Larvicidal effect of crude extracts of some marine plants (mangrove and seagrasses) on mosquitoes of *Culex pipiens*

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ABSTRACT

Marine halophytes (mangroves and sea grasses) were collected from the Red Sea coast of Egypt and tested for their mosquitocidal activities. The toxicities of mangroves *Avicennia marina* & *Rhizophora mucronata*, and *seagrasses Thalassodendron ciliatum*, *Halodule uninervis* and *Halophilia* ***s****tipulacea* were examined against lab. Strain of 3rd instar larvae of *Culex pipiens*. Results showed that extracts of seeds and leaves of *Avicennia marina* were more effective than other parts of the same plant as well as of the leaves of *Rhizophpra mucronata* against 3rd instar larvae of *Culex pipiens* mosquito. On the other hand, comparison of the toxicities of the three species of sea grasses showed that crude extract of *Halodule uninervis* was more active against 3rd instar larvae of mosquito *Cu pipiens* than crude extracts of *Thalassodendron Ciliatum and Halophilia stipulacea*. In view of these results, the purified active compounds from the most effective samples found in our studies could be effective in killing mosquito larvae or repelling adult female mosquitoes in an economic and safe manner.

Keywords: Mangroves plants, sea grasses, Mosquito, Toxicity.

INTRODUCTION

During the last few decades synthetic chemicals have been used in large quantities to control insect pests as they are low-priced and effective (Thangam & Kathiresan, 1990). The synthetic insecticides are generally non target specific and can cause environmental damage due to their persistent nature. Throughout the world there is a long history of plant products being used for their insecticidal or repellent properties. Hence natural insecticides were realized to be ecofriendly and are given preference (Nazar *et al*., 2009).

In this context, a large number of terrestrial plants have been previously screened for mosquito larvicidal and/or repellent activities (Thangam & Kathiresan, 1990, 1994). Plant extracts have recently gained an importance of insect control, being considered safe to environment and less hazardous to non target biota (Gajendran & Ragupathy, 2003). Intensive work was conducted on the biological activity of plant extracts as natural sources of insecticides (Saleh *et al*, 1983, Thangam and kathiresan 1993). However, plant extracts may act as toxicants or repellents (Su, and Harvart, 1981, and Sharma, & Dhiman, 1993) or act as insect growth regulators (Bowers *et al*., 1972).

In vitro, assessment of the antibacteriophage, antibacterial and anticandidal activities as well as cytotoxicity were previously evaluated for both aqueous and ethanol extracts prepared from roots, cotyledons, leaves and stems of the mangrove *Avicennia marina* (Khafagi *et al., 2003*). Aqueous extracts of both shoots and roots of the seedlings demonstrated antibacteriophage activity using coliphage against *Escherichia coli* which indicates antiviral activity. Aqueous extracts also exhibited moderate cytotoxicity against the larvae of the brine shrimp *Artemia salina*, which demonstrates antiplasmodial and antimalarial activities. Seeds were found to be the most effective followed by leaves and flowers.

It has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values (Miles *et al.*, 1998). Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents. *Avicennia marina* has received some attention in determining its important chemical constituents. A naphthoforan compound with phytoalexin activity has been isolated (Sutton *et al*., 1985; Miles *et al*., 1998).

In vitro, antimalarial activity and cytotoxicity of *A. marina* have also reported previously by Sharaf *et al*., 2000. Recently, chemo-preventive activity (anti-tumor promoters) of some naphthoquinones and their analogs isolated from *Avicennia* plants was noted (Itoigawa *et al.*, 2001). The bark and roots of *A. marina* are known to contain the tannin lapachol (Tomlinson, 1994). The bark leaves and fruits of *A.marina* are used in folk medicine to treat skin diseases.

As there was no information on the mosquito larvicidal or repellent activities of marine plants (mangroves and sea grasses) inhabiting the Red Sea, the present study was conducted to evaluate the mosquito larvicidal activity of some of these plant extracts against those of *Culex pipiens*, the main vector of lymphatic filariasis in Egypt.

MATERIAL AND METHODS

1. Collection and preparation of samples

Marine halophytes (Mangroves and Sea grasses) were collected from Gharqana coast. Nabq protected area and Ras Mohammed national park at southern Sinai coast (Gulf of Aqaba) during November 2007. Sea grasses were collected from the seagrass beds during low tides from Gulf of Aqaba.

Mangrove leaves, stems, seeds and flowers of *Avicennia marina* and whole plant of *Rhizophora mucronata* were separately cleaned with many changes of seawater in order to remove epiphytes, shells and other extragenous matter and were immediately transferred to separate polythene bags and placed on ice till return to the laboratory. Each species was again cleaned in running tap water and further once with distilled water and shade dried under room temperature (28±2ºC) for further use.

Different species of seagrasses *Halodule uninervis*, *Halophilia stipulacea* and *Thalassodendron ciliatum*) were chosen duo to their bundance and biomass. The collected samples were packed in plastic bags and transported to the laboratory. Immediately they were washed with fresh water for removing sand, epiphytes and any extraneous matter; they were then dried in shade for five to seven days then powdered using a pestol and mortar.

2. Extraction of plant material

Extraction was carried out with ethyl acetate solvent at ambient temperature. The extracts were freed from solvent under reduced pressure, the residue obtained are finally dried under vacuum evaporator and used for in vitro screening of antimosquitocidal activity.

3- Antimosquitocidal activity

-Tested insect

A colony of *Culex pipiens* was established in the laboratory, where mosquito larvae were firstly collected from small ponds under leaking irrigation faucets in West- Qanttara. They were reared in enamel pans (30cm, in diameter and 10cm in height) and fed daily upon a mixture of dried powder bread, yeast and dried milk in the ratio of 2:1:1, respectively.

Emerging adults were successively reared under room temperature (27 ± C º) in Plastic cages of 30×30×30cm, and fed on 10% sucrose solution which was offered in a piece of sponge suspended by a wire thread from the roof of the cage. Larvae of the 3th instar were mounted and identified. After inbreeding for several generations, 5 rafts were introduced into each enamel containing the nutrient solution mentioned above. Homogeneous larvae of 3rd instar were isolated subsequently for running bioassay tests.

-Tested procedure

The method of WHO (1975) was followed for testing mosquito larvicides. In order to study the toxicity of the studied plant extracts preliminary screening tests were carried out at a concentration level of 1000 ppm (w/v). This was accomplished by dissolving 0.5mg of each tested crude extract in 10 ml of solution and placed in a 100ml glass beaker marked at 50 ml volume.

Twenty five 3rd instar mosquito larvae of *Culex pipiens* were transferred to a beaker in least quantities of water by means of a small dropper. Then the solution level was adjusted to a 50 ml. Five to seven concentrations were tested. Larval mortality was counted after 24, 48, 72 hours and exposed to log probit regression analyses (Unkelbach, 1985). This process was repeated in three other beakers for each tested extract. Control experiments were carried out alongside other treatments where the same solution was used alone without plant extract, and then examined for calculating the percentage of mortalities:

$$\% \text{ corrected mortality} = \frac{\% \text{ test kill- } \% \text{ control kill} \times 100}{100\text{- } \% \text{ control kill}}$$

Only promising extracts (i.e. of mortality equals to 50/or more) were subjected to detailed toxicity studies where their LC_{50} and LC_{90} values were determined. This was accomplished according to the well established methods (e.g. WHO, 1975). In this respect, a different range of concentrations of each concerned extract was prepared in order to obtain mortalities ranging from 20% to 90%. At least 4 replicates were usually carried out for each tested concentration to prepare the LC-p line according to Finney (1955).

RESULTS

The results presented in table 1 revealed regression lines of toxicity with slopes ranging from 1.58 to 1.89 for different parts of *Avicennia marina* and 1.89 for *R. mucronata* leaves. The LC_{50} values of seeds and leaves of *Avicennia marina* were 3.97 ppm. For flowers and stems LC_{50} values were 4.35 and 7.02 ppm, respectively. The Lc_{50} value of *Rhizophpra mucronata* was 7.02 ppm. Results shown in (table 1 and fig. 1) clearly indicated that crude extracts of seeds and leaves of *Avicennia marina* were more effective than that of other parts of the same plant as well as of *R. mucronata* against 3rd stage larvae of *Culex pipiens* mosquito.

Table 1: Comparative toxicities of two mangrove plants *Avicennia marina and Rhizophora mucronata* against 3rd stage mosquito larvae of *Culex pipiens*.

Lethal conc.	**Extract conc. (ppm) for plant parts of *Avicennia marina***				***Rhizophora mucronata***
	Seeds	**Flowers**	**Leaves**	**Stems**	
LC_{10}	2.21 (0.40-12.39)	2.07 (0.25-17.20)	2.21 (0.40-12.39)	3.08 (0.20-13.20)	3.08 (0.20-13.20)
LC_{20}	2.70 (0.69-10.59)	2.67 (0.53-13.37)	2.70 (0.69-10.59)	4.09 (1.71-9.75)	4.09 (1.71-9.75)
LC_{50}	3.97 (1.96-8.04)	4. 35 (2.24-8.45)	3.97 (1.96-8.04)	7.02 (4.60-11.05)	7.02 (4.60-11.05)
LC_{90}	7.14 (4.59-11.12)	9.13 (3.59-23.22)	7.14 (4.59-11.12)	15.99 (1.89-135.31)	15.99 (1.89-135.31)
Slope	1.58	1.78	1.58	1.89	1.89
CHI^2	0.03<3.85	0.01<3.85	0.03<3.85	0.00<3.85	0.00<3.85

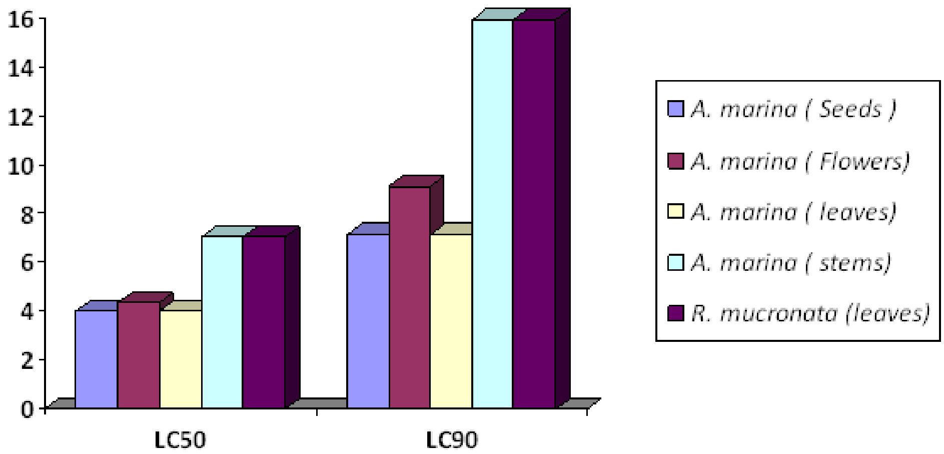

Fig. 1: Comparative toxicities of *Avicenna marina* (leaves, seeds, flowers, stems and *Rhizophora mucronata* leaves.

Results of the effectiveness of the crude extracts of three plant species of sea grasses *Thalassodendron ciliatum, Halophilia stipulacea*, *Halodule uninervis* on 3rd stage mosquito larvae of *Culex pipiens* are shown in table 2.

Table 2: Comparative toxicity between three sea grass species *Thalassodendron ciliatum*, *Halodule uninervis*, and *Halophilia stipulacea* against 3rd mosquito larvae of *Culex pipiens* .

Lethal conc.	Extract conc.(ppm) for each sea grass species		
	Talassodendron ciliatum	*Halophilia stipulacea*	*Halodule uninervis*
LC_{10}	2.07 (1..25-17.20)	3.03 (1.34-6.85)	2.21 (0.40-12.29)
LC_{20}	2.67 (0.53-13.37)	3.49 (1.84-6.65)	2.76 (0.69-10.59)
LC_{50}	4.35 (2.24-23.92)	4.60 (3.32-6.36)	3.97 (1.96-8.04)
LC_{90}	9.131 (3.59-23.92)	6.965 (5.21-9.32)	7.143 (4.59-11.12)
Slope	1.78	1.38	1.58
CHI^2	0.01<3.85	0.00<3.85	0.03<3.85

The results revealed regression lines of toxicity with slopes of 1.78, 1.38 and 1.58 respectively for the laboratory strain of *Cu. Pipiens*. The LC_{50} value of *H. uninervis* was at 3.97 ppm followed by *T. ciliatum* and *H. stipulacea* at 4.35 and 4.60 ppm respectively. Results in (table 2 and fig. 2) clearly showed that crude extracts of *H. uninervis* by ethyl acetate was more effective than that of *T. ciliatum* and *H. stipulacea* against 3rd stage larvae of *Culex pipiens* mosquito.

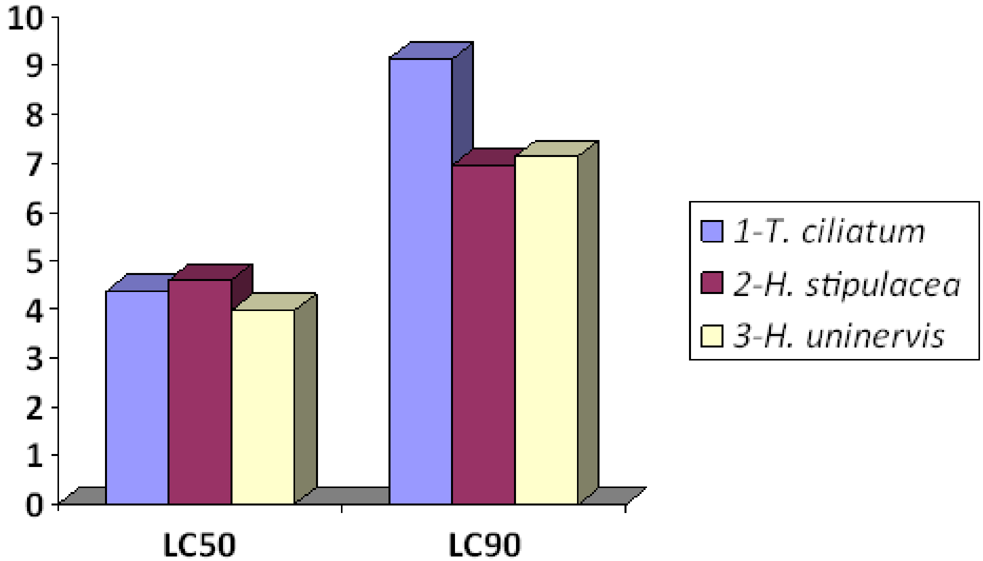

Fig. 2: Comparative toxicities for the sea grasses *Thalassodendron ciliatum*, *Halodule uninervis*, and *Halophilia stipulacea* against 3rd mosquito larvae of *Culex pipiens.*

DISCUSSION

Culex pipiens is the most widely distributed mosquito species in the world. Hoogstraal *et al*. (1977) stated that mosquitoes in Egypt are vectors of malaria, various forms of filariasis and numerous arboviruses like dengue and yellow fevers. Thangum and Kathiresan (1996) in Parangipettai studied a large number of marine plants as insecticidal and /or repellent activities against mosquito in India, their study was the first to investigate sea weeds, seagrasses and mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Study of environmental hazards in using synthetic insecticides against mosquito was also conducted by Bahgat *et al* (2001) by using spinosad which is produced from soil Actinomycete. Our results showed that extracts of seeds and leaves of *Avicennia marina* were more effective than other parts of the same mangrove plant as well as of the mangrove *R. mucronata*. The results also displayed that some extracts of *H. stipulacea* are more susceptible against *Culex pipiens* larvae.

There have been numerous studies on the mosquito larvicidal activity of terrestrial plants (Kathiresan and Thangam, 1987). Subsequently, the mosquito larvicidal activity of the seaweeds *Plocamium telfairiae* and *Laurencia nipponica* was reported by Watanabe *et al.* (1989; 1990) who isolated Mosquito larvicidal compounds. Ours was the first study on the mosquito larvicidal activity of Egyptian marine plants. Effective repellent compounds, like dimethyl phthalate which are available in the market are very costly and can give protection only for a short period of one or two hours (Kalyanasundaram *et al.*, 1986).

In view of these results, the purified active compounds from the most effective samples found in our studies could be effective in killing mosquito larvae or repelling adult female mosquitoes in an economic and safe manner. This finding would be useful in the field of mosquito control without polluting the environment.

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Arabic Summary

تأثير مستخلصات بعض النباتات البحرية (أشجار الشورى والحشائش البحرية) على يرقات بعوض كيولكس بيبينس *Culex pipiens*

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تم تجميع عينات لبعض النباتات البحرية (المانجروف والحشائش البحرية) من سواحل البحر الأحمر المصرى بغرض دراسة تأثير سميتها البيولوجية. وخلال هذه الدراسة تم اختبار تأثير المستخلصات الخام لنباتات المانجروف *(Avicennia marina & Rhizophora mucronata)* والحشائش البحرية *(Thalassodendron ciliatum, Halodule uninervis and Halophilia stipulacea* على يرقات الطور الثالث لبعوضة *Culex pipiens*.

بينت النتائج أن مستخلصات كل من بذور وأوراق المانجروف *Avicenia marina* هى الأكثر فاعلية من بين الأجزاء الأخرى لنفس النبات وايضا اكثر فاعلية من اوراق المانجروف *Rhizophora mucronata*.

وعلى الجانب الاخر بالنسبة لمستخلصات الحشائش البحرية فقد اوضحت النتائج ان مستخلص الحشيش البحرى *Halodule uninervis* هو الأكثر فاعلية على يرقات الطور الثالث من بعوض *Culex pipiens* عن الأنواع الأخرى من الحشائش البحرية التى تم اختبارها.

في ضوء هذه النتائج، يمكن للمركبات النقية من العينات الأكثر فعالية التي وجدت في دراساتنا أن تكون فعالة في قتل يرقات البعوض أو ضد البعوض البالغات بطريقة اقتصادية وآمنة. وبهذه النتيجة يكون مفيدا في مجال مكافحة البعوض دون تلويث البيئة.