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Original Research Article

Larvicidal potential of *Khaya senegalensis* seed oil against *Dermestes maculatus* Degeer

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Abstract

Purpose: Fishes are highly vulnerable to insect pest attacks during processing and storage resulting in physical, financial, and nutritional losses from these infestations. The prevailing tropical climate's humidity encourages the infestation of dermestids. Hence, post-harvest losses due to *Dermestes maculatus* infestation pose a significant challenge in the preservation of smoked-dried fish, necessitating alternative pest control strategies. The study focused on the examination of the insecticidal potential of *Khaya senegalensis* seed oil against the larvae of the hide beetle, *Dermestes maculatus* on smoked-dried African mud catfish, *Clarias gariepinus*.
Methods: Oil was extracted from pulverized seeds of *Khaya senegalensis* using n-hexane by Soxhlet method. The Refractive index, Specific gravity, Acid, Iodine, Peroxide, and Saponification values of the oil were determined by Standard Methods. Parameters assessed were repellency and larval mortality at 24, 48, 72, and 96 hour exposure time. The doses of the seed oil administered were $0.003mLg^{-1}$, $0.009mLg^{-1}$, $0.027mLg^{-1}$, $0.081mLg^{-1}$, and $0.243mLg^{-1}$. Thirty late (5^{th}) instar larvae of *D. maculatus* were exposed to each of the treated fish in triplicate including a control devoid of oil treatment.
Results: The repellent activity of the oil was dose-dependent, with significant repellency observed at concentrations of 0.081 mLg^{-1} and 0.243 mLg^{-1}. Mortality rates of *D. maculatus* larvae increased with oil concentration and exposure duration, with up to 85% mortality recorded at the highest dose after 96 hours ($p<0.05$).
Conclusion: This findings suggest that *K. senegalensis* seed oil has strong potential as a sustainable, plant-based insecticide for protecting stored fish products. Further research should focus on isolating active compounds and assessing field applications.

Keywords: Repellency, Larvicidal, Smoke-dried fish, Dermestes Larvae, Mortality

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INTRODUCTION

It is well known that dried animal skin, fur, fish, meat, and other foods high in protein are destroyed by coleopteran beetles. In many developing nations, the main pests responsible for post-harvest losses of cured fish are blowflies (Diptera: Calliphoridae and

Sarcophagidae) and hide beetles (Coleoptera: Dermestidae and Cleridae).[12, 13] It is impossible to overstate how vulnerable fish are to insect pest attacks during processing and storage. Physical, financial, and nutritional losses could result from these infestations. [12] The tropical climate's humidity encourages the infestation of dermestids, which enter their substrate indoors, look for a mate, mate, create a crack in the substrate, and lay eggs. The larvae that emerge from these situations are typically incredibly destructive and cause a significant decline in the quantity and quality of dried fish.[5, 20]

Smoked-dried fish is an essential protein source in many developing countries, particularly in Africa, where it plays a crucial role in food security and nutrition. However, post-harvest losses due to insect infestations, particularly by *Dermestes maculatus*, significantly reduce the quality and quantity of stored fish. *D. maculatus* larvae bore into dried fish products, causing extensive damage that results in financial losses for small-scale fish processors and traders.[8] Chemical insecticides are commonly used to mitigate these losses, but concerns over environmental contamination, insect resistance, and human health hazards have necessitated the search for safer, eco-friendly alternatives.[7, 15]

Plant-derived oils have gained attention as potential bioinsecticides due to their biodegradability, low toxicity to humans, and efficacy against a wide range of pests. [16] Among these, *Khaya senegalensis* seed oil remains largely underexplored despite the well-documented pesticidal properties of its related species within the Meliaceae family. The plant parts or even the whole plant are themselves utilized in the treatment of illnesses. [10] Marked activity of the stem bark extract of *K. senegalensis* against different isolates of MRSA at the concentration tested has also been reported. [9] While previous studies have focused on neem (*Azadirachta indica*) and other conventional botanical insecticides, this study presents novel insights into the bioinsecticidal potential of *K. senegalensis* seed oil against *D. maculatus*. The study evaluates the physicochemical properties of the oil, its repellent activity, and its insecticidal efficacy, providing a scientific basis for its application in protecting stored fish.

By investigating the dose-dependent repellency and toxicity of *K. senegalensis* seed oil, this research contributes to the growing body of knowledge on plant-based pest control strategies. The findings offer an innovative approach to post-harvest fish protection that is sustainable, cost-effective, and safe for both consumers and the environment.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the Fisheries Research Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria at ambient temperature of between 25 - 30ºC.

Sources of Plant Materials

The Ahmadu Bello University Main Campus Samaru, Zaria is where the seeds of *K. senegalensis* used in this study were gathered from fruiting trees (Latitude: 11° 03' 60.00" N Longitude: 7° 41' 59.99" E). At Ahmadu Bello University's Herbarium Unit of the Biological Sciences Department in Zaria, the plant was correctly recognized and verified with a specimen voucher number 900081.

Processing of plant materials

After being extracted from their pods, the seeds of *K. senegalensis* were sun-dried for 17 days before being crushed with a crusher and pestle. After being ground, it was sieved and kept in pre- fresh cellophane bags with label.

Extraction of oil by Soxhlet extraction method

To extract the oil, 40g of the seed powder was weighed into a different muslin fabric and added separately to the soxhlet chamber. 350 millilitres of n-hexane was added to the round-bottom flask as the extraction solvent. The extraction was done at 60 - 80ºC until the solvent in the soxhlet chamber became transparent. After disassembling the soxhlet apparatus, the contents of the round-bottom flask were moved to a rotating evaporator, which was used to remove extra solvent from the oil. Before being utilized for the bioassay, the extracted seed oil was kept in a labeled bottle in a cold environment.

Determination of the Physicochemical Properties of the Seed Oils

The physicochemical properties of the seed oil of *Khaya senegalensis* determined were Acid, Iodine, Peroxide, and Saponification values. Others include the Refractive index and Specific gravity by Standard Methods. [2, 3]

Determination of Acid value

A solvent mixture of 25ml Diethyl ether was neutralized with 0.1M ethanolic KOH using phenolphthalein as indicator. About 4g of oil was dissolved in the neutralized solvent mixture and titrated with 0.1N KOH.

Acid Value = 56.10 x T x (V2-V1)
Mass of test oil (g)
Where:
T = Molar concentration of ethanolic KOH used
V1 = Volume of KOH solution used for the blank test
V2 = Volume of KOH solution used for the test oil

Determination of Iodine value

Exactly 0.2g of each oil sample was weighed into quick-fit conical flasks; 15ml of CCL_4 and 25ml Wij's reagent was added into each flask and placed in the dark for 1 hour. After the set time, 20ml of 10% KI and 150ml distilled water was added. The resulting mixture was titrated against 0.1M $Na_2S_2O_3$ using 1% starch solution as indicator. A blank test was carried out simultaneously under the same conditions.
Iodine Value = 12.69 x T x (V1-V2)
Mass of test oil (g)
Where:
T = Molar concentration of $Na_2S_2O_3$
V1 = Volume of $Na_2S_2O_3$ solution used for the blank test
V2 = Volume of $Na_2S_2O_3$ solution used for the test oil

Determination of Peroxide value

Four grams of test oils was weighed into quick – fit conical flasks and 10ml chloroform was added to each flask to dissolve the oil samples. Then 15ml acetic acid and 1ml 5% KI solution was added. The mixture was kept in the dark for 5 minutes after which 75ml of water was added and titrated with 0.002N $Na_2S_2O_3$.

$$\text{Peroxide Value} = \frac{(V \text{ X } T)}{m}$$

Determination of Saponification value

Two grams of the test oil was weighed into a quick-fit conical flasks and 25ml of 0.5M ethanolic KOH was added. Boiling chips were also added, then a reflux condenser was fitted and the mixture refluxed for one hour. After 1 hour, 0.5ml of phenolphthalein was added and titrated with 0.5M HCL solution. A blank test was carried out simultaneously.
Saponification Value = 56.10 x T x (V2 – V1)
Mass of test oil (g)
Where,
V2 =Volume in ml of standard hydrochloric acid required for the blank
V1 =Volume in ml of standard hydrochloric acid required for the sample
T = Molar concentration of the standard hydrochloric acid

Determination of Specific Gravity

The specific gravity of the oils was determined using the specific gravity bottle at 25ºC. The specific gravity bottle was weighed empty (W1) after which the oil sample was carefully poured into the specific gravity bottle and weighed (W2). The same step was repeated with distilled water.
Weight = W2 – W1
Where W2 = Weight of oil sample + weight of specific gravity bottle
W1 = Weight of specific gravity bottle
The specific gravity of oil= $\frac{\text{Weight of oil held in specific gravity bottle}}{\text{Weight of water held in specific gravity bottle}}$

Determination of Refractive index

The refractive index of the oil was determined using an Abbe Refractometer with temperature control set at 25ºC. The double prism was opened with the help of the screw head and a drop of oil was placed on the prism. The prism was firmly closed and water was made to circulate through the instrument. The instrument was allowed to stand for a few minutes before taking the reading. The prism is cleaned between readings by wiping off oil with a cotton pad moistened with ethyl alcohol/toluene/petroleum ether and dried. When temperature correction was necessary the formula below was used:
R = R1 + K1(T1 – T)
Where,
R = Reading of the refractometer reduced to the specified temperature T˚C
R1 = Reading at T1C
K = constant 0.000365 for fats and 0.000385 for oils
T1 = temperature at which the reading R1 is taken and
T = specified temperature (generally 40˚C)

Collection and Treatment of Insects

The larvae of *D. maculatus* were properly identified under X40 magnification of a stereo microscope using pictorial keys. [11] The larvae were acclimatized for 24 hours in the laboratory before being subjected to bioassay. The insects were obtained from infested fish pieces purchased from Sabon – Gari market (11º13'N and 07º52'E), Zaria.

Collection of Smoke – Dried Fish

We bought smoked catfish, a specimen of *Clarias gariepinus* weighing 17–20g, at the Sabon–Gari market in Zaria.

Pilot Study

To ascertain the concentration range of the oil to be used for the conclusive testing (bioassay), range-finding experiments were carried out. Using five

nominal concentrations (0.001 to 0.234 mLg^{-1}), this was accomplished by adding the oil to the heat-sterilized fish in triplicate and then placed individually in Kilner jars. The previous concentration is multiplied by three to determine the subsequent concentration. Next, each jar was filled with thirty (30) *D. maculatus* late instar larvae in triplicate. The setup was examined at 24, 48, 72, and 96 hours to determine the range of total and minimal/zero mortality. Five concentrations were selected for the bioassay test between the entire and zero mortality found in this investigation.

Bioassay

The smoke-dried fishes were heat sterilized in the oven set at 60±2ºC for one hour and then allowed to cool. After cooling to room temperature, each fish was weighed and tagged. From the pilot study conducted, the following application levels $0.003mLg^{-1}$, $0.009mLg^{-1}$, $0.027mLg^{-1}$, $0.081mLg^{-1}$, and $0.234mLg^{-1}$ of the oils were determined. Oil-treated fishes were introduced into separate Kilner jars for the larvicidal test. Thirty (30) late (5^{th}) instar larvae of *D. maculatus* were introduced into the jars and the entire setup was replicated three times. Observations were recorded at 24, 48, 72, and 96 hours of exposure time. The repellent effects of the oils were tested in triplicates using rectangular glass containers of 18cmx12cmx10cm dimension. A treated and an untreated fish were placed at opposite extremes in the container and 30 late instar larvae were introduced at the mid-point of it. The setup was left for 24 hours at which time larvae found within a 1cm radius of the treated fish were considered repelled.

Statistical Analysis

All repellent and mortality data were subjected to Analysis of Variance (ANOVA) at $p< 0.05$ to determine significant differences between treatments. Least Significant Difference (LSD) was employed to separate the means using IBM SPSS Statistics 24. Mortality data were subjected to Probit Analysis to determine the median lethal concentration (LC_{50}) of all the seed oils using MS Excel 2010.

RESULT AND DISCUSSION

Physicochemical Properties of the Seed Oils

The seed oil of *K. senegalensis* had a relative density of 0.97, refractive index of 1.47, saponification value of 192.26, peroxide value of 4.75, Iodine value of 88.43 and Acid value of 2.71 as reflected in Table 1.

The relative density of 0.96 and refractive index of 1.47 indicate that the oil is light and less viscous compared to palm oil, which may enhance its ability to spread over treated surfaces effectively. The obtained saponification value (192.26 mg/KOH/g) is within the standard range for vegetable oils, suggesting its stability and potential for long-term use in pest control. [14] The peroxide value (4.75 meq/kg) is below the standard limit of 10 meq/kg, which implies that the oil has a low tendency for oxidation and remains stable during storage. [21] The iodine value of 88.43 Wij's indicates a high degree of unsaturation, which could affect its shelf life and susceptibility to rancidity. [19] The acid value of 2.74 KOH/g suggests a relatively low level of free fatty acids, implying that the oil is less prone to hydrolytic rancidity. [4] These properties collectively highlight the suitability of *K. senegalensis* seed oil for application in pest control without significant risk of rapid degradation.

Toxicity (Repellent and Mortality) effect of *Khaya senegaensis* seed oil

Khaya senegaensis seed oil demonstrated strong repellency against the larvae of *D. maculatus* ($p<0.05$). $0.003mLg^{-1}$, $0.009mLg^{-1}$ and $0.027mLg^{-1}$ did not show any repellent effect while 0.081 mLg^{-1} and 0.243 mLg^{-1} demonstrated noticeable repellency of the larvae. A similar trend was observed concerning larval mortality which was directly proportional to increasing exposure time as shown in Table 2 and Figure 1.

The repellent effect of *K. senegalensis* seed oil increased with dosage, which is likely due to the presence of bioactive compounds with insecticidal properties. This observation aligns with the findings of a report that plant-derived oils often act as anti-feedants and deterrents against insect pests. [18] The strong repellent activity at higher concentrations (0.081 mLg^{-1} and 0.243 mLg^{-1}) suggests that the oil contains secondary metabolites such as monoterpenes, sesquiterpenes, β–caryophyllene, diterpenes, and volatile terpenoids, which have been documented as effective insect repellents. [16] These compounds may interfere with olfactory receptors in insects, thereby inhibiting host recognition and feeding behaviour.

The median lethal concentration (Lc_{50}) of *K. senegalensis* seed oil was determined by probit plot and was computed to be $0.084328mLg^{-1}$ (Figure 2).

Table 1: Physical properties of seed oils of *Khaya senegalensis* used for the experiment

Seed oils	Relative Density	Refractive Index	Saponification value	Peroxide value	Iodine value	Acid value
K. senegalensis	0.96	1.47	192.26	4.75	88.43	2.71
FAO Standard (2009)	0.910-0.916	1.4677 - 1.4707	184 - 196	10 - 15	75 - 94	0.6 - 10

Table 2: Repellent effect of *Khaya senegalensis* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period

Application(ml)	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.003mlg^{-1}	25.67	4.33	-60.00	I
0.009 mlg^{-1}	18.33	11.67	-22.22	I
0.027 mlg^{-1}	15.00	15.00	0.00	I
0.081 mlg^{-1}	4.67	25.33	68.89	V
0.243 mlg^{-1}	0.00	30.00	100.00	VI

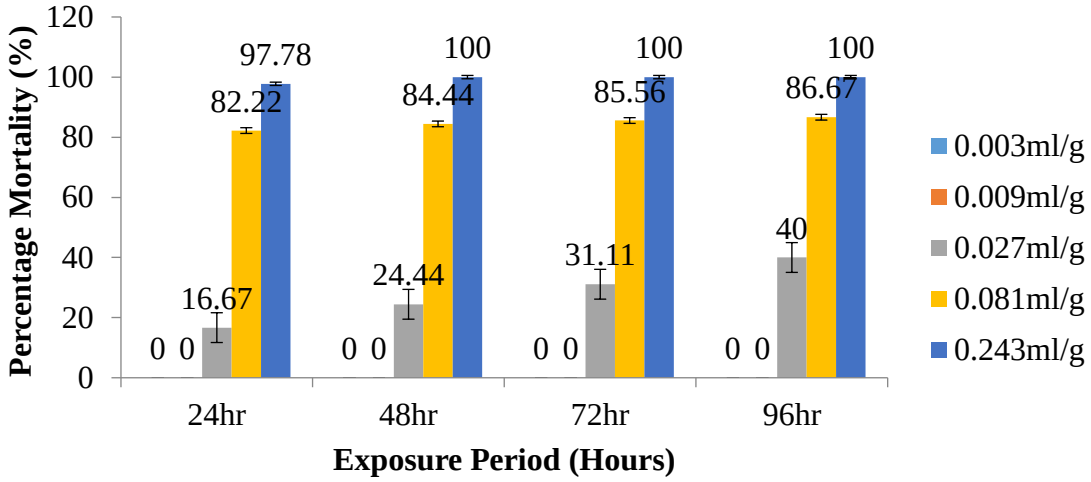

Figure 1: Mortality effect of *Khaya senegalensis* seed oil on *Dermestes maculatus* larvae

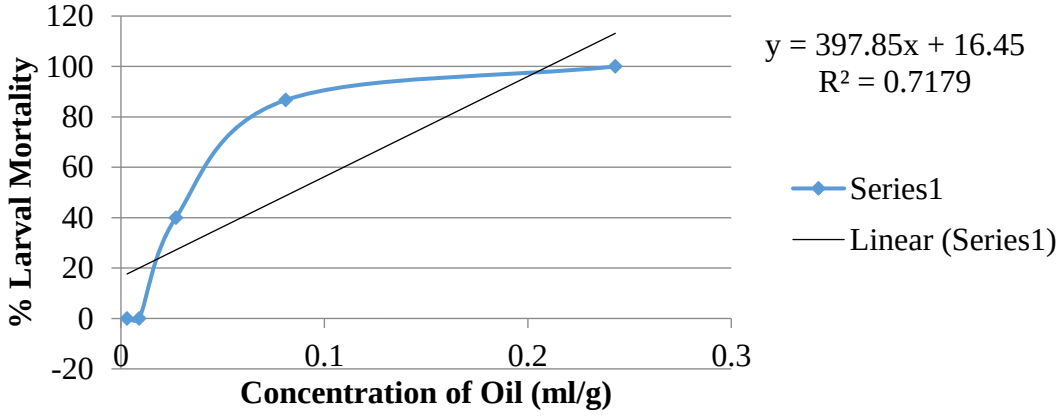

Figure 2: Median Lethal Concentration (Lc_{50}) of the Seed Oil of *Khaya senegaensis*

The probit plot revealed that 0.084328mLg^{-1} *K. senegalensis* seed oil resulted in 50 percent mortality of *D. maculatus* larvae. This mortality of *D. maculatus* larvae was also dose-dependent, with a significant increase in larval mortality observed with increasing exposure time ($p<0.05$). This supports the hypothesis that prolonged contact enhances the toxicity of the oil, likely through cuticular absorption or spiracle blockage leading to suffocation. Similar findings have reportedly demonstrated that plant oils from *Vitellaria paradoxa*, *Ricinus communis*, *Carthamus tinctorius*, and *Sesamum indicum* caused high mortality rates in *D. maculatus* larvae on smoked-dried fish. [8] The present study also aligns with observed findings that *Corchorus olitorius*, *Solanum nigrum*, *Lycopersicon esculentum*, and *Telferia occidentalis* seed oils had potent insecticidal effects against *D. maculatus*. [17]

The toxic action of *K. senegalensis* seed oil is likely attributed to both contact and stomach poisoning. As demonstrated in other studies, plant oils may block insect spiracles, leading to asphyxiation and eventual death. [1] The bioactive compounds present in the oil could also act as neurotoxins or digestive inhibitors, leading to starvation and mortality. [7] This mechanism is similar to the fumigant toxicity observed in *Zingiber officinale* (ginger) essential oil, which exhibited strong insecticidal activity against *D. maculatus* due to the synergistic effects of its bioactive components. [6, 22] Additionally, *Thymus vulgaris* (white thyme) essential oil caused significant ($p<0.05$) mortality of *D. maculatus* larvae, reinforcing the hypothesis that plant-based oils can serve as effective biopesticides. [15]

The findings of this study confirm that *K. senegalensis* seed oil is an effective larvicide and repellent against *D. maculatus*, offering a sustainable and eco-friendly alternative to synthetic insecticides. Its physicochemical stability ensures prolonged efficacy, making it a viable option for post-harvest protection of smoked-dried fish. Given the widespread availability and traditional medicinal use of *K. senegalensis*, its adoption for pest management could be well-accepted among local communities.

CONCLUSION

The results of this study provide strong evidence that *K. senegalensis* seed oil is an effective larvicide against *D. maculatus*. Its repellent and toxic effects on larvae highlight its potential as a bioinsecticide for protecting smoked-dried fish from infestation. Given that *K. senegalensis* is commonly used for medicinal purposes, its application in post-harvest fish protection is likely to be well-accepted by consumers and small-scale fish merchants. Additionally, the physicochemical properties of the oil suggest that it is stable and safe for use, posing minimal environmental and health risks. Future studies should explore the isolation and characterization of the active insecticidal compounds in the oil and evaluate their efficacy under field conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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