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LARVICIDAL ACTIVITY OF *GLIRICIDIA SEPIUM* AGAINST *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Formulating phytochemicals in lieu of the synthetic ones for the control of mosquito menace is the imminent need of the day. Hence an investigation was carried out to explore the activity of different extracts of *Gliricidia sepium* against fourth instar larvae of *Culex quinquefasciatus*. The larval mortality was observed after 24 h of exposure. All the extracts showed moderate larvicidal effects; however the highest larval mortality was found in petroleum ether extract (LC_{50}= 61 ppm).This has revealed the presence of highly potential ergastic materials in *Gliricidia sepium* which can be exploited for increasing the mortality rate in mosquito larvae. As the source plant is very common and the formulation is not costly, the present study may open new vistas towards finding out an environmentally safe and effective larvicide of the mosquito. Further this could boost up amelioration of the mosquito menace which is very acute in South India.

KEYWORDS : Phytochemicals, Larvicide, Mosquito, Culex, Gliricidia

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INTRODUCTION

Mosquitoes are well known as vectors of serious human diseases such as malaria, filariasis, dengue and other viral diseases. *Culex quinquefasciatus* is a vector of lymphatic filariasis a widely distributed tropical disease with around 130 million people infected worldwide and 44 million people having common chronic manifestations[1]. It is reported that *C.quinquefasciatus* infects more than 100 million individuals worldwide annually[2]. Human filariasis transmitted by *C.quinquefasciatus* is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries[3].

It is established that repeated use of synthetic larvicide results in the development of resistance in mosquitoes; and hence effective ecofriendly phytochemicals are the need of the day. Plant world comprises a store house of bio chemicals that could be tapped for use as insecticides and they are the richest source of renewable bioactive organic chemicals. Total number of plant chemicals may exceed 400,000; of these 10, 000 are secondary metabolites whose major role in the plant is reportedly defensive[4]. The naturally occurring pesticides thus appear to have prominent role in the development of future commercial pesticides not only for agricultural productivity but also for safety of environment and public health[5].

Gliricidia sepium (jacq.) Kunth ex steud belonging to Fabaceae is a semi deciduous tree with broad canopy of leaves. It grows best in tropical, seasonally dry climates. The tree thrives in deep and well drained soils. *Gliricidia* is remarkable with imparipinnately compound leaves clustered towards the apex of branches. Leaves bear a prominent pulvinus and 5-8 leaflets on each side. The racemose cluster of pinkish white flowers is borne cauliflorously. Prominent zygomorphy, vexillary condition, brownish anthers on the staminal tube and the black colour of the mature legumes are very characteristic of the genus.

As *Gliricidia* appears to be a multipurpose tree in the light of various reports published, the present endeavour is intended to elucidate its impact on the growth of the larvae of *Culex quinquefasciatus.*

MATERIALS AND METHODS

(i) Rearing of mosquitoes:

Larvae were collected and kept at 27 ± 2 °C temperature at lab room. They were fed with a diet of finely ground biscuits and yeast (3:1). Pupae emerged were transferred to new trays containing tap water placed in screened cages(30×30×30 cm).When adults emerged they were fed with chicken blood and 10 % glucose solution. Glass petridishes with tap water lined with filter paper was kept inside the cage for oviposition[6].

(ii) Plant collection and extraction

Fresh mature healthy leaves were collected from Vagamon at Idukki district, in Kerala state during the month of November to January. They were cleaned, cut into small pieces, dried in shade and ground well in electric mixer .The extracts were prepared from the fine powder by employing a soxhlet apparatus using petroleum ether, hexane, acetone, methanol and water successively as solvents. For 50 gm of powder 250 ml of the solvent may be added. The extracts were evaporated in a rotary vacuum evaporator at 40 °C to dryness and stored at 4 °C in an air tight bottle for further analysis[7].

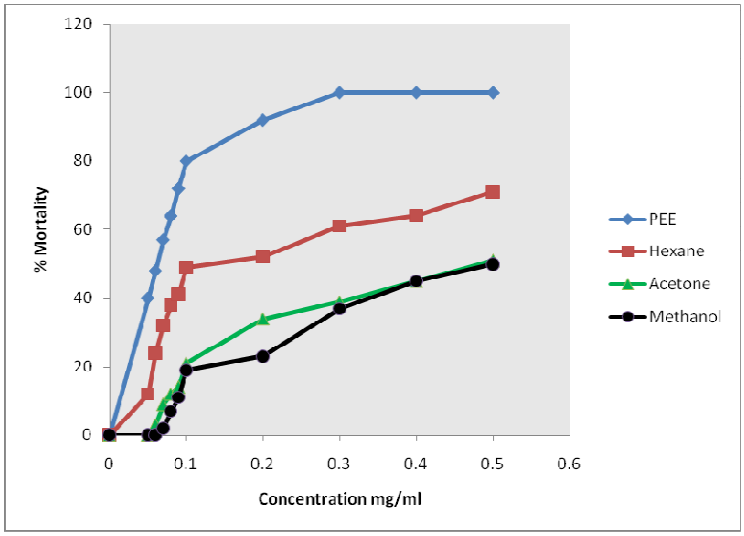
(iii)Larvicidal bioassay

500mg of extract was dissolved in 100 ml of water. Various concentrations 0.05-0.5 mg/ml were made from this stock solution. The concentration of Dimethyl sulfoxide (DMSO) used as emulsifier in the assay was 1 %. Controls were also used for each assay which involved treating the larvae with 1 % DMSO in water. Twenty five, 4th instar larvae were released into 250 ml glass beaker containing 100 ml solutions. Four replicates were setup for each concentration. Larvae were counted as dead when they were not

coming to the surface for respiration or probe insensitive[8]. Observation on mortality of the larvae was recorded after 24 h of continuous exposure. The dead larvae in four replicates were combined and expressed as percentage of larval mortality for each concentration.

RESULTS

This work demonstrates the potency of *G. sepium* in the control of mosquito larvae. It was observed that larvae become slowly inactive after ten hours and began to fall towards the bottom of the beaker. The dose dependent effect of leaf extracts on the mortality percentage of fourth instar larvae of *Culex quinquefasciatus* is represented in Graph 1. The data were recorded and statistical data regarding LC_{50} with 95 % confidence limit values were calculated using EPA probit analysis program version 1.5(Table 1). The LC_{50} values of *G.sepium* against fourth instar larvae of *C.quenquefasciatus* were 0.061, 0.160, 0.428, and 0.451 mg/ml for Petroleum ether, Hexane, Acetone and Methanol extracts respectively. Water extract shows no mortality up to 4mg/ml. No mortality was observed in control; DMSO didn't exert any adverse effect.



Graph 1
Effect of various extracts of *Gliricidia sepium* on the mortality percentage of fourth instar larvae of *Culex quinquefasciatus*

Table1
LC_{50} values with 95 % confidence limit of plant extracts of Gliricidia sepium.

Extract	LC_{50} (mg/ml)	LFL	UFL
Petroleum ether	0.061	0.055	0.066
Hexane	0.16	0.118	0.3
Acetone	0.428	0.32	0.677
Methanol	0.451	0.343	0.69
Water	No mortality up to 4 mg/ml		

DISCUSSION

The common plant *Gliricidia* has recently gained much attraction as a multipurpose genus, especially the species sepium. Apart from being a source of green manure its use as a rodenticide has aroused attention in farming and storage. It is a fast growing nitrogen fixing tree used throughout the tropics for the many edaphic services and also as fallow tree to improve degraded land. *Gliricidia* is an important source of green manure, fodder and fuel wood. The toxic effect of *Gliricidia* are well known in central America where the leaves or the ground bark, mixed with cooked maize are traditionally used as a rodenticide[9] .There have also been reports of toxicity and growth inhibition in other monogastric animals including poultry[10] and rabbits[11]. The oil extracted from the bark of *Gliricidia* exhibited pronounced activity against microorganisms comparable at par with standard antibiotics according to Reddy and Jose (2010). Thus *Gliricidia* is considered by many to be the second most important multipurpose tree legume after *Leucana leucocephala* in the humid tropics.
Above all *Gliricidia sepium* is selected for the present study primarily on the basis of ethno botanical information gained from the field survey conducted in different belts in Kerala state. Significant information, among other things was pertaining to its use as mosquito repellent. As explicit from table1 in the present study out of the various solvents used for making the extract, the petroleum ether extract is seen to be more effective in its larvicidal activity.

Long before the advent of synthetic pesticides and insecticides, plants as a whole or their decoctions were traditionally known to be used to repel the pests related to farming and storage. As their efficacy was very poor, the synthetic brands have suddenly appeared and gradually conquered the entire agricultural world. Of late mosquitoes have invaded the entire peninsula inflicting untold miseries to the human. Now it seems that mosquitoes regard Kerala as its paradise because of special eco-social aspects besides edaphic and climatic factors. Due to, increasing awareness about the resistance developed and implied hazards to environment and health, the attention is now diverted towards developing eco-friendly phytochemicals. In these context high potentials reserved incognito in the ergastic materials of *Gliricidia sepium* capable of increasing the mortality of mosquitoes seems to deserve exploration. It may open a path for the commercial and industrial production of mosquito larvicide from *Gliricidia sepium*. The investigation for isolation, purification and characterization of the potential biochemical and for its impact on non-target organisms is in steady progress.

CONCLUSION

The findings of the present studies, therefore suggest the use of *Gliricidia sepium,* as a local resource in controlling mosquito larvae. Our promising results may provide new tools for effective control approaches against *Culex quinquefasciatus* larvae. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for the development of ecofriendly chemicals for control of insect vectors**.**

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