

# BIOPESTICIDE POTENTIAL OF SELECTED PLANT EXTRACTS AGAINST CALLIMETOPUS CAPITO PASCOE ATTACKING ALSTONIA MACROPHYLLA WALL. IN BOTOLAN, ZAMBALES

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## Abstract

This study evaluated the potentiality of different plant extracts as a botanical insecticide against 5th instar larvae and adult M.T.B. Mango twig borer (*Callimetopus capito* Pascoe) attacking batino (*Alstonia macrophylla*) seedlings. Nine (9) treatments were tested under laboratory conditions, and the three superior botanical extracts were further assessed using cage assay. The experiment was set up utilizing a Completely Randomized Design (C.R.D.) with five replications. All Methanol-based plant extract solutions in the laboratory assay revealed moderate to high toxic effects to 5th instar *C. capito* P. larvae. However, *Annona squamosa* (T2), *Albizia procera* (T5), *Carica papaya* (T7), and *Annona muricata* significantly obtained the highest mortality rate as compared to other treatments. Whereas it was observed in adult *Callimetopus capito* P., the highest mean mortality was obtained in *A. squamosa* (T2), *C. papaya* (T7), *A. procera* (T5), and *G. sepium* (T8). Hence, applying other plant extracts to adult *C. capito* also reduces mortality. Cage experiment revealed that *A. squamosa* effectively controlled adult *C. capito* up to 50% through contact action. Applying the botanical extracts as stomach poison did not manifest control against *C. capito*; instead, partial repellence was observed. The result of the study proved that crude plant extracts could become an effective botanical insecticide in the protection against 5th instar larvae and adult *C. capito* Pascoe attacking *A. macrophylla* seedlings.

**Keywords:** Crude Plant Extracts, Botanical Insecticide, Contact Action, Highly Toxic, Cage Experiment, *Alstonia Macrophylla*

## INTRODUCTION

Massive production of seedlings in the nurseries faced several unavoidable pests and disease problems, causing damage to seedlings and out-planted trees. The existence of insect pests in forest tree species and other vulnerable crops is significant. However, one of the current pests of indigenous batino (*Alstonia macrophylla* Wall.) trees observed in production nurseries and out-planted seedlings is the *Callimetopus capito* Pascoe or commonly referred to as mango twig borer (M.T.B.). It was reported recently that *Callimetopus capito* P. was observed attacking seedlings of *Callimetopus capito* P., damaging the young twigs and, eventually, the death of young shoots that further suppressed the growth of seedlings. *Callimetopus capito* P. is one of the severe mango pests trees in Luzon (Adoro et al., 2008). To date, there are no studies conducted yet to determine and evaluate potentially safe control measures using botanicals against *C. capito* to protect *A. macrophylla*. The *Alstonia macrophylla* Wall, commonly known batino is identified with metallophytic properties, which is highly ecologically valuable. Thus, conservation is vital. In Antamok, Benguet's mining-waste area, batino seedlings survived in harsh conditions and had robust growth (ERDB, 2012). The root component of *Alstonia macrophylla* in Brookes Point, Palawan, has high Fe content than the soil classifying it as a phytostabilizer; thus, it could be a suitable species for phytoremediation

(Claveria et al., 2010). The existence of forest plant species with metallophytic ability to tolerate extreme metal concentrations commends them for revegetation of mines and metal-contaminated sites (Whiting et al., 2004). Thus, using metallophytes for phytoremediation is an innovative way of addressing the environmental impacts of mining (Claveria et al., 2010). The use of synthetic pesticides is the usual control method in forest nurseries. Insect pests are effectively controlled at some times but may gain resistance to pesticides. Further, the use of synthetics is detrimental to health as well as sacrificing ecological soundness. Using plant extracts as botanical pesticides primarily reduced harmful environmental effects, especially on humans, while giving comparable results to pest control. Hence, this study was undertaken to evaluate the potentiality of different plant extracts as botanical pesticides against the attack of M.T.B. to *A. macrophylla* that will lead to the development of pest protection and management program using botanicals.

## **MATERIALS AND METHODS**

### **Experimental site and plant material**

The experiment was carried out in the experimental research area of President Ramon Magsaysay State University, Botolan Campus, Porac, Botolan, Zambales. *Alstonia macrophylla* was used as the tested indigenous tree seedlings.

### **Experimental condition**

For this experiment, a two-year-old *Alstonia macrophylla* seedling served as the host plant of *C. capito* P. during the testing in laboratory and cage experiments. One (1) mL of diluted plant extract for each treatment utilizing eight plants and one control was tested against larvae and adult stage of *C. capito* attacking *A. macrophylla* seedlings for laboratory. Also, for the cage experiment, three treatments were separately tested through the stomach and the contact action of the botanical extracts against adult *C. capito*.

### **Experimental design**

This study was laid out using a Completely Randomized Design (C.R.D.) for both assays. For the larvicidal assay, nine treatments were conducted with five replications, and each replication was represented by five individual larvae/or adults. For the field assay, four treatments were set up. There were five (5) replications where each cage was represented as a replicate. Each cage was set with five (5) *A. macrophylla* seedlings that served as the feeding host of *C. Capito* during the entire experimentation process. The 1m height x 0.5 m<sup>2</sup> width metal cage was covered with a fine white mesh screen. Five adult *C. Capito* regardless of sex, was released into each cage and allowed to be acclimatized with the environment for 24 hours, ensuring that the mortality of the tested insects is attributed solely to the effect of the treatments evaluated.

### **Treatments**

The laboratory and in-situ (cage) assays with the following treatments in the laboratory, 1.) *Annona squamosa* L., 2.) *Dioscorea hispida* Dennst., 3.) *Albizia procera* (Roxb.) Benth.4.)

Lantana camara L., 5.) Gliricidia sepium Jacq.Kunth ex Walp., 6.) Annona muricata L., 7.) Carica papaya L., and 8.) Cymbopogon citratus Staph. And 9.) Control against Callimetopus capito Pascoe attacking Alstonia macrophylla Wall. in Botolan, Zambales. For the cage set-up, the three most effective plant extract against C. capito 5th larvae was selected for further screening using the adult stage of the insect.

## EXPERIMENTAL PROCEDURE

### Collection of larvae and adult Mango Twig Borers

The C. capito Pascoe larvae and adults attacking A. macrophylla seedlings were collected from the RMTU Botolan Campus, Zambales. Collection of larvae and adult C. capito was done early in the morning or late in the afternoon, thus protecting the insect from stress and reducing mortality. The collected infected twigs were checked for the presence of larvae. Other larvae were collected from mango twigs since mango served as the pest's primary host. The collected larvae were transferred to fresh twigs, placed in a clean bottle, and acclimatized before the experiment. Adult C. capito was artificially reared in a clean bottle with a new A. macrophylla twigs and kept in the laboratory until the conduct of the investigation.

### Collection and drying of plant materials

The plant materials were collected and cleaned to free them from soil and other debris. One (1) kilogram (kg) of leaves for each plant material were laid on a clean table and air-dried for three to five days to attain approximately 20% moisture content (MC). The number of days to drying depended on the characteristics of leaf thickness. After drying, the plant materials were checked to see if they had already attained the MC. Airdried leaves were chopped, ground, and sieved to obtain the powdered leaves and utilized as the plant material for extraction (Figure 1).

### Preparation of plant extracts using different solvents

A pre-trial was conducted to determine the most appropriate solvent for extracting compounds from plant materials. Three solvents, acetone, methanol, and distilled water, were tested. Methanol and acetone were used for their ability to remove a wide range of compound polarities (Silver, 2015). The bioactive compounds from plants were extracted using methanol since it is a polar solvent and can extract polar compounds and other nonpolar molecules in plants. Methanol and acetone have a low boiling point of 65 °C and 55 °C, respectively (Agrawal, 2014; Dos Santos, 2014). Twenty milligrams (mg) of powdered leaves mixed with 100 ml of each solvent were prepared to obtain 20000 parts per million (ppm) concentration solution. The extraction was done manually by stirring the mixture for 10 minutes (min), then soaked for 72 hours. After which, the mixture was filtered and lined with #1 Whatman filter paper and centrifuged at 4,000 rpm (1,790 xg) for 10 min to obtain the supernatant. The distilled water plant extracts were set aside. An improvised evaporator was used with a temperature ranging from 55 °C to 60 °C and 45 °C to 50 °C for methanol and acetone plant extracts, respectively monitored and maintained prevented, to the boiling point, and obtained the pure extracts. Extracts were set aside in a clean reagent bottle under normal temperature for treatment preparations. The fresh tubers of nami were washed, cleaned, thinly cut, and grind

20 grams (g) with 80 ml of the solvents tested and followed the same procedure of extraction in dried leaves (Figures 1 & 2)

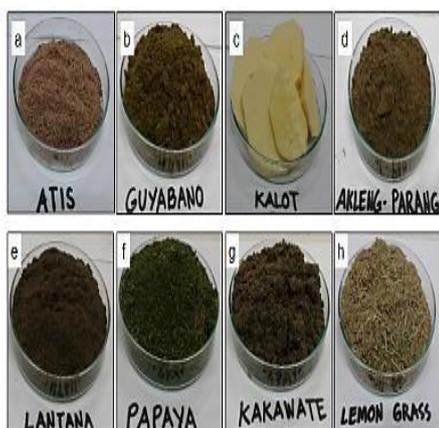


Figure 1 Powdered Plant materials of (a) Atis (*Annona squamosa*), (b) Guyabano (*Annona muricata*), (c) tubers of Nami (*Dioscorea hispida*), (d) Akleng-parang (*Albizia procera*), (e) Lantana (*Lantana camara*), (f) Papaya (*Carica papaya*), (g) Kakawate (*Girardinia septium*) & (h) Lemon grass (*Cymbopogon citratus*).

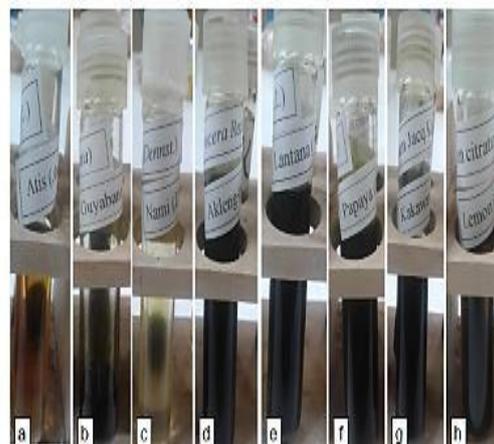


Figure 2 Plant extracts of (a) Atis (*Annona squamosa*), (b) Guyabano (*Annona muricata*), (c) tubers of Nami (*Dioscorea hispida*), (d) Akleng-parang (*Albizia procera*), (e) Lantana (*Lantana camara*), (f) Papaya (*Carica papaya*), (g) Kakawate (*Girardinia septium*) & (h) Lemon grass (*Cymbopogon citratus*).

### Application of test plant extracts

The prepared plant extracts (Figure 2) were used separately as treatments. Each treatment is composed of five replications, and each replicate is represented with five larvae as samples. Point five (0.5) milliliter (ml) of pure extract diluted to 0.5 ml of distilled water to have one (1) ml of solution and sprayed into each petri dish containing the larvae samples. Each treatment was replicated for the experiment using adult *C. capito* and represented each replicate with five adults. The extracted amount was sprayed for each adult bottle as a replicates sample. The three plant extracts that exhibited the best results in the laboratory assay using adult *C. capito* P. were subjected to the field cage experiment.

## DATA GATHERED

### A. Larvicidal assay for screening botanical insecticides

About 225 reared 5th instar larvae were evaluated for the larvicidal effect of the prepared plant extract solutions. A total of 675 larvae were utilized for the entire experiment. Five representative larvae were placed in a clean petri dish. One ml concentration of 50% distilled water and 50% botanical plant crude extract (1:1, v/v) was sprayed into the larvae using a calibrated hand sprayer with a spray rate of 0.20 mL/second. On the other hand, 1 mL of distilled water was separately sprayed on larvae and served as the control treatment. The response and effect of plant extracts on *C. capito* larvae were observed and recorded. The same procedures were done in the succeeding experiment. Two trials were done in this test to verify how effective are the plant extracts to larvae. The larvae mortality was recorded from 5 minutes after the plant extracts application up to 72 hours.

## **B. Laboratory Screening of Botanical insecticide using adult *C. capito***

Previously reared adult *C. capito* was placed in a clean bottle with a single fresh twig of *A. macrophylla*. One mL of each plant extract solution was sprayed into the insect and allowed the solution to dry in the insect's body for about 5 min. After which, constant monitoring of the insect response to the tested plant extract solution until 72 hours was evaluated. The three plant extract solution that gave the best result was utilized in the succeeding experimentation.

## **C. Evaluation of effective botanical insecticide using cage method.**

The three (3) botanical insecticides that gave the best results and were effective against *C. capito* during laboratory assay were further tested under natural conditions using the cage method utilizing adult *C. capito* P. as the test insect. Each treatment had five replications, and each cage represented a replicate. Each cage was placed with five two years old *A. macrophylla* seedlings and served as the host plant of *C. capito* P. during the testing. The stomach and contact action of the botanical extracts were separately tested against adult *C. capito* as follows:

**For stomach action.** Twenty (20) mL of the prepared botanical extract solution were sprayed on the seedlings using a 30 ml calibrated hand sprayer and allowed to stick into the plant for 5 min. Afterward, five adult *C. capito* were released into each cage, allowing them to feed on the *A. macrophylla* seedlings. The repellency behavior and mortality of *C. capito* P. were monitored and recorded after 3 min, 5 min, 15 min, and an hour after insect release, then every 4 hours after that until 72 hours. The ambient temperature was monitored and recorded every reading period, which ranged from 26-30 degrees Celsius.

**For contact action.** Five *C. capito* adults were released into the cage and allowed to acclimatize under cage conditions for 24 hours. After which, 20 mL of the prepared botanical extracts were sprayed on the seedlings using a 30 ml hand sprayer. The repellency behavior and mortality of *C. capito* P. were monitored and recorded after 3 min, 5 min, 15 min, and an hour after insect release, then every 4 hours after that until 72 hours. The ambient temperature was monitored and recorded every reading period, which ranged from 26-30 degrees Celsius.

## **Statistical Analysis**

The significance of treatments was interpreted and analyzed using Analysis of Variance (ANOVA). Further, the significant differences between treatment means were computed using DMRT at a 1% significance level.

## **RESULTS AND DISCUSSION**

### **Selection of plant extracts preparation for bioassay**

The activity of crude plant extracts is often attributed to the complex mixtures of active compounds. The preliminary screening revealed the potential larvicidal activity of plants extracted using three (3) different solvents. One hundred percent (%) larval mortality was observed in methanol-based extracts, while 84% and 0 % mortality were observed using acetone and water, respectively (Table 1).

**Table 1: Preliminary Screening of Larvicidal Activity of Plants Using Different Extraction Solvents against 5<sup>th</sup> Larval Instar of *Callimetopus capito*.**

Plant species	Plant part used	% Mortality*		
		Distilled Water	Acetone**	Methanol***
<i>T<sub>2</sub> - Annona squamosa</i>	Seeds	0	84 ± 8.944	100
<i>T<sub>3</sub> - Annona muricata</i>	Leaves	0	40 ± 14.142	60 ± 14.142
<i>T<sub>4</sub> - Dioscorea hispida</i>	Tuber	0	0	0
<i>T<sub>5</sub> - Albizia procera</i>	Leaves	0	60 ± 20.00	100
<i>T<sub>6</sub> - Lantana camara</i>	Leaves	0	40 ± 14.142	60 ± 14.142
<i>T<sub>7</sub> - Carica papaya</i>	Leaves	0	80 ± 14.142	84 ± 8.944
<i>T<sub>8</sub> - Gliricidia sepium</i>	Leaves	0	40 ± 14.142	64 ± 8.944
<i>T<sub>9</sub> - Cymbopogon citratus</i>	Leaves	0	40 ± 14.142	60 ± 20.00

\*mean value of 5 replications

\*\**sd* = 11.072

\*\*\**sd* = 9.640

Given the result of the preliminary testing, plants were extracted using MeOH and used in the succeeding experimentation. The larvicidal assay (Table 2) was done with 5th instar *C. capito* larvae using eight (8) plant extracts and control. All the plant extracts showed moderate to highly toxic effects on *C. capito* larvae. Among the plant extracts, *Annona squamosa* (T<sub>2</sub>), *Carica papaya* (T<sub>7</sub>), *Albizia procera* (T<sub>5</sub>), and *Annona muricata* (T<sub>3</sub>) were significantly found with the highest mortality of 80%, 64%, 60%, and 52%, respectively after 38-72 hours exposure ( $F_c = 85.3870 > F_{tab} = 3.04$ ). Fifth instar *C. capito* larvae weakened upon contact with the *A. squamosa*, *C. papaya*, *A. procera*, and *A. muricata* extract solution and did not immediately arrest the insect larvae exposed to *D. hispida*, *L. camara*, *C. citratus*, and the control.

This observation conforms to the studies conducted for some Anopheles insects. Several studies have shown that leaf and bark extract of *A. squamosa* has antifeedant and larvicidal activities against other insects, such as Anopheles species (Kikampa et al., 2009; Saxena et al., 1993; Kamaraj et al., 2010;). Various studies on the insecticidal activity of the seed extracts of *A. squamosa* are attributable to annonins (i.e., annonin I = squamocin), adjacent bis-tetrahydrofuran (T.H.F.) ring acetogenins (Leatemia, 2004).

**Table 2: Percentage (%) Mortality of *C. capito* 5<sup>th</sup> Instar Larvae and Time of Exposure to Selected Plant Extracts in Trial 1**

Plant extract treatment	Exposure time hours(hr.)	% Mean mortality	Transformed mean value
T <sub>1</sub> - Control (distilled water)	72	0	0.000 f
<i>T<sub>2</sub> - Annona squamosa</i>	67	80 ± 14.142	1.897 a
<i>T<sub>3</sub> - Annona muricata</i>	70	52 ± 10.954	1.708 abcd
<i>T<sub>4</sub> - Dioscorea hispida</i>	72	28 ± 10.954	1.421 e
<i>T<sub>5</sub> - Albizia procera</i>	65	60 ± 14.142	1.768 abc
<i>T<sub>6</sub> - Lantana camara</i>	72	32 ± 10.954	1.482 e
<i>T<sub>7</sub> - Carica papaya</i>	54	64 ± 16.733	1.793 ab
<i>T<sub>8</sub> - Gliricidia sepium</i>	38	40 ± 14.142	1.577 bcde
<i>T<sub>9</sub> - Cymbopogon citratus</i>	72	24 ± 8.944	1.396 e

*sd* = 11.218

The *Callimetopus capito* P. larvae responded and exhibited mortality at a different exposure time than the average time when the larvae were mainly arrested. It showed that *Gliricidia sepium* (T8) had the shortest exposure time of 38 hr. but with less mortality of 40 % compared to other plant extracts with a longer exposure time of 67 hr., 54 hr., and 65 hr., but with high mortality of 80%, 64%, and 60% in *Annona squamosa* (T2), *Carica papaya* (T7), and *Albizia procera* (T5) respectively. Other plant extracts such as *Cymbopogon citratus* (T9), *Lantana camara* (T6), *Dioscorea hispida* (T4), and distilled water (T1) had 72 hrs exposure with 24 %, 32%, 28%, and 0% mortality.

The same procedure was utilized on the second trial (Table 3) to test the prepared MeOH-based plant extracts' effect on *C. capito* larvae. Consistently observed, similar to the first trial, *A. squamosa* (T2) showed the highest toxicity among tested plant extract solutions with an average mortality rate of 72%. In addition, *C. papaya* (T7), *A. procera* (T5), and *G. sepium* (T8) were found to be equally effective, with an average mortality of 60%, 56%, and 52%, respectively. On the contrary, the application of *A. muricata* (T3), *D. hispida* (T4), *L. camara* (T8), and *C. citratus* (T9) was found to be less effective as compared to other botanical extracts.

**Table 3: Percentage (%) Mortality of *C. capito* 5<sup>th</sup> Instar Larvae and Time of Exposure to Selected Plant Extracts in Trial 2**

Treatment	Exposure time hours (hr.)	% Mean mortality	Transformed mean Value
T <sub>1</sub> - Control (distilled water)	72	20 ± 8.944	1.301 f
T <sub>2</sub> - <i>Annona squamosa</i>	65	72 ± 10.954	1.850 a
T <sub>3</sub> - <i>Annona muricata</i>	71	32 ± 17.889	1.460 e
T <sub>4</sub> - <i>Dioscorea hispida</i>	72	28 ± 10.954	1.420 e
T <sub>5</sub> - <i>Albizia procera</i>	64	56 ± 16.733	1.730 abc
T <sub>6</sub> - <i>Lantana camara</i>	72	36 ± 8.944	1.540 cde
T <sub>7</sub> - <i>Carica papaya</i>	54	60 ± 14.142	1.770 ab
T <sub>8</sub> - <i>Gliricidia sepium</i>	67	52 ± 10.954	1.710 abcd
T <sub>9</sub> - <i>Cymbopogon citratus</i>	65	44 ± 21.909	1.520 cde

sd = 13.492

Several reports and studies concerning the effects of *A. squamosa* against insect larvae of the diamondback moth *Plutella xylostella* L. (Leatemia et al., 2004) and *Culex quinquefasciatus* (George et al., 2005). Various studies also proved the topically applied seed extract caused a steady decline of all free amino acids in freshly emerged 5th-instar larvae of *Dysdercus koenigii*. (Kotkar et al, 2002). The crude methanolic seed extracts of *A. squamosa* were also ~10 times more active as a feeding deterrent of third-instar *Trichoplusia ni* (Hübner) larvae in a leaf disc choice bioassay. They were more toxic through feeding (LC50 = 167.5).

The results in laboratory tests trials conform to the various studies on the laboratory and field tests of *Annona squamosa* L. seed extracts that were effective against crop pests like spotted stem borer, *Chilo partellus*, leafhopper *Nilaparvata lugens*, *Spodoptera litura*, *S. frugiperda*, *Helicoverpa armigera*, hairy caterpillar, *Spilosoma oblique*, Brinjal spotted leaf beetle, *Henosepilachna vigintioctopunctata*, cotton ball worm, *Dysdercus koenigii*, semi-looper

*Achaea janata* Linn. and Aphids (Khalequzzaman et al., 2006). The results of these studies only proved that MeOH seed extract of *A. squamosa* was most effective in controlling insect larvae (Figure 3).

#### LABORATORY ASSESSMENT OF BOTANICAL INSECTICIDES USING ADULT *C. CAPITO*

Among the plant extract solution tested (Figure 4), *A. squamosa* (T2) followed by *C. papaya* (T7), *A. procera* (T5), and *A. muricata* (T3) manifested significantly comparable control against *C. capito* adult with an average mortality of 72%, 68%, 60%, and 48%, respectively. Among the four, *A. squamosa* outstood as it killed 72% of tested adult borers in the shortest period possible. The mortality caused by these plant extracts was significantly different from the other treatments. Hence, applying other plant extracts to adult *C. capito* also affects them with lesser mortality.

**Table 4: Percentage (%) Mortality of adult *C. capito* and time of exposure to selected plant extracts of *C. capito*.**

Treatment	Exposure time hours (hr.)	% Mean mortality	Transformed mean value
T <sub>1</sub> - Control (distilled water)	72	0	0.000 f
T <sub>2</sub> - <i>Annona squamosa</i>	50	72 ± 22.803	1.837 a
T <sub>3</sub> - <i>Annona muricata</i>	67	48 ± 10.954	1.672 abcd
T <sub>4</sub> - <i>Dioscorea hispida</i>	72	44 ± 16.733	1.517 de
T <sub>5</sub> - <i>Albizia procera</i>	49	60 ± 20.00	1.758 abc
T <sub>6</sub> - <i>Lantana camara</i>	61	24 ± 8.944	1.361 e
T <sub>7</sub> - <i>Carica papaya</i>	49	68 ± 17.888	1.823 ab
T <sub>8</sub> - <i>Gliricidia sepium</i>	48	36 ± 16.733	1.517 de
T <sub>9</sub> - <i>Cymbopogon citratus</i>	72	28 ± 10.954	1.421 e

*sd* = 15.626

Table 4 showed the shorter exposure time of adult *C. capito* to plant extracts resulted in high mortality of insects as in T2, T5 and T7 with 50, 49, and 49 hours of exposure, respectively.

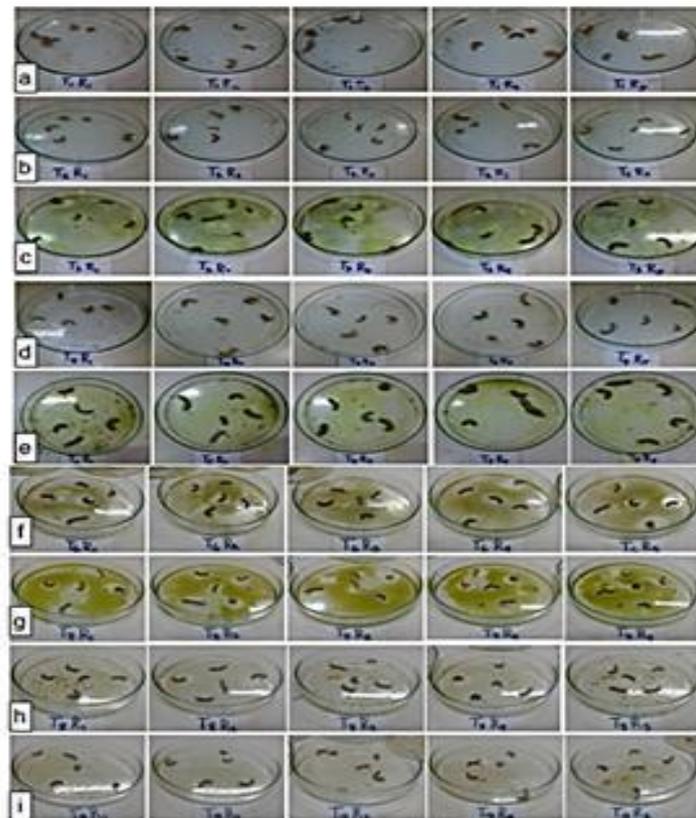


Figure 3. Larvicidal Assay of Botanical Plant Extracts with 9 Treatments and 5 Replicates ; a) Water, b) Atis (*A. squamosa*), c) Guyabano (*Annona muricata*), d) tubers of Nami (*Dioscorea hispida*), e) Akleng-parang (*Albizia procera*), f) Lantana (*Lantana camara*), g) Papaya (*Carica papaya*), h) Kakawate (*Gibberichia septum*) & i) Lemon grass (*Cymbopogon citranus*).

### Evaluation of effective botanical insecticide using cage method

The first three plant extracts that gave superior results in adult *C. capito* assay were tested and applied in the cage experiment. These were the *A. squamosa* (T2), *C. papaya* (T7) and *A. procera* (T5) plant extracts and the control (T1).

Some literature mentioned that, for a botanical pesticide to be effective, it must control at least 50% of the pest population for a given exposure time. For the first trial (Table 5), *A. procera* (T3) killed 64% of the treated *C. capito*, which has a comparable effect with *A. squamosa* (T2) and *C. papaya* (T4), with an average mortality of 48%. Given the environment simulating the prevailing weather condition and conditions appropriate for insect development, *A. procera* extract solution gradually killed the insect, as it took 51.76 hours before it died. On the other hand, the shortest time exposure is needed to kill 48% of the adult insects using *C. papaya* extracts.

**Table 5: Percentage (%) Mortality of Adult *C. capito* and Time of Exposure to Selected Plant Extracts under Contact Action in Trial1**

Treatment	Average exposure time (hours)	% Mean mortality	Transformed mean value
1-Distilled Water	72	0	0b
2- <i>Annona squamosa</i>	53.2	48 ± 10.954	1.67a
3- <i>Albizia procera</i>	51.76	64 ± 26.077	1.78a
4- <i>Carica papaya</i>	34.87	48 ± 17.889	1.65a

sd = 18.306

**Table 6: Percentage (%) Mortality of Adult *C. capito* and Time of Exposure to Selected Plant Extracts under Contact Action in Trial 2**

Treatment	Exposure time (hours)	% Mean mortality	Transformed mean value
1-Distilled Water	72	0	0c
2- <i>Annona squamosa</i>	33.87	52 ± 22.804	1.672a
3- <i>Albizia procera</i>	17.5	24 ± 8.944	1.361b
4- <i>Carica papaya</i>	25.66	28 ± 10.954	1.421ab

sd = 14.234

In the second trial, *A. squamosa* showed a superior effect in controlling adult *C. capito*. 52% of the sprayed adult insect died after 33.87 hours of exposure to the solution. *A. procera* showed the shortest exposure time to kill the insect; however, 24% of the treated insects only died.

Among the four treatments, *A. squamosa* plant extracts had effectively caused the death of adult *C. capito* over the other plant extracts and control. This result showed that the longer exposure of adult *C. capito* to *A. squamosa* extracts revealed the highest mean mortality of 52 % but a comparable effect on those affected with *Carica papaya*. The result further implied an instantaneous effect through contact action might not be possible using botanical plant extracts. It took effect progressively through the release or chemical reaction of compounds to the insect's body, causing the death of insects.

The application of four treatments in the field, the distilled water (T1) *A. squamosa* (T2), *A. procera* (T3), and *C. papaya* (T4), resulted to mean values of 2.2353, 1.4712, 1.4712 and 0.7071 with 27, 19, 34 and 72 hours exposure respectively (Table 7). The % mean mortality obtained in T1, T2, T3, and T4 were 0, 8.0, 4.0, and 4.0 %, respectively. Based on the analysis of variance (ANOVA), these results revealed that the application of botanical extracts 5 minutes before the release of adult *C. capito* to cages did not affect the mortality of insects. These results

showed that effective against the control of adult *C. capito*. Figure 6 shows some of the damages in *A. macrophylla* seedlings caused by *C. capito* P.

**Table 7: Percentage (%) Mortality of Adult *C. capito* and Time of Exposure to Selected Plant Extracts under Stomach Action**

Treatment	Exposure time (hours)	% Mean mortality	Transformed mean value
1-Distilled Water	72	0	0
2- <i>Annona squamosa</i>	27	8.0 ± 11.00	2.2353
3- <i>Albizia procera</i>	19	4.0 ± 8.90	1.4712
4- <i>Carica papaya</i>	34	4.0 ± 8.90	1.4712

sd = 9.614/

**Table 8: Percentage (%) Repellency of Adult *C. capito* Under Contact Action in trial 1.**

Treatment	% Mean repellency	Transformed mean value
1-Distilled Water	0	0d
2- <i>Annona squamosal</i>	80 ± 14.142	1.79c
3- <i>Albizia procera</i>	64 ± 16.733	1.89ab
4- <i>Carica papaya</i>	84 ± 8.944	1.92a

sd = 13.273

After applying selected plant extracts to adult *C. capito*, the insect was repelled from *A. macrophylla* seedlings. The insects repelled due to botanical extracts' toxic and unfavorable effects on insects. The highest repellency of 84% was obtained from applying *C. papaya* extract solution (Table 8). At the same time, *A. squamosa* and *A. procera* received 80% and 64% repellence, respectively.

Various studies proved that papaya leaf extract is an effective insecticide for aphids with an LC50 value of 87.0 ppm. And to *A. gossypii*, *E. vittella*, *P. puncticollis*, *B. tabaci* (Zobayer & Hasan, 2013), fall armyworm, and *Sitophilus zeamai* (Muzemu, 2013). *C. papaya* plant extract effects after 24 h of exposure. However, the highest larval and pupal mortality is found in the leaf extract of methanol papaya against the first- to fourth-instar larvae (Kovendan et al., 2012). These insecticidal effects are attributed to the toxic compounds in papaya leaf extract alkaloids, such as carpaine papain and cyanogenic glycosides (De Conceicao, nd).

**Table 9: Percentage (%) Repellency of Adult *C. capito* Under Contact Action in Trial 2**

Treatment	% Mean repellency	Transformed mean value
1-Distilled Water	0	0
2- <i>Annona squamosal</i>	52 ± 17.888	1.727ab
3- <i>Albizia procera</i>	60 ± 20.000	1.767a
4- <i>Carica papaya</i>	60 ± 14.142	1.757ab

sd = 17.343

Table 9, trial 2 shows the percentage repellency of adult *C. capito* under contact action revealed that after batino seedlings were applied with extracts of *C. papaya* (T4) and *A. procera* (T3) had equally obtained the same actions. The highest % mean repellency was obtained in T3 and T4 with 60%, while 52% was only in T2. Whereas no insects were repelled when applied with distilled water (T1). Extracts of seeds of *A. squamosa* had repellent and anti-oviposition properties when applied to *Ceratitis capitata* (Epino, 1991). The *A. squamosa* was more toxic through feeding at LC50 = 167.5 ppm.

**Table 10: Percentage (%) Repellency of Adult M.T.B. Under Stomach Action**

Treatment	% Mean repellency	Transformed mean value
1-Distilled Water	0	0d
2- <i>Annona squamosa</i>	76 ± 26.077	1.86abc
3- <i>Albizia procera</i>	60 ± 31.623	1.72abc
4- <i>Carica papaya</i>	76 ± 26.077	1.86abc

sd = 27.925

The *A. macrophylla* seedlings sprayed with *A. squamosa* (T2), and *C. papaya* (T4) extracts obtained the highest mean repellency of 76%, with a mean value of 1.86 over T3 and T1. Whereas *A. procera* (T3) and Distilled water (T1) obtained the least mean repellency of 60% and 0% with a mean value of 1.72 and 0, respectively. However, adult M.T.B. repelled all plant extracts when sprayed inside the cages 5 minutes after the release of insects to *A. macrophylla* seedlings. These results showed that plant extracts contained feeding deterrent compounds that prevent insects from feeding into the plant.

## CONCLUSION

Extracts of *Annona squamosa* (T2), *Carica papaya* and (T7), and *Albizia procera* (T5) were significantly found to be highly toxic to M.T.B. 5<sup>th</sup> instar larvae and adults that caused their high mortality. However, treatments *A. muricata* (T3), *D. hispida* (T4), *A. muricata* (T5), *L. camara* (T6), and *G. sepium* (T8) exhibited moderate to low toxicity effects as compared to

distilled water (T1) with zero impact. In the cage experiment, the stomach mode of action was not significantly effective in arresting adult M.T.B. whereas, the application of plant extracts as stomach poison showed less effectiveness against the insect pest.

## RECOMMENDATION

The usage of botanical plant extracts in the protection against M.T.B. (*Callimetopus capito* Pascoe) attacking batino seedlings should be applied under the following conditions;

1. To fully utilize the result of this study, the active alkaloid causing the death of larvae and adult *C. capito* P. should be tested in the scientific laboratory;
2. Establish the minimum concentrations of botanical insecticide using the same plant extracts.
3. Further study on the toxicity of botanical plant extracts against *C. capito* P. should be conducted since the indigenous forest tree batino is being massed produced not only in the province but also in the region because of its metallophytic properties;

## References

1. Adoro, L. C. , Domingo, F.A., Baysa, J.M., dela Crus, B.N., Melegrito, C.P., Roxas, A.C., Lutap, L.A., Dacayo, N.A., Farin, E.N., Garcia, S.S., & Ico, R.R. (2008). Bio-ecology and control of mango twig borers *Niponoclea capito* (Pascoe) and *N. albata* (Newman), Coleoptera, Cerambycidae. PCAARRD Highlights Pp 89-92. DOST.
2. Agrawal, D. (2014). Why is methanol generally used as a first solvent for extraction purpose to look for bioactives in medicinal plants? Indian Institute of Petroleum (I.I.P.). Retrieved from <https://www.researchgate.net/post/>. Retrieved on November 15, 2015.
3. Claveria, R., De los Santos, C., Teodoro, K., Rellosa, M., & Valera, N. (2010). The Identification of Metallophytes in the Fe and Cu Enriched Environments of Brookes Point, Palawan and Mankayan, Benguet and their Implications to Phytoremediation. *Science Diliman* 21(2): 1-12. National Institute of Geological Sciences. UP, Diliman 1101, Q.C.
4. De Conceicao, P. (nd.). The Properties of Papaya Leaf insecticide. Retrieved from [http://www.ehow.com/list\\_6790220\\_properties-papaya-leaf-insecticide.html](http://www.ehow.com/list_6790220_properties-papaya-leaf-insecticide.html). Retrieved on December 2015.
5. Dos Santos, P.C. (2014). Why is methanol generally used as a first solvent for extraction purpose to look for bioactives in medicinal plants?. Instituto Nacional de Investigação Agrária e Veterinária (INIAV). Retrieved from <https://www.researchgate.net/post/>.
6. Ecosystems Research and Development Bureau. (2012). Rehabilitation and ecological restoration R and D for marginal and degraded landscapes and seascapes: A research compendium of rehabilitation strategies for mining and volcanic debris-laden areas. ERDB-DENR, College, Laguna.
7. Epino, P. (1991). Insecticidal activity and physiological property of *Annona squamosa* L. seed extracts againsts the Mediterranean fruitfly, *Ceratitis capitata*. Thesis (PhD) University of Hawaii at Manoa. 1991. Retrieved from <http://scholarspacea.manoa.hawaii.edu/handle/10125/9770>.
8. George,S.&Vincent,S. (2005). Comparative efficacy of *Annona squamosa* L inn.and *Pongamiaglabra* Vent. to *Azadirachta indica* A. Juss against mosquitoes. *J Vect Borne Dis* 42, 159–163.

9. Kamaraj C, Abdul Rahman A, Bagavan A, Abdur Zahir A, Elango G, Kandan P, et al. (2010). Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus*(Diptera: Culicidae) Trop Biomed. 2010; 27:211–9.
10. Khalequzzaman, M. & Shajia, S. (2006). Insecticidal activity of *Annona squamosa* L. seed extracts against the red flour beetle, *Tribolium castaneum* (herbst) J. bio-sci. 14: 107-112, 2006 ISSN 1023-8654, Rajshahi 6205, Bangladesh.
11. Kihampa C, Joseph C, Nkunya MH, Magesa SM, Hassanali A, Heydenreich M, et al. Larvicidal and I.G.R. activity of extract of Tanzanian plants against malaria vector mosquitoes. J Vector Borne Dis. 2009; 46:145–52.
12. Kotkar, H.M., Mendki, P.S., Sadan, S.V., Jha, S.R., Upasani, S.M., & Maheshwari, V.L. (2002). Antimicrobial and pesticidal activity of partially purified flavonoids of *Annonasquamosa*. PestManagSci.58(1), 33-7.
13. Kovendan, K., Murugan, K., Kumar,A., Vincent, S., Hwang, J. (2012). Bioefficacy of larvicidal and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). 110(2), 669-78. PMID: 21750871.
14. Leatemia , J.A. & Isman, M.B. (2004). Insecticidal activity of crude seed extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. Entomology phytoparasitica 32(1), 30-37.
15. Muzemu, S., Chitamba, J., Mutetwa, B. (2013). Evaluation of *Eucalyptustereticornis*Tagetesminuta and *Carica papaya* as stored maize grain protectants against *Sitophiluszeamais* (Motsch.) (Coleoptera: Curculionidae). Agriculture, Forestry and Fisheries 2(5), 196-201 doi: 10.11648/j.aff.20130205.13.
16. Saxena RC, Harshan V, Saxena A, Sukumaran P, Sharma MC, Kumar ML. Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. J Am Mosq Control Assoc. 1993; 9:84–7.
17. Whiting, S. N.,Reeves, R. D.,Richards, D.,Johnson, M. S.,Cooke, J. A.,Malaisse, F.,Paton, A., Smith, J. A. C.,Angle, J. S.,Chaney R. L., Ginocchio, R., Jaffre, T. Johns, R., McIntyre, T., Purvis, O. W.,Salt, D. E., Schat, H., Zhao, F. J.& Baker, A. J. M. (2004). Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. Restoration ecology 12(1), 106-116. Doi:10.1111/j.10612971.2004.00367.x
18. Zobayer, N., Hasan, R. (2013). Effects of Manually Processed Bio-pesticides on Crop Production and Pest Managements in Okra (*AbelmoschusEsculentus* (L.) Moench). Journal of natural sciences research.3(8). Retrieved from <http://iiste.org/Journals/index.php/JNSR/article/view/6913>.