



FULL LENGTH ARTICLE

Larvicidal properties of two asclepiadaceous plant species against the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae)

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Abstract Certain mosquito species are important vectors of fatal human diseases, among which *Anopheles arabiensis* is known to be associated with malaria transmission in different tropical and subtropical areas. Since chemical control of mosquitoes was linked with numerous drawbacks, like resistance development, the search for effective environmentally sound alternatives is urgently needed. Therefore, it was aimed by this study to evaluate some extracts prepared from two asclepiadaceous plants, viz., *Solenostemma argel* “Hargel” (seeds and leaves) and *Calotropis procera* “Usher” (leaves and flowers), as natural larvicides against *An. arabiensis*. The main parameters included bioassays of treatments for knockdown and residual effects, besides phytochemical analysis of the tested extracts. The results revealed variable groups of secondary metabolites in the two plants, with *S. argel* seemed to be the richest one. Hence, *S. argel* extracts caused higher larval mortalities than those of *C. procera*. This could be ascribed to some potent secondary metabolites in the former plant, which needs further studies. Almost all the high concentrations of *S. argel* extracts exerted the highest knockdown effect (90% mortality) after 24 h, which were comparable with those obtained by two standard insecticides. The highest doses of petroleum ether and water extracts of this plant also manifested significantly higher residual effects than the other extracts after three days following treatments, but were surpassed by the chemical insecticides thereafter. However, *S. argel* seed petroleum ether extract at 0.5% was the most effective of all botanicals up to three weeks of exposure. This extract needs to be evaluated under field conditions for proper exploitation as mosquito larvicide.

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1. Introduction

Several mosquito species of the genera *Anopheles*, *Culex* and *Aedes* are vectors of various human diseases (Brown, 1972). *Anopheles arabiensis* is the most important species associated with the transmission of malaria disease in more than hundred countries worldwide (WHO, 2002). Therefore, one of the

approaches for controlling mosquito borne diseases is the interruption of disease transmission through mosquito control or avoiding mosquito bites. Plant products of potentials as insecticides or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at community level (Potter and Beavers, 2005). Some botanical extracts such as nicotine obtained from *Nicotiana tabacum* leaves, alkaloidal anabasin and lupinine extracted from *Anabasis aphylla*, rotenone from *Derris elliptica* and pyrethrums from *Chrysanthemum cinerifolium* flowers have been used as natural insecticides even before the discovery of synthetic organic insecticides (Campbell et al., 1993).

Nevertheless, the discovery and use of synthetic persistent chemicals not only overshadowed the use of plant products, but also become the major tactic for mosquito control nowadays. However, chemical control of vectors had started in limited areas prior to the Second World War in 1940s (Potter and Beavers, 2005), but chemicals have managed to replace traditional control methods all over the world. Accordingly, the first chemical used against mosquitoes in Sudan during 1950–1965 was the benzene hexachloride (BHC), which was utilized as residual spray. During the period 1970–1974 temephos (Abate) was used as larvicide in limited areas (Haridi et al., 1975). In 1975 malathion (Cythion) was used extensively (Akood, 1980), but has been substituted by fenitrothion (Sumithion) since 1980. Chlorpyrifos (Dursban) and fenthion (Mercaptophos) were the two main larvicides used against the urban mosquito, and now replaced by diazinone (Alfatox) (Abdel Gadir, 1993). In the 90's malathion has been reused again in the country (Azami et al., 1996).

However, the extensive and indiscriminate uses of pesticides have resulted in serious draw backs, the most important of which was the evolution of mosquito resistant strains as well as toxicity hazards to man, livestock and wild life (Bay, 1976). Hence, a high level of adult malathion resistance in *An. arabiensis* was reported earlier from Sudan (Hemingway, 1983). A study in Ethiopia by Yewhalaw et al. (2011), proved that *An. arabiensis* was resistant to an array of insecticides, including permethrin, deltamethrin and malathion. Moreover, mosquito resistance to the four classes of insecticides was documented in Sudan and other countries (El Gadal et al., 1985; WHO, 1992; Ranson et al., 2001, 2009; Matambo et al., 2007). However, residues of some persistent chemicals in the environment have subsequently disturbed the ecosystem (Hill, 1989). Investigations in Sudan have indicated the presence of measurable amounts of organochlorines and organophosphates in surface water. Also, marine area in the Red Sea has suffered pesticide contamination as a result of desert locust control (UNESCO, 2000).

Based on the above mentioned and many other drawbacks of pesticides, researchers all over the world are working hard to find environmentally safe alternatives. They resorted again to plant extracts as potent sources of natural biocides (Ahmed et al., 1984). Botanical biocides are relatively harmless to non-target organisms and present little risks to users and consumers (Satti et al., 2004). Several botanical derivatives have shown selective actions against certain pests through a variety of biological activities, including production of behavioral modifying chemicals (insect growth regulators) such as pheromone analogs, repellents, attractants and antifeedant, besides the direct toxicant effects (Bower et al., 1976). Roark (1947) described approximately 200 plant species with insecticidal values, while Sukumar et al. (1991) listed and discussed 344

plant species that only exhibited mosquitocidal activities. In Sudan, promising results were achieved in this field, where more than twenty plant species in sixteen families, including members of Asclepiadaceae, were listed to be effective at variable levels as mosquito larvicides (Kehail and Bashir, 2004; Satti et al., 2010). However, the rich flora in Sudan as well as in other tropical countries is still waiting for thorough investigations to be exploited as natural biocides. Hence, laboratory studies were carried out to evaluate the larvicidal properties of two asclepiadaceous plant species (viz., *Solenostemma argel* and *Calotropis procera*) against the mosquito, *Anopheles arabiensis*, an important malaria vector in Sudan.

2. Materials and methods

2.1. Rearing of *Anopheles arabiensis*

Eggs of *A. arabiensis* were collected from stagnant water pools near the White River (Elozozab area), Khartoum, during the rainy season in July using a dipper. The stages of *An. arabiensis* were distinguished from those of *Culex* species due to morphological differences (Gillett, 1971; Potter and Beavers, 2005). The eggs were transferred to a glass container with clean water and brought to the laboratory to start the rearing and mass culturing according to the WHO (1975, 1992).

First instar larvae were reared in a glass container (40 × 40 × 40 cm) with tap water, and fed with a diet of Brewer's yeast and wheat flour until they reached the fourth instar. Pupae were transferred to an open glass Petri dish containing tap water and enclosed in a glass cage (40 × 40 × 40 cm), covered with muslin cloth to prevent the escape of adults. Emerging males and females were fed on 10% honey diet, which was kept in a bottle hung to the cage. In each bottle a thin cotton thread was inserted in the honey solution and extended to the tip of the bottle in order to facilitate feeding of adults. This kind of food is utilized for flight and metabolism. Since *Anopheles* females need a full blood meal for laying eggs, they were provided with Albino rats (*Rattus norvegicus*) placed in resting cages. Glass Petri dishes with 70 ml tap water covered with filter paper were placed inside the cage for oviposition. After two days, the females started to lay eggs on the surface of the wet filter paper; hence, eggs were taken from the Petri dishes to the adult rearing cage to start new culture of *An. arabiensis*. By doing so, 4th instar larvae of the second generation were provided for the different bioassay tests.

2.2. Preparation of plant materials and extracts

Different botanical parts of two asclepiadaceous plant species, viz., *Solenostemma argel* (leaves and seeds) and *Calotropis procera* (leaves and flowers), were investigated under laboratory conditions for their larvicidal effects against *An. arabiensis*. Fresh samples were collected from the Khartoum State, during autumn season, washed thoroughly with clean water and dried under room temperature. Dry samples were ground into fine powder using an electric blender. However, the powders required for each experiment were prepared in the same day of extractions (water and organic).

Regarding water extract, two hundred grams of powder from each plant sample was mixed with 1000 ml of distilled water in a conical flask. The contents were thoroughly stirred

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