Chlorophyll Content, Cell Size, Growth and Resistance to Coconut Scale Insect (*Aspidiotus rigidus* Reyne) of Buri Palms (*Coryphautan* Lam.) as Influenced by Different Hoagland Concentrations

Lourdes B. Tayrus[1] Mark Joseph J. Buncag[1,2*]

[1]*Faculty, Environmental Science, Forestry and Graduate Departments, College of Forestry and Environmental Studies, Mindanao State University Main Campus, Philippines*

[2]*Associate Member, Social Sciences Division, National Research Council of the Philippines (NRCP), Philippines*

Abstract *- Buri palms (Coryphautan Lam.) is the third most important non-timber forest resource of the Philippines because of its multiple uses. It is now being threatened by the coconut scale insect (Aspidiotus rigidus Reyne) epidemic due to its similarity to coconut palms. In order to protect this very important resource, this research was carried out to determine the effect of different Hoagland concentration on the growth and resistance of buri palms to coconut scale insects. Chlorophyll content was determined using Apogee CCM-200 plus Chlorophyll meter. Cell size of the mesophyll layer and leaf thickness was measured using Image J (1.43m) software. Similarly, shoot length, root length, leaf area and plant biomass was measured. Coconut scale insect count was also performed. Chlorophyll content and leaf thickness were unaffected while cell sizes of the mesophyll layer were significantly increased with varying Hoagland concentration. Interestingly, insect count was significantly affected by the treatment. Plants grown in 50% Hoagland solution gave the highest insect population at 71, twice as much as that of 100% concentration with only 36. Treating the plant with 100% Hoagland solution induced the formation of raphides crystals of calcium oxalate on the leaf surface. This provided an added protective layer on the leaf that made it less preferred by the insect. Therefore, improving the nutrition of the plant is a better and safer alternative in reducing the susceptibility of buri palms to coconut scale insect attack.*

Keywords: *Chlorophyll count index, coconut scale insect, experimental design, Aspidiotus rigidus, Coryphautan, Hoagland's solution, plant nutrition, calcium oxalate*

I. INTRODUCTION

Buri (*Coryphautan*Lam) is regarded as the third most important non-timber forest product in the Philippines. It is a perennial, monocarpic plant [1] that is economically significant for its multiple uses. The most important product is the buntal fibers [2] used extensively in making export quality and environment-friendly hats, bags, placemats, and braids. These products are exported to Australia, Japan, United Kingdom, United States of America, Spain and Germany [3] with a gross worth of about $53 million [4]. Though not listed in the IUCN threatened species, as one of the major dollar earners of the country, this palm should be protected.

The major problem of the buri industry is the inadequacy of the supply of raw materials. There are no known records of buri plantation in the Philippines. Thus, most of the supply comes from the wild. In addition, buri is a slow growing plant which could only be propagated by seeds. This palm undergoes a grass stage for two to three years and it takes eight to ten years before it can be harvested [5]. Now, one alarming threat to the industry is the coconut scale insect (*Aspidiotus rigidus* Reyne*)* infestation. It is an epidemic spreading to the countryside that has ruined tens of thousands of coconut plantation. Being similar to coconut palms and their proximity with one another, buri palms are also vulnerable to insect infestation. Once infested, the leaves wither, reducing the capacity of the plant to photosynthesize leading to its death. The infestation is further promoted by the El Niño phenomenon characterized by prolong droughts due to climate change [6,7]. It was a serious pest reported in 1947 in Sangi Island, Indonesia and Mindanao, Philippines [8,9]. Another concern was the highly polyphagous nature of the pest [10]. It does not only feed on palms [11] but also other tropical crops like rubber, cacao, mangosteen, and banana.

There are several strategies developed to solve the insect problem: biological [12-14], cultural [15], chemical [16] and systemic control [17]. Among the possible solutions, the latter technique has gained more support because of its 80 – 90% success rate [6]. In this method, a potent pesticide would be injected directly to the stem of the palm. As the insects feed on the leaves, it would siphon the chemicals from the plant's sap leading to their death. However, because the chemicals would be absorbed by the plant, humans are at risk because we consume the sap, shoots and nuts of the palms. A safer way of reducing the susceptibility of Buri palms to scale insect attack is of paramount importance.

Studies showed that nutrient application does not only affect the growth of the plant but could also improve the plant's resistance to pests and diseases [18]. Plants have ways to defend itself from the attacks of pest [19] and it would be more effective when it is at the peak of its health. This study was conducted to test this hypothesis. Although there have been a number of studies concerning the use of nutrition on plant growth, there is still no study on how nutrition would affect the resistance of buri palms to coconut scale insect infestation.Thus,the study aimed to determine the effect of different Hoagland concentration on the growth and resistance of buri palms (*Coryphautan* Lam.) to coconut scale insects (*Aspidiotus rigidus* Reyne). Specifically, the study was aimed to determine the effect of different Hoagland solution on the chlorophyll count, cell size, leaf thickness, shoot length, root length, leaf area and plant biomass of buri palms, and on how this nutrient concentrate would influence the proliferation of the coconut scale insect.

II. MATERIALS AND METHODS

Species description and geographical distribution

Buri (C*oryphautan* Lam.) belongs to the Arecaceae family, the tallest and largest among the native palms growing in the Philippines [1]. It is widely distributed in the Provinces of Camarines Sur, Sorsogon, Capiz and Quezon [20]. It thrives in second-growth forests, near or along coastal plains and even in established coconut plantations [21]. It is also common in India, Sri Lanka, and Bangladesh and through other parts of Southeast Asia to tropical Australia.

Material preparation

Buri wildlings coming from the same mother were collected. The collected seedlings were potted immediately using sterilized topsoil in 5" x 8" polyethylene bags. Acclimatization was done for about one month following standard nursery preparation and silvicultural practices such as hardening and leaf pruning.

Main Treatment

The presence (+) and absence (-) of the insect were the main treatment of the experiment. Nine (9) samples were exposed to the insect while the other nine (9) were protected from it. Both set-ups were covered with mosquito nets to prevent the insect from transferring from one set-up to the other.

Hoagland application

The different Hoagland solution mixture was presented in Table 1. Hoagland was used because it could easily be absorbed by the plant [22]. The set-up was maintained in the screen house with natural light. Average temperature ranged from 27.22°C- 29.16°C with relative humidity of 67% [23]. Freshly prepared Hoagland solutions were added to the plants every time the level of the solution reached halfway to ensure the continued supply of the nutrients. In addition, six (6) drops of $FeCl_3$ were added three times a week to regulate the soil pH.

Table 1. The Hoagland's solution used in the study is composed of different chemicals combined to give 1.0L of full nutrient concentration.

CHEMICALS	AMOUNT(ml)
KNO_3	6.0
$CaNO_3$	4.0
$(NH_4)\ H_2PO_4$	1.0
$MgSO_4.H_2O$	2.0
Trace Element (H_2BO_3,$MnCl_2.6H_2O$,$CuSO_4.5H_2O$, $ZnMoO_4$,$7H_2O$,H_2MoO_4)	1.0
$FeCl_3$(0.5% solution)	6 drops(applied 3 times weekly

Chlorophyll content measurement

Chlorophyll content was measured using the Apogee Chlorophyll Meter (Apogee CCM-200 plus). This instrument provides the absorbance of wavelengths that were used to calculate the Chlorophyll Concentration Index (CCI). Average of 3 measurements was used in the evaluation.

Anatomical examination

A cross-section of a fully expanded young leaf were prepared using free-hand sectioning and observed under a light microscope. Leaf anatomy was described in terms of mesophyll, vascular and dermal tissues [24]. Digital images were taken and from these images, leaf thickness and cell size (mesophyll layer) were measured using the latest digital image software (Image J 1.43m).

Growth parameters

Initial measurements of shoot and root length in centimeters, leaf area in square centimeters were taken. Monthly measurements were performed for shoot length and leaf area. After three (3) months, final measurements of the individual growth parameters (root, shoot, and leaf area) were conducted including plant biomass.
Growth performance between the two treatments (with and without insect) was then compared by considering their percent differences e.g.:
Percent difference = (with insects - without insects)/without insects x 100%

Population count

After the treatment, the total number of scale insect was determined using a dissecting microscope. Average count of the insect was used in the analysis.

Statistical analysis

The research design considered two (2) main treatments (with [+] and without [-] insect) and three (3) sub-treatments (0%, 50%, 100% Hoagland solution). Hence, a 2 x 3 x 3 factor-factorial experiment in a Randomized Complete Block Design was used in the analysis where replication was also considered as a factor. Evaluation was performed at $\alpha = 95\%$.

III.RESULTS AND DISCUSSIONS

Morphological Attributes

The general appearance of buri seedlings treated with different Hoagland concentrations is shown in Figure 1. Shoot tip chlorosis and early senescence in young leaves were evident particularly in 0% Hoagland solution. In addition, signs of calcium deficiency as exhibit by the unexpanded leaves [25] were also observed in 0% Hoagland especially in the samples exposed to the scale insect. For 50% concentration, yellowing of the leaves was noticed. Finally, plants in the 100% solution were greener and fully expanded.

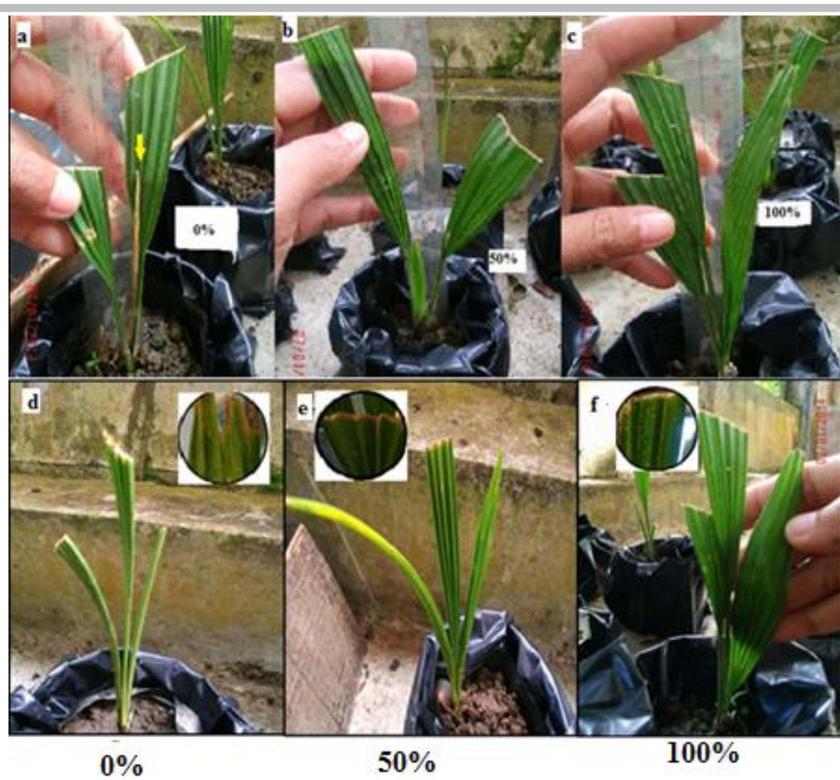

Figure 1.The appearance of buri seedlings grown under 0% (*column I*), 50% *(column II)* and 100% (*column III*) Hoagland solution concentrations.***Notes****: upper row (a, b, c)=without insects and lower row(d, e, f) =with insects (A. rigidus);arrow-death of shoot*

Influence of the treatments on Chlorophyll Content, Cell Size, and Leaf Thickness

Figure 2 depicts the response of chlorophyll content, cell size and leaf thickness on the different Hoagland solution for both treatments (with [+] and without [-] insect). A slight increase of the individual parameters was noticed as nutrition was enhanced except for the leaf thickness of samples with insect. In increasing the Hoagland concentration, nitrogen was improved [22]. Nitrogen is an essential element to plant growth, yield, and quality because of its role in cation-anion balance of the xylem and vacuoles [26]. More nitrogen would mean greener and healthier shoots which was clearly observed in the experiment.

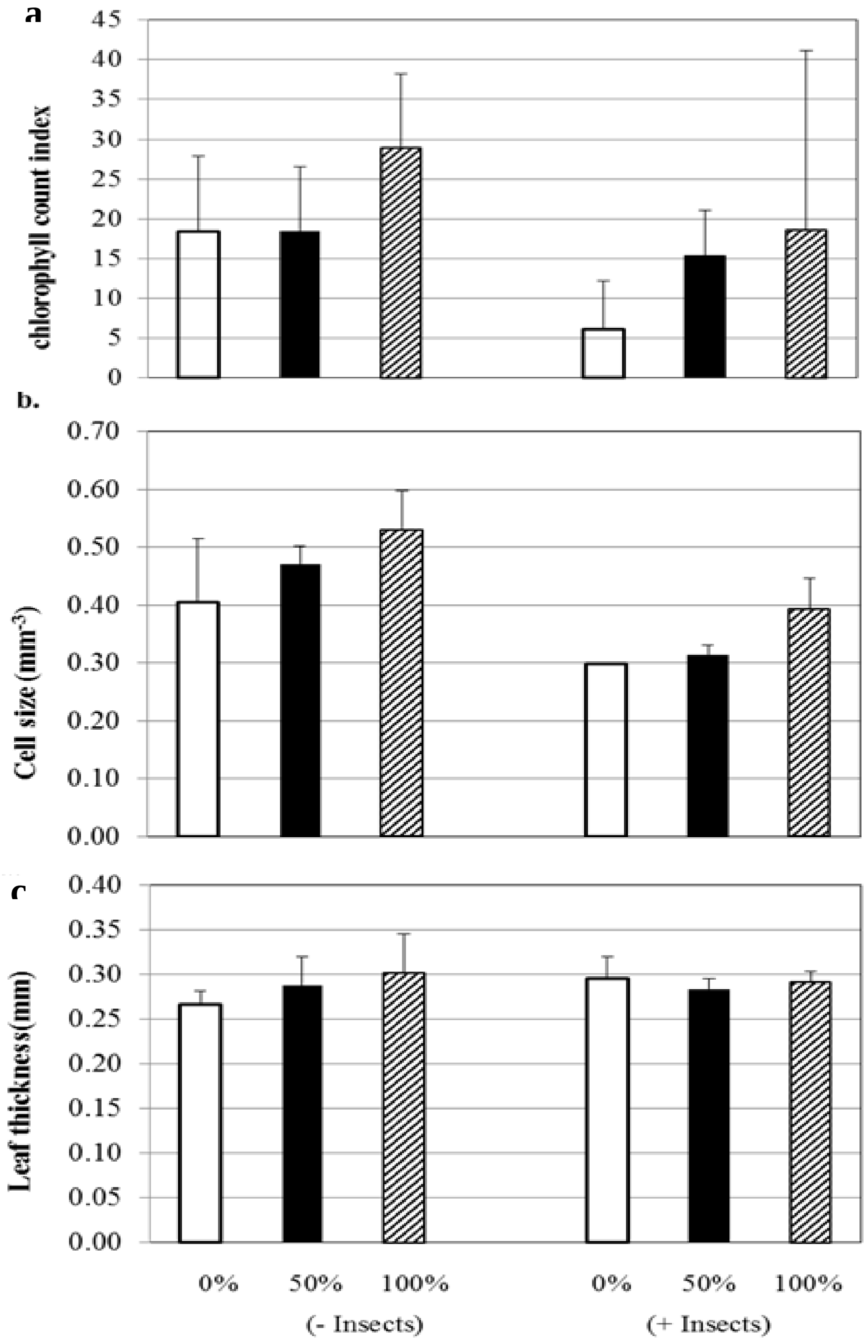

Figure 2. Chlorophyll count(***a***), cell size ***(b)***and leaf thickness **(*c*)** as influenced by nutrient concentrations and introduction of scale insects in buri seedlings after three months.

Influence of the treatments on the different growth parameters

Buri palms, just like any other palms, undergo grass/rosette stage [27]. At this time, the plant does not produce any stem or trunk. This was the reason why the plants were non-responsive to the Hoagland treatment at first. Growth and development could only be detected through their shoots and roots. A slight interaction was also observed in the samples without insects particularly on the shoot length, leaf area and root length (Fig. 3). The shoot length and leaf area of the samples with insect were significantly reduced reaching almost zero (0) at 0% Hoagland solution. The leaf and shoots of these plants failed to expand clearly exhibiting the symptoms of calcium deficiency. This was further exacerbated by the insect attack leading to the very low value of the 0% Hoagland solution. Interestingly, among the parameters measured, only root length of the samples without insect gave a very pronounced result. Samples treated with 100% Hoagland gave a value that was twice as much as of the 0% and 50% Hoagland. In fact, when the shoot-root ratio was considered, 100% Hoagland gave a root-shoot ratio of 0.97 as compared to the 0.65 ratio for both 0 and 50%. Apparently, the ammonium incorporated in the Hoagland solution provided the essential nutrients needed by the roots. This resulted to longer and healthier roots. Consequently, this would mean that the plant will be stronger to carry its weight when given 100% Hoagland concentration [28].

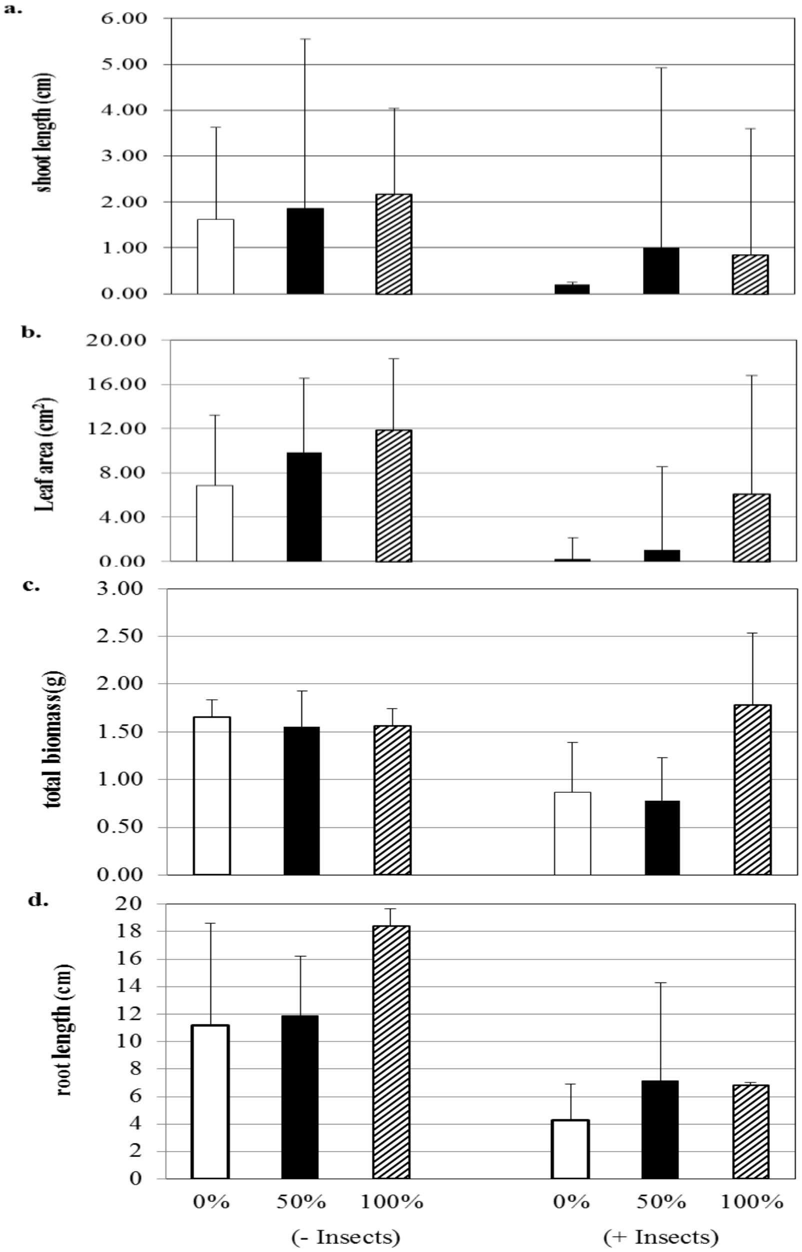

Figure 3.Growth increment in shoot length (***a***)*and* leaf area(***b***), together with total biomass (***c***) and root length (***d***)with and without insects under different Hoagland concentrations.(n=3: bar=standard deviation)

The coconut scale insect (Aspidiotus rigidus **Reyne)**

The *Aspidiotus rigidus* Reyne (Figure 4) is a voracious pest equipped with a proboscis used to suck the sap of plants resulting to the discoloration of its leaves [11]. The insect inserts its 0.5 mm long proboscis to penetrate even up to the chlorophyll- rich mesophyll layer of the leaves (Figure 5). These insects inject toxins from their salivary glands that could also destroy the chlorophyll pigments, therefore, reducing the ability of the plants to photosynthesize [10]. Mesophyll cells are normally dark green in color indicating the abundance of chlorophyll. These were visibly reduced by the presence of the insects while the plants with 100% concentration contained higher amounts of these green pigments (Figure 6).

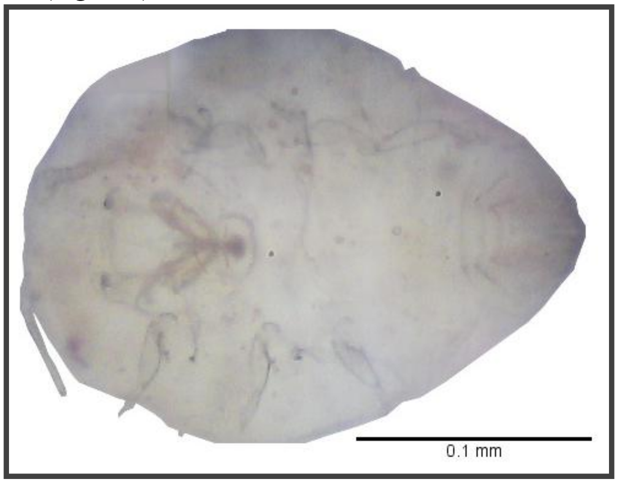

Figure 4. The adult coconut scale insect (Aspidiotus rigidus Reyne).

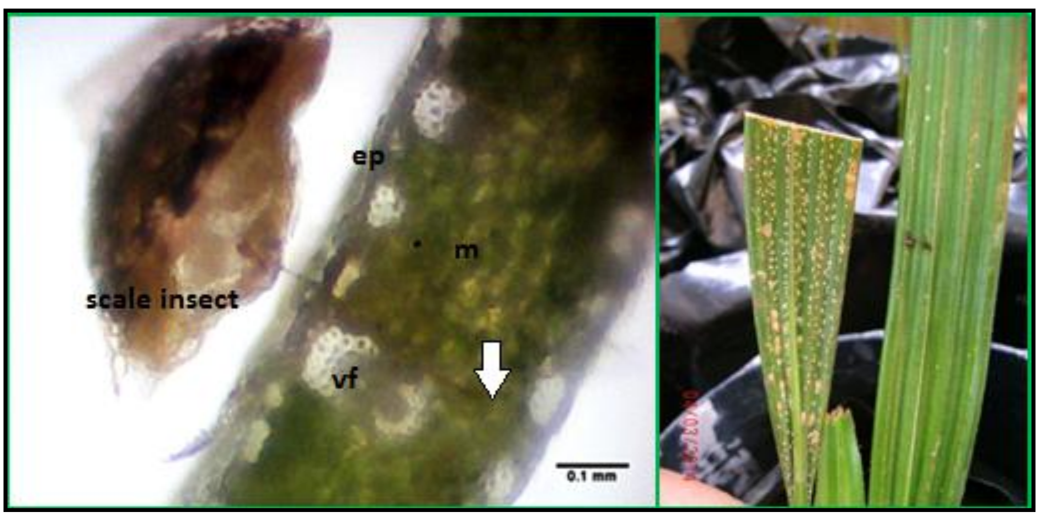

Figure 5.The mode of proboscis insertion (***a***) by *A. rigidus* along mesophyll layer of buri leaf (***b***). *Note:ep-epidermallayer;vf-vascularfiber;m-mesophyl layer containing chlorophyll; arrow-proboscis*

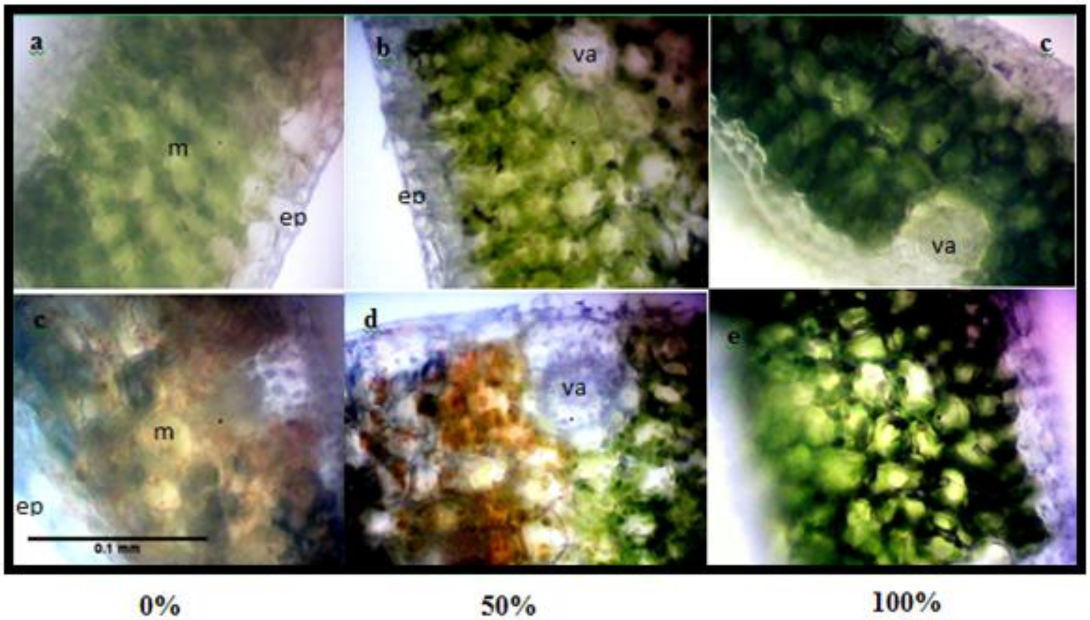

Figure 6. Variations in cross-sections of buri leaf tissue showing appearance of chloroplasts as affected by presence or absence of *A. rigidus* in seedlings grown under 0 (*columnI)* ,50(*column II)*and 100% (*column III)* nutrient concentrations respectively. *Notes : upper row(a, b, c) =without insects and lower row(d, e, f) =with insects); Epidermal layer(ep),mesophyll layer(m),vascular bundles(va).*

***Influence of Hoagland solution on Aspidiotus rigidus* Reyne.**

Figure 7 showed the influence of the Hoagland solution on the population of the scale insect. Among the three concentrations, 50% gave the highest insect count at 71. Interestingly, it was two times higher than those plant treated with 100% with 36 insects and 0% concentration with only 11. In a way, the insects tend to have avoided the plants with 0% concentration. This was but logical because the insect would also need nutrients for them to grow and survive. Depriving the plant with nutrients would starve the insects as well. However, it was interesting to note why the nutrient- rich plants that are more palatable to the insects were not the main preference of the pest?

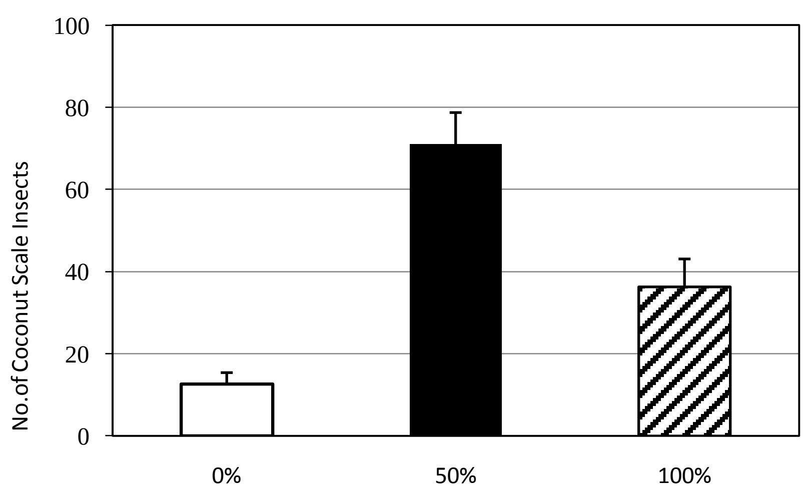

Figure 7**.** Population counts of *A. rigidus* in buri seedlings under different nutrient concentrations after three months.

Closer inspection of the leaves revealed the abundance of raphides crystals on the leaf surface of the plants treated with 100% Hoagland solution for both with or without insects (Fig. 8) which was absent in the 0 and 50% Hoagland. Palms have the inherent ability to adapt to the changing environment by producing defense mechanisms such as calcium oxalate crystals [29]. These crystals could be observed in the vegetative and reproductive organs of monocots [30]. They cause a stinging sensation that irritates and inflames the skin of would be attackers. Therefore, these crystals provided an added layer of protection to the plant against the attack of the insects. This was clearly observed in the study. Apparently the healthier the plant, the more resistant it was against the scale insect attack.

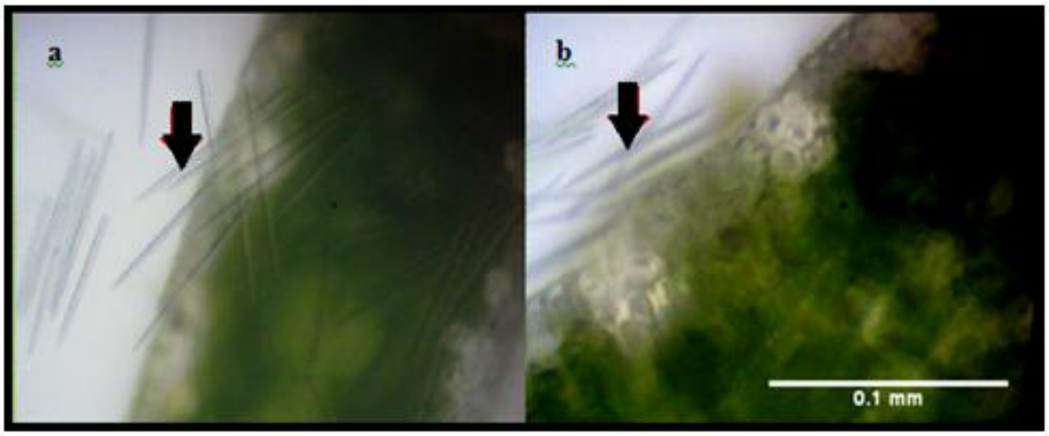

Figure 8. The calcium oxalate crystals (raphides) formed on the leaf surface of seedlings grown at 100% Hoagland concentration without insects (*a*) and with insects (*b*).Note*: arrow-raphides*

Statistical result

Statistical analysis (Table 2) proved that the growth of the buri palms was primarily affected by the presence of the insects at α = 95%. Varying the Hoagland concentration in a way improved growth; however, this was only secondary to the influence of the insect. Finally, the interaction of these two treatments was not significant. Nonetheless, their intertwining role on the growth of the plant could not be discounted.

Table 2. Summary of F-Statistics for the different growth parameters.

	PARAMETERS	F-COMPUTED WITH OR WITHOUT INSECT (A)	NUTRIENT CONCENTRATION (B)	INTERACTION (A X B)
1.	Chlorophyll count	2.95ns	1.81ns	0.31ns
2.	Cell size	22.92**	5.29**	0.28ns
3.	Shoot length	29.70**	3.50 ns	0.85 ns
4.	Leaf area	8.11**	1.65ns	0.13ns
5.	Total Biomass	6.05**	2.93ns	3.38ns
6.	Root length	12.59**	1.71ns	0.86ns
7.	Leaf thickness	0.09ns	0.16ns	0.27ns

Note: **- significant at α=95% level, ns- not significant

Calculations on percent difference (Table 3)revealed that the highest effect of insect infestation was on the shoot length and leaf area increment with 98.62% and 97.80% change, respectively. Both of these values were observed in palms grown in 0% Hoagland concentration. Apparently, only leaf thickness was not significantly affected by the insect infestation. This was regardless of how much Hoagland solution was given to the palms.

Table 3. The Percent difference of the different parameters on seedlings without *Aspidiotus rigidus* to thosewith insects as observed for three-month period.

PARAMETERS (Average Values)	WITHOUT INSECTS Hoagland Conc.			WITH INSECTS Hoagland Conc.			PERCENT (%) DIFFERENCE		
	0%	50%	100%	0%	50%	100%	0%	50%	100%
1. Cellular Characterization									
a. Leaf thickness(mm)	0.27	0.28	0.28	0.28	0.27	0.29	5.42	3.23	2.39
b.Cell size (mm^{-3}) (mesophyll layer)	0.40	0.47	0.53	0.30	0.31	0.39	26.27	33.25	25.93
c. Chlorophyll Content(CCI)	18.37	18.42	28.92	6.16	15.33	18.62	66.49	16.77	35.61
2. Biometrics									
a. Shoot Length Increment(cm)	1.62	1.87	2.17	0.02	1.00	0.84	98.62	46.43	61.02
b. Leaf Area Increment(cm^2)	6.89	9.87	11.88	0.15	1.01	6.11	97.80	89.76	48.54
c. Total Biomass(g)	1.65	1.55	1.56	0.87	0.78	1.78	47.58	49.68	14.10
d. Root Length(cm)	11.16	11.88	18.40	4.27	7.15	6.81	61.76	39.81	62.99

This study was focused only on buri palms and on the use of Hoagland solution. Using other palms such as coconut and rattan is recommended for further studies. In addition, commonly used fertilizers e.g, organic and inorganic; can be used instead of Hoagland solution because they are cheaper and readily available.

IV. Conclusion

Chlorophyll count, mesophyll size, and leaf thickness were slightly affected by the coconut scale insect and Hoagland solution. Similarly, shoot length, leaf area, root length and total biomass were minimally affected probably due to the grass/rosette stage of the plant. The most important finding of the study was it was able to reveal the presence of raphides crystals of calcium oxalate at the surface of the leaves treated with 100% Hoagland solution. These crystals provided an extra protective layering on the leaf surface of the palm making the plant less preferred by the insect. Therefore, improving the nutrition is a safer alternative in reducing the susceptibility of buri palms to coconut scale insect attack.

V. Recommendations

A safer alternative of reducing the infestation of coconut insect on buri palms was obtained from the current study. Providing the plants with enough nutrients allowed it to develop a defense mechanism that made it less preferred by the insect. It is recommended that this kind of alternatives would be considered first before using procedures that would put humans at risk.

VI. Acknowledgements

The authors would like to thank the Department of Science and Technology (DOST) particularly the Accelerated Science and Technology Human Resource and Development Program for the financial assistance extended during the course of the study and the Forest Biological Science Department of the College of Forestry and Natural Resources, University of the Philippines Los Baños for use of their greenhouse and other laboratory equipment.

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