Laboratory efficacy of metabolites of *Lagenidium giganteum* (Couch) on *Anopheles stephensi* (Liston) after filterations by Column Chromatography

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**ABSTRACT**

This study was conducted in laboratory to evaluate the efficacy of filtered extracellular metabolites of *Lagenidium giganteum* against all the four instars of *An. stephensi* larvae. Fungal colonies have been cultured in PYG broth and after 15 days of culturing the fungus, metabolites have been filtered twice by whatman filter paper. These metabolites were again filtered by column chromatography and by rang syringe filters. Filtered metabolites were then used against all instars of *An. stephensi* larvae. The bioassays were conducted at five significantly different concentrations (1.68, 1.99, 2.17, 2.30, 2.40 ppm). The results suggest significant mortality on first three instar larvae and very low on fourth instar larvae.

Key words: *Lagenidium giganteum*, Column Chromatography, *Anopheles stephensi*, Secondary metabolites, Larvae

**INTRODUCTION**

Attitudes regarding malaria during the past century have fluctuated between hope during the period when worldwide control efforts seemed in reach and despair as problems seemed to mount inspite of massive research efforts. When one looks over the global distribution of malaria in the 21st century, it is realized how widespread the disease has been and how its distribution has diminished during the past 150 years.\(^1\)

Malaria spread by mosquito and mosquitoes are medically significant vector and transmit parasites and pathogens which continue to have a devastating impact on human's population. Therefore it becomes a necessity to control mosquitoes to control diseases they may spread. The control of
mosquito larvae is one potential answer in vector control. One potential alternative approach to the use of chemical pesticides is the use of biolarvicides.

Although studies\textsuperscript{2-5} have indicated on the efficacies of \textit{L. giganteum} by zoospores on \textit{An. stephensi}, \textit{Cx. quinquefasciatus} and \textit{Ae. Aegypti}, but there is no study on the efficacy by the secondary metabolite of \textit{L. giganteum} not this is screened for the efficacy in tropical conditions such as semi-arid zone of India, which is contributing significantly to vector borne diseases.

\textbf{\textit{Lagenidium giganteum} Couch (Oomycetes: Lagenidiles),} is biological control agents of pest mosquitoes and has been evaluated in a variety of habitats including rice fields, seepage, ditches and irrigated pastures and fields\textsuperscript{2-8}. The present investigation was designed to test the toxicity of purified metabolite of \textit{L. giganteum} against four instars of \textit{An. stephensi} in the laboratory conditions.

\textbf{MATERIAL AND METHODS}

\textbf{Fungal}

The fungal strain of \textit{L. giganteum} was obtained from the Institute of Microbial Technology (MTCC-719) Chandigarh India. Fungal colonies of \textit{L. giganteum} were cultured in 250 ml conical flasks containing 100 ml of PYG broth (1.25 g. peptone and yeast extract, 3.0g. glucose and 1000 ml deionized water with conductance of 1.0 µmho).

\textbf{Mosquito}

The colonies of \textit{An. stephensi} were maintained in a laboratory at a temperature of 25±2 °C, relative humidity of 70±5% and photoperiod of 14:10 (L: D). Larvae of \textit{An. stephensi} were maintained in separate containers and were generally not fed during the experiments.

\textbf{Filtration and Chromatography}

A cell free culture filtrates has been obtained by filtering the broth through successive whatman no.1 filter papers after incubation. Thereafter, the metabolites were separated by Column Chromatography (HP 20). In an experiment, 1.2 g. of crude metabolite dissolved in small amount of methanol (1 ml) and was chromatographed on a silica gel (100-200 mesh size). Elution was done with methanol and deionized water dilutions (1:1) and filter twice, then 0.5 ml fractions were collected from ratio. These were again filtered by Rang Syringe Filter Holder for 0.2 µm (pore size) with leur lock outlet (Ranbaxy Fine Chemicals Ltd.).

\textbf{Bioassay}

The efficacy of filtered metabolites of the \textit{L. giganteum} was assessed against first, second, third and fourth instars of \textit{An. stephensi}. The larvae were placed in separate containers (60×40×20 cm.) containing microbe free deionized water. The experiments were conducted separately for different instars of \textit{An. stephensi} at five selected test concentrations (1.68, 1.99, 2.17, 2.30, 2.40 ppm.). Twenty larvae of each stage were separately exposed to 100 ml of test concentrations. Different test concentrations of metabolites in 100 ml
were prepared by adding the fungal filtrate to water in 250 ml beakers. The culture media at each concentration without fungal filtrates was added as control. The mortality was scored after 24, 48 and 72 hours of the exposure. The experiment was replicated thrice to record variation in the results. Values for the 50, 90 and 99% toxic concentrations (LC50, LC90 and LC99 respectively) with 95% fiducial limits were estimated and slopes were derived using probit analysis.

RESULTS

The significant differences in mortality on An. stephensi mosquito larvae were noted at different concentrations of extracellular metabolites. There was 100% mortality recorded for the first instar larvae by L. giganteum metabolite. After exposure of that metabolite to the second and third instar larvae it has shown 50% and 90% mortality. However, the fourth instar of An. stephensi appeared to have lesser mortality (less than 32%).

The probit regression lines for each of the larval stages of An. stephensi were recorded. The fiducial limits for the probit equations depict the value of LC50 and LC90. All are found statistically significant (p<0.05).

DISCUSSION

In present work, L. giganteum filtered metabolites found to be effective against larvae of An. stephensi. The first instar found much effective but fourth instar found less effective. The crude metabolites of L. giganteum have been tested against three species of mosquito larvae and depict comparisons of larval infection rates under laboratory conditions however, in present work we have tested efficacy on An. stephensi by crude metabolite, filtered by Column Chromatograph.

The mechanism, where many elements playing an important role in maintaining the vitality of the mosquito larvae decreased and some of the heavy metal elements accumulated after the infection of L. giganteum. This might be responsible for part of the killing mechanism to Cx. quinquefasciatus larvae. Some studies have shown larvicidal activity against all larval stages of An. stephensi and Cx.

| Table 1. Mortality due to Lagenidium giganteum in all four instars of An. stephensi |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **First - instar**              | **Second - instar**             | **Third - instar**              | **Fourth - instar**             |
| Probit equation                 | First - instar                  | Second - instar                 | Third - instar                  | Fourth - instar                 |
| LC50                            | 100 % dead                      | 0.224+2.268x                    | 0.085+2.286x                    | 32 % dead                       |
|                                | 11.11                           | 11.07-11.14                     | 10.2-9.95                       |                                 |
|                                | 48.69                           | 47.2-49.0                       | 32.10                           |                                 |

x is a log10 concentration in ppm.


*K. quinquefasciatus* by crude metabolites of *Chrysosporium tropicum* however, in our studies based on filtered extracellular metabolites. On other hand it appeared to be significant to control larvae of *An. stephensi*.

Investigation of the toxicity of *L. giganteum* filtered metabolites can be further extended with the test of the isolated molecules on mosquito larvae. This is yet to be tested on natural population of mosquito larvae, which might be useful for community.

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