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Rearing of *Rhynchophorus phoenicis* Fabricius (Coleoptera: Dryophtoridae) last instar larvae on three artificial substrates in Kisangani, Democratic Republic of the Congo

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ABSTRACT

Insects have been a particularly popular resource for human and animal nutrition since the turn of the century, owing mostly to population increase and the ever-increasing need for animal protein. A five (5) months experimental study was carried out at the Yangambi Laboratory of Applied and Functional Entomology in Kisangani to explore a variety of artificial substrates for the mass production of African palm weevil (*Rhynchophorus phoenicis*) larvae. The larvae were reared in plastic boxes with three types of substrate: T1, based on ripe plantains; T2, based on brewery flour; and T3, based on Makololo flour, which was used as the energy component and made up 60% of the substrate. Palm oilcake and soybean, which accounted for 40% of the substrate, supplied protein. Each treatment was divided into two (2) groups (A and B), this subdivision being based on the presence or absence of soybean meal. 0.8 kg of substrate for each box was given *ad libitum* every two weeks (14 days). The adults used were captured after trapping in the forest. Broodstocks were randomly assigned to 42 groups (boxes), randomly assigned to six (6) feeding treatments, using a completely randomised design (CRD) with seven (7) replicates. After feeding trial, the results showed that the number and weight of last instar larvae differed between treatments. From the various treatments, an average of 398 last instar larvae LIV, rearing for 32 to 37 days and weighing between 1.56 and 2.22 g were collected. The substrate based on ripe plantain ($T1_A$) produced the highest average number of larvae compared to others ($p<0.05$), while the substrate based on brew ($T2_B$) produced LIV with higher average weights ($p<0.05$).

Keywords: *Rhynchophorus phoenicis*, larvae, artificial substrates, agro-food waste, valorisation, rearing, Kisangani.

RÉSUMÉ

Elevage des larves de dernier stade de *Rhynchophorus phoenicis* Fabricius (Coleoptera : Dryophtoridae) sur trois substrats artificiels à Kisangani en République Démocratique du Congo

Depuis le début de ce siècle, les insectes sont devenus une ressource particulièrement appréciée pour l'alimentation humaine et animale en raison notamment de la croissance démographique et de la demande toujours croissante de la protéine animale. Une étude expérimentale de cinq (5) mois a été menée au laboratoire d'Entomologie Appliquée et Fonctionnelle de l'Institut Facultaire des sciences Agronomiques de Yangambi à Kisangani, pour explorer quelques substrats artificiels pour la production massive des larves de charançon du palmier africain (*Rhynchophorus phoenicis*). L'élevage a été réalisé dans des boîtes en plastique, avec trois types de substrats, T1 : à base des bananes plantains mûres, T2 : à base de la farine de drèche et T3 à base de farine Makololo considérées comme composantes énergétiques qui représentaient 60% de substrats. La protéine a été apportée par le tourteau palmiste et la farine de soja qui constituait 40% de substrats. Chaque traitement était subdivisé en deux (2) groupes (A et B), cette subdivision était basée sur la présence ou l'absence de farine de soja. 0,8 kg de substrat pour chaque boîte ont été donnés *ad libitum* toutes les deux semaines (14 jours). Les adultes utilisés étaient capturés après piégeage en forêt. Les géniteurs ont été regroupés au hasard dans 42 groupes (boîtes), répartis au hasard entre six (6) traitements alimentaires, en utilisant un plan complètement randomisé (CRD) avec sept (7) répétitions. Après l'essai d'alimentation, les résultats ont montré que le nombre et le poids des larves de dernier stade variaient selon les traitements. Un nombre total moyen de trois cent nonante-huit (398) larves de dernier stade LIV, de durée d'élevage de 32 à 37 jours et pesant entre 1,56 et 2,22 g ont été récoltés à partir des différents traitements utilisés. Le substrat à base de banane plantain mûre ($T1_A$) a donné le meilleur résultat pour le nombre moyen des larves obtenues comparé à d'autres ($p<0,05$), tandis que celui à base de drèche ($T2_B$) a permis l'obtention des larves de dernier stade en poids moyens plus élevés ($p<0,05$).

Mots-clés : *Rhynchophorus phoenicis*, larve, substrats artificiels, déchets agroalimentaires, valorisation, élevage, Kisangani.

INTRODUCTION

Insects are high-nutritional-value foods (Finke, 2002; Rumpold and Schluter, 2013a, b). Insects have been eaten for a very long period, and there are over 2,000 species consumed globally, primarily in developing countries (Ramos-Elorduy, 1997; Gahukar, 2011; van Huis, 2013). However, despite a substantial surge interest in recent years (Riekkinen et al., 2022), entomophagy remains marginal in industrialised countries, notably in the West (Dumont and Provost, 2019). By 2050, the world's population will exceed 9 billion (Leridon, 2020). To meet the demands of this growing population, we must identify new protein sources or increase the availability of those we already have. Consider insect farming as a possible solution to food security challenges. Insects are ubiquitous and multiply rapidly (Monzenga, 2015), and their farming has the potential to reduce greenhouse gas emissions (Kim et al., 2020). In the Democratic Republic of Congo, the work of Monzenga (2015) and recently Monzenga et al. (2022) has established the presence of three *Rhynchophorus* species in the Kisangani region, as well as the potential of rearing them in the laboratory conditions. Several studies in tropical Africa have proved the nutritional value of *Rhynchophorus sp.* larvae (Lenga et al., 2012; Malaisse, 1997). These larvae are often eaten as a delicacy in most parts of Africa and some parts of Asia. They are the main pests of oil palms or Raphia and are often obtained from these plants, which are the main larval rearing substrates for their production (Onyeike et al., 2005). Given the use of these plant species for other purposes (production of palm oil, wine, etc.), the search for and availability of artificial substrates to ensure the production of these larvae would be the best way of preserving the plant biodiversity thus threatened. The present study was initiated to explore artificial substrates developed from certain agri-food waste products for the production of last-stage larvae of the African palm weevil (*Rhynchophorus phoenicis*).

MATERIALS AND METHODS

Study area

The trials of the present study was carried out at the Laboratoire d'Entomologie Appliquée et Fonctionnelle of the Institut Facultaire des Sciences Agronomiques de Yangambi (LENAF-IFA), Kisangani agro-campus in the Democratic Republic of Congo. The geographical coordinates of the site, measured with a GPSMAP 64SX, were: 0°30'46.99" latitude North, 25°9'50.53" longitude East and an altitude of 404 m. The average monthly temperature was 27°C. Our study covered the period from October 2022 to March 2023.

Obtaining broodstocks

The adult insects were collected in the forested fallow area of Akodale, which is located on the right bank of the Tshopo River 12 km from Kisangani city at 0°34'N latitude and 25°08'E longitude, at a height of 450 m (Monzenga, 2015) in Tshopo province, DR Congo. These adults were kept in perforated plastic jars (29 cm length, 19 cm wide, and 13.5 cm deep) and fed sugarcane pieces.

Installation of rearing substrates

To ensure good larval nutrition, the substrates were formulated using local ingredients (mostly agri-food waste). Carbohydrates, proteins and minerals were the elements on which the choice of the various ingredients was based. Ripe plantain, brew and Makololo (the blackish surface layer of cassava chips) were chosen as the energy components, while palm oilcake and soybean flour were used as the protein components. Minerals were provided by ash from snail shells. The energy and protein components were 60% and 40%, respectively. The quantities used were 0.48 kg and 0.32 kg for the energy and protein components respectively, giving a total weight of 0.8 kg of substrate for each box. The energy component was considered as the main component, thus determining the substrates. In our study, three artificial substrates were developed: ripe plantain-based substrate **(T1)**, brew-based substrate **(T2)** and Makololo-based substrate **(T3)**. Each treatment was subdivided into 2 groups, based on the presence or absence of soybean flour. The different treatments are following: $\mathbf{T1_A}$: substrate based on ripe plantain and palm oilcake; $\mathbf{T1_B}$: substrate based on ripe plantain, palm oilcake and soybean; $\mathbf{T2_A}$: substrate based on brew and palm oilcake; $\mathbf{T2_B}$: substrate based on brew, palm oilcake and soybean meal; $\mathbf{T3_A}$: substrate based on Makololo and palm oilcake and $\mathbf{T3_B}$: substrate based on Makololo, palm oilcake and soybean meal.

The ripe plantain was boiled and then pounded in a home-made mortar, while the brew and Makololo powders were prepared in boiled water by mixing them with the palm oilcake and soybean flour (Fig. 1).

Figure 1. Substrates based on plantain (A), brew (B) and Makololo (C)

Substrate infestation

This experiment was carried out in 19 x 11.5 x 10.5 cm plastic boxes containing three pairs of adults' *R. phoenicis*. Each rearing substrate received 7 duplicates (the plastic boxes) of each developed treatment, for a total of 42 boxes. Females oviposited on pieces of split sugar cane put on the rearing substrate. All of the plastic boxes holding the rearing substrates and *R. phoenicis* couples were stored at room temperature.

Larvae monitoring and collecting

To keep the substrates wet, they were irrigated (with water) with a watering can. The rearing substrate was replaced after 14 days, when the environment had degraded due to the high degree of larval activity. Potential predators of the larvae were rigorously removed throughout growing, up to stage LIV, the final larval stage of this beetle. Finally, the larvae were collected at the final larval cycle. Following harvesting, a sample of 30 larvae per substrate was obtained to assess their weight. A total of 180 larvae from various substrates were weighed in the laboratory using a precision electronic scale made in China. The duration of the rearing cycle was also investigated.

Statistical data analysis

The last instar larvae (LIV) harvested after rearing and the types of substrates used during rearing were recorded in Microsoft Excel 2010 software, which enabled us to calculate the means and standard deviations. An analysis of variance (ANOVA) followed by Student's t-test was performed to compare the average weight of the larvae obtained and the type of substrate used, analysed using Gaph Pad Prism5 software (Graph Pad Software, San Diego, California, USA) and a Bonferroni post hoc multi-comparison test was performed at the 5% significance level.

RESULTS

Total number of larvae per treatment

The results after evaluating the number of larvae produced in each diet show that production (number of larvae) varied according to the type of diet used (Fig. 2).

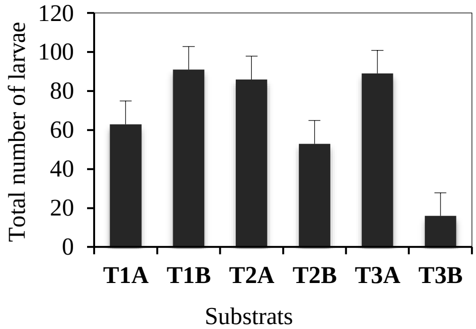


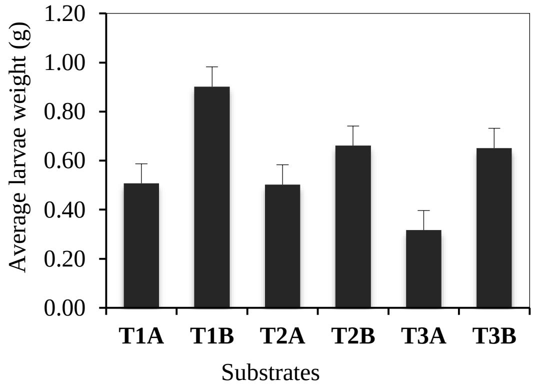
Figure 2. Average number of larvae per diet

Last instar larvae production was highest on the substrate composed of ripe plantain + palm oilcake + soybean meal with 91 larvae, i.e. an average of 13 ± 1.53 larvae per rearing box, whereas the lowest production was obtained on the treatment composed of Makololo, palm oilcake and soybean on which 16 larvae were harvested, i.e. an average of 2.33 ± 2.08 last instar larvae per box. A comparison of the number of larvae harvested per treatment shows that the mature plantain substrate produced a total of 154 larvae, compared with 139 for the brew substrate and 105 for the Makololo substrate. The numerical differences observed could be explained partly by the quality of the substrates used and partly by the larval attacks (Fig. 3C) observed during rearing, despite our efforts to limit them.

Figure 3. Larvae in ripe plantain-based substrates (A), last instar larvae (B) and attacked larvae (C)

Average weight of larvae after 14 days of substrate infestation#

The average weight of larvae 14 days after infestation was significantly affected by the type of diet ($p<0.0001$) (Fig. 4).



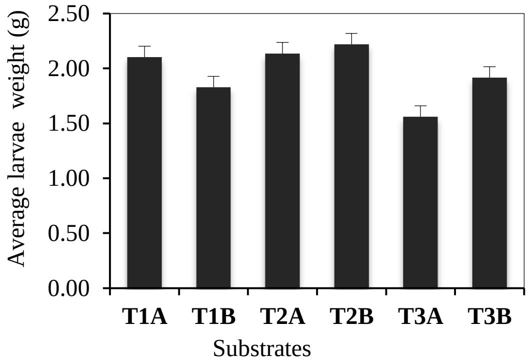
#One-Way ANOVA comparing mean larvae weights 14 days after infestation between the 6 treatment groups used (p<0.0001

Figure 4. Average weight of larvae 14 days after infestation

This figure shows that the young larvae from the banana + oilcake + soybean diet had a higher average weight than the others, i.e. 0.90 ± 0.31g, followed by those from brew + oilcake + soybean and Makololo + oilcake + soybean with 0.66 ± 0.41g and 0.65 ± 0.33g respectively. It appears that young larvae from substrates containing soybean had higher average weights than those whose diets did not contain soybean. These results showed highly significant differences for larvae reared on banana + oilcake + soybean compared with those on diets based on Makololo + oilcake ($p<0.0001$) as well as banana + oilcake + soybean and brew + oilcake ($p<0.0001$). Similarly, significant differences were observed between larvae produced with banana + oilcake + soya and Makololo + oilcake + soybean ($p<0.05$); and with brew + oilcake + soybean and Makololo + oilcake ($p<0.05$). When the average weights of these larvae (obtained 14 days after infestation) were compared to those obtained on banana-based diets (with or without soybean), there was a huge significant difference ($p<0.0001$). The similar observation was found amongst larvae raised on Makololo-based diets ($p<0.05$).

Last instar larvae weight average#

The results in Fig. 5 reveal that there was no significant difference in the weight of the larvae and the type of substrate used in the rearing ($p=0.0794$). This figure shows that the average weight of the last instar larvae was higher for larvae from a substrate based on brew + oilcake + soybean ($T2_B$), i.e. 2.22 ± 0.69g, whereas the lowest average weight was recorded for larvae from Makololo + oilcake ($T3_B$), which produced an average of 1.56 ± 0.52g.



#the weights of the last instar larvae collected across the six treatment groups were compared using one-way ANOVA (p=0.0794), and the treatments were compared using Bonferroni's Multiple Comparison Test (p>0.05)

Figure 5. Last instar larvae average weight based on substrate

Duration of rearing cycle

The duration of the rearing cycle varied according to the substrates, but the incorporation of soybean into the diet did not influence this duration (Fig. 6).

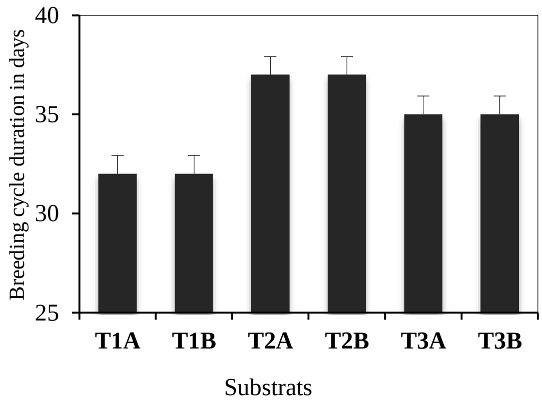


Figure 6. Rearing cycle duration

Rearing duration was at least one month on all diets. The larvae were harvested 32±0.58 days after infestation on the ripe plantain-based diet, whereas this duration was 37±1.15 and 35±2.0 days respectively on the brew and Makololo-based diets.

DISCUSSION

The results of this study highlight the possibility of producing *R. phoenicis* larvae from substrates derived from agri-food waste. Several researchers have investigated the possibility of producing *Rhynchophorus sp.* larvae on artificial substrates with the aim of limiting the use of plant species (Rananavare et al., 1975; Zagatti et al., 1993) and reducing competition with human food. Francardi and Benvenuti (2010) evaluated several artificial substrates as alternatives for rearing *R. cruentatus* larvae. A study in Kumasi, Ghana, attempted to use artificial diets containing varying levels of palm oil, with various combinations of agro-wastes, including banana and pineapple fruit waste and millet waste in the rearing of *R. phoenicis* (Quaye et al., 2018). The results of our study showed that larvae reared on different diets had an average weight of 3±0.51 g overall. This shows that our diets need to be improved (Monzenga et al., 2017) in order to increase the average weight of last instar larvae. Similarly, Giblin-Davis et al. (1989) showed that *R. cruentatus* larvae gained less weight when fed an artificial diet than when fed a natural substrate. However, our results are better than those of Al-Ayedh (2011) who produced last instar *R. ferrugineus* larvae weighing on average 0.01g on a semi-synthetic diet. Food quality directly influences larval nutrition, survival and development (Tammaru, 1998). In a study on the evaluation of the growth of *R. phoenicis* in a controlled environment on agricultural products and by-products, Masika et al. (2023) produced larvae with mean weights of 2.01±0.38g, 1.50±0.05g and 3.17±0.61g respectively on substrates composed of maize meal, palm kernel cake and wheat bran. The last instar larvae produced in this study had higher average weights than those of these authors, with the exception of the larvae harvested on $T3_A$. This shows that our diets were of good quality. Our results are similar to those of Giblin-Davis et al (1989) who reported average weights of between 3.5 and 5 g. The duration of larval development (duration of the rearing cycle) can be affected by substrate quality (Kaakeh, 2005) whereas our study showed relatively short durations on all diets. In his study, Berthier (1986) harvested the last instar larvae of *R. ferrugineus* 120 days after infestation of artificial substrate. Salama and Abdel-Razak (2002) obtained last instar larvae after 58 days on sugarcane, banana and artificial diet. However, it took Karmataka (2009) 55.69 days to collect *R. ferrugineus* last instar larvae on sugarcane. We find that our substrates significantly reduced rearing time, indicating that they were nutrient-rich as previous studies have shown that insect development time varies with rearing substrate (Oonincx et al., 2015). The larval stage is the longest period of insect development. Gabr et al, (2022) recorded an estimated average larval development time of 79.21 ± 12.994 for *R. cruentatus*. The duration of the larval stage in *R. ferrugineus* larvae varies from 25 to 105 days (Thomas, 2010), whereas it was 86.30 days for larvae fed with ground sugarcane and additives and 128.35 days for larvae fed with ground maize + additives (Abdel-Hameid (2019). The duration of the larval stage can be affected not only by the quality of the rearing substrate but also by the species.

CONCLUSION

The aim of this study was to produce the last instar larvae of *R. phoenicis* on artificial substrates from agri-food waste. *R. phoenicis* adults were trapped in the forest and kept in perforated plastic jars, where they were regularly fed sugarcane pieces. The diets were formulated using ripe plantain, brew and Makololo as energy components and palm oilcake and soybean as protein components. Snail shell ash and lemon juice were added to provide minerals and limit fungal attack, respectively. The experiments were carried out in plastic boxes in which the diet and three pairs were infested with sugar cane (*Saccharum officiranum*) as an oviposition substrate. The number of larvae and the weight of the larvae depended on the diets tested. The diet based on ripe plantain was the best in terms of the number of larvae produced and reduced the duration of the rearing cycle. On the other hand, the diet based on brewer's grains stood out in terms of the average weight of the last instar larvae obtained.

Acknowledgments: N/A

Conflicts of interest
The authors declare no conflict of interest.

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