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Comparative Study of Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) Reproduction Reared Using Different Diets

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Abstract: *Rhynchophorus ferrugineus* or known as red palm weevil (RPW) is a damaging pest of coconut and many other palms. Due to its damaging nature to palms, many efforts have been taken to develop control methods of the pest which originated from laboratory studies. The use of wild-captured RPW from the field for laboratory trials would cause inconsistencies due to many factors. Thus, rearing them under laboratory condition is a must. Rearing techniques used by previous workers are laborious, requires frequent maintenance and sometime produced insufficient samples for testing. Some of the ingredients used also could not be acquired easily. Therefore, a study was conducted to compare RPW reproduction using different alternative diets in order to produce better technique for continuous rearing as preparation before laboratory studies. This study was done by preparing two types of alternative diets, namely Diet A using cassava+chicken feed+young coconut husks as the main ingredients and Diet B using sugarcane, for rearing the weevil. Two pairs of RPW were used on each diet and observations were done daily. Numbers of RPW were recorded for every stage until adult emergence. Development time and adult longevity were also recorded. The experiment was done for two generations and replicated five times. Results of the study revealed that Diet A increased RPW fertility per female ($P<0.0001$), shortened the developmental time ($P<0.05$), and requires less maintenance as compared to Diet B. However, RPW longevity was the same in both diets ($P>0.05$) as it could have been dependent on the freshness of the diets over time. Thus, the results of this study suggested that Diet A is a better option as compared to Diet B for RPW rearing under laboratory condition.

Keywords: Red Palm Weevil, *Rhynchophorus ferrugineus*, Mass-Rearing, Diets, *Cocos nucifera*

1. Introduction

Rhynchophorus ferrugineus or red palm weevil (RPW) is an invasive pest of coconut and palms in many countries around the world. In Malaysia, RPW was first reported in 2007 attacking coconuts palms in Terengganu [1] and has spread throughout Peninsular Malaysia. RPW also found on other palms such as date palms (*Phoenix dactylifera*), sago palm (*Metroxylon sagu*), nipah palm (*Nypa fruticans*), ornamental palm (*Bismarckia nobilis*) and others. Several methods have been applied to control its infestations including chemical control through trunk injection, pheromone traps and cultural practices [2, 3]. Unfortunately, the infestations still exist and due to this, efforts were taken to develop other control strategies such as biological controls which originated from numerous laboratory trials [4-7] that requires a lot of tested samples. However, non-standardized and insufficient tested samples collected from the field will affect trial results. Thus, continuous rearing of RPW in the laboratory is ought to be done.

Nutrient supply plays major role in mass-rearing of insect pests for any laboratory trials. The main nutrient components such as carbohydrates, proteins, fibres, vitamins and others are vital in helping in the insect's development to complete its life cycle. Therefore, suitable diet selections which contains all of these nutrients should be taken into considerations. Several diets for RPW rearing have been developed by many workers [8-10]. However, some of the existing rearing techniques are laborious and requires regular maintenance [11], and some of the ingredients used for artificial diets are expensive and not easily available [12]. Thus, this study was carried out to improve rearing technique of RPW by comparing the effect of different type of diets on RPW development.

2. Materials and Methods

2.1. Source of RPW

RPW were collected from infested palms and bucket traps loaded with pheromone (Ferrolure ™) around Kuala Terengganu. The collected adults were later identified according to sex and reared in laboratory [11]. Upon adult emergence, the adults were isolated, fed and prepared (within 24 hours) to be used in the experiment.

2.2. Diets Preparation

Two (2) types of diets were used in this experiment; 1) alternative diet (Diet A) and 2) sugarcane (Diet B). The alternative diet was prepared as follows:

1) 0.4kg grind cassava tubers (source of carbohydrate)
2) 0.4kg chicken feed powder (source of protein)
3) Distilled water (source of moisture)
4) 1kg young coconut husk (source of fibre)

Cassava tubers were chosen as the main ingredient as it contains the highest amount of carbohydrates as compared to other crops [13]. Chicken feed powder was used as main source of protein which contributed the highest nutrient (21%) of the feed [14]. Distilled water played major role in providing moisture to the diet, while coconut husk served as fibre which could also help RPW in cocoon construction. These ingredients were also easily available in local market and farm.

The cassava tubers were peeled and chopped into small pieces (Figure 1a). They were later grind with distilled water and poured into plastic basin (130cm diameter x 17cm height). Chicken feed powder was weighted and mixed into the basin (Figure 1b). Young coconut husks were divided equally into two (2) portions. One portion was chopped into dices and put into the same basin (Figure 1c). All ingredients were mixed (Figure 1d). Another portion of young coconut husks were peeled and arranged on top of the mixed ingredients (Figure 1e).

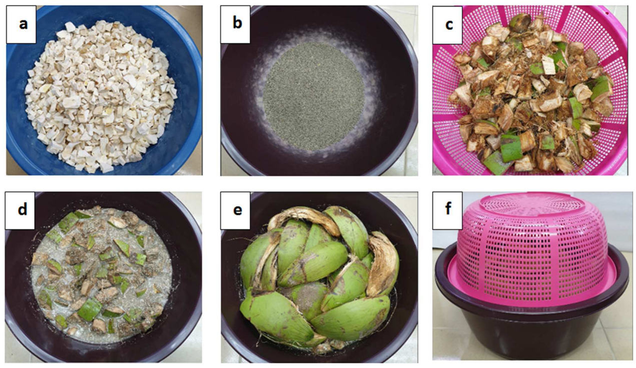


Figure 1. *Preparation of Diet A (a) peeled and diced cassava; (b) chicken feed powder; (c) diced young coconut husks; (d) mixing of the ingredients; (e) arrangement of young coconut husks; (f) covered rearing container.*

The sugarcane diet (Diet B) (Figure 2) was prepared by cutting fresh sugarcane into 15cm segments (for egg laying and development of early instars larvae) and 30cm segments (for late-stage larvae and pupae). The egg laying sugarcane were split meanwhile small holes were made at the end of the other sugarcanes for larvae entry-points. All sugarcanes were replaced every two days to avoid fungus contamination.

Figure 2. *Preparation of Diet B using sugarcane; (a) split sugarcane for egg laying; (b) unsplit sugarcane segments for developing larvae and pupae construction; and (c) rearing container.*

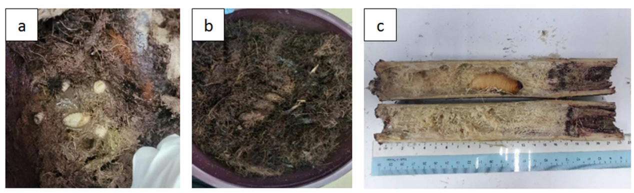


Figure 3. *(a) RPW larval stage in Diet A; (b) Pupal stage in Diet A; (c) Larvae in Diet B.*

2.3. Experimental Design

Two pairs of newly emerged RPW adults were starved for two hours before released into each diet container and allowed to mate and fed. Observations were done daily until adult emergence for two RPW generations. The experiment was arranged in Complete Randomized Design (CRD) with 5 replications.

2.4. Data Collection and Analysis

Data on number of eggs, larvae, pupae and adults per female (Figure 3) from each generation, development time, and adult longevity were recorded. Data on hatchability, pupation, emergence and fertility were calculated as follows:

% hatchability= (Number of eggs hatched / Number of eggs laid) x 100

% pupation=(Number of pupae formed / Number of larvae) x 100

% emergence= (Number of adults emerged / Number of pupae) x 100

% fertility=(Number of adults emerged / Number of eggs laid) x 100

All data were analysed using t-test as well as 2-factorials ANOVA, and LSD after normality test on SAS 9.4. Data visualizations were done using GraphPad Prism 8.

3. Results

Results of the study show significantly higher percentage of each RPW developmental stage when fed with Diet A as compared to Diet B for both generations (P<0.05) except on mean fecundity of the 1st generation (P=0.11) (Figure 4), even though Diet A recorded higher fecundity. The two-factorial analysis also showed significant results for both factors (generations and diets) as well as interaction between factors (P<0.05) (Figure 5).

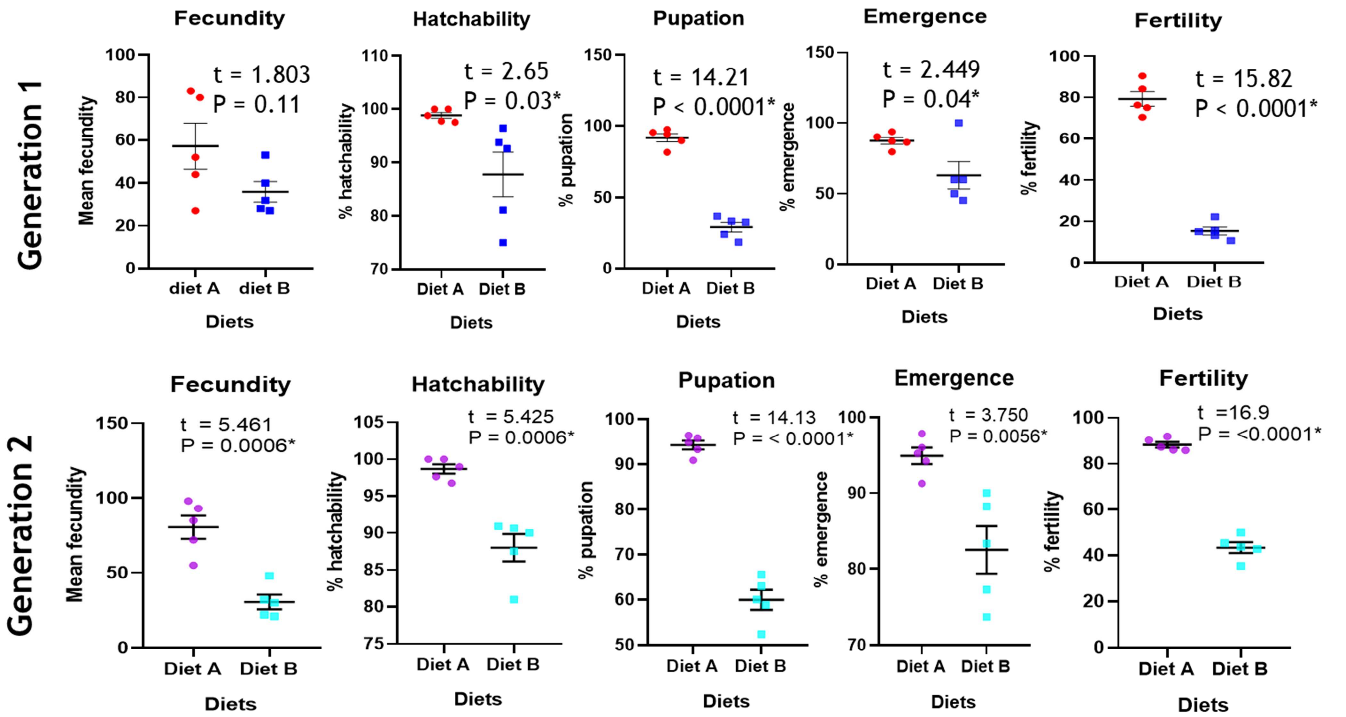
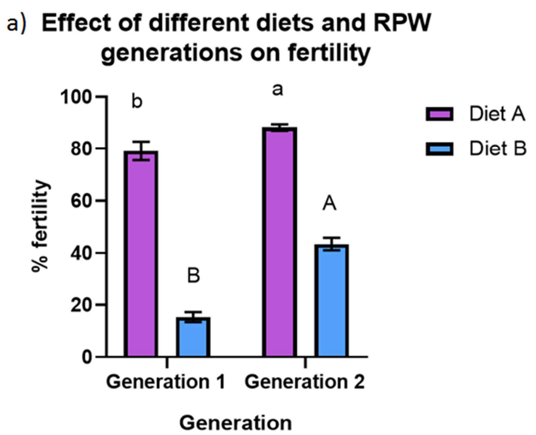


Figure 4. *Effect of different diets on fecundity, hatchability, pupation, emergence and fertility of 1st and 2nd RPW generation. Error bar showing standard error of mean (SEM). Asterisks indicate significant results at p≤0.05.*



b)

Source	F value	P value
Generations	59.27	<0.0001*
Diets	505.68	<0.0001*
Generations x Diets	15.63	0.0011*

Figure 5. *(a) Fertility percentage (%) of 1st and 2nd RPW generations reared on different diets. Lettering showing significant difference in treatments and similar letter showed no-significant difference computed with P ≥ 0.05. Error bar showing standard error of mean (SEM). (b) ANOVA table.*

Aside from that, the RPW also take significantly shorter time to complete its developmental time for two stages when reared with Diet A as compared to diet B (P<0.05) (Figure 6) with 33.4±1.3 days (Diet A) vs. 73.0±1.5 days (Diet B) for larvae-pupae stage and 27.2±0.6 days (Diet A) vs. 31.4±0.7 days (Diet B) for pupae-adult stage. On the other hand, the egg-larvae developmental time for the two diets are not statistically different (P=0.545) with 3.2±0.4 days in Diet A and 3.6±0.5 days in Diet B (Figure 6a). In total, RPW reared on Diet A required 63.8±1.9 days to complete its life cycle, meanwhile Diet B took about 108±1.8 days (Figure 6d). However, adults' longevity shows no difference between the two diets with P = 0.171 (Figure 7).

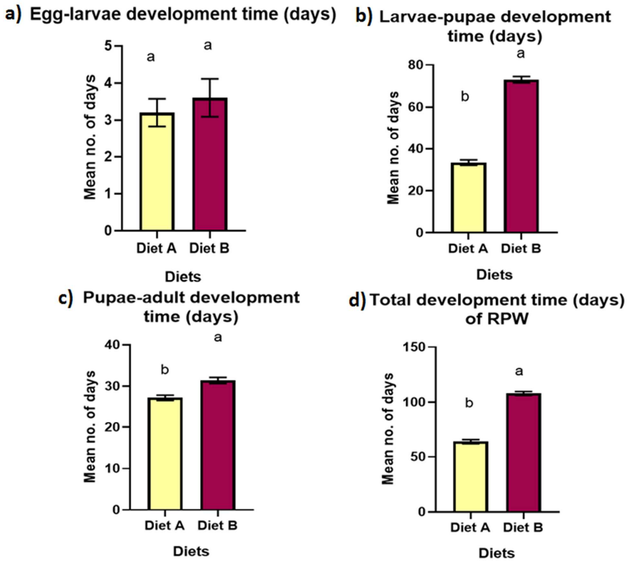


Figure 6. *Developmental time (days) of RPW during each stage; (a) egg-larvae, (b) larvae-pupae, (c) pupae-adult; and (d) the whole life cycle on different diets. Lettering showing significant difference in treatments and similar letter showed no-significant difference computed with P > 0.05. Error bar showing standard error of mean (SEM) of five replicates.*

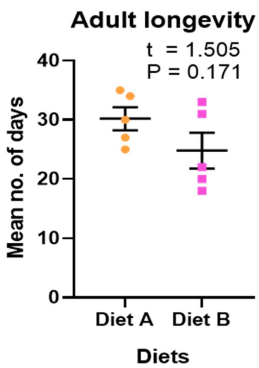


Figure 7. *Adult longevity of RPW reared on different diets. Single asterisks showing significant difference in treatments computed with P ≤ 0.05. Error bar showing standard error of mean (SEM) of five replicates.*

4. Discussions

In this study, the similar fecundity of RPW between the two diets in the 1st generation could have been contributed by the parents' diet. Even though the parents were starved a few hours before the experiment, the fact that the parents were fed on same diet prior to the experiment might have affected their eggs production. This is supported by Dind et al. in which they explained the complexity of transgenerational effect of parental diet on offspring production [15]. On the other hand, the difference in fecundity of the 2nd generation RPW would be resulted from different nutritional value of both diets fed by the parents. Macartney et al. mentioned that protein consumption by female insects would enhance fecundity [16], and this could have expressed in Diet A by the addition of chicken feed powder that contain high proteins which is about 20.66%-21.05% as well as other essential nutrients such as carbohydrates, vitamins and others [14]. Meanwhile, only 4.71% of crude protein was reported in sugarcane [17].

In the present study, hatchability, pupation and adult emergence recorded higher percentages (>90%) for Diet A in both generations. Typically, insect eggs such as RPW contain nutrients to support embryogenesis and first instar emergence [18] which contributed by the parents' feeding activity. As for the larvae development, it was found that the larvae consist of seven instars in Diet A and nine instars in Diet B. The use of its natural host as one of the ingredients in Diet A could have explained the fewer instars. This is agreed by Ju et al. who found that larvae have fewer instars and shorter development time on suitable host plants [19]. Moreover, addition of cassava in Diet A which contains high amount of carbohydrates has played major role in larvae activity and development. Aside from that, higher pupation percentage in Diet A is attributed by the availability of fibers [20]. Presence of coconut fibers from the husk has helped in cocoon construction which able to retain moisture and speed up pupal development [21]. The emergence of RPW could have been affected by the properties of fiber that constructed the cocoons. From a study conducted by Danso, coconut fibers appeared to be smoother in texture with highest tensile strength as compared to sugarcane and oil palm fibers [22]. Fiber strength is important to provide durability of the cocoon and protection to the pupae [23].

Aside from that, RPW development time which serve as an indicator to determine the suitability of a diet to RPW growth can be affected by the structure of the diet. Most of the ingredients in Diet A were prepared manually prior to the mixing, thus giving the appearance of highly viscous with lumpy-looking diet which make it easier for RPW to lay eggs and feed on. This condition has shortened the feeding process and speed up its development time as compared to solid and compact appearance of diet B. This is supported by Ainatun et al. where they found that hard and rigid textured food such as sago (in this case sugarcane) was the least preferred diet compared to other food with softer texture [24]. This is because the food texture affects boring activities of the larvae [25]. In this study, diet B requires frequent replenishment as the food would easily contaminate with fungus. However, the frequent diet changing activity and larvae transfer would hinder cocoon constructions by RPW which later prolonged the pupae-adult development time.

5. Conclusion

Selection of suitable diet for RPW mass-rearing is vital to ensure continuous supply of the live samples to be used in laboratory trials. Thus, this study suggested that Diet A which consists of cassava tubers, chicken feed and coconut husks as main ingredients is suitable for the RPW continuous rearing as compared to Diet B which use sugarcane only. However, further study should be conducted while considering other factors such as mating frequencies, nutritional status of the diets as well as diets freshness. Moreover, more parameters should also be considered including size of adults and egg loads, to understand its development.

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