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Toxorhynchites Rutilus Rutilus Larvae: A Potential Biological Control Agent for Malaria in Uganda

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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 larvivorous effects of *Toxorhynchites rutilus rutilus* (Giant mosquito) larvae which prey on larvae of smaller mosquitoes, against the fourth instar larvae of Anopheles mosquito. *T. rutilus rutilus* larvae were reared in the laboratory and larvicidal assays were done to determine their effectiveness to eat and reduce the *Anpopheles* larvae population in the laboratory. The *T. rutilus rutilus* larvae cleared all the 120 Anopheles larvae within 24 h. All the 120 Anopheles larvae in each the control vessels remained alive and active during the test period. Researchers concluded that the giant mosquito larvae are potential biological agents for control of malaria in Uganda.

Key words: Biological agents, *Toxorhynchites rutilus*, larvivorous, malaria, mosquito, Uganda

INTRODUCTION

Malaria is a well known disease worldwide with >2 billion people living in malaria endemic regions of the world. It infects 300-500 million people annually, especially to the vulnerable pregnant women and children under 5 years. About 1.2-2.7 million deaths occur annually due to malaria and it contributes to 25% of all childhood deaths in Africa (CDC, 2011; WHO, 2009). And >360 million clinical cases (90%) and 1.0 million death occur annually in the sub-Saharan Africa (Marshall and Taylor, 2009; Walker, 2002).

Malaria is a complex disease caused by protozoan parasites belonging to the genus *Plasmodium* of which *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are the most common (WHO, 2009; DFID, 2010; PMI, 2010; UmoH, 2010). The *P. falciparum* is responsible for the most severe form of the disease and mortality in Africa (WHO, 2009; DFID, 2010). The parasites are transmitted by female mosquitoes of the genus *Anopheles* of which *Anopheles funestus Giles* and members of the *Anopheles gambiae Giles complex* are the commonest in sub-Saharan Africa (Marshall and Taylor, 2009; Farenhorst *et al*., 2008; Walker and Lynch, 2007). They are intermediate hosts of several important human diseases including malaria, yellow fever, dengue, Japanese encephalitis and filariasis (DFID, 2010). The burden of these diseases has led to the development of a number of vector control measures worldwide. The methods include environmental management with temporary or permanent removal of Anopheline larval habitats through physical means, larviciding with chemical or biological agents and use of insecticides on the adult Anopheles mosquitoes (Walker, 2002; Walker and Lynch, 2007).

In Uganda, malaria is endemic in 95% regions of the country with 63% of the population exposed to high malaria transmission (UmoH, 2010) and 25% to moderate levels while 12% live in areas with low or unstable transmission that are epidemic prone (UmoH, 2010). It is estimated that about 70-100,000 deaths occur in children under 5 years of age annually and between 10 and 12 million clinical cases seek treatment country-wide. All the four Plasmodia species occur in Uganda with *P. falciparum* contributing to 90-98% of the parasite population. *P. malariae* is the second, accounting for 1-3% mono-infection but mainly appears as a mixed

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infection with *P. falciparum* in 16% of childhood infections in endemic areas. Both *P. vivax* and *P. ovale* are rare and do not exceed 1-1.5% of malaria cases (UmoH, 2010). The predominant and main malaria vector in the country being *Anopheles funestus* and *Anopheles gambiae* (DFID, 2010; PMI, 2010; UmoH, 2010).

In Uganda, a number of methods are used in the malaria vector control, targeting both the larval and adult Anopheles mosquitoes. The commonest method involves environmental management with temporary or permanent removal of Anopheline larval habitats through physical means, larviciding with chemical or biological agents and use of insecticides on the adults (Walker and Lynch, 2007). These interventions eliminate vector breeding sites, changing natural habitats and improving human dwelling by reducing mosquito population while creating minimal adverse environmental and social impacts. The physical methods involve marsh alteration, filling, grading and drainage of any water reservoirs, vegetative plantings that repel mosquitoes around homes and house screening (Walker, 2002). The electrical gadget like green light lamp and many others have also been used in mosquito control (Atkinson, 2005; Cottee, 2011).

Chemicals have been very useful in malaria control globally. They eliminate or reduce the malaria vector population by killing the larvae (larvicides) and the adults (insecticides). They have been effectively used in both the urban and peri-urban areas worldwide (Walker, 2002). The mosquito vector control against adult mosquitoes utilise insecticide through Indoor Residual Spraying (IRS) and use of Insecticide Treated Bednets (ITNs) (DFID, 2010; Howard *et al*., 2011). The chemical methods are effective, can be organized quickly and can produce results at relatively low cost if used efficiently. However, emergence of insecticide resistance, environmental impacts, rising costs of IRS and logistical constraints have stimulated renewed interest in other methods of malaria vectors control using biological agents especially in many African countries (Farenhorst *et al*., 2008; Howard *et al*., 2011; Overgaard, 2006; Weill *et al*., 2003). Biological control methods utilize many organisms that are enemies of adult Anopheles mosquitoes and their larvae. Biological control refers to the introduction or manipulation of the organisms in order to suppress vector populations (Walker, 2002). They help regulate the Mosquito populations naturally through predation, parasitism and competition. Among the organisms reported include larvivorous fish, invertebrate predators like Toxorhynchites, mammals like bats, nematodes, protozoa, fungi and bacteria (Walker, 2002). The use of aquatic plant Azolla and carnivorous plants have also been recognized as potential biological control agents (Walker and Lynch, 2007). Work is also in progress on the use of genetically engineered sterile mosquitoes to interrupt multiplication of mosquitoes (Marshall and Taylor, 2009; Catteruccia *et al*., 2005; Knols *et al*., 2006; Breasbois, 2002). The advantages of biological control agents in comparison to chemical controls include their effectiveness at relatively low doses, safety to humans and non-target wildlife including natural predators of adult and larval stages of the mosquitoes, low-cost of production in some cases and lower risk of resistance development (Walker, 2002). Also these agents can effectively be used in community-based malaria prevention programmes including the rural poor though they tend to be more specific in terms of which mosquito species they can control and the habitats.

Other biological agents with potential vector control have been recognized. Various agents have been used against dengue vectors that cause dengue fever (Collins and Blackwell, 2000; Schreiber, 2007; Steffan *et al*., 1980; Shaalan and Canyon, 2009). Among these agents are the elephant (or Treehole) predatory giant mosquitos of the genus *Toxorhynchites*. They are the largest mosquitoes (Diptera: Culicidae) in the world and appear brightly colored. *Toxorhynchites* are found primarily in tropical forested areas. The taxonomy has 88 Toxorhynchites species and subspecies (Goettle and Adler, 2011). There are two main subspecies of Toxorhynchites that appear as brilliantly colored day-flying mosquitoes including *Toxorhynchites rutilus rutilus* and *Toxorhynchites rutilus septentrionalis* (Goettle and Adler, 2011; Jones and Schreiber, 1994). Adult predatory mosquitoes feed on nectar and other naturally occurring carbohydrate sources but never take blood meals. The large larvae of the giant mosquitoes are found in the water in tree holes, old tires and other containers where they prey on living macro invertebrates such as mosquito larvae of other species (Cannibal mosquitoes) (Meredith, 2011). These giant mosquitoes have low population numbers in the wild that make them highly susceptible to insecticides. The larvae have been recognized as potential to reduce pest and disease-bearing mosquitoes in places where the Anopheles mosquitoes breed. Each giant mosquito larvae may eat as many as 400 larval mosquitoes during its larval development (Jones and Schreiber, 1994). The combination of carnivorous larvae and innocuous adults is very attractive as biological control. They have been reported to successfully control dengue vectors, *Aedes egyptie* in Japan, Southeast Asia, the Caribbean and the United States (Jones and Schreiber, 1994). In this study, six giant larvae were discovered developing in the field Anopheles mosquito larvae culture. It was observed that the Anopheles larvae were completely cleared from the culture leaving only the giant larvae. The giant larvae were monitored to adulthood and they were identified as *Toxorhychites rutilus rutilus* larvae by the Entomologist. The study was then designed to investigate the larvicidal activity of the giant larvae as a biological agent against Anopheles larvae and this could contribute to the integrated malaria control programme in Uganda.

MATERIALS AND METHODS

This study was designed to test the hypothesis that *T. rutilus rutilus* larvae preys on Anopheles larvae in mosquito breeding vessels and therefore potentially reduce the population of mosquitoes around human dwellings. Anopheles mosquito larvae were reared and the 4th instar stages were transferred into vessels that contained the *T. rutilus rutilus* larvae. The survival of the larvae was determined every 12 h for 36 h and compared with survival in the control vessel that did not contain the *T. rutilus rutilus* larvae.

Study design: An experimental study was designed to test larvicidal efficacy of larvae of the giant mosquitoes of the specie of *T. rutilus rutilus* found in Uganda. The *T. rutilus rutilus* larvae are big with very strong mandibles that are capable of catching and engulfing Anopheles mosquito larvae. Test experiments were done with vessels that contained six *T. rutilus rutilus* larvae while the control tests had no *T. rutilus larvae* deployed in the vessels.

Rearing Anopheles larvae: Plastic rectangular containers (15×20 cm) were filled with culture medium and placed outside the department in partial shade. White filter paper was folded and placed on the water surface to provide the mosquitoes with resting place while laying their eggs. The set up was observed for 2 weeks to monitor the hatching of the eggs into larvae. Initially, the water used was collected from the swamp as larval rearing medium but later used distilled water successfully. The larvae were identified in the Department of Biology, School of Biological Sciences, Makerere University as Anopheline. The assays were done using the 4th instar mosquito larvae.

Rearing toxorhynchites larvae: Five plastic containers, filled with culture distilled water with folded filter study placed as above were positioned in shady the bushes near the department. Observations were made every 5 days to monitor the hatching of the larvae. After 2 weeks, six giant larvae identified as *T. rutilus rutilus* were seen swimming in one of the containers. The other four vessels had many Anopheles larvae swimming freely while the one containing the giant larvae had very few Anopheles larvae. The giant larvae were used in the larvicidal experiment.

Larvicidal bioassays: Two containers or vessels (test and control) 10×15×6 cm were filled with distilled water. Bread crumbs was added in the containers as feeds for the larvae. Here 120 4th instar Anopheles larvae were counted into each of the containers. Six *T. rutilus rutilus* larvae were then introduced into the test container while none was introduced in the control container. Live larvae in each container were counted every 12 h for 36 h. The survival of the Anopheles larvae in both the control and test containers were noted and recorded. The 6 giant larvae were observed daily until they developed into adults that were identified by the Entomologist in the department of Biology in the School of Biological Sciences, Makerere University.

RESULTS

At 12 h, only 40 Anopheles larvae were remaining in the test vessel. After 24 h all the Anopheles larvae had been cleared in the vessel by the giant larvae in the test group. In the control group, all the 120 Anopheles larvae were still living after 12, 24 and 36 h of observation as shown in Fig. 1. The trend of the survival of the Anopheles larvae against time in both the test and control groups showed a significant decrease in the Anopheles larvae in the test group and all were cleared after 24 h while in the control group, the trend remained constant after 36 h of observation as shown in Fig. 1.

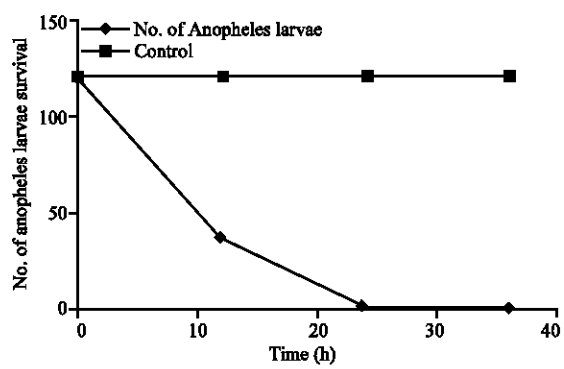

Fig. 1: Larvicidal effects of *T. rutilus rutilus larvae* on Anopheles larvae. The giant mosquito larvae ate all the Anopheles mosquito larvae within 24 h. All Anopheles mosquito larvae in the control vessel were alive by the end of the experiments

DISCUSSION

The larvicidal effect of *T. rutilus rutilus* on the Anopheles larvae were investigated in culture for 36 h. The results show that *T. rutilus rutilus* larvae exhibited a strong cannibalistic activity against Anopheles larvae. The *T. rutilus rutilus* larvae has been reported to exhibit both carnivorous and larvivorous activity against invertebrates and larvae of other free water living animals (Schreiber, 2007; Shaalan and Canyon, 2009; Meredith, 2011). Each *T. rutilus rutilus* larva has been reported to consume about 400 larvae during their developmental life and hence the observed effect in the study (Goettle and Adler, 2011; Jones and Schreiber, 1994). They have been used as a potential biological agent against various disease vectors (Schreiber, 2007; Shaalan and Canyon, 2009) and have been reported to successfully control dengue vectors, *Aedes egypti* in Japan, Southeast Asia, the Caribbean and the United States of America (Jones and Schreiber, 1994). The *T. rutilus rutilus* species of giant mosquitoes have been reported to be friendly organisms as the adults do not feed on blood as is the case with Anopheles mosquitoes (Schreiber, 2007; Goettle and Adler, 2011). They instead feed on nectar and other naturally occurring carbohydrate sources (Schreiber, 2007; Goettle and Adler, 2011). The adult *T. rutilus rutilus* mosquitoes are naturally few in the wild and thus limiting their use as potential biological agent against various disease vectors and they are usually found in the tropical forested areas (Schreiber, 2007; Goettle and Adler, 2011; Jones and Schreiber, 1994). However, attempts have been made to multiply the giant mosquitoes in Japan in order to utilize them as biological agent against *Aedes eagypti* (Jones and Schreiber, 1994). The large larvae are found in the water in tree holes, old tires and other containers where they prey on living microorganisms (Cottee, 2011). The role of *T. rutilus rutilus* against Anopheles mosquitoes has not been reported. However, they can be reared in mass numbers so that massive predatory larvae are produced. The results from the study on the larvivorous activity of the giant mosquito larvae on Anopheles mosquito larvae has provided evidence of their larvivorous or cannibalistic effect since they preyed on all the Anopheles mosquito larvae in the container. The giant mosquitoes can be incorporated in the integrated malaria control programmes, especially in the endemic and severely affected malaria regions mainly in the sub-Saharan Africa.

CONCLUSION

The *T. rutilus rutilus* larvae of the giant species of mosquitoes completely preyed on the Anopheles mosquito larvae in the vessel and this indicated that the giant mosquito larvae exhibits both cannibalistic and larvivorous activity against Anopheles larvae in culture. They have a biological potential as an agent in controlling Anopheles larvae, thus interrupting the developmental life cycle of the Anopheles mosquitoes, thus reducing the mosquito population around the human dwellings and hence reducing the malaria burden in the worst affected regions of sub-Saharan Africa. Further research is also needed to develop this potential biological agent that can be incorporated in the integrated malaria control programmes.

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