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CHRYSOSPORIUM TROPICUM EFFICACY AGAINST *ANOPHELES STEPHENSI* LARVAE IN THE LABORATORY

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ABSTRACT. The keratinophilic soil fungus *Chrysosporium tropicum* was evaluated as a biological control agent against *Anopheles stephensi* larvae in the laboratory. Culture filtrates of *C. tropicum* were found to be toxic to *An. stephensi* larvae at various concentrations. The ranges of the 50% lethal concentration (LC_{50}) values of fungal filtrates were 16.60–17.78, 12.02–12.88, and 34.67–35.48 µl/ml against 1st-, 2nd-, and 3rd-stage larvae, respectively. The ranges of LC_{99} values were 38.90–63.10, 12.02–213.80, and 74.13–109.65 µl/ml against 1st-, 2nd-, and 3rd-stage larvae, respectively.

KEY WORDS *Chrysosporium tropicum, Anopheles stephensi,* biological control of mosquitoes, mosquitocidal fungi

INTRODUCTION

The development of resistance by mosquito populations to chemical insecticides requires alternative approaches for sustainable vector control. Also, growing awareness of environmental hazards of synthetic chemicals has promoted development of novel, alternative methods for mosquito control. The future of biological control of mosquitoes depends on additional research, especially that leading to the discovery of new species and strains of effective organisms (Legner and Sjogren 1984). Fungi and fungus-derived products are highly toxic to mosquitoes, yet have low toxicity to nontarget organisms. Accordingly, the use of entomophagous fungi and their derived products may be a promising approach for biological control of mosquitoes (Kirschbaum 1985).

A number of microbes and microbial products have been tested for use in controlling vector populations. Extracellular secondary metabolites from many fungi have been screened for larvicidal activity against mosquitoes (Vijayan and Balaraman 1991). Crude extracts of tolypin from the fungus *Tolypocladium niveum* were tested against mosquito larvae in the laboratory and found to be toxic (Matha et al. 1988). The role of extracellular chitinase from *Trichoderma virens* has also been tested as a biological control agent (De La Cruz et al. 1992).

The fungus *Lagenidium giganteum* has been widely tested and used against mosquito larvae (Brey 1993). Lord and Fukuda (1990) found that *Leptolegnia* sp. readily infected larvae of *Anopheles quadrimaculatus* Say, *Culex quinquefasciatus* Say, and *Aedes aegypti* (L.).

In this study, the efficacy of culture filtrates of *Chrysosporium tropicum* was tested in the laboratory against 1st-, 2nd-, and 3rd-stage larvae of *Anopheles stephensi* Liston larvae. This was done in an attempt to discover substances in addition to chitinase that can be extracted from keratinophilic fungi.

MATERIALS AND METHODS

Fungal colonies were obtained by placing sheep hair moistened with deionized water (0.01 µmho conductivity) in petri dishes containing soil (Srivastava et al. 1996). Under conditions of 30 ± 2°C, relative humidity 90 ± 5%, and daily photoperiod of 14:10 (L:D), fungal colonies appeared within 10–15 days. Fungal colonies were transferred to 250-ml conical flasks containing 100 ml of Richard's broth (25 g sucrose, 5 g potassium nitrate. 2.5 g dihydrogen phosphate, 1.25 g magnesium sulfate, 0.01 g ferric chloride, 500 ml distilled

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Table 1. Toxicity estimates of *Chrysosporium tropicum* against 3 larval stages of *Anopheles stephensi.*[1]

	Larval instar		
	First	Second	Third
Probit equation	Y = −0.65 + 4.49X	Y = 1.54 + 3.17X	Y = −3.97 + 5.87X
LC_{50} (µl/ml)	16.98 (16.60–17.78)	12.3 (12.02–12.88)	34.67 (34.67–35.48)
LC_{90} (µl/ml)	30.2 (26.92–37.15)	28.18 (25.70–28.18)	56.23 (51.29–75.86)
LC_{99} (µl/ml)	46.77 (38.90–63.10)	53.7 (12.02–63.10)	83.18 (74.13–109.65)

[1] Fiducial limits in parentheses.

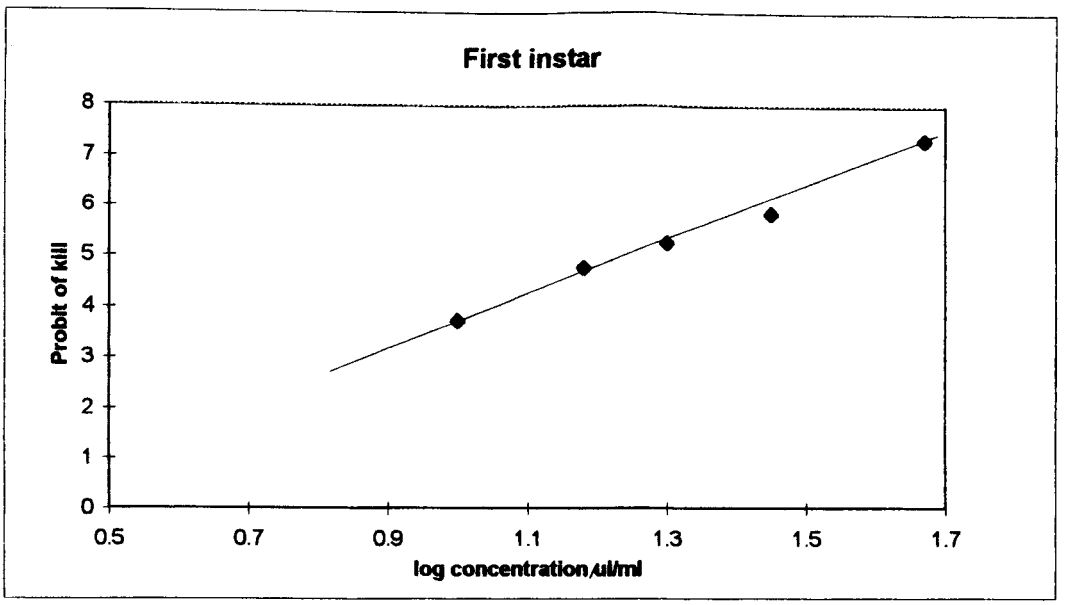

Fig. 1. Relation between probit of kill and log concentrations of *Chrysosporium tropicum* culture filtrate, showing probit regression line in 1st-stage larvae of *Anopheles stephensi* in laboratory.

water) using sterilized inoculating needles. The fungus-containing broth was incubated at 27 ± 2°C for 15 ± 2 days. A cell free culture filtrate was obtained by filtering the broth through Whatman No. 1 filter papers after incubation. The Mycology Division of the Botany Department in the Dayalbagh Educational Institute verified the presence of *C. tropicum* in the filtrate.

Anopheles stephensi larvae were maintained in deionized water in the laboratory at 25 ± 2°C and a daily photoperiod of 14:10 (L:D). Larvicidal activity of the culture filtrates of *C. tropicum* was assessed at different concentrations against 1st-, 2nd-, and 3rd-stage larvae of *An. stephensi.* Twenty larvae of each stage were placed separately in 100-ml test concentrations and were not fed during the ex-

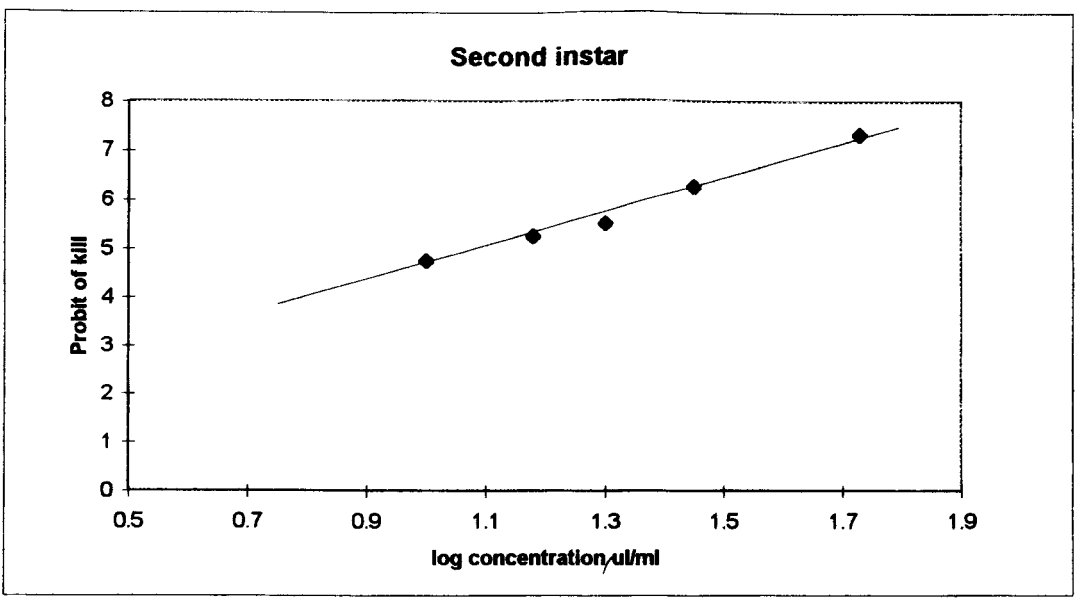

Fig. 2. Relation between probit of kill and log concentrations of *Chrysosporium tropicum* culture filtrate, showing probit regression line in 2nd-stage larvae of *Anopheles stephensi* in laboratory.

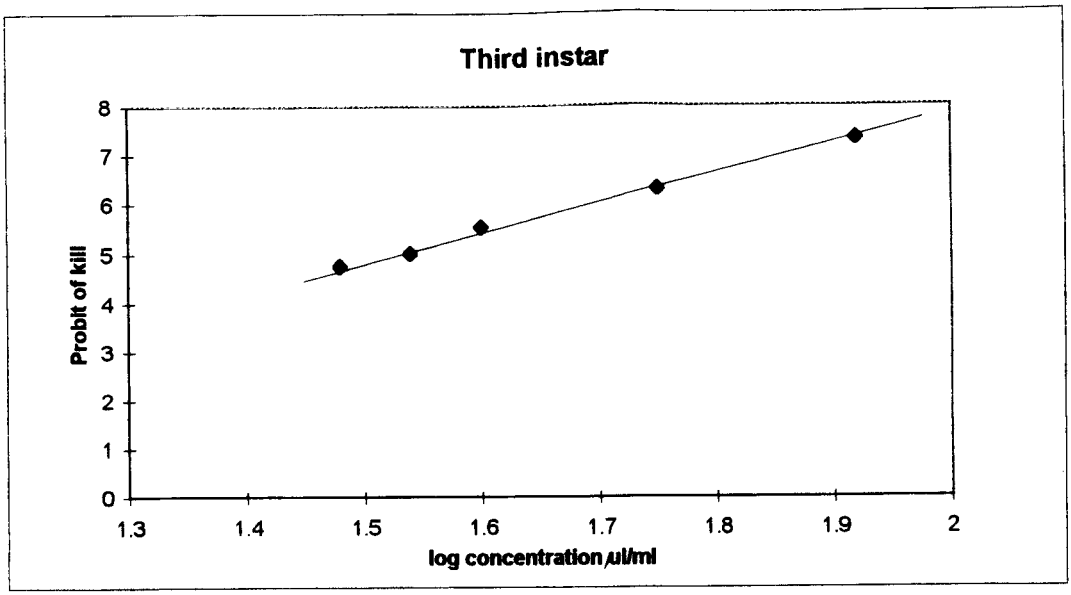

Fig. 3. Relation between probit of kill and log concentrations of *Chrysosporium tropicum* culture filtrate, showing probit regression line in 3rd-stage larvae of *Anopheles stephensi* in laboratory.

periments. As controls, culture media at each concentration without fungal filtrate was added. Mortality was scored after 24 h exposure of the larvae to the test concentrations. The experiment was replicated 3 times. Values for the 50, 90, and 99% lethal concentration (LC_{50}, LC_{90}, and LC_{99}, respectively) with 95% fiducial limits were estimated, and slopes were derived using probit analysis (Finney 1981).

RESULTS AND DISCUSSION

In the present study, culture filtrates of *C. tropicum* were found to be toxic for all larval instars of *An. stephensi* tested. Estimates of LC_{50}, LC_{90}, and LC_{99} for the metabolites against the 3 larval stages tested are shown in Table 1. The culture filtrate proved to be most toxic to 2nd instars of *An. stephensi.* Third instars were affected only at the highest concentrations of culture filtrate.

Probit regression lines for each of the 3 larval stages are shown in Figs. 1–3. The fiducial limit for the probit equation shows that the values of LC_{50}, LC_{90}, and LC_{99} are all statistically significant.

Other studies in India by Vijayan and Balaraman (1991) involved comprehensive screening of fungal metabolites. The LC_{50} values of fungal metabolites from 17 species of fungi were in the range of 7–83 µl/ml, whereas in our study, LC_{50} values against 3rd-stage larvae of *An. stephensi* ranged from 34.67–35.48 µl/ml. This would place *C. tropicum* in the midrange of toxicity for the various fungi tested by Vijayan and Balaraman (1991). Matha et al. (1988) found that a crude extract of tolypin produced 100% mortality in *Cx. pipiens, Ae. aegypti,* and *An. maculipennis* at a concentration of 0.1 mg/ml. Our results show comparable toxicity for *C. tropicum* against *An. stephensi.*

It is hoped that further studies on the isolation and identification of chitinophilic agents present in culture filtrates of *C. tropicum* will be more toxic than the filtrates we tested. If more toxic metabolites are found, this may present excellent opportunities for producing improved materials through modern methods of molecular biology.

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REFERENCES CITED

Brey PT. 1993. Variation in larval susceptibility to *Lagenidium giganteum* in the mosquito species. *J Invertebr Pathol* 62:1–8.

De La Cruz J, Hidalgo GA, Lora JM, Benitez T, Toro JAP, Lobell A. 1992. Isolation and characterisation of three chitinases from *Trichoderma harzianum. Eur J Biochem* 206:859–867.

Finney DJ. 1981. *Probit Analysis* 3rd edition Cambridge, United Kingdom: Cambridge Univ. Press.

Kirschbaum JB. 1985. Potential implication of genetic engineering and other biotechnologies to insect control. *Annu Rev Entomol* 30:51–70.

Legner EF, Sjogren RD. 1984. Biological mosquito control furthered by advances in technology and research. *Mosq News* 44:449–455.

Lord JC, Fukuda T. 1990. A *Leptolegnia* (Saprolegniales) pathogenic for mosquito larvae. *J Invertebr Pathol* 55: 130–132.

Matha V, Weiser J, Olejnicek J. 1988. The effect of tolypin in *Tolypocladium niveum* crude extract against mosquito and black-fly larvae in the laboratory. *Folia Parasitol* 35:379–381.

Srivastava JN, Ghawana VK, Kumar A. 1996. Biodegradation of wool by *Trichophyton simmi* and *Aspergillus niger*. *Mycoses* 39:483–487.

Vijayan V, Balaraman K. 1991. Metabolites of fungi and actinomycetes active against mosquito larvae. *Indian J Med Res* [A] 93:115–117.