
Controlling *Planococcus citri* in Rambutan (*lat*) culture with aqueous extracts

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Abstract It was found that plant extracts work in the control of *Planococcus citri* (mealy bug) in rambutan crops, specifically those corresponding to *Petiveria alliacea* and *Momordica charantia*, which managed to reduce the density of the mealybug population to a maximum of 1 individual per leaf, per panicle and for every 1000 fruits observed. The application of these aqueous plant extracts at the juvenile stage of the fruit managed to keep the mealybug population under control in rambutan crops, which translates into a great reduction in damage and a greater quantity of fruits available for marketing. In addition, plant extracts increased the number of phenolic compounds in the panicles from 0.003 to 0.006 mg EAG, per gram of panicle, which benefits the trees, as they are defended as metabolites against pests and diseases. It is mentioned that plant extracts could offer an alternative to replace synthetic insecticides.

Keywords: Extracts, Pest, Control, Rambutan, Mealybug

Introduction

Rambutan (*Nephelium lappaceum* L.) is a tropical tree with round or ovoid fruits with a yellow or reddish pericarp, depending on the variety, and has long spindles and a white edible aril (Hernández-Hernández *et al.*, 2019). Its maturation pattern corresponds to non-climacteric fruit (Caballero-Pérez *et al.*,

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2011), as they are harvested when they are ripe for consumption and have optimal external appearance. Due to its morphological and physiological characteristics, it is a highly perishable fruit, because its shelf life does not exceed seven days after being harvested, with noticeable dehydration occurring in the spines and pericarp, with oxidation happening easily and producing a dark and undesirable appearance, thus limiting its marketing for fresh consumption (Hernández-Arenas *et al.*, 2010).

In Mexico, rambutan (*Nephelium lappaceum* L.) is cultivated in five states: Chiapas, Oaxaca, Tabasco, Michoacán and Nayarit (SAGARPA, 2016). The región of Soconusco, Chiapas, has over 2000 cultivated hectares (Osorio-Espinoza *et al.*, 2019), which includes 716 producers of this exotic fruit. Backyard horticultural systems are used and are a main and economic pillar that will strengthen many family nuclei if more than 8 ha are planted (Pérez *et al.*, 2014). On the other hand, the production methods and techniques are based on chemical control and are usually monocultures, which favors the deterioration of the productivity and quality of the fruit. In addition, there is also infestation by insects, such as the mealybug (*Planococcus citri*), which is considered a pest in the agricultural system (Villatoro, 2016).

The mealybug (*Planococcus citri*) belongs to the order Hemiptera and the family Pseudococcidae, with 2200 species around the world (Ben-Dov *et al.*, 2013). This insect feeds on the phloem and can reduce the vigor of the plant, the quality of the fruit and the yield of the crop (Pérez *et al.*, 2017).

The damage it generates to rambutan trees happens in all stages of tree development, causing crop losses and rejection of the fruit meant for export. Damage includes weakening, discoloration of the leaves, which is accompanied by necrosis at the edges. These insects feed on the sap of the plant or tree, where they can transfer viruses and toxic compounds. Some species excrete honeydew, which promotes the proliferation of sooty mold, thus reducing the quality of the fruit and causing economic losses (Quesada-Sojo and Rivera-Méndez, 2016). The type of damage and symptoms caused by these insects are characteristic of most members of the Pseudococcidae family (Palma-Jiménez *et al.*, 2019). Therefore, the use or application of plant extracts is a viable alternative for pest control that has less negative impact on the environment (Esquivel-Rivera *et al.*, 2022).

The use of plant extracts is considered important for pest control. Extracts of plant origin are characterized by the presence of secondary metabolites, which are part of the defense mechanism of plants, and are grouped into nitrogenous, phenolic, and terpenoid compounds. These compounds provide important properties to the extracts, such as antiviral, antimicrobial, insect repellent, which

allow them to be used to protect crops and to increase food quality and production (Akkoc *et al.*, 2019; Delgado *et al.*, 2012).

The use of plant extracts, as an alternative insecticide, is a form of control that does not generate the problems caused by chemical synthetic insecticides, which cause environmental imbalances in crops, affecting plants and animals present in the ecosystem. Conventional insecticides contaminate water resources, trigger resistance in insects and leave toxic residues for humans. The use of plants with insecticidal properties is a very old practice. Up until the discovery of synthetic organic insecticides in the first half of the last century, substances extracted from vegetables were widely used to control insects (Akkoc *et al.*, 2019; Pérez *et al.*, 2017).

The aqueous extracts of *Allium sativum*, *Ocimum basilicum*, *Momordica charantia*, *Dysphania ambrosioides* and *Petiveria alliacea* L, on rambutan aim to reduce the population density of the mealybug and keep their number below the economic threshold, which would lead to avoiding drastic damage to the rambutan crop. It is known that the extract of *Allium sativum* has been used for the controlling of various pests, including biological control of the mealybug (Cázares *et al.*, 2014).

To protect crops in modern agriculture, plant extracts can be a feasible pest management method for multiple crops and may contribute towards reducing the use of synthetic chemical insecticides (Rangel-Guerrero *et al.*, 2018). Integrating alternative products, such as the use of plant extracts, in the ecological management of insect and vector pests is crucial in current Integrated Pest / Vector Management programs that seek to reduce environmental impact without altering crop properties (Granados-Montelongo *et al.*, 2021; Pérez *et al.*, 2017). The objective was to evaluate the control of *Planococcus citri* (mealy bug) with aqueous plant extracts in rambutan cultures.

Materials and methods

Sampling site

The study area is located in the "Toluquita" Canton in the Municipality of Tapachula, Chiapas; Mexico (14° 58'13.001" N, 92° 14'2" W). The temperature in the municipality varies from 21°C to 33°C, occasionally dropping below 20°C or even rising over 35°C. Study was carried out between 6:00 am and 11:00 am. The piece of land was undertaken with 1.5 hectares, where the age of the trees ranged between 7 and 10 years. The trees are planted with the planting design tri boliadoa to distance of 5 m × 5 m between trees.

Experimental design

The experiment was completely randomized Design (CRD) adapted method. The experiment is made up of 12 treatments and a control, where each treatment is made up of 5 experimental units (plants), for a total of 60 experimental units. The treatments were tagged with plastic tape to differentiate each extract, *Allium sativum* as E.A, *Ocimum basilicum* as E.AL, *Momordica charantia* as E.C. A, *Dysphania ambrosioides* as E.E and *Petiveria alliacea* as E.ZO. The treatment with water was tagged as (+), where the treatment with Cypermethrin was identified as (-). The concentrations used in the study are shown in Table 1.

Table 1. Treatments: *Allium sativum* (E.A), *Ocimum basilicum* (E.AL), *Momordica charantia* (E.C. A), *Dysphania ambrosioides* (E.E) and *Petiveria alliacea* (E.ZO), treatment with Cypermethrin

Treatments	Concentrations (% v/v)	Amount of plant extract (L)	amount of amount adherent (Honey solution 0.001%) (mL)	of water amount (L)	final volume (L)
E. E	20	3	150	11.85	15
E. E	10	1.5	150	13.35	15
E. A	20	3	150	11.85	15
E. A	10	1.5	150	13.35	15
E. AL	20	3	150	11.85	15
E. AL	10	1.5	150	13.35	15
E. C. A	20	3	150	11.85	15
E. C. A	10	1.5	150	13.35	15
E. ZO	20	3	150	11.85	15
E. ZO	10	1.5	150	13.35	15
C	100	0	150	14.85	15

Obtaining plant material and preparation of aqueous extracts

The biological material *Allium sativum*, *Ocimum basilicum*, *Dysphania ambrosioides*, were purchased at the "San Juan" market, located in Tapachula, Chiapas. The other materials were donated. *Petiveria alliacea* was provided Mr. Angel Gallardo, producer of Tuxtla Chico and *Momordica charantia* was donated

by the producers of Ejido Carrillo Puerto, found in the municipality of Tapachula, Chiapas; Mexico.

Firstly, the biological materials were washed and dried, after which the extracts were obtained by a modification of the method described by Pérez *et al.*, (2017), whereby the weight of the sample and amount of solvent were modified. The plant material of the washed and dried, the weight was 1.25 kg, which cut into 3 cm² fragments, which were then added to a container, to which 4.75L of boiling water were introduced, thus ensuring a 1: 4 ratio. Materials were let stand at room temperatura (28°C) for 24 h in a dark room without exposure to light. Finally, the extracts were filtered with Whatman paper to obtain particle-free extracts.

Application of extracts

The application began after the insect larvae of interest was detected on rambutan leaves. The products were applied by using 20 L manual backpack sprayers, using working volume of 15 liters. The adherent used was *M. solani* honey, at a concentration of 0.01% v/v. The solution was applied on the leaf area, including the underside and the top of the leaves, and were also applied on the rambutan fruits. The solution volume applied per tree was 3 liters. A total of 8 applications were performed during the investigation, where each application occurred at 14-days intervals, for total time of 20 weeks.

Response variables

Sampling was carried out for each tree every 7 days after spraying. The presence of *Planococcus citri* was identified by prior consultation of the reported technical sheet of characteristics and morphology (Francis *et al.*, 2012). On each rambutan tree, floral panicles and four branched were marked (one for each cardinal point) for inspection throughout the treatment. Each marked branch contained cluster of fruits. Each branch was observed later in the experiment to identify the presence of the mealybug in the leaves, floral panicles, fruits, where the level of infestation and the efficacy of the extracts was assessed (National Service of Agrarian Health, 2006).

Determination of total phenols

Fresh samples weighing 500 mg were taken from the main branch of the panicle were obtained, after which analyses were carried out in triplicate with the Folin-Ciocalteu colorimetric method according to Kupina *et al.*, (2018).

A mixture containing 10 µl of the ethanolic extract, 990 µl of distilled water and 100 µl of the Folin–Ciocalteu reagent was prepared. After 5 min, add 600 µl saturated sodium carbonate in 1M NaOH were added to the sample. After one hour, the absorbance was measured at 725 nm in a spectrophotometer. The result was expressed in mg of soluble phenols per gram of fresh sample (mg g⁻¹ MF), using a standard curve of gallic acid as reference.

Analysis of results

All the obtained data was subjected to an analysis of variance (ANOVA) and a Pearson analysis was used to find a correlation between the phenolic compounds and the number of pest insects in INFOSTAT® statistical software.

Results

The population dynamics of mealybug on the leaves in the rambutan crop is presented in Figure 1. Two weeks after the first application, it was observed for both concentrations (10 and 20 %), that the number of this insect's larvae are present at an average value of less than 2.0 for most the treatments. This trend was observed in general, regardless of the type of extract. The presence of his larva was only seen in the treatments involving *P. alliacea* and *D. ambrosoides*.

After the second application (3rd week of study) of the 10% v/v aqueous extracts, the average value of mealybugs on the rambutan leaves remained at 2.0 for the next 12 weeks, reducing its value after 15 week, after the last application made. Treatments that received 20% v/v of the plant extracts, a similar controlling effect of the pest was observed in regards to the 10% extracts, where the average value remained at 2.0. This was observed after the 3rd week of the study and remained at that level for all of the applications throughout the 18 weeks of the study. In cipermethin treatments, the average value fluctuated between a minimum of 2.0 and a maximum of 12.0 larvae.

It is statistically observed that there were highly significant differences between all of treatments (F: 733.44, gl: 5.0, p<0.0001), but not between concentrations (F: 2.0, gl: 2.0, p= 0.1357). In general, the best treatments that maintained in all the evaluations carried out an average value of less than 2.0 for mealybugs in rambutan leaves were: *M. charantia* and *P. alliacea* in both applied concentrations. As for the *D. ambrosoid* extract, an average number below 2.0 larvae was found for all examinations only when a concentration of 20 % was used.

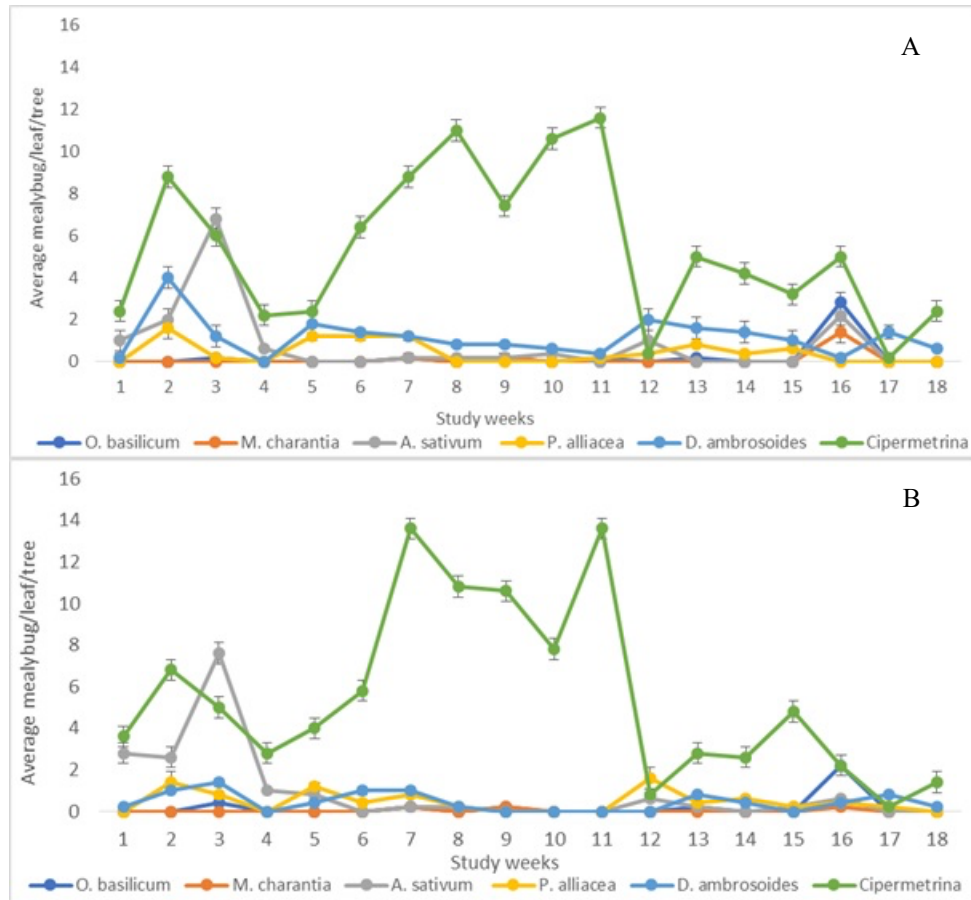


Figure 1. Population dynamics of mealybug on leaves, with treatments of aqueous extracts in the period of 18 weeks of study. (A: 10%. B: 20% v/v)

As for floral panicles, the effect on the mealybug population induced by the applied biological treatments at a concentration of 10 % v/v was sustained with the exception of *A. sativum* (week 3 and 18) and *M. charantia* (week 10) aqueous (Figure 2). The 20 % v/v concentration, the *A. sativum* extract increased the average number of mealybugs for sampling weeks 3, 17 and 18. The insect population for the other aqueous extract treatments remained below of 2.0. The cypermethrin treatments showed that the average value fluctuated between a minimum of 2.0 and a maximum of 12.0.

A comparison between treatments allowed to observe that there were statistical differences between them (F: 113.53, gl: 5.0, $p < 0.0001$), but not between the concentrations of aqueous extracts (F: 0.75, gl: 2.0, $p = 0.4715$). The treatments were averaged values of less than 2.0 mealybugs per rambutan panicle

which found at all times for both used concentrations of *O. basilicum*, *P. alliacea* and *D. ambrosoides*. Only in the *M. charantia* extract (20 %) were found an averaged values lower than 2.0.

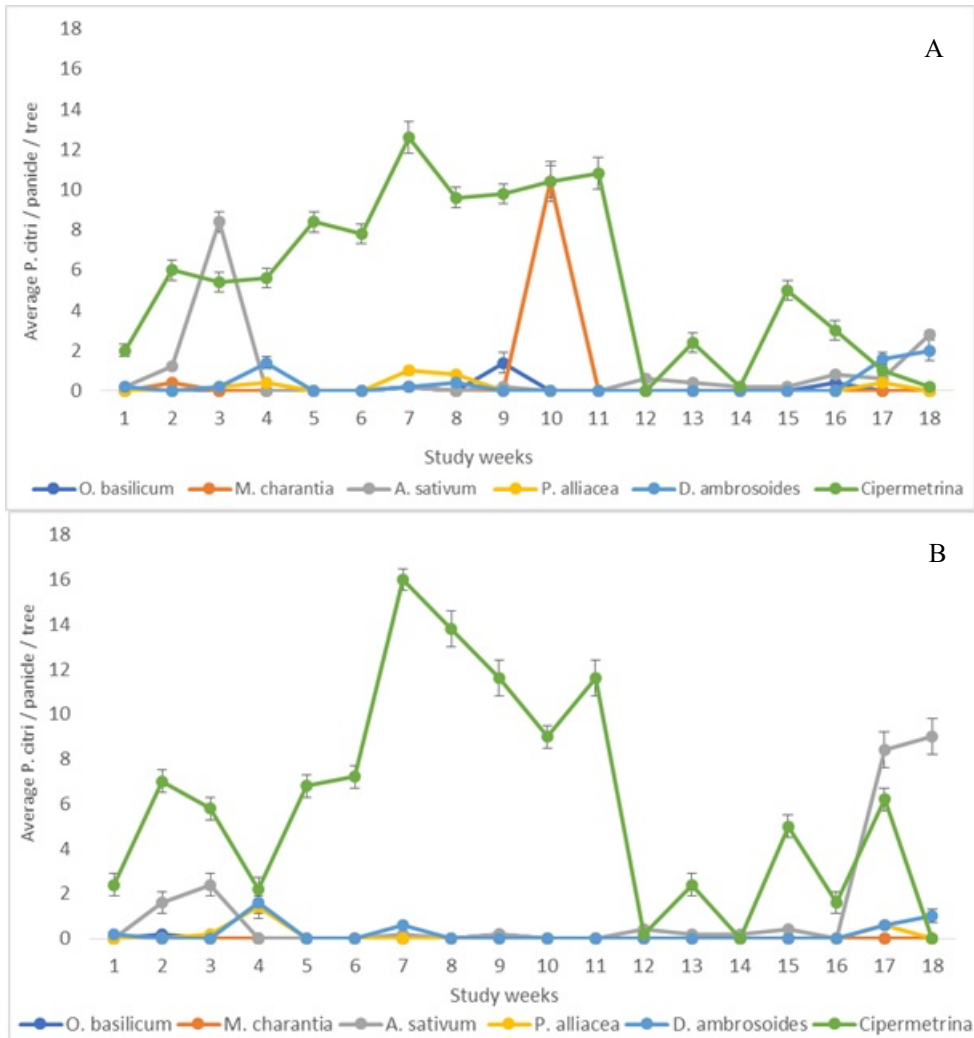


Figure 2. Population dynamics of mealybugs with treatments of aqueous extracts in panicles, in the period of 18 weeks of study. (A: 10%. B: 20% v/v)

The best aqueous extract treatments, where an average number of mealybugs per rambutan fruit cluster below 2.0 was observed at all times and at both concentrations, were: *M. charantia*, *P. alliacea* and *D. ambrosoides* (Figure 3 y 4). The statistical differences were found between treatments (F: 161.84, gl: 5.0, $p < 0.0001$), and between the concentrations used (F: 3.83, gl: 2.0, $p = 0.0222$).

Average values higher than 2.0 of mealybugs per fruit cluster were only observed when applying the aqueous extracts of *A. sativum* and *O. basilicum*, regardless of concentrations. They were observed at week 12 and 18 at a concentration of 10%, and at weeks 2, 12, 16, 17 and 18 at a concentration of 20%.

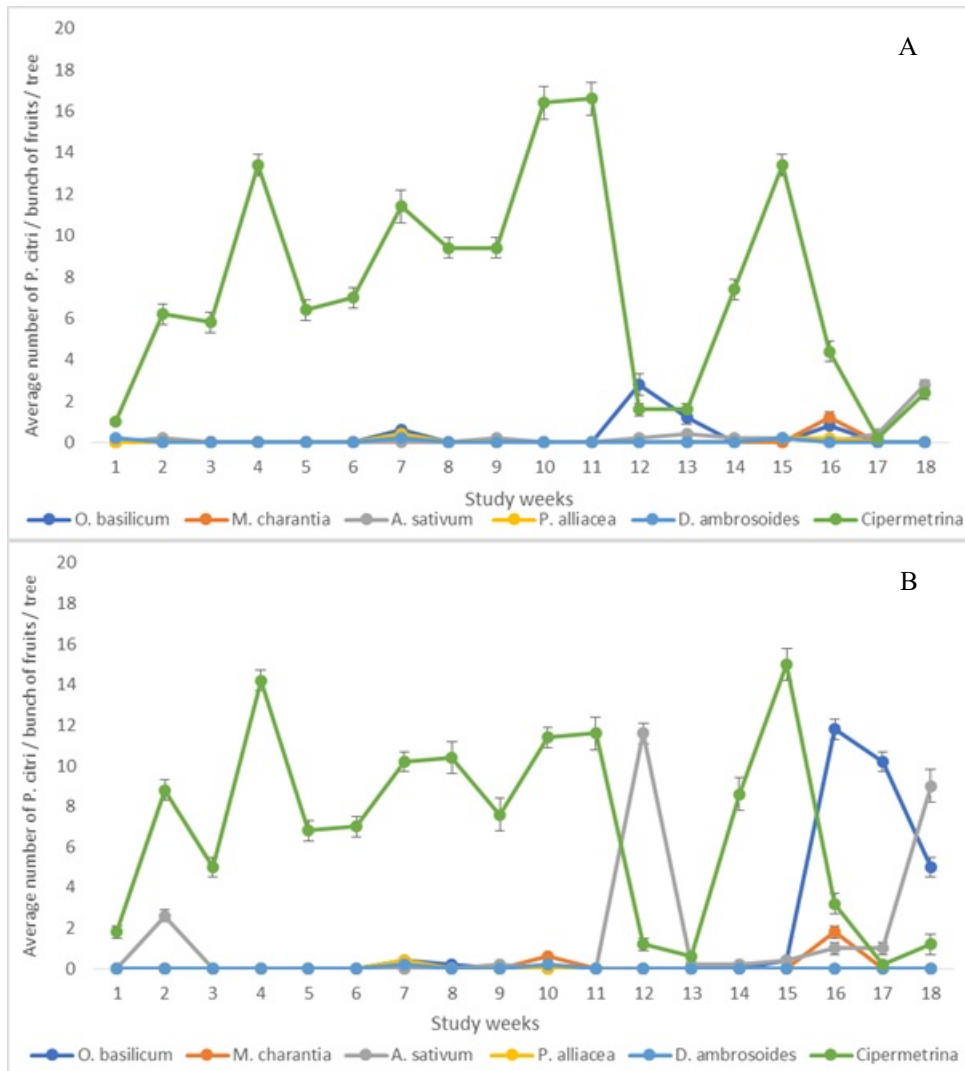


Figure 3. Population dynamics of mealybug with treatments of aqueous extracts in fruits, in the period of 18 weeks of study. (A: 10%. B: 20% v/v)



Figure 4. Rambutan fruits of different treatments. (A) Cypermethrin Treatment, showing the damage to the fruit generated by *Planococcus citri*. (B) 20% *Dysphania ambrosioides* extract. (C) *Momordica charantia* extract 10%

Induction of phenolic compounds was observed a week after the application for all treatments (Figure 5). For all biological treatments, with the exception of the control, it was observed that the concentration of phenolic compounds would be firstly increased after application and then decreased, regardless of the concentration applied.

The concentration of phenolic compounds was never completely reduced in any of the treatment. An increase in phenols was observed that after each application, which was cumulative as previously induced, thus it showed an increasing dynamic in the concentration of these compounds in the tissue of the rambutan panicle.

The 10 % concentration, only for the aqueous extract of *A. sativum* did the induction dynamics of phenolic compounds remained below the concentration found in the other aqueous extracts. The 12th week found that *M. charantia* and *P. alliacea* treatments stood out, where values were higher than 0.005 mg of EGA which detected in the panicle tissue, while the other treatments remained below this concentration.

When an aqueous extract concentration of 20 % was used, it was observed that the *M. charantia*, *O. basilicum* and *P. alliacea* treatments reached a maximum concentration above 0.005 mg EGA of phenolic compounds. The *A. sativum* and *D. ambrosioides* treatments were below this concentration. Only in the control treatment was increased in phenolic compounds which observed between week zero (0) and week 5, after which the reduction was observed. However, it was noticed that a slight increasing was occurred at 11th week. The concentration of phenolic compounds in the panicle did not exceed the concentration of 0.003 mg of EGA at any time.

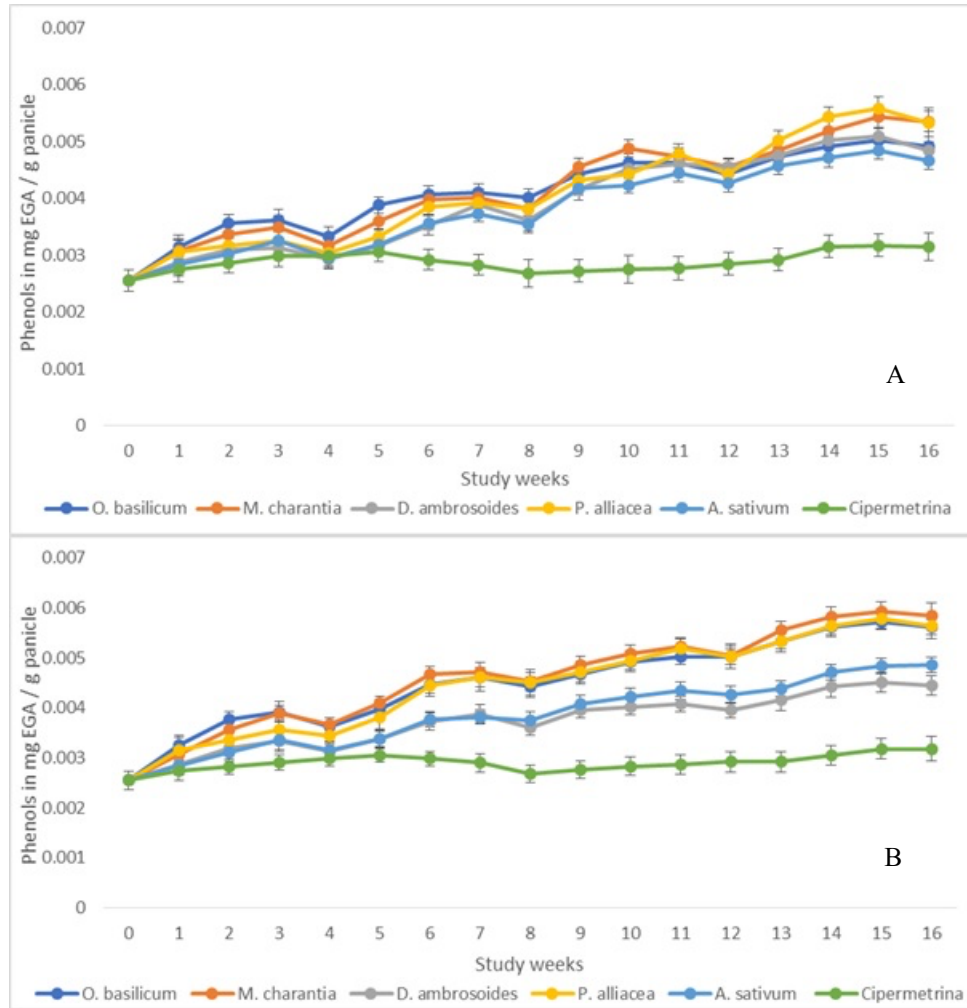


Figure 5. Phenols production dynamics of treatments of aqueous extracts in panicles, in the period of 16 weeks of study. (A: 10%. B: 20% v/v)

It was statistically differed between treatments (F: 1616.4, gl: 5.0, $p < 0.0001$), whereby the *M. charantia* treatment was the highest average production of phenolic compounds. A correlation was observed between phenolic compounds and the number of mealybugs in the panicles. This correlation was higher than the relationship found between phenolic compounds and mealybugs in rambutan fruits (Table 2).

Table 2. Pearson correlation values between phenol concentrations and mealybug values in panicles and fruits in the rambutan crop

Treatment	Concentration	Phenols - panicle	phenols frutos	p-value
P. Alliacea	10	-0.58	-0.55	<0.0001
P. Alliacea	20	-0.60	-0.62	<0.0001
D. Ambrosoides	20	-0.58	-0.52	<0.0001
D. Ambrosoides	10	-0.52	-0.48	<0.0001
M. Charantia	10	-0.60	-0.56	<0.0001
M. Charantia	20	-0.64	-0.62	<0.0001
A. Sativum	20	-0.54	-0.52	<0.0001
A. Sativum	10	-0.50	-0.48	<0.0001
O. Basilicum	10	-0.50	-0.54	<0.0001
O. Basilicum	20	-0.54	-0.58	<0.0001
Cipermetrina	2	-0.33	-0.24	<0.0001
Cipermetrina	1	-0.34	-0.22	<0.0001

Discussion

The plant extracts showed efficiency for the control of *Planococcus citri*, albeit some concentrations induced better results than others. Extracts act as growth regulators, by altering the function of insect hormones as reported Figueroa *et al.*, (2019). They also act as repellents due to bad odor or irritating effects, causing the death of insects by starvation (Vera *et al.*, 2016).

The trees that were treated with both concentrations of the *Momordiga charantia* extract (10% and 20%), generated a very significant decrease in the presence of *Planococcus citri* in the leaves, flowers and fruit of the rambutan, thus showing an insecticidal activity. This is in agreement with the results reported by Jaramillo *et al.* (2019), who determined that *Mordiga charantia* has several groups of secondary metabolites of acaricidal and insecticidal interest, such as flavonoids, phenol alkaloids, among others.

The *Ocimum basilicum* extract showed good result in the control of the pest during the flowering stage at both concentrations. During the fruit development stage, it controlled the population of *Planococcus citri* at a concentration of 10 %. This agrees with Torres and Roldán (2015) who indicated that *O. basilicum* alcoholic extracts have an insecticidal and larvicidal and adulticidal effect for *A. aegypti* at a concentration between 15-20 %. This effect was attribute to the presence of alkaloids, glycosides, flavonoids, triterpenes, recins, and tannins thus contributing to their death.

Allium sativum and *Petiveria aliacea* extracts were less effective in the control of *P. citri* as compared to the extracts of *Momordiga charantia*. This is

comparable to what Martínez and Rivera (2008) mention, indicating a discontinuity in the controlling effect by *A. satium* when its concentrations are greater than 1%. This suggests that determining the maximum action dose and lethal dose for a good biocide or repellent potential is highly important, as a concentration outside of the determined range will not have the expected results for pest control, (Zelaya-Molina *et al.*, 2022).

The *Dysphania ambrosioides* extract showed the best results for pest control. During the flowering and fruit development periods, using this extract at a concentration of 20% resulted in almost total absence of *Planococcus citri*. This agrees with Vázquez *et al.* (2007), who indicated that the effects of the secondary metabolites in the *Dysphania ambrosioides* extract produce alteration on the development of the insect and its feeding habits. Also the presence of irritating compounds in the extract tend to repel the pest. According to Bernardino *et al.* (2019), in order to achieve adequate pest management, it is necessary to diagnose and evaluate them. Thus, necessary information will be available to decide the most appropriate control method. Likewise, Zelaya Molina *et al.* (2022), proposed that the use of plant extracts for pest control is recommended when attempting to avoid problems caused by the mismanagement of conventional pesticides.

On the other hand, the reduced number of mealybug individuals in the rambutan fruits clusters, leaves and panicles, suggests that each of the aqueous extracts that were used exerted a repellent and pesticidal action, due to its components. It is noteworthy that the trees receiving biological treatment were practically free of this insect at a certain time of evaluation. Secondly, the increase in individuals in the treatment where cypermethrin was used as a control product could be the result of the repellent protection that each extract provided to the rambutan trees, making them less attractive or providing a non-preferential aroma for insects. *P. citri*. It is likely that this effect forced these insects to migrate to the other trees.

In regards to last paragraph, it is considered that the production of phenolic compounds in the plant tissue was elicited by the presence of the aqueous extracts. The components contained in these extracts acted as insect repellents and as defense inducers against pests in the exposed trees. This is similar to what was reported by War *et al.* (2012), who indicates that the phenolic compounds produced by plants participate in direct and indirect defense mechanisms that are activated by the damage caused by herbivores. On the other hand, Celaya-Michell *et al.* (2018) report that the compounds contained in the plant extracts will induce defense mechanisms against pest insects. Depending on the type of components, the compounds may show insecticidal activity, as in the case of phenols and flavonoids.

According to Sobhy *et al.* (2022), the exogenous induction of defense mechanisms in plants activates the biosynthesis of tannin, flavonoids and phenolic compounds, which promote resistance against pest. War *et al.* (2012), indicated that Plants activate volatile defenses with make the target plant less attractive, thus avoiding damage from herbivores when attacked by these insects, provided they receive appropriate stimulation.

The aqueous plant extracts exerted control on the infestation of *Planococcus citri* in the rambutan crop, as maximum average values of 1.0 insects was observed, both per leaf and per panicle for each rambutan tree. The maximum average value of insects per fruits cluster per tree was 2.0. The aqueous plant extracts where minimum values of zero (0) were obtained for leaves and panicles were those from *M. charantia* and *O. basilicum*. In fruits cluster, the best aqueous extracts to control this pest were *P. alliacea* and *D. ambrosoides*.

All aqueous plant extracts treatments induced the production of phenolic compounds, but the best the treatments were thus originating from *P. alliacea*, *M. charantia* and *O. basilicum*.

Finalilly, aqueous plant extracts can be considered for replacing agrochemicals and chemical insecticides, which in turn could contribute toward improving crop health and causing environmental impact.

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