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Posted Date: 12 May 2023

doi: 10.20944/preprints202305.0964.v1

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The Effects of Biological and Physical Factors on Controlling the Destructive Palm Pest *Rhynchophorus ferrugineus* Olivier

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Simple Summary: Red palm weevils (RPWs), *Rhynchophorus ferrugineus* Oliver, are a promising source of low-cost, high-quality protein commonly cultivated in Southeast Asia. In recent years, a correlation in the increased weevil cultivation and the damage to palm plants can be observed due to the uncontrolled growth rate of the weevils leading to economic loss in palm industries. Therefore, it is important to develop a sustainable, organic, and cost-effective approach to control the weevil's metamorphosis rate to reduce the plant invasion by insects. RPWs' growth are related to internal factors such as the growth hormones, mutualistic relationship with gut microbiota and external factors such as host plants and the surrounding environment. In this study, we provide a novel approach to control RPW's metamorphosis rate by using local herbs that are related to their internal factors and altering their pupation nature related to their external factors.

Abstract: Red palm weevils (RPWs) are a major pest to coconut and palm trees, causing significant economic losses in many countries. The current control method involves using insecticides, which are unsustainable and environmentally damaging. Thus, it is necessary to develop an eco-friendly and sustainable approach to mitigate the RPW problem. Our studies have revealed that feeding RPWs with food containing specific herbs can affect the weevils' mutualistic relationship with gut microbiota or their growth hormones, resulting in a notable reduction in the metamorphosis rate at the pupation stage. For instance, the addition of Kra-jeab (a roselle *Hibiscus sabdariffa*) and Kra-pao (a holy basil *Ocimum tenuiflorum*) led to a reduction of 76.7% and 75.6%, respectively, in the metamorphosis rate, while combining the two herbs resulted in a higher reduction rate of 86.7%. In addition to these findings, altering their pupation nature by reducing the fiber length used in RPW feed to less than 1 centimeter can stop the weevil's pupation entirely. These findings demonstrate the potential application of biological and physical factors to reduce RPWs' metamorphosis rate and control their destructive impact on palm industries, thus providing a promising solution for the conflict between palm and insect farmers.

Keywords: red palm weevil; pest invasion; metamorphosis; gut microbiota; growth hormone; pupation nature

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1. Introduction

The red palm weevil, sago palm weevil, or the Asian palm weevil, also known scientifically as *Rhynchophorus ferrugineus* (Olivier) is a species of insect from the family Curculionidae [1]. This insect species is widely recognized as a significantly destructive pest to plants belonging to the family Arecaceae (palms), including but not limited to date palms, sago palms, talipot palms, coconut palms, and oil palms, as they feed and nest within these trees. The larvae of RPWs develop within the host's young shoots, while adults create a nest to lay eggs, which often leads to the death of the plant. The insect species is commonly found in Southern Asia and Melanesia, though they can be found causing damage in countries such as Saudi Arabia [2,3].

The adult female RPW is capable of laying approximately 30 white, elongated, ovoid-shaped eggs per day. After 2-5 days in this stage, the egg will hatch and emerge as a first instar larva [4]. The body of the newly hatched larvae displays a white, segmented, and legless appearance with reddish-brown coloration on the heads. In their natural habitat as a pest, the larvae undergo approximately 10-11 molting cycles while nesting and feeding inside the trunks of their host plant until they are fully developed after 1-3 months and ready to construct a cocoon and enter pupation. As they develop, the coloration of the larvae will gradually change to a darker hue, with the body becoming brownish-yellow, and weighing approximately 5 grams. The larval stage is when weevil farmers collect their cultivated weevils for the market. If the RPWs are not collected during this stage for commercial purposes, the fully developed larvae will utilize available surrounding fibers from their environment, such as coconut fibers, fibers from their host plant, or fibers from their feed to construct a cocoon. The ovoid-shaped cocoon will encase the entire body of the larva, allowing it to undergo metamorphosis and develop into a dark brown pupa with physical characteristics similar to that of an adult RPW [1]. The pupal stage of RPWs lasts for a duration of 14-21 days, after which the insect emerges from the cocoon as an imago [4]. The RPW imago displays wide variations in their coloration and patterns among individuals, with base colors ranging from reddish-brown to brownish-black. The male RPWs possess shorter snouts than their female counterparts, and both sexes have hairy snouts. The morphological difference between RPW males and females are crucial for identifying the sex and play an essential role in the reproductive process, as they enable the female weevils to use their elongated snouts bore into the soft tissue of the host plant for oviposition [1]. The average time period a RPW will stay in the imago stage is around 98 days [4].

Since the RPW is a devastating pest to the palm industry, in coconut palms (*Cocos nucifera*), RPWs start their infestation when the adults bore into the top parts or the stem of the host tree, where they undergo their entire life cycle until they are mature imagoes and are ready to spread to other trees. The female RPWs use their elongated snouts to bore into the stem of the host plant and lay their eggs in cracks and scarred parts of the stem, or in parts that have already been bored by other insects that are also pests to palm plants such as the coconut rhinoceros beetle (*Oryctes rhinoceros*). Once the RPW eggs hatch, the larvae will tunnel into the heart of the palm plant, causing major damage inside the trunk of the tree and cutting off the connection between the roots and the tops of the infested tree. This damage disrupts the plant's transport of water and nutrients to the tops, causing them to rot and fall. This damage could be fatal to the plant since these RPW damage cases eventually lead to the death of the host plant causing major damage to the palm industry. In Thailand, a total of two species of RPW are found, namely the species in the study, *Rhynchophorus ferrugineus*, and the other RPW species of *Rhynchophorus vulneratus*, both of which are considered major pests to the palm industry [1]. Since they are destructive pests, the farmers found a way to manage the RPW infestation problem by consuming and cultivating the larvae of the red palm weevil as an alternative protein source. By consuming the larvae, farmers are not only able to manage the RPW pest problem but also create a sustainable and profitable business benefiting them.

The larvae of RPWs are recognized as a nutritious food source and cultivated for human consumption due to their high nutritional value [5]. They serve as an eco-friendly and alternative source of protein that can help alleviate the world's food shortage problem [6,7]. In comparison to farm animals, cultivating RPWs has numerous benefits, including reducing greenhouse gas emissions that contribute to global warming, such as reducing carbon dioxide by up to 730,000 million kilograms per year and reducing methane by up to 30 trillion kilograms per year [8,9]. It also reduces the space needed, since insects can be raised in plastic basins [10]. However, the increase in RPW cultivation has led to the release of adults into the environment, resulting in pest problems that cannot be controlled in numerous economic plants, namely plants in the family Arecaceae [11].

The growth of RPWs is influenced by various factors. Internal biological factors such as growth hormones and gut microbiota [12,13]. According to previous research, gut microbiota located in RPW's digestive track produces enzymes that help digest cellulose [13], which affects the metamorphosis of RPWs. To slow down their metamorphosis cycle, we found that roselle (*Hibiscus sabdariffa*), garlic (*Allium sativum*) and turmeric (*Curcuma longa*) can reduce the growth of bacteria found in the RPW digestive track [14–16]. Additionally, some plants, namely mulberry (*Morus alba*), basil (*Ocimum basilicum*), and holy basil (*Ocimum tenuiflorum*) contain small amounts of juvenile hormone-like secondary metabolites, called phytojuvenoid, which maintain insects in an immature state until the appropriate developmental stage for reproduction is reached [17,18].

In nature, many plants contain certain compounds that can affect the symbiotic relationship between the RPW and its gut microbiota, and some plants can affect the insect's hormone levels. These plants can be used as a biological factor to control the metamorphosis of the weevil larvae. The RPW has a mutualistic relationship with its gut microbiota, which produces enzymes that aid in digesting cellulose, providing the insect with the necessary nutrients for growth and development, thus affecting its metamorphosis [13]. Plants such as roselle (*Hibiscus sabdariffa*), known locally in Thai as “Kra-jeab”, could slow down the RPW’s metamorphosis cycle since it can reduce the growth of RPW gut microbiota, particularly those of the family Enterobacteriaceae, which is a significant percentage of the insect’s gut microbiota [14,15]. Garlic (*Allium sativum*), and turmeric (*Curcuma longa*), known in Thai as “Kra-thiam” and “Kha-min”, respectively, have also been known to be able to reduce the growth of general bacteria found in the RPW’s gut [16].

In addition, animals in the phylum Arthropoda have been known to produce hormones that regulate their metamorphosis cycle. The juvenile hormone is an example of these hormones, which slow down growth and increases molting, thus regulating metamorphosis. Plants commonly found in Thailand, such as the leaves of mulberry (*Morus alba*), basil (*Ocimum basilicum*), and holy basil (*Ocimum tenuiflorum*), known in Thai as “Bai-Mon”, “Ho-ra-pha”, and “Kra-pao”, respectively, have been reported to contain small amounts of compounds called phytojuvenoid, which are juvenile hormone-like secondary metabolites, having similar properties to the juvenile hormone, thus also able to slow down the metamorphosis of RPWs [17,18]. This suggests that these plants may be able to be used as a natural regulator of the RPW metamorphosis cycle.

External factors such as host plants and environmental conditions also influence the growth of RPWs [19]. According to previous research, different-sized coconut fiber also affects the rate of their pupation, where the length of fiber affects the pupation ability of insects [20].

As certain physical factors also play a role, the availability of surrounding fibers can affect the construction of the RPW's cocoon when it enters pupation, which is essential for the proper progression of the metamorphosis cycle, and any disruption in this process can have significant consequences on the metamorphosis of the RPW. As previous research has indicated, the fully developed larvae of RPWs rely on available fibers from the surrounding environment to construct a cocoon, and the availability of these fibers can significantly impact their ability to do so [20]. This behavior of the RPW could potentially slow down its metamorphosis cycle if there is less fiber available.

As RPWs become adults, their reproduction rate becomes costly to control, making the development of effective control methods critical. This study highlights the potential for a sustainable, organic, and cost-effective method to control RPW growth by manipulating their gut microbiota, growth hormones, and altering their pupation nature for RPW farmers.

2. Materials and Methods

Red palm weevil larvae (RPWL) were obtained in the second instar stage from a commercial farm in Nonthaburi, Thailand, and then transported to a decontaminated laboratory at Bangkok Christian College. Ninety larvae were placed in each plastic basin and reared at a controlled temperature range of 27-30°C and relative humidity of approximately 70-80% with a completely randomized design used for all experiments with 3 replicates. The larvae were fed with a diet composed of the grounded trunk of the sago palm tree (*Cycas revoluta*), water, coconut fiber, and commercial pig feed in a ratio of 30:50:1:5, respectively, and the diet was replaced every 14 days to avoid spoilage, similarly to the method of Chinarak et al. [5]. This diet was used as the control feed.

The researchers conducted regular monitoring of the developmental stages of RPWL every two days, including the tracking of larval development and pupation until all RPWL in the control basin had entered pupation. The number of larvae entering pupation was recorded every two days, allowing us to calculate the average metamorphosis rate of the treated RPWs.

2.1. Effects of plants on RPWL gut microbiota

To investigate the effects of plants on RPWL gut microbiota, three groups of experimental diets were used. Each experimental diet consisted of a 1:15 mixture of a supplemental plant and the control RPW feed. To examine the effects of plants on inhibiting the mutualistic relationship of RPWL and their gut microbiota, *Allium sativum, Hibiscus sabdariffa,* and *Curcuma longa* were added to the feed of the RPW and used as supplemental plants. From the first day of supplementation of plants, the number of larvae entering pupation was recorded every two days until all RPWL in the control basin had entered pupation. The extended data, which includes the tracking of all RPWL in all basins entering pupation, can be found in Appendix A, Table A1.

2.2. Effects of plants on RPWL juvenile hormone level

To investigate the effects of plants on increasing the RPWL juvenile hormone level, three groups of experimental diets were used. Each experimental diet consisted of a 1:15 mixture of a supplemental plant and the control RPW feed, similar to the method of section 2.1. To examine to effects of plants on increasing the level of juvenile hormone of the RPWL, the plants used were altered to *Morus alba, Ocimum basilicum,* and *Ocimum tenuiflorum* and added to the feed of the RPW to be used as supplemental plants. From the first day of supplementation of plants, the number of larvae entering pupation was recorded every two days until all RPWL in the control basin had entered pupation. The extended data, which includes the tracking of all RPWL in all basins entering pupation, can be found in Appendix B, Table A2.

2.3. Mixed effects of plants on RPWL gut microbiota and juvenile hormone level

To compare the effectiveness of plants that inhibit the mutualistic relationship and plants that increase the juvenile hormone level, three groups of experimental diets were used. Each experimental diet consisted of a 1:15 mixture of a supplemental plant and the control RPW feed, similar to the method of sections 2.1 and 2.2, but for comparison, the most effective plants from the previous two experiments were added to the feed of the RPW both individually and combined and used as supplemental plants. From the first day of supplementation of plants, the number of larvae entering pupation was recorded every two days until all RPWL in the control basin had entered pupation. The extended data, which includes the tracking of all RPWL in all basins entering pupation, can be found in Appendix C, Table A3.

2.4. Effects of coconut fiber length on RPWL pupation rate

To investigate the effects of coconut fiber length on decreasing RPWL pupation rate, four groups of experimental diets were used. Coconut fiber length was measured and individually cut for each experimental diet. To examine the effects of coconut fiber length on RPWL pupation rate, coconut fibers were cut to lengths of 15, 10, 5, and less than 1 centimeter and added in with the feed of the RPW to be used in each experimental diet. From the first day of coconut fiber combined with the weevil feed, the number of larvae entering pupation was recorded every two days until all RPWL in the basin with the feed combined with 15-centimeter coconut fibers had entered pupation. The extended data, which includes the tracking of all RPWL in all basins except the basin combined with coconut fibers less than 1 centimeter in length entering pupation, can be found in Appendix D, Table A4.

2.5. Statistical Analysis

One-way ANOVA was used for statistical analysis. The data were analyzed for mean and standard deviation. Difference between means were analyzed according to Least Significant Difference (LSD).

3. Results

3.1. Effects of plants on RPWL gut microbiota

Results showed that red palm weevil feed containing *Hibiscus sabdariffa* had the greatest effect on slowing down the metamorphosis rate of RPWL, as evidenced by only 23.3% of the treated larvae pupating, which is 76.7% less when compared to the number of RPWL pupating in the control group. Red palm weevil feed containing *Curcuma longa* as the supplemental plant was the next most effective in slowing down RPWL metamorphosis, with 26.7% of the treated larvae pupating, followed by feed containing *Allium sativum* as the supplemental plant at 32.2% of the treated larvae pupating. (For the extended data - see Appendix A, Table A1.)

Table 1. The rate of RPWL metamorphosis classified by the plant that affects RPW gut microbiota.

Number of days	Number of RPWL pupating classified by the plant			
	Control group	*A. sativum*	*H. sabdariffa*	*C. longa*
0	0	0	0	0
2	0	0	0	0
4	4	0	0	0
6	5	0	0	0
8	3	5	2	0
10	11	0	0	3
12	22	0	4	4
14	24	14	6	5
16	21	10	9	12
Total	90	29	21	24
SD	9.82	5.33	3.32	4.03
%	100.0*	32.2*	23.3*	26.7*

* statistically significant at the 0.05 level

3.2. Effects of plants on RPWL juvenile hormone level

Results showed that red palm weevil feed containing *Ocimum tenuiflorum* was the most effective in slowing down the metamorphosis rate of RPWL, as evidenced by only 24.4% of the treated larvae pupating, which is 75.6% less when compared to the number of RPWL pupating in the control group. Red palm weevil feed containing *Ocimum basilicum* as the supplemental plant was the next most effective in slowing down RPWL metamorphosis, with 27.8% of the treated larvae pupating, followed by feed containing *Morus alba* as the supplemental plant at 28.9% of the treated larvae pupating. (For the extended data - see Appendix B, Table A2.)

Table 2. The rate of RPWL metamorphosis classified by the plant that affects RPWL hormone level.

Number of days	Number of RPWL pupating classified by the plant			
	Control group	*M. alba*	*O. basilicum*	*O. tenuiflorum*
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	0	0	0	1
8	5	3	1	0

10	11	4	5	3
12	25	5	8	5
14	27	8	8	5
16	22	6	3	8
Total	90	26	25	22
SD	11.64	3.06	3.42	2.96
%	100.0*	28.9*	27.8*	24.4*

* statistically significant at the 0.05 level

3.3 Mixed effects of plants on RPWL gut microbiota and juvenile hormone level

When both *Hibiscus sabdariffa* and *Ocimum tenuiflorum* were combined and added to RPW feed, they had the greatest effect on slowing down the metamorphosis rate of RPWL, as evidenced by only 13.3% of the treated larvae pupating, which is 86.7% less when compared to the number of RPWL pupating in the control group. Red palm weevil feed containing only *Hibiscus sabdariffa* as the supplemental plant was the next most effective, with 32.2% of the treated larvae pupating, followed by feed containing only *Ocimum tenuiflorum* as the supplemental plant at 35.6% of the treated larvae pupating. (For the extended data - see Appendix C, Table A3.)

Table 3. The rate of RPWL metamorphosis classified by the plant that affects the mutualism of RPWs' gut microbiota and plants that affects the hormone level.

Number of days	**Number of RPWL pupating classified by the plant**			
	Control group	*H. sabdariffa*	*O. tenuiflorum*	*H. sabdariffa* + *O. tenuiflorum*
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	2	3	2	0
8	11	1	0	0
10	13	5	7	2
12	24	6	7	1
14	22	6	5	4
16	18	8	11	5
Total	90	29	32	12
SD	9.86	3.11	4.10	1.94
%	100.0*	32.2*	35.6*	13.3*

* statistically significant at the 0.05 level

3.4 Effects of coconut fiber length on RPWL pupation rate

Results showed that red palm weevil feed containing coconut fiber with a length of less than 1 centimeter was the most effective in slowing down the metamorphosis rate of RPWL, as evidenced by only 0.0% of the larvae pupating. Red palm weevil feed containing coconut fiber with a length of 5 centimeters was the next most effective, with 28.9% of the larvae pupating, followed by feed containing coconut fiber with a length of 10 centimeters and feed containing coconut fiber with a length of 15 centimeters at 36.7% and 100.0% of the larvae pupating, respectively. (For the extended data - see Appendix D, Table A4.)

Table 4. The rate of RPWL metamorphosis classified by the length of coconut fiber.

Number of days	Number of RPWL pupating classified by the length of coconut fiber			
	15 centimeters	10 centimeters	5 centimeters	<1 centimeter
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	1	1	0	0
8	1	2	1	0
10	2	0	1	0
12	7	3	2	0
14	8	1	0	0
16	13	5	1	0
18	26	11	8	0
20	32	10	14	0
Total	90	29	32	0
SD	11.19	4.02	4.52	0.00
%	100.0*	36.7*	28.9*	0.0*

* statistically significant at the 0.05 level

4. Discussions

We conducted an investigation to determine the impact of various biological and physical factors on the metamorphosis rate of RPWs, for the development of a sustainable, organic, and cost-effective approach to control the insect's metamorphosis rate, with an ultimate goal of reducing RPW invasion problems and major destruction in palm plantations. The current method for controlling RPW pest problems involves the use of harmful and toxic insecticides, which are unsustainable and environmentally damaging. Therefore, we were interested in developing an eco-friendly and sustainable approach to mitigate the RPW problem.

Our findings revealed that plants with inhibitory properties on the mutualistic relationship of RPWs and their gut microbiota can effectively reduce the metamorphosis rate of RPWL. Our results indicate that *Hibiscus sabdariffa*, when combined with weevil feed showed the most significant reduction in RPWL metamorphosis rate, with only 23.3% of the treated larvae entering pupation, which is 76.7% lower than the RPWL in the control group, which had a pupation rate of 100%. The metamorphosis rate of the RPWLs was followed by feed containing *Curcuma longa* and *Allium sativum* as the supplemental plants, respectively. Both of these plants have been proven to also be effective, as the results revealed that they could also reduce the metamorphosis of the RPWL, evidenced by the 26.7% and 32.2% of the treated larvae pupating, which is 73.3% and 67.8% lower than the RPWL in the control group, respectively. This finding is supported by the previous research of Habineza et al. [13], which demonstrated that disruptions in the mutualistic relationship of RPWs with their gut microbiota could affect their growth, thus slowing their metamorphosis rates due to the decreased production of enzymes.

Additionally, the results of this study revealed that plants containing phytojuvenoid compounds, which have the properties of increasing the juvenile hormone level in RPWs can also reduce the metamorphosis rate of the weevil larvae. Our results showed that *Ocimum tenuiflorum*, when combined with weevil feed was the most effective in slowing down the RPWL metamorphosis rate, with only 24.4% of the treated larvae entering pupation, which is 75.6% lower than the control group which had a pupation rate of 100%. The metamorphosis rate of the RPWL were followed by feed containing *Ocimum basilicum* and *Morus alba* as the supplemental plants, respectively. Both of these plants have been proven to also be effective, as the results revealed that they could also reduce the metamorphosis of the RPWL, evidenced by the 27.8% and 28.9% of the treated larvae pupating, which is 72.2% and 71.1% lower than the RPWL in the control group, respectively. This finding is consistent with the previous studies by Khyade et al. [17] and Jaiswal and Srivastava [18], which reported that insects exposed to secondary metabolites with hormonal activity similar to juvenile hormone (phytojuvenoid) would exhibit slower metamorphosis rates.

Furthermore, our results showed that the use of the combination of plants that inhibit the mutualism of gut microbiota and plants containing phytojuvenoid compounds, which have properties of increasing the juvenile hormone level showed the best results in reducing the metamorphosis rate of RPWL. The combination of *Hibiscus sabdariffa* and *Ocimum tenuiflorum in* the weevil feed produced the most significant reduction in the RPWL metamorphosis rate, with only 13.3% of the larvae treated with the combination entering pupation, which is 86.7% lower than the control group, which had a pupation rate of 100%.

Moreover, we conducted an investigation to determine the physical factor that affects the metamorphosis rate of red palm weevils. Our findings revealed that reducing the length of coconut fibers in the feed that the RPWL use for constructing their cocoons to enter pupation can effectively slow down their metamorphosis rate. The results of this study showed that weevil feed containing coconut fibers less than 1 centimeter in length can slow down red palm weevil metamorphosis rate the best, as seen by the least number of larvae pupating at 0.0%. The metamorphosis rate of the RPWL were followed by feed containing coconut fiber with lengths of 5, 10, and 15 centimeters at 28.9, 36.7, and 100.0%, respectively. This finding is consistent with the previous studies by Norzainih et al. [20], which reported that when RPWs receive the ideal length of coconut fibers, they will be able to construct a cocoon and enter pupation, thus having a higher metamorphosis rate.

In conclusion, our findings suggest that the combined use of *Hibiscus sabdariffa* and *Ocimum tenuiflorum* in the weevil feed, along with the reduction of the coconut fiber length in the weevil feed to less than 1 centimeter were best to be used in slowing down RPW metamorphosis rate to reduce their pest problems.

5. Conclusions

In conclusion, as red palm weevils are a major destructive pest to palm plants, through our study, we have developed a sustainable, organic, cost-effective, and efficient approach to control the metamorphosis rate of red palm weevils, with an ultimate goal of reducing the problem of the pest. We experimented by adding plants to the weevil feed and reducing the coconut fiber in their feed. As a result, we were successfully able to slow down the metamorphosis of red palm weevils using plants that inhibit the mutualism of their gut microbiota and plants that contain phytojuvenoid compounds, which have properties of increasing the juvenile hormone level. We were also able to slow down their metamorphosis rate by reducing the length of coconut fibers in the feed that the larvae use for constructing their cocoons. This approach has the potential to provide a more sustainable and environmentally friendly strategy for mitigating RPW invasions in palm plantations when compared to the conventional method of using harmful insecticides. Though our research proved to be successful, further research is required to validate these findings and explore the long-term effectiveness of this approach.

Author Contributions: Conceptualization, S.A., T.C., C.K. and W.B.; Data curation, S.A. and T.C.; Formal analysis, S.A., T.C., C.K. and W.B.; Investigation, S.A. and T.C.; Methodology, S.A., T.C., C.K. and W.B.; Project administration, S.A. and T.C.; Supervision, C.K. and W.B.; Visualization, S.A. and T.C.; Writing – original draft, S.A. and T.C.; Writing – review & editing, S.A., T.C., C.K. and W.B. All authors will be informed about each step of manuscript processing including submission, revision, revision reminder, etc. via emails from our system or assigned Assistant Editor.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We owe our gratitude to several individuals and departments whose contributions made this research possible. We first want to thank Bangkok Christian College for their generous support, which provided us with the resources and facilities to make our research possible. We are also grateful for the senior members of the BCC community for their invaluable guidance and insights. We would also like to thank our colleagues and peers, whose expertise and assistance throughout this project were immeasurable. Finally, we express our heartfelt appreciation to our families and loved ones for their unwavering support and understanding during this journey. Without their encouragement, this accomplishment would not have been possible.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The rate of RPWL metamorphosis classified by the plant that affects RPWs' gut microbiota.

Number of days	Number of RPWL pupating classified by the plant			
	Control group	*A. sativum*	*H. sabdariffa*	*C. longa*
0	0	0	0	0
2	0	0	0	0
4	4	0	0	0
6	5	0	0	0
8	3	5	2	0
10	11	0	0	3
12	22	0	4	4
14	24	14	6	5
16	21	10	9	12
18	-	15	10	13
20	-	13	11	11
22	-	11	8	9
24	-	9	7	9
26	-	6	6	8
28	-	4	7	6
30	-	3	4	5
32	-	-	3	2
34	-	-	4	2
36	-	-	4	1
38	-	-	2	-
40	-	-	3	-
Total	90	90	90	90
SD	9.82	5.60	3.47	4.43
%	100.0	100.0	100.0	100.0

Appendix B

Table A2. The rate of RPWL metamorphosis classified by the plant that affects RPWL hormone level.

Number of days	Number of RPWL pupating classified by the plant			
	Control group	*M. alba*	*O. basilicum*	*O. tenuiflorum*
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	0	0	0	1
8	5	3	1	0
10	11	4	5	3
12	25	5	8	5
14	27	8	8	5
16	22	6	3	8
18	-	9	10	10
20	-	12	13	12
22	-	12	12	11
24	-	9	10	8

26	-	8	7	7
28	-	6	5	5
30	-	4	4	5
32	-	2	3	4
34	-	2	1	3
36	-	-	-	3
Total	90	90	90	90
SD	11.64	4.01	4.38	3.80
%	100.0	100.0	100.0	100.0

Appendix C

Table A3. The rate of RPWL metamorphosis classified by the plant that affects the mutualism of RPWs' gut microbiota and plants that affects the hormone level.

Number of days	Number of RPWL pupating classified by the plant			
	Control group	*H. sabdariffa*	*O. tenuiflorum*	*H. sabdariffa* + *O. tenuiflorum*
0	0	0	0	0
2	0	0	0	0
4	0	0	0	0
6	2	3	2	0
8	11	1	0	0
10	13	5	7	2
12	24	6	7	1
14	22	6	5	4
16	18	8	11	5
18	-	9	12	6
20	-	12	14	5
22	-	11	11	6
24	-	9	8	6
26	-	8	7	7
28	-	5	6	8
30	-	4	-	6
32	-	3	-	7
34	-	-	-	5
36	-	-	-	6
38	-	-	-	4
40	-	-	-	3
42	-	-	-	5
44	-	-	-	4
Total	90	90	90	90
SD	9.86	3.84	4.77	2.63
%	100.0	100.0	100.0	100.0

Appendix D

Table A4. The rate of RPWL metamorphosis classified by the length of coconut fiber

Number of days	Number of RPWL pupating classified by the length of coconut fiber			
	15 centimeters	10 centimeters	5 centimeters	<1 centimeter
0	0	0	0	0
2	0	0	0	0

4	0	0	0	0
6	1	1	0	0
8	1	2	1	0
10	2	0	1	0
12	7	3	2	0
14	8	1	0	0
16	13	5	1	0
18	26	11	8	0
20	32	10	14	0
22	-	13	13	0
24	-	14	12	0
26	-	12	11	0
28	-	9	7	0
30	-	6	6	0
32	-	3	5	0
34	-	-	5	0
36	-	-	3	0
38	-	-	1	0
Total	90	90	90	0
SD	11.19	5.12	4.82	0.00
%	100.0	100.0	100.0	0.0

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