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Larvicidal Potency of *Dioscorea sansibarensis* Leaf Extract against Vector Mosquitoes: *Anopheles gambiae* s.s. and *Culex quinquefasciatus*

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Abstract

Mosquitoes are responsible for transmission of illnesses of public health importance including malaria, lymphatic filariasis, dengue, chikungunya, and many other diseases caused by viruses. Vector control using synthetic insecticides has been the cornerstone for management of vector-borne diseases. However, the chemical based interventions have not been sustainable due to emergency of resistance against insecticides among disease vectors. Plant based mosquitocidal products can be potential alternative tools in vector control. Therefore, the present study aimed at exploring the larvicidal properties of *Dioscorea sansibarensis* leaf extract against malaria and lymphatic filariasis vectors; *Anopheles gambiae* s.s. and *Culex quinquefasciatus*. The larvicidal activities of *Dioscorea sansibarensis* were assessed following WHO test procedures. Ethanol leaf extract of *Dioscorea sansibarensis* was evaluated against all the four instar larvae stages of *An. gambiae* s.s and *Cx. quinquefasciatus* susceptible laboratory colonies. The highest larvicidal potency was shown against the 4th instar stages of both species with the LC_{50} values of 60.915 ppm and 80.700 ppm for *Cx. quinquefasciatus* and *An. gambiae* s.s., respectively. The respective LC_{95} values for *Cx. quinquefasciatus* and *An. gambiae* s.s. were 168.898 ppm and 249.295 ppm. This implies that the extract can be applied as mosquito larvicide should its impact on non-targeted species be established.

Keywords: *Dioscorea sansibarensis*; vectors, mosquitoes, Zanzibar yams, Dar es Salaam, Tanzania.

Introduction

Mosquitoes transmit a variety of well-known vector-borne diseases, including malaria, filariasis, encephalitis, yellow fever, chikungunya, dengue and many other arboviral infections, causing mortalities and morbidities across the globe. Vector-borne diseases constitute over 17% of all infectious diseases, causing more than 700,000 deaths annually (WHO 2020a). Malaria alone caused approximately 229 million cases and 400,000 deaths in 2019, and the highest burden was recorded from WHO-African region which accounted 94% of all the cases (WHO 2020a, 2020b). Mosquitoes in the *Anopheles* genera are responsible for transmission of malaria parasites, *Anopheles gambie* s.l. being the main vector in Tanzania. *Culex quinquefasciatus* on the other hand, transmits *Wuchereria bancrofti* responsible for lymphatic filariasis. Lymphatic filariasis is the second most common vector-borne parasitic disease after malaria accounting to 120 million global cases annually (Jones et al. 2018). In Tanzania, the burden of malaria remains high with 14–18 million new cases and approximately 120,000 deaths reported each

year (Makundi et al. 2007). Vector control plays a central role in the prevention of malaria and other vector-borne diseases. The main vector control methods involve synthetic chemicals in form of Indoor Residual Sprays (IRS), Insecticide Treated Nets (ITNs)/Long Lasting Insecticidal Nets (LLINs) and larvicides. The past decade saw a significant decrease in incidences of vector-borne diseases as a result of massive scale up of the chemical based interventions (IRS and ITNS/LLINs) (Ashley et al. 2018, WHO 2020b). Malaria cases for example, decreased from 238 million in the year 2000 to 218 million cases in 2015, nevertheless, recent records suggest rebound of the disease (Khatib et al. 2018, WHO 2020b). Despite the massive scale up of ITNs/LLINs and IRS, the sustainability of these interventions is faced by several challenges, including: vector resistance to insecticides, change in vector feeding and biting behavior, outdoor malaria transmission and adaptation of mosquito to polluted environments (Killeen et al. 2016, Antonio-Nkondjio et al. 2018). Early biting of *An. gambiae* s.s. between 18:00–21:00 hours has been reported, this occurs when people are not under protection of ITNs/LLINs (Githeko et al. 1996, Wamae et al. 2015, Sougoufara et al. 2020). Similary, early biting of *Cx. quinquefasciatus* was recorded in the coastal region of Nigeria where a good number of females were trapped between 1800-1900 hours (Uttah et al. 2013). It was further reported that the circadian biting peak of *Cx. quinquefasciatus* was between 18.00 and 20.00 hours outdoors (Uttah et al. 2013), and this means that vectors are less likely to be controlled by the current interventions (LLINs/IRS) that target vectors indoors. The increasing trend of vector resistance to insecticides used in LLINs and IRS (Ranson et al. 2011, Nardini et al. 2012, Antonio-Nkondjio et al. 2018, Sougoufara et al. 2020), the environmental issues and toxicity of the chemical based insecticides that spill over to the food chain, and their impacts on non-targeted species (Sharma et al. 2016), have led to exploration of environmentally friendly, non-persistent and species specific plant products which are locally available.

In that regard, researchers have transformed their interests towards the development and uses of botanical products for controlling mosquitoes and other insects which are considered safe and environmentally friendly alternatives (Isman 2000). Phytochemical products have a long history of uses and proven evidence of efficacy as antimicrobial, antioxidants, anti-inflamatory, insecticidal, and repellants (Obidiegwu et al. 2020). Furthermore, several studies have explored and established the efficacy of botanical extracts and essential oils for vector control (Kalimuthu et al. 2012, Kweka et al. 2008, Kovendan et al. 2013). Nevertheless, the potential of *Dioscorea sansibarensis*, the commonly used plant by farmers against crop pests, to control mosquito vectors has not been explored.

The plant (*Dioscorea sansibarensis*), also known as Zanzibar yam, is native to Tanzania and one of the largest and most widely distributed species of the genus *Dioscorea* L. in the coastal zones. It is the climber which produces both bulbils and underground tubers. The tubers/yams of various *Dioscorea* spp are used for food in many countries, with therapeutic values (Obidiegwu et al. 2020), other species have been characterized as toxic. *Dioscorea sansibarensis* vegetative parts are evergreen with broad leaves but always undamaged by chewing insects; the destructive herbivores also avoid feeding on them (Mauti et al. 2019). Although the potential of *Dioscorea sansibarensis* to control crop pests has been reported (Price et al. 2016, Kumar et al. 2017, Mauti et al. 2019), information concerning the usefulness and promising uses of the plant against disease vectors is lacking. Owing to the increasing level of vector resistance against synthetic insecticides (Antonio-Nkondjio et al. 2018), it is fundamental to explore the plant based natural products that would complement the existing vector control interventions. Therefore, this study explored the larvicidal potency of *Dioscorea sansibarensis* crude extract against

the two vector species for possible screening of anti-mosquito agents.

Materials and Methods

Collection of plant materials

The leaves of the plant *Dioscorea sansibarensis* were collected from the small forest behind the Zoology-Botany buildings, Mwalimu Nyerere Campus, University of Dar es Salaam, Tanzania in June, 2020. One bucket of the leaves was collected by hand picking, the plant leaves were authenticated by a taxonomist from the Department of Botany, University of Dar es Salaam, where the voucher specimen (FMM 3910b) is deposited.

Extract preparation

The leaves of *Dioscorea sansibarensis* were washed with distilled water, and air dried under shade for seven days. The air-dried materials were powdered, and 1 kg powdered plant material was soaked in 3.0 L of ethanol for 24 hours then filtered by using cotton wool to obtain the filtrates. The filtrates were re-soaked for another 24 hours and taken to a rotary evaporator at 300 rpm for vaporization of ethanol, the remnant was the crude extract. The crude extract was stored in a refrigerator at 4 °C ready for larvicidal bioassays. Plant extract preparation was executed by modifying the published procedure (Kovendan et al. 2013, Mauti et al. 2019). A stock solution (1%) was prepared by diluting 200 mg of extract with 20 ml ethanol. From this stock solution various concentrations were prepared for subsequent larvae bioassays, low concentrations of extract were tried to establish the lethal dose, and for this reason the concentrations were converted to ppm by using the formula 1 mg/L = 1 ppm. The experiments for preparation of plant extracts were carried out at the Institute of Traditional Medicine of the National Institute for Medical Research, Tanzania.

Larvae rearing

The susceptible laboratory larvae were used for this study. The larvae were obtained from the Ifakara Health Institute (Bagamoyo branch) where the susceptible colonies are maintained. The first larvae instars of both *An. gambie* s.s. and *Cx. quinquefasciatus* were collected, fed on tetramine fish food and maintained at 27 °C and 70% relative humidity (Philbert and Ijumba 2013, Philbert et al. 2017). All the four larvae instar stages were involved in assessments of plant extract pontency.

Larvae bioassays

The larvicidal potency of *Dioscorea sansibarensis* leaf extract was assessed at the following concentrations: 25 ppm, 50 ppm, 75 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm for all the four instar stages of both *An. gambie* s.s. and *Cx. quinquefasciatus*. Mosquito larvae were separated in batches of 20. Each batch was transferred into a 250 ml beaker containing a corresponding volume of water and a stock solution. Each bioassay had four replicates carried out concurrently at the same conditions and a control which contained water and ethanol as a solvent to make corresponding test volumes (100 ml). The procedure was repeated for each extract concentration (25 ppm, 50 ppm, 75 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm), and for all the four larvae instar stages (I–IV) for both species. The dead larvae were recorded 24 hours after exposure. The larvae were touched gently using a plastic pipette and were considered dead in the absence of any signs of movement. Bioassay experiments were conducted following the WHO test procedure (WHO 2013).

Data analysis

The mean larvae mortality rate were computed and subjected to probit analysis for calculating the lethal concentrations at 50% and 95% (LC_{50} & LC_{95}) and other statistics at 95% fiducial values of upper confidence limit (UCL) and lower confidence limit (LCL) values, and paired sample t-test was computed to establish differences in mortality values between *An. gambie* s.s. and *Cx. quinquefasciatus* larvae.

Results

The assessment of larvicidal potential of *Dioscorea sansibarensis* leaf extract by ethanol solvent carried out against the four instar stages of *An. gambiae* s.s. and *Cx. quinquefasciatus* are presented in Figures 1 and 2. The mortality rates are directly proportional to the concentrations of the extract across the four larvae instar stages of both species. Complete larval mortality (100%) occurred at the highest concentrations between 300 and 400 ppm, and the lowest mortality (2.5% and 12.5%) occurred at the lowest concentrations (25 and 50 ppm) for *An. gambie* s.s. and *Cx. quinquefasciatus*, respectively.

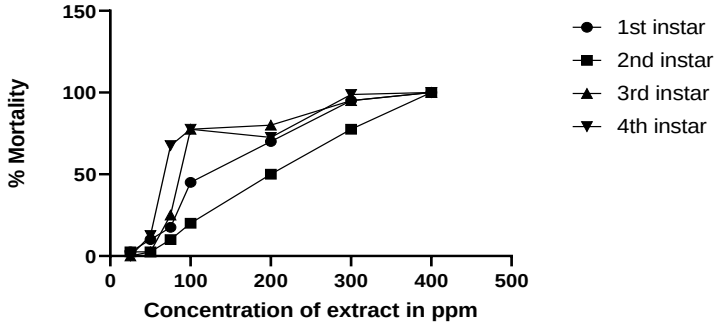

Figure 1: Percentage mortality of different larvae stages of *An. gambiae* s.s. against various concentration of *Dioscorea sansibarensis* leaf extracts.

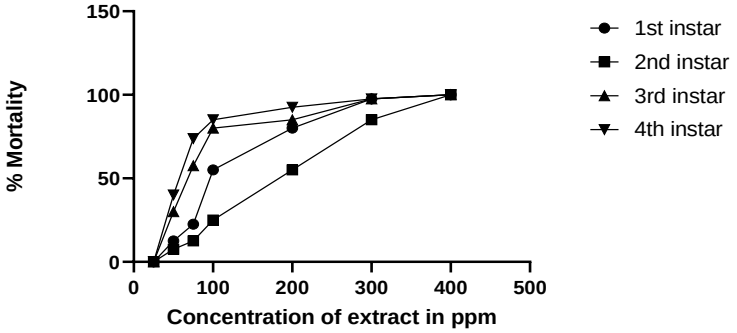

Figure 2: Percentage mortality of different larvae stages of *Cx. quinquefasciatus* against various concentrations of *Dioscorea sansibarensis* leaf extracts.

The results showed that 24 hours exposure of the larvae could result in 100% mortality, irrespective of the developmental stage (Table 1). The control groups did not result in any larvae mortality after 24 hours. The lethal concentration to cause 50% mortality of the larvae ranged from 50.879 to 167.529 ppm for *Cx. quinquefasciatus* and 66.290 to 215.795 ppm for *An. gambie* s.s. with higher values recorded against second instar stages of both species. The lethal concentration of 95% larvae mortality was within the fiducial range values of 134.580–526.518 ppm and 249.295–919.045 ppm for *Cx. quinquefasciatus* and *An. gambie s.s.*, respectively. The study revealed the highest larvicidal potency of the extract against the 4th instar stages for both species with the mean concentrations of LC_{50} of 80.700 ppm and 60.915 ppm for *An. gambie* s.s. and *Cx. quinquefasciatus*, respectively (Table 2).

Table 1: Percentage mortality of different larvae stages of *An. gambiae* s.s. and *Cx. quinquefasciatus* mosquitoes exposed to various concentrations of *Dioscorea sansibarensis* ethanolic leaf extract

Larvae stage	**Concentrations in ppm**	***An. gambiae* s.s.** **% mortality ± SD[a]**	***Cx. quinquefasciatus*** **% mortality + SD[a]**	**P -value**
1st instar larvae	25	2.5 ± 5	0.0	0.0175
	50	10 ± 5	12.5 ± 0	
	75	17.5 ± 0	22.5 ± 5	
	100	45 ± 5	55 ± 5	
	200	70 ± 19.15	80 ± 5.77	
	300	95 ± 8.16	97.5 ± 8.16	
	400	100 ± 5.16	100 ± 5	
	Control	0 ± 0	0 ± 0	
2nd instar larvae	25	2.5 ± 5	0 ± 0	0.0488*
	50	2.5 ± 5	7.5 ± 5	
	75	10 ± 8.16	12.5 ± 9.57	
	100	20 ± 0	25 ± 5.77	
	200	50 ± 8.16	55 ± 5.77	
	300	77.5 ± 5	85 ± 5.77	
	400	100 ± 0	100 ± 0	
	Control	0 ± 0	0 ± 0	
3rd instar larvae	25	0 ± 0	0 ± 0	0.1046
	50	2.5 ± 5	30 ± 8.16	
	75	25 ± 5.77	57.5 ± 9.57	
	100	77.5 ± 5	80 ± 0	
	200	80 ± 8.16	85 ± 5.77	
	300	95 ± 5.77	97.5 ± 5	
	400	100 ± 0	100 ± 0	
	Control	0 ± 0	0 ± 0	
4th instar larvae	25	0 ± 0	0 ± 0	0.0871
	50	12.5 ± 5	40 ± 14.14	
	75	67.5 ± 9.57	73.8 ± 4.78	
	100	77.5 ± 5	85 ± 5.77	
	200	72.5 ± 13.3	92.5 ± 5	
	300	98.8 ± 2.5	97.5 ± 5	
	400	100 ± 0	100 ± 0	
	Control	0 ± 0	0 ± 0	

* = significant at 95% CI; paired sample t–test; SD[a] = standard deviation for the mean of 4 replicates. The overall interaction was significant (p = 0.0011), with significantly higher mortality in *Cx. quinquefasciatus* than *An. gambiae* s.s. two-tailed t = 3.658, df = 28.

Table 2: Larvicidal potential of the leaf ethanol extract of *Dioscorea sansibariensis* against the four instar stages *An. gambiae* s.s. and *Cx. quinquefasciatus* after 24 hours exposure time

Mosquito species	Instar stage	LC_{50} (ppm)	95% Fiducial limits	LC_{95} (ppm)	95% Fiducial limits
An. gambiae s.s	1	118.363	104.111-134.725	343.186	279.084-456.422
	2	169.659	135.839-215.795	500.129	355.494-919.045
	3	101.229	88.630-115.268	254.135	208.677-337.740
	4	80.700	66.290-96.391	249.295	191.017-380.607
Cx. quinquefasciatus	1	105.099	96.493-114.478	273.140	237.194-326.528
	2	152.944	139.888-167.529	433.374	371.365-526.518
	3	72.653	65.759-79.808	218.095	187.299-265.042
	4	60.915	50.879-71.150	168.898	134.580- 240.931

Discussion

The crude ethanol extract of *Dioscorea sansibariensis* has a significant larvicidal potency against the two important vector mosquitoes *Cx. quinquefasciatus*, and *An. gambiae* s.s. In the present study, the larvicidal effects of the plant extract against the four instar larvae stages varied according to the concentrations used. The larvae mortality increased with increasing concentrations and the maximum mortality (100%) was attained between 300 and 400 ppm extract concentrations for both species. The LC_{50} values ranged from 60.915 to 152.944 ppm for *Cx. quinquefasciatus* and 80.700 to 169.659 ppm for *An. gambiae* s.s. The highest larvicidal activities were observed for the 4th instar stages of both species. These results are comparable to other previous studies (Sakthivadivel and Daniel 2008, Bagavan et al. 2009). The assessment of the toxicity of six medicinal plant extracts, *Acacia nilotica, Jatropha Cxrcas, Citrullus colocynthis, Withania somnifera* (leaves), *A. mexicana* (leaves and seeds) also resulted in an LC_{50} value of less than 100 ppm against 3rd instars of *Cx. quinquefasciatus, An. stephensi*, and *Ae. aegypti* (Sakthivadivel and Daniel 2008). Similary, the leaf extracts of the plants *Citrus sinensis, Ocimum canum, Rhinacanthus nasutus* and *Ocimum sanctum* when tested against larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* gave the LC_{50} values ranging between 21.6–88.15 ppm and 39.32–109.12 ppm, respectively (Bagavan et al. 2009). Similar findings were also reported when the larvicidal activity of ethanol *Cadaba indica lam* leaf extract was investigated against *Ae. aegypti* which gave the LC_{50} value of 143.75 ppm (Kalimuthu et al. 2012).

Several studies have shown that plants are sources of bioactive compounds that can be used to develop environmentally safe anti-vector and anti-pest agents which are cheap and user friendly. The screening of local medicinal plants for mosquitocidal agents from plants have been attempted in many countries some decades ago (Crobsy 1971, Berenbaum 1985). Nevertheless, the applications of these products for vector control at field operation level are limited. The scale up of synthetic insecticides in terms of ITNs and IRS led to decreased malaria cases in most malarious countries (O'Meara et al. 2010, Ashley et al. 2018), but recent reports suggest the disease resurgence (WHO 2020b). This increase is attributed to vector resistance to insecticides, parasite resistance to anti-malaria drugs as well as change of vector behavior with more species now biting earlier before bed time. Early biting of both malaria and lymphatic filariasis vectors have been reported from many countries (Githeko et al. 1996, Uttah et al. 2013, Wamae et al. 2015, Killeen et al. 2016, Sherrard-Smith et al. 2019), thus the need for new vector control alternatives to complement the existing ones is now pertinent than ever.

The control of diseases vectors has been responsible for shrinking the map of many vector-borne diseases, and the need for the

integrated vector control methods (IVM) using both insecticides and non-insecticide based methods is emphasized (Wilson et al. 2020). The overreliance on the chemical based interventions that replaced the traditional methods such as environmental management, habitat modification/manipulation making them unsuitable for larvae development has proved abortive. Moreover, several attempts to screen medicinal plants for antimosquito products including ovicides, larvicides, adulticides and repellants have not been scaled up for possible applications in the fields. Many studies are laboratory based and the findings remain of academic interests. The *Dioscorea sansibarensis* leaf extract showed potency against all the four instar larvae stages of the main vector species investigated. These are preliminary findings that need to be further investigated to characterize the phytochemical compounds and functional groups to establish the impacts of the product against other untargeted species for possible scale up. This calls for increased investment in vector control interventions that will utilize indigenous knowledge and local products other than synthetic chemicals.

Conclusion

The findings of this study clearly showed the larvicidal efficiency of *Dioscorea sansibarensis* against *An. gambiae* s.s. and *Cx. quinquefasciatus* vector mosquitoes. Although the safety of the product to humans and other untargeted species remains unknown, the plant is suitable candidate for development of mosquitocidal product. Extensive studies on this plant against other mosquito stages (eggs, pupa and adults), its repellency properties, longevity, bioactive compounds and their modes of action are required.

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Declaration of interest

The author declares no competing interests.

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