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LABORATORY EVALUATION OF *TRICHOPHYTON AJELLOI*, A FUNGAL PATHOGEN OF *ANOPHELES STEPHENSI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT. *Trichophyton ajelloi*, a fungus isolated from soil, caused high larval mortality in *Anopheles stephensi* and *Culex quinquefasciatus* in the laboratory. The efficacy and toxicity of *T. ajelloi* against *An. stephensi* and *Cx. quinquefasciatus* were evaluated at 6 different concentrations. At different concentrations of *T. ajelloi*, chitin synthesis in larval cuticle was adversely affected. Larvae of 3rd-stage *An. stephensi* were more susceptible to *T. ajelloi* than were larvae of 1st, 2nd, and 4th stages as calculated by probit analysis. Larvae of *Cx. quinquefasciatus* were relatively resistant to infection by *T. ajelloi*.

KEY WORDS Biological control, fungi, *Anopheles stephensi, Culex quinquefasciatus, Trichophyton ajelloi*

INTRODUCTION

Major obstacles to the control of the mosquito vectors of malaria include the emergence and spread of pesticide resistance in vectors, environmental pollution and toxicity to nontarget organisms by broad-spectrum synthetic insecticides, and their costs. The drawbacks associated with synthetic insecticides have led to the search for alternative vector control methods, including biological control (Kirschbaum 1985). Biological control agents that have been evaluated include strains of spore-forming bacteria (*Bacillus sphaericus* and *Bacillus thuringiensis israelensis*). Also, some strains of deuteromycetous as well as oomycetous fungi have proved effective in field trials (Des Rochers and Garcia 1984, Mulla et al. 1984, Mittal et al. 1985, Frances and Pinnock 1990). Among entomopathogenic fungi, *Lagenidium giganteum* is a promising biological control agent of mosquito larvae (Federici 1981, Lacey and Undeen 1986). Additionally, the deuteromycetous fungi *Tolypocladium cylindrosporum* and *Culicinomyces clavisporus* have generated some interest as potential biocontrol agents of mosquito larvae (Gardner and Pillai 1987). To counter resistance developed in larvae due to continuous use of microbes, the discovery and development of new fungal strains can be helpful (Elena et al. 1997, Wirth and Georghiou 1997). Among 7 fungal species isolated from soil and studied in the laboratory, *Trichophyton ajelloi* (Vanbreuseghem) was found to infect *Anopheles stephensi* (Liston) and *Culex quinquefasciatus* Say. *Trichophyton ajelloi* is a common saprophytic fungus and is keratinophilic in nature. It is an imperfect fungus and its reproduction is asexual. Two types of spores are present: macroaleuriospores and microaleuriospores. The absence of studies on *T. ajelloi* and its apparent toxicity for mosquitoes prompted us to undertake the study discussed in this paper.

MATERIALS AND METHODS

Colonies of *An. stephensi* and *Cx. quinquefasciatus* 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 *An. stephensi* and *Cx. quinquefasciatus* were maintained in separate containers and were not fed during experiments. Larvae were placed in double-distilled microbe-free water at pH 7.00 and a conductance of 1.0 μmho. To counteract evaporation, water was added daily. Glassware (Borosil) was sterilized by autoclaving at 20 psi for 20 min. Twenty mosquitoes of either 1st, 2nd, 3rd, or 4th instars of each species were added to 750-ml beakers containing 500 ml test concentrations in 5 replicates. Controls consisted of 5 replicates containing 20 larvae of each instar in beakers containing 500 ml of microbe-free double-distilled water. Bioassays were run at 6 different concentrations chosen to produce larval mortalities between 20% and 95% for calculating LC_{50}, LC_{90}, and LC_{99} values. A wet colony of *Trichophyton ajelloi* of defined weight in 500-ml beakers was placed in a rotary shaker at 75 rpm for 1 h for homogenous mixing. Mortality readings were taken after 72 h and corrected according to Abbot's formula (Abbot 1925). The LC_{50}, LC_{90}, and LC_{99} values were calculated by using log/probit analysis (Finney 1981) with the help of SPSS and MS Excel 97 software.

From soil collected on the grounds of the institute, *Trichophyton ajelloi* was isolated by the hair baiting technique with peacock feathers as bait (Ajello et al. 1965). The feathers were then moistened with distilled water and placed in petri dishes lined with moist filter paper. Petri dishes were incubated at 24 ± 2°C in incubators. After 7 days, fungal colonies grown on the feathers were transferred with an inoculation needle to petri dishes containing Sabourad's dextrose agar (SDA). Chloramphenicol was supplemented at 50 μg/ml to SDA medium as bacteriostatic agent (Gardner and Pillai

Table 1. Probit equation and susceptibilities of different instars of *Anopheles stephensi* against *Trichophyton ajelloi* (LC values in mg/liter and 95% fiducial limits in parentheses).

Instar	Probit equation	LC_{50}	LC_{90}	LC_{99}
1st	3.984 + 3.006X	2.17 (2.10–2.20)	5.81 (5.72–5.91)	12.94 (12.69–13.10)
2nd	4.419 + 2.560X	1.68 (1.63–1.70)	5.33 (5.30–5.39)	12.60 (12.50–12.79)
3rd	4.921 + 2.920X	1.06 (1.02–1.07)	2.92 (2.83–2.96)	6.66 (6.58–6.77)
4th	3.785 + 2.249X	3.47 (3.36–3.57)	12.86 (12.64–13.04)	37.50 (36.49–37.95)

1987). *Trichophyton ajelloi* was identified (Srivastav et al. 1996) by the Mycology Division of the Botany Department of the Dayalbagh Educational Institute. After 15 days of growth, *T. ajelloi* colonies were tested for toxicity against *An. stephensi* and *Cx. quinquefasciatus.*

RESULTS AND DISCUSSION

Significant mortality differences from infection with *T. ajelloi* were observed among instars of *An. stephensi.* Comparison of LC_{50}, LC_{90}, and LC_{99} values (Tables 1–3) among different instars of *An. stephensi* showed that 3rd-stage larvae were about twice as susceptible as 1st-stage larvae, whereas 2nd-stage larvae were nearly 2-fold as tolerant (LC_{90}, 5.33 mg/liter; LC_{99}, 12.60 mg/liter) as were 3rd-stage larvae (LC_{90}, 2.92 mg/liter; LC_{99}, 6.66 mg/liter). The LC_{50}, LC_{90}, and LC_{99} values for 4th-stage larvae of *An. stephensi* showed them to be about 3-, 4-, and 6-fold as tolerant as 3rd-stage larvae, respectively. The minimum effective dosage (LC_{99}) of 3rd-stage larvae was 6.66 mg/liter, whereas in 4th-stage larvae, it was 37.50 mg/liter. Other LC values and probit equations are presented in Tables 1–3 and in Fig. 1.

Many workers (Lacey et al. 1988, Frances and Pinnock 1990, Vijayan and Balaraman 1991, Weiser et al. 1991, Hoti and Balaraman 1992, Sandhu et al. 1993, Kerwin et al. 1994) have tested the toxicity of fungi for various mosquito species, but not all species can tolerate high temperatures. Although we have not yet tested *T. ajelloi* in the field, our laboratory results suggest this fungus may be effective at temperatures as high as 45°C, making them more suitable for tropical climates. Frances and Pinnock (1990) found that, in Australian environments with temperatures as high as 30°C, *T. cylindrosporum* and *Culicinomyces clavisporus* produced high levels of toxicity. On the other hand, Frances (1991), investigating *Crypticola clavulifera,* encountered temperature limitations.

Vijayan and Balaraman (1991) screened numerous species for suitability in tropical climates and found one fungus, *Paecilomyces* spp., with significant toxicity for mosquitoes. This species produced mortality at 3 μl/ml, although the fungus was tested only against 3rd-stage larvae, and no probit analysis or slope for tests was provided. In the present study, we had similar results against 4th-stage larvae.

Hoti and Balaraman (1992) observed more than 75% mortality and Weiser et al. (1991) demonstrated >80% mortality in mosquitoes from *Tolypocladium terricola* after 8 days. In the present study, we achieved this level of mortality after only 3 days, and *T. ajelloi* were found growing on the cuticular region of the larvae of both species.

In terms of the different susceptibilities of various larval stages, our results agree with those of Woodring and Kaya (1992), who demonstrated that 4th-stage larvae of *Culex* spp. are less susceptible to *L. giganteum* than are other instars.

We observed that larvae of *An. stephensi* are more susceptible to *T. ajelloi* than are larvae of *Cx. quinquefasciatus.* However, the deuteromycetous fungus *C. clavisporus* is more effective against *Culex* spp. than against *Anopheles* spp., and *T. cylin-*

Table 2. Probit equations and susceptibilities of different instars of *Culex quinquefasciatus* against *Trichophyton ajelloi* (LC values in mg/liter and 95% fiducial limits in parentheses).

Instar	Probit equation	LC_{50}	LC_{90}	LC_{99}
1st	3.179 + 3.398X	3.43 (3.35–3.49)	8.18 (8.03–8.30)	16.60 (16.33–16.86)
2nd	3.123 + 3.634X	3.28 (3.21–3.32)	7.40 (7.31–7.49)	14.30 (14.26–14.51)
3rd	2.589 + 4.859X	2.76 (2.72–2.79)	5.06 (4.94–5.11)	8.29 (8.11–8.34)
4th	3.238 + 2.553X	4.90 (4.86–5.03)	15.60 (15.53–16.02)	40.00 (38.38–41.56)

Table 3. Grand probit equation of all instars of *Anopheles stephensi* and *Culex quinquefasciatus*.

Instar	*An. stephensi*	*Cx. quinquefasciatus*
1st	3.984 + 3.006X	3.179 + 3.398X
2nd	4.419 + 2.560X	3.123 + 3.634X
3rd	4.921 + 2.920X	2.859 + 4.859X
4th	3.785 + 2.249X	3.238 + 2.553X

drosporum also was less effective against *Anopheles* spp. (Frances and Pinnock 1990). These findings differ from our results with *T. ajelloi.* This difference may be because of differences in microenvironment and water quality among these studies.

Lacey et al. (1988), with *Metarhizium anisopliae,* demonstrated mortality within 24 h after using a wetting agent. We have not tested the effect of wetting agents in the present investigation.

On the basis of the present results, *T. ajelloi* appears to deserve additional evaluation in the field as a mosquito control agent. The U.S. Environmental Protection Agency has registered (USEPA registration nos. 56984-1, 56984-2, 56984-3) 3 formulations of *L. giganteum* (Kerwin et al. 1994) for control of mosquito larvae in wildlife ponds. Pilot studies for the use of mycelia of this fungus showed reduction of mosquito larvae for >2 months. These results encourage us to continue to evaluate entomopathogenic fungi as an alternate strategy for controlling the density of mosquito larvae in tropical countries.

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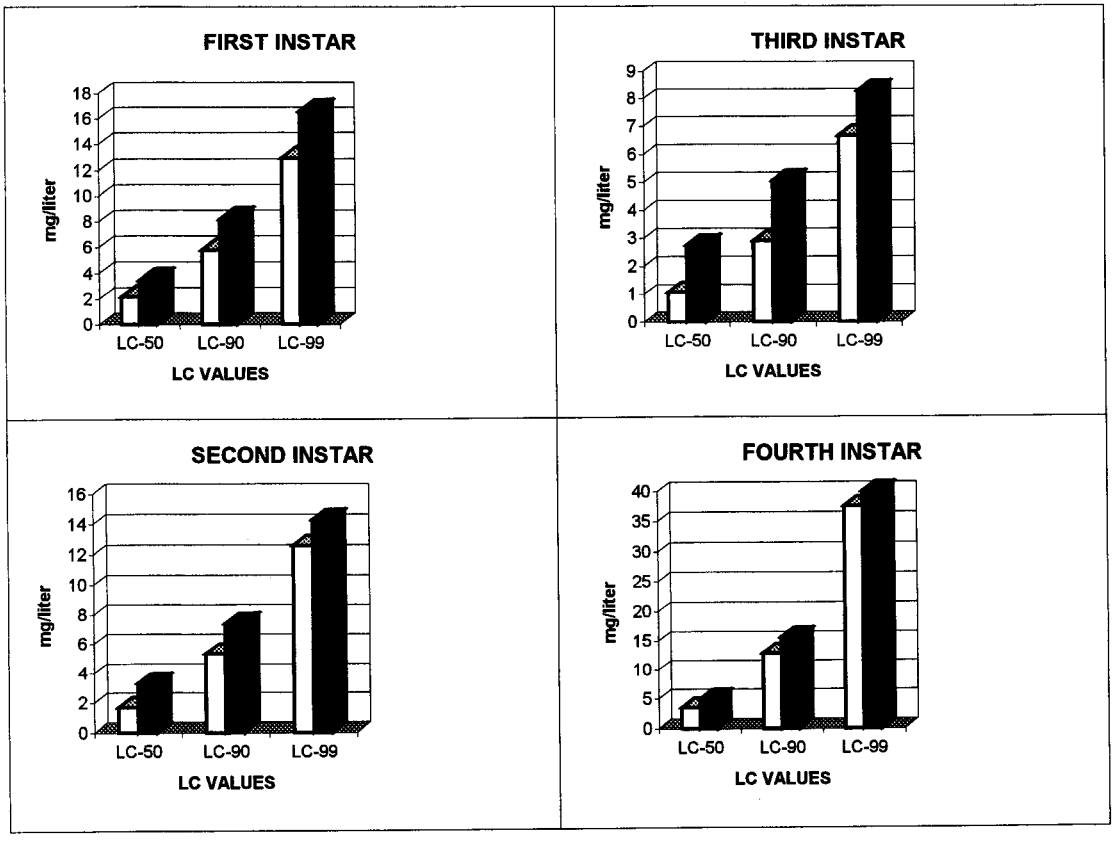

Fig. 1. LC_{50}, LC_{90}, and LC_{99} values for various instars of *Anopheles stephensi* and *Culex quinquefasciatus* treated with *Trichophyton allejoi.* Solid bars = *Cx. quinquefasciatus,* open bars = *An. stephensi.*

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