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Natural Extracts as Eco-Friendly Larvicides against *Aedes aegypti* Mosquito, Vector of Dengue Fever Virus in Jeddah Governorate

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Severe human diseases are spread by mosquitoes, causing millions of deaths every year. Many well-known and severe problems have been caused by the indiscriminate use of synthetic chemical insecticides, such as the residual insecticides for humans and environment and high operating cost in addition to the possibility of developing insect resistance. The larvicidal and delayed effects of the body wall extract of *Holothuria scabra* and leaves extract of *Acalypha fruticosa* against 4th instar larvae of mosquito, *Aedes aegypti* were evaluated. Ethanolic extract of *H. scabra* recorded more larvicidal efficiency (LC50, 79.31 ppm) than A. *fruticosa* leaves extract (152.86 ppm) by about 1.93 folds. Morphological features showed abnormalities on the larval and pupal stages with *H. scabra* and to less extent with *A. fruticosa*. Therefore, it is possible to build on the results of this study to use these two extracts to control of A. *aegypti* mosquitoes and in line with recent trends in adopting combat methods that are safe on humans and the environment.

Keywords: *Aedes aegypti*, Dengue fever, *Acalypha fruticosa*, *Holothuria scabra*, Natural larvicides**.**

Mosquitoes belong to (Diptera: Culicidae) are a category of insects that pose the greatest threat to human and veterinary health as vectors of diseases, more than any other insect group.

The mosquito *Aedes aegypti* is one of this category that shares a similar ecological niche with human. Globally, mosquitoes represent a major public health problem. They are estimated to spread diseases to more than 700 million individuals annually and are currently expected to be responsible for the deaths of a round one person in 17 people[1].

Aedes. aegypti is the principal transmitter of Dengue, Chikungunya, Yellow fever and Zika viruses. This mosquito species is well suited to humankind. Their females get blood meals through biting the mammals and digesting it inside their bodies to obtain the eggs. The control of A. *aegypti* is difficult task because they able to lay their eggs in many places even those of low quantity of water.

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The eggs have the ability to survive months in the dry conditions and they hatch as soon as water is available. Furthermore, they have been developed resistance against commonly used insecticides [2,3,4].

Extensive application of pyrethroid and organophosphate insecticides to monitor all types of mosquito has resulted in an acceleration of the level of resistance created. In addition to resistance, other adverse effects may be associated with certain mosquiticides, including harmful effects against non-targeet species, environmental problems and human health concerns[5].

Surveys of appropriate alternatives to conventional insecticides have shown that phytochemicals are a good choice in terms of relative protection, global availability and low cost. Therefore, screening for locally available medicinal plants for mosquito control may be an option that is cheaper than costly imported products to improve local jobs and public health[6].

Chemicals extracted from natural sources have recently been predicted to be weapons of potential mosquito control programs as they are shown to be eco-friendly and largely non-toxic to humans and other mammals and biodegradable[7].

These natural metabolites exhibit significant bioactivities including antidiabetic, antioxidant, antibacterial, anticancer and antiinflammatory activities[8,9] and several of these natural products can be used for derive a unique drug agents[10].

The marine ecosystem is an enormous and incredibly rich source of biological and chemicals products of natural origin. Many of these substances have beneficial medical and pharmaceutical properties. A large number of marine bioactive metabolites with specific properties have recently been isolated and characterized[11]. Natural biologically active constituents such as steroids, terpenoids, alkaloids, sterols and other metabolites are formed by the marine biota[12].

Recently, bioassay work was designed to evaluate the larvicidal effect of methanol, chloroform, ethyl acetate and aqueous extracts of macro algae *Codium edule* against *Aedes aegypti* larvae. The findings indicated that Chloroform fraction exhibited the most larvicidal activity with LC50 value of 19.54 ppm[13]. In this manner the present work aimed to study the possibility of control of *Aedes aegypti* by extracts of *Holothuria scabra* as a marine animal and of *Acalypha fruticosa* as a terrestrial plant under the laboratory conditions.

MATERIALS AND METHODS

Mosquito colony

The study requires a sufficient number of larvae to carry out the biological evaluation experiments and for this purpose, The mosquito *Aedes aegypti* strain was brought from the Jeddah Municipality laboratory to combat public health pests, Kingdom of Saudi Arabia. The mosquito colony was established under laboratory controlled conditions (temperature 27±2 °C, Relative humidity 75± 5 % and 10-14 light-dark periods) in the dengue research unit at King Abdulaziz University, where adult insects were kept in Canvas cages and daily provided with 10% sucrose solution. The females mosquitoes were fed with adequate blood meals from a pigeon and then mosquito eggs were obtained, which were immersed in ceramic dishes with sizes of 20-30 cm half-filled with dechlorinated water. Mosquitoes raring continued for a number of generations according to the method of (Mahyoub, 2013)[14]. The hatching larvae were fed daily by mixture of yeast powder: dry bread powder: skimmed milk powder in 1:1:1 proportions[15].

Sea cucumber and plant materials

Sea cucumber *Holothuria scabra* was collected from AL-kharrar lagoon, Red Sea (west of Saudi Arabia) during July 2019. The animal was identified by marine biology department-King Abdulaziz university. The fresh leaves of plant *Acalypha fruticosa* were collected from AL-Baha region (south of Saudi Arabia) during August 2019 and the plant was classified by taxonomist, department of biology, faculty of Science, King Abdulaziz university. The *H. scabra* body wall and the leaves of *A. fruticosa* were washed by water and left in shade area at room temperature until become dry, then grinded using electrical blender to obtain fine powder.

Preparation of extracts

The extraction was performed by soaking 100 gm fine powder of both organisms in sufficient amount of 70 % ethanol in shaking machine for overnight. The supernatant was filtered using whatman filter paper, the extraction was repeated three times and the combined solvent was subjected to rotary evaporator at 45 °C. The dry extract (6.45 gm of *H. scabra* and 8.67 gm of *A. fruticosa*) were kept under -4°C until start the experiments[16].

The bioassay experiments

The mosquito fourth instars larvae were treated with a series of concentrations of both plant and sea cucumber extracts under laboratory conditions at a temperature of 27 ± 2 ºC and a relative humidity of 75 ± 5%. Standard method of immersion was used[1]. The tests were carried out in a beaker containing 100 ml of water and five replicates were used for each concentration where each single contains 20 larvae in addition to five replicates for control. Taking into account the supply of larvae with food to avoid starvation factor. Mortality rate were recorded during the period from the start of the experiment until pupation and adult emergence.

Statistical analysis

The mortality percentages of larvae were calculated for each concentration. The results were analyzed using LDP line software to derive statistical values and constants[17] at 95% confidence intervals and a significant level of 0.05 based on the degree of probability Probit analysis and calculating LC50 and LC90 together with the lower and upper confident limits and the inclination of toxicity line and Chi square according to Finney 1971[18].

Record of deformities

The morphological features of treated larvae have been compared to control media during the course of lethal experiments. Any noticeable difference in appearance between control and treated was reported as deformity. The deformities were described according to their resemblance to those previously seen in the literature[19].

RESULTS AND DISCUSSION

Bioactive products from natural origin with insecticidal properties have been used in the recent pest control of different insect pests and vectors[20].

The mosquito-human relationship is related to many diseases. Serious diseases such as malaria, arboviral encephalitis, dengue fever, chikungunia fever, west Nile virus and yellow

Table 1. Effect of sea cucumber *H. scabra* and plant *A. fruticosa* on *Aedes aegypti* 4th instar larvae

Conc. (ppm)	Mortality (%)	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	$(Chi)^2$	slope
H. scabra					
50	35	79.31(65.4 - 92.6)	314.12(260.4 - 402.6)	2.2	2.144
100	58				
200	79				
300	87				
500	98				
A. fruticosa					
50	18	152.86(130.3- 177.7)	782.34(588.3- 1174.2)	0.367	1.807
100	39				
200	57				
300	71				
500	82				

Table 2. Index compared *Holothuria scabra* with *Acalypha fruticosa*

Line name	LC50	Lower limit	Upper limit	RR
Holothuria scabra	79.31	65.49	92.61	1.927
Acalypha fruticosa	152.86	130.30	177.73	

fever are transmitted by mosquitoes. These diseases cause significant mortality in human and livestock around the globe[16].

Results of toxicity test of *H. scabra* and *A. fruticosa* extracts on the 4th larval instar of *A. aegypti* were listed in Table (1 & 2) and Figure (1, 2 and 3). It is clear that the mortality percent increased with increasing extracts concentrations. *H. scabra* extract was found to be more bioactive than *A. fruticosa* extract where LC50 values were (79.31, 152.89) ppm and LC90 values (314.12, 782.34) ppm, respectively.

Both extracts caused reduction in the pupation percentage. The pupation decreased as the extract concentration increased. Moreover, the toxic effect of *H. scabra* had been extended to the

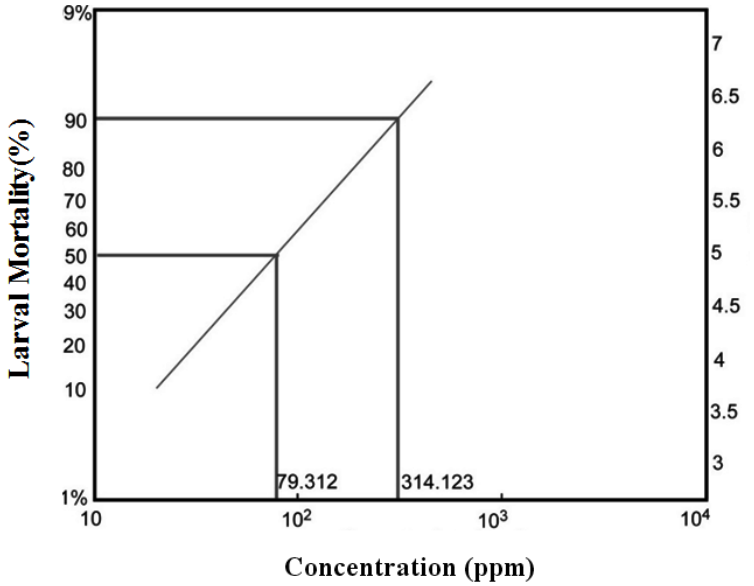

Fig. 1. Relationship between *H. scabra* extract and larval mortality percent of *A. aegypti*

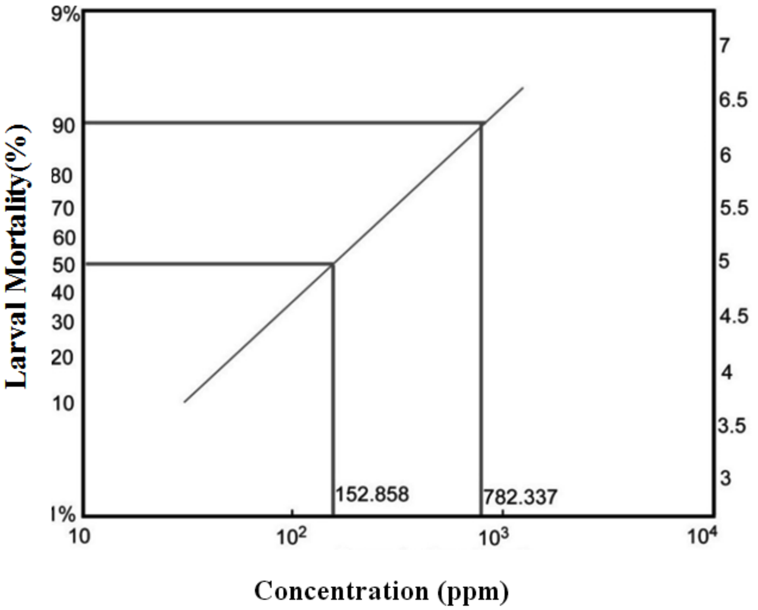

Fig. 2. Relationship between *A. fruticosa* extract and larval mortality percent of *A. aegypti*

pupae and adults and this effect also observed with *A. fruticosa* but to less extent. These effects of both extracts manifested in deformities noticed under microscope (Figure 4&5). In addition, the extracts caused decline in the emergence of adults and this reduction depended on the extract concentration. Such findings are consistent to some extent with previous outcomes of (Sharma et al., 2006)[21].

Strong activity of *H. scabra* may be attributable to their high saponin content. High larvicidal activity of saponin extract has been proven in earlier study with LC50 11.7 ppm against *Culex pipiens*[20]. Also dose of 35 ppm of saponin extract of *Balanites aegyptiaca* fruit mesocarp inhibited fifty percent of the population of treated larvae, this would definitely help to significantly decrease the mosquito density[22]. Earlier reports suggested the bioactivity of saponins as a naturally mosquito larvicidal agent; however, no investigation has been done on saponins regarding their effect on mosquito adult emergence[23].

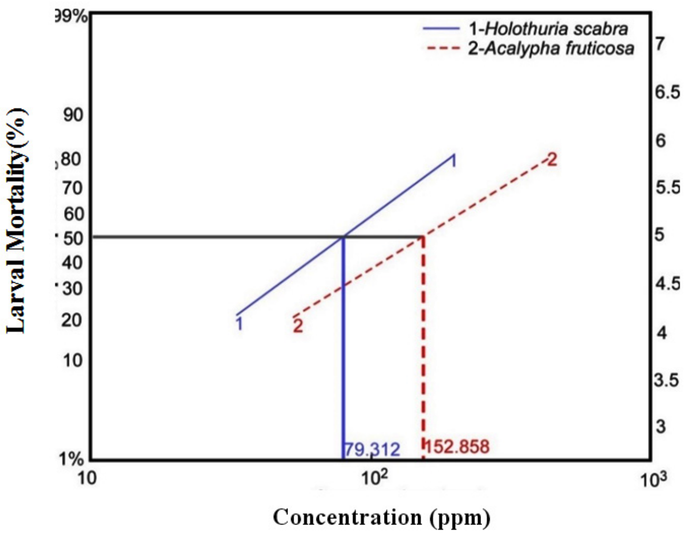

Fig. 3. Relationship between *H. scabra* and *A. fruticosa* extracts and larval mortality percent of *A. aegypti*

Fig. 4. (a) larvae shows dark melanization (b) dwarf of larval body (c) Normal larvae (larvae treated with A. *fruticosa*)

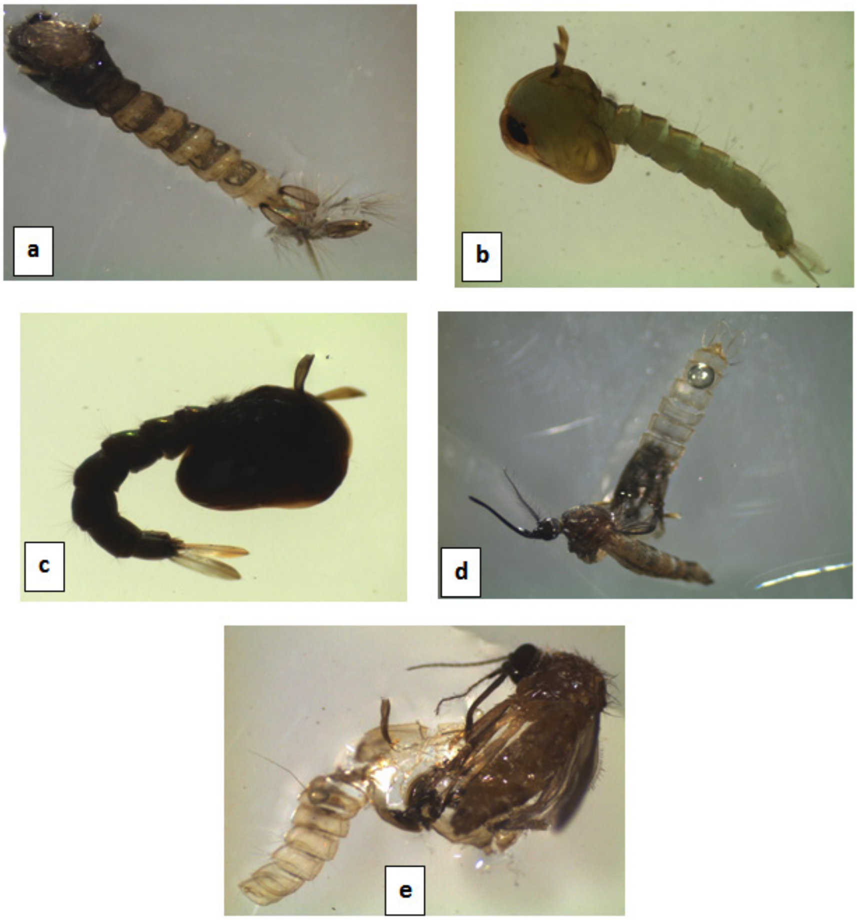

Fig. 5. (a, b) albino case (c) normal pupae (d, e) partly emerged adult with attached pupal case (larvae treated with *H. scabra*)

These observed and reported deformations on the larvae treated with the sea cucumber extract may be due to the fact that it contains many valuable valid active chemical constituents such as saponins (mentioned above) and terpenes, this results are in line with previous study on Sea cucumber *Holothuria atra* extract against *A. aegypti*[24]. On the other hand phytochemicals found in *A. fruticosa* can contribute collectively or independently in larvicidal action.

In general, the death of treated larvae could be attributable to the failure of the moulting bodies to swallow adequate volume of air during ecdysis to break the old cuticle and extend the new one, or to the effect of the plant extract to inhibit larvae body metamorphosis which may be based on the hormone control disturbance and lead to an imbalance of the growth processes and larval deaths[19, 25].

CONCLUSION

The current investigation showed high bioactivity of sea cucumber *H. scabra* and plant *A. fruticosa* extracts against larval stage of *Aedes aegypti* mosquito. Such findings could be provide an opportunity to develop alternatives to organophosphorous, pyrothroids and other artificial insecticides that are very costly and environmentally hazardous.

So these extracts represent candidates that can be used in mosquito control without harming the surrounding environment in addition to their less cost. We recommend to do further chemical investigation to isolate the active ingredients and preparation for their commercialization.

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Conflict of interest

The authors declare no conflict of interest.

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