

# Bio-activity of the essential oil of *Syzygiumn aromaticum* L against *Dermestes maculatus* DeGeer 1774 (Coleoptera: Dermestidae) infesting smoke-dried *Clarias gariepinus* Burchell 1822

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## Abstract

In this study, essential oil of *S. aromaticum* (EOSA) obtained by steam distillation using Clevenger apparatus was evaluated against *Dermestes maculatus* infesting smoke-dried *C. gariepinus* by residual contact method under laboratory conditions ( $26 \pm 2^{\circ}\text{C}$  and  $75 \pm 5\%$  RH). Four concentrations (2.5, 5, 7.5 and 10%) of EOSA were separately applied on fifteen grams of *C. gariepinus*. Newly (0 -24 hours) emerged adults and 3<sup>rd</sup> instar larvae of the pest were exposed to the treated samples for a period of 5 days. Results showed that 80% larval mortality was recorded in 7.5 and 10% concentrations of EOSA at 24 HAE, which increased to 70 – 100% with extending the exposure period to 48 hours in 5 – 10% concentrations. The essential oil resulted in 50 – 100% adult mortalities at 24 HAE. The LC<sub>50</sub> of EOSA against adults *D. maculatus* was 2.72 and 1.80% at 24 and 48 hours, respectively, whereby the median lethal time (LT<sub>50</sub>) required by 5.0 and 7.5% concentrations of EOSA to kill 50% of the adult are; 17.15 and 10.94 hours respectively. Similarly, the LC<sub>50</sub> of the EO against larvae of the pest were 4.87 and 3.14% with LT<sub>50</sub> of 29.70 and 13.10 hours at the same period of time and concentrations. Oviposition, egg hatchability and adult emergence of *D. maculatus* were positively inhibited by EOSA. Therefore EOSA could be used as a protectant to smoke-dried *C. gariepinus* against the infestation of *D. maculatus*.

**Keywords:** *C. gariepinus*, *D. maculatus*, essential oil, infestation, pesticides, *S. aromaticum*

## Introduction

Fish makes a significant contribution to nutrition by providing readily available dietary nutrients to a large number of the people worldwide [1], it serves as a source of income generation, poverty alleviation, foreign exchange earnings and provision of raw materials for the animal feed industry [2]. It is composed of essential amino acids, notably lysine, methionine, isoleucine and served as a source of vitamins A, B, D, E, K and respectable amount of minerals such as phosphorus, calcium, iron, iodine, fluorine and magnesium [3]. Nigeria have an estimated annual per capita fish consumption of 17.5 kg, and a projected fish demand of 3.61 million metric tonnes [4]. However due to perishable nature of fishes especially in tropical climates, smoke-drying is the most common preservation method adopted by local fish mongers in Nigeria and Africa, to ensure all season availability and meet diversified consumers taste [5]. Hence about 80% of the captured fish are consumed smoked dried and the remaining 20% is consumed either as fresh, salted, sun dried or fried [5]. *Clarias gariepinus* Buchell (1822) is the most widely cultivated and smoke dry fish in Nigeria, because it's highly nutritious, economically important and easily cultured [4]. Nevertheless, smoke-dried *C. gariepinus* have been reported of being infested by pests especially *Dermestes maculatus* (Deeger 1774) L, after harvest, during transport and or storage [2-4]. Therefore different synthetic pesticides were used and found effective in the control and management of the infestation. However these chemicals were scientifically

discovered harmful to human health, non-target organisms and the environment at large [5, 7]. This elucidate the interest of formal testing insecticidal activities of various forms of plant materials against the infestation of storage pests [6]. Therefore the aim of this study is to evaluate the bio-activity of *S. aromaticum* essential oil against *D. maculatus* infesting smoked dried *C. gariepinus*.

## Materials and Methods

### Collection and preparation of plants materials

Dried buds of *S. aromaticum* were purchased from Katsina Central market. Samples were then washed using tap water in the laboratory and air dried under shade for a period of five days. The dried buds were then kept in a well labeled plastic container [7].

### Extraction of the essential oil

The essential oil was extracted by steam distillation method, using Clevenger apparatus with some modifications. Seventy five grams (75 g) of the dried buds were taken in a 1000 mL round bottom flask, and 750 mL of water was added. The flask was heated on heating mantle for a period of three hours. The volatile oil was extracted and it was evaporated along with the water vapor, through the connection tube and passed through condenser, Distillate was collected in a separation funnel. The oil was drained into a beaker followed by passing over anhydrous sodium sulfate to remove excess water and then kept in a dark sealed vials at 4<sup>o</sup>C [8].

### Collection and rearing of *Dermestes maculatus*

Naturally infested smoked dried *C. gariepinus* obtained from dry fish vendors at Katsina central market, was used as the initial source of *D. maculatus* culture for this study. The infested sample was taken to postgraduate laboratory of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK), in a plastic jars covered with muslin cloth. The insects were sorted, identified and confirmed in the insectary of the Department, using the observable morphological features with the aid of hand lens and dissecting microscope. Un-infested fish sample and the experimental jars were disinfested in dry air oven at 60°C for 60 minutes to kill all possible insect pests and their eggs that might be present and later air dried [9]. Twenty pairs of identified beetles were introduced into three different rearing containers containing 100 g disinfested smoked dried fish sample and water soaked cotton wool, to serve as source of food as well as meeting the water requirement for oviposition. The rearing containers were then covered with muslin cloth, to prevent escape of the insects and or entrance of unwanted insects. The set up was then kept in an incubator at a temperature of 28 ± 2°C and 65 ± 5% relative humidity (R.H) for a period of two weeks to allow oviposition and larval emergence. After larval emergence, the adults were removed and the newly emerged larvae were used to conduct Larvicidal bioassay and further reared the remaining to adult which were used for adulticidal bioassay [10].

### Larvicidal bioassay

To test the toxicity of *S. aromaticum* essential oils (EOSA) against larva of *D. maculatus*, residual contact method maintained by [7] was adopted. Four stock solutions (2.5, 5.0, 7.5 and 10% concentrations) were prepared using acetone as a solvent. After which 5ml of each of the oil solutions were thoroughly sprayed on 15 g of disinfested smoked dried fish samples using a sprayer, each placed in different labeled transparent plastics container. The treated samples were air-dried for 60 min to allow acetone evaporation. Same grams of fish sample, treated with acetone only served as a control. Ten 3<sup>rd</sup> instar larvae of *D. maculatus* were released on each treated fish flesh in the containers and cover with perforated lead fitted with muslin cloth to allow aeration and to prevent entrance of some insects from outside. All treatments and control were replicated three times and kept in an incubator at 28± 2°C and 65 ± 5% R.H. Larval mortalities were recorded in every 24 hrs for 4 days and mean larval mortality was calculated

### Adulticidal bioassay

Adulticidal activity of *S. aromaticum* against adult *D. maculatus* was tested using residual contact toxicity method [7]. Fifteen grams (15 g) of smoked dried fish were thoroughly treated with 5 ml of each concentration of the prepared oil solutions, treated fish samples were then placed in labeled transparent plastic containers. A control was also prepared by spraying 5ml of acetone only on the same grams of smoked dried fish. Each of the treatments and control were replicate three times. The treated samples were air-dried for 60 min to allow the solvent evaporation, before releasing five pairs of newly emerged (0-24 hours old) *D. maculatus* into each container and covered with a lid perforated and pitted with net

from inside, to allow aeration, prevent the insects escaping and intrusion of other insects from outside. All treatments and control were replicated three times and kept in an incubator at 28 ± 2°C and 65 ± 5% R.H. Mortalities were recorded every 24 hours for five days, the insects were judged dead when they failed to respond to pin probe and mortalities were express in percentages.

### Oviposition, egg hatchability and adult emergence bioassay

To determine the effect of *S. aromaticum* essential oils on oviposition, egg hatchability and adult emergence, a method of [11] was adopted. A 5ml measure of each concentrations (2.5, 5.0, 7.5 and 10%) of the EO was sprayed individually to 15 g muscle of smoked dried fish in transparent plastic container and thoroughly mixed with the aid of a glass rod. The fish muscles were air dried for 1 hour after which ten pairs of newly emerged (0 – 24 hrs) *D. maculatus* adults were introduced into each container and covered with a lid perforated and fitted with net from inside, to allow aeration, prevent insects escaping and or intrusion of other insects from outside. The containers were kept at room temperature and a water soaked cotton wool was placed into each container to meet the water requirement for oviposition. Same weight of fish muscle treated with acetone only served as control and all containers were replicated three times. After three days all dead and alive adult beetles were removed from the treated and control containers, the number of eggs laid were counted using hand lens and soft entomological brush. Percentage reduction of egg laid was calculated using the formula below:

$$\% \text{ Oviposition deterrence} = \frac{NEC - NET}{NEC} \times 100$$

Where NEC = Number of eggs laid in the control and NET = Number of eggs laid in the treated samples.

Same weight of *C. gariepinus* and EO concentrations was prepared to evaluate the egg hatchability in treated fish samples. Forty (40) newly laid eggs were placed on 15 g of treated fish samples in each of the containers and kept in an open air shelf at laboratory conditions. Number of larvae emerged was recorded 48, 72 and 96 hours after treatment. Egg hatchability was determined using the following formula.

$$\text{Egg Hatchability (\%)} = \frac{\text{Number of Eggs Hatch}}{\text{Number of Eggs Introduced}} \times 100$$

Another set of containers were prepared to assess the effect of the EO on adult emergence of *D. maculatus*. Thirty 3<sup>rd</sup> instar larvae were introduced into each container containing 15 g of *C. gariepinus* treated with concentrations of the EO. A transparent plastic container with same weight of fish sample treated with acetone only served as a control. Both treated and control were replicated three times and kept at a laboratory conditions. The number of larva reaching adult stage were recorded in both treated and control containers after four weeks and the percentage reduction in adult emergence of F<sub>1</sub> progeny or inhibition rate (IR) was calculated according to the method described by [11].

$$\text{Inhibition Rate (\%)} = \frac{C_n - T_n}{C_n} \times 100$$

Where; C<sub>n</sub> is the number of emerged insects in the control and T<sub>n</sub> is the number of emerged insects in the treated containers.

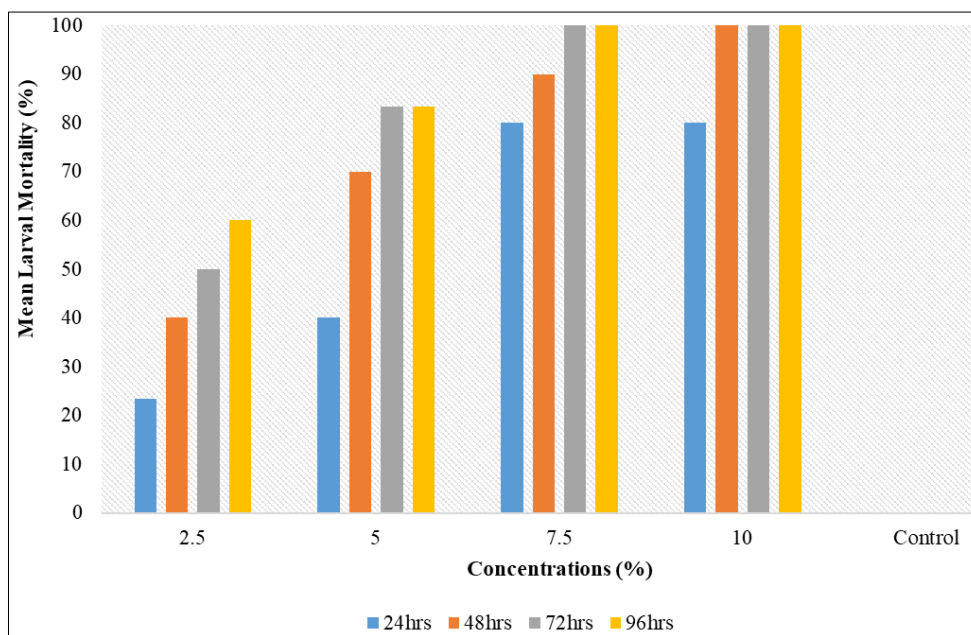
**Data analysis**

Mortality data were subjected to one way Analysis of Variance (ANOVA) at *p* < 0.05. Probit analysis was used to determine median lethal concentration (LC<sub>50</sub>) and lethal time (LT<sub>50</sub>) of EOSA against larvae and adult of *D. maculatus* using SPSS version 26.

**Results**

**Larvicidal activity of EOSA against *D. maculatus***

Result shows that highest (80.00%) larval mortality was recorded in both 7.5 and 10.0% concentrations 24 hours after exposure (HAE) and lowest (23.30 - 40.00%) mortality was observed in 2.5 and 5.0% concentrations, respectively. Meanwhile, with extending the exposure period to 48 hours, the larval mortality increased to 70.00 and 90.00 - 100% in *C. gariepinus* treated with 5% and 7.5 - 10% concentrations of EOSA. The results further indicated that the mortality increased to 50.00 and 83.30% in fish sample treated with 2.5 and 5.0% concentrations, respectively 72 - 96 HAE (Figure 1).

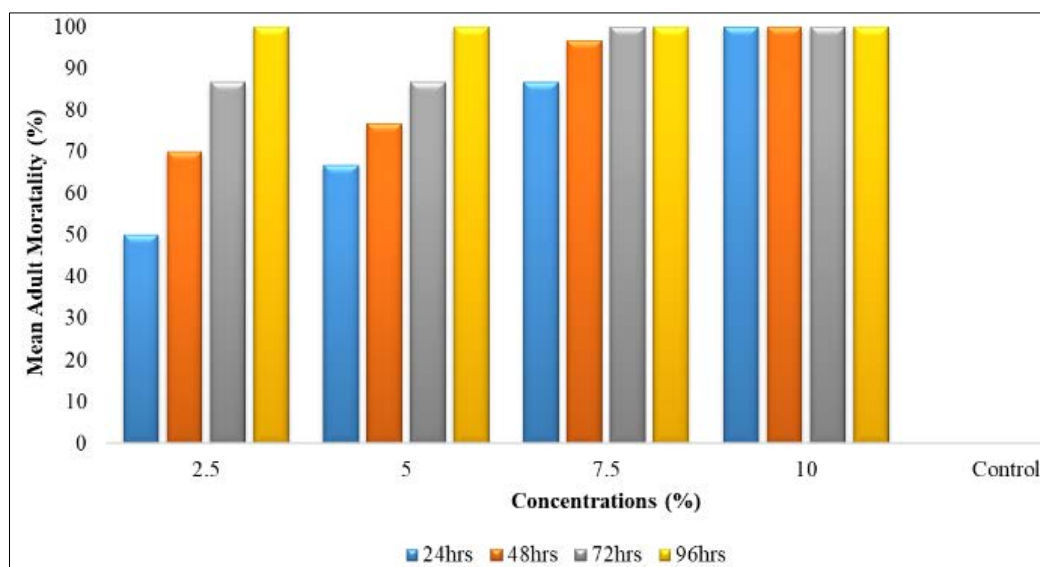


**Fig 1:** Mean Mortality of *D. maculatus* Larvae in Varying Concentrations of EOSA

**Adulticidal Activity of EOSA against *D. maculatus***

Result of adult mortality of *D. maculatus* exposed to concentrations of EOSA is presented in Figure 2. The mortality varied with varying concentrations of essential oil and exposure period. At 24 HAE 50, 60, 90 and 100% mortality was recorded in fish treated 2.5, 5.0, 7.5 and 10%

concentrations, respectively. However, when the exposure period was extended to 48 - 72 hours, 70.00 - 100% mortalities were recorded in *C. gariepinus* treated with 2.5 - 7.5% concentrations of EOSA. Moreover, complete (100%) adult mortality was recorded in all concentrations of EOSA at 96 HAE (Figure 2).



**Fig 2:** Adult Mortality of *D. maculatus* in Varying Concentrations of EOSA

### Median lethal concentrations (LC<sub>50</sub>) and median lethal time (LT<sub>50</sub>) of EOSA against *D. maculatus*

The concentrations of EOSA required to kill 50% of adult *D. maculatus* were 2.72, 1.80 and 0.99% at 24, 48 and 72 hours, respectively (Table 1). For the larval stage, the LC<sub>50</sub> of EOSA varied between 2.60 and 4.87% at the same period of time. On the other hand 2.5, 5.0, 7.5 and 10% concentrations required 26.40, 17.15, 10.94 and 7.00 hours to kill 50% adult of *D. maculatus*. Meanwhile, the LT<sub>50</sub> of the EOSA against larvae of the pest ranged from 13.50 to 69.04 hours at the same concentrations.

**Table 1:** Median Lethal Concentrations (LC<sub>50</sub>) and Median Lethal Time (LT<sub>50</sub>) of EOSA against Adults and Larvae of *D. maculatus*

Exposure period (Hrs)	LC <sub>50</sub> (%)		LT <sub>50</sub> (Hours)		
	Adult	Larvae	Concentration s (%)	Adult	Larvae
24	2.72	4.87	2.5	26.40	69.04
48	1.80	3.14	5.0	17.15	29.70
72	0.99	2.60	7.5	10.94	13.10
			10	7.00	13.50

**Table 2:** Oviposition Deterrence, eggs hatchability and adult emergence of *D. maculatus* in *C. gariepinus* treated with EOSA

Concentrations (%)	Mean No. of Eggs Laid	Oviposition Deterrence (%)	Mean No. of Eggs Hatched	Mean Egg hatchability (%)	Inhibition Rate (%) in Adult Emergence
2.50	9.66	89.94	2	5.00	96.66
5.00	7.33	92.36	1	2.50	96.66
7.50	0.00	100.00	0	0.00	100.00
10.0	0.00	100.00	0	0.00	100.00

### Discussion

The essential oil of *Syzygium aromaticum* was evaluated against *D. maculatus* under laboratory conditions using residual contact method. The EO was discovered effective and observed to be concentration and time of exposure dependent. EOSA showed a significant effect against adults and larvae of *D. maculatus*, hence the concentrations required to kill 50% of the pest (LC<sub>50</sub>) were generally promising but higher against larvae than against the adults. Similarly the time required to kill 50% of the pest (LT<sub>50</sub>) is significantly higher in larvae than in adults. Meanwhile, EOSA substantially impaired oviposition of *D. maculatus* at lowest concentrations, significant number of the pest where dead and the mating ability of those remain was affected therefore few eggs were laid and total oviposition deterrence was observed in *C. gariepinus* treated with highest concentrations of the EO and significantly affected egg hatchability of the pest. Therefore few adults were emerged in the lower concentrations and no emergence in the higher concentrations. These results agrees with the findings of [12] that complete larval mortality of *T. castanum* was recorded in clove essential oil within 24 hours of exposure using residual method. Similarly the result was in agreement with the findings of [13] who reported 100% mortality of *Sitophilus zeamais* and *A. obtectus* were recorded in 17.9 and 35µL g-1 concentrations of the essential oil of *S. aromaticum* 48 h after treatment. The result also support the findings of [14] who reported that the essential oils of *S. aromaticum* exhibited insecticidal activities similar to Deltamethrin in the control of *Callosobruchus maculatus*, whereby increments in the oil dosage proportionately decreased the growth rate, offspring emergence was almost abolished and oviposition was significantly impaired when parents were exposed the

### Oviposition deterrence, eggs hatchability and adult emergence of *D. maculatus* in *C. gariepinus* treated with EOSA

Results of oviposition, egg hatchability and adult emergence of *D. maculatus* on *C. gariepinus* treated with concentrations of EOSA are shown in Table 2. The results highlighted that oviposition deterrence was 75 and 92% in *C. gariepinus* treated with 2.5 and 5% concentrations of EOSA, respectively, while complete (100%) deterrence was recorded in *C. gariepinus* treated with 7.5 and 10% concentrations of EOSA (Table 2). Furthermore, the result showed that a few number of eggs were hatched in lower concentrations and none was hatched in the fish samples treated with higher concentrations of EOSA. Moreover, the results indicated adult emergence was positively inhibited in almost all concentrations of the EOs tested (Table 4.5). Adult emergence was significantly (96.66%) inhibited in *C. gariepinus* treated with 2.5 and 5% concentrations of EOSA, while complete (100%) inhibition was recorded in fish treated with 7.5 and 10% concentrations of the EO.

concentrations of the oil. Similarly [15]; reported that oil extracts of *Corchorus olitorius*, *Solanum nigrum*, *Lycopersicon esculentum* and *Telferia occidentalis* were found capable of controlling different stages of *D. maculatus* on treated smoked dried *C. gariepinus* during storage at concentrations of 2, 4, 6 and 8%. All oils were significantly (p<0.05) effective in killing all adults, eggs, larvae and pupae.

### Conclusion

In view of the findings of this study, essential oil of *S. aromaticum* could be used as an alternative to synthetic pesticides in the management of *D. maculatus* infesting smoke-dried *C. gariepinus*.

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