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Larvicidal Activity of Aquatic Carnivorous Plants on Anopheles Mosquito Larval Stages

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Abstract: Malaria is still a major killer disease in tropical Africa, contributing 10% to the overall disease burden. The Plasmodium parasites are mainly transmitted by the mosquitoes *Anopheles gambiae* and *A. funestus* in Uganda. Approaches for malaria vector control include denial of breeding ground near human dwellings, use of insecticide treated nets and chemical spraying. Chemical approaches raise challenges of vector resistance, non-target specificity and ecological and human health concerns. In view of these draw backs, this research explored the effects of carnivorous plants of the genera; *Aldrovanda vesiculosa* (*A. vesiculosa*) and *Utricularia reflexa* (*U. reflexa*) against the fourth instar larvae of Anopheles mosquito. *A. vesiculosa* and *U. reflexa* are aquatic carnivorous plants that derive some or most of their nutrients from trapping and consuming animals or protozoans, typically insects and other arthropods. Aldrovanda and Utricularia grow in water and have bladder traps that suck in prey by generating an internal vacuum. Some of the prey may include mosquito larvae from the breeding sites. The plants were collected from the wild and allowed to grow in the laboratory. Larvicidal bioassays were done to determine their effectiveness to ingest and reduce the population of mosquito larvae in the laboratory. The carnivorous plants remarkably reduced the larvae population in 5 days. All the 20 larvae in each of the control vessels remained alive and active during the test period. It was concluded that aquatic carnivorous plants are potential biological agents for ecologically sound and sustainable strategy for controlling malaria in Uganda.

Key words: Carnivorous plants, larvicidal, malaria, anopheles mosquito, transmitted, dwettings

INTRODUCTION

Malaria is Africa's major cause of mortality in those <5 years of age and constitutes 10% of the overall disease burden (WHO, 2009a). Mosquitoes of the genus Anopheles is the most important malaria vector in sub-Saharan Africa (White, 1974). The most common species that transmit malaria in Uganda are *Anopheles gambiae s.s* and *Anopheles funestus* Giles. Current mosquito vector control methods are based on insecticide or chemical larvicide applications in breeding sites, Indoor Residual Spraying (IRS) and Insecticide Treated Bed Nets (ITNs). Anopheles larval control has been shown to suppress malaria in tropical Africa (Walker and Lynch, 2007). Biological larval control methods using mosquito infective agents (fungi, nematodes and bacteria), plants that cover water surfaces and prevent multiplication of larvae (e.g., *Azolla* sp.) and larvae-eating fish have also been used to control mosquito populations around human dwellings (Walker, 2002). Research is also in progress on the use of genetically engineered sterile mosquitoes to interrupt multiplication of mosquitoes (WHO, 2009b). However, insecticide resistance and lack of target specificity pose serious threats to sustainable insecticide-based vector control in many African countries. Insecticides also pose serious environmental and human health concerns. Furthermore, it is found that these control methods are not sufficient on their own to eliminate or reduce malaria burden (Walker and Lynch, 2007). There is therefore increasing need to develop Novel Vector Control Methods that can complement or replace existing intervention tools.

Aquatic carnivorous plants that grow in Uganda include 19 species of Utricularia and one species of Aldrovanda (*A. vesiculosa*) (Katende and Lye, 1998). These carnivorous plants trap and kill their prey like mosquito larvae by ingenious pitfalls and traps as a source of nitrogen required for protein synthesis. They have modified leaves that trap insects by bladder trays that suck the insects (Slack and Gate, 2000). The aquatic

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plants (Aldrovanda and Utricularia) are suited for capturing mosquito larvae because they can be viewed as motile if the water is a little agitated hence, increasing their chances of finding the prey. The natural habitats of these species include still or slow flowing water. These properties imply potential for aquatic carnivorous plants to be used as biological control agents against Anopheles larvae. When the aquatic carnivorous plants feed on mosquito larvae, they are described as larvivorous.

In this study, it was hypothesized that aquatic carnivorous plants may control mosquitoes and reduce their population in and around human dwellings by destroying their larvae in breeding places. The aquatic plants, deployed in breeding sites were expected to ingest larvae, thereby reducing the mosquito population and hence human/vector contact. No study has been carried out to explore the possibility of using these plants as biological agents to control malaria. Therefore, the present study was designed to explore the possibility of using live *A. vesiculosa* and *U. reflexa* to control Anopheles larvae under laboratory conditions. Researchers are reporting the results of larvicidal bioassays that screened these plants as a possible strategy for malaria control in Uganda. The larvicidal activity of these plants, on the survival of Anopheles larvae in the laboratory was investigated. The residual effects of these plants on Anopheles larvae in a second set of experiments where the plants were transferred to new containers with fresh larvae was also determined.

MATERIALS AND METHODS

This study was designed to test the hypothesis that the larvivorous plants may be able to trap and kill Anopheles larvae in mosquito breeding vessels and therefore, reduce the population of mosquitoes around human dwellings. Mosquito larvae were reared and the 4th instar stages were transferred into vessels that contained the plants. The number of living larvae in each of the test containers was determined every 12 h and compared with survival in the control vessels that did not contain the carnivorous plants.

Study design: This study was a laboratory experimental study designed to test larvicidal efficacy of *A. vesiculosa* and *U. reflexa*, some of the aquatic carnivorous plants growing in Uganda. These plants have leaves modified into organs that trap their prey. They both have modified leaves which can be triggered to close into vacuoles (bladders) by touch and chemical stimulation of their sensitive hairs. They trap their prey that is then digested by enzymes released into the bladders. They are both submerged floating aquatic plants making them suitable for engulfing mosquito larvae. Test experiments were done with vessels that contained the live plants while the control tests had no plants deployed in the vessels.

Plant collection: Field trips were made to the swamps where the herbarium specimens had been originally collected. The aquatic plants were collected from Mabamba swamp, on the shores of lake Victoria located in Wakiso district in Southern Uganda. Both *U. reflexa* and *A. vesiculosa* were found floating on water along the edges of the channel. Whole plants were scooped out and kept in 5-10 L buckets half filled with water from the swamp. The plant samples were transported to the Botany Herbarium Garden in Makerere University for monitoring growth and survival rate. Some plant samples were taken to the Department of Pharmacology and Therapeutics for larvicidal bioassays. Verification of plant identification was done in the Herbarium. About 500 mL of water samples from the collection sites was also collected in clean and dry plastic bottles for chemical analysis.

Rearing anopheles mosquito larvae: Plastic rectangular containers (15×20 cm) were half-filled with distilled water and placed outside the Department of Pharmacology and Therapeutics in partial shade near the laboratory animal house. The rabbits in the animal house acted as mosquito attractants. White filter paper was folded and placed on the water surface to provide the mosquitoes with resting place while laying their eggs as described by Matovu and Olila (2006). The set up was observed for 2 weeks to monitor the hatching of the eggs into larvae. Initially, water collected from the swamp was used as larval rearing medium but later distilled water was used successfully. The larvae were identified in the Department of Zoology, Entomology Unit in the School of Biological Sciences, Makerere University as Anopheline. The bioassays were done using the 4th instar mosquito larvae.

Larvicidal bioassays: Three paired containers, 10×15×6 cm were filled with distilled water. Bread crumbs was added in the containers as feeds for the larvae. Individual plants of about 10 cm length were placed in each of the three test containers while the other three control containers were left without any carnivorous plant as controls. Twenty 4th instar larvae were counted and placed in each of the six containers using plastic pipettes. Live larvae in each container were counted every 12 h for 5 days. After 1 week, the tests were repeated with the same carnivorous plants but with fresh sets of larvae. This was to determine the residual activity; the period the plants would remain effective against the mosquito larvae when they are outside their natural habitat. The mean numbers of the live larvae in the test vessels were compared to those in the control vessels using the Student's t-test.

RESULTS AND DISCUSSION

Larvicidal tests were done by exposing the mosquito larvae to the plants in plastic containers. The survival of the larvae was observed and recorded every 12 h for 5 days. The experiments were done in triplicate and the mean number of live larvae was obtained and compared to the control experiments.

Both plants were observed actively ingesting Anopheles larvae in their bladders. The number of live larvae in the test containers progressively reduced and by the 5th day there was no more larvae in the Utricularia test while only one of the three vessels of Aldrovanda had three remaining larvae. All the 20 larvae in each of the control vessels remained alive and active during the test period and some had pupated. The results are shown in Fig. 1. The results in Fig. 1 and 2 show very rapid decline in the number of mosquito larvae in the test containers. Unlike in the control experiments, the carnivorous plant deployment registered high mosquito larval mortality caused by their larvivorous activity. *U. reflexa* was particularly more effective at ingesting and killing the mosquito larvae than *A. vesiculosa*. The difference in survival rate of the Anopheles mosquito larvae between treatments with carnivorous plants and the control experiments was statistically significant ($t = -4.754$, $p = 0.005$ for *A. vesiculosa*; $t = -5$, $p = 0.004$ for *U. reflexa* and $t = -3.384$, $p = 0.028$ for *A. vesiculosa*; $t = -3.896$, $p = 0.018$ for *Utricularia reflexa*) in the plants freshly and in the residual larvicidal test, respectively. These results suggest that *A. vesiculosa* and *U. reflexa*, aquatic carnivorous plants may eliminate Anopheles mosquito larvae from breeding sites. Most of the larvae in the test containers did not survive >72 h while majority of the larvae in the control containers survived and had started pupating by the end of the experimental period. The larvicidal property of these plants could be useful in reducing the populations of Anopheles mosquitoes around human dwellings and thereby control malaria transmission when they are deployed in mosquito breeding sites. While this study demonstrated the efficacy of the plants to control the population of Anopheles larvae under the laboratory conditions, it is still not known if the plants can survive in the natural mosquito breeding sites. The composition of the breeding site water may differ from the swamp water or distilled water that was used to rear larvae and perform the bioassays. It is therefore necessary to determine the larvicidal effects of these plants in different ecological sites where the Anopheles mosquitoes breed. The scope of this research was limited in the laboratory. It is suggested that the effectiveness of the plants in controlling the mosquito populations should be done under field conditions using the method of Zairi and Lee (2005). This study is planned in the second phase of the grant.

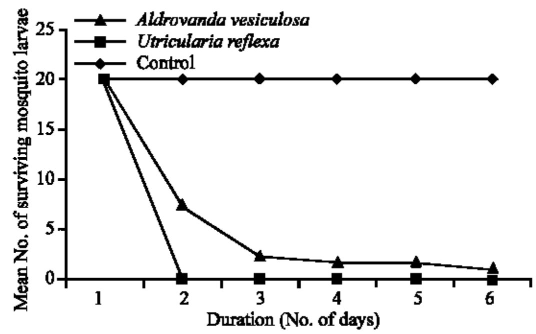

Fig. 1: The larvicidal activity of fresh *Aldrovanda vesiculosa* and *Utricularia reflexa* on Anopheles larvae. *Utricularia reflexa* killed the larvae within 2 days while *Aldrovanda vesiculosa* reduced the larvae to <20% within 3 days. All larvae in the control tests were alive by the 6th day

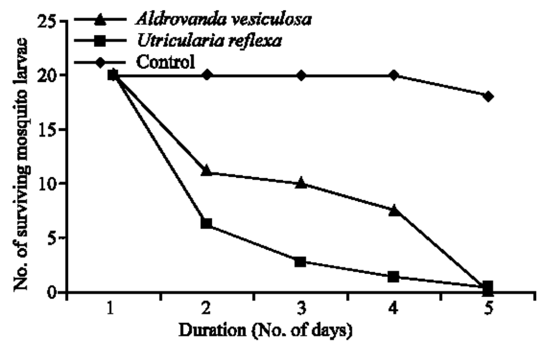

Fig. 2: The residual larvicidal activity of *Aldrovanda vesiculosa* and *Utricularia reflexa* on Anopheles larvae. The graph shows that both plants were still showed significant larvicidal activity after staying in the laboratory for 7 days. The number of Anopheles larvae was reduced to zero within 5 days of exposure to the plants

Larval control of vector Anopheles mosquitoes is a well-proven preventive method that deserves renewed consideration for malaria control particularly in sub-Saharan Africa (Walker and Lynch, 2007). The Malaria Control Policy in Uganda is now based on indoor residual spraying, insecticide treated nets or early case detection and treatment (Matovu, 2005). But increasing resistance of malaria parasites to antimalarial drugs and vector resistance to insecticides (Berg, 2009; Walker, 2002), inadequate health care systems, population displacements, widespread poverty and declining community acceptance have reduced the effectiveness of these approaches. At the same time, ecological changes driven by deforestation (Krupnick and Kress, 2003), human migration and unmanaged urbanization have increased the densities of human hosts and vector breeding sites in the country. Given the variability of malaria parasites, the vector and the vulnerability of human populations in Uganda, there is need for a range of malaria control approaches including selective and sustainable vector control. Although, unlikely to replace insecticide-based adult mosquito control, improved non-chemical larval control methods could offer sustainable supplements to other malaria vector and disease control efforts (Killeen *et al*., 2000). These findings suggest that *A. vesiculosa* and *U. reflexa* could be used to control malaria, especially in urban areas where the human density is high relative to the number of breeding sites.

The study demonstrated that the carnivorous plants significantly reduced the population of Anopheles larvae under laboratory conditions. They also demonstrated significant residual larvicidal effects on the larvae. The plants may have a potential in the integrated management of malaria by controlling the density of malaria vectors around human dwellings.

CONCLUSION

Aquatic carnivorous plants, growing in Uganda are capable of ingesting Anopheles mosquito larvae, thereby reducing their populations. While it is still too early to think of integrating carnivorous plants in malaria control strategy in Uganda, researchers recommend further research on this biological strategy in controlling malaria. The researchers plan field trials and pilot testing. The field trials would establish the efficacy of these plants in reducing the population of mosquitoes in different ecological mosquito breeding sites.

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