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ORIGINAL PAPER

Efficacy of *Chrysosporium tropicum* metabolite against mixed population of adult mosquito (*Culex quinquefasciatus*, *Anopheles stephensii*, and *Aedes aegypti*) after purification with flash chromatography

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Abstract *Chrysosporium tropicum is* a keratinophilic fungus and an effective mosquito control agent. This fungus was grown in Richards broth at 27±2°C and a relative humidity of 75%±5% for 15±2 days. Filtration was done with Whatman number 1 filter paper, column chromatography, and flash chromatography. Adulticidal efficacy was performed against a mixed population of mosquitoes including *Culex quinquefasciatus, Anophelese stephensii, Aedes aegypti* at five different concentrations 5:5, 6:4, 7:3, 8:2, 9:1 by adding fungal filtrate of flash chromatography to methanol in different ratio (metabolite/methanol). The experiment was conducted in the cage with an area of 2×2×3 ft. The mortality in mosquito population was recorded after 8 hours of exposure, and adulticidal activity was tested by probit analysis. The LC_{50} was determined to be 4.9921 ml. Results of present study confirm that metabolites of *C. tropicum* can be utilized as alternative biological control agents for adult mosquitoes.

Introduction

Mosquitoes are a vector agent that carries disease causing virus and parasite from person to person without catching the disease themselves. *Culex quinquefasciatus* is a common man-biting mosquito, and it serves as the vector of lymphatic filariasis and Japanese encephalitis in India. Viral diseases as yellow fever and dengue fever are transmitted by *A. aegypti*. Mosquito alone spreads some of the vector borne diseases like Dengue, Japanese encephalitis, filariasis, yellow fever, malaria, and chikungunya. In addition to personal protection and educating the public, the most successful method of minimizing the incidence of mosquito borne disease is to eradicate and control the mosquito vector, which is performed principally by systematic treatment of the breading places through a combination of environmental management and application (Rozendaal 1997). The synthetic adulticide were effective but have now been discarded or not in use due to its deleterious effect on ecosystem; also, the continuous use of insecticide against mosquitoes has led to a substantial increase in physiological resistance in mosquitoes (Hargreaves et al. 2000). It is reported that *A. aegypti* shows resistance to insecticides like permethrin and temephos (Ponlawat et al. 2005).

The use of biological control agent can reduce the drawback associated with insecticide (Krischbam 1985). There is a consensus among workers that entomopathogenic fungus could be exploited more effectively as natural resource. Recent developments in this field show that certain funguses are virulent to adult mosquitoes. The potential of *Metarhizium anisopilae* isolates against *C. quinquefasciatus* are evaluated by altering the method of incubation (Lecy et al. 1988). Among them, *Metarhizum anisopilae, Beauveria tenella, Leginidium gigantium, Chrysosporium lobatum*, and *Chrysosporium tropicum* are studied widely for use against mosquito larvae (Balaramn et al. 1979; Federici 1981; Lecy et al. 1988; Vyas et al.

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Table 1 Probit equation and susceptibility of mixed population of adult mosquitoes against extracellular metabolites after purification with flash chromatography

S.No.	Concentration metabolite/methanol	Log dose x	% Mortality	Probit mortality	Probit equation[n]	χ^2	LC_{50}
1	5:5	0.666	43.75	4.8414	$Y = 6.3796x + 28.427$	1.538	4.9921 ml
2	6:4	0.778	52.94	5.0728			
3	7:3	0.845	64.70	5.3772			
4	8:2	0.903	66.6	5.4287			
5	9:1	0.954	70.58	5.5388			

2007; Mohanty and Prakash 2008). Moreover, that fungus has the distinct advantage of being able to recycle in stagnant water, injecting multiple and overlapping generations of mosquitoes (Legner 1995).

Recently, insect pathogenic deuteromycetes fungi were tested in the laboratory as against the adults of *Anophelese* and *Culex* sps., and the effective one was *M. anisopilae* (Scholte et al. 2004), and also, the spores of entomopathogenic fungus *Fusrium pallidoroseum* were found to be effective against adults of female *C. quinquefasciatus* (Mohanty et al. 2008) This is the first report of *C. tropicum* used against adult mosquito population. In the present investigation, we have evaluated the adulticide activity of metabolite of fungus *C. tropicum* against adult mosquitoes after purification of metabolites. This experiment validates and reveals the efficacy of extracellular metabolites of *C. tropicum* against adult mosquitoes.

Materials and methods

Culture and growth condition

The fungal strain of *C. tropicum* was obtained from the Institute of Microbial Technology (MTCC-2838), Chandigarh, India. Fungal colonies were cultured in a 250-ml conical flask containing 100 ml of Richard's broth (50.0 g sucrose, 10 g Pot. nitrate, 8.0 g potassium dihydrogen phosphate, 2.5 g magnesium sulfate, 0.02 g ferric chloride, and 1000 ml deionized water). The fungus was incubated under static condition at 27±2°C for 15±2 days.

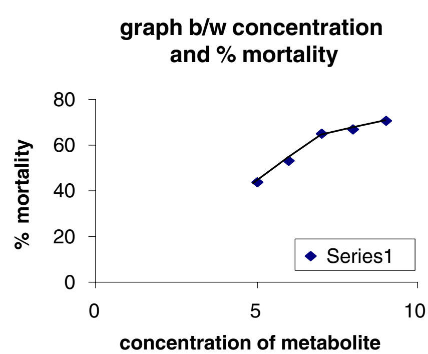

Fig. 1 Graph between the % mortality and different concentrations of metabolites after purification with flash chromatography

Isolation of extracellular metabolite

The cell-free culture filtrate was obtained by filtering the broth through successive Whatman number 1 filter paper. The resultant was then subjected to column chromatography, which were then tested against adult mosquitoes. The active ratio was then again purified with flash chromatography.

Flash chromatography

This resultant ratio was then subjected to flash chromatography through automated flash chromatograph. In a typical experiment, the sample was prepared by 4 ml sample in 1 ml solvent (methanol/deionised water, 9:1) then mixes it in 10 g silica (for flash chromatography). Swirl the mixture until the solvent evaporates; this sample is then loaded to prepecked column of automated flash chromatograph and then run the system. Fractions of 5 ml were collected each time elution was done with methanol and deionized water in the ratio of 9:1.

Bioassay

To test the adulticidal activity of *C. tropicum* metabolites, 40 pupae were collected 20 for test and 20 for control each time. After metamorphosis of the beaker containing adult mosquitoes placed in a cage area of (2×2×3 ft.), the test was conducted after each filtration and the best results were seen after flash chromatography. For the different test, concentrations of filtered metabolite and methanol were prepared and then sprayed on adult mosquitoes. Mortalities were determined after 1, 2, 4, 6, and 8 hours of exposure. During the experiment, no food was offered to the adults. The efficacy study of mixed adult population of mosquito viz. *C. quinquefasciatus, A. aegypti,* and *Anophelese stephensi* were analyzed by probit analysis (Finney 1971). The relation between probit and log concentration was established as probit equation (Fig. 1).

Results

The efficacy study shows highest mortality at 9:1 (metabolite/methanol) ratio after 8 hours of exposure. The highest mortality rate was 70.58% corrected with control. The LC_{50} value was recorded 4.9921 ml, which was calculated with probit analysis (Table 1).

During the experiment, *C. tropicum* was used as mosquito adulticide for the first time and found significantly effective. The colony of the selected fungi was maintained in their specific media for certain period of time. The culture filtrates were obtained by filtering process after incubation periods through Whatman filter paper and purified by column chromatography, then by flash chromatography, and then this purified metabolite was tested against adult mosquitoes. The test concentrations (5:5, 6:4, 7:3, 8:2, and 9:1) were prepared by mixing metabolite with methanol in different ratios.

The LC value of metabolite of *C. tropicum* after flash chromatography reported in the present studies was found effective against adult mosquitoes. The chi-square value (1.538) of all purifications confirmed the adequate representation of experimental data. The results show that as the concentration of metabolite increased, the efficacy of metabolite also increased.

Discussion

Unlike other mosquito control agents, the entomopathogenic fungi are unique because fungi have the ability to directly infect the host insect by penetrating into the cuticle and do not need to ingest by the insect to cause disease. There are preferential advantages when we use fungi as biocontrol agent for mosquitoes. The fungi have very narrow range, and considerable progress has been made in recent years in development of environmentally benign spores and mycelium-based biocontrol agent for the mosquito population. Fungal biocontrol agents have reduced inputs of harmful synthetic chemical pesticide in agriculture, horticultural, and forest system (Strasser et al. 2000). Amer and Mehlhorn (2006) have studied the effect of plant oil extract under different storage condition against third instar larvae of *A. aegypti* and showed around 100% mortality. Michaelakins et al. (2008) studied the activity of naturally occurring napthoquinones and derivatives against culex pipenes with 100% mortality. The effect of three citrus species and enantiomers of α- and β-pipenes were also studied against third instar larvae of culex pipenes (Michaelakins et al. 2008). These studies were based on plant extract aginst mosquito larvae.

A number of entomopathogenic fungi have been so far used effectively to control mosquito vector for the last few decades. McCray et al. (1973) showed that fungus can successfully infect and kill larvae of *A. aegypti* and *C. quinquefasciatus* with 100% mortality. The spores of *M. anisopilae* and *B. bassina* were also observed as beneficial insecticide (James and Lighthart 1994). A study with the spores of *C. lobatum* also shows 100% mortality to each instar larvae of *C. quenquefasciatus* (Mohanty and Prakash 2007) However, in these studies, the spores of fungus were used but not the metabolite, whereas the present investigation is based on metabolite of the fungus. Govindrajan et al. (2005) have tested extracelluler secondary metabolite of different soil fungi against late third instar larvae of culex quinquefasciatus. Priyanka and Prakash (2001) have also tested the *C. tropicum* metabolite against mosquito larvae of *A. stephensi* and the third instars larvae of *C. quenquefasciatus,* respectively. They reported first instars larvae were most succeptible with LC_{50} value of 38.9 μl/ml compared with fourth instar, which is 122.6 μl/ml. Vyas et al. (2007) tested the metabolite of *L. gigantium* against the first instars larvae of *C. quenquefasciatus, A. aegypti, and A. stephensi* with 100% mortality. However, all these tests were performed against the larvae of mosquito not on adult. The present investigation shows the effect of metabolite of *C. tropicum* against adult mosquitoes with 70% mortality.

Mohanty et al. (2008) have tested the efficacy of the spores of *F. pallidoroseum* against adult mosquitoes. But again this study is based on the spore, whereas the present investigation is based on purified metabolite. This is the first report in which the effect of fungal metabolite against adult mosquito population has shown.

Hence, it can be concluded that the use of extracellular metabolites of the fungi may provide better technology alternatives for controlling large population of mosquito larvae and adults. We could now believe that any widely used chemical insecticide will sooner or later encounter the resistance problems and raised a question: what measures should be taken to counter these problems? The solution depends on more advancement in researches, especially in the discovery and development of new species and candidate metabolites from effective organisms for sustainable control of mosquitoes.

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