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Recovery of Coconut Trees from Scale Insect Infestation with Salt and *Trichoderma* Microbial Inoculant (TMI)

ABSTRACT

This study showed the role of Trichoderma Microbial Inoculant (TMI) and salt in the fast recovery of mature coconut trees damaged by coconut scale insect, Aspidiotus rigidus Reyne, and in the growth of coconut seedlings. Four treatments with three replicates were laid out in a randomized complete block design. Treatments made were: T0 with 0 fertilizer, 0 TMI; T1 with full dose N, P, K fertilizers, NaCl, 0 TMI; T2 with ½ dose fertilizers + NaCl, + TMI; and T3 with NaCl + TMI. Fertilizers and salt were split delivered in T1. Ten 1-m tall coconut seedlings (Laguna Tall Variety) per replicate treatment were interspersed with the mature trees in each block. About 25 g TMI per tree and 15 g TMI per seedling were applied together with fertilizers. Numbers of green, yellow, and brown leaves, inflorescences, and fruits produced per mature tree were monitored for 27 months. New and mature green leaves were counted as measure of coconut seedling growth. Mature trees in T3 had significantly higher number of green leaves and fruits compared to other treatments. T3 seedlings had significantly higher number of mature green leaves than those of other treatments. Both mature trees and seedlings in T3 exhibited the fastest growth. These results highlighted the importance of salt and TMI for productivity of coconut trees and seedlings.

Keywords: *Aspidiotus rigidus, coconut, coconut scale insect, recovery from CSI infestation, Trichoderma microbial inoculant*

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INTRODUCTION

The coconut industry plays a vital role in the Philippine economy. For one, the country is the world's top exporter of coconut products. For another, the industry is also a leading dollar earner among the country's agricultural exports and earned an annual average of US$1.58 billion from 2009 to 2011 (*PEF 2016*). Data from the Philippine Statistics Authority (PSA) show that the performance of 18 non-traditional coconut products export in January 2019 registered a new high, amounting to more than USD $100 million for that month alone. If this trend continues, the total coconut export products can be more than US$2 billion (*UCAP 2019*).

Around 3.56 million hectares, comprising around 25% of the total agricultural lands in the country, are planted to coconut. As many as 3.5 million farmers and farm workers are engaged in coconut cultivation, thus making the coconut industry the lifeblood in rural communities. However, coconut farmers are considered the poorest among the poor in the country (*PEF 2016*).

The coconut industry is faced with serious difficulties and challenges caused by the impacts of climate change among others, foremost of which is pest infestation. The coconut armored scale insect (*Aspidiotus rigidus* Reyne) has become a leading coconut pest problem. This pest is a voracious sap sucking insect that feeds on the vascular system of the leaflets such that its heavy population on the palm leaf causes it to rapidly turn yellow and eventually brown and nonfunctional. Trees infested with this pest do not bear fruits. In the Philippines, this pest problem started in Tanauan, Batangas in 2011 and spread rapidly in the Southern Tagalog provinces including Laguna. This pest is effectively dispersed by wind, wherein scale insect from an infested tree can be dispersed up to 400 m per month in the direction of the prevailing wind. By 2012, around 780,000 trees were affected in the region. Severely affected areas had to declare a state of calamity due to loss of income and livelihood (*PCAARRD 2015*).

The pest is regarded as an invasive species in Luzon although it may be native to Mindanao. Thus, it takes time to build up the population of its natural enemies. The government hence responded and launched a science-based coconut scale insect integrated pest management (CSI-IPM) protocol to control the pest problem.

In July 2014, typhoon Glenda (international name: Rammasun), with initial 85 kph gusts that developed into a super typhoon with maximum sustained winds of 140–170 kph near the center and gusts of up to 170 kph passed over the pest-affected Southern Tagalog provinces. The combined effects of control measures applied and the typhoon's destructive effects on the insect caused pest populations in these areas to decrease. The population build-up of its natural enemies also cannot be discounted as a factor in its population decline.

Surviving coconut trees need assistance to recover from the severe damage caused by the infestation, and to rehabilitate plantations to regain lost productivity. Studies on enhanced productivity of the coconut trees are needed to address these challenges to maintain the Philippines' leadership in coconut export products and to alleviate the coconut farmers' and workers' poverty. Meanwhile, the pest has spread to other areas of the country, i.e., Visayas and Mindanao (*Garcia 2017*) and remains a major pest problem of coconut trees.

This study focused on the recovery of previously infested coconut trees and on the rehabilitation of the coconut plantation by planting new coco seedlings. *Trichoderma* microbial inoculant (TMI) was used as a means of hastening this process concomitant with the application of different levels of mineral fertilizers and salt. *Trichoderma* is a beneficial fungus that is known worldwide as a biological control agent of crop fungal pathogens and a growth promoter. It is also used for priming inoculated plants to develop induced systemic resistance to pests and increased resistance to abiotic and biotic stresses (*Hermosa et al. 2012; Contreras-Cornejo et al., 2016; Stewart and Hill 2014*). In a local study of scale insect infestation on lanzones (*Lansium domesticum*) seedlings, *Trichoderma* was found to induce systemic resistance in lanzones against the scale insect (*Silva et al. 2019*). This study focused on the ability of *Trichoderma* to enhance growth, recovery, and productivity of scale insect-infested coconut trees and seedlings.

There are several mechanisms on how rhizosphere-competent *Trichoderma* species promote growth and help manage crop diseases (*Misra and Ansari 2021*). *Harman* (*2000*) defined rhizosphere-competent as "the ability of a microorganism to grow and function in the developing rhizosphere". *Harman et al.* (*2004*) reported that in corn, there is improved root development as the fungus colonizes the root surfaces and penetrates the epidermis and cortex. It then enhances root development and promotes deeper rooting, thereby leading to greater nutrient uptake, including (but not limited to) nitrogen.

Enhanced solubilization of soil nutrients and increased root hair formation have also been observed. *Cuevas et al.* (*2012*) showed that TMI can control club root disease in crucifers, thereby producing marketable Class A heads of previously infected plants. Accordingly, there was formation and proliferation of new secondary roots, which enabled the crops to absorb needed nutrients. *Hermosa et al.* (*2012*) also reported that *T. harzianum* can solubilize several plant nutrients. The colonization of cucumber roots by T. asperellum has also been shown to enhance the availability of P and Fe to plants, with significant increases in dry weight, shoot length and leaf area. *Cuevas* (*2006*) reported that *T. pseudokongii*, when present in sufficient population in the soil, resulted in more mineral nutrients (i.e., P and Zn) available for plant use that increased growth and yield of rice and tomato.

This study aimed to validate the efficacy of *Trichoderma* Microbial Inoculant (TMI) in hastening the growth and yield of mature coconut trees previously infested with coconut scale insect, *Aspidiotus rigidus* Reyne, and in establishing the higher growth rate of newly transplanted coco seedlings. This objective was demonstrated in the effect of TMI on promoting good crop growth through new leaf production and early bearing of fruits, and through the better growth of transplanted coconut seedlings in comparison with the untreated ones. This study shows how TMI can be usd in the sustainable management of crops post infestation.

MATERIALS AND METHODS

The research was conducted in the Institute of Crop Science (formerly Department of Horticulture Perennial Crops Division) of the College of Agriculture and Food Science, University of the Philippines Los Baños in Laguna, Philippines specifically inside the two-hectare coconut germplasm plantation at the International Rice Research Institute (IRRI) experimental station in Purok Boot, Brgy. Putho-Tuntungin, Los Banos, Laguna. No written document was found that provided information on the varieties of coconut planted on the site and on the ages of the trees. On pest management, based on an interview, the undersurface of the leaves of all trees in the site were sprayed with detergent solution (70 g detergent powder in 16 L tap water per tree) from September 2014 to November 2014 as a control measure against scale insects.

Laguna is a suitable area for coconut cultivation. It is considered an intermediate coconut growing province, with adequate rainfall year-round and with only 3–4.5 dry months. On the average, areas under this category have an annual yield of 1.5–2.5 t copra or in nut term

6,750–11,250 nuts per hectare before the scale insect infestation (*PCA 2011*).

The research was conducted from February 2015 to May 2017 for a total of 27 months monitoring and observations. The site was divided into three blocks representing three replicates. All the four treatments, consisting of 20 trees per treatment, were randomly laid out in each block (**Figure 1**). All trees were numbered and color-coded according to the treatments. Initial data such as soil fertility status of the site were collected (**Table 1**).

The amount of fertilizer applied was computed for fruit-bearing trees based on the recommendations of *Magat and Canja* (*2010*) for multi-nutrient fertilizers equal to 14%N, 5%P_2O_5, 20% K*2*O, 15% Cl, 4.5% S and 0.02% B. The study site has mean soil organic matter content (SOM) of 3.81% and mean soil pH of 5.65 (**Table 1**). With this SOM level, S and B are no longer limiting as explained by *Kaiser* (*2018*) and *Yermiyahu et al.* (*2001*). The applied mineral fertilizers were computed to give 14% N, 5% P_20_5, 20% K_2O + 15% NaCl (common table salt). This rate was computed on an annual nutrient need per tree on the assumption of 123 trees per hectare (9×9 square planting). The computed amounts of fertilizer requirements for N, P, K were split for twice a year application for T1.

Treatments

The following were the treatments made for this study:

T0 – control, no mineral fertilizer applied, no *Trichoderma* inoculation
T1 – full dose mineral fertilizers - N, P, K, NaCl, 0 TMI
T2 – ½ dose mineral fertilizers – N, P, K, NaCl + TMI
T3 – NaCl + TMI

In each replicate block, all trees were color-coded according to the treatments and were numbered 1-20. The colors were as follows: T0 – white, T1 – red, T2 – green, T3 – yellow.

Trichoderma microbial inoculant (TMI) in powder form was applied at the rate of 25 g per tree. TMI was developed by the senior author and consists of two strains of *Trichoderma ghanense* Doi (formerly identified as *T. pseudokoningii* Rifai) and one strain of UV-irradiated *T. harzianum* Rifai mixed in equal proportions. These species of *Trichoderma* are naturally occurring free-living fungi that are components of soil and litter (root environments). One gram of TMI powder contains 1.6 × 108 colony-forming units (CFU) of *Trichoderma* spp. The powder has moisture content of less than 10%. With the use of TMI, only 50% dose of mineral fertilizer N, P, K is recommended based on

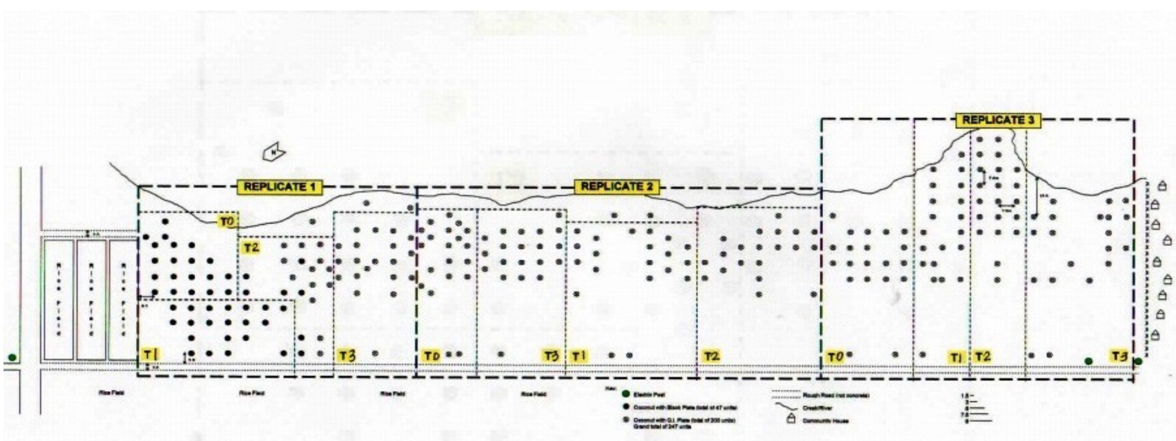

Figure 1. Field lay-out of treatments in the experimental site at the Institute of Crop Science (ICS) of the University of the Philippines Los Baños coconut germplasm collection in IRRI experimental station (Sitio Boot, Brgy. Putho-Tuntungin, Los Baños, Laguna, Philippines).

Table 1. Soil fertility status of the coconut plantation study site based on analysis of composite samples taken from each block (data presented are means of two composite samples).

Treatment	pH	% OM	P (ppm) Bray	K meq $100g^{-1}$ soil
T0 (0 chemical fertilizer, 0 TMI)	5.6	3.88	1.8	0.80
T1 (full dose chemical fert N, P, K, NaCl, 0 TMI)	5.4	4.00	2.6	0.78
T2 (½ dose chemical fert N, P, K, + NaCl + TMI)	5.8	2.98	11	2.27
T3 (NaCl + TMI)	5.8	4.45	14	1.17
Average	5.65	3.81	7.35	1.255

the results of *Cuevas* (*2006*) and *Cuevas et al.* (*2012*) where lower dose of mineral fertilizers with TMI have significantly higher yield than the full dose in rice and vegetable cropping.

All fertilizers were applied on a constructed ring furrow one-meter radius from the base of the trunk. In T2, TMI powder was broadcasted on the soil of the furrow before mineral fertilizers were applied and then covered with soil. In T3, only TMI powder and salt were applied in the ring furrow.

Ten one-meter-tall coconut seedlings (Laguna tall variety) per treatment were planted interspersed among the mature trees on June 2015. The seedlings were sourced from the Institute of Plant Breeding (IPB) nursery of the University of the Philippines Los Baños. The amount of fertilizers applied was based on the recommendations of *Canja and Magat* (*2006*) for seedlings at field planting, which are approximately 14-5-20 (N, P205, K20) + 100g NaCl. In T2 and T3, the level of NaCl was reduced by half following the principle of reducing fertilizer amounts with the use of TMI. Seedlings also require less amount of fertilizer. Reduction of applied salt concentration was not done in mature coconut trees since it was already established by *Magat et al.* (*1975*) that areas far from the sea are deficient in chlorine.

Figure 2. White tagged trees T0 – Control, 0 input, showing the spherical pattern of canopy development of fully mature coconut trees with few fronds in a horizontal position (*Foale et al. 1993*). Note the vertically oriented young fronds. Photograph taken on June 8, 2015.

Figure 3. Coconut trees under various treatments as shown by the color tags at the trunk. Trees have yellow turning brown, drooping fronds in contrast to the vertically oriented young fronds. Fronds with at least 50% yellow leaflets were categorized as yellow, while those with at least 50% brown leaflets were considered brown and senesced (photograph taken on July 24, 2017).

Data Gathered

All coconut fronds were counted whether drooping (pointed downward) or upright (90° from the perpendicular) and categorized as green, yellow or brown. According to *Foale et al.* (*1993*) in coconut trees beyond 15 years, the lower fronds begin to droop induced by a heavy load of fruit, and form a 'skirt' and the crown assumes a spherical shape (**Figure 2**). Not all leaflets within a frond senesce at the same time. Fronds with at least 50% yellow leaflets were considered yellow fronds, while those with at least 50% brown leaflets were considered brown and senesced (**Figure 3**). The direction of the fronds was also considered in classifying them. Green fronds were upright, though some were drooping (**Figure 2**), while yellow and brown fronds were drooping (**Figure 3**).

The leaves under each category were counted per tree in February 2015 as the initial data and then at 10-week interval after the 1st fertilizer application on March 26, 2015. All the 240 trees were evaluated (20 trees per replicate with three replicates and four treatments). Starting June 2015 and at monthly intervals, the number of trees with inflorescence per treatment were countedand duly recorded. The number of fruits present in each tree was also monitored at monthly intervals. The survival of transplanted coconut seedlings and the number of new leaves they produced were monitored at bi-monthly intervals. Observed insect pests (e.g., *Brontispa longissima*) attacking the leaves were counted.

Statistical Analyses

The data were first analyzed using Wilk-Shapiro (W-S) Test for normality and Levene's Test for homogeneity of variance. Tukey's test was done to compare treatment means. Non-parametric tests were used when the data did not satisfy the assumptions for the parametric tests. Multivariate analysis and Pearson correlation were also conducted.

RESULTS AND DISCUSSIONS

Growth and Fruit Production of Mature Coconut Trees

Monitoring of the vegetative growth of mature coconut trees was based on number of green, brown, and yellow leaves. All green fronds drooping or upright were counted. Yellow and brown leaves mostly drooping were likewise counted. Productivity was measured in terms of the number of flowers, number of fruits and weight of harvested mature fruits.

The trend on growth of the mature trees in all treatments were monitored in terms of number of upright green fronds per tree during the 1st 14-month observation period (**Figure 4**). The data were gathered every 10 weeks from February 2015 to April 2016. A typical S-shaped growth curve was observed.

From February to July 2015, growth in all treatments was very minimal (lag phase). Trees in all treatments exhibited growth (measured by the mean number of green leaves both upright and drooping, per tree) increasing from July 2015 to January 2016. *Foale et al.* (*1993*) reported that as the palm begins to produce fruit, physical pressure between the frond bases begins to force the fronds downwards, usually the outer (older) whorls begin to droop induced by heavy weight of fruits. The photograph of T1 trees taken on June 8, 2015, most of the trees had green leaves, with the outer whorls drooping while the innermost whorls were vertically oriented (**Figure 2**). The highest number of green leaves was in January 2016. This phenomenon is highly understandable since the trees just had heavy infestation of scale insects for about three years. Control treatment also showed growth since the site has good fertility status with average soil OM of 3.8% (**Table 1**). Growth stabilized from January to March 2016 (stable phase) and then slowed down. However, growth in T0 (without fertilizer application) was much slower compared to those with fertilizer treatments.

The number of green leaves per tree in T3 (salt + TMI) was significantly higher than those trees with fertilizer treatments and control (T0, T1, T2) during the March and April 2016 monitoring (**Table 2**). Before these monitoring periods (February 2015- January 2016), there were no significant differences in the number of green leaves in all treatments (**Table 3**). It can be clearly seen from these results that T3 had much faster growth compared to all other treatments as indicated by more developing leaves. The number of yellow leaves per tree in T0 was not significantly different from those of the other treatments (T1-T2) (**Table 2**). T3 had significantly higher yellow leaves.

Yellowing of coconut fronds is not always due to old age and therefore senescence. *Deepa et al.* (*2016*) reported that in India, the phenomenon of yellowing of fronds was due to root wilt caused by phytoplasma. This type of yellowing starts for inner whorls, then middle whorls to the outer whorls followed by shedding of immature nuts, and drying of inflorescence. In addition, *Oropeza et al.* (*1991*) also reported the incidence of lethal yellowing of coconut fronds caused by mycoplasma-like organisms (MLO) that killed millions of coconut trees in Yucatan Peninsula. *Watson et al.* (*2016*) also reported yellowing of all coconut foliage in Luzon, Philippines due to scale insect infestation. The scale insect encrusts the lower leaflet surfaces which blocked the stomata and resulted in stopping of photosynthesis. The coconut fronds turn yellow and dry, resulting to the death of the trees.

In this study, yellowing of the fronds was probably due to old age and therefore were senescent. More yellow fronds were observed in April 2016. As shown in the photographs (taken on July 24, 2017) the fronds that turned yellow and eventually brown were those in lowest whorls and drooped while the young leaves in the inner whorls were green and vertically oriented (**Figure 3**). This is in comparison to the photograph taken on June 8, 2015 (**Figure 2**), where most of the tree fronds were green whether drooping or oriented in other directions.

Chan and Elevitch (*2006*) and *Sebastian* (*2011*) stated that coconut fronds senesce about 2.5 years after unfolding. *Sebastian* (2011) mentioned that the lower fronds have reduced light interception since the younger and vertically oriented leaves that have higher leaf area index (LAI) shade the lower leaves and make them parasitic to other leaves. Senescence is a way of nutrient recycling whereby nitrogen from the lower fronds were transported elsewhere, chlorophylls were dissolved, leaving only the carotenoids, thus, the fronds turn yellow and eventually brown and the whole frond drops. The

Table 2. Effect of treatments on number of coconut leaves based on two sampling periods in March and April 2016.

Treatment	Mean number of Leaves		
	Green Leaves	Brown Leaves	Yellow Leaves
T0 (0 chemical fertilizer, 0 TMI)	8.77 b	0.693 b	7.493 ab
T1 (full dose chemical fert N, P, K, NaCl, 0 TMI)	10.02 b	0.907 ab	4.507 b
T2 (½ dose chemical fert N, P, K, + NaCl + TMI)	10.21 b	1.493 a	5.40 b
T3 (NaCl + TMI)	15.38 a	1.107 ab	8.907 a

* In a column means followed by a common letter are not significantly different at 5% level by Tukey's test.

Table 3. Multivariate analysis of treatment means on number of green, yellow and brown leaves, number of flowering trees and number of fruits per coconut tree and interaction of mean number of green leaves and mean number of flowering trees and interaction of mean number of green leaves and mean number of fruits, February 2015 to January 2016.

Treatment	Green Leaves*	Yellow Leaves	Brown Leaves	Flowering Trees	Fruits	Green Leaves × Flowers	Green Leaves × Fruits
T0 0 chemical fertilizer, 0 TMI	8.49 a	6.283 ab	0.67 b	6.61 b	55.500 b	6.61 b	55.500 b
T1 full dose chemical fert N, P, K, NaCl, 0 TMI	9.55 a	4.91 a	1.028 ab	10.444 ab	68.111 b	10.444 a	68.111 b
T2 ½ dose chemical fert N, P, K, + NaCl + TMI	9.561 a	5.2 ab	1.406 a	12.222 a	101.389 b	12.222 a	101.389 b
T3 NaCl + TMI	6.88 a	6.417 a	1.267 a	12.833 a	191.00 a	12.833 a	191.00 a

* In a column means followed by a common letter are not significantly different at P=0.05

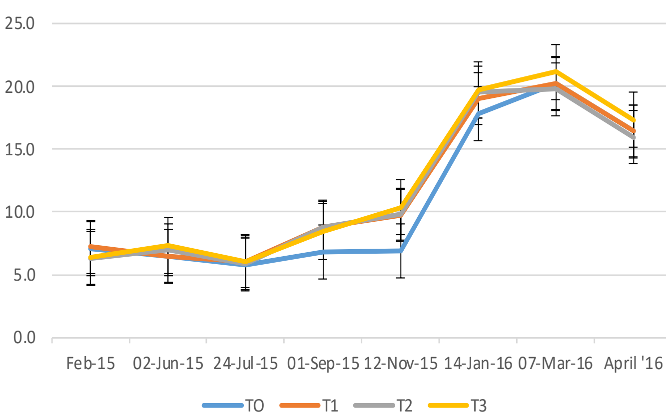

Figure 4. Growth of mature coconut trees in IRRI experimental site measured in terms of number of green leaves both upright and drooping monitored every 10 weeks from February 2015 to April 2016.

two photographs in were taken two years apart (**Figures 2** and **3**). The drooping fronds were all green (**Figure 2**). These drooping fronds were yellow turning brown, an event supported by the statements of *Chan and Elevitch* (*2006*) and *Sebastian* (*2011*) (**Figure 3**).

It can be interpreted that in T3, there is faster senescence of leaves but there is also much faster production of green leaves. Those leaves that senesced were immediately replaced by new ones, indicating faster growth. The senescent leaves then dry up and turn brown. The number of brown leaves in T3 was not significantly different from other treatments.

The growth status of mature coconut trees under different treatments after one year of fertilizer applications (February 2015 to January 2016) was statistically analyzed by multivariate analysis. Growth was measured in terms of number of green leaves per tree and number of flowering trees and number of fruits per tree. The results of multivariate analysis are presented in Table 3. Data show that there were no significant differences in the mean number of green leaves in all treatments in the period under consideration. T2 and T3 (both with TMI) had significantly higher mean number brown leaves compared to T0, but not significantly different from T1 though T1 and T0 had no significant differences. This result seemed to show the effect of hastening of senescence probably indicating more rapid growth in T2 and T3.

In this study, inflorescence were monitored starting in June 2015 until March 2016. *Chan and Elevitch* (*2006*) stated that floral primordia are initiated 12 months before spadix emerges but the number is influenced by growth conditions such as weather and nutrition months prior to emergence. *Banzon and Velasco* (*1982*) stated that if the treatment is designed to affect the number of female florets, it must be applied at least two years before nuts are harvested.

The floral primordia in the site were initiated a year before applications of fertilizers were started. However, we believe that fertilizer treatments done especially TMI and NaCl influenced the final number of florets that developed into fruits. The weather conditions during the duration of the study were favorable, thus, had same effects on all treatments.

T3 had significantly higher mean number of fruits compared to other treatments. Also, in the analysis showing the interaction of number of green leaves and fruits, T3 had significantly higher number compared to T0 and all other treatments. In this interaction analysis, all other treatments (T0, T1, T2) were not significantly different from each other. These results are evidences of the significantly faster growth rate or recovery of the coconut trees under T3 from the infestation of scale insects. Analysis of interaction of green leaves and number of flowers also shows that treatments with fertilizers (T1, T2 and T3) also had significantly higher number compared to T0. This shows the effect of fertilizers in hastening flowering.

Correlation matrix shows the growth dynamics observed in the field. Correlation of the different parameters analysed (**Table 4**), was based on the data collected from February 2015 to January 2016 (**Table 3**). There was significant increase in number of green leaves per tree in all treatments through time (r=0.734). On the other hand, there was significant continued decrease in number of yellow and brown leaves through time (r=-0.535). As leaves senesced, new leaves were formed. There was also high significant correlation in number of brown leaves and number of trees with flowers. There were more trees with green leaves and more brown leaves that were flowering. (r=0.422). As old leaves senesced, new leaves were formed. On the other hand, there was significant correlation between number of yellow leaves with number of fruits (r=0.301). As leaves turned yellow, there were more fruits produced. Interaction of green leaves with flowers was significantly correlated with trees with green leaves and fruits (r=0.509) reflecting the importance of functional leaves (green) in filling up the endosperm of the seed (coconut meat).

The above results on mature coconut trees tend to show that one year after heavy infestation of scale insects, T3 (salt + TMI) had an advantage in having complete recovery much faster than other fertilizer treatments and the control (**Tables 3** and **4**). TMI in T3 was responsible for this early recovery. T1 and T2 both have salts but still registered significantly lower green leaves and fruits than T3. *Cuevas* (*2006*) had already demonstrated this beneficial effect of *Trichoderma pseudokoningii* now, *T. ghananse*, a component of TMI, in tomato. The presence of *T. pseudokoningii* in the soil fertilized with chicken manure resulted in the release of mineral elements K, P, Ca and Zn in the soil during the vegetative stage of tomato and then promoted early flowering and fruiting resulting to higher yields. In addition, *Indriyanti et al.* (*2020*) showed that *Trichoderma* metabolites cause increase in number of coconut leaves, which is consistent with the results shown in this study.

The much faster recovery of T3 (TMI + salt) compared with T2 (½ dose mineral fertilizer + salt + TMI) may be due to the higher efficacy of TMI in a soil environment with less amount of nutrients. *Harman* (*2000*) reported that in corn inoculated with T. harzianum T22, maximum yields were obtained with 38% less nitrogen. *Cuevas* (*2006*) also showed that in a field trial in rice cropping, treatment with *T. pseudokongii* application at 0 N fertilizer yielded grain significantly higher than that treated with 90 kg N ha^{-1} without the fungus. Crops applied with the fungus, had greater uptake of P and Zn that resulted to higher yield. *Florentino et al.* (*2018*) reported that the best biostimulation effects from the Trichoderma treatments were observed in green leafy vegetables when grown under low N availability. *Harman* (*2000*) and *Harman et al.* (*2004*) attributed this action of *Trichoderma* to the ability of the fungus to induce development of more and deeper roots which enhanced the capability of the crop to access more nutrients from the soil. As demonstrated by *Cuevas et al.* (*2012*) TMI can initiate good root growth in crucifers.

Table 4. Correlation of different parameters observed in the coconut trees recovering from scale insect infestation.

Parameter	Time	Green Leaves	Yellow Leaves	Brown Leaves	Trees with Flowers	Fruits	Green Leaves × Flower	Green Leaves × Fruit
Time	1							
# Green leaves	.734**	1						
# Yellow leaves	-.535**	-.769**	1					
# Brown leaves	-.265*	-0.035	0.116	1				
Trees with Inflorescence	-0.124	0.071	0.011	.422**	1			
No. of fruits	-0.163	-0.182	.301*	0.06	0.509	1		
Green Leaves × Flowers	-0.124	0.071	0.011	.422**	1	0.509	1	
Green Leaves × Fruits	-0.163	-0.182	.301*	0.06	0.509	1	.509*	1

* significant at 5%

On the second year of monitoring (March 2016 - April 2017), it was observed that more trees produced flowers which then matured into fruits. During the March 2016 sampling there was no significant difference in mean number of inflorescences in all treatments. However, in April and June 2016, significant number of inflorescences was counted in trees under T2 and T3 (both treated with TMI) compared to T1 and T0. In September 2016, the number of inflorescences in T3 was not significantly different from T0. In the previous sampling in June 2016, T3 had the highest number of inflorescences which then matured into fruits and, therefore, not counted as inflorescence anymore but as fruits. In all sampling periods, T0 had the significantly lowest number of inflorescences among all the treatments. On the last sampling in March 2017, T2 and T3 had significantly higher number of inflorescence than the control (T0) (**Table 5**).

The much faster growth of coconut seedlings in T3 (TMI + ½ dose salt) as manifested in significantly higher mean number of mature leaves is again evidence of the influence of TMI in hastening growth (**Table 5**). This effect of the inoculant in promoting growth in seedlings has been recorded in vegetables. Vegetables treated with TMI have much higher seedling height and dry weights than the control treatments. The same observation was recorded in rice by *Banaay et al.* (*2012*). These observations can be attributed to the efficacy of *Trichoderma* in initiating good root development and in solubilizing nutrients which were efficiently utilized by the crops (*Macias-Rodriguez et al. 2020; Sood et al. 2020*).

Data show that there was continuous production of fruits as more florets were fertilized and became fruits. There were no significant differences observed but it can be clearly seen that T0 had the lowest rate of increase in the number of fruits produced, which can be expected since the crops did not receive any external nutrient inputs and the fastest rate of increase and highest number of fruits were those from T2 and T3, both with TMI inoculation (**Table 6**).

The mean total weights of the four different treatments were increasing at every sampling period. The harvest at each sampling period was significantly higher than the previous with the last harvest on April 2017 having the highest mean weight of the harvested nuts (**Figure 5**). These results are evidences that the trees have completely recovered from the infestation.

Growth and survival of coconut seedlings as affected by treatments

Coconut seedlings transplanted interspersed among mature trees last June 2015 were also subjected to the same treatments as the mature trees. The transplanted seedlings were heavily attacked by *Brontispa longissima*. The growth and survival of the seedlings as well as the number of the pest per seedling per treatment were monitored bi-monthly.

Table 5. Mean number of coconut inflorescences by sampling dates (n= 20 plants per replicate; replicates=3).

Treatment	Mean Number of Inflorescences					
	Sampling Months (2016-2017)					
	Mar '16	Apr '16	Jun '16	Sept '16	Oct '16	Mar '17
T0 (0 chemical fertilizer, 0 TMI)	9.00 a	9.00 b	9.00 b	16.33 b	17.67 b	17.67 b
T1 (full dose chemical fertilizer N, P, K, NaCl, 0 TMI)	19.67 a	17.67 b	17.67 b	39.00 a	32.00 a	39.00 ab
T2 (½ dose chemical fertilizer N, P, K, + NaCl + TMI)	20.33 a	32.00 a	39.00 a	34.67 a	34.67 a	42.33 a
T3 (NaCl + TMI)	24.00 a	34.67 a	42.33 a	16.33 b	37.00 a	47.00 a

* Means followed by a common letter are not significantly different at 5% by Tukey's test.

Table 6. Mean total number of coconut fruits per treatment from June 2015 to January 2016.

Observation Date	Mean Total Number of Fruits per Treatment			
	TO (0 chemical fertilizer, 0 TMI)	T1 (full dose chemical fertilizer, 0 TMI)	T2 (½ dose chemical fertilizer + TMI)	T3 TMI + Salt
Jun. 2, 2015	40.7	41.7	23.0	30.0
Jul. 24, 2015	33.0	34.7	31.7	37.3
Sep. 1, 2015	32.3	55.7	67.3	64.3
Nov. 12, 2015	66.3	87.3	138.0	134.7
Jan. 14, 2016	119.0	180.0	271.3	247.3
Rate of increase in number of fruits per month	11.1	19.8	35.4	31.0

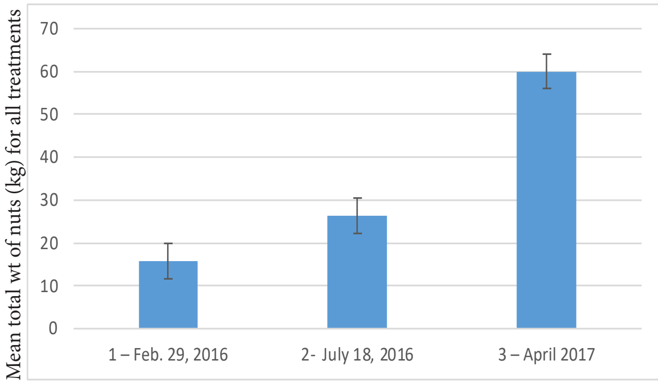

Figure 5. Graph showing harvested nuts increased with harvest dates as measured by mean total weights (kg) of nuts for all treatments.

Brontispa longissima Gestro (Coleoptera: Chrysomelidae) is one of the important coconut pests in Indonesia as reported by *Hosang et al.* (*2004*). However, *Ayri and Ramamurthy* (*2012*) believed that this pest is a native of Indonesia that has spread to India and other Asian countries, an indication that it is an invasive pest of coconut. Both larvae and adults attacked coconut leaves, particularly unfolded leaves. They feed on the soft tissues of the youngest leaf in the throat of the palm, both adult and larvae typically feed on abaxial and adaxial epidermis of the leaves (*Ayri and Ramamurthy, 2012*). These beetles attack all ages of coconut, although more damage is found in coconut plantation between four to five years old, especially in drying areas. Severe damage of this pest would kill the palms. They avoid light and stay inactive inside the still folded heart leaf during daytime and actively fly and attack coconut plants at night. Female lays an average of 50-100 eggs per day (*Hosang et al. 2004*). It is also known as the two-colored coconut leaf beetle, or the coconut hispine beetle. It is also found in Australasia and Pacific Islands attacking not only coconut palm but also several other cultivated and wild palms (*Singh and Rethinam 2004*).

Hosang et al. (*2004*) reported that spraying the entomopathogenic fungi (*Metarhizium anisopliae* var. *anisopliae* and *Beauveria bassiana*) on the affected coconut crops reduced the pest population at about 90.37-95.0%. *Singh and Rethinam* (*2004*) also reported that introduction and enhancement of parasitoids *Asecodes hispinarum* and *Tetrastichus brontispae* have proved very effective.

Growth of the coconut seedlings was measured by production of new and mature leaves. Seedlings do not shed their old leaves yet so leaves were not yet classified into yellow and brown leaves. The mean number of new leaves was not significantly different among treatments though T0 has the lowest number of new leaves. However, in terms of mean total number of mature leaves, the highest number was exhibited by seedlings under T3, significantly higher than T0. But mean number of mature leaves in T1, T2 and T3 were not significantly different from each other. However, number of mature leaves in T1 and T2 was not significantly different from T0. The data show that coconut seedlings under T3 exhibited the fastest growth. (**Table 7**).

Results obtained from T3 in both mature trees and seedlings tend to show that Cl- is the limiting nutrient in the study site and TMI enhanced the absorption of Cl- that fostered faster growth and early flowering and fruiting. The importance of chlorine in growth and yield of coconut trees has been established by *Magat et al.* (*1975*). The Philippine Coconut Authority (PCA) recommends the use of common table salt (NaCl) as source of chlorine especially in areas farther inland or away from sea salt spray (*PCA 2000*). *Braconnier and Bonneau* (*1998*) reported that in coconut, Cl- is essential in stomatal conductance especially during dry periods. Cl- enabled stomata to increase leaf transpiration, resulting to increase in evapo-transpiration and maintaining a high level of net assimilation. *Chen et al.* (*2010*) stated that Cl- is an essential micronutrient of higher plants and participates in several physiological metabolism processes. Its functions in plant growth and development include osmotic and stomatal regulation, evolution of oxygen in photosynthesis, and disease resistance and tolerance. *Raven* (*2016*) reported that chlorine is an essential micronutrient and multifunctional beneficial ion in oxygenic photolithotrophs. Intracellular Cl− acts

Table 7. Mean number of new, mature and total coconut leaves in seedlings per treatment on April 27, 2017.

Treatment	Mean new leaves/ treatment	Mean total mature leaves per treatment	Mean total number of leaves per treatment
T0 - 0 chemical fertilizer, 0 TMI	5.67 a	41.33 b	47.00 a
T1 – full dose chemical fertilizer N, P, K, NaCl, 0 TMI	11.33 a	58.00 ab	69.33 a
T2- ½ dose chemical fertilizer N, P, K, + NaCl + TMI	11.33 a	63.67 ab	75.00 a
T3 – NaCl + TMI	11.00 a	69.00 a	80.00 a

* Means followed by a common letter are not significantly different at 5% by Tukey's test.

Table 8. Average counts of *Brontispa*-infested coconut seedlings in all treatments from August 2015 to January 2016.

Sampling Date	T0 0 Fertilizer, 0 TMI	T1 (full dose chemical fertilizer + salt, 0 TMI)	T2 (½ dose chemical fertilizer + salt + TMI)	T3 salt + TMI
Aug. 5, 2015	0	0	0	0
Nov. 12, 2015	3	2	1.3	1
Jan. 18, 2016	4	4	2.3	2

Table 9. Mean number of dead coconut seedlings and leaves infested with *Brontispa longissima* as of April 2017.

Treatment	Seedling Mortality*	Leaves Infested with Brontispa longissima*
T0 – 0 chemical fert , 0 TMI	0.33	3.67
T1 - full dose chemical fertilizer + salt, 0 TMI	1.33	2.33
T2 - 1/2 dose chemical fertilizer + salt+ TMI	0.00	1.00
T3 - salt +TMI	0.33	1.67

* No significant differences at 5%

by regulating cell turgor and volume, including stomatal and pulvinar nastic movements.

Data show that the *Brontispa longissima* infestation started in November 2015. Differences were not significant among treatments but a trend can be observed that those seedlings treated with TMI (T2 and T3) had lower *Brontispa* infestation (**Table 8**). The data for 2017 showed the highest mean mortality of seedlings occurred in T1 and one each in T0 and T3, although differences were not statistically significant (**Table 9**). No mortality occurred in T2. Highest incidence of infestation also occurred in T0 followed by T1 and lowest at T3 and T2, both inoculated with TMI. This study is the first report of the possible control of this pest by *Trichoderma*.

The induced systemic resistance effect of *Trichoderma* in protecting the coconut seedlings from insect pests (that are previously seen and reported for *Trichoderma* spp.) was not clearly manifested in this study, although there is a trend observed that those seedlings treated with *Trichoderma* had less *Brontispa* infestation. This may be due to the short duration of the study. Coconut trees are perennial plants and have long gestation period and therefore needs longer observation period. The possibility of *Trichoderma*-mediated induced systemic resistance against insect pests is worth pursuing since there is already a previous report for TMI-induced systemic resistance against the Lanzones scale insect, *Unaspis mabilis* (*Silva et al. 2019*). The same mechanism may work with coconut against *Brontispa longissima*. Furthermore, *Poveda* (*2021*) has reviewed and enumerated several mechanisms by which *Trichoderma* spp. are able to either directly or indirectly control insect pests. Likewise, *Monte* (*2023*) summarized a novel multitrophic fungus-plant-animal-microbiome interaction that leads to a *Trichoderma*-mediated biocontrol of insect pests.

CONCLUSIONS AND RECOMMENDATIONS

At the end of 27 months of study on the effect of *Trichoderma* microbial inoculant (TMI) and mineral fertilizers on growth, development and fruiting of coconut mature trees, and on growth of coconut seedlings, it can be concluded that on coconuts grown in relatively fertile soil such as on the study site, TMI with the addition of salt (NaCl) hastens recovery from scale insect infestation measured in terms of significant increase in the number of green leaves. Flower induction and fruit formation happened within one year, much faster than the other treatments and with greater number of inflorescence and fruits. Control treatment was able to recover only after two years of favorable climatic conditions.

Coconut seedlings treated with TMI + salt (T3) also exhibited significantly more vigorous growth measured in terms of higher mean number of mature leaves than the other treatments. TMI treatment seems to promote some degree of resistance or protection for seedlings from the attack of *Brontispa longissima*. The trend established seem to show that TMI-treated seedlings suffered less infestation and less mortality compared to seedlings that were not inoculated, although differences were not significant.

It is recommended that a longer duration for the study be conducted and the effect of *Trichoderma* on *Brontispa longissima* infestation be investigated.

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