



## Larvicidal Effect of Castor Plant (*Ricinus communis* Linn) on *Anopheles* Mosquito

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### Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

### Article Information

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### ABSTRACT

**Aims:** Mosquito-borne diseases still remain a major health problem and their control depends largely on preventive measures against the vectors. The control of mosquito at larvae stage is considered effective way of vector management. Hence, this study aimed at evaluating larvicidal activities of methanol, N-hexane leaf extract and seed oil of castor plant (*Ricinus communis* Linn) against 2<sup>nd</sup> and 4<sup>th</sup> instar of *Anopheles* mosquito larvae.

**Place and Duration of Study:** This research work was carried out in the Department of Microbiology, Federal University of Technology, Akure, Nigeria.

**Methodology:** The castor leaf and seed oil were extracted with soxhlet extractor using 98% N-hexane and methanol. Different concentration of the extracts were prepared (10.0-100.0 mg/L), also, a test concentration ranging from 200-1,000 ppm were prepared with distilled water, and mortality was recorded after 24 hours of larvae exposure to extracts.

**Results:** In 2<sup>nd</sup> instar larvae, there was a significant difference ( $p < 0.05$ ) in the LC<sub>80</sub> and LC<sub>95</sub> values of the extracts, Castor seed oil extract has the lowest values. Castor seed oil extract has the highest mean larval mortalities of  $97.60 \pm 1.63\%$  and high larvicidal activity at the lowest concentration of 200 mg/l. In 4<sup>th</sup> instar larvae of *Anopheles* mosquito after 24 hours of exposure, there was a significant difference ( $p < 0.05$ ) in the LC<sub>80</sub> and LC<sub>95</sub> values of the extracts, Castor seed oil has the lowest LC<sub>80</sub> and LC<sub>95</sub> values. Castor oil has the highest mean larvae mortality of  $97.60 \pm 1.00\%$  and larvicidal activity with high mortality at lowest concentration of 200 mg/l.

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**Conclusion:** Conclusively, the results revealed that extracts of *Ricinus communis* possesses larvicidal properties against *Anopheles* mosquito larvae and might be a potential biological control measure.

**Keywords:** Mosquito-borne diseases; larvicidal activities; castor plant extract; larvae mortality; 2<sup>nd</sup> and 4<sup>th</sup> larvae instar; LC<sub>80</sub> and LC<sub>95</sub> value.

## 1. INTRODUCTION

Throughout the world, mosquitoes are responsible for the transmission of several human diseases and are by far the most important of the blood sucking arthropods giving annoyance to and causing diseases in humans. Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. About 3,500 species of mosquitoes have been described worldwide, WHO has described the mosquito as "public enemy number one" and reported mosquito borne diseases across globally infecting more than 700,000,000 people every year. Mosquitoes are passing pathogens of life threatening diseases like malaria, West Nile Virus disease, yellow fever, dengue, chikungunya, filariasis and encephalitis [1,2].

Mosquitoes are vector of malaria which has been ranked amongst the world's the most prevalent tropical vector borne communicable disease. An estimated 300-500 million people are infected with malaria annually, resulting in 1.5 to 3 million deaths [3].

The present recrudescence of this disease is due to the higher number of breeding places in society and increasing resistance of mosquitoes to current commercial insecticides [4]. Mosquitoes develop genetic resistance to synthetic insecticides [5]. Control of mosquitoes is something of utmost importance in the present day with rising number of mosquito borne illnesses. Early efforts to control arthropod pest and disease vectors relied primarily on the application of broad spectrum pesticides. This approach often cause undesirable side effects including environmental pollution, health hazards, the development of insecticide resistance and sometimes sudden increase in old, or even new and worse pests [6].

The best method to control mosquito-borne disease is to control the vectors thereby bringing about interruption in disease

transmission [7]. All *Anopheles* larvae lack the respiratory siphons used as breathing tubes in most other mosquito genera, and therefore the larvae lie parallel to the water surface in order to breathe. *Anopheles* mosquitoes develop through four larval sizes or instars before pupating [8]. Larvae are very small in the first instar and increase in size until reaching 5 to 6 mm by the completion of the fourth instar [8]. They feed on organic matter and algae [8]. At each instar, the larvae molt, shedding their exoskeletons, or skin to allow for further growth. Control of mosquitoes in the larval stage is considered an efficient way in the integrated vector management [9]. Due to the dramatic increase in resistance of mosquito to failliar chemicals in the absence of new compounds, better alternative means of control are sought, to combat some of these problems, various studies on natural plant products against mosquito vectors have been indicated as a possible alternative to synthetic insecticides [10,11,12]. Many researchers have reported on the effectiveness of plant extracts against mosquito larvae [13].

Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, active against specific target-insects and are biodegradable [14]. *Ricinus communis* (Castor bean plant) is a small wooden tree which grows to about 6 meters in height widespread throughout tropical regions as ornamental plants. Stems of *R. communis* have Anticancer, antidiabetic and antiprotozoal activity [15]. The castor bean *Ricinus communis* (Euphorbiaceae) has shown a great potential as a source of insecticidal molecules against several insects [13,16,17,18,19], the aerial parts of *R. communis* extract have been shown to possess insecticidal activity against a wide range of haematophagus insects [20]. Further research shows that *R. communis* extract has larvicidal effects with 100% killing activities for *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* larvae [15]. There is need to explore substances of natural origin because they are likely to lower the high cost of treatment,

reduce resistance of drugs and insecticides and also reduce environmental pollution caused by insecticides. Therefore, this study aimed to verify the larvicidal activity of Castor plant (*R. communis*) on *Anopheles* mosquito larvae.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plants

Young seeds and leaves of castor plant (*R. communis* Lim) were harvested at North gate area of Federal University of Technology, Akure and authenticated by the experts at the Department of Crop, Soil and Pest Science, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure and later transported to the Department of Microbiology, Federal University of Technology, Akure, Nigeria.

### 2.2 Preparation of Plant Materials

The harvested leaf samples were washed in running water, petioles and major leaf veins were removed and the remaining was cut into small pieces and air dry under the shade. 10 g of dried leaf was ground in a mortar with a pestle. The leave powder was kept at room temperature in a well labeled bag until use. Ripe castor seeds were sundried and then oven dried at 40°C for 72 hours. 500 g of seeds obtained after manual decortications were grounded using mortar and pestle to form paste, then stored at 4°C.

### 2.3 Extraction of Castor Seeds Oil

Castor seed paste obtained was mixed with N-hexane in a soxhlet extractor to extract the oil. The excess solvent (N-hexane) was removed under reduced pressure of 22-26 mmHg and the oil was placed in a desiccator to remove any remaining water and the stored at 4°C. The percentage oil extracted was also determined.

### 2.4 Extraction of Castor Leaves

500 grams of the previously prepared plant powder were extracted in Soxhlet apparatus with N-hexane and methanol at 60-80°C until there was discoloration of the leaf powder. The liquid extracts were cooled and filtered with Whatman filter paper. The filtrates were evaporated under reduced pressure of 22-26 mmHg at 45°C and the residues obtained were stored at 4°C.

## 2.5 Mosquito Larvae Culture

*Anopheles* mosquito larvae were collected from stagnant waters, uncovered septic tanks and ponds. They were selected and identified using physical characterization.

## 2.6 Preparation of Stock Solution

The extracts were dissolved in Dimethyl sulphoxide (DMSO) to prepare stock solution. Homogeneous suspension was obtained by gentle shaking or stirring. Different concentration of the extracts were prepared (10.0-100.0 mg/L), also, a test concentration ranging from 200 to 1,000 ppm were prepared with distilled water.

## 2.7 Larvicidal Bioassay

Larvicidal effect of the N-hexane, methanol leaf extracts and seed oil of castor plant were determined by using WHO standard. The experiment was setup in six groups, group 1-3 were 2nd instar larvae, group 4-6 were 4th instar larvae. In each group, 100 larvae were stocked for each treatment (N-hexane, methanol plant extracts, seed oil of castor plant and control). All were setup in triplicates, the number of dead larvae at the end of 24 hours was recorded as percentage mortality. During the treatment, no food was offered to larvae to avoid the possibility of feed reaction with the extract.

$$\text{Percentage Mortality (\%)} = \frac{\text{total number of dead larvae at the end of 24 hours}}{\text{total number of larvae stocked}} \times 100$$

For calculation of LC<sub>80</sub> and LC<sub>90</sub>, different concentrations of extract were used, however the concentration at which 80% and 90% of the larvae dies off after 24 hours were recorded as LC<sub>80</sub> and LC<sub>90</sub> respectively.

LC<sub>80</sub>= concentration at which 80% of larvae dies off, LC<sub>95</sub>= concentration at which 95% of larvae dies off.

## 2.8 Statistical Analysis

The result were presented in a descriptive statistic and means were separated using Duncan's' new Multiple Range test and significant level is taken at 95% Confidence level. The analysis was carried out using SPSS version 20 software.

### 3. RESULTS AND DISCUSSION

#### 3.1 Larvicidal Effect of Castor Plant Extracts on the 2<sup>nd</sup> Instar Larvae of *Anopheles* Mosquito after 24 Hours of Exposure

Table 1 shows the LC<sub>80</sub> and LC<sub>95</sub> and larval mortalities of N-hexane and Methanolic castor leaf extract and seed extract (oil) on 2nd instar larvae of *Anopheles* mosquito larvae after 24 hours of exposure. There was a significant difference ( $p < 0.05$ ) in the LC<sub>80</sub> value of the extracts, Castor oil has the lowest LC<sub>80</sub> value (201.4±0.12 mg/L), and however, there were no

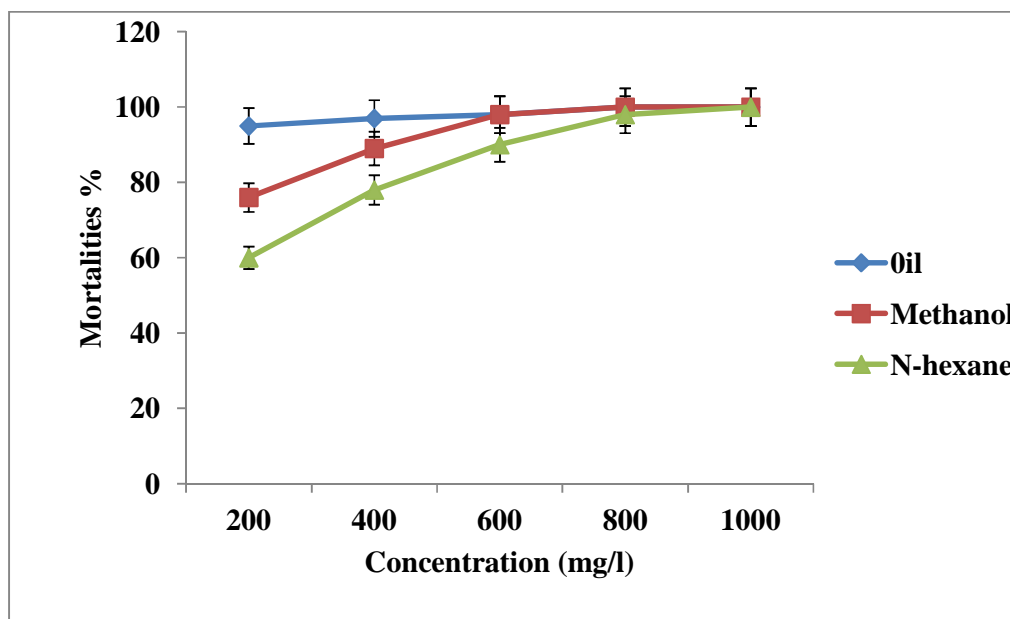
significant difference ( $p > 0.05$ ) in the LC<sub>95</sub> value of Methanolic extract and oil of Castor plant. Castor oil has the highest mean larvae mortality of 976.0±1.63 after 24 hours of exposure. Fig. 1 shows the larvicidal effect of Castor plant extract against 2nd instar larvae of *Anopheles* mosquito after 24 hours of exposure, 100% mortality was observed in all extract, the larvicidal activity of oil has high mortality at 200 mg/l, 400 mg/l, 600 mg/l and 800 mg/l, methanolic extract has high mortality at 600 mg/l, 800 mg/l and 1000 mg/l followed by N-hexane extract, the mortality was high at 800 mg/l and 1000 mg/l. However, there was no mortality in the control.

**Table 1. Larvicidal effect (LC<sub>80</sub> and LC<sub>95</sub>) of the Castor plant extracts on the 2nd instar larvae of *Anopheles* mosquito after 24 hours of exposure**

Castor plant extract	LC <sub>80</sub> (mg/l)	LC <sub>95</sub> (mg/l)	Mean larvae mortality (%±SE)	95% CI of Mean mortality	
				Lower bound	Upper bound
Leaf N-hexane	534.0±0.04 <sup>c</sup>	624.9±0.03 <sup>b</sup>	848.0±1.91 <sup>a</sup>	814.5	895.5
Leaf Methanol	248.8±0.31 <sup>b</sup>	438.6±0.02 <sup>a</sup>	928.0±1.63 <sup>b</sup>	924.0	958.3
Seed Oil	201.4±0.12 <sup>a</sup>	431.4±0.21 <sup>a</sup>	976.0±1.63 <sup>c</sup>	954.0	985.9

Values are means ± SE. Values in the same column carrying the same superscript are not significantly different at ( $p > 0.05$ ) using Duncan's New Multiple Range test.

Key: mg/l= milligram per Litre, LC<sub>80</sub>= concentration at which 80% of larvae dies off, LC<sub>95</sub>= concentration at which 95% of larvae dies off, CI= confidence interval



**Fig. 1. Larvicidal effect of Castor plant extracts against 2nd instar larvae of *Anopheles* mosquito after 24 hours of exposure**

Key: mg/l= Milligram per Litre

### 3.2 Larvicidal Effect of Castor Plant Extracts on the 4<sup>th</sup> Instar Larvae of *Anopheles* Mosquito after 24 Hours of Exposure

Table 2 shows the LC<sub>80</sub> and LC<sub>95</sub> and larval mortalities of N-hexane and Methanolic castor leaf extract and seed extract (oil) on 4th instar larvae of *Anopheles* mosquito after 24 hours of exposure. There was a significant difference (p<0.05) in the LC<sub>80</sub> value of the extracts, Castor oil has the lowest LC<sub>80</sub> value (259.2±0.11 mg/L), and however, there was a significant difference (p<0.05) in the LC<sub>95</sub> value of Methanolic extract, N-hexane extract and Castor oil. Castor oil has the highest mean larvae mortality of 976.0±1.00 after 24 hours of exposure. Fig. 2 shows the larvicidal effect of Castor plant extract against 4th instar larvae of *Anopheles* mosquito after 24 hours of exposure, 100% mortality was observed in all extract, the larvicidal activity of oil has high mortality at 400 mg/l, 600 mg/l and 800 mg/l,

methanolic extract has high mortality at 800 mg/l and 1000 mg/l followed by N-hexane extract, the mortality was high at 1000 mg/l.

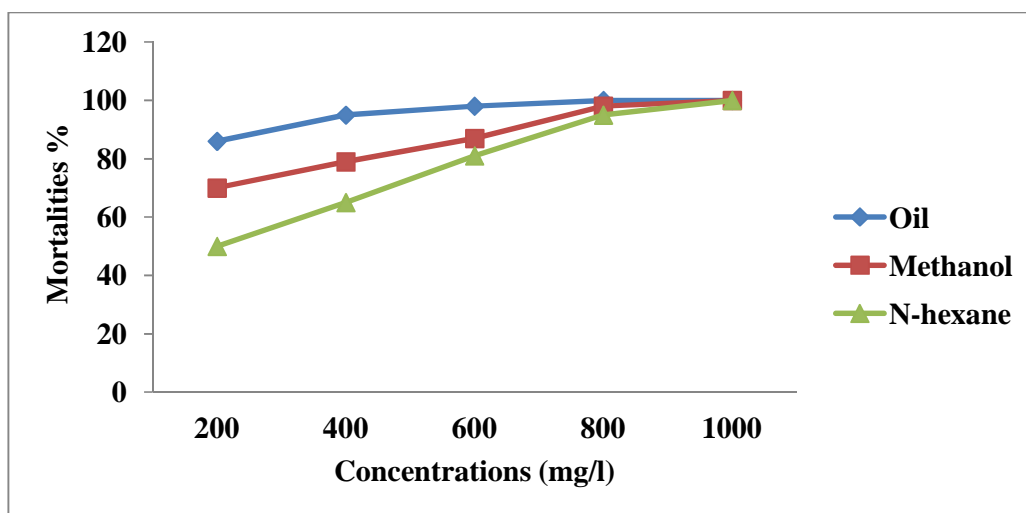
Control of mosquitoes in the larval stage is considered an efficient way in the integrated vector management [9]. Due to the, various studies on natural plant products against mosquito vectors have been indicated as a possible alternative to synthetic insecticides [10,11,12]. Many researchers have reported on the effectiveness of leaf extracts against mosquito larvae [21]. Plants are rich in bioactive chemicals, active against specific target-insects and are biodegradable [14]. In this study, it was revealed that castor plant could be toxic to larvae of *Anopheles* mosquitoes, methanol, N-hexane extracts and seed oil of castor plant has larvicidal effect, this may be due to the presence of toxic ricin in the castor plant, that cause acute cell death by inactivation of ribosomal RNA and inhibiting protein synthesis [22,23,24].

**Table 2. Larvicidal effect (LC<sub>80</sub> and LC<sub>95</sub>) of the Castor plant extracts on the 4th instar larvae of *Anopheles* mosquito after 24 hours of exposure**

Castor plant extract	LC <sub>80</sub> (mg/l)	LC <sub>95</sub> (mg/l)	Mean larvae mortality (%±SE)	95% CI of mean mortality	
				Lower bound	Upper bound
Leaf N-hexane	624.0±0.12 <sup>c</sup>	815.0±0.33 <sup>c</sup>	840.0±1.63 <sup>a</sup>	814.0	874.0
Leaf methanol	407.5±0.02 <sup>b</sup>	695.7±0.21 <sup>b</sup>	928.0±1.00 <sup>b</sup>	899.1	930.9
Seed oil	259.2±0.11 <sup>a</sup>	608.9±0.01 <sup>a</sup>	976.0±1.00 <sup>c</sup>	949.1	980.9

Values are means ± SE. Values in the same column carrying the same superscript are not significantly different at (p>0.05) using Duncan's New Multiple Range test.

Key: mg/l= milligram per Litre, LC<sub>80</sub>= concentration at which 80% of larvae dies off, LC<sub>95</sub>= concentration at which 95% of larvae dies off, CI= confidence interval



**Fig. 2. Larvicidal effect of Castor plant extracts against 4th instar larvae of *Anopheles* mosquito after 24 hours of exposure**

Key: mg/l= Milligram per Litre

LC<sub>80</sub> and LC<sub>95</sub> and larvae mortality of N-hexane and Methanolic castor leaf extract and seed extract (oil) on 2nd and 4th instar larvae of *Anopheles* mosquito after 24 hours of exposure revealed that Castor oil has the lowest LC<sub>80</sub> and LC<sub>95</sub> value and the highest mean larvae mortality after 24 hours of exposure this has also been reported by [18,25,26], they reported that castor bean oil exhibit high toxic effect on 1st, 2nd, 3rd and 4th instars larvae of *Anopheles* mosquito, this may be because castor bean oil contain three toxic proteins Ricin A, B and C and one ricinus agglutinin which are more concentrated in the seed than other parts of castor plant [27].

Also, in this study 2nd instar larvae of *Anopheles* mosquito were more susceptible to castor plant extract with high mortality at low concentration of extracts, the varying susceptibility and mortality rate on 2nd and 4th instar larvae of *Anopheles* mosquito are probably due to differences in the physiological characteristics of the two instars. This result agrees with the findings of Pushpanathan et al. [28], who reported that 2<sup>nd</sup> instar larvae of *Culex quinquefasciatus* were more susceptible than 3rd instar, and the later was more susceptible than 4th instar larvae.

#### 4. CONCLUSION

The castor bean leaf methanol, N-hexane extract and seed oil are potential source of molecules with larvicidal properties against instars of *Anopheles* mosquito larvae at different concentrations. It is therefore recommended that extracts from castor plant could be developed into a good larvicidal agent to put mosquito-borne diseases under control, also, purification of these extracts and identification of bioactive components are important for proper use in insect vector control management. Also, effect of Ricin on human and other animals needs to be carried out to establish the safety of castor leaf as larvicidal.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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