Journal of the American Mosquito Control Association, 19(4):404–407, 2003

LABORATORY EFFICACY TESTS FOR FUNGAL METABOLITES OF *CHRYSOSPORIUM TROPICUM* AGAINST *CULEX QUINQUEFASCIATUS*

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ABSTRACT Efficacy of fungal metabolites of *Chrysosporium tropicum* was evaluated against *Culex quinquefasciatus* larvae in the laboratory to determine their larvicidal activity at 6 concentrations, with a mortality range of 10–95%. Efficacy of *C. tropicum* was analyzed by probit analysis procedures. The LC_{50} (concentration lethal to 50% of the population) values were 38.9, 63.7, 79.0, and 122.6 µl/ml for the 1st, 2nd, 3rd, and 4th instars, respectively. The LC_{90} values were 79.5, 95.6, 136.9, and 174.5 µl/ml against the 1st-, 2nd-, 3rd-, and 4th-stage larvae, respectively. Lethal concentrations of fungal metabolites were significantly different among the 4 instars. The 1st-stage larvae were most susceptible, whereas 4th instars were least susceptible to *C. tropicum* metabolites. The larvicidal potential of *C. tropicum* metabolites warrants field trials against various species of mosquitoes.

KEY WORDS *Chrysosporium tropicum, Culex quinquefasciatus,* metabolites, lethal concentration, efficacy

INTRODUCTION

Entomopathogenic fungi potentially are a target-specific, cost-effective, and environmentally compatible means of controlling mosquitoes. Also, fungi may be compatible with some existing chemical larvicides (Orduz and Axtell 1991). *Lagenidium giganteum* Couch (Oomycetes: Lagenidiales) is a promising biological control agent of mosquito larvae and the only fungal control agent approaching operational use in mosquito control (Patel et al. 1990, Kerwin et al. 1994). Another Oomycetes fungi, *Leptolegnia* sp. and *Crypticola clavulifera* Humber et al. were found to be pathogenic against mosquitoes (Lord and Fukuda 1990, Frances 1991). Among Deuteromycetes fungi, some species of the genus *Tolypocladium* have shown their effectiveness against mosquito larvae (Weiser 1991). In an earlier study, *Trichophyton ajelloi* Vanbreuseghem was found to infect *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say larvae (Mohanty and Prakash 2000).

Fungal-derived products, particularly extracellular metabolites and fungal extracts, have been investigated to assess their mosquito larvicidal properties (Vijayan and Balaraman 1991, Badran and Aly 1995). Some of the isolated fungal entomotoxic agents, including tolypin, beauvericin, and NK374200, have provided effective toxicity against target mosquitoes (Matha et al. 1988, Zizka and Weiser 1993, Morino et al. 1995). Recently, the larvicidal activity of *Chrysosporium tropicum* Carmichael metabolites against *Anopheles stephensi* larvae in the laboratory (Priyanka et al. 2001) has been examined. The present study was undertaken to evaluate the bioefficacy of fungal metabolites of *Chrysosporium tropicum* against *Cx. quinquefasciatus.*

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MATERIALS AND METHODS

Colonies of *C. tropicum* were isolated from the soil, using sheep hair as bait. Sterilized sheep hair was moistened with deionized water and placed in petri dishes containing soil. At 27 ± 2°C, relative humidity of 90 ± 5% and a daily photoperiod of 14:10 h (L:D), fungal colonies appeared within 10–15 days (Priyanka et al. 2001). The Mycology Division of the Botany Department of Dayalbagh Educational Institute verified the fungus as *Chrysosporium tropicum.* Fungal colonies were transferred with a sterilized inoculating needle to 250-ml conical flasks, containing 100 ml of autoclaved Richard's broth. In the laboratory, *C. tropicum* was maintained at 27 ± 2°C in Richard's broth. After 15 ± 2 days of incubation, culture filtrates were obtained by filtering the fungal culture broth through Whatman no. 1 filter paper. The filtrate was used to examine the larvicidal activities of *C. tropicum.*

Larvae of *Cx. quinquefasciatus* were maintained under laboratory conditions at 25 ± 2°C and a photoperiod of 14:10 h (L:D). Larvae were placed in containers (60 × 40 × 20 cm) containing microbe-free deionized water with conductance of 1.0 µmho.

Toxicity of the *C. tropicum* metabolites was tested against 1st, 2nd, 3rd, and 4th instars of *Cx. quinquefasciatus* at 25 ± 2°C. Different test concentrations of 100 ml were prepared by adding the fungal filtrate to water in 250-ml beakers. Bioassays were conducted separately for all 4 instars at 6 selected test concentrations of fungal metabolites covering the mortality range of 10–95% (Table 1). To test the larvicidal activity of *C. tropicum* metabolites, 20 larvae of each stage were separately exposed to 100 ml of test concentrations and were not fed during the experiment. Similarly, controls were run to test the natural mortality, except respective concen-

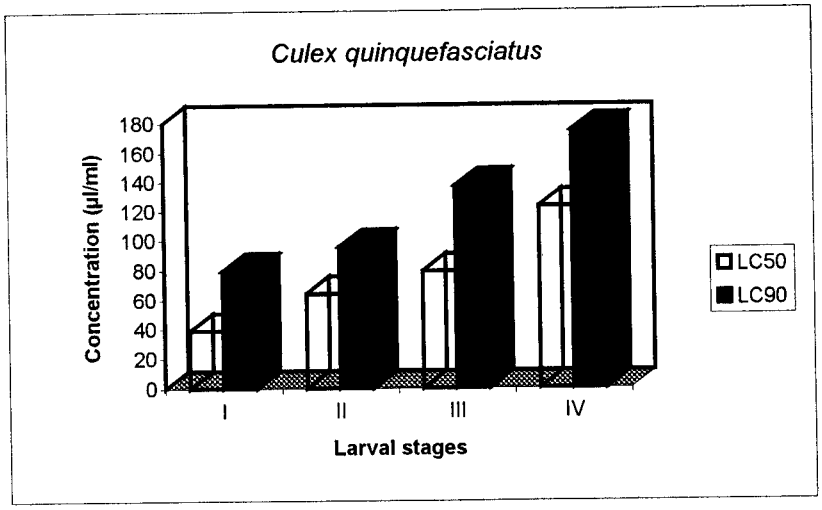

Fig. 1. Comparison of LC_{50} and LC_{90} values of fungal metabolites for the 1st, 2nd, 3rd, and 4th instars of *Culex quinquefasciatus*.

trations of culture medium were used instead of fungal filtrate. Mortality was scored after 24 h of exposure. The experiments were replicated thrice to validate results.

Efficacy of *C. tropicum* against *Cx. quinquefasciatus* larvae was analyzed by probit analysis (Finney 1971). The concentrations producing 50 and 90% mortality in larvae (LC_{50} and LC_{90}, respectively) were calculated with their fiducial limits at 95% confidence level. The relationship between probit

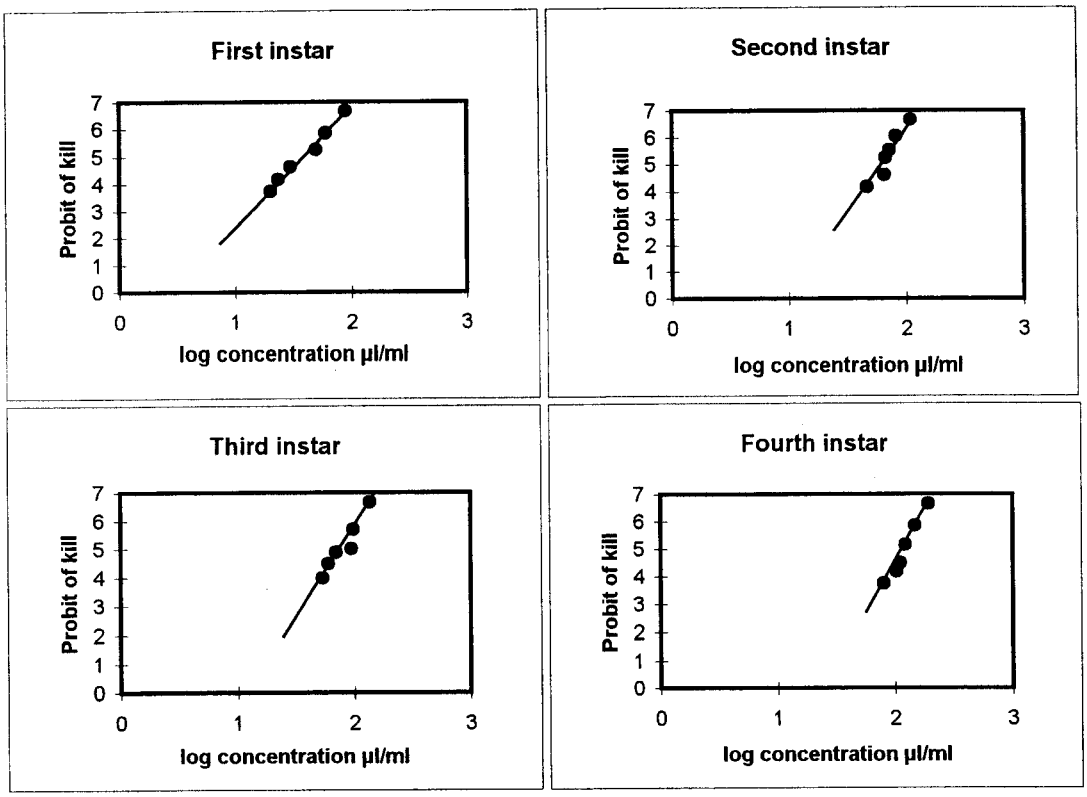

Fig. 2. Probit regression lines depicting relation between probit of kill and log concentration of *Chrysosporium tropicum* metabolites for all larval stages of *Culex quinquefasciatus*.

Table 1. Six concentrations of *Chrysosporium tropicum* metabolites used against *Culex quinquefasciatus* larvae.

Instar	Concentration (µl/ml)					
	1	2	3	4	5	6
1st	20.0	23.3	30.0	50.0	60.0	90.0
2nd	46.7	65.0	66.7	72.5	82.5	110.0
3rd	53.3	60.0	70.0	95.0	98.3	136.7
4th	80.0	103.3	111.7	123.0	150.0	193.3

and log concentration was established as probit equations and probit regression lines were drawn for each of the larval stages. The larval mortality was corrected to natural mortality recorded in controls, if any, using Abbott's formula (Abbott 1925). Differences between observed and expected mortality were tested by Chi-square test at a 95% confidence level (Finney 1971). To compare the lethal concentrations of fungal metabolites among 4 larval stages of *Cx. quinquefasciatus,* the analysis of variance F test (ANOVA F test) was used.

RESULTS AND DISCUSSION

The soil fungus *C. tropicum* showed larvicidal activity against all larval stages of *Cx. quinquefasciatus,* and the fungus appeared to be keratinophilic. Toxicity of *C. tropicum* metabolites is shown in Table 2 and Fig. 1. The LC_{50} and LC_{90} values (Table 2) were lower for the 1st instars than for the other 3 instars, whereas these values were higher for the 4th instar than for the others. The ANOVA F test established a significant difference ($F = 3.61$; df = 3,20; $P < 0.05$) in lethal concentrations among 4 instars of *Cx. quinquefasciatus.*

Calculated χ^2 values at 4 df were 0.82, 3.39, 4.77, and 1.52 for the 1st, 2nd, 3rd, and 4th instars, respectively. All these values of χ^2 were lower than the critical value of χ^2 at the 0.05 significance level. Therefore, the results from the Chi-square test were not statistically significant at the 95% confidence level, which suggested that there was no significant difference between expected and observed data. Small values of χ^2 confirmed the adequate representation of probit regression lines (Fig. 2) for the experimental data.

Observed lethal concentrations have shown the degree of susceptibility to fungal metabolites among the four larval stages of *Cx. quinquefasciatus* in the order of instar I > instar II > instar III > instar IV. However, larvae of *Cx. quinquefasciatus* appear to be less susceptible than those of *Anopheles stephensi* Liston (Priyanka et al. 2001).

In the present study, *C. tropicum* metabolites have provided a promising larvicidal potential against early larval instars of *Cx. quinquefasciatus.* These laboratory results suggest that field trials should be carried out to determine the efficacy of these fungal metabolites under more natural conditions.

ACKNOWLEDGMENTS

We are grateful to S. S. Bhojwani, Director, Dayalbagh Educational Institute, for providing laboratory facilities. We also thank J. N. Srivastava for his help in fungal identification. All India Council for Technical Education provided financial support through a grant to Soam Prakash (212/Biotechnology/1998). Priyanka thankfully acknowledges the M. C. R. E. I., Agra for granting the research scholarship.

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Table 2. Lethal concentrations of *Chrysosporium tropicum* metabolities with their fiducial limits (in parentheses) at 95% confidence level against 4 larval instars of *Culex quinquefasciatus.*

Instar	Probit equation	LC_{50} (µl/ml)	LC_{90} (µl/ml)
1st	$Y = -1.553 + 4.122x$	38.9 [33.3–45.5]	79.5 [63.9–116.2]
2nd	$Y = -8.091 + 7.257x$	63.7 [57.6–68.9]	95.6 [85.4–118.3]
3rd	$Y = -5.194 + 5.372x$	79.0 [70.6–89.4]	136.9 [105.7–186.3]
4th	$Y = -12.458 + 8.359x$	122.6 [121.8–124.1]	174.5 [156.2–211.5]

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