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RESEARCH ARTICLE

STUDIES ON LARVICIDAL ACTIVITY OF *GLORIOSA SUPERBA* ON THE DEVELOPMENTAL STAGES OF THE CHICKUNGUNYA MOSQUITO, *AEDES AEGYPTI*

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ABSTRACT

In the present study the larvicidal effect of botanical insecticide, *Gloriosa superba* against *Aedes aegypti* was administrated. The plant extract showed higher mortality in lower doses and it exhibit better protection against the different developmental stages of *Aedes aegypti*. Based on the probit analysis,the LC_{50} values and 95% upper and lower fiducial limit and regression values of the plant extract on *Aedes aegypti* were also noticed. The treatment of seed extract against III and IV instar larvae of *Aedes aegypti* showed that III instar larvae are more susceptible than IV instar larvae. These morphogenetic abnormalities are commonly caused by botanical extract and the disturbance from the growth regulating hormones. It is because the active principle compounds such as terpenoids, alkaloids, flavonoids, phenols and tannins present in this plant. Activity of alkaline phosphatase has been drtermined during different hours of larval development of *Aedes aegypti*. The activity has been determined during III instar mould to IV instar mould with a period of 24 hours in *Aedes aegypti*, activity increased from 35ppm to 40ppm. It is therefore suggested that *Gloriosa superba* derivatives can be effectively used as a biocide for vector control operations.

INTRODUCTION

Mosquitoes are vectors of serious human diseases and are the single most important group of insects in terms of public health importance (WHO 2010). The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss and social disruption such as malaria, lymphatic filariasis and viral diseases is well recorded (Becker *et al*., 2003). Mosquito borne diseases have an economic importance, including losses in commercial and labour outputs, particularly in countries with tropical and subtropical climates (Fradin and Day 2002). *Aedes aegypti,* the main carrier for viruses that cause dengue, hemorrhagic and yellow fever is found majorly in tropics and subtropics. There is no effective vaccine against dengue, and thus the only way of significantly lowering the incidence of this disease is through mosquito control (Malavige *et al*., 2004). The use of insecticidal sprays and other chemicals for killing the adult mosquitoes will affect the natural environmental conditions. Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, air and water, so there is a constant need for developing biologically active plant materials as larvicides which is expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment (Prabakar and Jabansan 2004). In an attempt to resolve these problems, attention to insecticides of natural origin, particularly plant derived product, has been recently revived (Chaithong *et al*., 2006). In early historical times man used plant extract and compounds to control certain pests and disease causing insects. Plants are rich source of bioactive organic chemicals and synthesize a number of secondary metabolites that serve as insecticides, antifeedant, oviposition deferrents, adulticides, repellents, growth inhibitors (Mittal and Subbarao 2003). Phytochemicals obtained from the huge diversity of plant species are important sourse for safe and biodegradable chemicals, which can be screened for mosquitocidal activities. The natural products, especially plant derivatives called bioinsecticides are now emerging as a viable component of Integrated Pest Management (IPM) strategies in view of their pesticidal potency as well as efficiency on pest and safety to parasitoids and predators (Kumbhar *et al*., 2000).

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Phosphatases

The role of acid phosphatase in processing of yolk during development was first recognized by (Lemanski and Aldoroty 1974) working with amphibian embryos. In addition acidification of yolk platelets during xenopus (Fagotto and Maxfield 1994), sea urchin (Mallaya *et al*., 1992), and tick development (Fagotto 1991) was demonstrated to correlate with yolk utilization. Genetic variations of alkaline phosphatases in late instars of *Drosophila melanogaster* has been studied by starch gel electrophoresis (Harper and Armstrong 1973). They found that the secretory tissue involved with the maintainense of the sperm, contained the

most intensive activity of all the tissue that were examined. The importance of phosphatase in biochemical transformation has lead to several studies of acid and alkaline phosphatase activity during insect metamorphosis. Hence the present study was carried out to control the mosquito population as well as to study the phosphatase enzyme in different larval stages (III and IV) in *Aedes aegypti* by using the seed extract of *Glorioa superba*.

MATERIALS AND METHODS

For the present study the acetonic extract of seeds of *Gloriosa superba* was used to determine the groups of secondary metabolites present in it and to test the efficiency and insecticidal effects against III and IV larval stages of the mosquito *Aedes aegypti.*

Rearing and maintanance of the mosquitoes

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute of Communicable Diseases (NICD) at Mettupapalyam and were placed separately in sterilized glass throughs containing two liters of unchlorinated tap water, (Size 18cm diameter and 9cm height) under laboratory conditions.

Rearing of larvae, pupae and adult

After 24 hrs freshly hatched larvae were collected and maintained in separate containers with tap water (capacity 2liters); glucose biscuit and yeast (2:1) were given as the source of food. The mosquito colony was maintained at 7.0 - 8.5 pH, 28±2^{o}C temperature and 14:10 light and dark photo period cycle. Pupae were isolated from the culture and were allowed to emerge into adults in the mosquito net cage (42 cm x 30cm x 30 cm). Emerged adults were fed with 10% sucrose solution socked in cotton wick. Pigeon blood was given as a source of food for female mosquitoes as detailed by Meola and Readio (1987). Different batches of adults were maintained in the cage by introducing sufficient number of pupae. A small container with water was kept inside the cage to facilitate the female to lay the eggs. The eggs in the container were removed carefully and allowed to hatch. Eggs were laid singly and placed on the dry surface water. Usually the eggs are laid early in the morning. They are oblong shaped. The incubation period of the normal eggs were 48 hours. The plastic trays were examined every six hours (6, 12, 18 and 24 hours) and the number of larvae was recorded. Glucose biscuit was given as a source of food for the larvae.

Preparation of phytochemical extract

The seeds collected from the field were brought to the laboratory, washed with tap water followed by distilled water and were dried under shade. The dried seeds were ground to fine powder. The powdered seeds (100 gm) were extracted with acetone (300 ml) by using soxhlet apparatus for 8 hours (Vogel 1978). The extract was concentrated in a vacuum evaporator at 45^{o}C under low pressure. After complete evaporation of the solvent the concentrated extract was collected and stored in separate glass vessels at 4^{o}C in refrigerator for further experiments. 25 gm of the concentrated extract of dried seed of experiment plant were dissolved in 750 ml of acetone and kept as stock solution and were used to prepare the desired concentration for exposure of the mosquito larva.

Preliminary phytochemical studies

The extract was subjected to determine the presence of groups of secondary metabolites present in the plant materials such as alkaloids (Ciulci, 1994), flavanoids (Sofowara, 1993), glycosides (Gokhale *et al*., 2008), saponins (Brain and Tum, 1975), tannins (Mace and Gorbach, 1963:Ciului, 1994), steroids (Ciulci, 1994), phenols (Krishnamoorthy, 1998).

Larvicidal bioassay

The larvicidal bioassay was done using standard WHO protocols (W.H.O. 1996). To obtain the different concentrations of test medium, the stock solution were dissolved in water and then mixed thoroughly with the dry ingredients of the diet. Twenty freshly moulted third and fourth instars larvae of *Aedes aegypti* were exposed to different desired concentrations of plant extracts. At each tested concentrations 2 to 5 trails were made and each trail consist of four replications. Controls were maintained using respective solvents along with the experiment. Mortality of different developmental stages of treatment and control over a period of 24 hours was observed. The dead larvae were identified when then failed to move after probing with a needle in the siphon of cervical region, mori bund larvae were those incapable of rising to the surface or showing the characteristic diving reactions when the water was disturbed. Mortality in control was negligible in calculation.

Phosphatase

Enzyme source

Freshly moulted III and IV instar larvae were sacrificed and the cuticle was homogenized in buffer A with help of a mortar and pestle in ice cold condition. The homogenate was centrifuged at 10,000 rpm for 5 minutes and the clear supernant was freeze dried using speed vaccum concentrator and evaporater (savant USA) and stored. Buffer A was prepared by dissolving 20% sucrose, 1 mM d isopropyl phosphofluoridate (DPF) and a trace amount of phenyl thioueia (PTU) in 50mM of phosphate buffer (PH 6.9).The buffer contained DPF (1Mm) and phenyl thiourea to prevent the activity of the protease and tyrosinase respectively.

Assay of alkaline phosphatase activity in the III and IV instar larvae of *Aedes aegypti*

The assay medium consisted of 0.75 ml of citrate phosphate buffer, tris HCL buffer (0.01;P^{H} 10.5) for alkaline phosphatase, and 0.75 ml of substrate disodium phenyl phosphate (0.01M) and to which 100 µl of the enzyme source was added .Before the addition of the enzyme, the solution containing the buffer and the substrate was brought to room temperature. After the

addition of the enzyme, the contents were mixed well and allowed to stands for 45 minutes. After the incubation period 0.675 ml of the Folin Ciocalteus reagent was added to stop the reaction followed by the addition of 1.0 ml of 20% of sodium carbonate solution and the intensity of colour development was measured at 680 nm. Control was maintained along with experimental tubes by adding all the reagents and denatured enzyme or 100 µl of buffer in the place of enzyme.

Table 1. Results of preliminary phytochemical screening of *Gloriosa superba* seed using acetone extract

Secondary metabolites							
Alkaloids	Flavonoids	Glycosides	Saponins	Tannins	Steroids	Phenols	Terpenoids
+	+	-	-	+	-	+	+

+ indicates the presence of compounds and - indicates the absence of compounds

Table 2. LC50 values of acetone seed extract of *Gloriosa superba* on iii and iv instar of *Aedes aegypti*

Larval stages	% of mortality					95% Fiducial limit (ppm)		LC_{50} (ppm)	Regression equation
	30 ppm	35 ppm	40 ppm	45 ppm	50 ppm	LM	UM		
III instar	27	39	51	60	72	34.62	43.15	34.62	Y= -39+2.22x
IV instar	19	36	50	71	87	36.19	44.62	40.47	Y= -84.2+3.42x

Table 3. Effect of *Gloriosa superba* seed extract on the activity of alkaline phosphatase in III and IV instar larvae of *Aedes aegypti* (OD/100 µG protein/MIN)

S.NO	CONTROL	III INSTAR	CONTROL	IV INSTAR
1	0.679	0.575	0.559	0.489
2	0.656	0.539	0.570	0.470
3	0.680	0.540	0.578	0.478
4	0.667	0.548	0.582	0.482
5	0.668	0.529	0.500	0.464
Mean	0.670	0.546	0.578	0.456

RESULTS AND DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. Numerous workers have tested the efficacy of several plant extract using different mosquito species (Samuel *et al*., 2012). There is a number of naturally occurring compound that posses plant protection properties. The botanical extracts from the plant, leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. The preliminary screening is a good mean of evaluation of the potential larvicidal effects and the highest mortality was observed in the acetone seed extract of *Gloriosa superba* was observed under laboratary conditions and is presented in Table 1. In the present study effect of acetone seed extract of *Gloriosa superba* were experimented against III and IV instar larvae of *Aedes aegypti* and the percentage of mortality was calculated. Plants are rich source of bioactive compounds that can be used to develop environmemtally safe pest managing agents. The plant tested in our study are used for pest control in various parts of the world. The percentage of mortality values for III and IV instar larvae of *Aedes aegypti* treated with various concentrations of the seed extract of *Gloriosa superba* and LC_{50} values were presented in Table 2. Highest mortality 0f 87% and 72% was recorded for third and fourth instar larvae of *Aedes aegypti* at 40ppm concentration. *Aedes aegypti* larvae exhibited very little susceptibility to lower doses and the mortality of 27% and 19% was reported at the concentration of 30ppm. The highest sensitivity of III instar larvae of *Aedes aegypti* to the seed extract of *Gloriosa superba* was also evident by the lowest LC_{50} values (LC_{50}=34.62ppm). Least susceptibility was shown by IV instar larvae of *Aedes aegypti* (LC_{5O}=40.47ppm) respectively. Similar trend of differential susceptibility of mosquito species to plant extract was reported earlier. The effective responce of mosquito in terms of malformations and mortality to plant extract of this plant extract exhibit that the studied mosquito is highly susceptible to the active compounds such as terpenoids, alkaloids, flavonoids, phenols and tannins present in this plant. Present study reveals that in *Aedes aegypti* during the growth and development the alkaline phosphatase activity raised steadily and reached maximum before the time of pupation. Alkaline phosphatase activity in the III and IV instar larvae was studied and the results are shown in Table III. Moreover further studies need to performed to recognize the mode of action between the extract and mosquito larvae. From the overall results it is clear that the seed extract of *Gloriosa superba* can be effectively used as a biocide in the control of mosquito *Aedes aegypti.*

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