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Mosquito larvicidal efficacy of extracellular secondary metabolites of soil actinomycetes against the malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae)

M. Govindarajan[1], A. Jebanesan[1*], D. Reetha[2] and T. Pushpanathan[1]

[1]Division of vector Biology, Department of Zoology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India
[2]Department of Microbiology, Faculty of Agriculture Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

Abstract

Larvicidal property of extracellular secondary metabolites of 64 actinomycetes isolated from soil samples of different natural and man-made habitats was tested against early third instar of *Anopheles stephensi.* Twenty three isolates of them showed positive larvicidal activity. Among the 23 isolates, four isolates of *Streptomyces* (A14, A21, A49, A63) and each one isolate of *Micromonospora* (A32) and *Actinoplanes* (A52) were found to be highly active against mosquito larvae with LC50 values ranging from 9 to 60 ppm. Among them, the isolate of *Streptomyces* A21 was found to be a highly potential larvicidal agent against *An. stephensi,* having the lowest LC50 value of 9.20 ppm.

Keywords: *Anopheles stephensi*, larvicidal activity, actionomycetes, secondary metabolites

INTRODUCTION

Mosquitoes, which transmit a number of diseases such as malaria, filariasis, dengue, Japanese encephalitis, etc. and cause millions of deaths every year, are the most important single group of insects in terms of public health (Fradin, 1998). Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance (Brown, 1986), undesirable effects on non-target organisms and fostered environmental and human health concerns (Hayas and Laws, 1991), which initiated a search for alternative control measures.

Microbial insecticides are being well considered as an alternative to chemical insecticides because of their selective toxicity and ready decomposability in the ecosystem. Also, unlike the inherent risks associated with the production process on synthetic insecticides, the manufacturing process of microbial products is safe, well contained and less polluting (Govindarajan *et al.*, 2005). For e.g. biological active griseulin which has nematicidal and mosquitocidal properties, was isolated from *Streptomyces* sp (Ishibashi *et al.*, 1994). The present study was undertaken to determine the efficacy of extracellular secondary metabolites of actinomycetes culture filtrates against early third instar of *Anopheles stephensi,* the urban malarial vector mosquito under laboratory conditions.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from 12 different natural and man-made habitats namely paddy fields, ponds, mangroves, garden lands, farmyard pits, grass lands, cesspits, canals, lakes, cattle sheds, coir pits and tree holes, in and around , Annamalainagar, Tamilnadu, South India (located at 11°, 24′ N latitude and 79°, 5′ E longitude 14.79 m above the sea level).

Cultivation of actinomycetes

The organisms were grown in 500ml conical flask containing 100ml of yeast extract malt extract glucose medium (YMG) (yeast extract : 4.0 g, malt extract : 10.0 g, glucose : 4.0 g, distilled water 1000ml) by inoculating one or two loopful of spores from slant culture and incubated on a rotary shaker at 250 rpm in 28° ± 2°C for 7-21 days. After the incubation period the mycelial mass was separated from the culture broth by centrifugation at 6000 rpm for 10 minutes. The culture broth was passed through Whatman No.1 filter paper and the pH was adjusted to 7.0 using 0.01 NaOH. This was examined for the larvicidal activity against early third instar of *An. stephensi*.

Test organism

An. stephensi larvae were reared in dechlorinated water and fed with dog biscuits and yeast powder at the ratio of 3:1. They were maintained at 28° ± 2°C, 70-85% RH and under 14L : 10D photoperiod cycles.

Bio assay

In the preliminary test, ten early third instar larvae were placed in a 100 ml sterile beaker containing 50 ml of ten times diluted (100 µl/ml) culture filtrate. The medium alone served as a control and each experiment was replicated thrice. The beakers were held at 28° ± 2°C and the larvae were fed with dog biscuits and yeast powder in the ratio of 3:1. Observation on larval mortality were made after 24 hours. Metabolites which caused more than 50 per cent larval mortality in the preliminary test were further evaluated at different concentrations to

*Corresponding author
email: *drjeban@rediffmail.com*

Table 1. Toxicity of extracellular metabolites of actinomycetes against the larvae of *Anopheles stephensi*

Isolate number	Actinomycetes genera	LC_{50} (ppm)	95% confidence limit (ppm)		LC_{90} (ppm)
			LCL	UCL	
A14**	*Streptomyces*	26.97 ± 1.07	21.35 ± 1.51	32.87 ± 0.79	54.86 ± 0.89
A21	*Streptomyces*	9.20 ± 0.74	4.95 ± 1.57	12.60 ± 2.32	19.92 ± 1.30
A32	*Micromonospora*	53.62 ± 0.63	36.80 ± 1.24	68.83 ± 1.62	109.43± 0.94
A49	*Streptomyces*	34.56 ± 1.26	18.30 ± 1.02	47.45 ± 0.93	74.04 ± 1.32
A52	*Actinoplanes*	59.38 ± 1.74	40.48 ± 1.62	77.10 ± 1.24	124.23± 1.69
A63	*Streptomyces*	31.65 ± 1.26	23.70 ± 1.72	42.56 ± 1.03	67.48 ± 1.56

A– Actinomycetes **Isolate Number
Each value (X±S.D.) represents mean of six replicates.

determine the LC_{50} values using tap water as substrate. Each test concentration was tested six times along with appropriate controls. LC_{50} values were calculated by using probit analysis (Finney, 1971).

RESULTS

Ninety six samples collected from twelve different habitats, which yielded 64 isolates of actinomycetes which were designated as A1-A64. Screening of those metabolites showed that 23 of the isolates were positive larvicidal activity against mosquito larvae. Of them, four isolates of *Streptomyces* (A14, A21, A49, A63) and one isolate each of *Micromonospora* (A32) and *Actinoplanes* (A52) exerted significant larvicidal activity. The LC_{50} values of these metabolites ranged between 9-60 ppm against *An.stephensi* (Table 1). Among the actinomycetes, the lowest LC_{50} value of 9.20 ppm was recorded for the *Streptomyces* isolate A21 and the highest value of 59.38 ppm for the *Actinoplanes* isolate A52 (Table 1).

DISCUSSION

Earlier Mishra *et al.* (1987) reported that *Streptomyces, Micromonospora, Actinomadura, Actinoplanes* and *Micropolyspora* possessed mosquitocidal activity against *Aedes aegypti*. They reported that the LC_{50} of the extracellular metabolites from them to range from 500-1000 ppm. Vijayan and Balaraman (1991) reported extracelluler secondary metabolites from 350 fungi and 94 actinomycetes and screened them for their larvicidal activity against *Cx. quinquefasciatus, A. stephensi* and *Ae. aegypti*. Of them 133 fungal metabolites and 35 from actinomycetes were found to be active. They further observed that two from *Streptomyces* sp. and one from *Paecilomyces* sp. were highly active with LC_{50} value of 1-3 microlitre / ml. Sundarapandian *et al.* (2002) studied mosquitocidal properties of indigenous actinomycetes against *Culex quinquefasciatus* and found the LC_{50} of the selected three actinomycetes to range from 14-25 ppm (*Streptomyces* 98-1, *Streptomyces* 98-2 and *Streptomyces* 98-3). So it is evident that soil actinomycetes produce various kinds of secondary metabolites that can kill mosquito larvae. Further investigations are currently underway to isolate specific metabolites which will yield a compound, which may posses mosquitocidal properties even at very low concentrations.

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