Larvicidal potency of marine actinobacteria isolated from mangrove environment against *Aedes aegypti* and *Anopheles stephensi*

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Abstract The marine soil samples were collected from different locations of Parangipettai mangrove ecosystem, Vellar estuary, southeast coast of India. Totally 30 different marine actinobacteria were isolated by serial dilution plate technique on starch casein agar medium. The isolated actinobacteria were investigated for their larvicidal activity against *Aedes aegypti* and *Anopheles stephensi* mosquitoes. *Streptomyces fungicidicus*, *S. griseus*, *S. albus*, *S. alboflavus* and *S. rochei* were identified as potential biocide producers. Based on the antimicrobial activity, five strains were chosen for larvicidal and pupicidal activity. Among the crude extracts tested, the *S. alboflavus* extract showed significant activity against *Ae. aegypti* (LC₅₀ 1.48 ± 0.09 and LC₉₀ 3.33 ± 0.22) and *An. stephensi* (LC₅₀ 1.30 ± 0.09 and LC₉₀ 3.13 ± 0.21). Five isolates have shown a most significant mortality rate of the *Ae. aegypti* and *An. stephensi* mosquito larvae. This is an ideal eco-friendly approach for the control of Japanese encephalitis vectors, *Ae. aegypti* and malarial vector *An. stephensi*.

Keywords Actinobacteria · *Aedes aegypti* · *Anopheles stephensi* · Bioassay · Mangrove ecosystem

Introduction

Mosquitoes are vectors of many vertebrate blood parasites. Mosquito borne diseases such as malaria, filariasis, dengue and viral encephalitis contribute to a larger proportion of health problems of developing countries. In recent years, as a result of changes in public health policy, social factors and development of resistance in mosquitoes as well as the pathogens they transmit, there has been resurgence in the incidence of mosquito borne diseases (Geetha et al. 2007). Vector control is recognized as an effective tool for controlling these tropical diseases. Synthetic insecticides have been used for several decades to control varied dipteran pests. However, the use of chemical insecticides has been greatly impeded due to the development of physiological resistance in the vectors, environmental pollution resulting in bio amplification of food chain contamination and harmful effects on beneficial non target animals. Therefore, there is a need for alternate, more effective and environmentally friendly control agents (Dhiman et al. 2010; Rajesh et al. 2014).

Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance (Ramanathan et al. 2002). At present, microbial insecticides are the main component of the bio-pesticide industry (Xie 1998; Shi 2000). Most of the pesticidal micro-organisms, however, have been isolated from entomopathogens and the terrestrial environment (Zhang 1996; Leonard and Julius 2000). Several varieties of microorganisms including fungi, actinomycetes, bacteria, viruses and nematodes that are antagonistic to insects have been reported as strategies to biologically control them. Balakrishnan et al. (2013) extracted mosquitocidal *Bacillus* sp, whose extracellular

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metabolites exhibited mosquito larvicidal and pupicidal activity, from the mangroves of Parangipettai, southeast coast of India.

Marine organisms exploiting aggressively in several countries with an apparent focus on bioactive compounds (Berdy 2005; Demain and Sanchez 2009). The first report on the marine actinobacteria was made by Nadson (1903) when he observed and documented those in the salt mud. Extensive surveys were conducted for distribution of marine actinobacteria in the sediments of North Sea and Atlantic Ocean (Weyland 1969; Mehlhorn et al. 2005; Amer and Mehlhorn 2006a, b; Rahuman et al. 2009a, b). These studies reported that the marine actinobacteria are the best sources for isolation of unique bioactive compounds compared to terrestrial ones. The actinomycetes are noteworthy as antibiotic and enzymatic producers, making three quarters of all known products; the *Streptomyces* species are especially prolific and can produce many antibiotic and other class of biologically active secondary metabolites. If we include secondary metabolites with biological activities other than antimicrobial, actinomycetes are still out in front, over 60 %; *Streptomyces* spp. accounting for 80 % of these (Hopwood et al. 2000). Actinomycetes play an important role in the biological control of insects through the production of insecticidally active compounds against the house fly *Musca domestica* (Hussain et al. 2002). Actinomycetes gave a good effect, shown as lowest pupal formation percentages of *Drosophila melanogaster* (Gadelhak et al. 2005). Dhanasekaran et al. (2010) found that the actinomycete isolates producing strong larvicidal activity against *Anopheles* mosquito larvae. However, actinomycetes were effectively used against *Culex quinquefasciatus* (Sundarapandian et al. 2002). Many actinomycete strains caused larval mortality, of the cotton leaf worm *Spodoptera littoralis*, ranging from 10 to 60 % (Bream et al. 2001). In addition, considerable lethal effect of some actinomycetes was observed on pupae. Due to this property, marine actinobacteria have received much attention in recent days. It has been proposed that these antimicrobials are used in competition between microorganisms, offering an advantage to the producer strains (Jensen et al. 2005; Karthik et al. 2010b). Balakrishnan et al. (2014) studied the biosynthesis of silver nanoparticles with leaf aqueous extract of *A. marina* provides potential source for the larvicidal activity against mosquito borne diseases. Keeping these points, the present study has been undertaken to isolate and screen the larvicidal compounds producing actinobacteria from sediment of Parangipettai mangrove (Southeast coast of India) and also to characterize the different isolates by analyzing biochemical and larvicidal spectrum of actinobacteria against *Ae. aegypti* and *An. stephensi* mosquitoes.

Materials and methods

Soil sample collection

The soil samples were collected randomly from different locations in the Parangipettai mangrove ecosystem (11°29' N; 79°47' E), southeast coast of India. The samples were collected in plastic bags and immediately transported to the laboratory. To increase the isolation of actinobacteria, six different types of media were selected. A tenfold serial dilution series was made and plated in triplicate on agar plates. The plates were incubated at room temperature (28 °C) and monitored periodically over 3 months for actinobacteria growth (Karthik et al. 2010a, b; Vijayakumar et al. 2007).

Isolation of actinobacteria

Starch casein agar (SCA) medium was prepared and sterilized at 121 °C in 15 lbs pressure for 15 min. Subsequently the sterile medium was supplemented with Amphotericin B 50 µg/l and tetracycline 20 µg/l to prevent the bacterial and fungal contamination. One gram of soil sample was serially diluted. 100 µl of the aliquots from the 10^{-5} was spread over the SCA plates and incubated at 28 ± 2 °C for 7 days. After the incubation period, the actinobacterial colonies were purified and maintained in starch casein agar medium for further investigations.

Mosquito culture

The eggs of *Ae. aegypti* and *An. stephensi* were collected in and around Parangipettai area with the help of 'O' type brush. The eggs were transferred to 18 × 13 × 4 cm-size enamel trays containing 500 ml of water for larval hatching and larvae were fed with brewer's yeast and dog biscuits. Pupae were transferred to plastic jars containing tap water which was placed in wooden mosquito cage (90 × 90 × 90 cm) where adults emerged. They were maintained and reared in the laboratory as per the method of Kamaraj et al. (2008).

Extraction of extracellular bio metabolites from actinobacteria

The pure actinobacteria isolates were inoculated into a 500 ml conical flask containing 200 ml of starch casein broth and incubated at 28 ± 2 °C in a shaker incubator for 7 days. The cell free culture filtrates (supernatant) were taken and dissolved in equal volume of ethyl acetate and mixed properly for 1 h in a separating funnel. The solvent extract was removed, dried properly, dissolved in DMSO and was used for larvicidal bioassay.

Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity by using the standard method (WHO 1996). Twenty five numbers of third instar larvae/pupae (*An. stephensi* and *Ae. aegypti*) were introduced into wax coated paper cups containing 125 ml of dechlorinated tap water and 1 ml of un-inoculated DMSO broth. At each tested concentration, one to five different concentrations were made for each actinobacteria species. The control mortalities were corrected by using Abbott's (1925) formula.

Corrected mortality

$$= \frac{\text{Observed mortality in treatment} - \text{Observed mortality}}{100 - \text{Control mortality}} \times 100$$

Percentage mortality

$$= \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/Pupae introduced}} \times 100$$

The LC₅₀ and LC₉₀ were calculated from toxicity data using probit analysis (Finney 1971).

Larvicidal effect of potent actinobacteria on *An. stephensi* and *Ae. aegypti*

During preliminary screening, the potent larvicidal actinobacteria was selected for further study. The larvae of *An. stephensi* and *Ae. aegypti* were collected and the 3rd instar larvae were used for bioassay tests. Totally 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28 ± 2 °C). The control was maintained with dechlorinated tap water. For bioassay tests, each 20 larvae were maintained in 249 ml of water. Different concentrations (10, 20, 30, 40 and 50 ppm) of actinobacterial extract were added. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates.

Characterisation and identification of actinobacteria

The selected actinobacteria showing larvicidal activity were further identified by the characteristics of colony morphology, microscopic observations by Cover slip culture technique and biochemical properties and identified up to generic level by the standard methods (Pridham et al. 1958; Shirling and Gottlieb 1996). The isolates from *Streptomyces fungicidicus*, *S. griseus*, *S. albus*, *S. alboflavus* and *S. rochei* that exhibited total mortality (100 %) were identified to species level based on morphological, cultural and physiological characteristics.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀ and LC₉₀, and other statistics at 95 % fiducial limits of upper confidence limit (UCL), lower confidence limit (LCL) and Chi-square values were calculated using the SPSS 16.0 version (Statistical software package) to find the regression equation values (Reddy et al. 1992). Results with *p* < 0.05 were considered to be statistically significant.

Results and discussion

Actinobacteria have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Biological control or 'biocontrol' is the use of natural enemies to deal with mosquito populations. There are several types of biological control including the direct introduction of parasites, pathogen and predators to target mosquitoes (Kenneth 1995) or by using the dead spores of varieties of the natural soil bacteria and actinomycetes which used to interfere in the digestion systems of larvae. Actinobacteria are a group of gram positive organisms producing a number of antibiotics and enzymes. In addition to antibiotics they also produce some biologically active secondary metabolites. These metabolites are found to be toxic to mosquito larvae. Hence control of vector borne disease is becoming difficult due to the increasing resistance of mosquitoes to insecticides (Balaraman 1995).

In this study, totally, 30 actinobacterial isolates including white, grey, brown, pink and yellow colour colonies were isolated from soil samples of Parangipettai mangrove ecosystem. Among them, 21 isolates showed larvicidal activity against *Ae. aegypti* and *An. stephensi* mosquito larvae, particularly, five isolates *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* were more toxic to the late third instar larva of *Ae. aegypti* and *An. stephensi*. The present study revealed that the larvicidal and pupicidal activities of some marine actinobacteria at low concentrations and short exposure time. These crude extracts have potential for the development of new and safe control products for *Ae. aegypti* and *An. stephensi*. As naturally occurring insecticides, these marine actinobacteria-derived materials could be useful as an alternative for synthetic insecticides controlling field populations of *Aedes* and *Anaphelos* spp. The genus and species of strains with good larvicidal and pupicidal activities against *Ae. aegypti* and *An. stephensi* were identified as *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* using cell wall composition analysis, morphological characteristics studies, nonomura key and Bergey's manual of determinative bacteriology (Table 1).

Table 1 Morphological characteristics of potential five marine actinobacteria

Characteristics	<i>S. fungicidicus</i>	<i>S. griseus</i>	<i>S. albus</i>	<i>S. rochei</i>	<i>S. alboflavus</i>
DAP type	L-DAP	L-DAP	L-DAP	L-DAP	L-DAP
Spore chains Rectiflexibiles	—	+	—	+	+
Spore chain Spirales	+	—	+	—	—
Spore mass color	Buff	Gray	Gray	White	White
Substrate mycelia color	White	Green	Brown	White	Violet
Diffusible pigment produced	—	—	Brown	—	—
Melanin on tyrosine agar	—	—	—	—	—
Antibiosis					
<i>Bacillus subtilis</i>	+	+	+	+	—
<i>Micrococcus luteus</i>	+	+	+	+	—
<i>Candida albicans</i>	—	—	—	—	+
<i>Saccharomyces cerevisiae</i>	—	—	—	—	+
<i>Streptomyces murinus</i>	—	+	+	+	—
<i>Aspergillus niger</i>	—	—	—	—	+
Enzymatic activity					
Lecithinase activity	+	—	—	—	+
Lipolysis	+	+	+	+	—
Pectin hydrolysis	—	+	—	—	—
Nitrate reduction	+	+	—	—	—
H ₂ S production	+	+	+	+	+
Hippurate hydrolysis	—	—	—	—	—
Elastin degradation	+	+	+	+	+
Xanthine degradation	+	+	+	+	+
Arbutin degradation	+	+	+	+	+
Sensitivity assay					
Neomycin (50 µg/ml)	—	—	—	—	—
Rifampicin (50 µg/ml)	+	+	+	+	+
Oleandomycin (100 µg/ml)	—	+	+	—	—
Penicillin G (10 i.u.)	+	+	+	+	+
Growth at 45 °C	+	—	+	+	+
NaCl (7 % w/v) growth	+	+	+	+	+
NaN ₃ (0.01 % w/v) growth	+	—	+	+	+
Phenol (0.1 % w/v) growth	+	+	—	+	+
Potassium tellurite (0.001 % w/v) growth	+	+	+	+	+
Thallos acetate (0.001 % w/v) growth	+	+	—	+	+
Utilization test					
DL- α -Amino-n-butyric acid	—	—	—	—	+
L-Cysteine	—	+	—	+	+
L-Valine utilization	—	—	—	—	—
L-Phenylalanine utilization	—	+	—	+	+
L-Histidine	+	+	+	+	+
L-Hydroxyproline	—	—	+	—	—
Sucrose	+	—	—	+	—
Meso-Inositol	+	—	—	+	—
Mannitol	+	+	+	+	—
L-Rhamnose	+	+	—	+	—
Raffinose	+	—	—	+	—
D-Melezitose	+	+	+	+	+
Adonitol	—	+	+	—	+
D-Melibiose	+	—	+	+	—
Dextran	+	+	—	+	—
Xylitol	+	—	—	+	—

+ denotes presence, — denotes absence

Table 2 Screening the biological effects of actinobacteria secondary metabolites against *Ae. aegypti*

Sample	Larval mortality (%)	Larval duration	% of pupation	Pupal mortality (%)	Pupal duration	Total mortality (%)
<i>S. fungicidicus</i>	100.0	–	–	–	–	100.0
<i>S. griseus</i>	100.0	–	–	–	–	100.0
<i>S. albus</i>	100.0	–	–	–	–	100.0
<i>S. rochei</i>	100.0	–	–	–	–	100.0
<i>S. alboflavus</i>	50.0	5.2 ± 0.55	33.3	100.0	–	100.0

Table 3 Screening the biological effects of actinobacteria secondary metabolites against *An. stephensi*

Sample	Larval mortality (%)	Larval duration	% of pupation	Pupal mortality (%)	Pupal duration	Total mortality (%)
<i>S. fungicidicus</i>	100.0	–	–	–	–	100.0
<i>S. griseus</i>	100.0	–	–	–	–	100.0
<i>S. albus</i>	100.0	–	–	–	–	100.0
<i>S. rochei</i>	100.0	–	–	–	–	100.0
<i>S. alboflavus</i>	66.7	5.7 ± 0.80	33.3	100.0	1.5 ± 0.70	100.0

Table 4 Larvicidal activity of crude extracts of *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* against third instar larvae of *Ae. aegypti* and *An. stephensi*

Sample	Mosquito species	Percentage adult mortality ± SD
<i>S. fungicidicus</i>	<i>Ae. aegypti</i>	65 ± 2.251
	<i>An. stephensi</i>	72 ± 2.244
<i>S. griseus</i>	<i>Ae. aegypti</i>	70 ± 2.260
	<i>An. stephensi</i>	75 ± 2.588
<i>S. albus</i>	<i>Ae. aegypti</i>	78 ± 2.598
	<i>An. stephensi</i>	82 ± 2.925
<i>S. rochei</i>	<i>Ae. aegypti</i>	71 ± 2.244
	<i>An. stephensi</i>	72 ± 2.244
<i>S. alboflavus</i>	<i>Ae. aegypti</i>	100 ± 0.000
	<i>An. stephensi</i>	100 ± 0.000

Dhanasekaran et al. (2010) reported that among 30 isolates of actinobacteria, only 23 isolates showed anti larval activity against *Anopheles* mosquitoes, and of the 23 isolates, only four isolates showed 100 % inhibition. Based on the morphological characteristics, the actinobacteria were identified as *Streptomyces* sp., *Streptosporangium* sp., and *Micropolyspora* sp. Similar type of work has been reported by many workers using *B. thuriangensis*

(Balaraman et al. 1979, 1983a, b; Manonmani and Balaraman 2001), *B. sphaericus* (Rajendran et al. 1991; Balaraman 1995), *B. thuriangensis* (Prabhakaran et al. 2000), *B. subtilis*, *B. thuringiensis*, *B. sphaericus* and *B. cereus* (Balakrishnan et al. 2013), fungus *Trichoderma viridae* (Geetha and Balaraman 2001) and actinobacteria (Dhanasekaran et al. 2010; Vijayakumar et al. 2007). The insect mortality may be due to the secretion of bioactive materials of actinobacteria against *Culex* mosquito species. This work has been reported by many researchers (Willoughby et al. 1987; Herbert 2010; Vijayakumar et al. 2010; El-Khawagh et al. 2011).

Based on the antimicrobial activity, *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* were retained and used for the screening of larvicidal and pupicidal activity against *Ae. aegypti* and *An. stephensi*. The obtained data exhibited that the isolates have lethal effects. Complete mortality of treated larvae with secondary metabolites of *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* was recorded while the other isolates cause mortalities ranged from 66.7 to 50.0 % (Tables 2 and 3). The crude extracts of marine actinobacteria often attributed to the complex mixture of active compounds. Larvicidal activities of crude extracts of five marine actinobacteria are presented in Table 4.

Table 5 LC₅₀, LC₉₀, and other statistical analyses of different crude extracts against third instar larvae of *Ae. aegypti* and *An. stephensi*

Sample	Mosquito species	LC ₅₀ ± SE (ppm)	(UCL–LCL)	LC ₉₀ ± SE (ppm)	95 % Fiducial limits (UCL–LCL)	χ ² (df = 4)
<i>S. alboflavus</i>	<i>Ae. aegypti</i>	1.48 ± 0.09	1.30–1.69	3.33 ± 0.22	2.96–3.85	Y = 8.63 + 2.03X
	<i>An. stephensi</i>	1.30 ± 0.09	1.11–1.49	3.13 ± 0.21	2.77–3.65	Y = 9.81 + 1.90X

LC₅₀ lethal concentration that kills 50 % of the exposed larvae, LC₉₀ lethal concentration that kills 90 % of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, χ² Chi-square, df degree of freedom significant at *p* < 0.05 level

The present results showed that, the extract of *S. alboflavus* was tested against the larvae of *Ae. aegypti* (LC_{50} 1.48 ± 0.09 and LC_{90} 3.33 ± 0.22) and *An. stephensi* (LC_{50} 1.30 ± 0.09 and LC_{90} 3.13 ± 0.21). The Chi square value was significant at $p < 0.05$ level. The mortality values were significantly greater than the values of control. Based on the preliminary screening results, 100 % larval mortality obtained crude extracts alone were selected and subjected to bioassay for larvicidal activity against *Ae. aegypti* and *An. stephensi*. The present observation showed that all extracts showed moderate significant toxicity while the crude extract of *S. alboflavus* caused 100 % mortality against the larvae of *Ae. aegypti* and *An. stephensi* in 24 h incubation (Table 5). The insecticidal activity of tetranectin (Ando 1983), avermectins (Pampiglione et al. 1985), faerifungin (Anonymous 1990), macrotetrolides (Zizka et al. 1989) and flavonoids (Rao et al. 1990) was reported, respectively, by *Streptomyces aureus*, *S. avermitilis*, *S. albidum*, *S. griseus*, and *Streptomyces* sp., and the larvicidal activity of *Micromonospora*, *Actinomadura*, *Actinoplanes*, *Micropolyspora*, *Nocardioopsis*, *Oerskonina*, *Thermomonospora*, *Sreptovercillium*, and *Chainia* was also reported. The metabolites of fungi and actinomycetes were studied against the ovicidal, larvicidal, and adulticidal activities of *C. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*. The metabolites of three actinomycetes have ovicidal activity, 35 actinomycetes have larvicidal activity, and no activity was shown against adults. Two *Streptomyces* sp. and one from *Paecilomyces* sp. were highly active with LC_{50} value of 1–3 μ l/ml (Vijayan and Balaraman 1991).

The percentages of third instar larvae/pupa of *Ae. aegypti* and *An. stephensi* with crude extracts of *S. fungicidicus*, *S. griseus*, *S. albus*, *S. rochei* and *S. alboflavus* are presented in Table 6. The crude extract of *S. alboflavus* exerted 100 % mortality at 50, and 10 ppm, a very high hatchability (100 ± 0.0 , 12 ± 1.18 and 10 ± 1.22 respectively), was recorded against third instar larvae of *Ae. aegypti* and *An. stephensi*. The crude extract of *S. alboflavus* exerted 100 % mortality at 40, 50 and at 10 ppm, a very high hatchability (100 ± 0.0 , 38 ± 1.72 and 23 ± 1.20 respectively), was recorded against pupae of *Ae. aegypti* and *An. stephensi*.

Recent studies have indicated that spinosad, a mixture of two tetracyclic macrolide compounds produced during the fermentation of a soil actinomycete, may be suitable for controlling a number of medically important mosquito species, including the dengue vector, *A. aegypti* (Antonio et al. 2009). A new antibiotic ichthyomycin produced by *Streptomyces* sp. strain 1107 was tested for its toxicity and effect on the ultra structure of tissues of larvae of *C. pipiens autogenicus*. The mortality of larvae after 24-h exposure was 90, 30, 0, and 0 %, and after 48 h was 100, 100, 87 and 0 %, respectively (Nair et al. 1989; Zizka et al.

Table 6 Screening the biological effects of different marine actinobacteria extracts against mosquito larvae of *Ae. aegypti* and *An. stephensi*

Sample	Mosquito species	Percentage of third instar larvae $\pm$ SD					Percentage of pupae $\pm$ SD					Control				
		Concentration (ppm)					Concentration (ppm)									
		10	20	30	40	50	10	20	30	40	50					
<i>S. fungicidicus</i>	<i>Ae. aegypti</i>	40 $\pm$ 1.10	42 $\pm$ 1.62	42 $\pm$ 1.62	67 $\pm$ 1.72	96 $\pm$ 1.78	12 $\pm$ 1.18	16 $\pm$ 1.12	32 $\pm$ 1.26	88 $\pm$ 1.17	92 $\pm$ 1.26	98 $\pm$ 0.0				
	<i>An. stephensi</i>	27 $\pm$ 1.30	32 $\pm$ 1.26	34 $\pm$ 1.76	69 $\pm$ 2.72	98 $\pm$ 1.32	10 $\pm$ 1.22	24 $\pm$ 1.78	40 $\pm$ 1.10	85 $\pm$ 1.64	81 $\pm$ 1.07	97 $\pm$ 0.0				
<i>S. griseus</i>	<i>Ae. aegypti</i>	16 $\pm$ 1.12	50 $\pm$ 2.60	52 $\pm$ 1.74	76 $\pm$ 1.62	71 $\pm$ 2.11	23 $\pm$ 1.20	27 $\pm$ 1.30	16 $\pm$ 1.12	72 $\pm$ 1.41	88 $\pm$ 1.17	97 $\pm$ 0.0				
	<i>An. stephensi</i>	32 $\pm$ 1.26	56 $\pm$ 1.62	48 $\pm$ 1.26	61 $\pm$ 1.76	80 $\pm$ 2.64	27 $\pm$ 1.30	23 $\pm$ 1.20	27 $\pm$ 1.30	76 $\pm$ 1.62	76 $\pm$ 1.62	98 $\pm$ 0.0				
<i>S. albus</i>	<i>Ae. aegypti</i>	30 $\pm$ 1.70	27 $\pm$ 1.30	31 $\pm$ 1.96	72 $\pm$ 1.41	90 $\pm$ 1.74	32 $\pm$ 1.26	30 $\pm$ 1.70	34 $\pm$ 1.76	84 $\pm$ 1.21	72 $\pm$ 1.41	96 $\pm$ 0.0				
	<i>An. stephensi</i>	16 $\pm$ 1.12	23 $\pm$ 1.20	38 $\pm$ 1.72	67 $\pm$ 1.72	84 $\pm$ 1.96	34 $\pm$ 1.76	32 $\pm$ 1.26	30 $\pm$ 1.70	80 $\pm$ 2.64	81 $\pm$ 1.07	98 $\pm$ 0.0				
<i>S. rochei</i>	<i>Ae. aegypti</i>	12 $\pm$ 1.18	23 $\pm$ 1.20	24 $\pm$ 1.78	50 $\pm$ 2.11	98 $\pm$ 1.32	42 $\pm$ 1.62	16 $\pm$ 1.12	50 $\pm$ 2.11	98 $\pm$ 1.32	69 $\pm$ 2.72	97 $\pm$ 0.0				
	<i>An. stephensi</i>	11 $\pm$ 1.25	32 $\pm$ 1.26	34 $\pm$ 1.76	56 $\pm$ 1.62	92 $\pm$ 1.32	30 $\pm$ 1.70	11 $\pm$ 1.25	67 $\pm$ 1.72	90 $\pm$ 1.74	97 $\pm$ 0.0	99 $\pm$ 0.0				
<i>S. alboflavus</i>	<i>Ae. aegypti</i>	10 $\pm$ 1.22	60 $\pm$ 1.20	76 $\pm$ 1.76	85 $\pm$ 1.64	100 $\pm$ 0.0	23 $\pm$ 1.20	67 $\pm$ 1.72	71 $\pm$ 1.72	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0				
	<i>An. stephensi</i>	12 $\pm$ 1.18	64 $\pm$ 1.12	82 $\pm$ 1.14	71 $\pm$ 1.72	100 $\pm$ 0.0	38 $\pm$ 1.72	76 $\pm$ 1.62	84 $\pm$ 1.96	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0				
$\pm$ SD standard deviation																

1991). The ovicidal activity of 21 hyphomycete fungi species against *A. aegypti* was reported. The reported fungi were *Paecilomyces carneus*, *P. lilacinus*, *P. marquandii*, *Isaria fumosorosea*, *Metarrhizium anisopliae*, *Penicillium* sp., *Beauveria bassiana*, and *Evlachovaea kintrischica*. These are the first kind of results to show the effects of entomopathogenic fungi against the eggs of *A. aegypti*, and they have potential as control agents of this vector (Luz et al. 2007). The screening results suggest that the crude extract of *S. albobflavus* is promising in mosquito control. Further studies on isolation of bioactive fraction/constituent may provide futuristic lead products for field application of mosquito control.

Conclusion

In conclusion, the present investigation clearly reveals the biodiversity and distribution of actinomycetes in different soil and their antilarval potentials of selected isolates. Actinobacterial metabolites showed more significant effect against *An. stephensi* and *Ae. aegypti* larvae. So it can be used as an alternative source of mosquito larvicides. Further studies are needed for identification of the active compounds that can be used in broad spectrum for controlling mosquitoes and also for the determination of the mode of action of these compounds. This investigation explores the importance of larvicidal actinomycetes as valuable resource for the discovery of novel insecticidal molecules.

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References

- Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–266
- Amer A, Mehlhorn H (2006a) Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res* 99:466–472
- Amer A, Mehlhorn H (2006b) Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res* 99:478–490
- Ando K (1983) How to discover new antibiotics for insecticidal use. In: Takahashi T, Yoshioka H, Misato T, Matusunaka S (eds) *Pesticide chemistry: human welfare and the environment. Natural products*, Pergamon, New York, pp 253–259
- Anonymous (1990) Biologically active KSB-1939L3 compound and its production-pesticide with insecticide and acaricide activity production by *Streptomyces* sp. culture. *Biotechnol Abstr* 9(19):58, Japan Patent, No. 273961, 1988
- Antonio GE, Sánchez D, Williams T, Marina CF (2009) Paradoxical effects of sublethal exposure to the naturally derived insecticide spinosad in the dengue vector mosquito, *Aedes aegypti*. *Pest Manag Sci* 65(3):323–326
- Balakrishnan S, Indira K, Srinivasan M (2013) Mosquitocidal properties of *Bacillus* species isolated from mangroves of Vellar estuary, Southeast coast of India. *J Parasit Dis*. doi:10.1007/s12639-013-0371-9
- Balakrishnan S, Srinivasan M, Mohanraj J (2014) Biosynthesis of silver nanoparticles from mangrove plant (*Avicennia marina*) extract and their potential mosquito larvicidal property. *J Parasit Dis*. doi:10.1007/s12639-014-0621-5
- Balaraman K (1995) Mosquito control potential of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sophaeicus*. *ICMR Bull* 25:45
- Balaraman K, Rao USB, Rajagopalan PK (1979) Isolation of *Metarrhizium anisopliae*, *Beauveria tenella* and *Fusarium oxysporum* (Deuteromycetes) and their pathogenicity to *Culex fatigans* and *Anopheles stephensi*. *Ind J Med Res* 70:718
- Balaraman K, Balasubramanian M, Manonmani LM (1983a) *Bacillus thuringiensis* H-14 (VCRC B-17) formulation as mosquito larvicide. *Indian J Med Res* 77:33
- Balaraman K, Balasubramanian M, Jambulingam P (1983b) Field trial of *Bacillus thuringiensis* H-14 (VCRC B-17) against *Culex* and *Anopheles* larvae. *Indian J Med Res* 77:38
- Berdy J (2005) Bioactive microbial metabolites—a personal view. *J Antibiot* 58:1–26
- Bream AS, Ghazal SA, El-Aziz ZKA, Ibrahim SY (2001) Insecticidal activity of selected actinomycetes strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent* 66(2a):503–544
- Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. *J Antibiot* 62:5–16
- Dhanasekaran D, Sakthi V, Thajuddin N, Panneerselvam A (2010) Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria. *Int J Appl Biol Pharm Technol* 1:374–381
- Dhiman RC, Pahwa S, Dhillon GP, Dash AP (2010) Climate change and threat of vector-borne diseases in India: are we prepared? *Parasitol Res* 106(4):763–773
- El-Khawagh MA, Hamadah KhSh, El-Sheikh TM (2011) The insecticidal activity of actinomycete metabolites, against the mosquito *Culex pipiens*. *Egypt Acad J Biol Sci* 4(1):103–113
- Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge, pp 76–80
- Gadelhak GG, El-Tarabily KhA, Al-Kaabi FK (2005) Insect control using chitinolytic soil actinomycetes as biocontrol agents. *Int J Agric Biol* 7(4):627–633
- Geetha I, Balaraman K (2001) Biomass and blastospore production in *Beauveria bassiana* (Bals) Vuill. as influenced by media components. *J Biol Control* 15(1):93
- Geetha I, Prabakaran G, Paily KP, Manonmani AM, Balaraman K (2007) Characterisation of three mosquitocidal *Bacillus* strains isolated from mangrove forest. *Biol Control* 42:34–40
- Herbert AK (2010) The spinosyn family of insecticides: realizing the potential of natural products research. *J Antibiot* 63:101–111
- Hopwood DA, Buttner MJ, Kieser T, Charter KF (2000) Antibiotic production by Streptomyces. *Pract Streptomyces Genet* 1:1–42
- Hussain AA, Mostafa SA, Ghazal SA, Ibrahim SY (2002) Studies on antifungal antibiotic and bioinsecticidal activities of some actinomycete isolates. *Afr J Mycol Biotechnol* 10:63–80
- Jensen PR, Mincer TJ, Williams PG, Fenical W (2005) Marine actinomycete diversity and natural product discovery. *Antonie Van Leeuwenhoek* 87:43–48
- Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G (2008) Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex*

- tritaeniorhynchus* Giles (Diptera: Culicidae). Parasitol Res 104(5):1163–1171
- Karthik L, Kumar G, Bhaskara Rao KV (2010a) Comparison of methods and screening of biosurfactant producing marine actinobacteria isolated from Nicobar marine sediment. IIOAB J 1(2):221–227
- Karthik L, Kumar G, Bhaskara Rao KV (2010b) Diversity of marine actinomycetes from Nicobar marine sediments and its antifungal activity. Int J Pharm Sci 2(1):199–203
- Kenneth TM (1995) Part 111: Interaction 31. A comparative Study of the ability of fish to catch mosquito larvae. Rice-fish culture in china. International Development Research Centre. ISBN 1-55250-313-5
- Leonard GC, Julius JM (2000) Review biopesticides: a review of their action, applications and efficacy. Pest Manag Sci 56:651–676
- Luz C, Tai MH, Santos AH, Rocha LF, Albernaz DA, Silva HH (2007) Ovicidal activity of entomopathogenic hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) under laboratory conditions. J Med Entomol 44(5):799–804
- Manonmani AM, Balaraman K (2001) A high mosquitocidal *Bacillus thuringiensis* var. *thompsoni*. Curr Sci 80:779
- Mehlhorn H, Schmahl G, Schmidt J (2005) Extract of the seeds of the plant *Vitex agnus castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. Parasitol Res 95(5):363–365
- Nadson GA (1903) Microorganisms as geological agents (Russian). From the Works of the investigation of the Slavian Mineral Waters, St. Petersburg
- Nair MG, Putnam AR, Mishra SK, Mulks MH, Taft WH, Keller JE, Miller JR, Zhu PP, Meinhardt JD, Lynn DG (1989) Faeriefungin: a new broad-spectrum antibiotic from *Streptomyces griseus* var. *autotrophicus*. J Nat Prod 52(4):797–809
- Pampiglione S, Majori G, Petrangeli G, Romi R (1985) Avermectins, MK-933 and MK-936, for mosquito control. Trans R Soc Trop Med Hyg 79:797–799
- Prabhakaran G, Padmanaban V, Balaraman K (2000) Comparative efficacy of UV screens in protecting the activity of a *Bacillus thuringiensis* formulation. J Biol Control 14:63
- Pridham TG, Hesseltn CW, Benedict RG (1958) A guide for the classification of Streptomycetes according to selected groups. Placement of strains in morphological sections. App Microbiol 6:52
- Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G (2009a) Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 104(6):1365–1372
- Rahuman AA, Bagavan A, Kamaraj C, Vadivelu M, Zahir AA, Elango G, Pandiyan G (2009b) Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 104(3):637–643
- Rajendran G, Sabesan S, Kuppusamy K, Balaraman K (1991) Evaluation of a *Bacillus sphaericus* formulation, Sphaeri fix for the control of *Mansoniodes* mosquitoes. Entomon 16:213
- Rajesh K, Dhanasekaran D, Tyagi BK (2014) Mosquito survey and larvicidal activity of actinobacterial isolates against *Culex* larvae (Diptera: Culicidae). J Saudi Soc Agric Sci. doi: 10.1016/j.jssas.2013.08.001
- Ramanathan A, Shanmugam V, Raghuchander T, Samiyappan R (2002) Induction of systematic resistance in ragi against blast disease by *Pseudomonas fluorescens*. Ann Plant Prot Sci 10:313–318
- Rao KV, Chattopadhyay SK, Reddy GC (1990) Flavonoides with mosquito larval toxicity—tangeratin, daidzein and genistein crystal production, isolation and purification; *Streptomyces* spp. Culture. Insecticide. J Agric Food Chem 38:1427–1430
- Reddy PJ, Krishna D, Murthy US, Jamil K (1992) A microcomputer FORTRAN program for rapid determination of lethal concentration of biocides in mosquito control. CABIOS 8:209–213
- Shi YF (2000) Advances of insecticidal microorganisms. Plant Prot 26:32–34
- Shirling EB, Gottlieb D (1996) Methods for characterization of *Streptomyces* species. Int J Syst Evol Bacteriol 16:312–340
- Sundarapandian S, Sundaram MD, Tholkappian P, Balasubramanian V (2002) Mosquitocidal properties of indigenous fungi and actinomycetes against *Culex quinquefasciatus* Say. J Biol Control 16:89–91
- Vijayakumar R, Muthukumar C, Thajuddin N, Panneerselvam A (2007) Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. Actinomycetologica 21:59–65
- Vijayakumar R, Murugesan S, Cholarajan A, Sakthi V (2010) Larvicidal potentiality of marine actinomycetes isolated from Muthupet Mangrove, Tamilnadu, India. Int J Microbiol Res 1(3):179–183
- Vijayan V, Balaraman K (1991) Metabolites of fungi and actinomycetes active against mosquito larvae. Indian J Med Res 93:115–117
- Weyland H (1969) Actinomycetes in North Sea and Atlantic Ocean sediments. Nature 23:858
- WHO (1996) Report of the WHO informal consultation on the evaluation on the testing of insecticides CTD/WHO PES/IC/96.1. p 69
- Willoughby OH, Hoy M, Conley J (1987) Toxicity of pesticides to western predatory mite. Calif Agric 41:12–14
- Xie MJ (1998) The perspective of the studies on microbial insecticides. J Liaoning Norm Univ Nat Sci 21:326–329
- Zhang DF (1996) Recent developments in research and utilization of microorganisms. J Agric Sci 24(1):44–46
- Zizka Z, Weiser J, Blumauerova M, Jizba J (1989) Ultra structural effects of macroterrolides of *Streptomyces grieseus* LKS-1 in tissues of *Culex pipiens* larvae—monactin, dinactin, triactin and nonactin preparation; insecticide activity. Cytobios 58:85–91
- Zizka Z, Weiser J, Popov KB (1991) Effect of ichthyomycin on the ultra structure of tissues of *Culex pipiens*. Cytobios 65(260):31–37