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BIOPOTENTIAL BEHAVIOR OF A HYDROPHYTE –EICHCHORNIA CRASSIPES AS MOSQUITO REPELLENT

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ABSTRACT Mosquitoes are the oldest human enemy and controlling them is of prime importance in recent years because of the numerous diseases caused by them. There is an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticide and drug resistance. The present study aims to evaluate the larvicidal activity of Eichhornia crassipes crude leaf extracts against the filarial vector Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti.

KEYWORDS : mosquito repellant, utilization, hydrophyte, eichhornia, larvicidal activity

INTRODUCTION

The world today is still plagued by a myriad of diseases and a number of these are caused by vector-borne. The mosquito, having a cosmopolitan distribution, except in the Antarctic regions is one of the most despised creatures in the animal kingdom with a horrific reputation of being a vector of several diseases. Vector-borne diseases are illnesses caused by pathogens and parasites in human populations. Mosquitoes represent a significant threat because of their ability to carry pathogens that cause diseases that afflict millions of people worldwide. Every year, more than one billion people are infected and more than one million people die from vector-borne diseases (Reuda *et al.,* 2008). About 3000 species of mosquitoes have been recorded worldwide, out of which more than 100 species are reported to be capable of transmitting diseases to humans (Rahuman *et al.,* 2009).

Around the world, the medical and economic burden caused by vector-borne disease continues to grow as current control measures fail to cope. There is an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticide and drug resistance (Govindarajan *et al.,* 2012). Mosquito control relies heavily on synthetic insecticide application. However, excess and injudicious application of synthetic insecticides has resulted in the development of resistance to these insecticides by mosquitoes and unwarranted toxic or lethal effects on non-target organisms, as well as environmental health problems. This phenomenon has triggered and urged the development of alternative techniques using natural products.

Eichhornia crassipes (Mart.) Solms commonly called water hyacinth is an aquatic perennial herb that belongs to the family Pontederiaceae. It is indigenous to the New World tropics and has its center of origin in Amazonia, Brazil with anthropogenic spread to other areas viz., Venezuela, parts of Central South America, the larger Caribbean islands, Egypt, India, Australia and Java. The fresh juice of this plant is used to treat fresh wounds and along with vinegar, it is being used in the treatment of septic wounds. The plant possesses antimicrobial, antioxidant, antitumor, anti-inflammatory and wound healing activity. The phytochemical constituents include anthraquinones, phenolics, alkaloids, flavonoids, sterols, anthocyanins, proteins, quinones, flavonoids, anthraquinones, carbohydrates, stigmasterol, campesterol, β-sitosterol and many phenalene compounds.

Eichhornia crassipes is also reported to exhibit insecticidal activity. In Bangladesh, the dried whole plant of water hyacinth has been used to ward off insects in animal sheds.

Considering the above-mentioned insecticidal activity particularly mosquitocidal, the present study is aimed to evaluate the larvicidal activity of *Eichhornia crassipes* crude leaf extracts against the filarial vector.

MATERIALS AND METHODS

Collection And Preparation Of Plant Extraction

The leaves of the *Eichhornia crassipes* were collected separately from the natural habitat of Vandalur, Chengalpattu district. Hydro-distillation process was separately performed for *Eichhornia crassipes* leaves using a Clevenger arm apparatus. The aqueous layer and organic layer were collected separately. Then the organic layer was allowed to dry over anhydrous sodium sulphate and the aqueous layer was extracted twice with dichloromethane. Finally, the combined solvents were evaporated and essential oil was obtained. Essential oil was weighed and stored in the refrigerator at 4ºC until it was used for the experiment.

Preparation Of The Test Solution

10% (v/v) of essential oils (*Eichhornia crassipes*, lemon and peppermint oils) containing ethanol solutions were mixed and prepared a stock solution. 0.3 ml of essential oil stock was mixed with 3 drops of Tween 80. Then ethanol was added until the volume was 3 ml. To prepare the control test solution, ethanol was added to 3 drops of Tween 80, until the volume was 3 ml.

Mosquito Larval Culture

To satisfy the enormous number of mosquitoes needed for the day-to-day bioassays, a colony is essential. The eggs and egg rafts of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* were collected from the stagnant water loggings in the nearby locality. Filter paper attached with eggs was dipped into a plastic tray containing 500 ml of de-chlorinated water for 30-40 min, time enough to allow for eggs to hatch into larvae. They were reared indoors at 28±2°C temperature and a 14:10 light and dark period cycle. The larvae were fed with a powdered mixture of fish food and yeast powder in 3:1 ratio. After five days of emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with a 100 g/L sucrose solution and allowed for blood feed using white albino for 2-3 h. A few days after having a blood meal, the gravid mosquito laid their eggs.

Larvicidal Activity

The test for the larvicidal effect of *Eichhornia crassipes* oil against mosquito larvae (*A. aegypti, C. quinquefasciatus* and *A. stephensi*) was conducted by WHO standard method.

Preparation Of Dosages

10ml of test solution was prepared by serial dilutions. It is expressed in volume per volume (v/v). Batches of 15 early 4th instar larvae of three mosquitoes (*A. aegypti, C. Quinquefasciatus* and *A. stephensi*) were transferred to big sized petri plate containing 100ml of distilled water and 1ml of test solution (10- 100ml). Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of 1 mL of the control test solution and 100ml of distilled water only for the larvicidal activity test. Different dosages of test solutions were prepared in the following ratio:

1. Dosage 1 - 1:1 (5ml of test solution+ 5ml of distilled water)
2. Dosage 2 - 1:2 (2.5ml of test solution + 7.5ml of distilled water)
3. Dosage 3 - 1:3 (1.25ml of test solution + 8.75ml of distilled water)
4. Dosage 4 - 1:4 (0.625ml of test solution + 9.375ml of distilled water)
5. Control

The above doses are used to make serial standard solutions of *Eichhornia crassipes* and 24 hours to observe their mortality.

After treatment, symptoms in treated larvae were observed and recorded immediately at time intervals and no food was offered to the larvae. At the end of 24 h, the larvae were considered dead, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the WHO's technical report series. The percentage of mortality was calculated with Abbott's formula,

$$\% \text{ Mortality} = \frac{\% \text{ Test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100\%$$

Preparation of the Mosquito Repellent Spray

Bio-Efficacy Testing of the Mosquito Repellent Spray

Bio-efficacy tests of the mosquito repellent spray were done. Outdoor and indoor field trials were conducted over two days from 8 am to 11 am by separately applying the mosquito repellent spray.

RESULTS

Extraction of *Eichhornia crassipes* oil

The simple oil distillation method was used for the extraction of oil (Figure 1). The yields of the extracts and the essential oils were determined as a percentage (w/w) using the formula,

$$\%\text{Yield of essential oil} = \frac{\text{Weight of essential oil}}{\text{Initial weight of plant material}} \times 100\%$$

Figure 1: E*xtracted Eichhornia crassipes* oil

Effects of Larvicidal Potential

The test solution of *Eichhornia crassipes* oil shows 100%, 90%, 73.3% & 40%, mortality rates after 4 hours of exposure in different dosages respectively (Figure 2).

After 8 hours it showed 100%, 93.3%, 90% &70% mortality rates in the above-mentioned concentrations respectively (Table 1).

Table - 1: Bioassay Experiment For Larvicidal Potential

Dilution	Concentration (gm/L)	No. of larvae (0 hrs)	% mortality rate (%)	No. of larvae after 8 hrs	Percentage mortality rate (%)
1:1	500	15	100	0	100
1:2	250	15	90	1	93.3
1:3	125	15	73.3	2	90
1:4	62.5	15	40	5	70

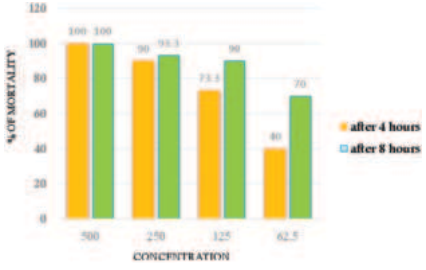

Figure 6: Mortality Rate

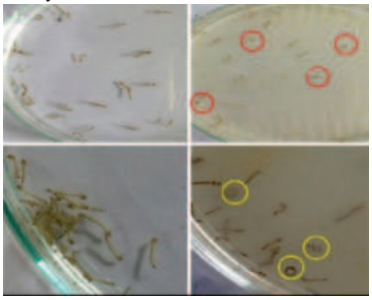

Figure 7: Effect of larvicidal activity

Effect Of Mosquito Repellency

The percentage of the mosquito repellency for test solutions of *Eichhornia crassipes* oil shown in Table 2 was calculated as below,

$$\% \text{ Mosquito Repellency} = \frac{C\text{-}N}{C} \times 100$$

where,

C= Number of mosquitoes aligned/left and aligned/bit when the solvent was used

N= Number of mosquitoes aligned/left and aligned/bit when the test solutions were used

Table 2: Mean Values Of Mosquitoes Repellency (%)

Formulated Dosages	Calculated Mean Value of Mosquitoes	Percentage Mosquito Repellency (%)
D1	1.00	93.75
D2	1.67	89.56
D3	2.33	85.44
D4	3.00	81.25
Control	0	0

Table 3: Bio-efficacy Test Results Of The Mosquito Repellent Spray

Time (am)	Day 1				Day 2			
	Indoor				Outdoor			
	Control	D1	Control	D1	Control	D1	Control	D1
8	18	0	15	0	17	0	15	0
9	13	0	14	0	16	0	14	0
10	07	0	09	0	11	0	08	0
11	04	0	05	0	08	0	03	0

According to the indoor and outdoor field trials which were carried out for four hours each day for two days, both the mosquito repellent spray has shown 100% mosquito repellency. The findings of this study could be varied to many factors such as the species of mosquito and the quality of the essential oil for mosquitoes. The quality of the essential oils depends on many factors such as plant species, growth conditions, maturity of plants, plant storage, plant preparation and methods of extraction.

CONCLUSIONS

Eichhornia crassipes essential oil showed higher mosquito repellent activities compared to plant extracts of the literature cited. The mosquito repellent spray which contained 1:1 ratio (v/v %) of the active ingredient, showed 100% mosquito repellency for outdoor and indoor field trials which were carried out for six hours each day for two days.

The present investigation reports the larvicidal potential and the mosquito repellency of *Eichhornia crassipes* essential oil was effective even at very low concentrations and could be a better alternative for chemical mosquito repellent sprays against diseases spreading mosquito larvae and mosquitoes.

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