Research Square

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Potential Health Benefits of Edible Larvae: Identification and Quantification of Bioactive Compounds with Nutritional Benefits

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Research Article

Keywords: Antioxidant, Entomophagy, Oryctes rhinoceros, Rhynchophorus phoenicis, Larvae

Posted Date: August 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10808504/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

Aim

The larvae of *Rhynchophorus phoenicis* (Mpose) and *Oryctes rhinoceros* (Makokolo) are widely consumed and commercially traded in African markets, yet their chemical composition and biological activities remain poorly characterized. This study aimed to contribute to the scientific valorization of these two beetle larvae in the Democratic Republic of Congo by investigating their microscopic features, phytochemical profile, mineral composition, and antioxidant activity.

Methods

Microscopic examination of larval powders was performed using Steinmetz reagent. Phytochemical screening was carried out by thin-layer chromatography, and total polyphenol and flavonoid contents were quantified by UV-Vis spectrophotometry. Mineral composition was determined by inductively coupled plasma optical emission spectrometry, and the *in vitro* antioxidant activity was evaluated through ABTS and DPPH radical scavenging assays on aqueous extracts.

Results

Microscopical analysis revealed insect-specific histological elements and plant-derived microscopic features in both larval powders, consistent with incomplete digestion of phytophagous material. TLC phytochemical screening confirmed the presence of flavonoids, phenolic acids, and terpenes in both species. Higher total polyphenol content was observed in *Oryctes rhinoceros* larvae, whereas *Rhynchophorus phoenicis* larvae were found to be richer in flavonoids. Mineral analysis identified nutritionally relevant macroelements, including calcium and magnesium, and microelements, such as zinc, selenium, and manganese, at concentrations near or above recommended daily intakes. Both larval extracts demonstrated significant radical scavenging activity, with *R. phoenicis* exhibiting more potent antiradical effects than *O. rhinoceros*.

Conclusions

The phytochemical richness, nutritional mineral profile, and potent antioxidant capacities of both larvae support their potential health benefits and therapeutic relevance for populations consuming them. Our findings provide a scientific basis for the integration of *R. phoenicis* and *O. rhinoceros* larvae into functional food strategies to mitigate food insecurity and oxidative stress-related diseases in sub-Saharan Africa.

1. INTRODUCTION

The global human population is projected to reach 10 billion by 2050, placing unprecedented pressure on food systems worldwide [1]. As demand intensifies, critical resources, including arable land and freshwater, are expected to become increasingly scarce due to climate change. Sub-Saharan Africa and Asia are expected to bear the greatest burden of these pressures, with food insecurity emerging as one of the most urgent consequences [2]. Conventional livestock farming, already operating near its ecological limits, is expected to be insufficient to meet the protein and micronutrient needs of rapidly growing populations [3]. In this context, entomophagy, the practice of consuming edible insects, has gained considerable scientific and policy attention as a nutritionally viable and environmentally sustainable alternative [4].

The Democratic Republic of Congo (DR Congo) is among the world's most biologically diverse nations, with extraordinary richness in both flora and fauna [5]. Yet much of this natural heritage remains underexplored and underutilized, particularly by rural communities who could benefit most from its valorization [6]. Indigenous foods in the region are also deeply intertwined with biodiversity, cultural identity, and pharmacological heritage, representing a repository of knowledge that merits systematic scientific investigation [7–8]. Recognizing the broader potential of this resource, the Food and Agriculture Organization declared in 2013 that edible insects represent a meaningful contribution to global food security and animal feed [9]. While insects have long been part of traditional diets across cultures, societal reluctance to consume them persists in certain regions. Nevertheless, innovations in insect farming are rapidly advancing, and evidence supports the integration of traditional entomophagic knowledge with modern nutritional science in both developed and developing countries [10].

From a nutritional standpoint, edible insects offer a compelling profile. They are rich in high-quality proteins and lipids, abundant in essential minerals such as iron, zinc, calcium, and phosphorus, and contain significant quantities of vitamins [11]. However, their nutritional composition is not static. As predominantly phytophagous organisms, the chemical and mineral compositions of insects depend on their diet, life stage, habitat, and species. This variability, spanning the metamorphic cycle and ecological context, highlights the importance of species-specific characterization. Despite these documented nutritional attributes, the biological activities of many edible insect species remain poorly characterized, and large-scale rearing policies to ensure sustainable harvest are largely absent, exposing wild populations to overexploitation [12]. Among all insect orders, Coleoptera represents the most consumed by humans globally, accounting for 659 inventory species and 31.2% of all edible insects [13]. In Africa, however, beetles occupy the third position in this hierarchy, reflecting distinct regional consumption patterns that warrant further investigation. The present study focused on two beetle species whose larvae are traditionally consumed in DR Congo: *Rhynchophorus phoenicis*, locally known as Mpose, and *Oryctes rhinoceros*, known as Makokolo. This study aims to evaluate the antioxidant capacity of larval extracts from both species and characterize their histological features and phytochemical fingerprints, contributing to the broader effort of valorizing Congo's edible entomofauna through evidence-based nutritional science.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Methanol and diethyl ether werepurchased from Merck (Germany), while formic acid, acetic acid, dimethyl sulfoxide, acetone, ethanol, and hexane were procured from VWR (USA). TLC plates (Silica Gel 60 F_{254}) were purchased from Merck (Germany). As chemical standards for phytochemical screening, quercetin, kaempferol, and rutin were used as flavonoid standards; rosmarinic acid, ferulic acid, and gallic acid as phenolic acid standards; thymol and menthol as terpene standards, purchased from Sigma-Aldrich (USA).

2.2. Insect Sampling

Larvae traditionally consumed in DR Congo, particularly in Kinshasa, were sampled from local markets. *Rhynchophorus phoenicis* (*Mpose*) larvaewere collected in November 2018, and *Oryctes rhinoceros* (*Makokolo*) larvae were collected in May 2019.Species identification was performed at the Department of Biology, Faculty of Sciences and Technology, University of Kinshasa. Larvae were frozen, thawed at room temperature, dried for 72 hours in an oven at 40°C, powdered using a blender, and then stored at 4 °C until analysis.

2.3. Botanical and Phytochemical Screening

2.3.1. Microscopic Analysis of Insect Sampling

The microscopic examination was carried out by placing 2 to 3 drops of Steinmetz reagent [14] in contact with a small quantity of powder on a slide. Observations were made with a VisiScope BL124 (VWR) microscope, andimages were captured using a Samsung S9 smartphone.

2.3.2. Phytochemical Screening of Insect Sampling

Analytical Thin Layer Chromatography (TLC) of 10 µL of a 10 mg/mL solution of methanolic and ethyl acetate extracts was carried out on normal-phase Silica Gel 60 F_{254} plates purchased from Merck (Germany), using different eluents for the identification of secondary metabolites [15].

2.3.3. Dosage of secondary metabolites

Dosage of Total Polyphenols

The total polyphenol content of the extracts was determined using the Folin-Ciocalteu method [16]. Expression of results: The amount of total polyphenols is expressed in mg Gallic Acid equivalent (GA E)/g dry matter using the following equation from the calibration curve: y = 10.089 x + 0.0742 and R2 = 0.9987, where x is the equivalent concentration of Gallic Acid (mg/g), and y is the absorbance.

Dosage of Total Flavonoids

The total flavonoid content ofthe extracts was estimated using a spectrophotometric method. Aluminum trichloride forms a yellow complex with flavonoids that absorbs at 415 nm [17]. Expression of results: The flavonoid content of the extracts is expressed in mg quercetin equivalent (QE)/g of the corresponding dry extract using the equation from the calibration line: y = 18.215x + 0.1498 with R2 = 0.944 where x is the equivalent concentration of quercetin (mg/g), and y is the absorbance.

2.3.4. Dosage of Mineral Composition

The determination of mineral content was carried out by the Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES) as described previously [18].

2.4. Cell-Free Antioxidant Assays

The extracts were solubilized in methanol; thus, their effect was compared to a control test performed with methanol alone. Antioxidant activity was assayed by spectrophotometric ABTS and DPPH methods, which were performed according to the method described by [16].

2.5. Statistical Analysis

Each concentration was tested in triplicate in each assay, and at least 3 different assays were performed. All results were expressed as mean values ± standard deviation (SD). The statistical analysis was performed with GraphPad 7.0 (GraphPad Software, San Diego, California, USA). Two-way analysis (ANOVA) and Student's paired t-test were performed; multiple comparisons of all data were performed using the "Tukey" Multiple Comparisons Test, and the level of statistical significance was considered at $p < 0.05$. The IC_{50} values were calculated with GraphPad Prism 7.0 by applying the function "log (inhibitor) vs. normalized response-variable slope" after converting the concentrations into their decimal logarithm.

3. RESULTS AND DISCUSSION

3.1. Botanical microscopic features

Powders obtained from larvae samples were treated with European Pharmacopoeia reagent andrevealed the following specific botanical microscopic characters for each sample. The microscopic elements observedincluded: histological characteristics specific to the insectin both larvae (Figs. 2 and 3); Unicellular hairs, fragments of parenchyma, fragments of cork, and sclereids (for powder from the *Rhynchophorus phoenicis* larvae) (Fig. 4); Fragments of fibers, isolated sclereids, fragments of sclerotic fibers, and pollen grains (for *Oryctes rhinoceros larvae* powder) (Fig. 5).

The microscopic analysis of plant drugs has long been a simple method of drug identification since ancient times [19]. Thus, each drug or group of drugs has its own microscopic characteristics that allow identification in the presence of other plant or animal material. Since the larvae of *R. phoenicis* (Mpose) and *O. rhinoceros* (Makokolo) are constantly feeding on palm debris, the objective of the microscopic examination of palm debris powder was to identify plant-specific features. As a result, we were able to

highlight elements characteristic of the insect, which were not relevant to this study (Figs. 2 and 3), but also characteristic elements of plants such as fiber fragments, pollen grains, sclereids, etc. (Figs. 4 and 5). This supports the hypothesis that by feeding on plant debris, especially from the palm tree. The larvae of *R. phoenicis* and *O. rhinoceros* do not completely digest the entire meal; for this reason, residues of the plant remain in the larvae, which may provide them with additional nutritional or therapeutic valuefor consumers. However, further microscopic examinations should be conducted to characterize the unidentified elements of the larvae.

3.2. Phytochemicals

3.2.1. TLC Phytochemical Screening

The results of the phytochemical screening carried out on the methanolic and ethyl acetate extracts of *R. phoenicis* and *O. rhinoceros* larvae powders are presented as follows: The TLC chromatographic analysis revealed the presence of phenolic compounds in various extracts of *Rhynchophorus phoenicis* and *Oryctes rhinoceros*. *Oryctes rhinoceros* larvae contained more flavonoids and phenolic acids compared to those of *Rhynchophorus*. Compared to the reference substances used, both larvae contained small amounts of flavonoids like quercetin or its derivatives (Fig. 6 and Fig. 7).

The presence of terpenes in thelarval extracts was also highlighted. The larvae of *O. rhinoceros* contained more terpenes than those of *R. phoenicis*.Based on control substances, *O. rhinoceros* larvae contained small amounts of menthol and thymol and not oleanolic acid (Fig. 8).

Chromatographic results did not reveal iridoids, coumarins, anthraquinones, anthocyanins, and tannins in both larvae. To the best of our knowledge, this work presents the first report of phytochemical screening of *O. rhinoceros* and *R. phoenicis* larvae. However, further chromatographic analyses shall be required to identify other compounds that might be under the limit of detection.

3.2.2. Metabolite Dosage

The results of flavonoid content and total polyphenols quantified in both species are shown in Table 1 below.

Table 1
Flavonoid content expressed in milligrams of Quercetin equivalent per 1g of the dry matter (mg QE/g DW) and total polyphenols expressed in milligrams of Gallic acid equivalent per 1g of the dry matter (mg GAE/g DW)

Samples	**Flavonoids (mg QE/g DW)**	**Polyphenols (mg GAE/g DW)**
Oryctes rhinoceros	30.87 ± 0.48	84.58 ± 5.49
Rhynchophorus phoenicis	64.89 ± 1.46	45.59 ± 1.27

Oryctes rhinoceros larvae contained a higher total polyphenol content than *Rhynchophorus phoenicis* larvae. Conversely, *R. phoenicis* larvae exhibited a higher flavonoid content than O. rhinoceros larvae, which explained their greater antiradical activity. The presence of substantial plant-derived phytochemicals in both larvae was attributed to the incomplete digestion of plant metabolites from palm debris. Consequently, consumption of these insects provided both larval nutritional benefits and plant-derived bioactive compounds such as flavonoids. To date, no prior study had evaluated these two larvae in this context.

3.3. Mineral Composition

The results of the composition of macro- and micro-mineral elements of the two larvae are shown in Tables 2 and 3.

Table 2
Macroelement content of *O. rhinoceros* and *R. phoenicis* larvae expressed in g/100g of dry matter (Mean, n = 3)

Macroelements	***Oryctes rhinoceros***	***Rhynchophorus phoenicis***
Calcium	2.2789 ± 0.0005	1.5793 ± 0.0002
Magnesium	4.7081 ± 0.0005	2.2386 ± 0.0003
Sodium	1.4686 ± 0.0020	1.0448 ± 0.0015

Table 3
Microelement content of *O. rhinoceros* and *R. phoenicis* larvae expressed in g/100g of dry matter (Mean, n = 3)

Microelements	***Oryctes rhinoceros***	***Rhynchophorus phoenicis***
Aluminium	0.0020 ± 0.0003	0.0006 ± 0,.00001
Cobalt	0.0002 ± 0. 00004	0.0042 ± 0.0005
Copper	0.0001 ± 0. 00004	0,0001 ± 0.00007
Iron	0.0126 ± 0.0007	0.0054 ± 0.0007
Manganese	0.0120 ± 0.0003	0.0048 ± 0.00006
Nickel	0.0012 ± 0. 00009	0.0014 ± 0.0004
Selenium	0.0005 ± 0.0001	0.0011 ± 0.0003
Zinc	0.0143 ± 0.0007	0.0138 ± 0.0008

Both larvae were rich in macroelements (calcium and magnesium) and microelements (iron, manganese, and zinc). Mineral analysis indicated that the iron content in both larvae was sufficient to meet the daily requirements of an individual, particularly pregnant women [20]. While iron and magnesium were present in substantial amounts in both species, selenium levels were comparatively low in *O. rhinoceros* larvae.

Rhynchophorus phoenicis larvae contained higher concentrations of selenium and zinc, whereas O. rhinoceros larvae exhibited higher amounts of calcium and sodium.

The presence of small amounts of selenium is an innovative feature of this work. The most recent studies on the mineral composition of these two larvae revealed only the presence of iron, zinc, magnesium, calcium, etc. [21–23]. Selenium is a trace element whose physiological importance has only been fully appreciated in the recent past. Its biological form consists essentially of the amino acid selenocysteine, a compound that does not exist naturally in a free state in the cell. Among the selenoenzymes currently identified in mammals, glutathione peroxidases constitute one of the lines of defense against attacks by oxygenated free radicals; tetraiodothyronine-5'-deiodinase activates the thyroid hormone. These two examples highlight the crucial importance of selenium, both in the prevention of damage to biological macromolecules and during development [24]. Potassium was not determined in either larva, but some previous studies detected fairly high potassium values in both larvae. Ehounou et al. [25] in a study found that *R. phoenicis* larvae contained a high level of potassium in the order of 75.26 ± 0.78 mg/ 100 g, while Omotoso [26] found a potassium level of 25.44 ± 0.31 mg/ 100 g in the larvae of *O. rhinoceros*. Calcium was present in both larvae at high levels relative to other minerals, measuring 2.2789 ± 0.0005 g/100 g (2278.9 mg/100 g) and 1.5793 ± 0.0002 g/100 g (1579.3 mg/100 g) for O. rhinoceros and R. phoenicis larvae, respectively. This corroborates the results from the literature [27, 25]

3.4. Antioxidant Activity Profiles of Edible Insects

The evaluation of antioxidant activity using ABTS and DPPH assays showed that all the extracts inhibited radicals, connected with their IC_{50} values (Table 4).

Table 4
IC_{50} values for ABTS and DPPH tests (µg / mL) of aqueous extracts of both larvae (mean ± standard deviation, n = 6)

Aqueous extracts	**ABTS**	**DPPH**
Oryctes rhinoceros	82.41 ± 6. 72	487.53 ± 160.94
Rhynchophorus phoenicis	27.67 ± 1.77	161.81 ± 18.49
Quercetin	1.42 ± 0.04	3.21 ± 0.99

The antiradical activities obtained were highly variable depending on the type of test. The IC50 values of the ABTS test were low compared to those of the DPPH test. This difference is explained by the complexity of the reaction mechanisms of each test. Indeed, ABTS reacts simultaneously with both hydrophilic and lipophilic compounds, while DPPH reacts only with hydrophilic compounds in the matrix being analyzed [16. *Rhynchophorus phoenicis* extracts exhibited lower IC_{50} values, reflecting a more potent radical-scavenging effect. Consequently, R. phoenicis larvae demonstrated greater activity than O. rhinoceros. This variation was attributed to differences in the qualitative and quantitative composition of secondary metabolites with antiradical properties in each extract. Nowadays, the literature does not

have data related to the evaluation of the antioxidant activity of these two larvae. Previous studies have shown that insects such as caterpillars have interesting antioxidant activities attributed to the various phytochemicals they contain from their nourishing plants [28].Polyphenols and flavonoids identified in both larvae were considered responsible for their antiradical activity. Dietary antioxidants contribute significantly to cellular defense against oxidative stress and its associated pathologies, suggesting a key protective role for these polyphenolic compounds. The antioxidant activity of polyphenols is based on several mechanisms, including direct scavenging of ROS, inhibition of ROS-generating enzymes, etc. The antioxidant activity of these two larvae could also be attributed to the presence of trace elements that are involved in the free radical defense mechanism. This is the case for selenium, zinc, and manganese. These mineral elements intervene as enzymatic cofactors in numerous reactions against free radicals [29]. Selenium, incorporated as selenocysteine in glutathione peroxidase, acts in antioxidant defense and thyroid hormone formation as a protective agent against cancer, muscle disease, coronary heart disease, and HIV. Selenium levels drop during oxidative stress. The recommended daily intake of Se varies from 60 µg/day for women to 70µg/day for men. These needs are estimated at 100 µg/day and 200 µg/day for pregnant and lactating women, respectively [24].

Another crucial aspect that could be attributed to the antioxidant activity of these two larvae is the presence of sulfur amino acids. Although not illustrated in this work, Huang et al. [30] studied the amino acid profile of *R. phoenicis* and *O. rhinoceros* larvae. It was found that these two larvae were an excellent source of essential and non-essential amino acids, including methionine and cysteine. Cysteine is of great importance in the metabolism of glutathione. In combination with selenium, it forms an enzyme called selenocysteine, which is the main form of selenium uptake in selenoproteins and therefore the main precursor of glutathione [24]. The level of reduced glutathione (GSH) is closely related to nutritional conditions, especially to a cysteine-rich diet. A major determinant of the rate of GSH synthesis is the availability of cysteine [24]. Cysteine normally comes from the diet. It is recommended to consume foods rich in sulfur compounds to fight free radicals. Another element that can be linked to the antioxidant activity of larvae, especially that of *Rhynchophorus phoenicis*, is Vitamin E. Vitamin E belongs to the family of tocopherols, natural lipophilic molecules provided by food. It exists in four forms: Alpha, Beta, Gamma, and delta tocopherols. Tocopherol is a powerful antioxidant substance due to its lipophilic character. It can limit the propagation of peroxidation by reducing membrane lipid peroxides into the corresponding alcohol. Indeed, Vitamin E is present in palm weevil larvae, and one study estimates the following levels: 35mg alpha-tocopherol per 100g and 9mg beta+gamma-tocopherol per 100g of dry matter. The recommended daily intake is 15mg [9]. Thus, the introduction of these two larvae in the diet of populations with dietary imbalance could contribute somewhat to food security on the one hand and, on the other hand, to the prevention of certain pathological situations associated with oxidative stress, such as Konzo which is a toxico-nutritional disease [31].

4. CONCLUSION

This study provided a comprehensive chemical and biological characterization of Rhynchophorus phoenicis and Oryctes rhinoceros larvae, two beetle species whose larvae are widely consumed in

Kinshasa, DR Congo. Microscopic examination revealed the presence of plant-specific microscopic elements in both larvae, supporting the hypothesis that phytophagous feeding behavior leaves residual plant material within the larval body, with potential nutritional and therapeutic implications for consumers. Phytochemical screening confirmed the presence of flavonoids, phenolic acids, and terpenes in both species. Oryctes rhinoceros larvae exhibited higher total polyphenol levels, whereas R. phoenicis larvae contained higher flavonoid levels, aligning with the stronger antiradical activity observed in R. phoenicis. Mineral analysis further revealed the presence of nutritionally relevant macro- and microelements, including calcium, magnesium, selenium, and zinc, at concentrations approaching or exceeding established recommended daily intakes. Antioxidant evaluation by ABTS and DPPH assays demonstrated potent radical-scavenging activity in both larval extracts, with *R. phoenicis* exhibiting more potent inhibitory effects, potentially attributable to its higher flavonoid content and the contribution of trace elements involved in enzymatic antioxidant defense. These findings establish a scientific basis for the integration of both larvae into human diets, particularly in sub-Saharan Africa, as functional food sources capable of contributing to food security and the prevention of oxidative stress-associated pathologies. Future investigations should prioritize the bioavailability of key minerals, notably iron, magnesium, and calcium, with particular attention to the potential of these larvae as nutritional supplements in formulations designed for young growing children.

Declarations

CONFLICT OF INTEREST

All the authors declare they have no conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge Mr Victoire Nsabatien of the University of Kinshasa’s Herbarium for the identification of larvae and Mrs Rose Gato Mboneza for the collection of larval samples.

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Figures



Figure 1

Images of *Oryctes rhinoceros* (A) *and Rhynchophorus phoenicis* (B) larvae used in this study

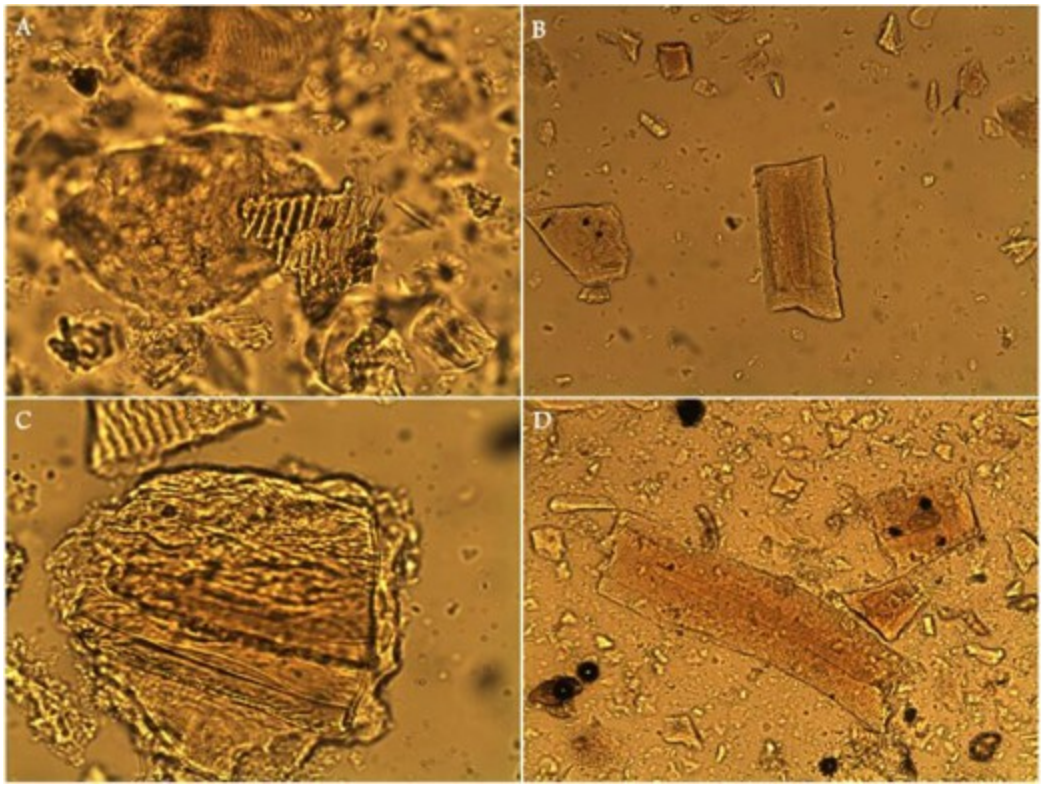


Figure 2

Species-specific histological characteristics of *Rhynchophorus phoenicis*

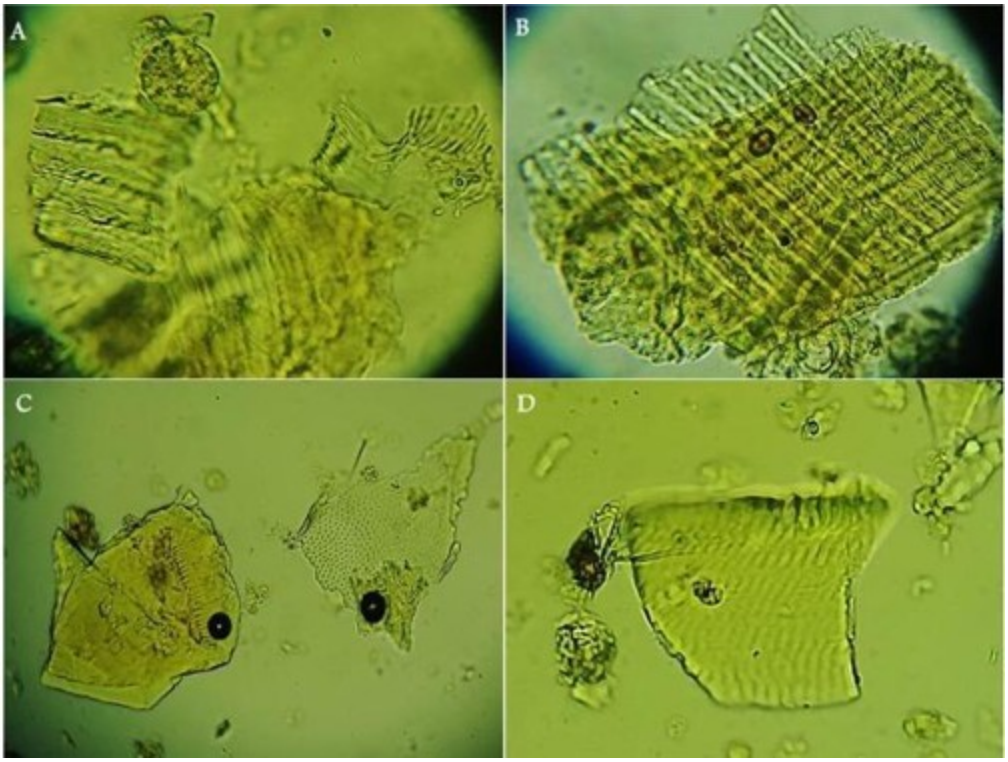


Figure 3

Species-specific histological characteristics of *Oryctes rhinoceros*

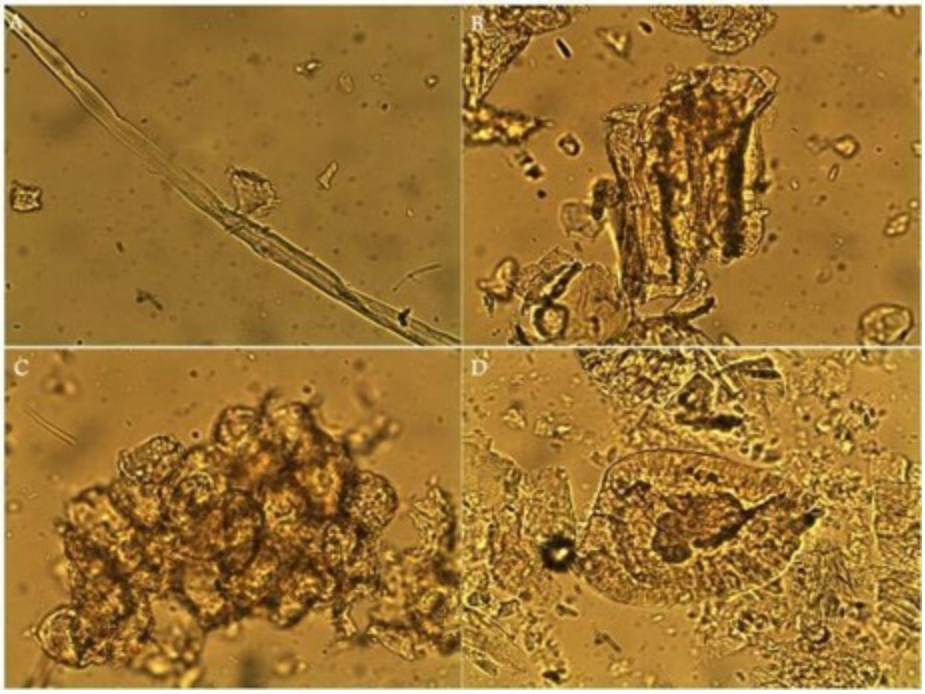


Figure 4

Single-celled hairs (A), Parenchyma fragments (B), Cork (C), Sclereids (D) of *Rhynchophorus phoenicis*



Figure 5

Fiber fragment (A), isolated sclereids (B), Sclerotia fiber fragments (C), Pollen grains (D) of *Oryctes rhinoceros*

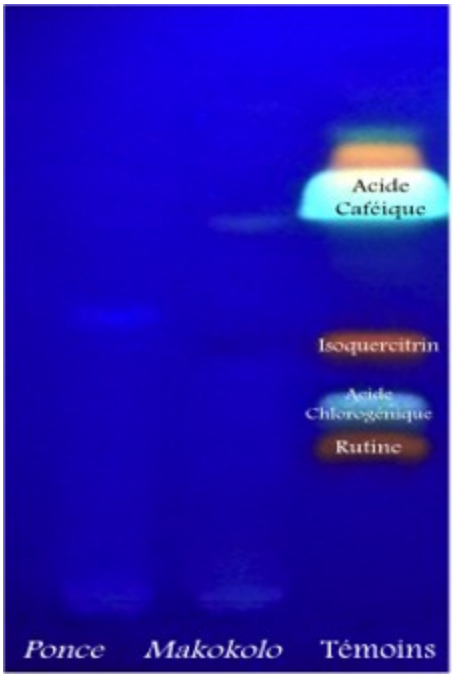


Figure 6

TLC chromatograms of methanolic extracts from the various insects in the presence of controls, developed on the mobile phase: ethyl acetate/ methanol/ water/ formic acid (20:2.5:2 :0.5; v/v/v) on Silicagel 60 F_{254} and revealed using Neu reagent at 365 nm.

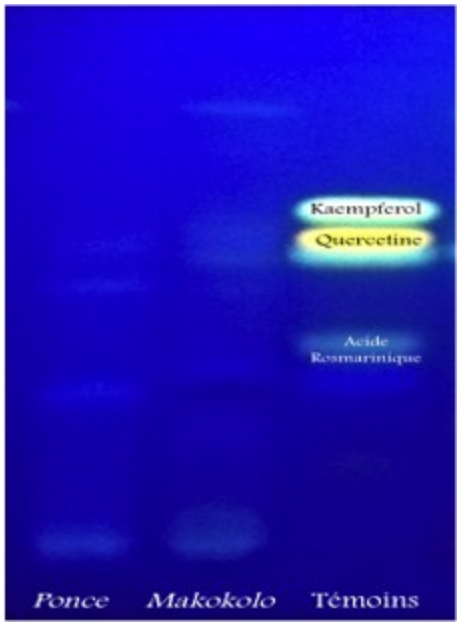


Figure 7

TLC chromatograms of methanolic extracts from the various insects in the presence of controls, developed on the mobile phase: dichloromethane/ formic acid/ acetone (85 :10 :20; v/v/v) on Silicagel 60 F_{254} and revealed using Neu reagent at 365 nm.

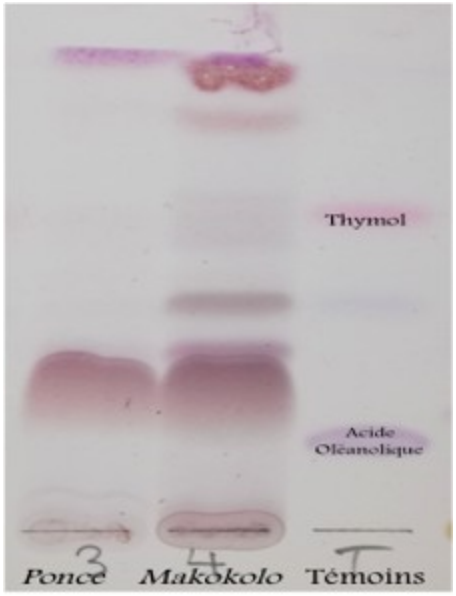


Figure 8

TLC chromatograms of the ethyl acetate extracts of the various insects in the presence of the controls, developed on the mobile phase: toluene/ethyl acetate/ (9 :1; v/v) on Silicagel 60 F_{254} and revealed using sulfuric anisaldehyde by heating plate to 100°C and observing in the visible range.