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Topic

**Evaluation of the insecticidal activity of *Oudneya africana*
from the Ghardaïa region**

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Dedication

First and foremost, I offer my sincere thanks and praise to Almighty God, who granted me the strength, courage, and patience to complete this humble work.

I dedicate this humble work to:

My dear mother and my father.

My siblings.

My grandparents, may God have mercy on them and grant them spacious gardens in Paradise.

All my family.

My dear friends.

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At the conclusion of this humble work, I would like to express my sincere thanks and gratitude to:

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To everyone else who contributed, my SINCERE thanks, respect, and gratitude.

Evaluation of the insecticidal activity of *Oudneya africana* from the Ghardaïa region

Summary:

The purpose of this study is to evaluate the insecticidal efficacy of *Oudneya africana* plant extracts from the Ghardaïa region as a natural and safe substitute for chemical pesticides are becoming less effective and have negative effects on the environment and health.

In this study the insecticidal activity of cold maceration extracts against *Tribolium castaneum* was examined using a bioassay. Activity was examined using both the contact and oral methods, and exposure times ranged from 30 minutes to 10 days.

The results revealed that the aqueous and methanolic extracts of *Oudneya africana* were insecticidal against *Tribolium castaneum*. The mortality seemed to increase at higher concentrations and longer exposure periods. The efficacy, however, showed high variability: when using contact application methods, the methanolic extract was more effective and had a quicker action compared to the aqueous extract. When considering ingestion methods, the efficacy of both extracts was relatively poor; however, the methanolic extract was slightly more potent and faster. Generally, the contact application method proved to be better in causing mortality for both extracts.

The findings reveal a significant variation in the toxicological profile of both extracts. The aqueous extract is more potent with regard to the dosage requirement for efficacy, as supported by the lower LD values, especially via ingestion. On the other hand, the methanolic extract has a faster action, as supported by the decreased LT values in contact and ingestion modalities. This suggests that while the aqueous extract might require a smaller concentration to cause mortality among pests, the methanolic extract causes deaths at a faster rate.

The results emphasize the potential of plant extracts in the biological control of *Tribolium*, highlighting an alternate, potentially beneficial way of developing a new and natural insecticide that may prove to be a better alternative to synthetics. However, extensive further studies need to be conducted to determine the individual active compounds that are accountable for this insecticidal activity.

Keywords: *Oudneya Africana*, *Tribolium castaneum*, insecticidal activity, Extract, Ghardaïa

Évaluation de l'activité insecticide d'*Oudneya africana* de la région de Ghardaïa

Résumé :

L'objectif de cette étude est d'évaluer l'efficacité insecticide des extraits de plantes d'*Oudneya africana* de la région de Ghardaïa, comme substitut naturel et sûr aux pesticides chimiques dont l'efficacité diminue et les effets néfastes sur l'environnement et la santé sont importants.

Dans cette étude l'activité insecticide des extraits de macération à froid contre *Tribolium castaneum* a été examinée par bio-essai, l'activité a été évaluée par voie orale et par contact, avec des durées d'exposition allant de 30 minutes à 10 jours.

Les résultats ont révélé que les extraits aqueux et méthanolique d'*Oudneya africana* étaient insecticides contre *Tribolium castaneum*. La mortalité semblait augmenter à des concentrations plus élevées et des périodes d'exposition plus longues. L'efficacité, cependant, présentait une grande variabilité : lors de l'application par contact, l'extrait méthanolique était plus efficace et avait une action plus rapide que l'extrait aqueux. Par ingestion, l'efficacité des deux extraits était relativement faible ; en revanche, l'extrait méthanolique était légèrement plus puissant et plus rapide. Globalement, la méthode d'application par contact s'est avérée plus efficace pour la mortalité des deux extraits.

Les résultats révèlent une variation significative du profil toxicologique des deux extraits. L'extrait aqueux est plus puissant au regard de la dose requise pour son efficacité, comme le confirment les valeurs de LD plus faibles, notamment par ingestion. En revanche, l'extrait méthanolique a une action plus rapide, comme le confirment les valeurs de LT plus faibles lors des applications par contact et par ingestion. Cela suggère que, si l'extrait aqueux pourrait nécessiter une concentration plus faible pour entraîner la mortalité des ravageurs, l'extrait méthanolique provoque des décès plus rapides.

Ces résultats soulignent le potentiel des extraits végétaux dans la lutte biologique contre *Tribolium*, mettant en évidence une voie alternative potentiellement bénéfique pour développer un nouvel insecticide naturel qui pourrait s'avérer une meilleure alternative aux produits de synthèse. Cependant, des études complémentaires approfondies doivent être menées pour déterminer les composés actifs responsables de cette activité insecticide.

Mots clés : *Oudneya Africana*, *Tribolium castaneum*, Activité insecticide, Extrait, Ghardaïa

تقييم النشاط المبيد للحشرات لنبات *الأودنيا الإفريقية* من منطقة غرداية

الملخص:

تهدف هذه الدراسة إلى تقييم فعالية مستخلصات نبات *حنة /الابل* في منطقة غرداية كبديل طبيعي وآمن للمبيدات الكيميائية التي أصبحت أقل فعالية ولها آثار سلبية على البيئة والصحة .

في هذه الدراسة، تم فحص النشاط المبيد للحشرات لمستخلصات النقع البارد ضد حشرة *خنفساء الطحين الحمراء* باستخدام اختبار حيوي. تم فحص النشاط باستخدام كل من طريقتي التلامس والفم، وتراوحت أوقات التعرض بين 30 دقيقة و10 أيام .

أثبتت النتائج فعالية المستخلصات المائية والميثانولية لنباتات *الأسنان الأفريقية* ضد *فطر خنفساء الطحين الحمراء*. وبدا أن معدل الوفيات يزداد مع التركيز ومدة التعرض. إلا أن الفعالية تفاوتت بشكل كبير: فقد كان المستخلص المائي أكثر فعالية عند التلامس، وكان مفعوله أسرع. كان كلا المستخلصين ضعيفين نسبياً عند الابتلاع؛ بينما أثبت المستخلص الميثانولي فعالية أكبر وأسرع تأثيراً. وبشكل عام، تبين أن التلامس أفضل من كلا المستخلصين في إحداث الوفيات .

أظهرت النتائج وجود فروق كبيرة في سمية كلا المستخلصين. بين المستخلص المائي نسبة جرة إلى تأثير أعلى، وهو ما يظهر من انخفاض قيم LD ، وبالأخص عند البلع. في حين أظهر المستخلص الميثانولي بدء تأثير أسرع وهو ما يبدو من انخفاض قيم LT عند اللمس والبلع. باختصار، هذا يعني أن المستخلص المائي قد يحتاج إلى تركيز أقل للقضاء على الآفة، بينما يسبب المستخلص الميثانولي هلاكاً أسرع .

تؤكد النتائج على قدرة المستخلصات النباتية في مكافحة الحويية لأفات حشرة *خنفساء الطحين الحمراء* ، موحية بنهج بديل قد يكون مجدياً في البحث عن مبيدات حشرية طبيعية جديدة تعتبر بدائل أفضل للمبيدات المصنعة. ولا بد من وجود حاجة إلى دراسات أشمل لعزل المركبات الفعالة الفردية المسؤولة عن هذا التأثير القاتل للحشرات .

الكلمات المفتاحية : ، *حنة /الابل* ، *خنفساء الطحين الحمراء* ، مبيد حشري فعال، مستخلص، غرداية.

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List of abbreviations

- % = percentage
- ANOVA = Analysis of Variance
- d = day (used in tables for exposure time)
- y = dependent variable in an equation (often representing mortality percentage)
- x = independent variable in an equation (often representing concentration or time)
- R^2 = Coefficient of determination (also written as R^2)
- LD₂₅ = Lethal Dose 25 (the dose that kills 25% of the test population)
- LD₅₀ = Lethal Dose 50 (the dose that kills 50% of the test population)
- LD₉₀ = Lethal Dose 90 (the dose that kills 90% of the test population)
- LT₂₅ = Lethal Time 25 (the time it takes for 25% of the test population to die)
- LT₅₀ = Lethal Time 50 (the time it takes for 50% of the test population to die)
- LT₉₀ = Lethal Time 90 (the time it takes for 90% of the test population to die)
- mg/kg = milligrams per kilogram
- µg/adult = micrograms per adult (insect)
- µg/cm² = micrograms per square centimeter
- mg/ml = milligrams per milliliter
- g = grams
- ml = milliliters
- °C = Degrees Celsius
- DMSO = Dimethyl Sulfoxide
- TFC = Total Flavonoid Content
- TPC = Total Phenolic Content
- CTC = Condensed Tannins
- GAE/g = Gallic Acid Equivalents per gram
- QE/g = Quercetin Equivalents per gram
- CE/g = Catechin Equivalents per gram
- HPLC-MS = High-Performance Liquid Chromatography-Mass Spectrometry
- *E. coli* = *Escherichia coli*
- *S. aureus* = *Staphylococcus aureus*
- °N = Degrees North (for latitude)
- °E = Degrees East (for longitude)
- m = meters
- mm = millimeters
- Bq/kg = Becquerels per kilogram (unit of radioactivity)
- % = percentage
- \$ = Dollars
- *T. castaneum* = *Tribolium castaneum*
- IPM = Integrated Pest Management

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INTRODUCTION

1. Introduction

Pests significantly affect worldwide food production resulting in heavy yield losses. Plant pathogens and pests are reported to inflict up to 40% yield losses in principal crops with economic losses of around US\$220 billion every year worldwide (**Wang, 2024**).

Tribolium castaneum, commonly known as flour beetles, are significant global pests of stored grains and processed food and cause economic losses through direct consumption and contamination. Their genetically well-developed species and laboratory culture ease make them central model species for the study of ecology, evolution, and pest management strategy (**Sokoloff, 1966; Via, 1999**).

The extended usage of chemical insecticides can lead to pest populations becoming resistant, hence complicating their control strategies. The use of pesticides can contaminate water and soil resources, impacting negatively on non-target organisms and disrupting the balance of ecosystems. The use of synthetic insecticides poses serious health hazards to both human beings and animals and can have both short-term and long-term health implications (**El-Deeb, 2021; Aboelhadid, 2023; Volpe, 2023**).

Biological control methods for *Tribolium castaneum* are increasingly recognized as potential alternatives for chemical pesticides. Biological control lessens the use of synthetic pesticides and therefore reduces the associated problems of resistance to such pesticides as well as their contamination effect on the environment. There are some plant extracts that have been tested for their efficacy on *Tribolium castaneum* to be beneficial in its control. (**Kouninki, 2007; Panezai, 2019**).

The Saharan plants are of late growing nationally conscious for their potential of biological pest control, particularly against arid land agriculture pests. Many Saharan plants contain bioactive chemicals such as flavonoids, alkaloids, and saponins which may play an insecticidal role by affecting the physiology of the pests. Thus, are natural alternatives to synthetic pesticides (**Gajger, 2021**).

The use of these plants within the frame of integrated pest management (IPM) encourages sustainable agriculture by reducing dependence on synthetic pesticides; hence, it is favorable to pest control as well as in maintaining ecological balance in the agro-ecosystem

(Douan, 2024). The use of Saharan plants in biological control thus, gives promising options for sustainable pest management in difficult ecosystems.

Oudneya africana, the native North African drought plant, has been traditionally used for medicinal treatments, disease cure, and as a food source under extreme conditions. Leaves and fruits of the plant are presently being studied to see if they exhibit any pharmacological activity, such as antioxidant and anti-inflammatory activity, since they have high phytochemical content **(Djemaoune et al., 2021; Hammouda et al., 1983).**

The objective of this study is to evaluate in vitro the insecticidal activity of methanolic and distilled water extracts of the Saharan plant *Oudneya africana* on the pest insect *Tribolium castaneum*, highlighting the effectiveness of extracts collected from the Ghardaïa region.

To achieve the objectives of this work, the document is structured as follows:

- The introduction presents the theme and objectives of the study.
- The first chapter presents the study area.
- The second chapter presents the methodology used in the study.
- The third chapter presents the results, including data on the effectiveness of the extracts and statistical analyses, followed by an in-depth discussion of the results obtained.
- The conclusion summarizes the main ideas addressed in the study.

CHAPTER I:

Bibliographic synthesis

CHAPTER I: Bibliographic synthesis

1. *Oudneya Africana* (Robert Brown (R.Br.) in 1826.)

The plant is drought-tolerant and preadapted to dry environments like the Sahara Desert. It has the impressive feature of being drought-resistant owing to antioxidative defense, to counteract oxidative stress under conditions of environmental stress (Talbi, 2015).

Other than this, its water leachates are allelopathic in nature and considerably inhibited the development of weeds like *Bromus tectorum* and did not significantly influence crops like wheat (Nasrine, 2014).

Besides its environmental adaptability, the plant is of great medicinal importance. Seed extracts have been shown to exhibit antimicrobial properties against pathogens like *Listeria monocytogenes* and *Escherichia coli*, indicating its possible use in traditional medicines (Hammami, 2009).

Apart from this, the plant is rich in flavonoid and phenolic compounds in its leaves, which are accountable for its strong antioxidant effect along with potential food preservative action (Hajlaoui, 2019).

1.1. Taxonomy

- Kingdom: Plantae
- Phylum: Angiosperms
- Class: Eudicots
- Order: Brassicales
- Family: Brassicaceae
- Genus: *Oudneya*
- Species: *Oudneya africana*

Oudneya africana, a member of the Brassicaceae family, is native to arid environments, particularly the Sahara Desert (Gómez-Campo, 1974).

1.2. Botanical Description

This short-shrub plant is about 1.5 meters in height and has a dense branching habit. It is leafy with leaves which are 20-50 mm long and 3-8 mm wide. It is also elongated in flower,

about 7-15 mm in length. It possesses petals ranging from 5 to 9 mm in length. Sepals measuring 5-6 mm are found in ascending order. Sepals are very thick, as much as 1.5 cm in thickness (Fig.5) (Jafri, 1977).



Figure 1: The shape of *Oudneya africana* (inaturalist.org,2023.)

1.3. Plant importance

This plant is distinguished by its economic, environmental, and medicinal value. It provides various fodder products that are utilized in traditional medicine. Environmentally, it helps prevent desert erosion and stabilize sand dunes. Medically, desert inhabitants have used it to treat numerous ailments, including digestive disorders, joint issues, colds, influenza, fever, and certain skin diseases (Drebel et al., 2010).

1.4. Chemical composition

The phytochemical composition of *Oudneya africana* is characterized by rich phytochemical compounds and active compounds. In the leaves, the plant is rich in total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins (CTC). In the ethanolic extract, TPC is highest at 661.66 mg GAE/g, TFC at 344.68 mg QE/g, and CTC at 90.18 mg CE/g. HPLC-MS revealed the main compounds to include quinic acid, chlorogenic acid, 4-O-caffeoylquinic acid, and rutin (Hajlaoui, 2019).

Furthermore, seeds of *Oudneya africana* have antimicrobial properties where protein extraction was revealed to have action against various pathogens including *E. coli* and *S. aureus* (Hammami, 2009).

The plant has a very efficient antioxidative defense system and is hence drought-tolerant (Talbi, 2015).

1.5. Habitat

Oudneya africana is a species of plant from the northern Sahara (Quézel and Santa, 1963).

It is found in sand and sandy ground. It is found as single plants along with *Stipagrostis pungens*. It is widespread in the northern Sahara (Chehma, 2005).

It is found in Algeria, Morocco, Tunisia, and Libya. It is found in M'Zab, El Goléa, Ouargla, and Biskra in Algeria. It colonizes desert steppes in North Africa (Ozenda et al., 1977).

2. Animal material

2.1. *Tribolium castaneum* (Red flour beetle)

The red flour beetle (*Tribolium castaneum*) is considered a serious pest that damages stored food products, especially in tropical and subtropical areas (Howe 1956, 1965; Sokolof 1972, 1974, 1977).

This insect causes significant harm to grain stocks in the Sahel region of Africa (Roorda et al., 1982).

Tenebrionidae is the family within the order Coleoptera with a size ranging from 2 to 80 mm and comprises about 20,000 species from 190 genera. They share the same rigid, thick exoskeleton, matt or shiny black (or dark, sometimes with iridescences), typical of all beetle taxa, and some subfamilies have relatively large, oval, or rounded eyes. Their antennae have 10 to 11 segments, and the members of the Tenebrionidae may have wings or be wingless (Balachowsky, 1962).

Several members of Tenebrionidae have been known as plant pest species, while many others infest food grains. It is interesting to know that from the genus *Tribolium*, *Tribolium*

confusum J. Duval and *Tribolium castaneum* Herbst are the important cosmopolitan and pestiferous species (Smith and Whitman, 1992).

2.2. Life cycle of *Tribolium castaneum*

Insects infesting goods stored for long periods of time have the typical life cycle: egg, which is laid by the fertilized female, hatching into worm-like larva. This larva goes through molts to reach its grownup form and finally undergoes pupal transformation to reach adulthood, which matures to reproduce soon after its egression. This is seen in both beetles and Lepidoptera (Cloutier, 1992).

Tribolium castaneum, popularly referred to as the red flour beetle, undergoes the interesting biological phenomenon of undergoing complete metamorphosis, where they have a four-part life cycle. This involves the preliminary egg phase followed by the larva phase, pupa phase, and finally the adult phase. A female red flour beetle deposits a staggering number of about 24 eggs each and every day, and the egg hatching period requires about 4 to 5 days before they hatch. However, for the larvae of this species, there are seven stages of growth, which are technically known as instars. This prolonged development period lasts about 70 to 83 days before they are formed into pupae. After they have been formed into pupae, they will remain there for about 6 to 9 days before they come out as full-grown adults. With all of this in mind, the overall life cycle of the red flour beetle is one of great variability and lasts from 164 to 194 days, and it's one that will fluctuate considerably depending on the particular environment under which they are growing. (Devi, 2015)

The female *T. castaneum* is known to produce a very high total of as much as 1,000 eggs all her life. This high fecundity is largely determined by many factors, ranging from the quality of the available food to the nature of the environment they are in. To better explain the nature of the situation, females under good feeding regimes where they mainly get to consume semolina have been seen to record the highest rates of fecundity, at 28.7 eggs per female (Skourti, 2020).

The reproductive process in females is inextricably linked with a diverse range of genetic elements and hormonal factors that cumulatively influence this fundamental biological process. Among the many determining factors that make up this complex system are the insulin signaling pathway and the TOR signaling pathway, both of which play fundamental roles in the complex and multifaceted process of oocyte maturation. (Parthasarathy, 2011)

2.3. Morphology of *Tribolium castaneum*

Tribolium castaneum, which is usually referred to as the red flour beetle, has a distinctive and readily identifiable morphological profile comprising its characteristic length and overall shape. With regards to dimensions, the adult members of the species have a length of about 3.6 mm and a width of about 1.1 mm, attributes which form principal identifying markers for the proper classification of the species in concern (Fig.6) **(Castro, 2023)**.

The shape is typically elongated and usually has a distinctly oval shape, with its surface not only smooth to touch but also having a gloss finish. Its surface is able to have a variety of different colors ranging from bright reddish-brown to very brown. The head of the beetle is proportionally small in comparison with the rest of its body, and is topped with large compound eyes readily visible to the eye. This small head is also provided with short antennae that are instrumental in heightening its sensory perception of the immediate environment. An important role is played by the elytra, the hardened forewings of the beetle, in protecting and enclosing the exposed abdominal surface lying below. Punctures fine as hairs adorn the surface of the elytra and serve to help create the beetle's distinctive and readily identifiable visual profile. Its legs are slender in form, carefully constructed for maximum mobility, enabling the beetle to move around its environment with much ease and success. **(Dia, 2018)**

Morphological studies have exhibited considerable differences in both size and shape within various populations, which is seen to be very much in association with the particular food available to these populations. This difference shows us that there is considerable morphological adaptability in such populations to suit their environment. **(Dia, anno Domini 2018)**

The morphology of *T. castaneum* plays a significantly important role in its survival and its amazing adaptability in different environments, particularly as a pest infesting and destroying stored grain products.

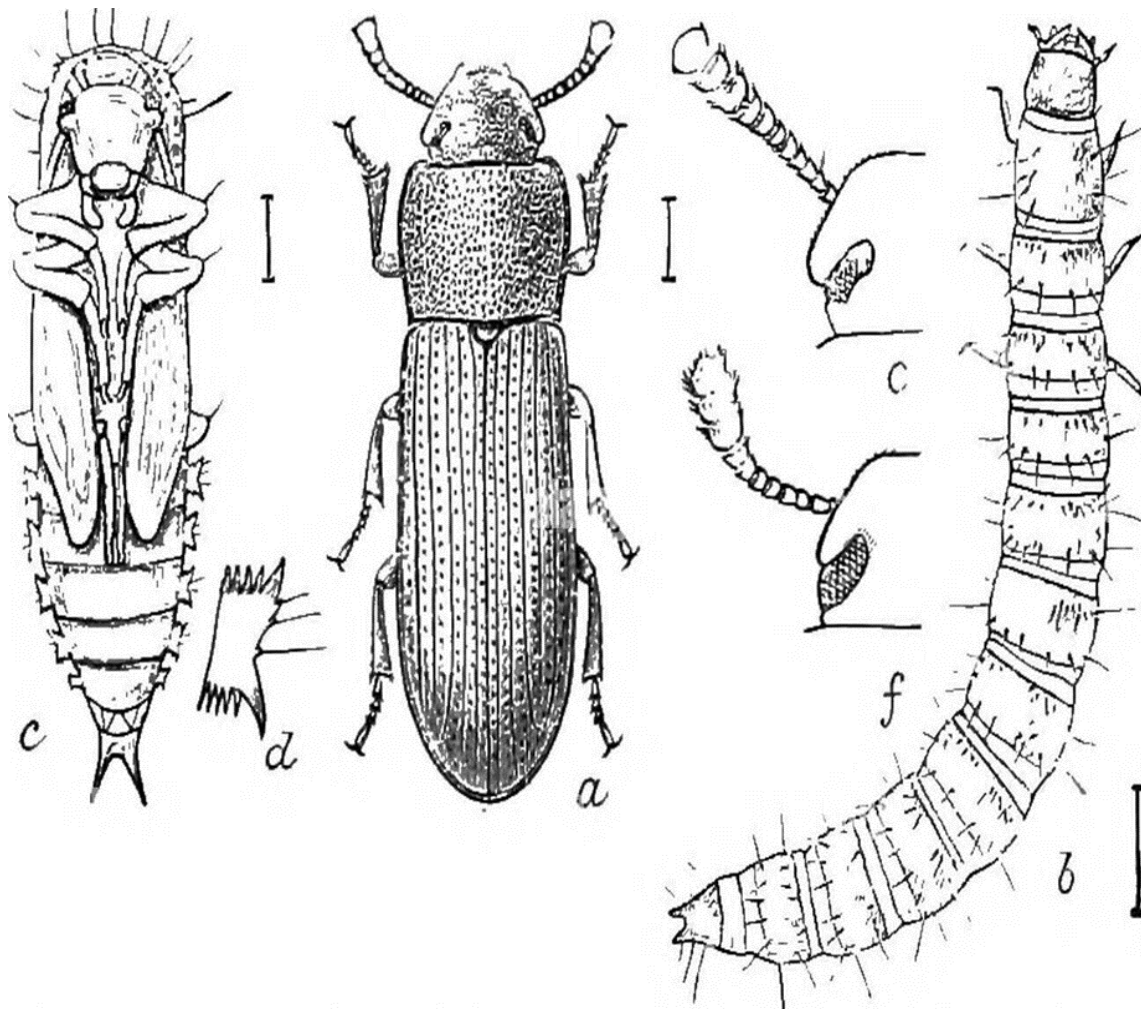


Figure 2: *The costaneum tribolium* in a close up (W.S. Blatchley, 1910)

CHAPTER II:

Material and methods

CHAPTER II: Material and methods

1. Study Objective

As part of biological control as an alternative to conventional methods, this study aims to evaluate the insecticidal effect of the plant *Oudneya africana* on a stored-product pest: *Tribolium castaneum*

2. Plant sample collection area (Ghardaïa, January 24 ,2025)

The *Oudneya africana* plant was collected on January 24, 2025, in Daya Ben Dahoua, located in Algeria's Ghardaïa region.

The area allocated for plant sample collection in Daya Ben Dahoua in Ghardaïa, Algeria (Fig.4), has unique ecological features contributing to rich vegetation diversity. It has a semi-arid type of climate, which has large temperature variations and little rainfall on average, thus having a corresponding effect on both vegetation quality and quantity present. The vegetation in the area consists of mostly fabaceae species, supplemented by anacardiaceae and poaceae representatives, which all share a significant ability to adapt themselves to the conditions prevailing around them.

The methods used in the process of harvesting in this area are important for the long-term sustainability of plant resources. The timing of harvesting can have a great impact on yield and quality, with specific optimal times determined for each species to maximize the yield of essential oil and biomass (Sönmez, 2019).

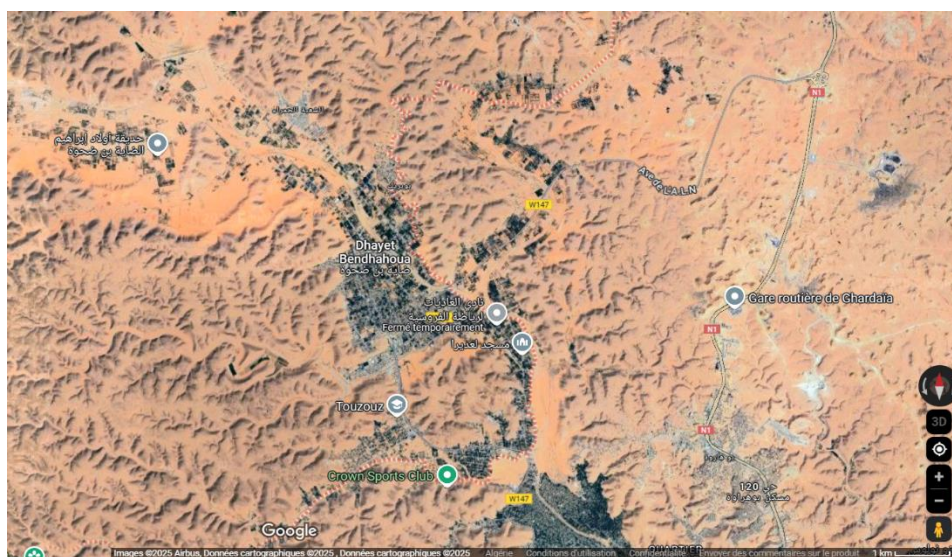


Figure 3: Geographic location of the collection region upstream of Daya Ben Dahoua (Google maps,2025)

2.1. Geographical Location of Ghardaïa Region

Ghardaïa is located in northern Algeria's southern region, bordering the northern edge of the Sahara Desert. The geographic coordinates for this location are approximately 32.48°N and 3.66°E, and it sits approximately 502 meters above sea level, Ghardaïa is located approximately 640 kilometers south of Algiers, the capital of Algeria, and its climate is a range from semi-arid to arid desert (Selloum, 2023; Gama, 2013).

As of 2024, Ghardaïa region in southern Algeria is bordered by many significant regions defining its geographical position (Fig.1):

- North: Bouira and M'Sila, important urban and farming centers.
- East: Ouargla, with its oil fields and typical desert landscapes.
- South: Laghouat, recognized as a solid regional center.
- West: El Menia, one of the area's socio-economic forces (Belaid, Alioua 2024).

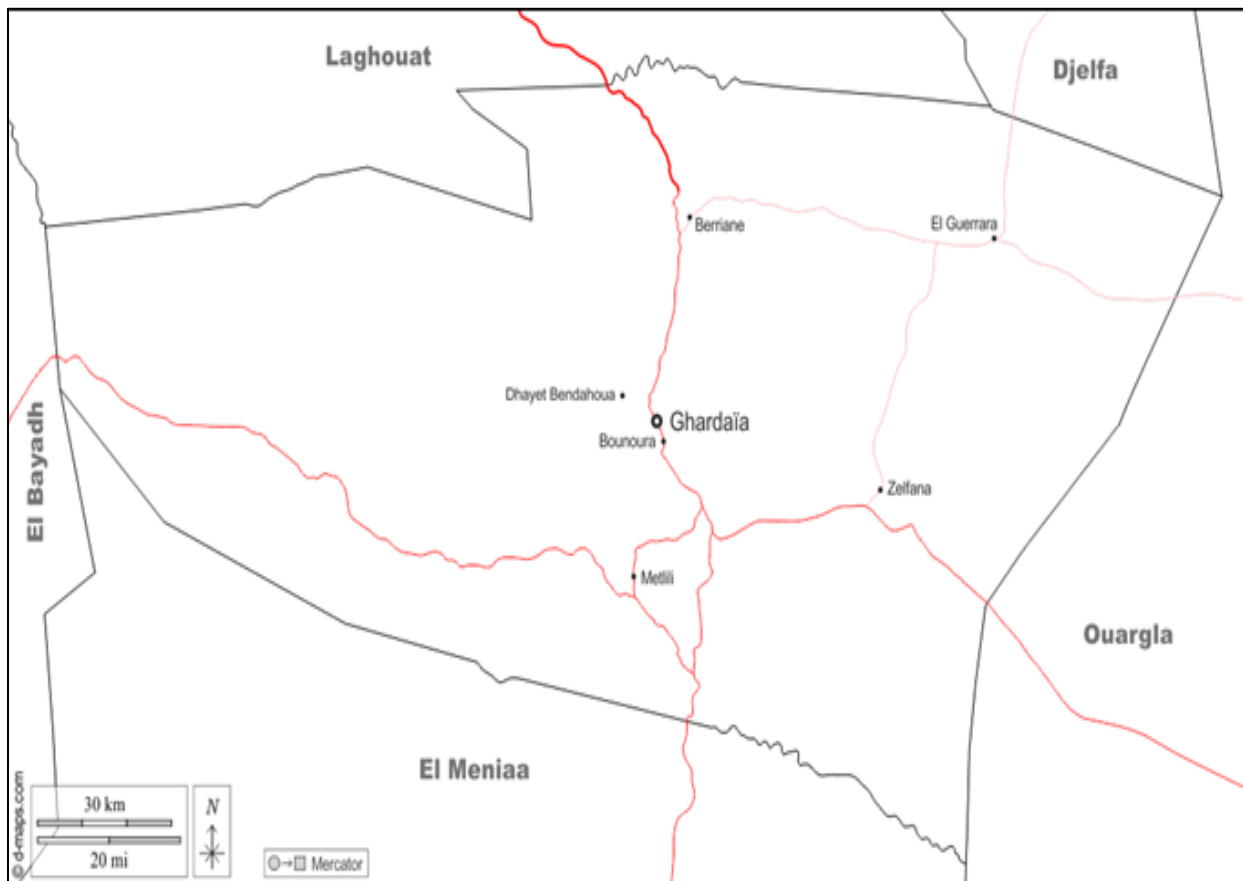


Figure 4 : Location of the Wilaya of Ghardaïa (d-maps.com,2025)

Being situated in the solar belt region, the region is blessed with abundant solar resources, hence making it an ideal place for the establishment of renewable energy projects (Belaid, 2025).

2.2. Topography and relief of Ghardaïa

Ghardaïa is located in the southern part of Algeria and is composed of varied topography consisting of mountains, valleys, and plains, thus having a considerable variation in elevation. The relief of Ghardaïa is not uniform, with some regions having heights of about 1,000 meters above sea level, especially in the bordering mountainous ranges, which exert a strong impact on the regional climate and ecosystems (Ammar, 2018).

The M'zab Valley that contains the city of Ghardaïa features a vast ecosystem of an oasis nature and a varied topographical configuration that has been shaped both naturally and anthropogenically. The valley's geographical features help support the region's agricultural economy in the production of date palms that prosper in the prevailing climatic conditions of the region (Gairaa, 2013).

The region's geographical position within the Sahara Desert is the reason why it is dry, influencing water and vegetation cover

The region receives high levels of solar irradiation that are important in facilitating the efficient harnessing of solar energy (Guermoui, 2018; Belaid, 2025).

2.3. Climate of Ghardaïa

Ghardaïa has a hot desert climate, with extremely high temperatures in summer and low annual rainfall, making it a typical example of a desert ecosystem (Boukhelkhal, 2016).

The mean temperatures of Ghardaïa can rise to approximately 40°C during the height of summer, and the nights during winter become rather cold, falling to approximately 5°C, resulting in substantial day-night temperature differences (Saadene, 2023).

The area is exposed to very little rainfall, with an average of approximately 100 mm annually; this rainfall takes place primarily from April to October, and it plays a fundamental part in agriculture along with the ecosystem (Mihoub, 2016).

Ghardaïa has two prevailing seasons: a long hot and dry summer, coupled with a comparatively mild winter, and these seasonal changes have an overwhelming influence on the local climate and agricultural practices (Belgaid et al, 2023).

The prevailing climatic conditions in Ghardaïa play a major role in determining the agricultural activities in the region, particularly in the management of water resources since the sparse pattern of rainfall necessitates the application of irrigation systems in the production of the date palm (Bouchakour, 2024).

In the Ghardaïa region, which is subject to a Saharan climate, analysis of the ombrothermic diagram shows that the dry season extends over nearly the entire year (Fig. 5). Rainfall is extremely low, and even nonexistent for several months, while temperatures remain high, especially during the summer season.

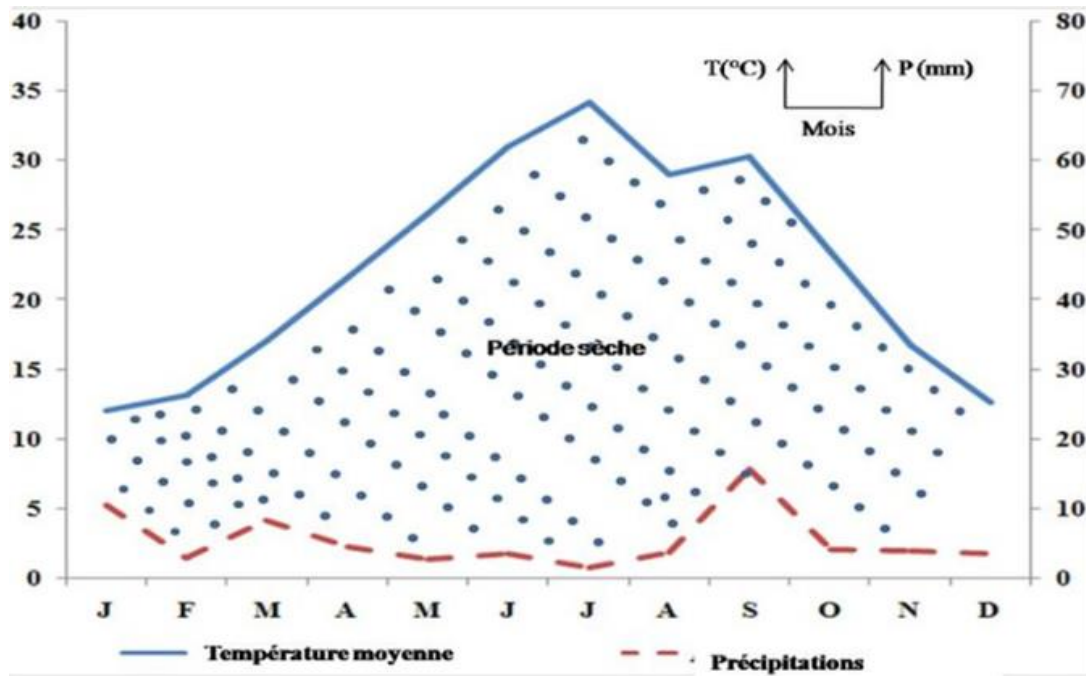


Figure 5: Bagnouls and Gaussen Ombrothermic Diagram (1953) for a 10-year period (2009-2018) of the Ghardaïa region (Zergoun,2020).

The Emberger climagram is used to identify the bioclimatic zone of a given region, based on an index known as the Emberger pluviothermic quotient (Q_2), defined by the following formula (Belli, 2012):

- Q_2 : Emberger's pluviothermic quotient
- P : mean annual precipitation (71.21 mm)
- M : average of the maximum temperatures of the hottest month (43.16 °C)
- m : average of the minimum temperatures of the coldest month (3.62 °C)

Figure 3 shows the Emberger climagram for the Ghardaïa region. Based on this index, with a calculated value of $Q_2 = 6.18$, Ghardaïa falls within the Saharan bioclimatic stage with mild winters.

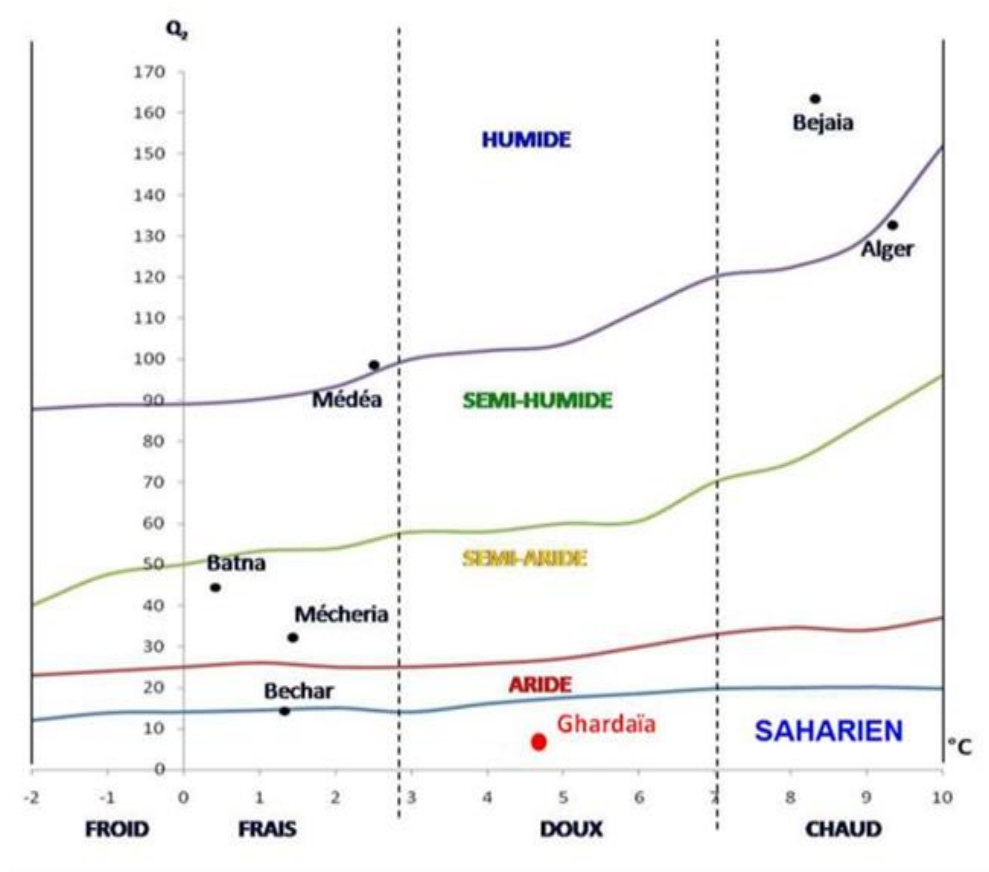


Figure 6: Ghardaïa's Bioclimatic Stage According to EMBERGER's Climagram (1998-2018) (Hamel, 2023).

3. Biological material

The choice to study *Oudneya africana* has a number of critical grounds on which it rests. Records in written form reveal numerous common applications as well as probable biological characteristics of the species, primarily in traditional medicine and combat of germs

Previous studies have shown an increasing interest in this plant species, more specifically in its efficacy in traditional medicinal uses

The phytochemical compounds of *Oudneya africana* are a central area of academic interest. The species has a notable diversity of phytochemical compounds containing flavonoids, terpenoids, and alkaloids, which are mainly related to pest control and antimicrobial action (Hammami, 2009; Djahafi, 2021; Abdeldjalil, 2025).

3.1 Preparation of the plant extract

3.1.1. Plant Collection

In order to prevent outside interference, the upper portion of the plant was cut (Fig.7), preserving the roots of the chosen plant for this study were gathered in Daya Ben Dahoua, Ghardaia, from its natural habitat, remote from populated areas and activities, in January 24, 2025.



Figure 7: The plants that was gathered to acquire the extract (**Original**)

3.1.2. Rinsing and Drying

The selected plants for extract preparation were isolated and washed thoroughly to remove impurities. They were then placed in an oven set at 30 degrees Celsius under ventilation for exactly 24 hours.

3.1.3. Grinding

After completing the plant drying process under ventilation and at the specified temperature, it was ground to obtain a fine powder. We acquired 350g of fine powder, as a preservation measure, this powder was placed in a clean, airtight container and stored in a safe and suitable place to maintain its quality.

3.1.4. Extraction

The plant extracts used for toxicity tests were prepared using one technique of maceration (Fig.8)

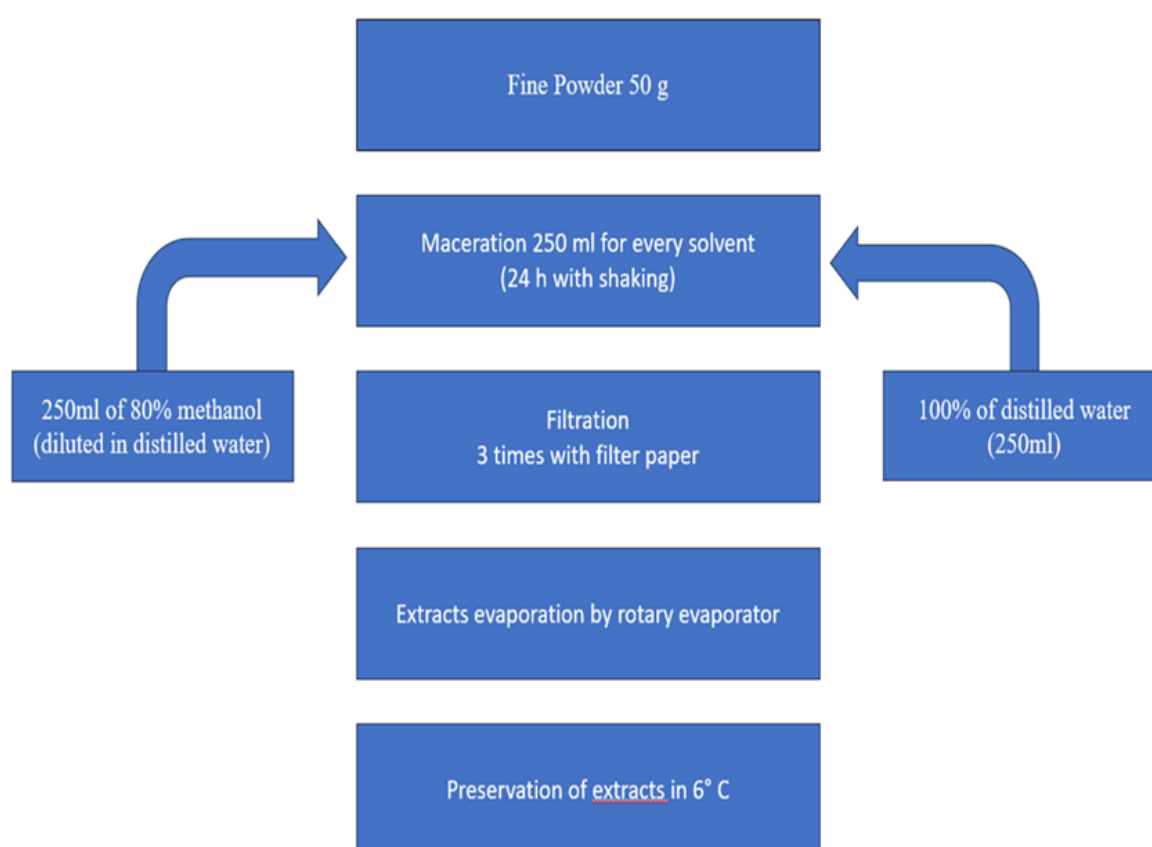


Figure 8: Extraction methodology

In the maceration method, two organic solvents with different polarities were used methanol and distilled water. Each solvent was used in a 250-ml volume to totally saturate 50 grams of ground plant material for 24 hours at room temperature and under continuous agitation using (Precise Shaking Incubator) (Fig.9).

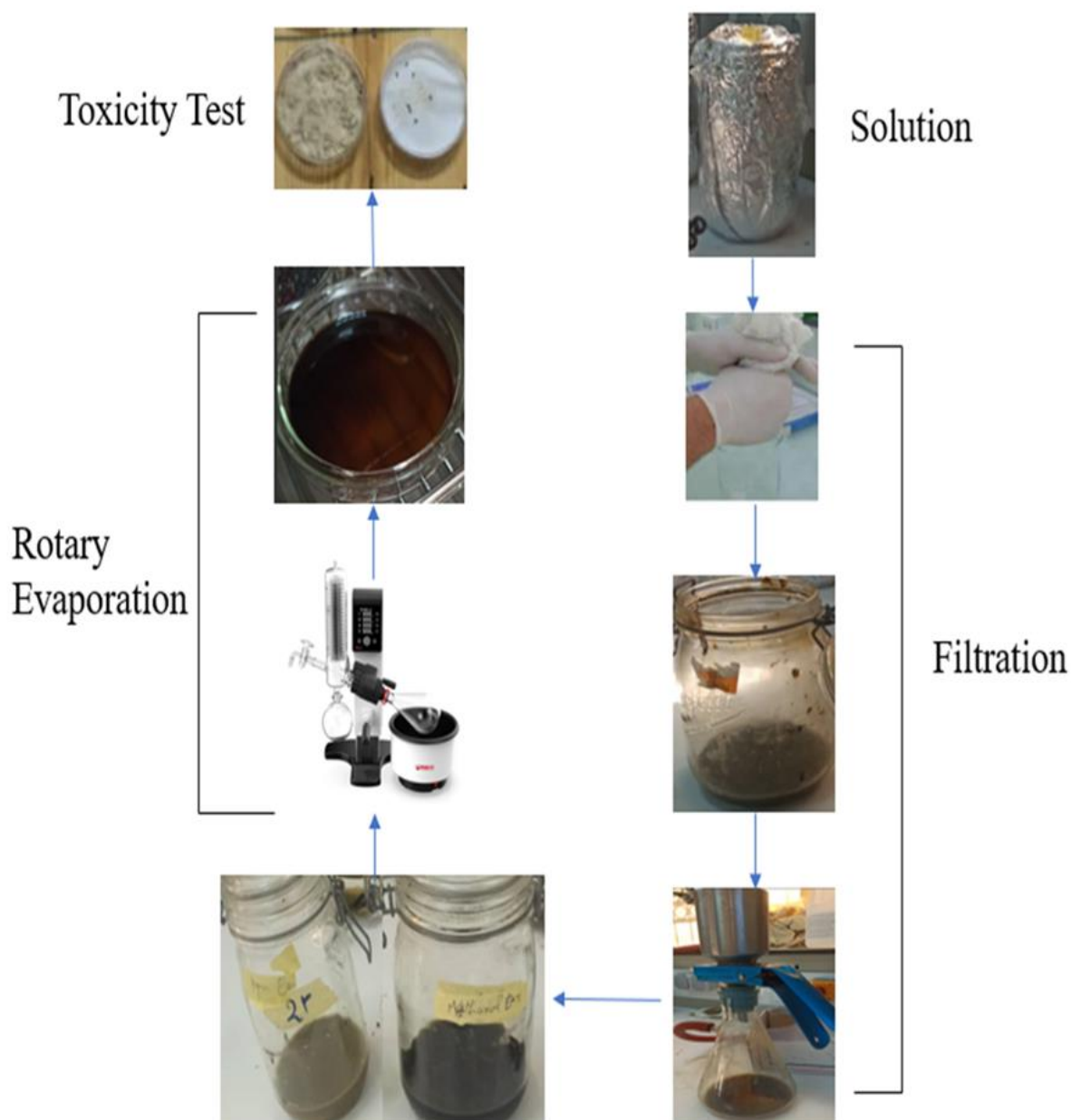


Figure 9 :The process of extraction stages

3.1.5. Filtration

The mixtures acquired after cold maceration were filtered three times to ensure filtration efficiency. Initially, filter paper was used, followed by vacuum filtration for a more thorough purification (Fig.10). After this, the filtered mixtures were placed in vials and labeled (Fig. 11).



Figure 10: The filtration process (Original)

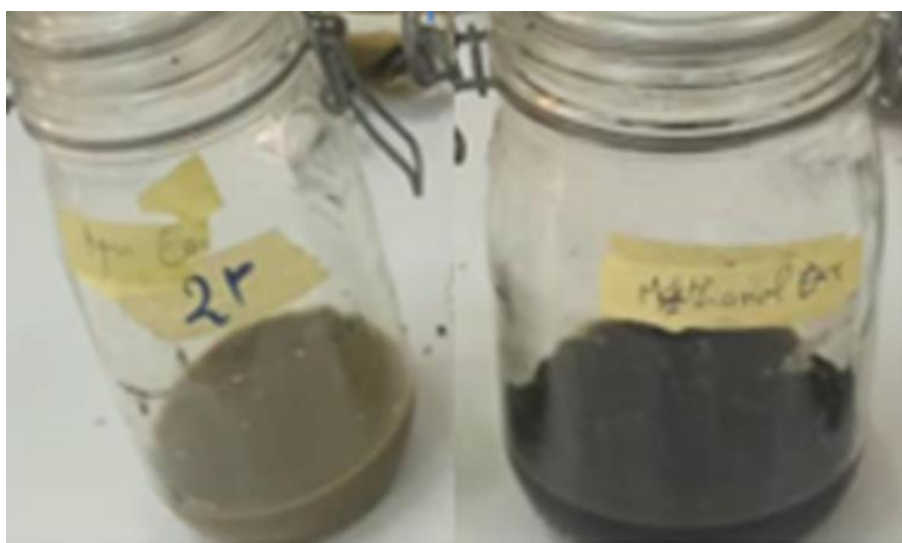


Figure 11: The aftermath of filtration (Original)

3.1.6. Extracts evaporation

A rotary evaporator was used to dry the extracts obtained after maceration at a temperature of 45 degrees Celsius (Fig12). The resulting dry extract was collected, weighed, labeled, and stored in small vials at a temperature of 4 degrees Celsius until use (Fig.13).



Figure 12: The rotary evaporator machine (Drawell Analytical,2023)



Figure 13: The extract after being evaporated (Original)

3.1.7. Extracts Yield

The percentage of the active compound dissolved in the organic solvent used for extraction, in relation to the weight of the plant material, is the yield of the plant extracts. The mass of the plant material used for the extraction is the basis for estimating these yields. The following formula was used to calculate the extraction yield (%) (Kemassi, 2014).

4. Toxicity test

We exposed adult *Tribolium castaneum* specimens to *Oudneya africana* at concentrations of 20mg/ml, 40mg/ml, and 60mg/ml using two methods: direct injection into the semolina and contact exposure, where the solution was applied to a surface (filter paper) (Fig. 14). This allowed the insects to come into contact with or ingest the treated surface. Mortalities were then monitored at fixed intervals (2, 4, 6, 12, 24, 48, and 72 hours, up to 10 days) to determine the insecticide's effectiveness.



Figure 14:The toxicity test that was applied on the insect (Original)

4.1. Direct injection

As part of the study's methodology, the extracts from the plant *Oudneya africana* were carefully made in three different concentrations precisely 20, 40 and 60 mg/ml. These different concentrations were then carefully combined with the semolina, making sure that they were dispersed equally throughout the mixture.

Different batches of semolina mixed with the extract were prepared in three groups (20, 40, and 60 ml/mg) with 10 adult red flour beetles (*Tribolium castaneum*) to ensure consistent experimental conditions. To reduce any variability and enhance the reliability of the results, three replicates were performed for each concentration.

4.2. Contact exposure

With this method, extracts obtained from *Oudneya africana* were used on the filter paper surface at the concentrations of 20, 40, and 60 mg/ml.

For this study, filter paper as a semi-permeable membrane separating small particulates from liquids and gases was used as the material to be modified.

Multiple batches of treated filter paper were prepared for each concentration. Each treated paper was then placed in a separate petri dish or container, and 10 adult *Tribolium castaneum* (red flour beetles) were carefully introduced onto its surface. To ensure the reliability of the results and account for potential variability, each concentration was replicated three times.

The beetles were then placed on this treated filter paper. Through direct contact with the paper and potential ingestion of any residue (if they investigated or fed on it), this approach aimed to assess the effect of the extract.

4.3. Control group

A control group was also established as part of the study, for both methods. The control group consisted of semolina, a solution of 5% DMSO (5 ml of dmsol, and 100 ml distilled water) and the same number of insects was added. The mortality rate of insects was monitored for a baseline measure, in turn allowing for the effective confirmations of the actions produced in the treatment groups of the *Oudneya africana* extracts. The number of dead insects in each group was counted and recorded at regular intervals, thereby allowing for the expressions of the efficiency of the extracts at different concentrations.

5. Analyzed toxicity parameters

In this study, three main indicators were used to assess the toxicity of the tested extracts: mortality rate, lethal doses, and lethal times.

5.1.Mortality rate

The mortality percentage was calculated based on the total number of exposed individuals, using the following formula (Kemassi et al., 2019; Rouari, 2022):

Observed mortality (%) = [(Number of individuals dead after treatment) / (Total number of individuals)] × 100

To account for the natural mortality observed in the control groups, a correction formula was applied (Kemassi et al., 2019):

Corrected mortality (%) = [(Mortality in treated group – Mortality in control group) / (100 – Mortality in control group)] × 100

This adjustment provides a more accurate estimation of the toxicity specifically induced by the treatment.

5.2.Lethal doses (LD₂₅, LD₅₀, and LD₉₀)

Lethal doses correspond to the concentrations causing 25%, 50%, and 90% mortality, respectively. Their determination is based on converting the corrected mortality rates into probit values and the extract concentrations into decimal logarithms. These values were then used to establish linear regression equations between the probit values and the log-transformed concentrations (Rouari, 2022; Kemassi et al., 2019).

5.3.Lethal times (LT₂₅, LT₅₀, and LT₉₀)

Lethal times represent the exposure durations required to reach 25%, 50%, and 90% mortality, respectively. These were determined through linear regression between the probits of corrected mortality and the logarithms of exposure durations. This approach allows for the comparison of the speed of action among different extracts based on standardized criteria (Rouari, 2022; Kemassi et al., 2019).

6. Data Processing

Statistical analysis of mortality data and other toxicity parameters was carried out using Microsoft Excel (version 2010). Significant differences in the effects of the plant extracts were evaluated using STATISTICA software (version 13) through an analysis of variance (ANOVA), followed by Tukey's HSD test. Data normality was verified beforehand, with the significance threshold set at $p < 0.05$.

CHAPTER III:

Results and discussion

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1. Results and discussion

The experiment in the laboratory was to test the insecticidal activity of extracts from samples of *Oudneya africana* from Ghardaïa in adult *Tribolium castaneum* specimens used as the model. The parameters used in the evaluation included the direct contact exposure as well as ingestion in the form of aqueous and methanolic solutions. Mortality levels were observed over a period of 10 days, and the resulting data were statistically analyzed with the aid of Microsoft Office Excel and STATISTICA software.

1.1. Extraction yield and characteristics of extracts

The yield of extraction is a measure of the success of the process used to convert raw dried botanical material into a concentrated dry extract rich in active ingredients, this is referred to as the ratio of the net weight of the resulting extract to the original weight of the botanical material used.

The extraction yields for *Oudneya africana* show distinct differences based on the solvent used. A yield of 6.315% for the aqueous extract, And the methanolic extract yielded 3.5% (Fig. 15 and table

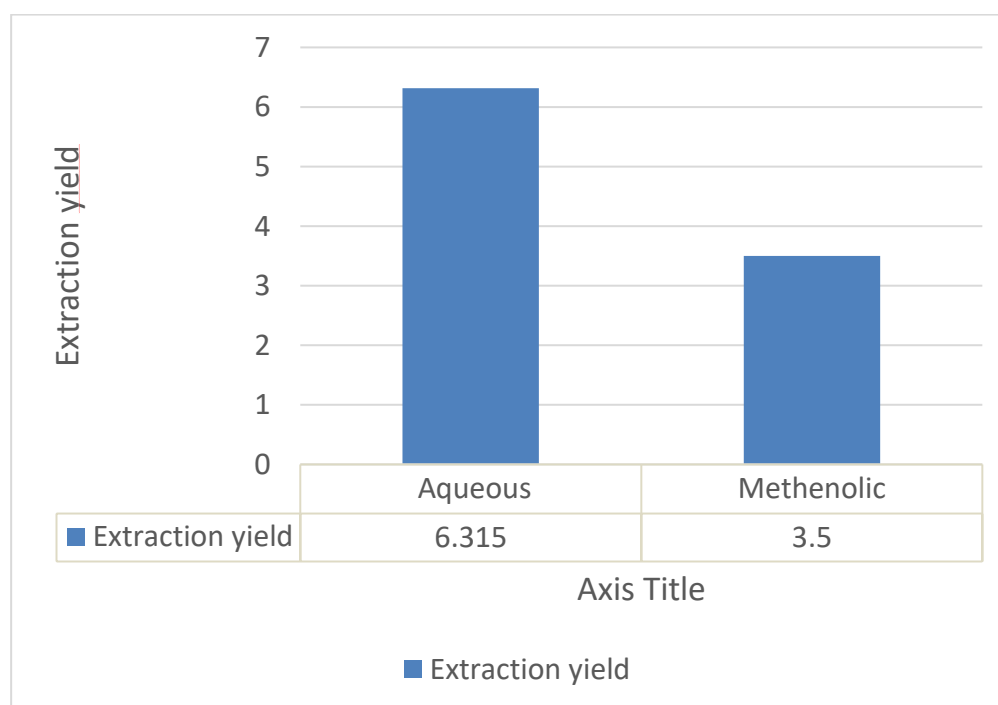


Figure 15 : Extraction yield of different organic extracts

Although the aqueous extract of *Oudneya Africana* yielded 6.315% and the methanolic extract 3.5%. It confirms that both extracts, despite their differing yields, contain a wide array of active compounds that can contribute to the observed biological efficacy, such as the insecticidal activity you tested against *Tribolium castaneum*. These quantitative and qualitative results, taken together, support the hypothesis that the diversity of chemical constituents in *Oudneya Africana* forms the basis of its broad therapeutic potential and its likely effectiveness as a biopesticide

Nabti and Belhattab (2016) found in their research that the extraction yields of *Oudneya Africana*, specifically focusing on methanolic and aqueous solvents. the research results: the aqueous extract yielded 10.15%, while the methanolic extract demonstrated a significantly higher yield of 29.55%.

Nabti and Belhattab (2016) confirmed in their research that *Oudneya Africana* generally contains alkaloids, flavonoids, quinones, coumarins, steroids, and tannins. Their analyses showed that the abundance of these compounds varied with the solvent used for extraction, with more polar compounds being more soluble in polar organic solvents like methanol and water. This diversity in the phytochemical composition underscores the presence of a significant number of secondary metabolites in *Oudneya Africana*, highlighting its broad therapeutic potential.

Table 1 : Organoleptic characteristics of the obtained extracts

Extract	Appearance	Color	Odor
Aqueous	Pasty	Dark brown	Unpleasant
Methanolic	Pasty	Light brown	Unpleasant

1.2.Toxicity

These graphs present (Fig. ,16,17,18 and 19) the toxicity of plant extracts by contact and ingestion against adult *Tribolium castaneum* insects was evaluated in the laboratory. The use of increasing doses of these extracts, within both aqueous and methanolic media, was shown to cause the mortality of treated individuals.

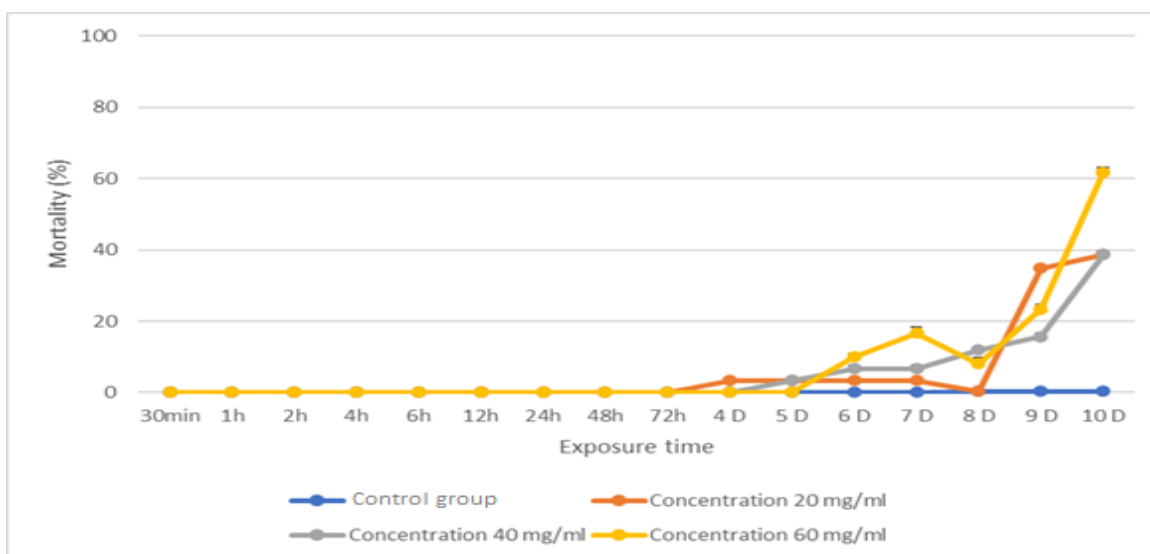


Figure 16: Toxicity of the aqueous extract applied by contact.

In Figure 16, The maximum mortality observed is around 62% after 10 days of exposure to the extract using a concentration of 60 mg/ml, all tested concentrations (20, 40, and 60 mg/ml) show no mortality during the first 72hours ,4th and 5th days of exposure. A noticeable increase in mortality for the 60 mg/ml concentration became apparent after 6 days, reaching 23% by day 9, and 62% by day 10. For the 40 mg/ml concentration, mortality was 0% until day 4, then showed 3% from day 5 to day 8 there was a slight increase reaching 12%, before increasing to 16% by day 9 and 39% by day 10. Similarly, the 20 mg/ml concentration also showed 0% mortality until day 4, maintaining 3% until day 7, then sharply increasing to 35% by day 9 and 39% by day 10.

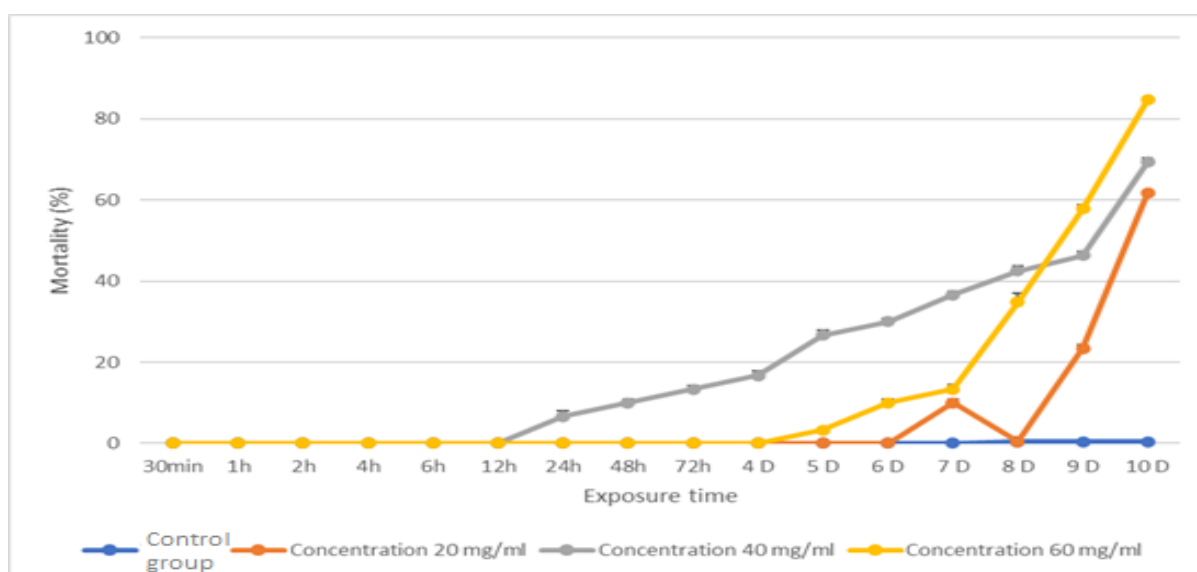


Figure 17: Toxicity of the methanolic extract applied by contact.

In Figure 17, The maximum observed mortality is around 85% after 10 days of exposure to the extract with the use of a concentration of 60 mg/ml, no mortality is recorded for the 20 mg/ml concentration until the 7th day of exposure. The 60 mg/ml concentration showed an initial mortality of 3% after 5 days, which quickly rose to 35% on the 8th day, 58% on the 9th day, and eventually 85% on the 10th day, The onset of mortality was earlier for higher concentrations; for instance, the 40 mg/ml concentration began to show effect after 24 hours (7% mortality), progressing to 37% by day 7, 46% by day 9, and ultimately reaching 69% by day 10, the 20 mg/ml concentration did not show any mortality until 7 days, recording a mortality of 10%, increasing to 23%, which spiked surprisingly to 62% by day 10.

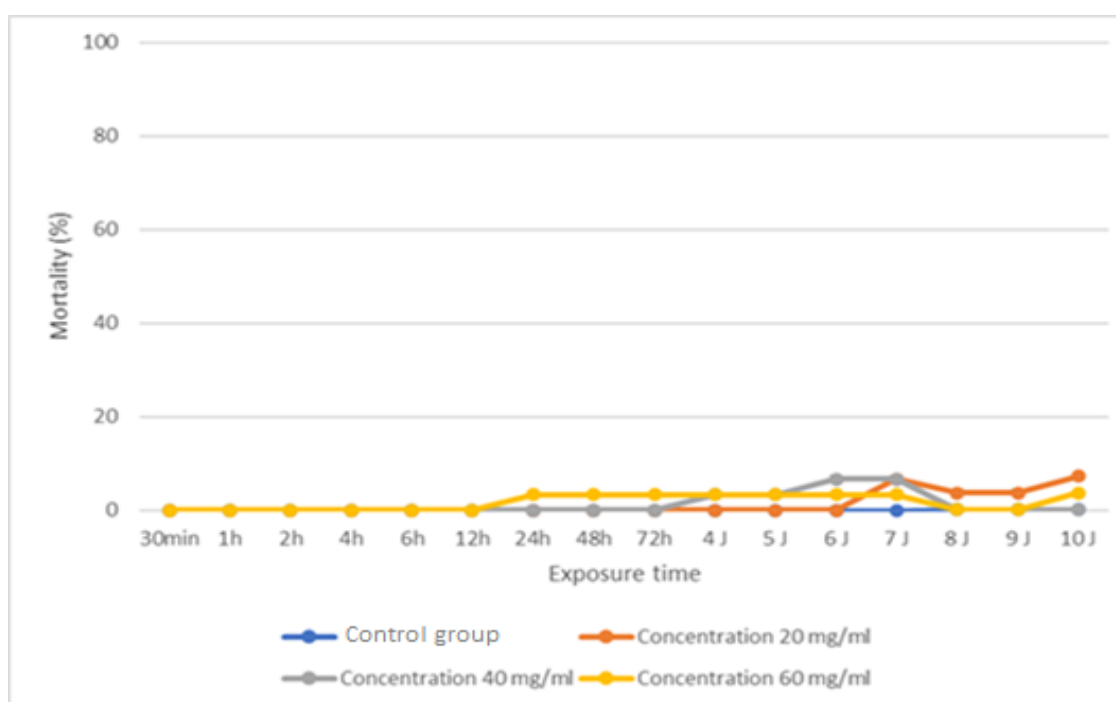


Figure 18: Toxicity of the aqueous extract applied by ingestion.

The maximum mortality observed is a very low 7% after 7 days of exposure to the extract using a concentration of 20 mg/ml (and also at 40 mg/ml after 6 and 7 days). In contrast, the insecticidal effect by ingestion appears to be limited, with some concentrations, such as 40 mg/ml and 60 mg/ml, showing very low or no mortality by 10 days despite showing transient effects earlier. Specifically, the 20 mg/ml concentration showed 0% mortality until day 7, then 7% day 7 and the same in day 10. The 40 mg/ml concentration showed 0% until day 4, then transiently reached 3% at day 4, 3% at day 5, 7% at day 6 and 7, before dropping back to 0% at day 8 and 0% by day 9 and 10. The 60 mg/ml dose had comparatively minor and transient effects, as can be inferred from the 3% mortality observed in the 24-hour period up to day 7,

before declining to 0% on days 8 and 9, and rising gradually to 4% by day 10. In general, this intervention contributed to largely low and highly varying mortality levels in the different concentrations tested.

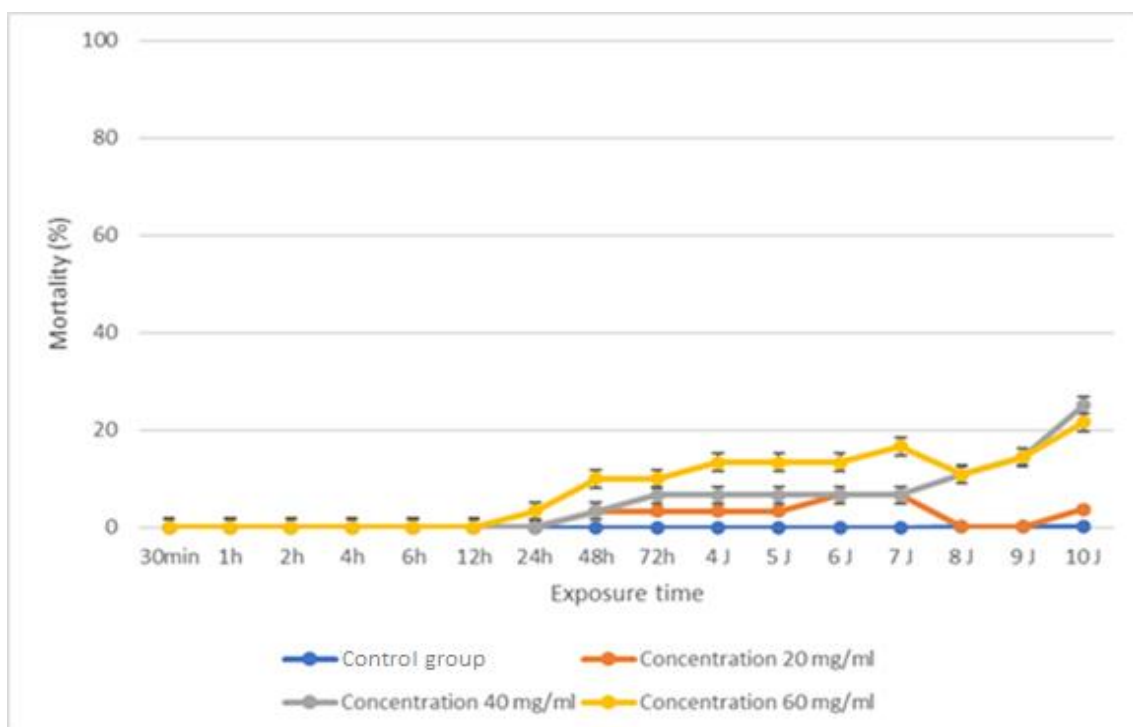


Figure 19: Toxicity of the methanolic extract applied by ingestion

The maximum mortality observed is 25% after 10 days of exposure to the extract using a concentration of 40 mg/ml. In contrast, the lowest concentration of 20 mg/ml shows very limited effect, not exceeding 7% mortality throughout the 10-day exposure period. The mortality rate of 60 mg/ml was initially observed at 24 hours to 3% and continued to increase progressively to 17% at day 7 and 22% at day 10. By contrast, the 40 mg/ml concentration became active from the first 24 hours with 3% and continued to increase progressively to 17% on day 7 before dropping a little on day 8 11%, and 14% on day 9 and increased up to 22% on day 10.

However, the lowest concentration of 20 mg/ml maintained a very limited effect, beginning at 48 hours (3%) and peaking at 7% from day 6 to day 7, before declining to 4% by day 10, thus not exceeding 7% mortality throughout the 10-day period.

The monitoring results of observed mortality rates in *Tribolium castaneum* flour beetles show that the studied plant has a varied insecticidal effect. in mortality rates ranging from low to high, with a clear increase observed as concentrations rise.

Based on the detailed results obtained, the following can be concluded regarding the two methods used to assess the toxicity of the extracts on adult *Tribolium castaneum*. Applying the extracts by the contact method demonstrates significantly higher insecticidal efficacy compared to the ingestion method.

This means that the active compounds in the extracts are more lethal when they directly contact the insect's body (perhaps absorbed through the cuticle or outer surface), or that these compounds may undergo rapid breakdown or detoxification within the insect's digestive system when ingested, reducing their effect. Deaths resulting from contact were significantly higher.

It was also found that the mortality rates of these flour beetles are directly and positively correlated with exposure time. This means that as the duration of insect exposure to the extract increases, mortality rates rise. Additionally, the significant differences in the toxicity of the various extracts increase over time, emphasizing the importance of exposure duration in determining insecticidal effectiveness.

There was a difference in the toxic effect between the methanolic and aqueous extracts. The insecticidal activity of both extracts, using the two methods, indicates the presence of certain phytochemical components and their effect on the biological activities of *Tribolium castaneum*.

The effectiveness of phenolic compounds as insecticides is attributed to their ability to reduce insect fertility and shorten their lifespan, (Al-Massarani et al., 2019). Secondary metabolites, especially those characterized by their richness in diverse functional groups, usually cause physiological disturbances in insects (Arab et al., 2018).

1.3.Lethal doses

Tables 2, 3, 4, and 5 group together the different lethal dose values, organized by time class (1 to 10 days), for the aqueous and methanolic extracts of *Oudneya africana*.

Table 2 : Lethal dose values of aqueous extract applied by contact

Exposure time	Equation	R ²	LD ₂₅ [mg/ml]	LD ₅₀ [mg/ml]	LD ₉₀ [mg/ml]
1 d	/	/	/	/	/
2 d	/	/	/	/	/
3 d	/	/	/	/	/
4 d	$y = -10.844x + 13.608$	0.8294	7.1772	6.2202	4.7378
5 d	$y = -9.681x + 13.3$	0.6611	8.4522	7.2003	5.3079
6 d	$y = 2.0102x + 1.1238$	0.9895	39.1720	84.7746	368.1403
7 d	$y = 3.0283x + 0.0531$	0.9712	25.7626	43.0087	113.9978
8 d	$y = 12.584x - 12.112$	0.7872	20.2424	22.8993	28.9533
9 d	$y = -1.3258x + 5.8306$	0.4298	13.6415	4.2315	0.4566
10 d3	$y = 1.8127x + 2.8147$	0.6611	6.8191	16.0528	81.8054

Table 3 : Lethal dose values of methanolic extract applied by contact

Exposure time	Equation	R ²	LD ₂₅ [mg/ml]	LD ₅₀ [mg/ml]	LD ₉₀ [mg/ml]
1 d	$y = 1.3135x - 0.3477$	0.0095	3615.790 6	11785.17 74	111520.4 201
2 d	$y = 1.3858x - 0.3669$	0.0095	2434.5539	7460.7256	62788.264 9
3 d	$y = 1.444x - 0.3823$	0.0095	1822.1980	5337.7050	41225.525 9
4 d	$y = 1.5081x - 0.3992$	0.0095	401.6115	1123.8959	7957.9707
5 d	$y = 11.537x - 10.846$	0.592	20.6573	23.6316	30.5221
6 d	$y = 13.209x - 12.578$	0.6954	19.0429	21.4170	26.7802
7 d	$y = 0.8383x + 3.115$	0.0619	27.8349	177.2549	5996.3063
8 d	$y = 16.123x - 15.54$	0.7996	17.0668	18.7913	22.5668
9 d	$y = 3.1589x + 1.1263$	0.9888	10.3019	16.8376	42.8666
10 d	$y = 1.9674x + 2.9987$	0.1492	4.7277	10.4047	46.6501

The tables above (Table 2 and 3) indicate the lowest lethal dose 25 (LD₂₅), lethal dose 50 (LD₅₀) and lethal dose 90 (LD₉₀) values for the contact application method are observed with the aqueous extract of *Oudneya africana*, followed by the methanolic extract of the same plant.

Based on the lowest LD₂₅ values, the aqueous extract showed a minimum LD₂₅ of 6.8191 mg/ml on day 10 (Table 2), while the methanolic extract reached its lowest LD₂₅ of 4.7277 mg/ml on the same day (Table 3).

When considering LD₅₀ values, the aqueous extract again demonstrated a lower toxicity threshold, with the minimum LD₅₀ recorded at 4.2315 mg/ml on day 9 (Table 2). In comparison, the methanolic extract exhibited a higher LD₅₀ value, with its lowest being 10.4047 mg/ml on day 10 (Table 3).

Similarly, analysis of LD₉₀ values revealed that the aqueous extract had a significantly lower LD₉₀ of 0.4566 mg/ml on day 9 (Table 2), whereas the methanolic extract's lowest LD₉₀ was 22.5668 mg/ml, observed on day 8 (Table 3).

Since lower LD₅₀ and LD₉₀ values are indicative of higher toxicity, these results clearly suggest that the aqueous extract exhibits greater toxic potential than the methanolic extract under contact application conditions.

The following figures represent the established correlations.

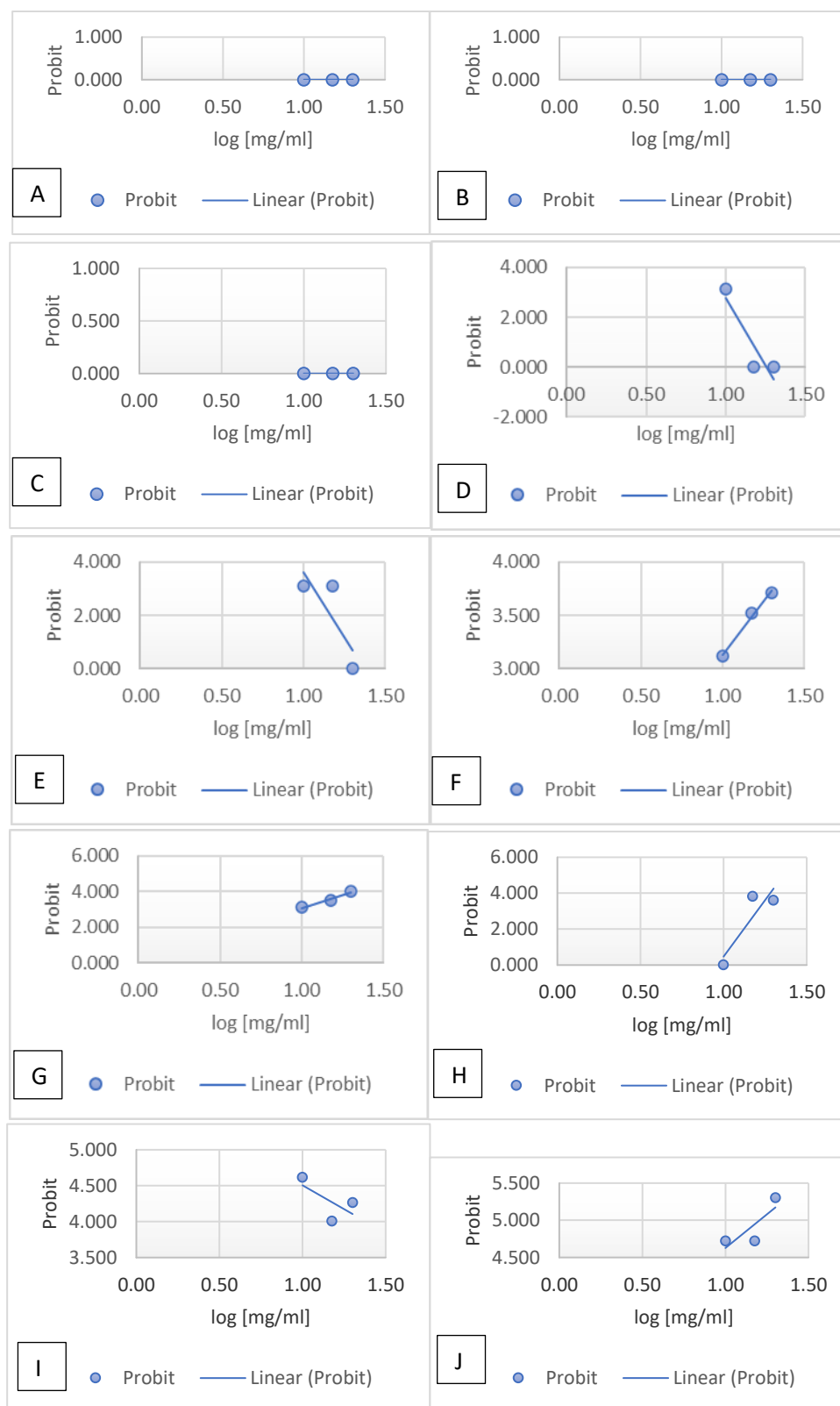


Figure 20: Correlation established for the aqueous extract applied by contact. (A : 1d, B : 2d, C : 3d, D : 4d, E : 5d, F : 6d, G : 7d, H : 8d, I : 9d, J : 10d).

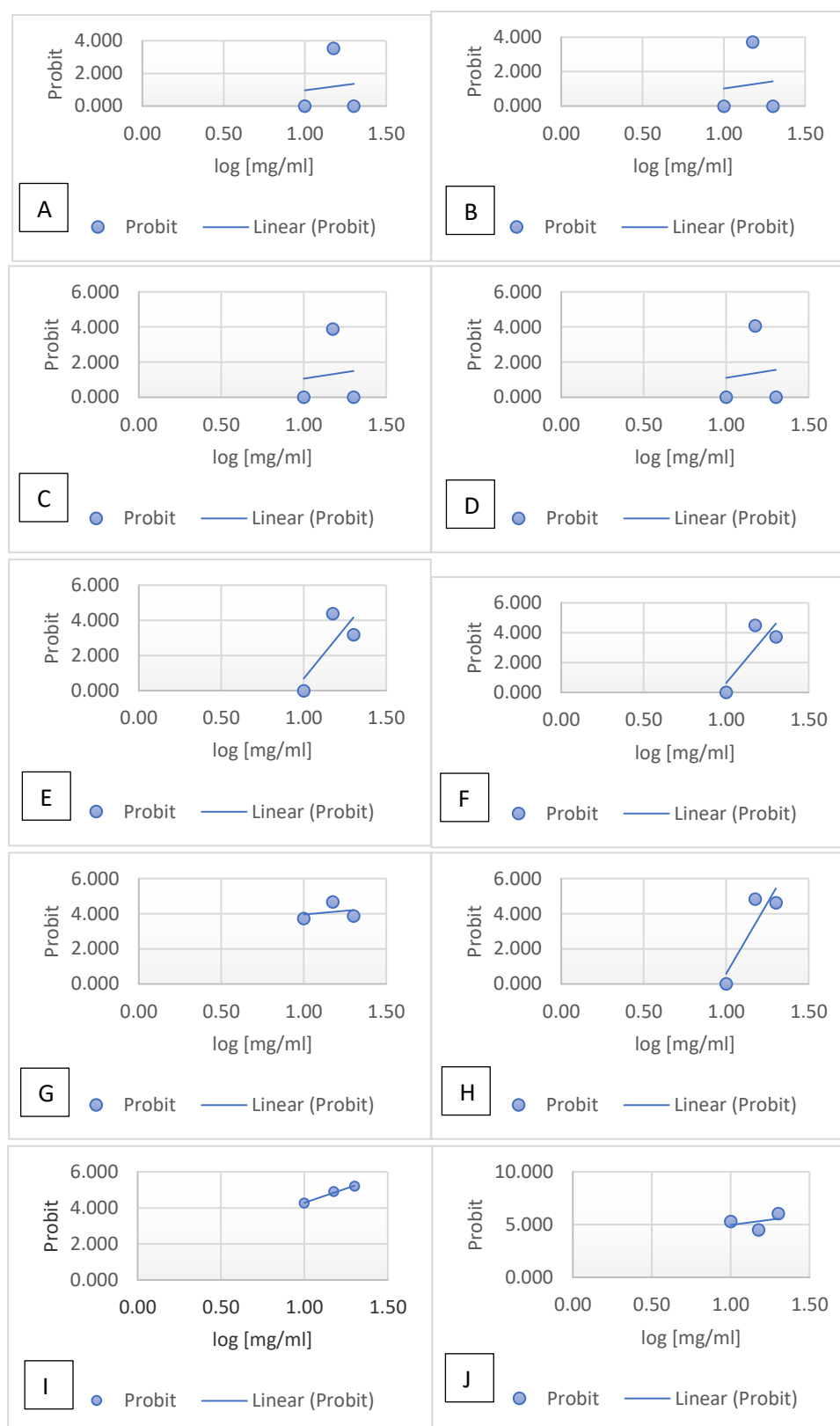


Figure 21: Correlation established for the methanolic extract applied by contact. (A : 1d, B : 2d, C : 3d, D : 4d, E : 5d, F : 6d, G : 7d, H : 8d, I : 9d, J : 10d).

Table 4 : Lethal dose values of aqueous extract applied by ingestion

Exposure time	Equation	R ²	LD ₂₅ [mg/ml]	LD ₅₀ [mg/ml]	LD ₉₀ [mg/ml]
1 d	$y = 9.681x - 10.181$	0.6611	31.5139	36.9933	50.1819
2 d	$y = 9.681x - 10.181$	0.6611	0.2798	0.3285	0.4456
3 d	$y = 9.681x - 10.181$	0.6611	31.5139	36.9933	50.1819
4 d	$y = 10.844x - 10.489$	0.8294	23.2377	26.8131	35.2022
5 d	$y = 10.844x - 10.489$	0.8294	23.2377	26.8131	35.2022
6 d	$y = 10.995x - 10.529$	0.7436	22.4424	25.8446	33.8040
7 d	$y = 10.995x - 10.529$	0.7436	22.4424	25.8446	33.8040
8 d	$y = -11.296x + 14.175$	0.8294	7.4456	6.4898	4.9974
9 d	$y = -11.296x + 14.175$	0.8294	7.4456	6.4898	4.9974
10 d	$y = -2.1671x + 4.7694$	0.028	1.6018	0.7827	0.2005

Table 5 : Lethal dose values of methanolic extract applied by ingestion

Exposure time	Equation	R ²	LD ₂₅ [mg/ml]	LD ₅₀ [mg/ml]	LD ₉₀ [mg/ml]
1 d	$y = 9.681x - 10.181$	0.6611	31.5139	36.9933	50.1819
2 d	$y = 1.8592x + 1.1637$	0.6611	50.2219	115.7216	566.1849
3 d	$y = 2.0102x + 1.1238$	0.9895	39.1720	84.7746	368.1403
4 d	$y = 2.4106x + 0.7027$	0.9989	31.8471	60.6273	206.2937
5 d	$y = 2.4106x + 0.7027$	0.9989	31.8471	60.6273	206.2937
6 d	$y = 1.0026x + 2.4697$	0.6611	71.0372	333.9937	6344.7385
7 d	$y = 1.5582x + 1.8854$	0.6611	36.8379	99.7344	663.1152
8 d	$y = 13.117x - 12.688$	0.8294	19.8196	22.3089	27.9393
9 d	$y = 13.628x - 13.183$	0.8294	19.2655	21.5893	26.8108
10 d	$y = 3.4401x - 0.0529$	0.7632	18.7456	29.4323	69.4204

The tables above (Table 4 and 5) indicate the lowest lethal dose 25 (LD₂₅), lethal dose 50 (LD₅₀), and lethal dose 90 (LD₉₀) for the ingestion application method are observed with the aqueous extract of *Oudneya africana*, which was followed by the methanolic extract of the plant.

Based on the lowest LD₂₅ values, the aqueous extract administered via ingestion exhibited the highest toxicity, with a minimum LD₂₅ of 0.2798 mg/ml observed on day 2 (Table 4). In contrast, the methanolic extract showed a much higher minimum LD₂₅ of 18.7456 mg/ml, recorded on day 10 (Table 5).

Regarding LD₅₀ values, the aqueous extract again demonstrated greater potency, with the lowest LD₅₀ recorded at 0.3285 mg/ml on day 2 (Table 4). The methanolic extract, on the other hand, showed its lowest LD₅₀ at 21.5893 mg/ml on day 9 (Table 5).

Similarly, analysis of LD₉₀ values confirmed this trend. The aqueous extract had a minimum LD₉₀ of 0.2005 mg/ml on day 10 (Table 4), whereas the methanolic extract's lowest LD₉₀ was significantly higher, at 26.8108 mg/ml on day 9 (Table 5).

Since lower LD₂₅, LD₅₀, and LD₉₀ values reflect greater toxicity, these results clearly indicate that the aqueous extract, when applied via ingestion, is significantly more toxic than the methanolic extract under the same mode of application.

The following figures represent the established correlations.

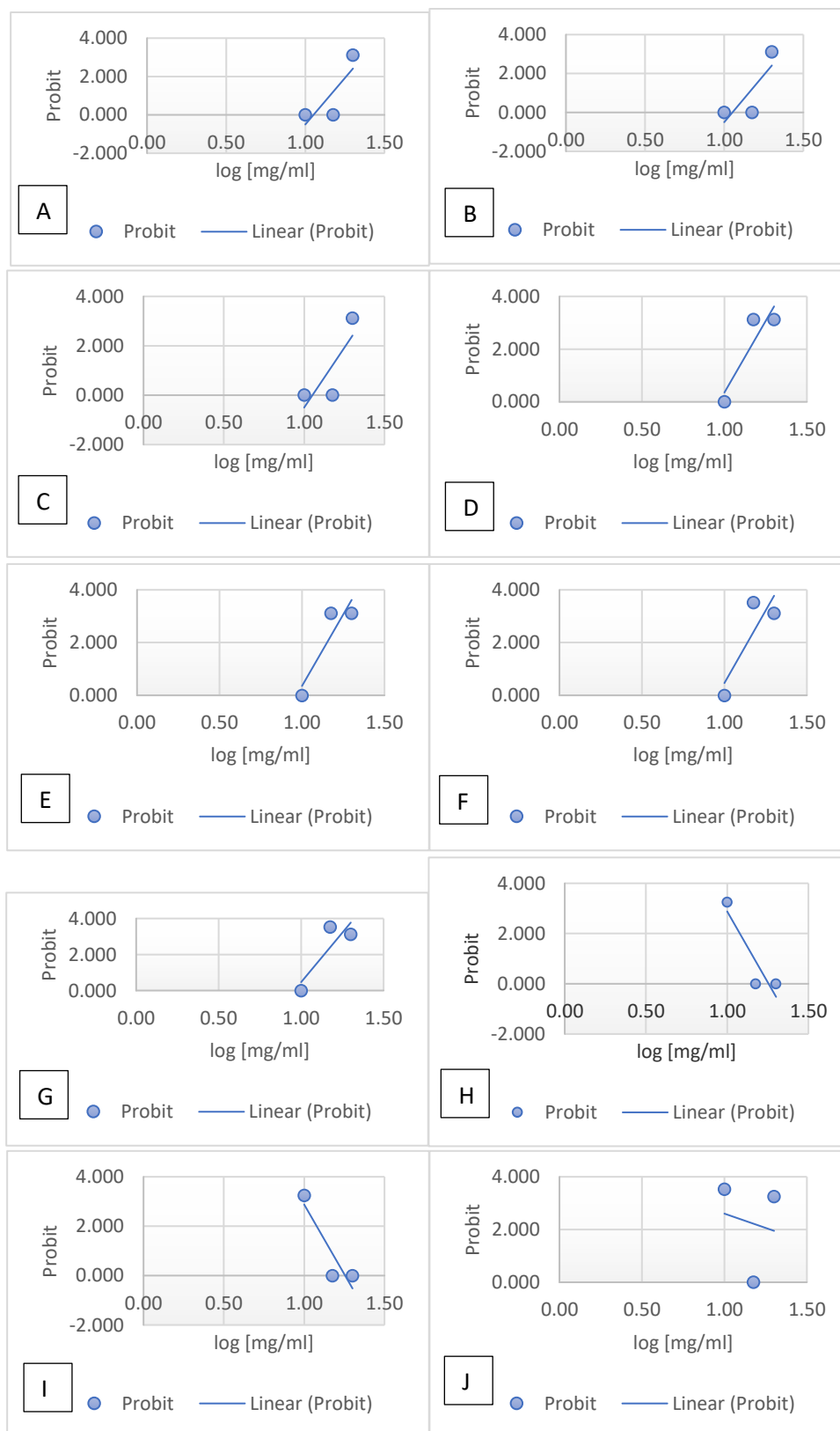


Figure 22: Correlation established for the aqueous extract applied by ingestion. (A : 1d, B : 2d, C : 3d, D : 4d, E : 5d, F : 6d, G : 7d, H : 8d, I : 9d, J : 10d).

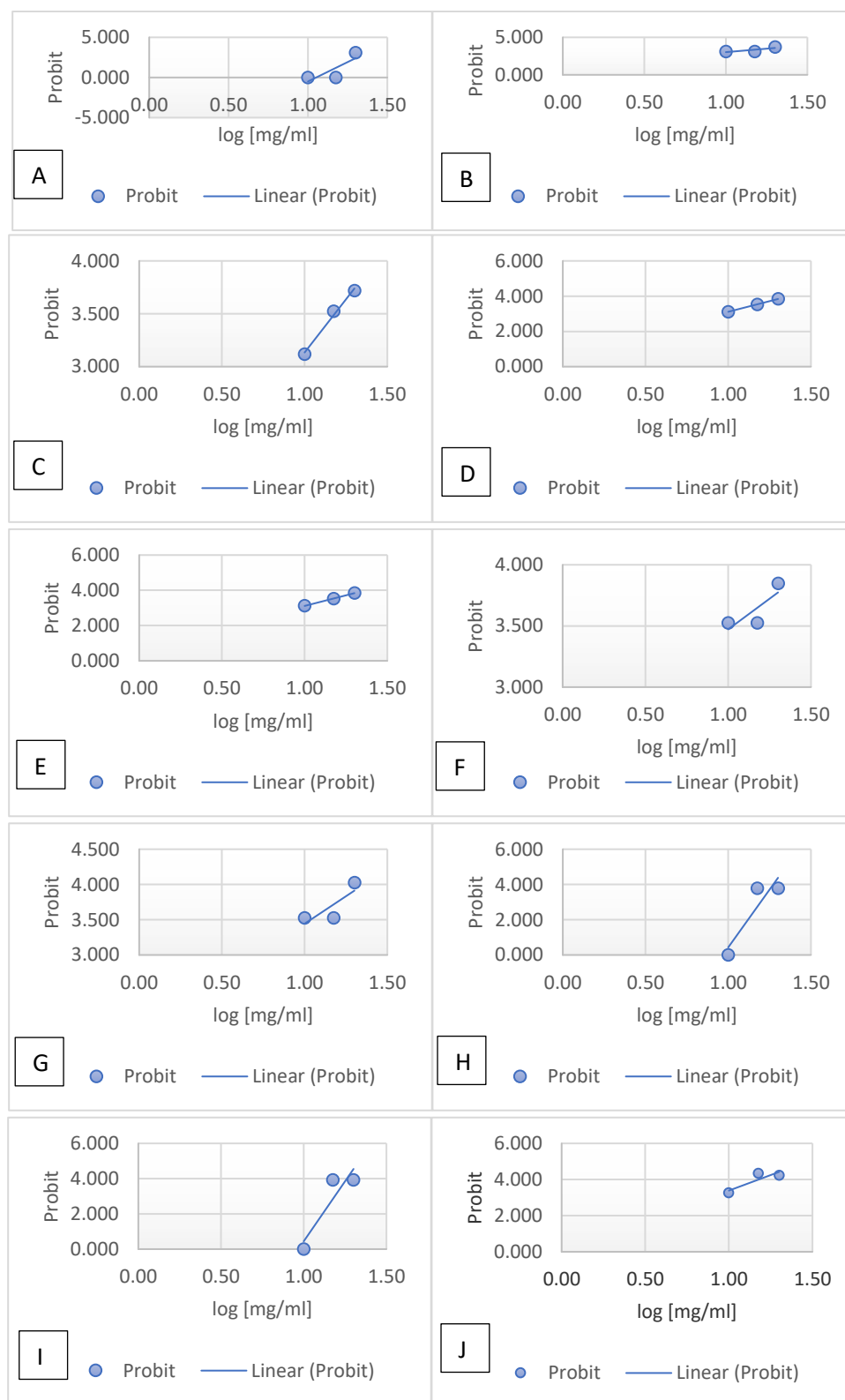


Figure 23: Correlation established for the methanolic extract applied by ingestion. (A : 1d, B : 2d, C : 3d, D : 4d, E : 5d, F : 6d, G : 7d, H : 8d, I : 9d, J : 10d).

1.4. Lethal times

Table 6 : Lethal time values of aqueous extract applied by contact

Applied Dose	Equation	R ²	LT ₂₅ [d]	LT ₅₀ [d]	LT ₉₀ [d]
20 mg/ml	$y = 1.4095x + 1.3022$	0.4622	139.7332	420.2266	3412.1326
40 mg/ml	$y = 1.5409x + 1.3532$	0.5273	84.9693	232.6317	1579.9747
60 mg/ml	$y = 1.4684x + 1.2442$	0.4265	125.5298	361.2018	2696.5535

Table 7 : Lethal time values of methanolic extract applied by contact

Applied Dose	Equation	R ²	LT ₂₅ [d]	LT ₅₀ [d]	LT ₉₀ [d]
20 mg/ml	$y = 0.9726x + 0.7881$	0.2365	4340.7963	21407.0720	445321.4949
40 mg/ml	$y = 2.2459x + 2.5847$	0.8709	5.9611	11.8967	44.2837
60 mg/ml	$y = 1.8258x + 1.5856$	0.5149	31.6906	74.1454	373.4585

The results obtained in (Table 6 and 7) show that (LT₂₅), (LT₅₀), (LT₉₀) values for the contact application method are generally observed with the methanolic extract of *Oudneya africana*, indicating its higher overall toxicity (faster action) compared to the aqueous extract. Based on the lowest LT₂₅ values:

In terms of LT₂₅ values, the methanolic extract demonstrated significantly higher toxicity under contact application, with the lowest LT₂₅ recorded at 5.9611 days at a concentration of 40 mg/ml (Table 7). In comparison, the aqueous extract showed a much higher LT₂₅ of 84.9693 days at the same concentration (Table 6).

For LT₅₀ values, the trend remained consistent. The methanolic extract exhibited a minimum LT₅₀ of 11.8967 days (Table 7), while the aqueous extract required a substantially longer period to reach 50% mortality, with the lowest LT₅₀ measured at 232.6317 days (Table 6).

Similarly, the LT₉₀ values further highlight the superior toxic efficiency of the methanolic extract. Its lowest LT₉₀ was 44.2837 days at 40 mg/ml (Table 7), whereas the

aqueous extract had a markedly higher LT_{90} of 1579.9747 days at the same concentration (Table 6).

As lower LT_{25} , LT_{50} , and LT_{90} values indicate faster action and greater toxicity, these results clearly demonstrate that the methanolic extract, when applied via contact, is significantly more toxic than the aqueous extract in terms of lethal time.

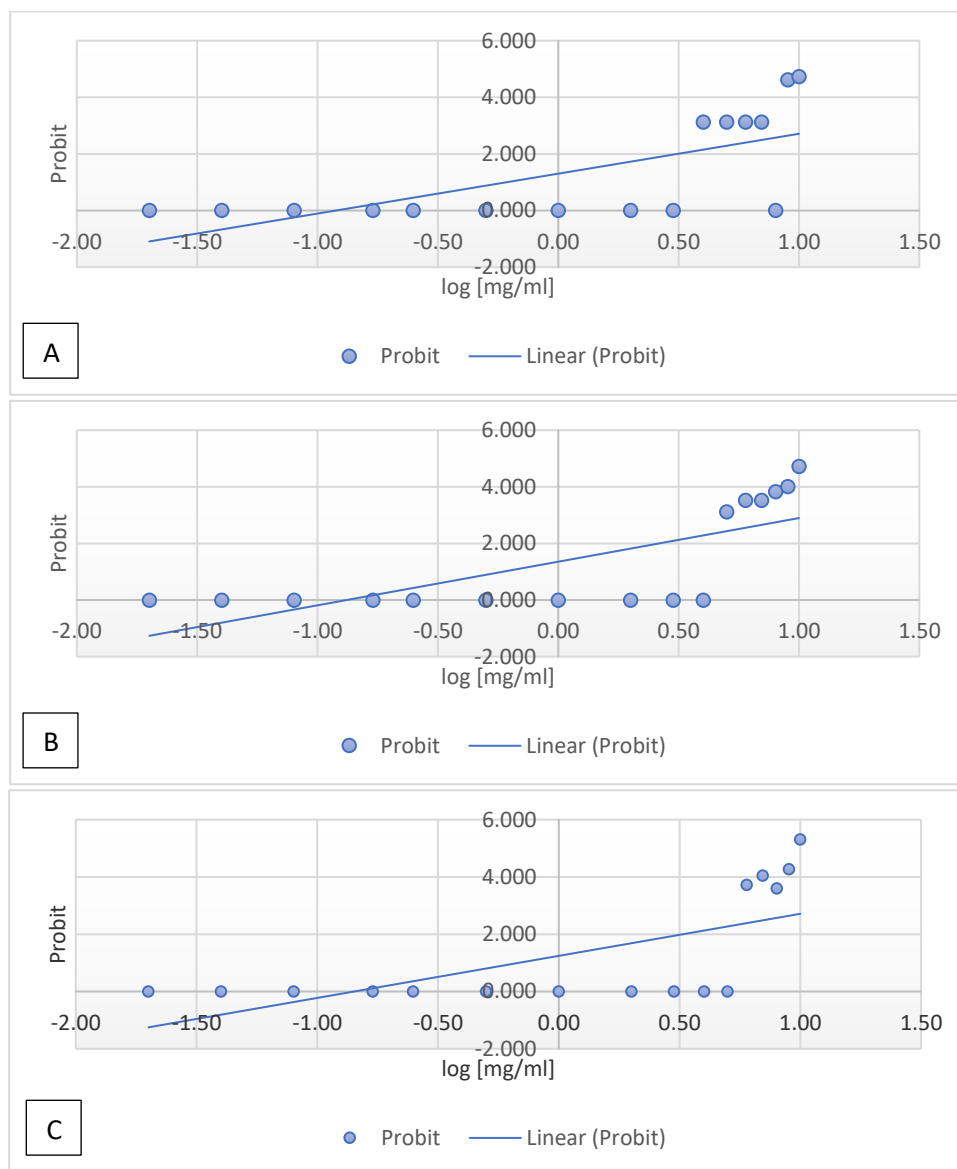


Figure 24: Correlation established for lethal times of aqueous extract applied by contact. (A: 20 mg/ml, B: 40 mg/ml, C: 60 mg/ml).

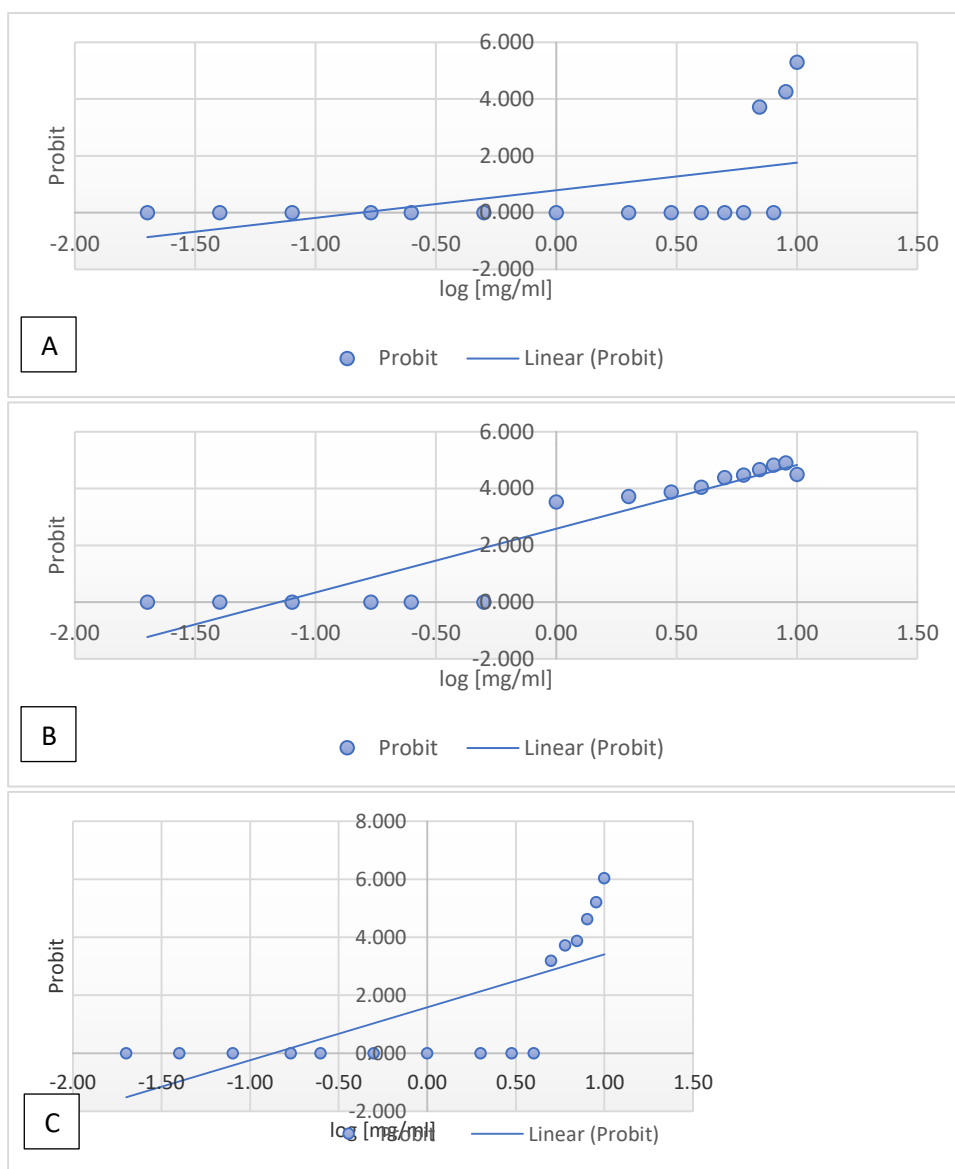


Figure 24: Correlation established for the lethal times of methanolic extract applied by contact. (A : 20 mg/ml, B : 40 mg/ml, C : 60 mg/ml).

Table 8 : Lethal time values of aqueous extract applied by ingestion

Dose applied	Equation	R ²	LT ₂₅ [d]	LT ₅₀ [d]	LT ₉₀ [d]
20 mg/ml	$y = 0.9735x + 0.8044$	0.3374	4144.4105	20408.447 3	423357.98 51
40 mg/ml	$y = 0.7497x + 0.7979$	0.2074	50817.786 0	402755.99 80	20656624. 6035
60 mg/ml	$y = 1.117x + 1.5192$	0.3893	325.6883	1306.7849	18362.052 2

Table 9 : Lethal time values of methanolic extract applied by ingestion

Dose applied	Equation	R ²	LT ₂₅ [d]	LT ₅₀ [d]	LT ₉₀ [d]
20 mg/ml	$y = 1.1787x + 1.3722$	0.4071	320.6130	1196.1829	14636.475 1
40 mg/ml	$y = 1.8722x + 1.9663$	0.8107	18.2133	41.7247	201.9059
60 mg/ml	$y = 1.9571x + 2.2929$	0.8547	10.9354	24.1669	109.2121

The results obtained in (Table 8 and 9) show that (LT₂₅), (LT₅₀), and (LT₉₀) values for the ingestion application method are generally observed with the methanolic extract of *Oudneya africana*, indicating its higher overall toxicity (faster action) compared to the aqueous extract.

Based on the lowest LT₂₅ values, the methanolic extract demonstrated markedly higher toxicity when administered via ingestion, with the lowest LT₂₅ recorded at 10.9354 days at a concentration of 60 mg/ml (Table 9). In contrast, the aqueous extract showed a considerably higher LT₂₅ of 325.6883 days at the same concentration (Table 8).

Looking at the LT₅₀ values, the methanolic extract again exhibited faster lethality, with a minimum LT₅₀ of 24.1669 days (Table 9). The aqueous extract, however, required much more time to reach the same mortality level, with a LT₅₀ of 1306.7849 days (Table 8).

This pattern is further supported by the LT₉₀ data. The methanolic extract reached its lowest LT₉₀ at 109.2121 days (Table 9), whereas the aqueous extract showed an extremely prolonged LT₉₀ of 18,362.0522 days at 60 mg/ml (Table 8).

Since lower LT₂₅, LT₅₀, and LT₉₀ values reflect faster mortality and therefore greater toxicity, these results clearly indicate that the methanolic extract, when applied via ingestion,

is significantly more toxic than the aqueous extract in terms of lethal time.

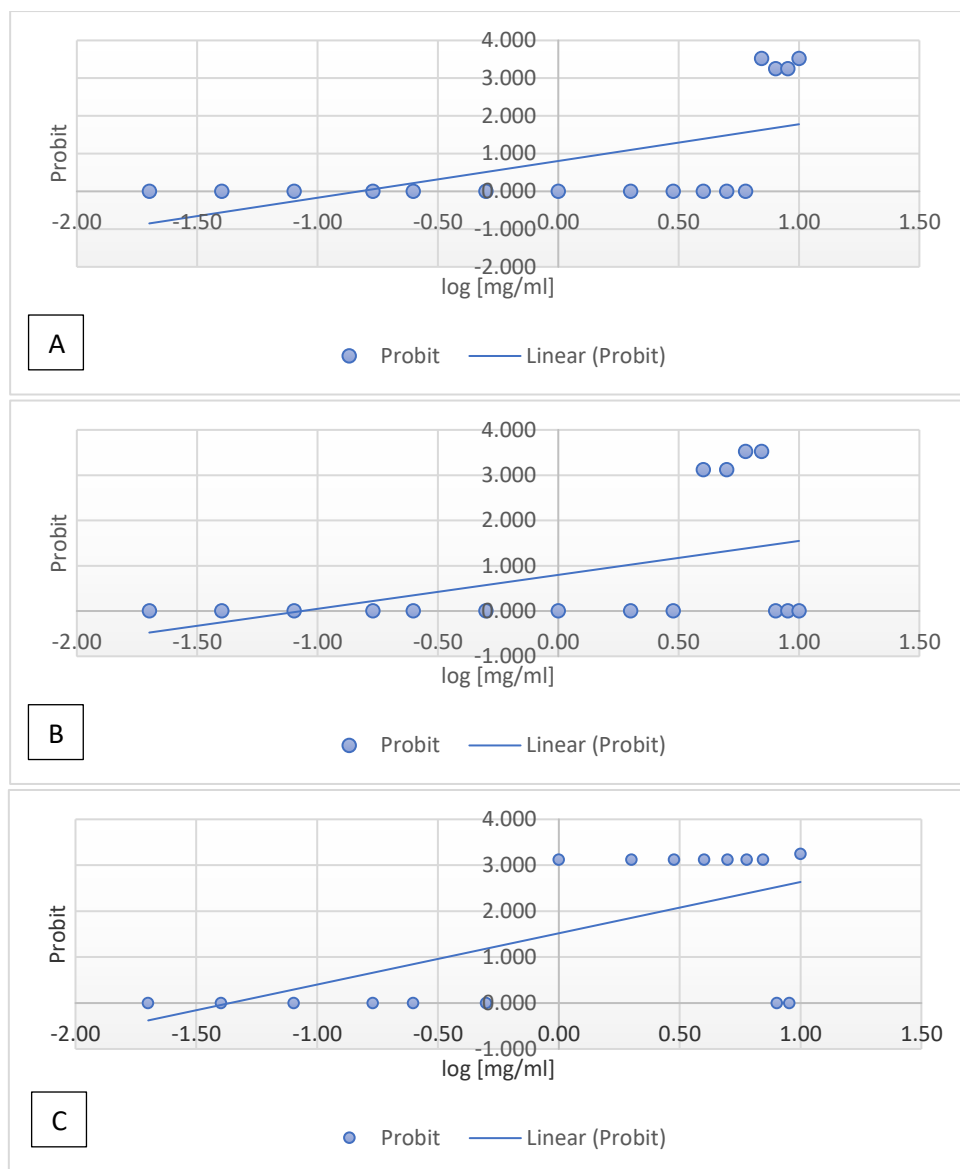


Figure 25: Correlation established for the lethal times of aqueous extract applied by ingestion. (A : 20 mg/ml, B : 40 mg/ml, C : 60 mg/ml).

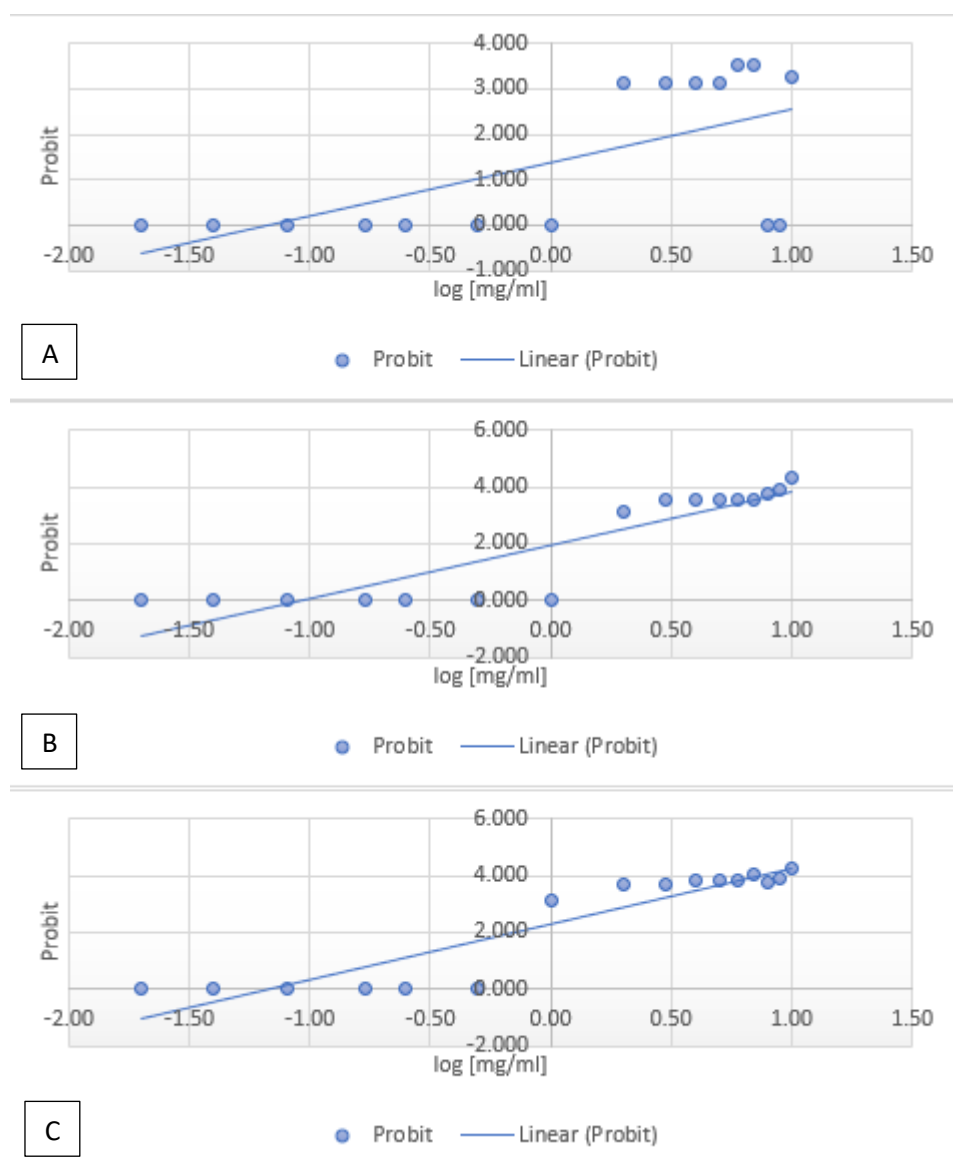


Figure 26: Correlation established for the lethal times of methanolic extract applied by ingestion. (A : 20 mg/ml, B : 40 mg/ml, C : 60 mg/ml).

The effectiveness of each organic extract was demonstrated through a dual assessment of its lethal dose (LD_{50} and LD_{90}) and lethal time (LT_{50} and LT_{90}) values.

For lethal dose (LD) values applied by contact, the aqueous extract demonstrated higher toxicity than the methanolic extract, as evidenced by its lower LD_{50} and LD_{90} values. Same goes for ingestion use, the aqueous extract was significantly more toxic, showing lower LD_{25} , LD_{50} , and LD_{90} values compared to the methanolic extract.

When considering lethal time (LT) values for contact application, the methanolic extract proved to be significantly more toxic (acting faster) than the aqueous extract, indicated by its lower LT₂₅, LT₅₀, and LT₉₀ values. This was consistent for ingestion use as well, with the methanolic extract demonstrating significantly higher toxicity (faster time to cause mortality) due to its lower LT₂₅, LT₅₀, and LT₉₀ values compared to the aqueous extract.

Several scientific papers consistently demonstrate the strong insecticidal potency of both aqueous and ethanolic plant extracts against the red flour beetle, *Tribolium castaneum*, offering a compelling alternative to chemical pesticides in a natural approach. Research by **Jameel et al. (2019)** introduced ethanolic and aqueous *Syzygium aromaticum* bud extracts as potent, with LD₅₀ values of 13.52 mg/kg and 20.47 mg/kg respectively, after 72 hours of external application, **Abdelgaleil et al. (2019)** showed *Ruta graveolens* ethanolic extract to be 401.78 mg/kg. The findings are supported by other researchers, with **Onuoha et al. (2020)** recording ethanolic *Cassia occidentalis* leaf extract to have a LD₅₀ and LD₉₀ of 2.45 µg/adult and 4.70 µg/adult respectively, **Akolade et al. (2017)** indicating *Vernonia amygdalina* ethanolic leaf extract's LD₅₀ of 0.50 mg/cm² as residual, respectively, and **Naim (2014)** indicating *Datura stramonium* ethanolic extract's LD₅₀ and LD₉₀ as 31.25 µg/cm² and 62.50 µg/cm² using filter paper impregnation. For aqueous extracts, while specific LD values may not always be indicated, experiments always indicate high mortality respectively, **Ahmed et al. (2014)** indicated 73.33% mortality against *Momordica charantia* leaf extract at 5% concentration respectively, and **Aregbesola et al. (2020)** recorded 80% mortality against *Ricinus communis* leaf extract at 10%, demonstrating extensive effectiveness, and with *Azadirachta indica* and *Artemisia annua* further displaying extensive insecticidal activity in proportion to concentration and duration of exposure, respectively (**Khattak et al., 2018; Ebadollahi, 2013**).

More studies have demonstrated the efficacy of botanical insecticides against the red flour beetle *Tribolium castaneum*. But for ethanolic and aqueous extracts of clove *Syzygium aromaticum* recorded LD₅₀ and LD₉₀ values ranging from 13.52 to 55.45 mg/kg (**Jameel et al., 2019**), respectively. the ethanolic extract of rue *Ruta graveolens* produced a LD₅₀ of 401.78 mg/kg (**Abdelgaleil et al., 2019**). Other extracts have also been effective, the ethanolic coffee senna *Cassia occidentalis* extract having LD₅₀ and LD₉₀ values of 2.45 and 4.70 µg/adult (**Onuoha et al., 2020**), respectively, and bitter leaf *Vernonia amygdalina* ethanolic extract having LD₅₀ and LD₉₀ values of 0.50 1.63 mg/cm² (**Akolade et al., 2017**), respectively. Jimsonweed *Datura*

stramonium ethanolic extract also had LD₅₀ and LD₉₀ values of 31.25 and 62.50 µg/cm² (Naim, 2014), respectively. Validating the wide capacity of these plant extracts to control this pest.

CONCLUSION

Conclusion

This study presents the serious problem caused by *Tribolium castaneum* as a widespread pest infesting stored products in Algeria. the dependence on chemical control measures is not effective because the insect quickly becomes resistant. This highlights the great need for safe alternatives such as biological control. The present in-vitro lab experiment was concerned with establishing the efficacy of Algerian Saharan plant *Oudneya africana* aqueous and methanolic organic extracts towards their insecticidal potential. The toxic effect of the extracts was measured by the Lethal Dose (LD) and Lethal Time (LT) determinations using two different routes of application: ingestion and contact.

The overall results were that the extraction yields were 6.315% for the aqueous extract and 3.5% for the methanolic extract. Generally, the extracts exhibited insecticidal activity in a variable manner, with mortality increasing alongside increasing concentration and exposure duration.

Under ingestion method the Lethal Dose LD the aqueous extract was significantly more toxic in terms of dose, with lower LD₂₅ values of 0.2798 mg/ml, LD₅₀ of 0.3285 mg/ml, and LD₉₀ of 0.2005 mg/ml. The methanolic extract, however, possessed LD₂₅ values of 18.7456 mg/ml, LD₅₀ of 21.5893 mg/ml, and LD₉₀ of 26.8108 mg/ml. This shows the aqueous extract is more potent in terms of dose for ingestion, requiring much lower concentrations to achieve the same rate of mortality.

Lethal Time (LT): Despite its higher dose potency, the methanolic extract showed a faster action. It recorded significantly lower LT₂₅ values of 10.9354, LT₅₀ of 24.1669d, and LT₉₀ of 109.2121d (at 60 mg/ml). The aqueous extract, conversely, recorded LT₂₅ values of 325.6883d, LT₅₀ of 1306.7849d, and LT₉₀ of 18362.0522d (at 60 mg/ml). This indicates the methanolic extract is faster acting via ingestion.

In Contact Method Lethal Dose (LD) The aqueous extract generally exhibited higher dose toxicity, especially at higher mortality rates, with LD₂₅ values of 6.8191 mg/ml, LD₅₀ of 4.2315 mg/ml, and LD₉₀ of 0.4566 mg/ml. The methanolic extract, on the other hand, recorded LD₂₅ values of 4.7277 mg/ml, LD₅₀ is 10.4047 mg/ml, and LD₉₀ was 22.5668 mg/ml. This suggests the aqueous extract is more potent in terms of dose for contact, despite the methanolic extract having a slightly lower LD₂₅.

Lethal Time (LT) The methanolic extract demonstrated significantly faster action via contact. It recorded lower LT_{25} values of 5.9611d, LT_{50} of 11.8967d, and LT_{90} of 44.2837d (at 40 mg/ml). In contrast, the aqueous extract recorded LT_{25} values of 84.9693d, LT_{50} of 232.6317d, and LT_{90} of 1579.9747d (at 40 mg/ml). This shows the methanolic extract is faster acting in the method of contact.

This work confirms that insecticides derived from plant molecules are effective and hold high potential, advising for the use of natural active substances of plant origin, these are considered less polluting, less harmful than chemical pesticides, and more sustainable.

The study highlights that both extracts possess insecticidal properties, but with varying mechanisms and speeds depending on the application method, this recommends the importance of choosing the most suitable extract based on the objective dose efficiency versus speed of action.

Conducting in-depth analysis and studies of the chemical groups responsible for the insecticidal activity in both extracts to identify the active compounds, along with a comprehensive chemical examination to accurately determine their components.

Performing additional studies to assess the toxicity of these extracts against other insect species beyond *Tribolium castaneum*, particularly the methanolic extract, which demonstrated rapid mortality in both application methods.

Verifying these results under realistic (in vivo) conditions to evaluate their effectiveness on a broader scale.

Study of the toxicity of the extracts used in this study against other insect species

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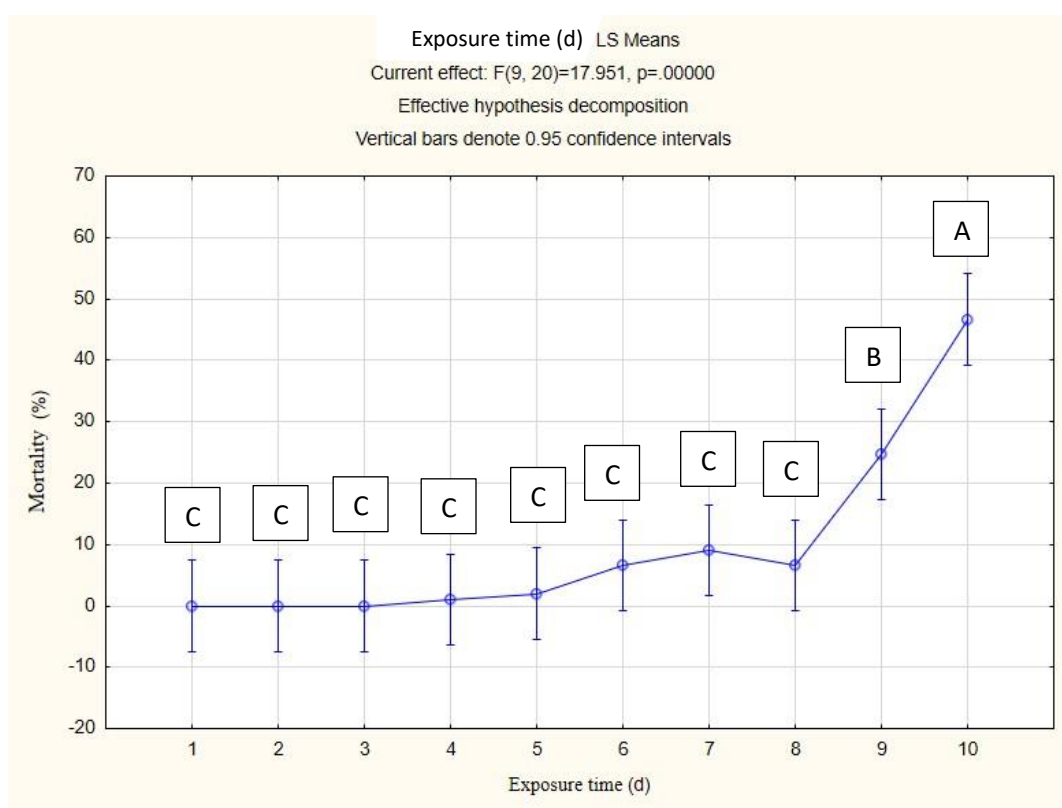
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APPENDICES

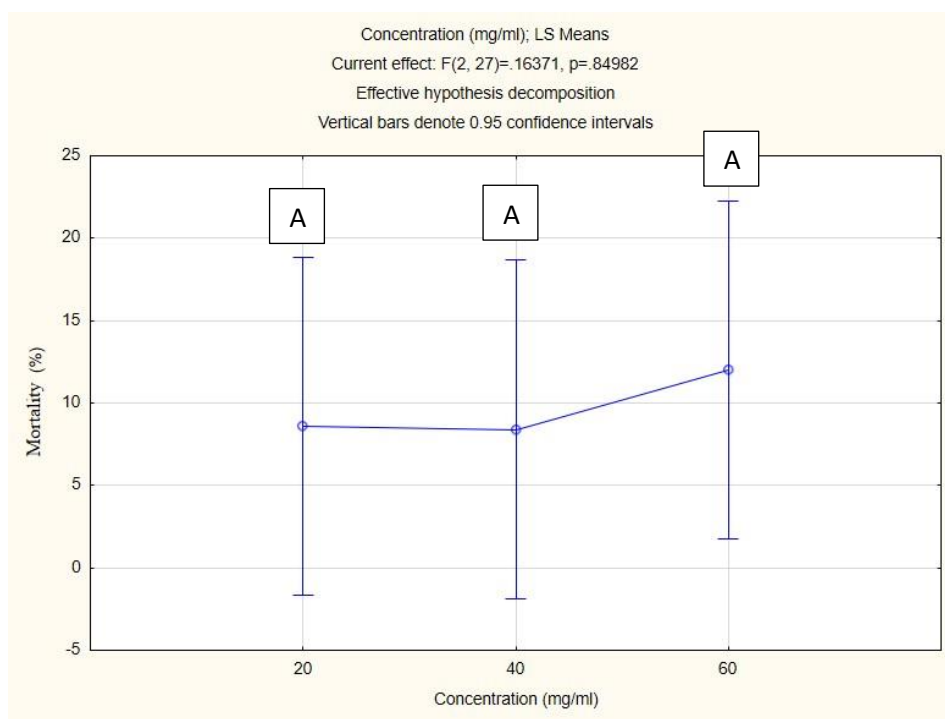
Appendices:

ANOVA test

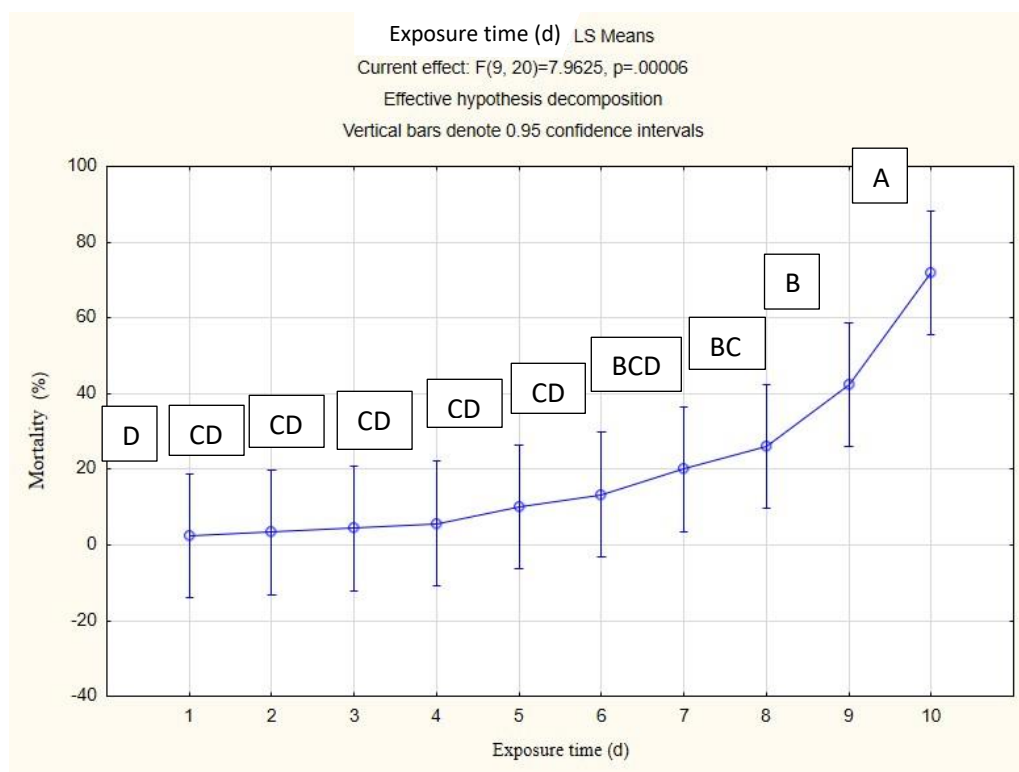
The ANOVA test was performed to analyze the differences in average mortality based on concentrations and exposure durations, using various extracts applied through different techniques. The results presented in the following figures indicate a highly significant difference between exposure times, with p-values well below the significance threshold for all treatments. In contrast, no significant difference was observed with respect to concentrations for the majority of extracts and techniques. A notable exception is the methanolic extract, for which a significant difference was detected based on concentrations, but not on exposure time.



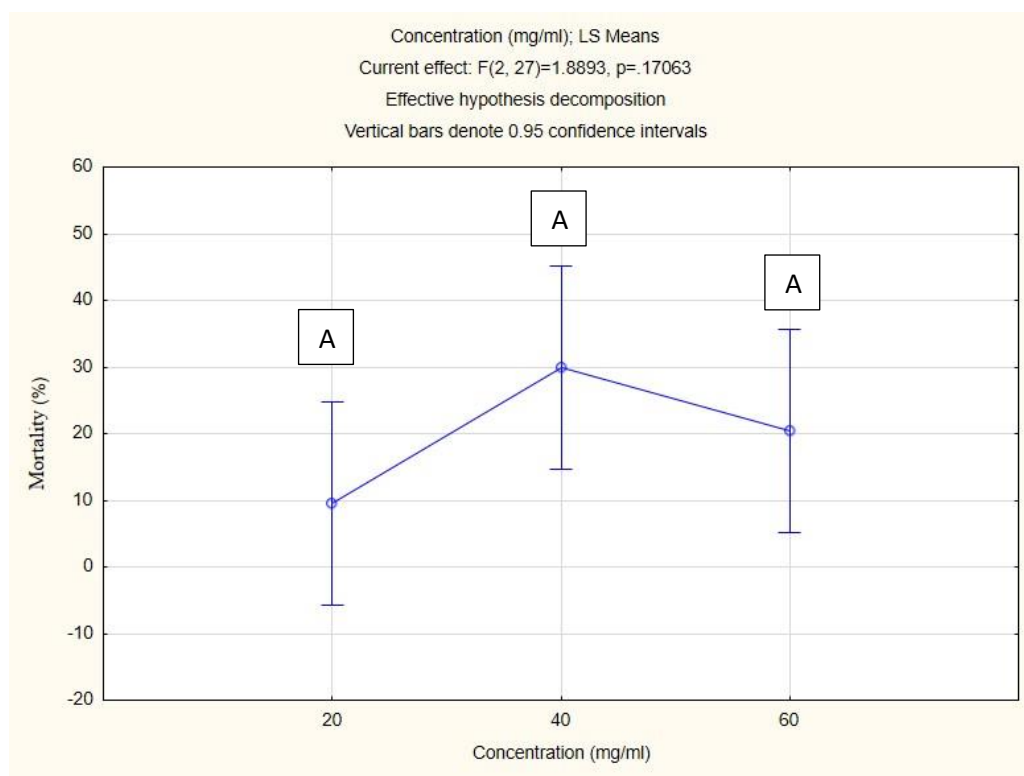
Annex 01: Interval graph of the ANOVA test for exposure durations using aqueous extract applied by contact



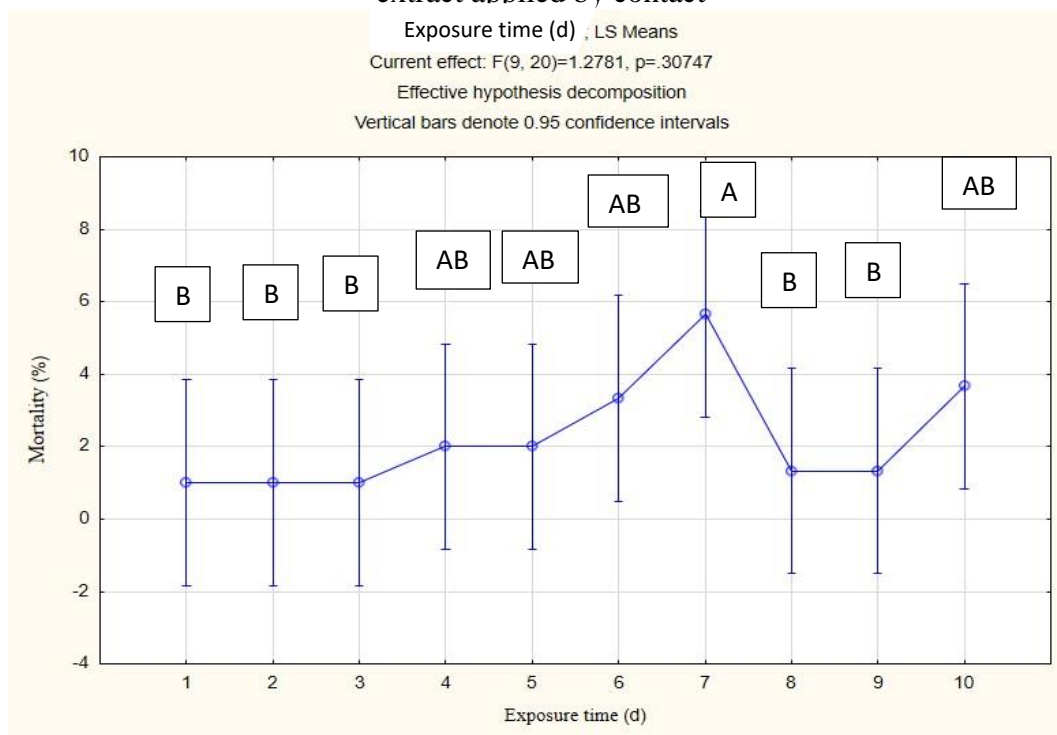
Annex 02: Interval graph of the ANOVA test for concentrations using aqueous extract applied by contact



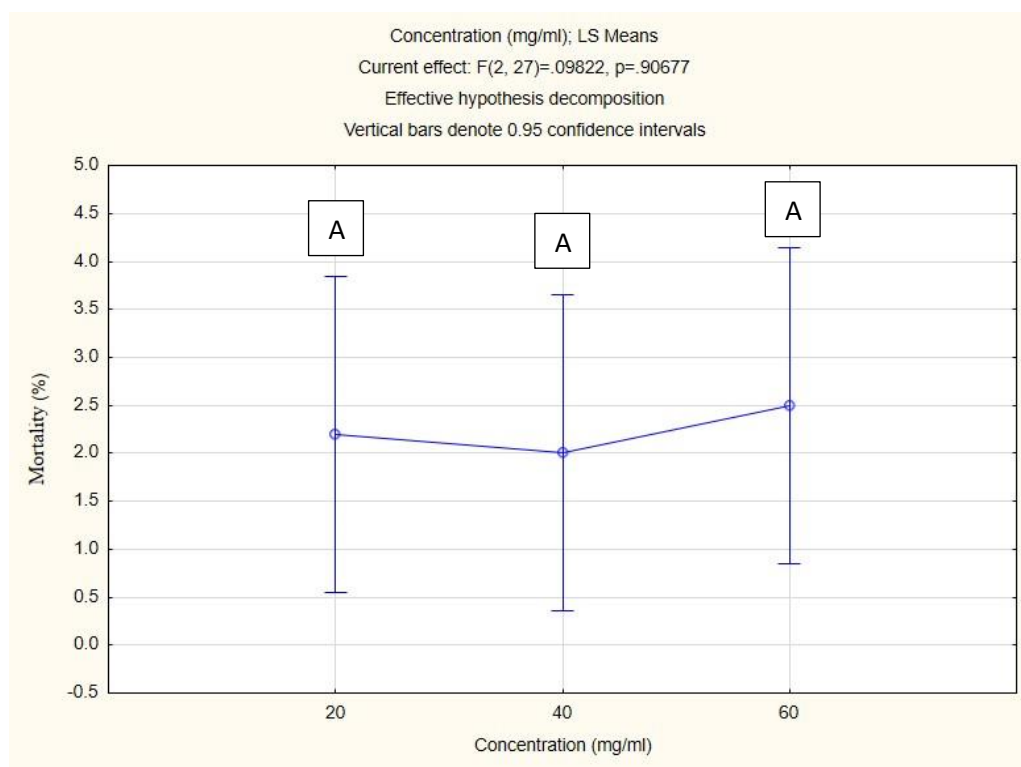
Annex 03: Interval graph of the ANOVA test for exposure durations using methanolic extract applied by contact



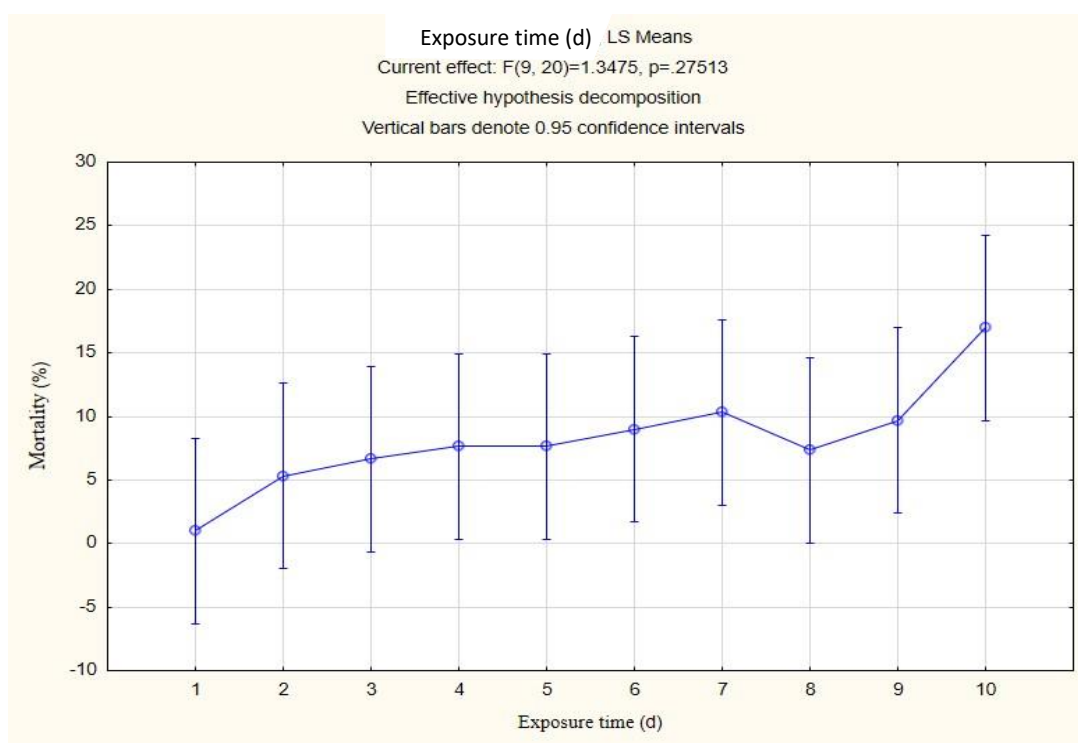
Annex 04: Interval graph of the ANOVA test for concentration using methanolic extract applied by contact



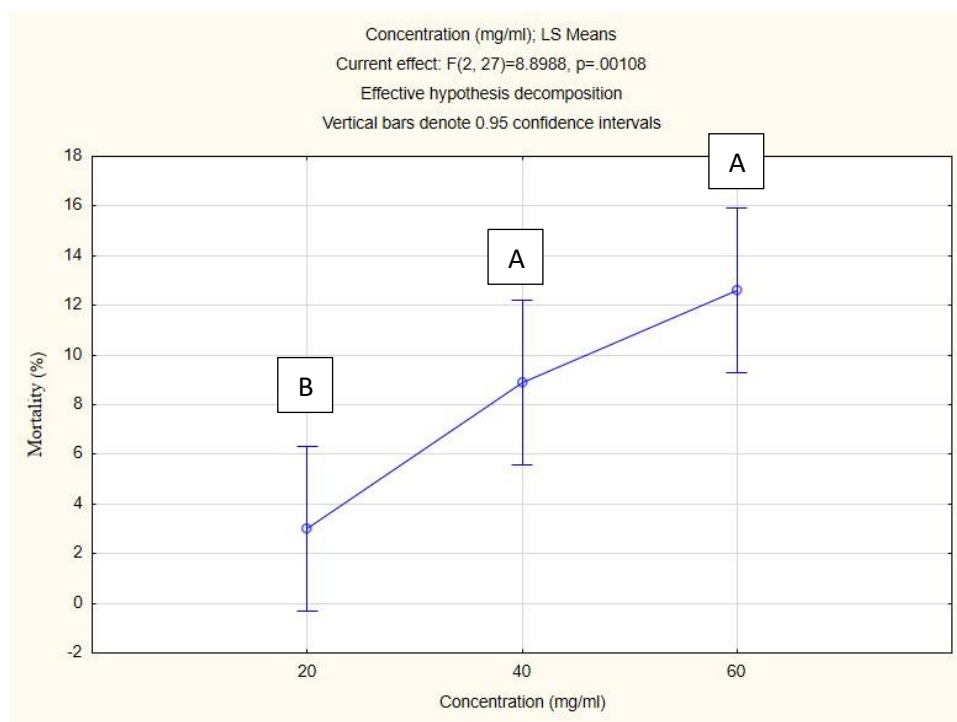
Annex 05: Interval graph of the ANOVA test for exposure durations using aqueous extract administered by ingestion



Annex 06: Interval graph of the ANOVA test for concentration using aqueous extract administered by ingestion



Annex 07: Interval graph of the ANOVA test for exposure durations using methanolic extract administered by ingestion



Annex 08: Interval graph of the ANOVA test for concentration using methanolic extract administered by ingestion

Evaluation of the insecticidal activity of *Oudneya africana* from the Ghardaïa region

Summary:

The purpose of this study is to evaluate the insecticidal efficacy of *Oudneya africana* plant extracts from the Ghardaïa region as a natural and safe substitute for chemical pesticides are becoming less effective and have negative effects on the environment and health.

In this study the insecticidal activity of cold maceration extracts against *Tribolium castaneum* was examined using a bioassay. Activity was examined using both the contact and oral methods, and exposure times ranged from 30 minutes to 10 days.

The results revealed that the aqueous and methanolic extracts of *Oudneya africana* were insecticidal against *Tribolium castaneum*. The mortality seemed to increase at higher concentrations and longer exposure periods. The efficacy, however, showed high variability: when using contact application methods, the methanolic extract was more effective and had a quicker action compared to the aqueous extract. When considering ingestion methods, the efficacy of both extracts was relatively poor; however, the methanolic extract was slightly more potent and faster. Generally, the contact application method proved to be better in causing mortality for both extracts.

The findings reveal a significant variation in the toxicological profile of both extracts. The aqueous extract is more potent with regard to the dosage requirement for efficacy, as supported by the lower LD values, especially via ingestion. On the other hand, the methanolic extract has a faster action, as supported by the decreased LT values in contact and ingestion modalities. This suggests that while the aqueous extract might require a smaller concentration to cause mortality among pests, the methanolic extract causes deaths at a faster rate.

The results emphasize the potential of plant extracts in the biological control of *Tribolium*, highlighting an alternate, potentially beneficial way of developing a new and natural insecticide that may prove to be a better alternative to synthetics. However, extensive further studies need to be conducted to determine the individual active compounds that are accountable for this insecticidal activity.

Keywords: *Oudneya Africana*, *Tribolium castaneum*, insecticidal activity, Extract, Ghardaïa

Évaluation de l'activité insecticide d'*Oudneya africana* de la région de Ghardaïa

Résumé :

L'objectif de cette étude est d'évaluer l'efficacité insecticide des extraits de plantes d'*Oudneya africana* de la région de Ghardaïa, comme substitut naturel et sûr aux pesticides chimiques dont l'efficacité diminue et les effets néfastes sur l'environnement et la santé sont importants.

Dans cette étude l'activité insecticide des extraits de macération à froid contre *Tribolium castaneum* a été examinée par bio-essai, l'activité a été évaluée par voie orale et par contact, avec des durées d'exposition allant de 30 minutes à 10 jours.

Les résultats ont révélé que les extraits aqueux et méthanolique d'*Oudneya africana* étaient insecticides contre *Tribolium castaneum*. La mortalité semblait augmenter à des concentrations plus élevées et des périodes d'exposition plus longues. L'efficacité, cependant, présentait une grande variabilité : lors de l'application par contact, l'extrait méthanolique était plus efficace et avait une action plus rapide que l'extrait aqueux. Par ingestion, l'efficacité des deux extraits était relativement faible ; en revanche, l'extrait méthanolique était légèrement plus puissant et plus rapide. Globalement, la méthode d'application par contact s'est avérée plus efficace pour la mortalité des deux extraits.

Les résultats révèlent une variation significative du profil toxicologique des deux extraits. L'extrait aqueux est plus puissant au regard de la dose requise pour son efficacité, comme le confirment les valeurs de DL plus faibles, notamment par ingestion. En revanche, l'extrait méthanolique a une action plus rapide, comme le confirment les valeurs de TL plus faibles lors des applications par contact et par ingestion. Cela suggère que, si l'extrait aqueux pourrait nécessiter une concentration plus faible pour entraîner la mortalité des ravageurs, l'extrait méthanolique provoque des décès plus rapides.

Ces résultats soulignent le potentiel des extraits végétaux dans la lutte biologique contre *Tribolium*, mettant en évidence une voie alternative potentiellement bénéfique pour développer un nouvel insecticide naturel qui pourrait s'avérer une meilleure alternative aux produits de synthèse. Cependant, des études complémentaires approfondies doivent être menées pour déterminer les composés actifs responsables de cette activité insecticide.

Mots clés : *Oudneya Africana*, *Tribolium castaneum*, Activité insecticide, Extrait, Ghardaïa

تقييم النشاط المبيد للحشرات لنبات *الأودنيا الإفريقية* من منطقة غرداية

المخلص:

تهدف هذه الدراسة إلى تقييم فعالية مستخلصات نبات *حنة* /*الابل* في منطقة غرداية كبديل طبيعي وآمن للمبيدات الكيميائية التي أصبحت أقل فعالية ولها آثار سلبية على البيئة والصحة .

في هذه الدراسة، تم فحص النشاط المبيد للحشرات لمستخلصات النقع البارد ضد حشرة *خنفساء الطحين الحمراء* باستخدام اختبار حيوي. تم فحص النشاط باستخدام كل من طريقتي التلامس والفم، وتراوحت أوقات التعرض بين 30 دقيقة و 10 أيام .

أثبتت النتائج فعالية المستخلصات المائية والميثانولية لنباتات *الأسنان الأفريقية* ضد *خنفساء الطحين الحمراء*. وبدأ أن معدل الوفيات يزداد مع التركيز ومدة التعرض. إلا أن الفعالية تفاوتت بشكل كبير: فقد كان المستخلص المائي أكثر فعالية عند التلامس، وكان مفعوله أسرع. كان كلا المستخلصين ضعيفين نسبيًا عند الابتلاع؛ بينما أثبت المستخلص الميثانولي فعالية أكبر وأسرع تأثيرًا. وبشكل عام، تبين أن التلامس أفضل من كلا المستخلصين في إحداث الوفيات .

أظهرت النتائج وجود فروق كبيرة في سمية كلا المستخلصين. بين المستخلص المائي نسبة جرعة إلى تأثير أعلى، وهو ما يظهر من انخفاض قيم DL، وبالأخص عند البلع. في حين أظهر المستخلص الميثانولي بدء تأثير أسرع وهو ما يبدو من انخفاض قيم TL عند اللمس والبلع. باختصار، هذا يعني أن المستخلص المائي قد يحتاج إلى تركيز أقل للقضاء على الآفة، بينما يسبب المستخلص الميثانولي هلاكًا أسرع .

تؤكد النتائج على قدرة المستخلصات النباتية في مكافحة الحبوبية لآفات حشرة *خنفساء الطحين الحمراء*، موحية بنهج بديل قد يكون مجديًا في البحث عن مبيدات حشرية طبيعية جديدة تعتبر بدائل أفضل للمبيدات المصنعة. ولا بد من وجود حاجة إلى دراسات أشمل لعزل المركبات الفعالة الفردية المسؤولة عن هذا التأثير القاتل للحشرات .

الكلمات المفتاحية : *حنة* /*الابل* ، *خنفساء الطحين الحمراء* ، مبيد حشري فعال، مستخلص، غرداية.