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Ovicidal effects of foliage extract of *Calotropis procera* Ait. and *Ricinus communis* L. against *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract
The study assessed the ovicidal properties of *Calotropis procera* and *Ricinus communis* leaf extracts as botanical insecticides against *Culex quinquefasciatus*, a Filariasis vector. Eggs mortality rates were measured after 5 days post-treatment, based on the number of unhatched eggs. Results showed *C. procera* extract to be more effective, with LC_{50} - LC_{90} values of 191.87 - 510.51 ppm and 961.612 - 1786.488 ppm, respectively. Both plants extracts show promise as ovicidal agents against *Culex quinquefasciatus*, highlighting the potential of plant-based insecticides in mosquito control. These results emphasize the significance of investigating natural alternatives for efficient vector control methods.

Keywords: *Culex quinquefasciatus*, ovicidal effect, *Ricinus communis*, *Calotropis procera*

Introduction

Lymphatic filariasis in Africa is primarily caused by the nematode, *Wuchereria bancrofti*, transmited by *Culex* mosquitoes and female *Anopheles*. This disease poses a substantial public health and economic burden in various tropical and subtropical areas globally. Lymphatic filariasis founds in about 39 of the 46 states that make up the WHO African Region. About 420 million people are expected to be at risk of the disease in the Region, which represent about 38% of the global burden (WHO 2004) [1]. Sudan falls within the Afro-tropical endemic region, with endemic disease prevalence particularly high in the southern states, as well as in Darfur and Blue Nile states in central Sudan (Satti & Abdel Nur,1974; Elsetouhy & Ramzy, 2003) [2, 3]. One effective strategy for disease control involves managing the vectors responsible for disease transmission. In the case of lymphatic filariasis in Africa, the main culprits are female mosquitoes belonging to the Anopheles and *Culex quinquefaciatus* species (WHO, 2004) [1]. By targeting and controlling these vectors, we can disrupt the transmission cycle of the disease and prevent further spread. This approach is crucial in reducing the burden of lymphatic filariasis and protecting public health in affected regions.

Culex quinquefasciatus is commonly found in tropical and subtropical regions, with a strong presence in Lagos (Nigeria) and Zanzibar (Tanzania) making up 90% and 95% of the mosquito species in these areas, respectively (Pedersen *et al*, 1999; Oduola and Awe, 2006) [4, 5]. The most dominant *Anopheles* species in the Sudan (White Nile state area) was *An. gambiae complex* and the most dominant *Culex* species were *Cx. quinquefasciatus* followed by *Cx. Univittatus* (Elmalih and Hassan, 2018) [6]. *Culex quinquefasciatus* and *Aedes aegypti* are key mosquito species within the *Culex* and *Aedes* genera found in Sudan (Rathor, 2000) [7]. *Culex quinquefasciatus* stands as the predominant mosquito species in the Khartoum region of Sudan, as noted by El Rayah and Abu Groun in (1983) [8].

In the wake of DDT's discovery, mosquito control has heavily leaned on synthetic organic insecticides. However, the rise of insect resistance, escalating costs, and environmental harm have spurred a quest for alternatives. Research on natural plant products as replacements for synthetic pesticides has emerged as a promising solution. These studies suggest that natural plant-based products could effectively combat mosquito vectors while addressing the drawbacks of synthetic chemicals. This shift towards natural alternatives signifies a significant step towards sustainable and eco-friendly mosquito control methods. (Mittal and Subbarao 2003; Shaalan, *et al*. 2005; Nath, *et al*. 2006; Promsiri, *et al*. 2006) [9, 10, 11, 12].

Recent global studies have demonstrated the potent ovicidal, larvicidal, and adulticidal properties of medicinal plant extracts against mosquito vectors (Promsiri, *et al*. 2006; Kumar, *et al*. 2012a; Prathibha, *et al*. 2014; Palanikumar, 2017; Hussain A, *et al*. 2023) [12, 13, 14, 15, 16]. These findings highlight the promising potential of natural remedies in combating mosquito-borne diseases.

Calotropis procera and Ricinus communis plants are native to tropical Africa, but it has naturalized in many tropical and subtropical areas around the world. *Calotropis procera* and *Ricinus communis* L., are growing naturally in many parts of Sudan. *Calotropis procera* (Usher) is a tree or shrub species that is widely distributed in Sudan. The number of *C. procera* plants in Sudan might be estimated to be about 1530666. (Hashim and Mohammed, 2011) [17].

Many studies have focused on studying the effect of plants extracts against mosquito larvae as natural pesticides, while few have focused on their effect on the hatching rate. This study aims to explore the ovicidal properties of crude aqueous extracts from *Calotropis procera* and *Ricinus communis* leaves against *Culex quinquefasciatus*, the Filariasis vector. This indigenous approach to mosquito control is environmentally friendly, economically feasible, and easily applicable by individuals and communities.

Materials and Methods

Rearing of Mosquitoes

Mosquito larvae were collected from breeding sites in the area surrounding the College of Agriculture and the College of Veterinary Medicine, University of Khartoum, located on the eastern bank of the Nile in the city of Khartoum Bahri. The larvae were bred in controlled laboratory conditions at the Department of Preventive Medicine, Faculty of Veterinary Medicine, Khartoum University, maintaining a temperature of 25-28 °C. Their diet consisted of finely ground powdered yeast to ensure optimal growth and development. Pupae were gathered daily and placed in small bowls filled with fresh water, then transferred to net cages measuring 30×30×30 cm for adult emergence. To sustain the adult mosquitoes, cotton soaked in a 10% sugar solution was provided in each cage. Periodically, adult mosquitoes were fed on pigeons for blood meals, with gravid females utilized for egg laying. Petri dishes equipped with moistened cotton or filter papers were included in each cage to facilitate egg laying.

Plant collection and extraction

Leaves of the plants *Ricinus communis* L., (Family: Euphorbiaceae) and *Calotropis procera* Ait. (Family: Apocynaceae) were collected from the area around the compous of the College of Agriculture and Veterinary Medicine, University of Khartoum, which located on the eastern side of Nile River in the city of Khartoum Bahri, Sudan. The leaves were collected during the plant's flowering stage, dried in the shade, and finely ground into powder. Five grams of each plant's powder was soaked in a separate 500ml container with 250 ml of distilled water. After 24 hours of occasional vigorous shaking, the solution was filtered and the residue washed multiple times with distilled water. The final volume was adjusted to 500ml to create a 1% stock solution, which was then diluted to the desired test concentrations.

Ovicidal assay

The ovicidal activity of *Ricinus communis* and *Calotrpis procera* extracts was tested using the method of Su & Mulla (1998) [18]. Freshly laid *Culex quinquefaciatus* eggs were exposed to varying concentrations of the extracts, with each concentration replicated three times. Dechlorinated tap water served as the control. The hatching rate was determined 5 days post-treatment by counting the number of hatched eggs. Egg mortality was calculated based on the number of unhatched eggs.

Statistical analysis

The data underwent probit analysis to determine the regression equation (Y = a + bx). LC50 and LC90 concentrations, along with fiducial limits (F.L.) and 95% confidence limits (C.L.), were calculated.

Results and Discussions

The efficacy of crude aqueous leaf extracts from *C. procera* and *R. communis* against *Cx. quinquefasciatus* eggs was evaluated. Statistical analysis revealed LC_{50} - LC_{90} values of 191.87-510.51 ppm and 961.612-1786.488 ppm, respectively. Notably, the extract from C. procera exhibited greater potency than that of R. communis against *Culex quinquefasciatus* eggs. Detailed data can be found in Table 1, Figure 1, and Figure 2. The correlation between egg mortality rates and extract concentration was found to be directly proportional in both plant species. These results are in line with previous studies, indicating a high level of consistency. In a study by (Ahmad Afaq, *et al.* 2023) [19] the potential of locally sourced aqueous plant extracts against *Aedes aegypti* mosquitoes was explored. The results were nothing short of impressive, with *Parthenium hysterophorus* extract proving to be highly effective, achieving a 100% success rate across all mosquito life stages. *Nicotiana tabacum*, *Fagonia indica*, and *Melia azedarach* followed suit in decreasing order of efficacy. The study also highlighted the significant impact of extract concentration and exposure time on *Aedes aegypti* egg hatching. Overall, the findings underscore the promising ovicidal effects of *P. hysterophorus* extract, even at low concentrations and extended exposure times.

The hatchability of *Ae. aegypti* eggs was significantly reduced when exposed to *Callistemon citrinus* leaf extract. The decrease in hatching rate was directly correlated with the concentration of the leaf extract (Palanikumar *et al*,2017) [15].

In a study examining the ovicidal properties of leaf extracts from four plant species - *Eugenia jambolana*, *Solidago canadensis*, *Euodia ridleyi*, and *Spilanthes mauritiana* - against three mosquito species, *Anopheles stephensi*, *Aedes aegypti, and Culex quinquefasciatus*, it was found that as the concentration of the extracts increased, the hatchability of eggs decreased. Furthermore, the hatchability was directly proportional to the number of eggs (Prathibha *et al*. 2014) [14].

The ovicidal potential of the crude extracts from five medicinal plants *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* against three species of mosquito, *An. stephensi*, *Ae. aegypti* and *Cx. tritaeniorhynchus*. showed 100% ovicidal activity against the three mosquito species (no hatchablity was recorded) at 200ppm concentration of all five plants (Dhanasekaran, S. *et al*. 2013) [20]. The toxicity of three species of plants against the different life stages of *Cx. Quinquefasciatus*, showed that the *Justicia adhatoda* was most effective one as larvicide, pupicide, and adulticide followed by *Artemisia scoparia*, then the *Ailanthus altissima* in the last against *Cx. Quinquefasciatus* (Hussain A. *et al* 2023) [16]. Aqueous leaves extracts of *Calotropis gigantea* showed moderate ovicidal effects at 1000 ppm concentration against *Cx. gelidus* and *Cx. tritaeniorhynchus* mosquitoes (Kumar, G., *et al*. 2012a) [13]. The aqueous leaf extract of *C. procera* against *Cx. Quinquefasciatus* and *An. arabiensis* larvae showed that, the lethal concentration that kill 50% and 90% (LC50–LC90) were 187.93–433.51, 218.27–538.27 and 264.85–769.13 ppm against 2nd, 3rd and 4th instar larvae, of *Cx. Quinquefasciatus*. And 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 ppm against 2nd, 3rd and 4th instar larvae of *An. Arabiensis* (Elimam *et al*., 2009a) [21]. The latex of C. procera demonstrated significant efficacy in inhibiting egg hatching and larval development of *Aedes aegypti* (Marcio V. *et al*., 2006) [22]. The study revealed that even a minute concentration of *C. procera* latex (0.1%) in water, which previously showed no mortality effect in larvicidal efficacy tests, was surprisingly effective in causing a significant mortality rate of 94.1% in *Aedes aegypti* eggs. This finding underscores the potent insecticidal properties of *C. procera* latex and its potential as a natural alternative for controlling mosquito populations (Manju *et al*, 2004) [23]. The aqueous extract derived from *C. procera* leaves exhibited potent ovicidal activity against *Cx. tritaeniorhynchus* and *Cx. gelidus* eggs, achieving a remarkable 100% inhibition of hatching at a concentration of 1000 ppm. Furthermore, the extract demonstrated a dose-dependent effect on both mosquito species. These findings underscore the potential of *C. procera* as a natural and effective means of controlling mosquito populations (Kumar G, *et al*. 2012b) [24]. The study consistently observed a 100% ovicidal activity of *Carica papaya* leaves extracts against *Cx. quinquefasciatus* at a concentration of 300 ppm. This finding remained consistent throughout the entire duration of the study (Anuskha and Dhivya 2017**)** [25]. A remarkable 100% ovicidal activity was demonstrated by *Cymbopogan citratus* oil at a concentration of 300 ppm against *Culex quinquefasciatus*. (Pushpanathan, T *et al*. 2006) [26]. Methanol extract of *Erythrina indica* against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatusn* showed 100% eggs mortality (no hatching) at 150, 200, and 250 ppm, respectively (Govindarajan and Sivakumar, 2014) [27]. The foliage extract of *Albizia lebbeck* showed hundred percent ovicidal activity at 250, 200, and 150 ppm against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*, respectively (Govindarajan and Rajeswary, 2015) [28].

The aqueous extracts from *R. communis* leaves exhibit significant larvicidal and oviposition deterrent effects against *An. arabiensis* and *Cx. Quinquefasciatus* mosquitoes. The 50% larvicidal activity was observed at concentrations of 403.65, 445.66, and 498.88ppm for 2nd, 3rd, and 4th larval instars of *An. arabiensis*, and 1091.44, 1364.58, and 1445.44ppm for 2nd, 3rd, and 4th larval instars of *Cx. quinquefasciatus*. These findings highlight the potential of R. communis as a natural and effective solution for mosquito control (Elimam *et al*., 2009b) [29]**.** The larvicidal properties of an aqueous extract from *R. communis* leaves demonstrated significant efficacy against *Aedes albopictus*, with ovicidal activity reaching 50% and 90% at concentrations of 149.57, 268.92 ppm and 155.57, 279.92 ppm for 2nd and 3rd instar larvae respectively (Waris M, *et al*. 2020) [30].

The ovicidal activity of *Ricinus communis* extracts against *Phlebotomus duboscqi*, showed that 250 µg/ml and 500 µg/ml of extract eroded all the chorionic membranes of the egg shell. While eggs hatching was inhibited to 7%, 9% and 26% at 500 µg/ml of aqueous, methanol and ethyl acetate extracts respectively (Samuel M. *et al*. 2016) [31].

The flavonoids extracted from the leaves of *Ricinus communis* L demonstrated significant insecticidal and ovicidal effects against *Callosobruchus chinensis* L (Coleoptera). These findings suggest that these compounds have the potential to be used as natural insecticides for pest control (Upasani *et al*, 2003) [32].. The essential oil extracted from the *Cymbopogan citratus* plant exhibited significant larvicidal and ovicidal activity against the *Culex quinquefasciatus* mosquito. Interestingly, the eggs displayed higher susceptibility to the plant extract compared to the larvae. A remarkable 100% ovicidal activity was observed at a concentration of 300ppm (Pushpanathan T. *et al*, 2006) [27]. The leaves extract of *Solanum trilobatum* L. showed zero hatchability (100% eggs mortality) at 100 ppm dose against *Culex quinquefaciatus* and *Culex tritaeniorhynchus* (Rajkumar and Jebanesan, 2004) [33].

Table 1: Ovicidal effects of foliage extract of *Calotropis procera* and *R. communis* against *Culex quinquefasciatus*

Plant species	LC_{50} ppm	LC_{90} ppm	Regression Equation	F.L with 95% C. L	r^2
Calotropis procera	191.867	510.505	Y=3.014X – 1.882	±2.373	0.930
Ricinus communis	961.612	1786.488	Y=4.758X – 9.193	± 2.076	0.975

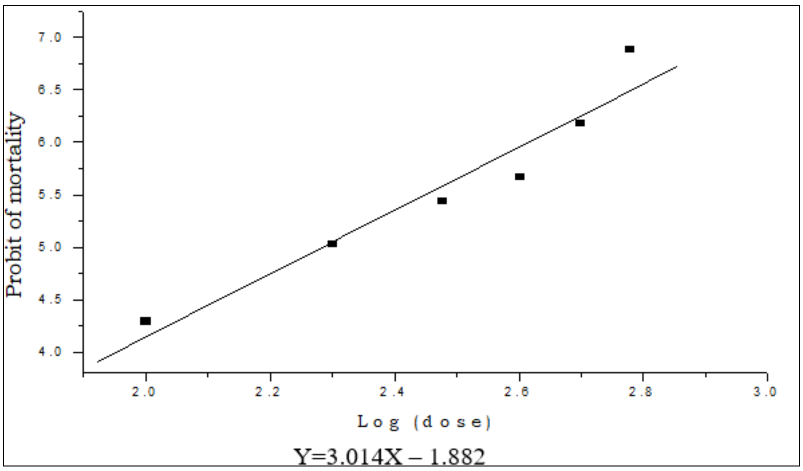

Fig 1: Ovicidal effects of foliage extract of *Calotropis procera* against *Culex quinquefsciatus* expressed as linear regression

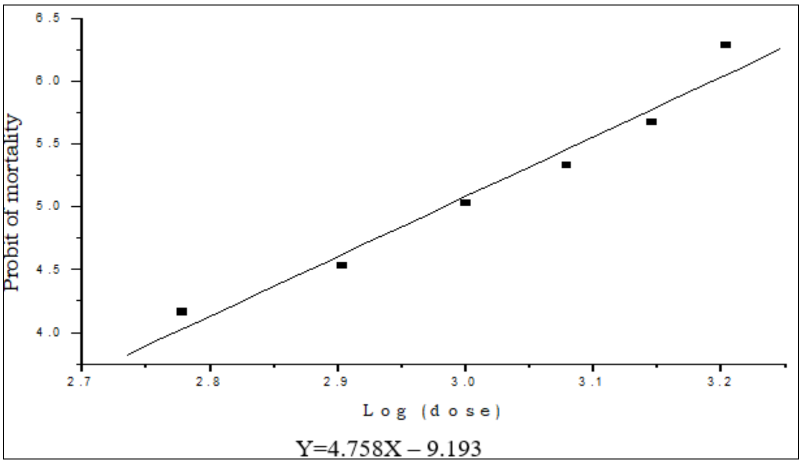

Fig 2: Ovicidal effects of foliage extract of *Ricinus communis* against *Culex quinquefsciatus* expressed as linear regression.

Conclusion

In conclusion, the study demonstrated that aqueous leaf extracts of *C. procera* and *R. communis* possess significant ovicidal properties against *Culex Quinquefasciatus*. The higher the concentration of the extract, the greater the mortality rate of eggs in both plant species. Notably, the extract from *Calotrpis procera* was more effective in inhibiting egg hatching compared to *Ricinus communis*. These plant extracts are environmentally friendly, readily available in Sudan, and easily extractable with water. Therefore, they hold promise for use in controlling mosquito-borne diseases as natural mosquitocides.

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Declaration of Conflicting Interests

The author(s) have disclosed that they have no conflicts of interest pertaining to the research, authorship, and/or publication of this article.

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