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Rearing and Larval Morphometrics of *Rhynchophorus phoenicis* (*Curculionidae*, *Coleoptera*) in Maniema Province, Democratic Republic of Congo

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Abstract

Bioassays were carried out to establish a suitable technique for the rearing of the palm weevil *Rhynchophorus phoenicis* larvae, the most abundant species of this genus in the region. Insects are common in the local diet. Locally available agricultural by-products were tested. Several morphometric parameters were recorded. Incubation of larvae on a mixture of cassava flour, coconut waste, sugar cane and plantain banana resulted in 65.1% of larvae producing cocoons and 97.8% of cocoons producing weevils. Larvae with small setae on the head capsule produce male weevils, while larvae without these setae produce females. When the feeds in the plastic boxes were packed up to 50% of the volume, the weevil survival rate at age 30 days significantly increased up to 86.7%, as compared to higher and lower box loading. Three innovative treatments based on agricultural co-products available in the region and a control (fresh stipe and oil palm kernel wood) derived from the best results of previous authors were used. On day 35, there was no significant difference between the weights and lengths of larvae fed on mixtures of coconut waste, corn meal and sugarcane and the control. Given the fact that large-scale production based on the host plant does not guarantee the conservation of the ecosystem, especially if we had to scale up to commercial production, we found an alternative feed. Our results will help improve the palm weevil rearing conditions, and the feed and they open up opportunities for the conservation of palm and raffia tree ecosystems.

Keywords

Insect Rearing, Larval Morphometrics, *Rhynchophorus phoenicis*

1. Introduction

Insect rearing for human food [1] is increasingly being studied as a sustainable solution to meet the growing food needs [2] of the global population [3]. In this context, the palm weevil *Rhynchophorus spp*, as a highly eaten insect [4], offers a promising exploitation opportunity [5]. In fact, insects are established as a popular and sustainable practice in human food, offering high nutritional value. In farming, insects are used to feed various animals, thereby reducing the pressure on natural resources. This approach represents a promising solution for meeting the nutritional needs of animals while promoting environmentally friendly food production [6]. Palm weevil larvae are significant sources of proteins, lipids, vitamins, minerals and fibers, all of which are essential for human and animal health and well-being. Its varied nutritional composition makes it a valuable food for growth, health and weight control. In some food cultures, palm weevil larvae are recognised as a key component in meeting overall nutritional requirements [2] [4] [7].

The use of agricultural by-products as feed for these insects can help to add value to these under-utilized resources, while offering an alternative source of protein for humans. Rearing palm weevils for food has multiple benefits [6]. Not only can it contribute to food security and the diversification of protein sources [8], but it could also offer a more environmentally [9] and resource-efficient alternative to traditional livestock farming [10]. Insect rearing, with its efficient feed conversion rate, requires fewer resources than livestock farming, thus reducing environmental impact while preserving biodiversity. By using organic waste, insect rearing reduces food waste and water and soil pollution. This sustainable approach enables more efficient use of natural resources for environmentally friendly food production [11]-[13]. Palm weevil larvae are generally reared for 30 - 45 days under appropriate environmental conditions.

This rearing practice is considered sustainable because of its low environmental impact, its use of organic substrates, its feed flexibility and its ability to be produced locally [14] [15]. Yet, the role of Rhynchophorus as part of the trophic chain and as a vector and pest which infests palm trees by feeding on the kernel tissues, causing internal damage and weakening the trees should not be underestimated. This infestation causes visible symptoms such as leaf wilting and dieback, which leads to the death of the palm trees. Moreover, by feeding on palm trees sap, Rhynchophorus spreads diseases, exacerbating economic losses [16]. Most likely, the rearing practice will remain minor as compared to natural palm weevil populations. Thus, the ecological processes in the oil palm ecosystems cycles will not be disrupted, even regarding larvae gatherers. Also, in order to avoid inbreeding, wild cocoons and larvae will be occasionally collected from the wild, based on empirical indicators of the rearing yield and carefully separating and grouping the breeders, to renew the genetic pool.

Previous studies involved the use of various substitute feeds such as dates, maize, palm kernel cake, sugarcane cake, pineapple cake, potato cake, blueberry flour, etc. The choice of sugarcane, cassava and maize meal, palm kernel cake, banana, rice bran, palm kernel and palm stalk as feed is justified by their availability in the environment, their low purchase cost, as well as the fact that these feed components have been used by tested several researchers. In most cases, production yields have been remarkable, though production varies with the study environment [14] [15] [17]-[20].

Some previous experiments evaluated larval development, production and survival of weevils and larvae using these alternative feeds. The researchers observed variations in the food preference and biological performance of weevils and larvae depending on the substitute feed used. Comparisons were made between the performance of weevils and larvae on natural and artificial feeds to study the impact of feeding on their life cycle. The experiments provided a better understanding of the nutritional requirements and preferences of different species of palm weevil, including *R. phoenicis*. The results highlighted significant differences in weevil adaptation to different feeds, which may have important implications for the sustainable management of species of the genus *Rhynchophorus.* It should be noted that not all feeds give good yields, as other feeds lead to high mortality and low production [5] [14] [15] [17]-[27].

Thus, the exploitation of agricultural by-products for *Rhynchophorus* rearing in Maniema province offers significant advantages. Effectively recycling this waste, not only reduces food waste, but also promotes sustainable, environmentally-friendly food production. However, challenges remain with regard to the rearing of *Rhynchophorus spp* for human food in Maniema province. Aspects such as optimal rearing techniques, necessary environmental conditions and post-harvest handling practices need to be investigated to ensure sustainability and food security. Palm weevil rearing in Maniema province is of crucial importance due to several key aspects. Currently, the larvae eaten are harvested in the wild, but this production is not sufficient to meet the needs of the community, due to the scarcity and seasonal fluctuations in the markets and their high prices. This is why it is essential to promote rearing to ensure sustainability. Furthermore, the rearing of *Rhynchophorus phoenicis* offers significant environmental advantages over traditional livestock farming. Such insect rearing requires fewer resources in terms of water and feed, while producing fewer greenhouse gas emissions; thus an environmentally-friendly practice. Moreover, the farming of edible insects can offer significant economic opportunities for local communities [28].

The overall aim of this paper is to explore the opportunities and challenges of rearing *Rhynchophorus spp* for human food. We aim to assess the feasibility of this practice, examine the technical aspects of its implementation, and discuss its potential as a sustainable food source. Specifically, this paper aims to identify agricultural co-products suitable for feeding *Rhynchophorus* to ensure healthy growth, and evaluate *Rhynchophorus spp.* rearing methods to optimize the production and quality of reared insects, and morphologically characterize reared *Rhynchophorus spp.* specimens. The hypothesis is that available agricultural co-products can provide a suitable nutritional source for rearing *Rhynchophorus spp* in a controlled environment and could improve larval growth performance, while ensuring the conservation of cultivated and wild palm and raffia in their ecosystems.

2. Study Area, Materials and Methods

2.1. Study Area

The current study was carried out in the city of Kindu (**Figure 1**), Maniema province in the East of the Democratic Republic of Congo. The city of Kindu covers an area of 101.295 km^2 with an estimated population of 719,895 inhabitants [29]. Maniema is characterized by a warm, humid equatorial climate in the north and a humid tropical climate in the south, with a transition zone in the center. Annual rainfall varies from 1300 mm in the south to 2300 mm in the north. Average temperatures range from 23˚C - 25˚C (N'sanda *et al.*, 2011). Two main plant formations cover Maniema: dense rainforest and savannah. Among the plant species, oil palm is an important crop in Maniema province, playing a crucial role in the local economy as a source of income for many families. Products derived from palm oil, such as palm oil and palm kernel oil, are widely used in food, cosmetics and handicraft industries. Oil palm production contributes to food security and poverty alleviation in Maniema province, providing jobs and economic opportunities for local communities. Raffia is another important resource in Maniema province, and it is used to make baskets, hats, ropes, and other traditional handicrafts. The sustainable exploitation of raffia would play an essential role in preserving biodiversity and local ecosystems. Local communities depend on raffia for their livelihoods, deriving income from the sale of finished products made from this natural raw material. Responsible management of oil palm and raffia resources is essential to ensure the economic and ecological sustainability of these sectors. Maniema province benefits from a rich hydrographic network. It is crossed from south to north by the Congo River, which drains the waters of several tributaries facilitating river traffic from Kindu to Ubundu/Kisangani, a very important route for supplying the province with manufactured products [30].

2.2. Materials and Methods

To start the study, larvae were collected in the wild. These larvae were incubated with two types of conditioning feed to facilitate their metamorphosis until breeder weevils developed. Then, the virgin weevils hatched in the laboratory enabled rearing bioassays to begin using three innovative treatments based on agricultural co-products available in the region and a control derived from the best results of previous authors [14] [18].

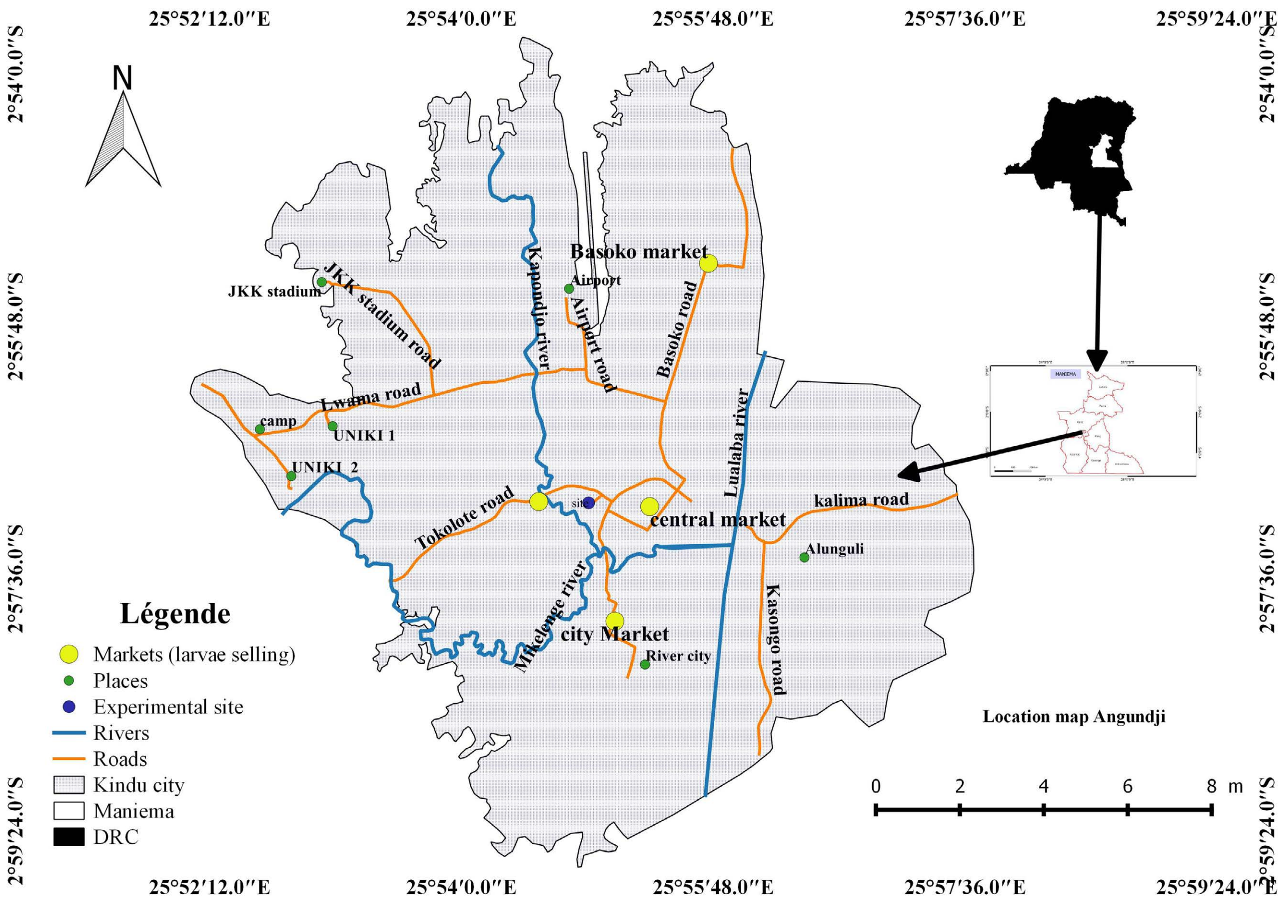


Figure 1. Kindu city, the study area.

2.2.1. Wild Larvae Collection Phase to Produce Virgin Weevil Breeder Stock

Eight hundred and forty (840) wild-collected larvae (Figure 2) were incubated at a rate of four hundred and twenty (420) larvae per feed type. At this preliminary stage, two types of feed were applied in 12 replicates to ensure that the larvae metamorphosed into weevils. Thus, 35 larvae were used per replicate in plastic boxes. The larvae were fed on substitute feeds until the emergence of the weevils. Cocoons were harvested from day 15 - 29 after incubation. This staggered harvest was justified by the fact that incubated larvae were not of the same size. Weevil harvesting was also staggered; a maximum duration of 60 days was observed from incubation to weevil harvesting. The following feed mixes were used at this stage: a) Cassava flour, coconut waste, sugar cane and plantain bananas; b) Rice bran, wood chips, palm kernel cake and banana trunks. The choice of these feeds is justified by their availability and very low purchase price. The aim of these treatments was to provide the larvae with the right conditions to promote metamorphosis [31].

2.2.2. Setting up Rearing Sites, Collecting Experimental Feeds and Loading Feeds in Plastic Boxes

Coconut waste, palm stipes and oil palm kernel woods were collected in the wild, while sugarcane, palm kernel cakes, rice bran and plantains were purchased at markets. Cassava and maize flour were collected at the mills (**Figure 3**).

Figure 2. Collection of wild larvae for the production of brood weevils.

Figure 3. Feeds collected.

The experimental set-up was installed in a fenced plot on avenue Musofu, quartier mission, commune Kasuku. 24 plastic boxes were placed on shelves in a completely randomized arrangement with three feed treatments and one control. Six boxs (3 blue and 3 green) were used per treatment (**Figure 4**).

T1: a mixture of rice bran, bananas and sugar cane

T2: a mixture of coconut waste, corn flour and sugar cane

T3: a mixture of cassava flour, palm kernel oilcake

T4: a control (mixture of fresh stipe and palm kenel wood).

Figure 4. Experimental setup and feed treatment (box length 29 × 42 × 28.5 cm); volume 25 liters.

2.2.3. Mating and Seeding of Experimental Boxes

Freshly emerged weevils were mated in plastic boxes (**Figure 5**). They were fed daily with sugarcane pieces [18].

Figure 5. Spawning feed, breeder stock and plastic box.

In the trial, 48 pairs were seeded in the substitution feeds. The weevils and their egg-laying feeds were placed in plastic boxes with holes drilled according to the treatments, then covered with a piece of mosquito netting to allow aeration and limit the attack of eggs and larvae by predators and parasites (**Figure 6**). Two pairs of weevils were seeded per box.

Figure 6. Rearing plastic boxes.

2.2.4. Rearing Monitoring and Larvae Production

The monitoring included the following activities: temperature monitoring (maintained between 25˚C - 30˚C), humidity control at 70%, regular checking of feed with addition if necessary, weekly watering of feed in case of apparent dryness, breeder stock monitoring, larvae control and development monitoring to determine optimal harvesting time. The domestication boxes were installed on a shelf. During the day, the shelf was covered with a tarpaulin to maintain shade, as palm weevil larvae prefer this environment. Every night, the tarpaulin was removed to promote aeration, and watering was carried out regularly. In our rearing facility, we carefully maintained a constant ambient temperature of between 20˚C - 30˚C to promote optimal larval development. We set up a standard photoperiod of 12 hours of light and 12 hours of darkness to simulate a natural day-night cycle, to satisfy the larvae's biological needs and make it easy for implementation in local farmers' conditions during the planned future extension phase.

The production (**Figure 7**) was evaluated in grams and kilograms on day 35. The experiment was terminated on day 35, the time when the cocoon formation started. The following variables were taken into consideration: number of larvae on 10 and 35 days, fresh weight (g), survival rate (%) and mortality rate (%), depending on feed treatments. Larval weights were measured using an Electronic Kitchen scale, with a 1kg capacity and 0.1 g accuracy.

Figure 7. Production of larvae.

2.2.5. Measurements of Reared Larvae

Fresh weight (g), total larval length and width (cm), larval color, and head capsule length and width were measured on days 15, 25 and 35 after seeding. Fresh weights were estimated on day 15, day 25, and finally day 35. Morphometrics were stopped on day 35 because by this stage some larvae had begun to form their cocoons. These parameters were evaluated in relation with feed treatments. Larval weights were measured with a 500 g capacity Digital Scale with an accuracy of 0.01 g on day 15th, and with a 1 kg capacity Electronic Kitchen Scale with an accuracy of 0.1 g on days 25 and 35. Total larval length and width, as well as head capsule length and width, were measured using a caliper (Figure 8).

Figure 8. Larval morphometrics.

2.2.6. Sexual Dimorphism of Larvae

After producing larvae in the laboratory using substitute feeds, we identified two categories of larvae based on the presence of setae on the head capsule. Some larvae had a head capsule with setae, while others had a head capsule without setae. Twenty larvae with setae on the head capsule and twenty larvae without setae on the head capsules were seeded in two separate boxes under feeds composed of cassava flour, coconut waste, sugar cane and plantains.

2.2.7. Influence of Box Loading Level (Height) on Weevil Life Cycle and Rearing Success

Following the observation of early weevil mortality in rearing boxes, a study was undertaken to identify the cause of this phenomenon and find solutions. The life cycles of 135 weevils were monitored for 30 days, using three different treatments and three replicates. Each replicate was seeded with 5 pairs of weevils. Survival and mortality rates were assessed on days 10, 20 and 30. The following treatments were used: T21: 90% box loading, T22: 50% box loading and T23: 20% tank filling.

2.2.8. Data Archiving and Statistical Analysis

The data collected was entered into an Excel 2013 spreadsheet, then exported and analyzed using Past 4.11 software. Averages of larval sizes by box loading level were compared using the non-parametric Kruskal-Wallis test in Jamovi 2.3.28 software, with a significance level of 5%. Descriptive analysis was performed on larval production and morphometric parameters to determine means, medians, standard deviation, minimum and maximum values and the coefficients of variation. Analysis of variance was performed to compare production averages and larval morphometric parameters. Principal component analysis (PCA) was also performed for morphometric parameters using Past 4.11 software, while path diagramming on JASP 0.18.3.0 facilitated the graphical linking of variables.

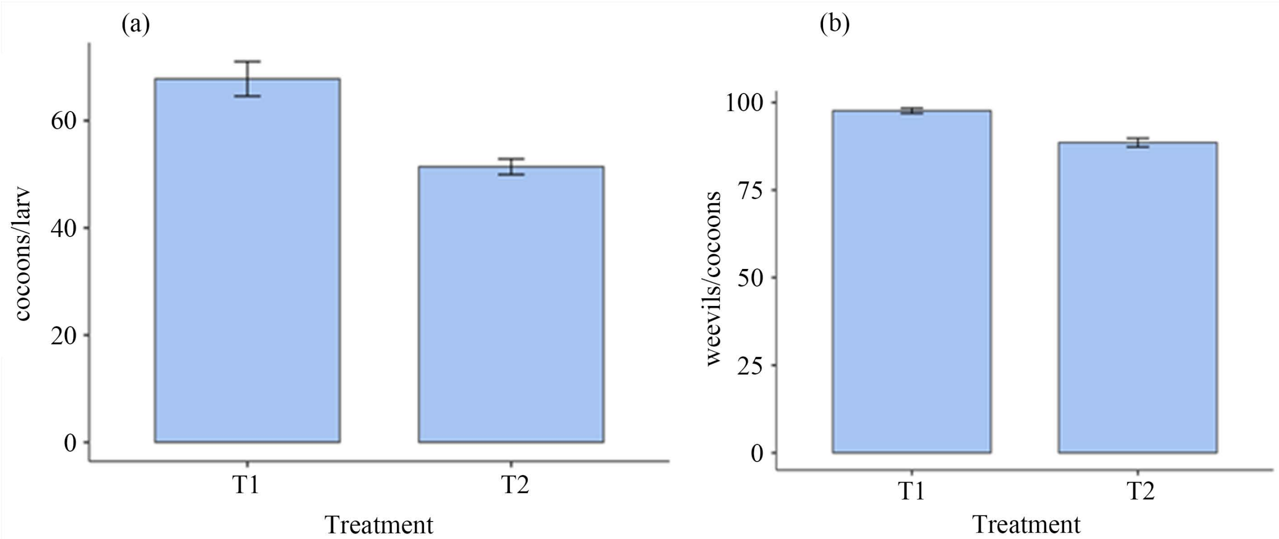
3. Results

3.1. Life Cycle and Proportions of Cocoons and Weevils Harvested after Larval Incubation

Here are some observations on the reproduction and life cycle: a) *Breeding stock and mating*: in July 21 - 22, 2024, after the eclosion from cocoons, the virgin weevils were sown in boxes (25 × 18 × 10 cm) which contained feed and sugarcane pieces appropriate for egg-laying. After a mating period of 24 hours, they were seeded in the rearing boxes all together with feed and the spawning sugar cane substrates. b) *Eggs* were observed 3 days after mating and larval hatching occurred on day 3 - 5. c) *Larval development*: the larvae stage varied from 35 - 90 days, influenced by moisture in the rearing medium. The humidity emanated from occasional watering. Larvae in a humid environment undergo slow metamorphosis; while larvae in a drier environment undergo an early metamorphosis. d) *Pupal* (*cocoon*) *phase and weevils life span*: the pupal phase lasts from 14 - 17 days and the life span of the weevils in the laboratory lasts from 25 - 70 days.

Table 1. Proportions of cocoons developed from larvae collected in the wild and proportions of weevils.

	Treatment	n	Mean	Median	SD	χ^2	df	P
Cocoons/larvae (%)	T1	12	67.8	64.6	11.17	14.6		<0.001
	T2	12	51.4	50	5.04		1	
Weevils/cocoons (%)	T1	12	97.6	98.5	2.65	16.1	1	
	T2	12	88.5	89.8	4.3			<0.001



Legend: T1, feed **1**, cassava flour, coconut waste, sugar cane and plantains and T2, feed **2**: rice bran, chips, oilcake and banana barter.

Figure 9. Means (± standard errors) of (a) proportions (%) of cocoons developed from incubated wild-collected larvae per box, and (b) proportions of weevils hatched from the cocoons (n = 12).

On average, the egg stage lasted 4.00 ± 1.00 days; the larval stage took 65.5 ± 27.5 days and the cocoon stage lasted 15.5 ± 1.5 days. Table 1 illustrates the descriptive statistics of the proportions of cocoons developed from larvae collected in the wild and the proportions of weevils hatched from the cocoons. Figure 9 shows the means and standard error bars for (a) the proportions of cocoons developed from larvae collected in the wild (which were incubated), and (b) the proportions of weevils hatched from the cocoons as breeders to start the rearing process. The Kruskal-Wallis test (Table 1 and Figure 9) shows that the proportions of cocoons harvested were significantly higher with the feed T1, a mixture of rice bran, banana and sugarcane (67.8% for T1 vs 51.4% for T2, a mixture of rice bran, chips, palm kernel cake and banana barter).

The hatching rate of weevils from these cocoons was 97.8% for feed 1 and 88.5% for feed 2. There were on average 22.8 ± 6.78 cocoons per 25 L box in the mixture of *cassava flour, coconut waste, sugar cane and plantain* vs 17.7 ± 5.57 cocoons per box in the mixture of *rice bran, chips, palm kernel cake and banana barter*. On average, the egg stage lasted 4.00 ± 1.00 days; the larval stage took 65.5 ± 27.5 days and the cocoon stage lasted 15.5 ± 1.5 days.

3.2. Influence of Feeds on Palm Weevil Production

Table 2, **Table 3** and **Figure 10(a)**, **Figure 10(b)** show that T4 performed highly significantly better than T2 regarding the hatching rates on day 10 and the survival rate of larvae on day 35 ($P < 0.001$). T3 and T1 yielded significantly fewer larvae from the eggs and provided much less larval survival than T4 and T2. T3 was the weakest treatment in this regard. Minimum and maximum survival rates were 52.4% and 82.6% for T1, 66.4% and 99.7% for T2, 20.0% and 59.4% for T3, and 86.4% and 98.5% for T4 respectively. Subsequently, the mortality was significantly lower for T4 followed by T2, while high mortality was observed for treatments T3 and T1. The minimum and maximum mortality rates for the different treatments were as follows: T4 (1.5% - 13.6%), T2 (0.3% - 33.6%), T1 (17.4% - 47.6%) and T3 (40.6% - 80.0%).

Table 2. Means and standard deviations of the proportions of numbers of larvae hatched/eggs on day 10 after seeding and larval survival rates (%) and mortality (%) on day 35 (n = 18).

	Treatments	n	Mean	Median	SD
Larvae on day 10	T1	18	69.3	72.0	20.6
	T2	18	125.9	117.5	36.5
	T3	18	26.3	25.0	16.1
	T4	18	181.0	180.0	8.4
Survival rate (%) based on day 10 larvae which reached day 35	T1	18	67.8	68.1	8.6
	T2	18	79.8	79.4	10.9
	T3	18	42.7	39.8	12.7
	T4	18	93.8	94.1	3.2
Mortality rate (%)	T1	18	32.2	31.9	8.6
	T2	18	20.2	20.6	10.9
	T3	18	57.3	60.2	12.7
	T4	18	6.2	5.9	3.2
Larvae/eggs (hatching rate %) estimated on day 10	T1	18	67.8	68.1	8.6
	T2	18	79.8	79.4	10.9
	T3	18	42.7	39.7	12.7
	T4	18	93.8	94.1	3.2

Legend: T1: 50% a mixture of rice bran, 30% banana and 20% sugarcane, T2: 30% a mixture of coconut waste, 50% corn flour and 20% sugarcane, T3: 50% a mixture of cassava flour, 30% oilcake and 20% sugarcane, T4: a control (mixture of fresh stipes 50% and oil palm kernel wood 50%.

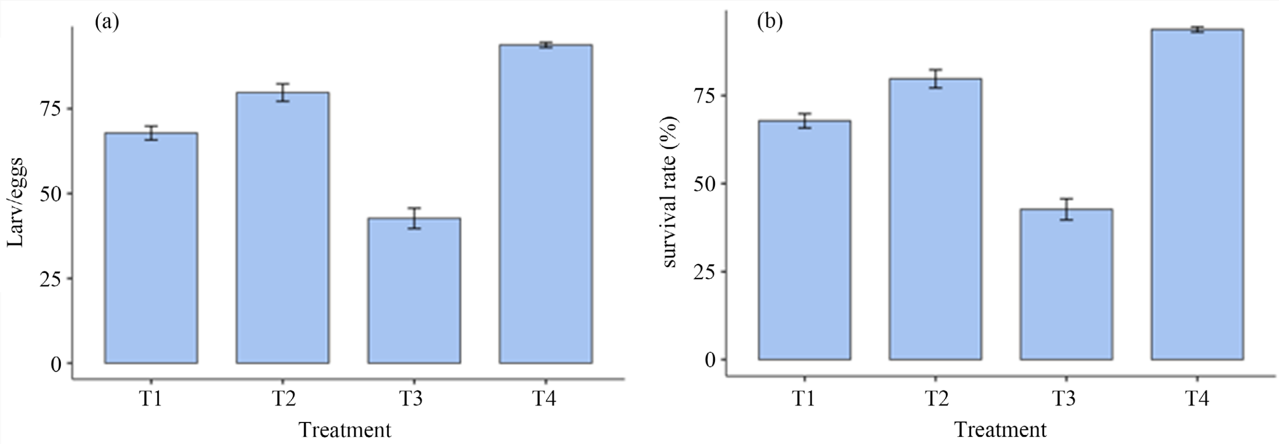


Figure 10. Means (standard error bars) of a) the proportions (%) of larvae hatched/eggs on day 10 after seeding and b) larval survival rates (%) per feeding treatment on day 35.

3.3. Sexing the Palm Weevils at Larval Stage: Dimorphism

After careful morphological observations of the larvae produced in the laboratory, it is now possible to differentiate the sex of *Rhynchophorus* at the larval stage. Larvae with small setae on the head capsule give rise to male weevils, while larvae without head setae, with a very smooth head capsule, give rise to female weevils (Figure 11). Twenty males developed from the twenty larvae with setae, while twenty females developed from the twenty larvae without setae.

Larva with setae on the head capsule | Larva without setae on the head capsule

Figure 11. Larval sexual dimorphism.

3.4. Influence of Box Loading Level on Weevil Lifespan and Rearing Success

The Kruskal-Wallis test proved that the box loading level had a highly significant influence on the survival rate and mortality of the weevils (Table 3 and Table 4). Loading the boxes up to 50% volume gave a very high survival rate for both sexes of weevils, although male weevils had a longer lifespan than females. In terms of mortality rate, filling the boxes up to 90% showed a high mortality rate, followed by filling the boxes up to 20%. Much lower mortality rate was observed when the boxes were filled up to 50%.

Table 3. The average survival and mortality rates of weevils on day 30 in relation to the loading levels of rearing boxes (%).

	Gender	T21	T22	T23	P
Weevils survival rate (%)	M	24.4 ± 4.44	86.7 ± 4.71	31.1 ± 5.87	<0.001
	F	13.3 ±4.71	73.3 ± 3.3	15.5 ± 5.55	<0.001
Weevil mortality rate (%)	M	75.5 ± 4.44	13.3 ± 4.71	68.9 ± 5.87	<0.001
	F	86.7 ± 4.71	26.7 ± 6.66	84.4 ± 5.55	<0.001

Legend: T21: 90% bin filling; T22: 50% bin filling and T23 20% bin filling.

Table 4. Descriptive statistics on day 35 of the morphometric parameters of larvae reared under various substitute feeds (n = 6).

Variable	Treatment	Mean	SD
Fresh weight (g)	T1	4.0	0.63
	T2	6.2	0.75
	T3	3.3	1.06
	T4	7.0	0.89
Total length (cm)	T1	5.1	0.08
	T2	5.4	0.05
	T3	4.4	0.29
	T4	5.5	0.14
Total width (cm)	T1	1.2	0.07
	T2	1.4	0.07
	T3	1.1	0.05
	T4	1.5	0.09
Length of head capsule (cm)	T1	0.6	0.04
	T2	0.8	0.04
	T3	0.6	0.00
	T4	0.9	0.06
Head capsule width (cm)	T1	0.4	0.04
	T2	0.6	0.04
	T3	0.4	0.00
	T4	0.6	0.04

3.5. Effects of Feed on the Growth Parameters of Reared Palm Weevil Larvae

3.5.1. Weight

Table 5 illustrates the (a) descriptive statistics of larval weight by treatment during the rearing process from day 10 to day 35 and b) significant Tukey Post Hoc comparisons. Table 5 and Figure 12(a), Figure 12(b) show that larvae fed on T4 and T2 were highly significantly bigger than those fed on T1 and T3 all along the experimental period. Figure 12 shows (a) the averages (with standard error bars) of larval fresh weights (g) by stage and by treatment and the pattern of growth under the various feeding treatments. The analysis of variance of larval fresh weight means by treatment and by stage and the treatment*stage interaction reveal significant differences. Figure 12 shows that treatment T4 provides the highest growth in fresh weight at each stage; similarly with T2 (days 15, 25 and 35).

Table 5. Larval weight by treatment from day 10 to day 35. Descriptive statistics of larval weight by treatment.

Days	Treatments	n	Mean (g)	Median (g)	SD	Minimum (g)	Maximum (g)
15	T1	6	0.8	0.8	0.09	0.6	0.9
	T2	6	1.0	1.0	0.09	0.9	1.1
	T3	6	0.2	0.2	0.02	0.2	0.2
	T4	6	1.5	1.4	0.28	1.3	2.0
25	T1	6	2.1	2.1	0.05	2.0	2.1
	T2	6	2.2	2.2	0.05	2.1	2.3
	T3	6	1.8	1.9	0.12	1.7	2.0
	T4	6	2.5	2.4	0.50	2.2	3.6
35	T1	6	4.0	4.0	0.63	3.0	5.0
	T2	6	6.2	6.0	0.75	5.0	7.0
	T3	6	3.3	3.5	1.07	2.1	4.5
	T4	6	7.0	7.0	0.89	6.0	8.0

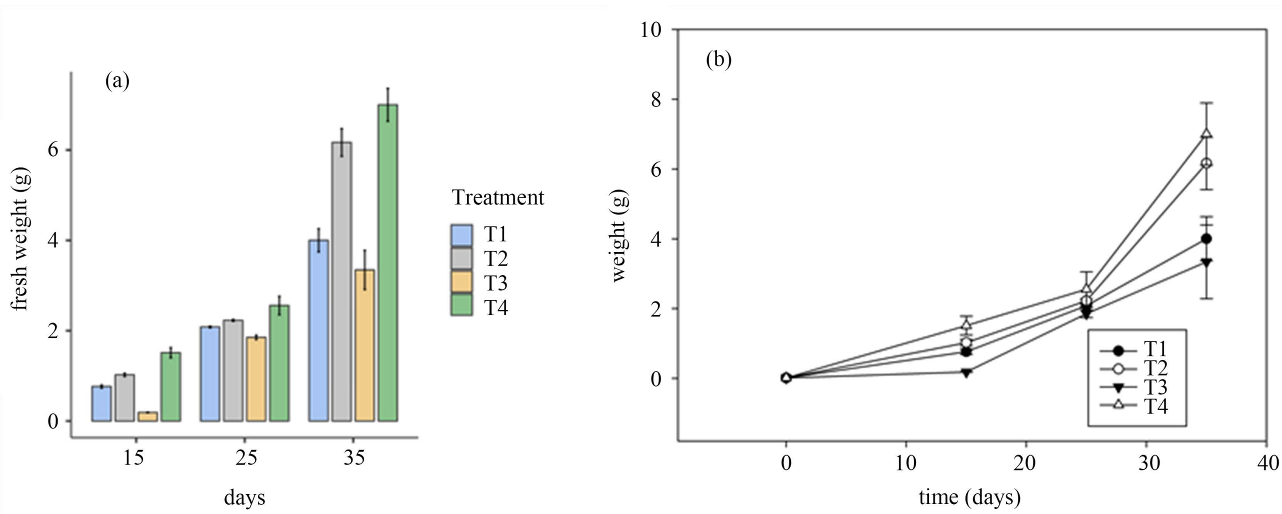


Figure 12. (a) Average individual larval weights by treatment over the experimental period and (b) Increment trend of larval weight by treatment since day 1 until day 35.

On day 35, T4 averaged 7.0 vs 6.2 g for T2. T4 reached a minimum of 6 g and a maximum of 8 g; vs a minimum of 5 g, a maximum of 7 g for T2. T1 and T3 produced smaller larvae with a maximum weight of 5 g and 4.5 g respectively on day 35; On the other hand, treatments T1 and T3 produced larvae with a maximum weight of 5 g and 4.5 g respectively on day 35.

Thus, from day 15 to day 35, larvae fed with a mixture of fresh stipe and oil palm kernel were heavier, followed by larvae fed with a mixture of coconut waste, corn meal and sugarcane. Larvae with very low fresh weights were observed when fed a mixture of cassava flour, palm kernel meal and sugarcane. The post-hoc test comparing means of fresh weight between treatments on day 35 shows that there was no significant difference between T2 (mixture of coconut waste, corn meal and sugarcane) and T4 (mixture of fresh stipe and oil palm kernel) ($p = 0.22$). On the other hand, when comparing T1 and T3 with T4, a highly significant difference was observed ($p < 0.001$***). This contrast was confirmed even with the extreme values. All of the bioassays were consistent, with low coefficients of variation (<25%); though T2 feed led to more consistent growth (cv = 2% - 12%) as compared to T4 (cv = 18% - 19%).

In the aftermath of the experiment (Figure 13), at harvest day 35, the larvae fed on treatments 2 and 4 were larger and statistically similar in weight and size (respectively $P = 0.42$ and $P = 0.25$). Larvae fed on feed treatment T1 and T3 were smaller and also similar in weight ($P = 0.97$); though larvae fed on T1 were longer ($P < 0.01$) than those fed on T3; but their respective widths were slightly similar ($P = 0.07$).

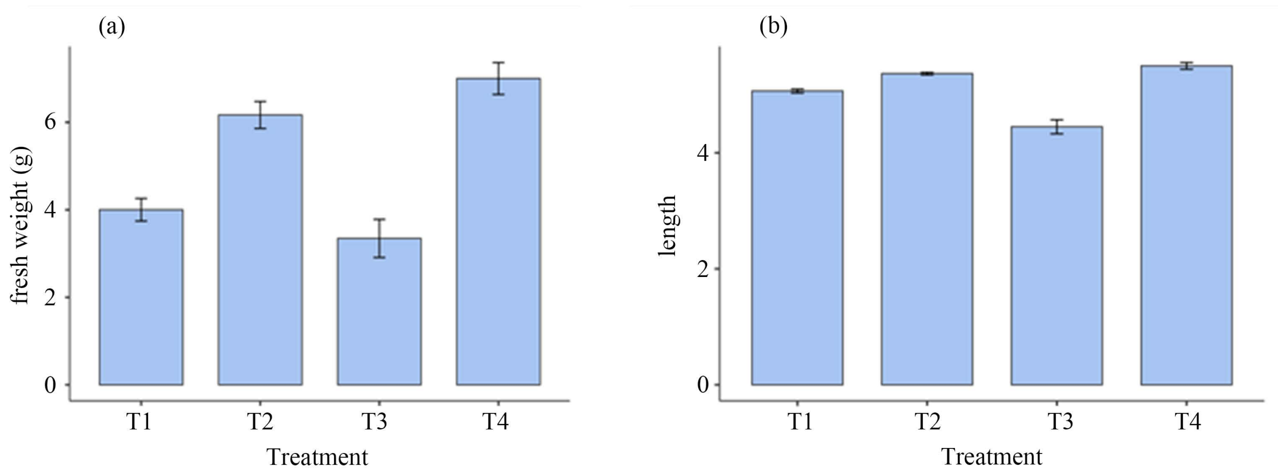


Figure 13. Means (standard error bars) of individual larval a) fresh weight and b) length at harvest time, day 35.

3.5.2. Larval Size: Length and Width

Figure 14(a), Figure 14(b) shows the averages (with standard error bars) of larval length and width in cm for the four different treatments (T1, T2, T3 and T4) at three growth stages (day 15, 25 and 35). Analysis of variance (ANOVA) of the means of total larval length as a function of treatment, stage and the treatment*stage interaction reveals significant differences. Figure 14 shows that treatment T4 and T2 yielded the longest and largest larvae at all growth stages.

The post-hoc test comparing total width averages on day 35 between T2 and T4 showed no significant difference ($p > 0.05$) as well (Figure 14). The larvae fed on T4 averaged 5.5 ± 0.14 cm vs 5.4 ± 0.05 cm for T2. On day 35, the extreme values kept a similar trend, with a min of 5.30 cm and a max of 5.70 cm for T4; vs a min.

of 5.30 cm and a max of 5.40 cm for T2. The size data were very consistent (cv = 1% - 9%) all along the experiment. Larvae fed on T1 and T3 were significantly shorter than those fed on T4 and T2.

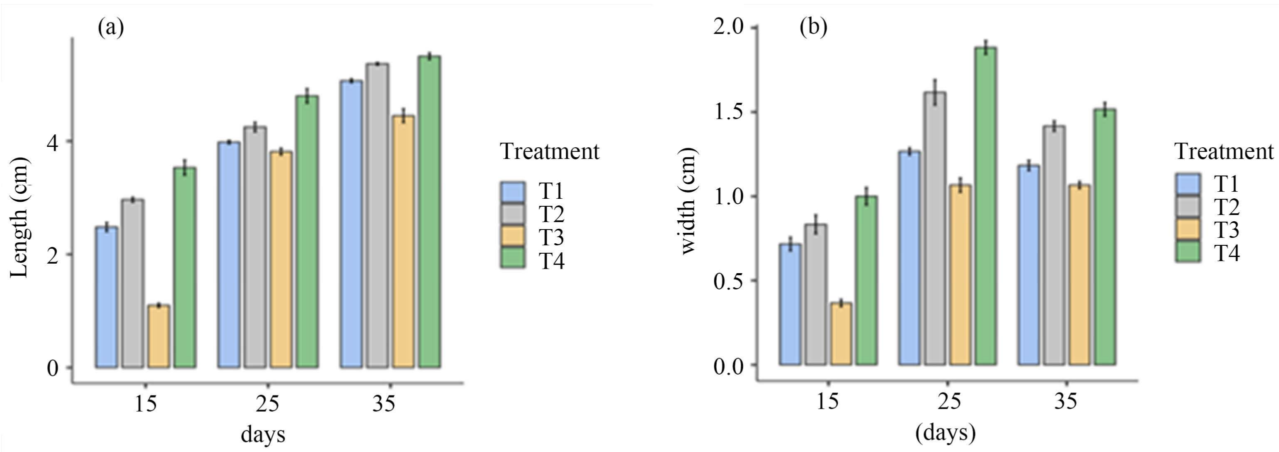


Figure 14. (a) Length and (b) width (cm) of larvae by treatment over the experimental period.

3.5.3. Size of the Head Capsule (cm)

On day 35, the head capsule for T4 averaged 0.9 ± 0.06 cm vs 0.9 ± 0.04 cm for T2 (P > 0.05, ns) and T1 and T3 produced smaller larvae in this regard (**Figure 15**).

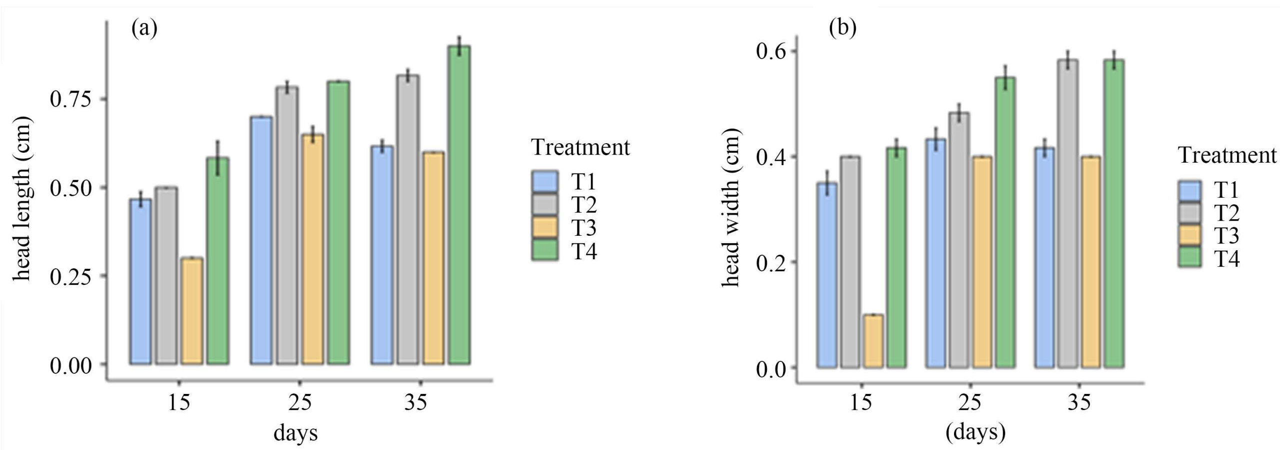


Figure 15. Average (a) length and (b) width of head capsule by treatment over the experimental period.

4. Discussion

The results of the rearing of the larvae collected in the wild, which were incubated to produce the breeder weevils, indicate that a mixture of cassava flour, coconut waste, and sugarcane and plantain banana promotes palm weevil metamorphosis. This mixture results in a success rate of 65.1% of larvae producing cocoons and 97.80% of cocoons producing weevils. [19] tested the metamorphosis of palm weevil larvae and produced 254 adults out of 449 larvae at stages 1 and 2 reared on sugarcane, *i.e.* 57%. **Figure 16** illustrates a simplified hypothetical model of the future rearing cycle.

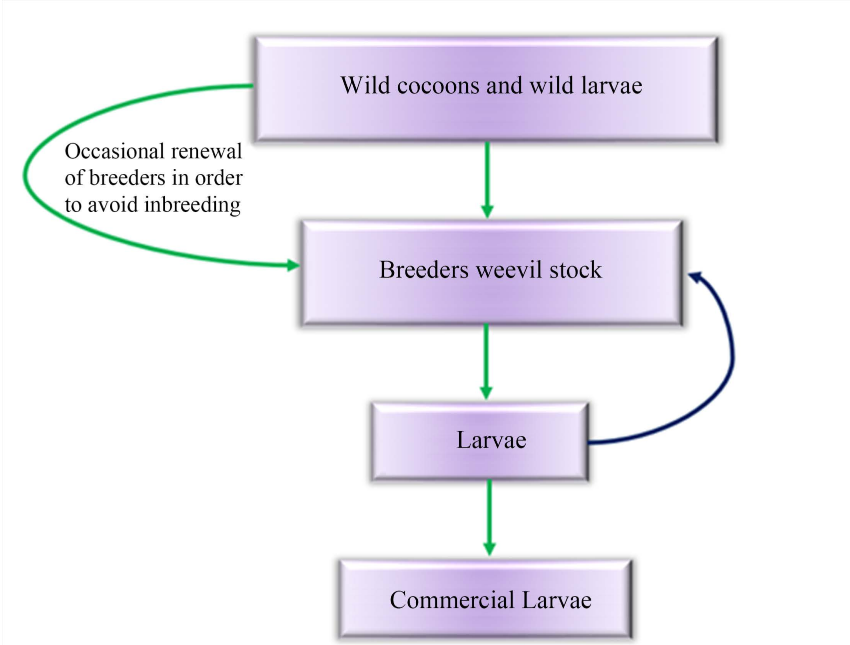


Figure 16. A model of the rearing cycle.

Regarding dimorphism, we observed that larvae with small setae on the head capsule give rise to male weevils; while those without setae and with a smooth head capsule give rise to female weevils. This finding is not visible in the scientific literature and contributes to the merit of the current paper.

Results on the effects of feed loading levels in the rearing boxes on weevil survival are optimal at 50% filling of the total volume. The mortality rate in the case of 50% filling is 19.7%, as opposed to 81.10% with 90% filling and 76.7% at 20% filling. The high mortality observed with 90% filling could be explained by the fact that weevils tend to move towards the fabric covering the box; rendering them immobile and leading to their death. Regular monitoring of spawners is crucial to mitigate this problem. Filling boxes down to 20% also results in high mortality due to lack of moisture conservation; leading to a rise in temperature that limits weevil lifespan. In this case, it is advisable to water the tanks almost every day. Thus, feed packing in rearing boxes plays a crucial role in palm weevil rearing. In fact, improved weevil lifespan leads to higher production of larvae.

With regard to weevil lifespan, male weevils live longer than females. Our result corroborates the findings of [22] which show that *R. ferrugineus* females have a shorter life cycle than males. The total longevity of females was 74 days in their study vs 94 days for males. In addition [25] stated that the lifespan of weevils is 97 ± 6.0 days. [26] showed that weevils undernourished with potatoes had a limited lifespan of 74.8 ± 2 days. [32] estimated that the average lifespan of *Rhynchophorus* males reared on a substitute feed was 94 days. After our experiments and verification of the results of other researchers, it is important to point out that the environment (temperature, moisture, and feed quality plays a crucial role in the lifespan of weevils. Regarding the effects of substitute feeds on *Rhynchophorus* *phoenicis* production, the preferred medium for *R. phoenicis* (control treatment which is a mixture of fresh stipe and oil palm kernel wood) performed well.

Reasons why a particular feed worked well may include the following: favorable chemical composition of the feed, providing essential nutrients for larval development, adaptability of the larvae to the specific feed, maintenance of optimum temperature and humidity conducive to larval growth, absence of inhibiting factors such as toxins or pathogens in the feed, positive interaction between the various feed ingredients, favoring an environment conducive to larval rearing, and the presence of positive phago-stimuli in the feed, favoring larval activity and growth, e.g., the mixture of coconut waste**,** corn flour and sugarcane generated a high yield due to its favorable characteristics. The feed maintained a normal temperature between 20˚C - 30˚C, accompanied by a relative humidity level of 70%**.** On the other hand, the feed composed of rice bran, ripe banana and sugar cane showed a low yield, mainly due to the fermentation of the banana. This fermentation led to excess water**,** resulting in the death of the larvae. On the other hand, treatment T3**,** containing cassava flour, palm oilcake and sugarcane, resulted in an excessively high temperature, fluctuating between 26˚C - 36˚C during fermentation. This high temperature is a limiting factor for larval survival in a warm environment like Maniema. The exact details of the chemical composition of each feed can be determined by laboratory analysis to assess the contents of proteins, lipids, carbohydrates, micronutrients and other specific components**.**

The larvae had different pigmentations depending on the feeds used in their rearing. Feeds containing cassava flour, palm kernel cake and sugar cane produced white larvae, while other mixtures produced yellow larvae due to variations in the pigments or chemical compounds present in the feed.

To promote the availability of larvae on the market and preserve the oil palm and raffia ecosystems, the use of a mixture of coconut waste, corn meal and sugarcane is crucial as it yields 125.94 ± 36.52 last instar larvae per 25 L rearing box; this represents a more stable range of 89.42 - 162.46 vs 172.55 - 189.45 in the control. The growth parameters (larval size, survival rate, metamorphosis) were similar in both feeding treatments.

In the study carried out by [18] who reared larvae on pieces of raffia stipe, an average of 131.7 ± 37.8 last instar per 25 L box was produced. Comparing these results with ours, it's clear that our best treatment significantly outperforms this yield. In addition, the work of [19] underlines the fact that rearing *Rhynchophorus* larvae on sugarcane offers optimum yield.

The survival rate of larvae in our trial varies significantly between treatments; with a higher rate under T4 treatment (93.8%), followed by T2 (79.8%) and T1 (67.8%). while T3 treatment shows a very low survival rate (42.7%). According to Tutondele *et al.* (2023) the survival rate in larval rearing was 73.3 ± 0.0 under wheat bran and 56.6% under corn flour vs 35.0% under palm kernel meal.

Mortality rates in our experiment are significantly lower for treatment T4 (6.2%), followed by T2 (20.2%). Higher mortality rates are observed for treatments T3 (57.3%) and T1 (32.2%). The results of [14] show higher mortality rates for palm kernel meal (65.0%) and corn flour (43.3%) and lower mortality rates for wheat bran (26.6%). Comparing our results with those of [14] who worked in Central D R Congo Province. Our best feeds show a very low mortality rate. Thus, the high mortality rate observed for treatment T1 containing 45% rice bran, 45% banana and 10% sugarcane is likely justified by banana fermentation which results in excess water, leading to the death of small larvae. On the other hand, treatment T3, a mixture of cassava flour, palm oilcake and sugarcane produces excess temperature up to 26˚C - 36˚C during fermentation; which is a limiting factor for larval survival in a warm environment like that of Maniema. Our results regarding the use of a mixture of cassava flour, palm oilcake and sugarcane are corroborated by those of [14] who highlighted the limitations of these feeds on *Rhynchophorus phoenicis*; notably the impact on larval weight which reverts to its initial values after two weeks. In addition. the results of [19] on *Rhynchophorus palmarum* confirmed that from an egg hatching rate of 77%, considerable mortality is observed during the early days of larvae, mainly due to larval cannibalism.

Our study on the influence of substitute feeds on the morphometry of *Rhynchophorus* larvae reared in the laboratory in Maniema province revealed that larvae fed with a mixture of fresh stipe and oil palm kernel had a higher fresh weight on 35 day (7 g), followed by those fed with a mixture of coconut waste, corn flour and sugarcane (6.2 g). [27] in his study on the rearing of *R. ferrugineus* on date palms found an average larval weight as low as 2.7 ± 0.08 g over a period of 35 days. In the research conducted by [18] with *R. phoenicis*, the use of young palm stipes yielded an average weight of 6.9 ± 0.98 g. The use of old stipes produced an average weight of 5.8 g after 30 days. Using palm kernel oilcake as a substitute over a period of 42 days, the average weight was 3.4 g; and with sugarcane, the average weight was 5.30 g after 34 days. Elsewhere, [15] found 3 - 6 g last instar larvae on day 30 on *Rhynchophorus phoenicis* in Kisangani, D.R Congo. Our results on fresh weight of larvae under our best feeds are, therefore, generally superior to those of the fore-mentioned studies; with the exception of [18] on *R. phoenicis* larvae reared on young stipes which are statistically similar as compared to the performance of the mixture containing coconut waste, corn flour and sugarcane. Monzenga's range is 5.9 - 7.93 g and ours is 5.4 - 6.91 g. The feed treatment T4 yielded the longest larvae; followed by T2. T1 and T3 yielded the shortest larvae. Larval length increases with time for all treatments. In terms of width on day 35, larvae in treatment T4 are the widest; followed by T2. T1 and T3 yielded the narrowest larvae. Head capsule followed the same trend, being longer in treatments T4 and T2; as opposed to T1and T3. For the length of *R. phoenicis* larvae, [7] reported an average length at last instar of 49.5 mm on *Rhynchophorus phoenicis* in Congo Brazzaville.

Alternative feeds offer a diversity of nutrients essential for the development of *Rhynchophorus phoenicis* larvae. Their greater accessibility and lower cost promote the sustainability of rearing. They will help preserve the ecosystem and improve resource management and enhance the value of local resources; while reducing environmental impact in the palm and raffia tree ecosystems. By opting for alternative feeds, farmers will reduce the pressure on oil palm populations and grant alternatives to livestock farming.

5. Conclusions

This research was carried out with the aim of establishing a suitable technique for rearing palm weevils in a region marked by considerable use of insects as human food. Locally available agricultural by-products were used in a controlled environment. Several experiments were carried out on the morphometry and sexual dimorphism of larval stages, and on the life cycles of palm weevils and on the production of *R. phoenicis*, the most abundant species of this genus in the region. The mixture of cassava flour, coconut waste, and sugar cane and plantain bananas gave good results on the metamorphosis of larvae collected in the wild until breeder weevils were produced at the adaptive stage. Larvae with small setae on the head capsule gave rise to male weevils; while larvae without these head setae gave rise to female weevils. This observation is a contribution to our research regarding the sexual dimorphism of palm weevil larvae. Given the fact that large-scale production based on the host plant (palm and raffia tree kernel) does not guarantee the conservation of the ecosystem, we found an alternative feed. Thus, the availability of larvae on the market while preserving the oil palm and raffia ecosystem is promoted. It is about using a mixture of coconut waste, corn meal and sugarcane. It has a yield similar to that of the control (mixture of fresh stipe and oil palm kernel). Additionally, we found that filling the feeds in the rearing boxes up to 50% of the container volume is very optimal and it boosts weevil lifespan. Thus, our results will help improve the rearing conditions for palm weevils, and the feed and they open up opportunities for the conservation of palm and raffia tree ecosystems.

Research perspectives include the determination of the chemical composition of each feed by laboratory analysis to assess the contents in proteins, to the level of amino-acids, lipids, carbohydrates, micronutrients and other specific components; as well as the nutritional values of the palm weevil larvae in the human diet. It will be relevant to conduct an economic study on the cost effectiveness of the various feeds and rearing techniques, especially the most promising ones. The planned future extension phase involves the implementation of these rearing techniques within local farmers' conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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