

## Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say

Govindarajan, M., Jebanesan, A. and Reetha, D\*

Division of Vector Biology, Department of Zoology,

\*Department of Microbiology, Faculty of Agriculture,

Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India.

**Abstract.** The culture filtrates of five different soil fungi viz., *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium falicum*, *Fusarium vasinfectum* and *Trichoderma viride* were tested for the larvicidal activity against third instar larvae of mosquito vector *Culex quinquefasciatus*. The concentrations of fungal culture filtrates used in the study ranged from 25 to 100 mg/L. The results showed that the larval mortality could be observed on 24 hours of exposure period. The LC<sub>50</sub> values of *A. flavus*, *A. parasiticus*, *P. falicum*, *F. vasinfectum* and *T. viride* were 38.34, 40.39, 44.97, 50.03 and 54.16 mg/L, respectively. Among the five different fungi, the culture filtrates of *A. flavus* was found to be more toxic than the other four species of fungi against *Cx. quinquefasciatus*.

### INTRODUCTION

Mosquitoes are unquestionably the most medically important arthropod vectors of disease. Mosquitoes transmit many dreadful diseases like malaria, filariasis, Japanese encephalitis and dengue fever affecting the socio-economical status of many nations (Service, 1983). Mosquitoes are also an important pest of humans, causing allergic responses that include local skin reaction and systemic reaction such as angioedema and urticaria (Peng *et al.*, 1999). *Culex quinquefasciatus* Say transmits filariasis and is predominantly found in the tropics and the warm temperate regions. There are already 26.42 million infected and 20-40 million chronic cases in India (Sharma, 2001). This species of mosquito and the incidence of filariasis are particularly in Chidambaram town, Cuddalore District of Tamilnadu, India.

Fungi and fungus-derived products are highly toxic to mosquitoes, yet have low

toxicity to non-target organisms. Accordingly the use of entomophagous fungi and their derived products may be a promising approach for biological control of mosquitoes (Kirschbaum, 1985). Extracellular secondary metabolites from many fungi have been screened for larvicidal activity against mosquitoes (Vijayan & Balaraman, 1991). Secondary metabolites are diverse natural products synthesized by cells that have stopped dividing (Bennett & Bentley, 1989). Microbial insecticides are being considered as alternatives to chemical insecticides because of their selective toxicity and ready decomposability in the ecosystem. Also, unlike the inherent dangers associated with the process of production of synthetic insecticides, the process for the manufacture of microbial products is safe, well-contained and less pollution (Misato, 1983). The present study has been undertaken to determine the efficacy of extracellular secondary metabolites of five fungal culture filtrates

against *Cx. quinquefasciatus* mosquito larvae under laboratory conditions.

## MATERIALS AND METHODS

### Sample collection

Five fungal species namely *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium falicum*, *Fusarium vasinfectum* and *Trichoderma viride* isolated from the various soil samples collected from Annamalai University campus, Annamalai Nagar, Tamilnadu were evaluated.

### Cultivation of fungi

Richards broth (25 g sucrose, 5 g potassium nitrate, 2.5 g hydrogen phosphate, 1.25 g magnesium sulphate, 0.01 g ferric chloride, 500 ml distilled water) was used to grow the fungi. A loopful of fungal growth from an agar slope was transferred to 100 ml of growth medium in a 250 ml conical flask and incubated on a rotary shaker at 110 rpm and at 30°C for 15 days. The culture was filtered through Whatman No-1 filter paper, the mycelial mass was discarded and the culture filtrate was used as test material for larvicidal activity. The filtrate was stored under refrigerator conditions.

### Test organism

A laboratory colony of *Cx. quinquefasciatus* was used for the

larvicidal activity. The larvae were reared in dechlorinated water and fed with dog biscuits and yeast powder at the ratio of 3:1. They were maintained at 28±2°C, 75-65% RH, under 142:10D photoperiod cycles.

### Bio-assay

Larvicidal activity of *Cx. quinquefasciatus* was assessed by using the standard method (WHO, 1996). Different concentrations (100, 75, 50 and 25 mg/L) of test sample were used. Uninoculated culture medium served as control. Twenty five early third instar larvae were introduced to each of the test solution as well as control. For each dose six replica were maintained at a time. The LC<sub>50</sub> value was calculated after 24 hours by Probit analysis (Finney, 1971).

## RESULTS AND DISCUSSION

The toxicity of the late third instar larvae of *Cx. quinquefasciatus* to culture filtrates of five fungi were noted and statistical data are presented in Table 1. The LC<sub>50</sub> values were 38.34, 40.39, 44.97, 50.03 and 54.16 mg/L for *A. flavus*, *A. parasiticus*, *P. falicum*, *F. vasinfectum* and *T. viride*, respectively. Among the five fungal species tested, *A. flavus* was highly toxic to larvae of *Cx. quinquefasciatus*, when compared to other four species of fungal culture filtrates.

Table 1. Efficacy of fungal secondary metabolites against the larvae of *Culex quinquefasciatus*

| Fungal species                 | LC <sub>50</sub><br>(mg/L) | Regression<br>equation | 95% confidential<br>limit (ppm) |       | χ <sup>2</sup> |
|--------------------------------|----------------------------|------------------------|---------------------------------|-------|----------------|
|                                |                            |                        | LCL                             | UCL   |                |
| <i>Aspergillus flavus</i>      | 38.34                      | Y = 13.40 + 0.93 X     | 13.73                           | 57.60 | 19.543*        |
| <i>Aspergillus parasiticus</i> | 40.39                      | Y = 9.40 + 0.97 X      | 28.22                           | 51.46 | 8.393*         |
| <i>Penicillium falicum</i>     | 44.97                      | Y = 7.60 + 0.93 X      | 31.13                           | 57.82 | 9.420*         |
| <i>Fusarium vasinfectum</i>    | 50.03                      | Y = 7.20 + 0.86 X      | 34.82                           | 64.77 | 9.419*         |
| <i>Trichoderma viride</i>      | 54.16                      | Y = 6.40 + 0.82 X      | 38.68                           | 70.23 | 9.198*         |

\* Significant at p < 0.05

LCL – Lower confidence limit

UCL – Upper confidence limit

Mishra *et al.* (1987) had observed mosquito larvicidal activity among 7 of 70 fungi. Priyanka *et al.* (2001) found that culture filtrate of *Chrysosporium tropicum* toxic to larvae of *Anopheles stephensi*. Vijayan & Balaraman (1991) involved comprehensive screening of fungal metabolites. The LC<sub>50</sub> values of fungal metabolites from 17 species of fungi were in the range of 7-83 µl/ml for the three major vector mosquitoes namely *Cx. quinquefasciatus*, *A. stephensi* and *A. aegypti*. Seleena & Lee (1994) reviewed the insecticidal activity of a Malaysian isolate of *Aspergillus niger*. Bioassays showed that *Ae. aegypti* larvae was the most susceptible with *An. maculatus* being the least susceptible while *Ae. aegypti* and *Cx. quinquefasciatus* were susceptible to the 72 h *A. niger* supernatant culture but not to the 24 h and 48 h culture.

In the present study, a wide variety of fungi that produce various kinds of mosquitocidal secondary metabolites were observed. So it is evident that soil fungi produce secondary metabolites that can kill mosquito larvae. Further investigations are currently underway to isolate specific metabolite which will yield compounds, that may possess mosquitocidal properties even at lower concentrations.

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