Gezira Journal Of Health Sciences 2016 vol.12(2)

EDITORIAL

Effect of Aqueous Extract of Cathedral Cactus (*Euphorbia trigona* Mill) on Larvae of *Anopheles arabiensis* (Diptera: Culicidae)

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Abstract:

Mosquitoes are considered as vector of malaria disease and some other endemic diseases in the world. There are some methods already been used for controlling mosquito; of which is using natural products. This study was conducted at Laboratories of Faculty of Engineering and Technology, University of Gezira, to evaluate the effect of cortex, spine and pith parts of cactus (*Euphorbia trigona*) on *Anopheles* mosquito larvae. The plant parts were collected from Wad Medani City, whereas, the mosquito larvae were collected from the breeding sites at Tayba village, Gezira State, Sudan. The plant parts (cortex, spines and pith) were shade dried away from the direct sunlight, grounded and then kept separately in small plastic sacks. From each plant part, a concentration of 1200 mg/L was used. The standards of WHO for testing toxicity of the toxic compound against mosquito larvae was followed. The mortality in *Anopheles* larvae were 48%, 37% and 62%, respectively, for *trigona* cortex, spine and pith. The results also showed that, the three used parts have a varied great impact on the survived larvae (morphological changes of skin color was in 82%, disconnecting of digestive tract was in 48%, and separation of some body parts was in 32%, after 48 hours of applying it). The study recommends adding these cactus parts as potential natural products for *Anopheles* larval control, and also running more sensitive tests to measure the environmental impact of these products, especially on human and on the aquatic fauna.

Introduction:

Mosquitoes can transmit more pathogens than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one". Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700 million people every year globally and 40 million of the Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, *etc*., in almost all tropical and subtropical countries and many other parts of the world (WHO, 1996). The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. In recent years, use of many of the former synthetic insecticides in mosquito control program has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Brown, 1986 and Russell *et al*., 2009). Thus, the Environmental Protection Act (EPA) has framed a number

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of rules and regulations to check the application of chemical control agents in nature (Bhatt and Khanal, 2009). Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites (Williams and Lemke 2002). Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage (Hunter, 2008). Many secondary metabolites are selected and optimized through evolution for use as "chemical warfare" agents against prey, predators, and competing organisms (Bhat *et al.*, 2005).

Roark (1947) described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al*., (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activity. Shallan *et al*., (2005) reviewed the state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research.

Cactus (plural: *cacti*, *cactuses*), is a member of the plant family Cactaceae within the order Caryophyllales. The name originally used by Theophrastus for a spiny plant whose identity is not certain (Johnson and smith1972). *Euphorbia trigona* also known as African milk tree, cathedral cactus (Timothy *et al*., 1991) and high chaparallis a perennial plant that originally comes from Central Africa. It has an upright stem that is branched into three or four sides. The stem itself is dark green with V-shaped light green patterns. The thorns are about 5 mm long and are placed in pairs of two on the stem's ridges. The drop shaped leaves grow from between the two thorns on each ridge. The plant has never been known to flower (James *et al*., 2011).

Objectives:

This study aimed to assess the larvicidal activities due to the cactus plant (*Euphorbia trigona*) on the mosquito larvae (*Anopheles*) and using various parts of the plant (cortex, spines and pith).

Materials and Methods:

The Study Area:

Wad Medani City which is located in the central parts of the Gezira State, Sudan. Tayba village was selected for sampling mosquitoes larvae (North Wad Medani City), whereas, cactus plant (*Euphorbia trigona*; Plate, 1) was brought from within Wad Medani City.

Methodology:

Larvae of *Anopheles arabiensis* were collected using dipping net with sufficient amounts of breeding water. Rearing and maintenance of mosquito larvae followed WHO (1996). The cactus plant was separated into three parts (cortex, spines and pith). Each part was shade dried at room temperature (26 ± 3^oC). The dried parts were grounded separately, using mortar and pestle and were then kept in small plastic bags for further works.

Experiments were started by preparing 10 plastic cups (size of 250 ml). Random samples (20 individuals) of *Anopheles* larvae of the third or early fourth instar were dropped to these cups which were filled with 250 ml tap water. 0.3 g of each grounded part of *trigona* plant was added individually in these cups that containing 20 *Anopheles* larvae and 250 ml water. This experiment was replicated thrice for each plant part (cortex, spines and pith). A control batch was included for comparison. These experiments were run in the Laboratories of Faculty of Engineering and Technology, at the room temperature (26$\pm$ 3^{o}C). Data of dead larvae were counted every 24 hours, for a period of 48 hours. A digital microscope provided with camera was used for documentation of observed changes, if any.

Figure (1): *Euphorbia trigona* plant

Statistical Analysis:

Data from the study experiment were collected and subjected to descriptive statistics and using Duncan's multiple range test (DMRT) tools.

Results:

Toxicity of *Euphorbia trigona* different parts on *Anopheles* larvae:

The toxicity of *E. trigona* cortex, spines and pith parts (at the concentration of 0.3 g/250 ml water; 1200 mg/L) on *Anopheles* larvae in terms of % mortality was represented in Table (1). At this concentration, and after 24 hours, the cortex powder produced 43.33% mortality on *Anopheles* larvae, while the mortality was 35.67% when spine powder was used, whereas the pith powder caused 54.6% mortality. Duncan's multiple range test (DMRT) revealed that, the difference observed in larval mortalities in respect to different plant parts was significant, i.e. the toxicities of the three parts were not similar against *Anopheles* larvae (the pith part was more toxic against

Anopheles larvae than the other parts, whereas, the spine parts has the lowest effect).

Table (1): Mean Percentage mortality of *Anopheles* larvae towards cortex, spine and pith (at 1200 mg/L of water) of *Euphorbia trigona* after 24 hours using Duncan's multiple range test (DMRT)

Treatment	Means
Cortex	43.33b
Spines	35.67c
Pith	54.0a

Means in the same column with same letter are not significantly different

The morphological changes in *Anopheles* larvae after 24 hours:

After 24 hours of applying each of the three cactus parts (cortex, spines and pith), some morphological changes in addition to the rate of the movement were monitored by using digital microscope provided with camera on the survived larvae (Table 2 and Plate 2). The change in the larval color was high in the larvae subjected to cortex part (55%), followed by those subjected to pith part (43%) and spine part (35%), approximately.

There was considerable number of larvae with detached digestive tract (13%, 5% and 10%, in those subjected to cortex, spine and pith parts, respectively). More work is needed to explain the changes observed. The movement of the survived larvae was noticed to be slow "relatively".

Table (2): Morphological changes (%) observed in *Anopheles* larvae towards cortex, spines and pith (at 1200 mg/L of water) of *Euphorbia trigona* after 24 hours

Morphological Change	Cortex	Spines	Pith
Larval color	55	35	43
Disconnection of the Digestive tract	13	5	10

Control larva

Larva with a changed color

Figure (2): The color change in the survived *Anopheles* larva after 24 hour

The morphological changes in *Anopheles* larvae after 48 hours:

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After 48 hours of applying each of the three cactus parts (cortex, spines and pith), some morphological changes (in color, connectivity of the digestive tract and separation of some body parts) in addition to the rate of the movement were monitored on the survived larvae (Table 3 and Plate 3). The change in the larval color was high in those subjected to cortex part (83%), followed by those subjected to pith part (65%) and spines part (55%). The survived larvae with disconnected digestive tract (cut) were 52%, 35% and 45%, respectively, in those subjected to cortex, spine and pith aqueous extracts. It was also noticed that, after 48 hours, some of the body parts of the larvae were cut off (disconnected), of which 17% of those subjected to cortex part, 5% of those subjected to spine part and 12% of those subjected to pith part). It was also noticed that, the larvae subjected to pith part were obviously swollen in comparison to the others, oldest larvae failed to pupate (Plate, 4) and all the survived larvae died. It was clear that, *E. trigona* parts killed some of *Anopheles* larvae after 24 hours and caused some morphological changes, and its effect extended after that period to hinder pupation and kill the rest of the survived larvae.

Table (3): Morphological changes (%) observed in *Anopheles* larvae towards cortex, spines and pith (at 1200 mg/L of water) of *Euphorbia trigona* after 48 hours

Change	Cortex	Spines	Pith
Color	83	55	65
Disconnected Digestive tract	52	35	45
Disconnected other body parts	17	5	12

Larva with a cut digestive tract (disconnected digestive tract)

Swelling larva treated with pith part

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Larva with disconnected head

Larva with disconnected paddles

Figure (3): Some morphological changes in the survived larvae after 48 hours

Larva which failed to pupate

Control pupa

Figure (4): The larva which failed to pupate

Discussion:

Alwan (2015) found that, the mortalities produced by *E. trigona* cortex and pith at the concentration of 1200 mg/L was 42% and 47%, respectively (which were not far from the obtained data of this study). Also, the study found that, cortex and pith of Wad Medani sample were rich in flavonoids, alkaloids, triterpenes, saponins, glycosides and steroids, but steroids are present in relatively more concentration in pith than in cortex (tannins were not detected in both parts).
Other Euphorbias attracting many scientists e.g. Geoff (2011), and Alonso and Santos, (2013) to run some studies on toxicity of Euphorbias on various organisms.

Conclusions:

At 1200 mg/L aqueous extract, and after 24 hours, the cortex, spines and pith parts of *E. trigona* produced 43.3, 35.6 and 54.6 % mean mortalities on *Anopheles* larvae. The movement of the survived larvae was noticed to become "relatively" slow. It was clear that, *E. trigona* parts killed some the *Anopheles* larvae after 24 hours and caused some morphological changes, but its effect extended after that period to hinder pupation and kill the rest of the survived larvae.

Recommendations:

The study recommends adding these parts to the potential natural products in *Anopheles* larval control, and also running more sensitivity tests to measure the environmental impact of these products, especially on human and on the aquatic predators.

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