J. Biol. Control, 22(1): 179-183, 2008

Larvicidal potential of fungi isolated from larval mosquito habitats against *Aedes aegypti*

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ABSTRACT: **Forty-six water and soil samples were collected from various larval mosquito habitats of four districts of Madhya Pradesh for isolation of fungal pathogens against *Aedes aegypti*. Five fungal isolates, namely, *Beauveria bassiana*, *B. nivea*, *Myrothecium roridum*, *Aspergillus flavus* and *Trichoderma harzianum*, were found to have potential against the third instar larvae. Larvae of *A. aegypti* were found to be more susceptible to *B. nivea* (LD_{50} value of $1.2x10^5$ conidia ml^{-1}). *M. roridum* yielded lowest LC_{50} value (67.61µl ml^{-1}) of extracellular metabolite, which was followed by *A. flavus* (107.15µl ml^{-1}). Highest LC_{50} value (631.0µl ml^{-1}) was obtained for *T. harzianum*. In case of *M. roridum*, larval mortality occurred due to toxic metabolites present in the sporodochia.**

KEY WORDS: *Aedes aegypti*, fungal pathogens, larvicidal potential, *Myrothecium roridum*, secondary metabolite, sporodochia

INTRODUCTION

Mosquito menace has been a major concern throughout the world and many control strategies have been or are being developed. Concern over the use of insecticides, insect resistance and their environmental impact has necessitated the development of alternative means of biological control (Lacey *et al.*, 2001; Scholte *et al.*, 2004). The search for effective mosquito pathogens that can be used in mosquito control operations has been going on for several decades. Both laboratory and field studies have been carried out on those fungi that appeared to have potential for operational use (Scholte *et al.*, 2004). Many species of fungi are currently being considered for use in microbial control of mosquito larvae (Sandhu and Sharma, 1994). Three genera of mosquito pathogenic fungi are generally considered important: *Lagenidium*, *Coelomomyces* and *Culicinomyces* (Federici, 1995). Secondary metabolites of fungi and actinomycetes are potential agents for the control of insects and mites (Berdy, 1984). The microbial metabolites including mycotoxins and antibiotics have been reported to cause retardation of growth and reduction in the size of pupae among insects and mites (Sundarapandian *et al.*, 2002). This study investigated the mosquito larvicidal potential of the fungi isolated from larval mosquito habitats.

MATERIALS AND METHODS

Sources of fungal cultures

A survey was conducted and sampling was done in four districts, *viz.*, Jabalpur, Seoni, Chinddwara and Narsinghpur of Madhya Pradesh in February 2006. Forty-six water and soil samples were collected from various larval mosquito habitats such as drains, ponds, ditches, streams, lakes and tree holes. One hundred and thirteen fungal cultures were isolated from the samples on malt extract peptone agar (MEPA) by employing pure culture technique. The media was supplemented with an antibiotic (chloramphenicol) to minimize bacterial contamination. Fungal cultures were maintained on MEPA slants and revived periodically. Identification of the fungi was done by using the keys given by Domsch *et al.* (1980) and Arx (1980).

Sources of mosquito larvae

Larvae of *A. aegypti* were reared in the Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of Biological Sciences, R.D. University, Jabalpur, as per standard methods (Sandhu *et al.*, 1993a). The larvae were kept in open trays exposed to light for a couple of hours during daytime. The water in the trays was routinely changed and larvae were fed with 0.1%(w/v) of sterilized yeast powder and dog biscuit in a ratio of 1:1.

Bioassay test by using spores/conidia and fungal metabolites

Fungal spores/conidia were harvested from the pure cultures in petri dishes by scraping with the sterile spatula. The spore mass was suspended in 20ml sterile water containing 0.05% Tween20 to form spore solutions. These spore solutions were used for bioassay tests. In the preliminary screening, ten 3rd instar healthy larvae were placed in a clean beaker containing 150ml of tap water. To each bioassay cup containing larvae, 10ml of spore suspension of different isolates was added. The bioassay cups were labeled with the culture accession numbers and larval feed was also added. The cups were held at 26 ± 2°C and larval mortality observations were made every day (Sundarapandian *et al.*, 2002). The isolates, which resulted in larval mortality were further evaluated for determination of LC_{50} values and larvicidal activity of their metabolites. The spore concentration was enumerated with the help of a haemocytometer (Neubauer).

Potential fungal isolates were grown in 100ml Richard's broth in 500ml Erlenmeyer flasks. Broth cultures were incubated for 14 days at 26 ± 2°C in a BOD incubator. After incubation, mycelial mass was separated from the culture broth by centrifugation at 6000rpm for ten minutes. The supernatant broth was passed through Whatman No.1 filter paper. This mycelia free culture filtrate was used to assess the larvicidal potential against 3rd instar larvae of *A. aegypti*.

Determination of LC_{50} values

The LC_{50} values of the fungal extracellular metabolites were determined through bioassay by applying 50-1000μl ml^{-1} of mycelia free culture filtrate in the bioassay cups. For determining LD_{50} value (conidia/ml), stock spore solutions were prepared and the number of spores ml^{-1} was determined by using a haemocytometer. For each isolate, five different concentrations of spores were tested to deduce the LD_{50} values. The LD_{50} values were calculated after converting the percentage mortality into probit values using probit regression analysis (Finney, 1971).

RESULTS AND DISCUSSION

It was observed that per cent larval mortality of *A. aegypti* increased with an increase in the dose. The larvae were more susceptible to *B. nivea*, which showed an LD_{50} value of 1.2×10^5 conidia ml^{-1} (Table 1). The per cent mortality varied from 40 to 80% depending on the conidial concentrations in the bioassay cups. The LD_{50} values for *B. bassiana*, *A. flavus* and *T. harzianum* were 4.8×10^6, 8.1×10^6 and 6.0×10^7, respectively (Table 1). Sandhu *et al.* (1993b) have shown the effect of *B. bassiana* and *Metarhizium anisopliae* against different instars of *Culex tritaenorynchus* and *A. aegypti*. The

washed conidia of *M. roridum* did not cause any significant mortality, due to the absence of toxic metabolites on their surface. The sporodochia of *M. roridum* consists of mass of dark green spores collected in green to blackish coloured fluid. It was confirmed by the results that in case of *M. roridum* larval mortality occurred due to a toxic metabolite present in the sporodochia. Microbes are used as alternatives to conventional broad-spectrum synthetic insecticides because of their selective toxicity and safety to the environment. The insecticidal secondary metabolites produced by entomopathogenic fungi have become a focus of interest for insect pathologists. The secondary

Table 1. Effect of fungal conidia/spores on third instar larvae of *Aedes aegypti* after 48h

Fungal Isolate	Dose (spores ml^{-1})	% Mortality	LD_{50} (Spores ml^{-1})	Slope	$\div^2$	Fiducial limits (spores ml^{-1})	
						Lower	Upper
Beauveria bassiana	$2.48x10^7$	90	$4.8x10^6$	1.37	3.52	$1.94x10^6$	$1.18x10^7$
	$1.98x10^7$	85					
	$1.48x10^7$	80					
	$9.90x10^6$	65					
	$4.95x10^6$	40					
Beauveria nivea	$3.65x10^5$	80	$1.2x10^5$	1.52	0.24	$1.15x10^5$	$1.38x10^5$
	$2.92x10^5$	70					
	$2.19x10^5$	60					
	$1.46x10^5$	50					
	$7.30x10^4$	40					
Aspergillus flavus	$2.05x10^7$	80	$8.1x10^6$	2.38	0.57	$6.08x10^6$	$1.04x10^7$
	$1.67x10^7$	70					
	$1.23x10^7$	65					
	$8.20x10^6$	45					
	$4.10x10^6$	40					
Trichoderma harzianum	$1.11x10^8$	80	$6.0x10^7$	1.44	1.69	$3.44x10^7$	$1.15x10^7$
	$8.88x10^7$	60					
	$6.66x10^7$	30					
	$4.44x10^7$	20					
	$2.22x10^7$	20					
Myrothecium roridum	$2.67x10^8$	05	-	-	-	-	-
	$6.03x10^7$	0					
	$4.39x10^7$	0					
	$2.34x10^7$	0					
	$1.55x10^7$	0					

The sign '-' indicates absence of mortality or nil results

metabolites of entomopathogenic fungi *Metarhizium*, *Beauveria*, *Tolypocladium* and *Fusarium* have potential insecticidal activity (Peeters *et al.* 1989). Vijayan and Balaraman (1991) have reported the metabolites of 17 fungi to be highly larvicidal and their LC50 values against the 3rd instar of *An. stephensi* and *Cx. quinquefasciatus* were in the range of 7–83 and 3–24μl ml^{-1}, respectively. Zizka and Weiser (1993) evaluated the effect of beauvericin against L4 larvae of *Cx. pipiens autogenicus* and reported that beauvericin caused 44% mortality at a concentration of 100μl ml^{-1}. Sandhu and Sharma (1994) evaluated the larvicidal potential of *B. bassiana*, *M. anisopliae* and *A. flavus* against *Culex pipiens*. They used fungal conidia and calculated LD_{50} (Conidia/ml) and LT_{50} values. But, in this study we have employed both fungal conidia and metabolites of the fungi that might be toxic to the mosquito larvae. The lowest LC_{50} value (67.61μl ml^{-1}) was obtained for *M. roridum*, followed by *A. flavus* (107.15μl ml^{-1}). The highest LC_{50} value (631.0μl ml^{-1}) was obtained for *T. harzianum*. LC_{50} values of 398.1μl ml^{-1}, and 512.9μl ml^{-1} were obtained for *B. bassiana* and *B. nivea*, respectively (Table 2.).

Therefore, it can be concluded that fungi isolated from soil and water of larval mosquito habitats can kill larvae of *A. aegypti*. Novel compounds can also be obtained from the specific metabolites by purification. After complete characterization and suitable formulation, these potential fungal isolates or their extracellular metabolites may be used in integrated pest management programs for *A. aegypti*.

Table 2. Efficacy of fungal metabolites against 3rd instar larvae of *Aedes aegypti*

Fungal strain	LC_{50} (μl ml^{-1})*	Fiducial Limits (μl ml^{-1})		χ^2 (n-2)	Regression Equation
		Lower	Upper		
Beauveria bassiana	398.1 ± 3.5	229.1	691.9	3.4164	0.559x + 2.0425
Beauveria nivea	512.9 ± 2.1	68.8	3821.2	0.7023	0.1808x + 3.155
Myrothecium roridum	67.61 ± 1.8	41.88	109.13	0.0313	6.8466x + 3.9368
Aspergillus flavus	107.15 ± 3.2	35.70	321.58	0.00074	5.83x + 2.5056
Trichoderma harzianum	631.0 ± 8.4	177.4	2242.6	0.4033	0.2808x + 2.946

*Values significant at P = 0.05 by Student's 't' test; values are means of three replicates.

extracellular metabolites in mycelia free culture filtrate for the determination of LD_{50} (Spores/ml) and LC_{50} values, respectively.

From the results (Table 2), it is evident that the extra-cellular metabolites of these isolates possess varying degree of larvicidal activity. The LC_{50} value of the extracellular metabolites of these five fungal isolates ranged from 67 to 631μl ml^{-1}. It was observed that the larvae in the beginning became very active exhibiting typical movements. Gradually the physiological and behavioral changes in the movements decreased and later on, the larvae died. In the controls, no such changes were observed. These changes may be attributed to the presence of some components in the extra-cellular

ACKNOWLEDGEMENT

We are grateful to the Head, Department of Biological Sciences, Rani Durgawati University, Jabalpur – 482001, Madhya Pradesh, for providing the facilities for research work. We also thank DBT for funding the project.

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(Received: 14.03.2007; Revised: 20.07.2007; Accepted: 25.09.2007)