratamientos, excepto el control de aplicación, consistieron en 3 diferentes concentraciones (200 %, 100 % y 50 %) del control positivo (detergente químico) y el consorcio entomopatógeno.
Resultados: se analizó el índice de mortalidad de cochinillas y la eficacia del consorcio. El tratamiento 3 (100 %) produjo la mayor tasa de mortalidad; mientras que el tratamiento 2, del control positivo, y el tratamiento 3, del consorcio entomopatógeno, no mostraron diferencias significativas en cuanto eficacia. Además, se analizó clorofila antes y después del tratamiento, el tratamiento 3 mostró diferencias significativas contra los tratamientos del control positivo.
Conclusiones: el consorcio de hongos entomopatógeno demostró ser una alternativa eficaz al insecticida químico para el control de *Planococcus citri*, debido a que se observó una mayor mortalidad de la plaga y una reducción significativa de la población con el uso del tratamiento frente al control negativo y el control positivo.

Palabras clave: Consorcio Entomopatógeno; *Planoccocus Citri*; Control Biológico; Invertebrados; Bioinsecticida.

INTRODUCTION

The citrus mealybug, *Planococcus citri,* is a globally recognized sap-sucking insect that originated in Asia but has spread worldwide.(1) It feeds on a wide variety of ornamental plants, fruit trees, and even citrus trees, hence its name.(2) This parasite excretes a sticky substance called honeydew, which attracts other organisms that feed on it, such as ants.(1,3,4)

In Guayaquil, Ecuador, in 2020, the first outbreaks of the pest were reported by the city council, creating a significant problem in recent years. The cochineal has spread aggressively, extending to a wide variety of plants in the city.(5) These insects are present in parks, homes, forests, and educational centers, affecting all green areas in different parts of the city. One of the affected areas is the María Auxiliadora campus of the Salesian Polytechnic University, where green spaces cover more than 50 % of its area. The damage caused hurts the growth of leafy plant species, also affecting their overall development.

Entomopathogenic fungi (EPFs) infect the vast majority of arthropods, including insects. They are natural rivals capable of wiping out entire populations of particular species belonging to that phylum.(6,7) Therefore, they are an integral strategy for the sustainable control of these organisms.(8) Because of their pathogenic capacity, they are used as a measure of pest biocontrol. These EPPs can be applied in combination with other fungal species, forming a consortium.

Among the most renowned consortia are *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecani, and Purpureocillium lilacinum*, which can work synergistically to grow and reproduce as *opportunistic* pathogens.

Insects become susceptible to these pathogens due to the infection caused by spores, which attach themselves to the outer surface of the organism's body and begin to germinate, decomposing the insect from within and causing its death.(9) Hyphae emerge from the lifeless body of the host and can remain in the upper tissues of the arthropod, where they sporulate again when environmental conditions are suitable.(10)

Due to their mechanisms of action, HEPs are emerging as a sustainable alternative for insect control, offering an advantage over chemical insecticides, as these arthropods have shown increasing resistance to them.(2,11,12) The objective of this study was to determine the effectiveness of a consortium of HEPs on mealybugs (*Planococcus citri*) located at the Salesian Polytechnic University's "María Auxiliadora" campus.

Justification

Plants are of fundamental importance because they help improve air quality by producing oxygen and mitigating climate change by acting as carbon sinks. Similarly, trees are vitally important because they stabilize the soil and prevent landslides. These are the main reasons for conserving these organisms.

The municipality, which is responsible for the maintenance and conservation of green areas in the city of Guayaquil, has sought solutions to combat the cochineal infestation in these public spaces. It has implemented a series of measures, such as releasing ladybugs and other natural predators of these insects, as well as cutting foliage, to reduce the infestation. The measures described above have had some success, but the cochineal infestation persists. Therefore, it is necessary to try other actions to combat this infestation in private spaces, such as the María Auxiliadora campus.

Green areas cover the María Auxiliadora campus of the Salesian Polytechnic University, and mealybugs infest a large percentage of these areas. Among the species identified by the authors of this study are *Planococcus citri, Orthezia sp.*, which is abundant in the university's citrus trees, and *Pseudococcus longispinus*.

The damage caused by Pseudococcidae hurts plant foliage because these insects suck the sap, not only weakening the plants but also maximizing the damage, leading to their death. This is because sap contains all the elements that plants need to grow, reproduce, and survive.[13,14,15] Due to their feeding habits, they excrete honeydew, which attracts ants, tending to aggravate the problem since ants are responsible for mobilizing scale insects, causing greater proliferation of the pest.

Although this study focuses on *Planococcus citri*, there is a wide variety of Pseudococcidae species that are also affecting the city and the María Auxiliadora campus. Still, the species covered in this study is more relevant because it is more resistant to insecticides, making it the perfect organism for testing pesticide efficacy.[16]

Planococcus citri shows greater resistance to insecticides and is used in Korea to evaluate the effectiveness of insecticides.[16] It will be an alternative to other species of mealybugs that affect the María Auxiliadora campus of the Salesian Polytechnic University and the city of Guayaquil.

The applicability of HEPs for scale insect control on the María Auxiliadora campus is a promising alternative. This is because it reduces the use of chemical insecticides, which can have adverse effects on the environment and human health. In addition, chemical insecticides often generate resistance. In contrast, biocontrol agents such as HEPs have a more significant impact due to their mechanisms of action, whereby insects become susceptible to infection when the spores attach to the outer surface of their bodies. Once these spores detect signals in the cuticle, the pathogen begins to germinate. During this process, a variety of enzymes are secreted that aim to break down the insect's exoskeleton, also known as its cuticle.

The combined action of these enzymes and the mechanical pressure generated by the germ tubes results in the rupture of the cuticle. Blastospores are produced and spread inside the insect, causing its death. Upon death, the fungal hyphae emerge and can remain in the upper tissues of the body, where they can sporulate again.[9] All these characteristics allow fungal consortia to be used as biocontrol measures against cochineal.

METHOD

Study site and geographical location

This research was evaluated under controlled conditions on the premises of the Salesian Polytechnic University in Guayaquil.

Sampling

Samples were obtained from trees on the university campus. Mealybugs were collected for identification and subsequent application of treatments.

Coccinella collection

To collect the entomological material, random sampling was carried out throughout the campus to identify scale insects with characteristics similar to *Planococcus citri*. To facilitate collection, a brush was used and the specimens were placed in a container for subsequent analysis.

Identification of Planococcus citri

A stereomicroscope was used to make a comparison with the taxonomic keys corresponding to the specimen based on the morphological characterization proposed by a study.

Collection of Planococcus citri

Once *Planococcus citri* was identified using a stereomicroscope, targeted sampling was carried out by selecting the areas that had been previously analyzed in which the arthropod was present. The plants with the largest pest populations were identified to facilitate the identification of their nests and the collection of specimens. This collection process was carried out over approximately one week.

Infection

To begin the infection process, the scale insects were viewed with a stereomicroscope to identify the species *(Planococcus citri)* and select live scale insects. The methodology of Buena et al.[19] was used, which recommends using gauze to apply the scale insects to the plant. They were separated into groups of 20 per gauze pad, with a total of three gauze pads per plant, resulting in a total of 60 mealybugs per plant. The infection process was completed in approximately three days.

Acclimation

Once the *Citrus × aurantiifolia* trees were infected with *Planococcus citri, we* waited two weeks for the insect to acclimatize to the new host plant, controlling the conditions to ensure the success of the acclimatization process.

Factorial design for evaluating the effectiveness of the entomopathogenic consortium on *Planococcus citri*

For the research, a complete factorial design with 3 factors (table 1) and 3 levels in treatments (table 2) and treatment concentration was used, with 1 level for the negative control, allowing all effects and interactions to be evaluated.

Factor 1: mortality: *Planococcus citri* (dependent variable)

Factor 2: treatments: T1, T2, and T3

Factor 3: treatment concentration: C1, C2, and C3

Table 1. Factor 2 Treatments

Treatment	Components
T1 (control)	Water
T2 (positive control)	Chemical insecticide (potassium detergent)
T3 (experimental unit)	Consortium (Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, and Purpureocillium lilacinum).

Table 2. Factor 3 Treatments

Factor 3: Concentration		T2	T3
C1	200 %	30,22 p/v	2,0x109 conidia/ml
C2	100 %	15,11 p/v	1,0x109 conidia/ml
C3	50 %	7,55 p/v	5,0x108 conidia/ml
Note: the concentration in T1 corresponding to the negative control is unique			

Factor 1 presents one level of mortality, factor 2 presents three levels for the three treatments, and factor 3 presents seven levels for the seven different concentrations, which is why 21 *Citrus* × *aurantiifolia* lemon plants between 4 and 6 months old are required for the application of the treatments in the experiment.

Application of treatments

For the application of the treatments to the plants, full protective equipment was used, including gloves, boots, mask, and goggles, to avoid any direct contact with the product. In addition, the application was carried out in an area with optimal ventilation to prevent cross-contamination (figure 1). After application, we waited one hour before returning the plants to the shelters built for their protection.

For the experiment, shelters were built to protect the plants from predators and external agents that could affect the sample. During this period, the plants were kept under controlled temperature conditions (24 °C) without direct light.

Figure 1. Application of treatments

Methodology for counting *Planococcus citri* mortality

Once the treatments were applied, mortality results were taken at 5, 10, and 15 days. *Planococcus citri* was counted manually; one leaf was selected from each plant at random for sampling and then observed under a stereomicroscope. The entire surface of the leaf was examined, including the front, back, and edge. The scale insects were divided into two groups, live and dead. The morphological criteria for classification were that live scale insects must be mobile with a soft body, while dead scale insects must be immobile with a rigid body.

Statistical analysis

A statistical evaluation of the treatments was performed by calculating the percentage of pest mortality using the formula mentioned. Percentage of mortality of the cochineals:

%M=(N° de insectos muertos)/(Total de insectos)×100

Efficacy was calculated in relation to the variable number of dead individuals (mortality) in a homogeneous population, for which it was decided to use the formula described by Schneider-Orelli (1947). Percentage of efficacy of the entomopathogenic consortium:

$$\%E = \%\, de\, mort.\, en\, muestra\, tratada - \%\, de\, mort.\, en\, muestra\, testigo\; 100 - \%\, de\, mort.\, en\, /\, muestra\, testigo\; (100)$$

Where:
%E= Percentage of efficacy.
%mort. in treated sample (Percentage of mortality of T3)
%mort. in control sample (Percentage of mortality of T1 or T2)

Analysis of variance (ANOVA)

IBM SPSS Statistics version 25 statistical software was used to perform the homogeneity, normality, and one-way ANOVA tests on the mortality data, and Google spreadsheets were used for the graphs.

Analysis of variance (ANOVA) is a fundamental statistical tool in biological research, as it allows the influence of more than two independent factors on a variable of interest to be compared.(17) In this research, the variable to be analyzed is mortality.

To perform the one-way ANOVA test, three requirements must be met. First, there must be more than two independent variables. Second, they must follow a normal distribution in each of the groups. The test to be used depends on the sample size. If the sample is less than 50, as in our case, the Shapiro test is used.- Wilk test is used. Third, there must be homogeneity of variances in each group, which can be confirmed with the Levene test.(17)

When interpreting the ANOVA test, the p-value must be known to determine whether it is less than 0,05 and accept the alternative hypothesis that there are significant differences between treatments. If it is greater than 0,05, the null hypothesis is accepted, demonstrating that there are no significant differences in the treatments.(17)

Chlorophyll

To measure chlorophyll, AMTAST digital equipment was used, which is a meter for the relative chlorophyll content in leaves (SPAD unit) with a chlorophyll measurement range of 0,0-99,9 SPAD. It is susceptible to a measurement time interval of less than 3 seconds.

Chlorophyll measurements were taken for all plants before and after the treatments were applied to check for significant differences in chlorophyll in any of the treatments.

The average of each of the values obtained by the chlorophyll meter in each of the treatments with their respective concentrations was calculated, and the normality and homogeneity tests mentioned above were applied to determine whether there were significant differences in chlorophyll in the treatments.

As the homogeneity requirement was not met, the one-way ANOVA test could not be performed, so Welch's ANOVA had to be applied, as it is not affected by unequal variances. For this analysis, Tukey's post hoc test was performed to compare the means and identify significant differences between the treatments.

The IBM SPSS Statistics version 25 program was used to perform the normality and homogeneity test for chlorophyll, and the *pingouin* library *(0.5.4)* of *Python* version (3.10) was used to apply Welch's ANOVA and Tukey's post hoc test.

RESULTS

Results of the coupling with *Planococcus citri* in *Citrus × aurantiifolia* lemons

The process of coupling the scale insects to the lemon plants lasted two weeks. After that period, several oothecae were observed on all plants, and molasses was also found, indicating that the population was active and established (figure 2).

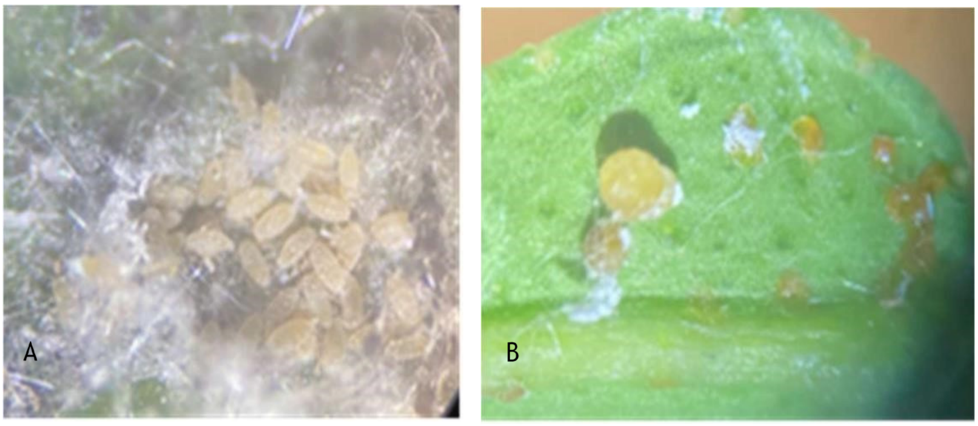

Figure 2. Scale insect infestation on the lemon plant; (A) Presence of nymphs and oothecae of Planococcus citri on the Citrus × aurantiifolia lemon plant. (B) Honeydew found on the leaves of the Citrus × aurantiifolia lemon plant

Mortality of *Planococcus citri* mealybugs

Treatment 3, corresponding to the entomopathogenic consortium composed of the species *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, and Purpureocillium lilacinum at* a concentration of 2,0×10^9 conidia/ml shows a total average mortality rate of 47,80 % over the 15 days, reaching its highest point on day 10 with a mortality rate of 86 %, as shown in figure 3.

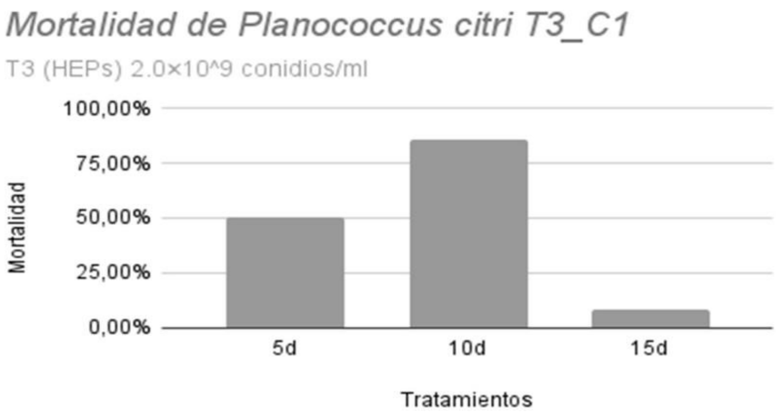

Figure 3. Mortality of *Planococcus citri* in treatment T3 (HEPs) at a concentration of 2,0×109 conidia/ml on days 5, 10, and 15

The experimental unit at a concentration of 1,0×109 conidia/ml in treatment 3 obtained an average mortality rate of 84 %, with the highest percentage obtained on day 10 with 96 %, as shown in figure 4.

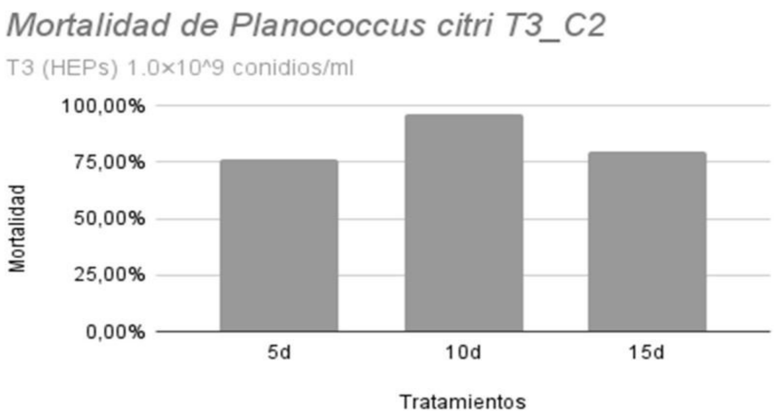

Figure 4. Mortality of Planococcus citri in treatment T3 (HEPs) at a concentration of 1,0 × 109/ml on days 5, 10, and 15

T3 at a concentration of 5,0 × 10^8 conidia/ml reached its maximum mortality on day 15 with 80 % and an overall average for all days of 71 %, as shown in figure 5.

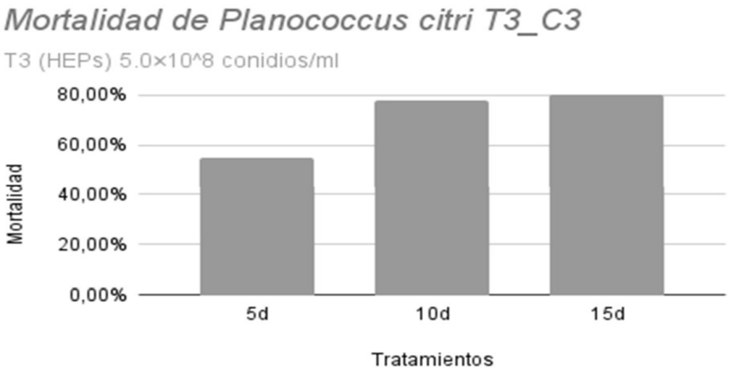

Figure 5. Mortality of Planococcus citri in treatment T3 (HEPs) at a concentration of 5,0 × 108 conidia/ml on days 5, 10, and 15

The highest mortality percentage was produced by treatment 3 with a concentration of 1,0x109 conidia/ml, reaching an individual average of 84 %, followed by the concentration 5,0x108 conidia/ml with 71 %; while the lowest percentage of treatment 3 was presented by the concentration 2,0x109 with an average of 47,80 %.

Analysis of the results corresponding to the mortality rate of *Planococcus citri when* evaluating treatment 3 at different concentrations of the experimental units over a period of 15 days reached a total average of 67,63 %, unlike treatment 1, which showed a mortality rate of 2,5 %.

Estimation of the efficacy of the entomopathogenic consortium treatments.

According to the formula described by Schneider Orelli for calculating the percentage efficacy of a treatment that follows a normal distribution, the percentage values of the efficacy of each of the T3 (HEPs) treatments in their three concentrations 2,0×109 conidia/ml, 1,0×109 conidia/ml, and 5,0×108 conidia/ml could be obtained, as described in figure 6.

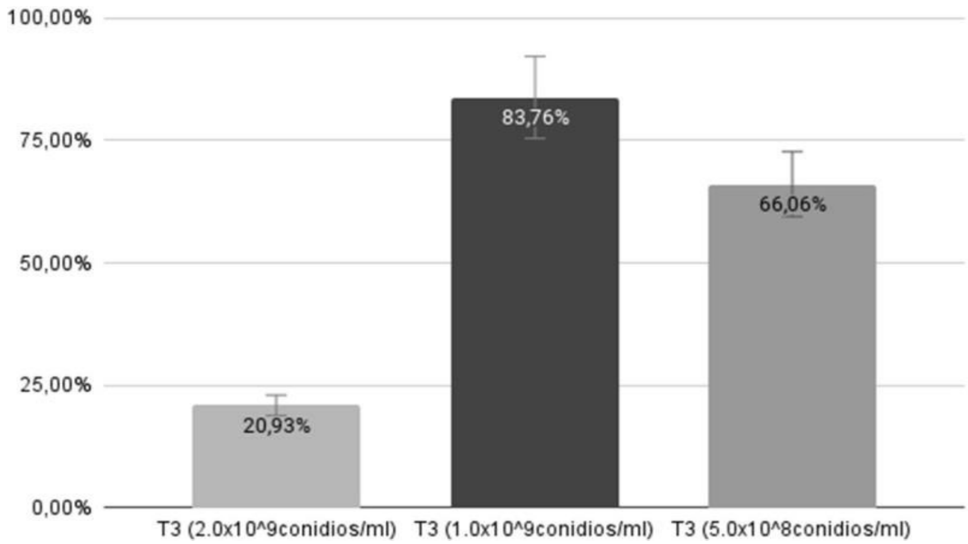

Figure 6. Efficacy percentages of treatment 3 concentrations

The T3 concentration of 1,0×109 conidia/ml showed the highest percentage of efficacy, reaching 83,76 % compared to the T1 negative control, followed by the $5,0\times10^8$ conidia/ml concentration with a value close to 66,06 %, and finally the $2,0\times10^9$ conidia/ml concentration had the lowest percentage of effectiveness with 20,93 %.

In addition, the percentage of efficacy of all T3 compared to T1 (negative control) and T2 (positive control) was calculated using the Schneider Orelli formula, and the final percentage of each of the treatments was obtained in order to determine the level of efficacy (figure 7).

The percentages showed results greater than 50 % in both cases. Compared to the negative control, T1 reached 70,74 % effectiveness of all T3 (entomopathogenic consortium) and 57,09 % effectiveness compared to T2 (chemical control).

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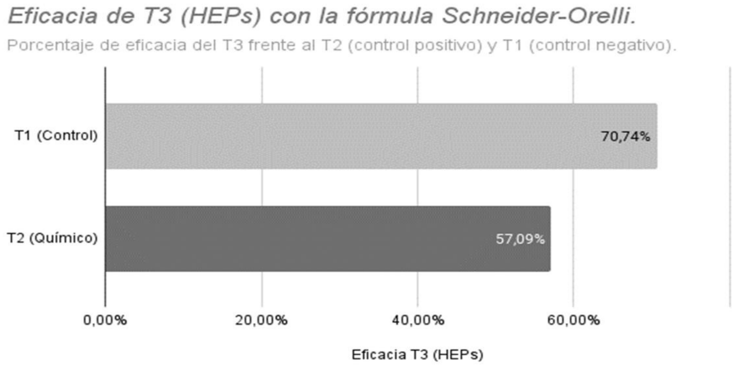

Figure 7. Percentages of efficacy of T3 compared to T1 and T2.

Comparison of the efficacy of treatments T3 and T2

To verify the efficacy of T3 (entomopathogenic consortium) through its mortality and compare it with the positive control T2 (chemical detergent) at 3 concentrations over a period of 15 days with sampling every 5 days, 18 readings were taken: 9 with the entomopathogenic consortium and 9 with the chemical detergent, as can be seen in table 3.

Table 3. Mortality percentage of the treatments

Day	T3_C1	T3_C2	T3_C3	T2_C1	T2_C2	T2_C3
5	0,50	0,76	0,55	0,46	0,17	0,35
10	0,86	0,96	0,78	0,51	0,59	0,67
15	0,08	0,80	0,80	0,70	0,23	0

Various statistical tests were performed, such as the Shapiro-Wilk test, and it was demonstrated that all treatments follow a normal distribution, as can be seen in figure 8. The Levene test was another statistical test performed to obtain a value of 0,355, verifying the homogeneity of the variances of each of the groups (figure 9).

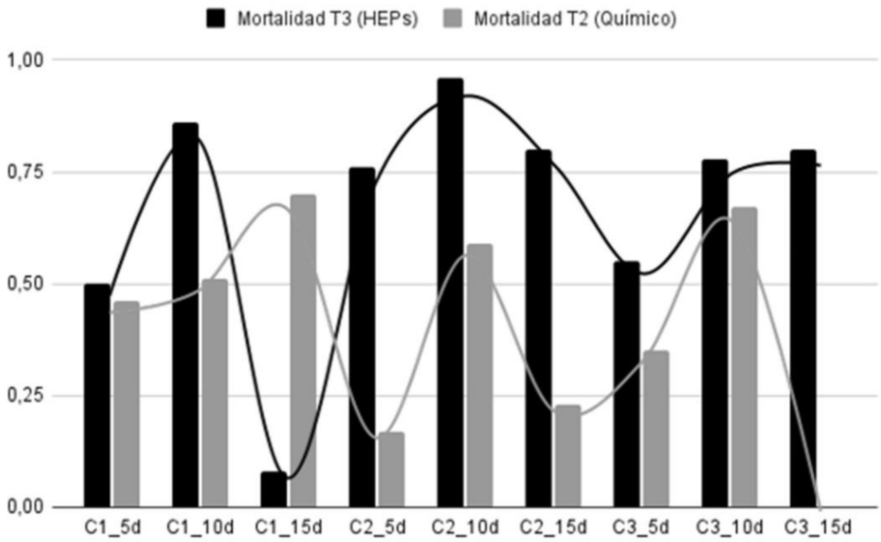

Figure 8. Normal distribution of treatments T2 and T3

ANOVA

Mortalidad

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.620	5	.124	2.052	.143
Within Groups	.726	12	.060		
Total	1.346	17			

Figure 9. Analysis of variance

In this case, in the analysis of variance, the value of (p= 0,143) showed that there are no significant differences between T2 and T3 in any of the concentrations, so the use of Tukey's post hoc test is ruled out.

Mortality of *Planococcus citri* mealybugs

The mortality of the observed cochineal insects in the control group (T1), as well as in the experimental group consisting of HEPs (T3), was quantified in three individual samples taken 5, 10, and 15 days after the application of treatment 3. For each sample, one leaf was collected from each infected plant. The following graphs show the mortality rates of the control group and the experimental group with the data collected.

In the control sample, throughout the 15-day experimentation period using the aforementioned method, mortality was only found on day 5 of the experiment, represented by 10 dead mealybugs, determining a 2 % mortality rate, as detailed in figure 10.

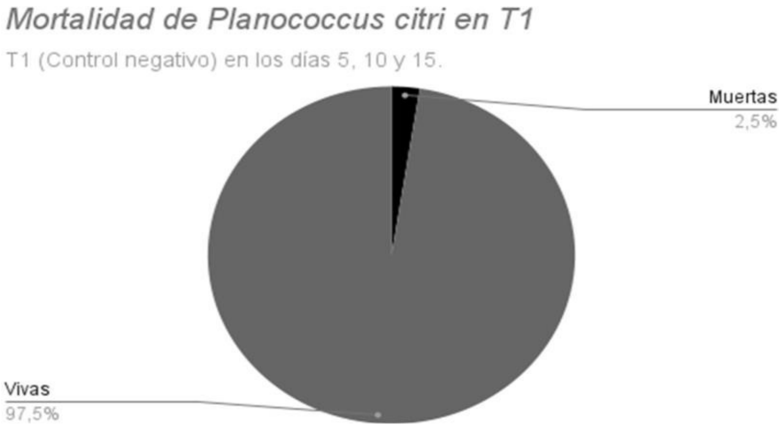

Figure 10. Mortality of Planococcus citri T1 versus T3 mealybugs

Comparison of chlorophyll levels in T1, T2, and T3 versus plants before experimentation

Eight treatments were classified: three for T3 (entomopathogenic consortium), three for T2 positive control (chemical detergent), one for T1 negative control, and one for all 24 trees in which chlorophyll levels were measured before any treatment.

A Welch ANOVA was chosen, and a Tukey test was also applied using the pingouin library (0.5.4) of Python (3.10). This method is used to adjust the degrees of freedom and sums of squares to detect differences in variances between non-homogeneous groups. The results indicate that there are significant differences between at least two treatment groups, with a p-value of 0,000717. In addition, the effect size (np2) is relatively high (0,787918), indicating that the treatments explain a large proportion of the variability observed in the data.

These results indicate specific comparisons between treatments where significant differences were found. The interpretation is made considering the p-value and other metrics such as the Bayes Factor (BF10) and the effect size (Hedges' g).

Treatment T3 at concentration C1 (2,0x109 conidia/ml) was found to show a significant difference from treatment T3 at concentration C3 (5,0x108 conidia/ml) with a p-unic = 0,009859, less than 0,05. This suggests that these treatments have a significantly different impact on the amount of chlorophyll present in trees with varying concentrations.

A significant difference is observed between treatment T3 at concentration C1 (2,0x109 conidia/ml) and treatment T2 at concentration C1 (30,22 p/v) with a value of (p-unic = 0,031713), which implies that the treatments have a significantly different impact on the amount of chlorophyll when compared to each other.

There is a significant difference between treatment T3 at concentration C1 (2,0 × 10^9 conidia/ml) and treatment T2 at concentration C2 (15,11 p/v) with a value of (p-corr = 0,012579).

In addition, a value of (p-corr = 0,008383) was found, demonstrating significant differences between T3 at concentration C1 (2,0 × 10^9 conidia/ml) and treatment T2 at concentration C3 (7,55 p/v). In turn, a significant difference was found in chlorophyll levels with a value of (p-unic = 0,049781) in treatment T3 at concentration C1 (2,0x109 conidia/ml) and the specimens before treatment.

Significant differences were found in chlorophyll levels between treatment T1 (negative control) and the samples before treatment.

DISCUSSION

Although several studies have focused on the biological control of mealybugs, most have concentrated on the use of a single fungal strain rather than an entomopathogenic consortium. The relevance of the previous statement lies in the fact that consortia exhibit interactions, both positive and negative, that affect their effectiveness as biocontrol agents. For example, a consortium composed of two strains of Beauveria bassiana showed 17 % higher mortality than treatments using individual strains; however, its mortality rate was low: 30 %. Metabolites were found in dead insects treated with the consortium that can suppress the caterpillar's immune system. In other research, it was determined that the combination of *Beauveria bassiana and Metarhizium anisopliae* was more virulent than using individual strains against insect pests of *Momordica charantia*.

HEP treatments on mealybugs under controlled laboratory conditions tend to show greater effectiveness than those analyzed in the field, due to the controlled conditions. Knowing this, the following is stated: the high mortality rate demonstrated by some of the treatments in our research is inferred to be caused by their correct application, limited exposure to their natural predators, and different external conditions, thus ensuring that the treatments acted on *Planococcus citri*. It should also be considered that all treatments were conducted under controlled temperature conditions (24 °C), which is a factor to consider since fungi do not develop adequately at unfavorable temperatures.[20]

It is suggested that this is the reason why experimental treatment 3 (HEPs) had a higher mortality rate compared to treatment T2 (chemical detergent). However, in terms of efficacy, they were statistically equal.

In the study by Buena et al.[19], mortality was demonstrated in only one of their products, with an overall average of 38,32 %, showing a higher level of mortality on day 14. The second and third products in that study did not show mortality in the cochineal (*Nipaecoccus nipae Maskell*) greater than 10 % and did not show significant differences from the control.

This contrasts with the results obtained in our research, which indicate that treatment 3, at all concentrations, causes mortality and shows significant differences compared to the control. The concentrations of 2,0x109 conidia/ml and 1,0x109 conidia/ml reveal a higher mortality rate on day 10, reaching 86 % and 96 % on that day, but with an overall average of 47,80 % and 84 %, contrary to the concentration of 5,0x108 conidia/of the consortium, which shows higher mortality on day 15, reaching 80 % and with an overall average for all days of 71 %. Our study, like that of Buena et al.[19], found that the mortality response of mealybugs, even in one of the treatments, peaked after day 10.

Similar to the results obtained, HEPs show high efficacy in a study based on the use of *Metarhizium anisopliae* and *Metarhizium robertsii* strains for the biological control of *Puto barberi* under laboratory conditions, resulting in percentages of 75,9 % and 85,4 %, respectively. At the same time, the application of these HEPs in seedbeds reduced the cochineal population by 55 % and 78 %. This shows that *Metarhizium robertsii* is more effective against this cochineal.[21]

Better results than those obtained are shown in the research by Karaca et al.[22] Under laboratory conditions, individual strains of *Beauveria bassiana*, *Paecilomyces fumosoroseus*, and *Paecilomyces lilacinus* are analyzed. It was found that the first strain showed 100 % mortality on day 5, while the second and third strains reached 82 % and 96 % mortality, respectively, on day 7.

A high concentration of chlorophyll is a sign of plant health and an indicator of the rate of photosynthesis.[23] Infestation by herbivores indirectly affects plant growth by suppressing photosynthetic activity. One study found that after 38 days with a high initial infestation of mealybugs of the species *Phenacoccus solenopsis*, of the genus Pseudococcidae, there was a 57,3 % reduction in the relative chlorophyll content in tomatoes.[24]

Recent studies have analyzed the endophytic capacity of HEPs. The work of Veloz-Badillo et al.[25] analyzes the endophytic capacity of *Beauveria bassiana,* finding no effects on chlorophyll concentration (in SPAD units). Meanwhile, research by Greenfield et al.[26] shows a negative contribution to the concentration of chlorophyll content in leaves after using different concentrations of *Beauveria bassiana and Metarhizium anisopliae*. On the other hand, after inoculation of the roots of *Triticum durum L. cv. Calero (*wheat) with *Metarhizium brunneum* showed an increase in chlorophyll concentration, but only in young leaves.[27]

This study shows different results, given that there are significant differences in the concentration of chlorophyll in leaves between treatment T3 at concentration C1, compared to the three treatments using the positive control (chemical detergent), and before treatment.

CONCLUSIONS

The highest mortality rate was produced by treatment 3 with a concentration of 1,0 × 10^9 conidia/ml, reaching an individual average of 84 % throughout the experiment. The consortium exhibits higher mortality compared to the negative control and the positive control.

High concentrations in treatment 3 showed a lethal effect in the short term, and low concentrations showed a higher degree of mortality on day 15, indicating a gradual lethal effect.

In terms of efficacy, treatment 2, corresponding to the positive control, and treatment 3, corresponding to the entomopathogenic fungal consortium, were found to be statistically equal throughout the experiment period.

The treatment corresponding to the entomopathogenic fungal consortium proved to be an effective alternative to chemical insecticides for controlling *Planococcus citri*, as higher pest mortality and a significant

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population reduction were observed with the use of the treatment compared to the negative control and positive control.

Treatment 3, concentration 2,0x109 conidia/ml, showed a significant increase in chlorophyll levels in all treatments 2, with treatment 3, concentration 5,0x108 conidia/ml, and with the percentage of chlorophyll before the application of the treatments, concluding that the presence of fungi may be a stimulating factor for chlorophyll synthesis.

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CONFLICT OF INTEREST
Authors declare that there is no conflict of interest.

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