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Larvicidal, ovicidal and repellent activities of *Streptomyces enissocaesilis* (S12-17) isolated from Western Ghats of Tamil Nadu, India

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Abstract
The aim of the present study was to isolate *Streptomyces enissocaesilis* (S12-17) from the soil sample collected from Venganayakkan region in Western Ghats of Tamil Nadu, India and check for its mosquito larvicidal, ovicidal and repellent activities. Isolation was performed by serial dilution using spread plate technique. Extract from *S. enissocaesilis* was checked for mosqutiocidal activity by using standard methods. Ethyl acetate and methanol extract presented good larvicidal activity with LC_{50} and LC_{90} values of 207.18, 635.21 and 282.30, 894.13 respectively, at 500ppm concentration against *Cx. quinquefasciatus*. Ovicidal activity of ethylacetate crude extract was 100% and methanol extract was 89% at 500ppm concentration against *Cx. quinquefasciatus*. The ethyl acetate extract gave repellency of 127.2 min and the methanol extract gave repellency 80.6 min at 5mg/cm^2 concentration against *Cx. quinquefasciatus*. Both the extracts was harmless to tested non-target organism *P. reticulata* The active strain S12-17 was identified using 16S rRNA sequencing; phylogenetic tree was constructed using MEGA4 software; the sequence was submitted in the GenBank (Accession No. KT827105.1). The present study clearly showed that the extracts from *S. enissocaesilis* were effective in controlling mosquito life stages. The mosquitocidal activities deserved in this study, encourage the search of new insecticides having better properties.

Keywords: *Cx. quinquefasciatus*, S. *enissocaesilis*, Repellent, Ovicidal, Larvicidal, *P. reticulate*

1. Introduction
Vector borne diseases are impediments to the health of the global population. Mosquitoes are one of the major vectors in India, they are responsible for transmitting a number of vector borne diseases (WHO; Muthu *et al.*) [1, 2].Vector-borne diseases like encephalitis, malaria, chikungunya, dengue, West Nile virus, and yellow fever cause significant damage to the economic, commercial and labor outputs (El-Sheikh *et al.*; Fradin and Day) [3, 4]. *Culex quinquefasciatus* is an urban vector in india which is responsible for transmitting nematode worm *Wuchereria bancrofti* responsible for filarial disease. About 23 million cases of symptomatic filariasis, 31 million microfilaremics and 473 million individuals potentially are at risk of infection (Rahuman *et al.*; Holder) [5, 6]. In 2008 the world health organization reported that more than 128 million people over 78 countries and 25 million peoples in India were infected by lymphatic filarial disease (Muthu *et al.*; WHO; NICD; Reegan *et al.*) [2, 7, 8, 9].
In recent years synthetic insecticides were used to control mosquitoes. Common synthetic insecticides such as pyrethroids, organophosphates, organochlorines and carbamates are used to kill mosquitoes in different stages. Increasing use of these chemical insecticides leads to deterioration of environment and public health with increased resistance to these substances (Tikar *et al.*; Llinás *et al.*; Mulyatno *et al.*; Shaalan *et al.*; Sutthanont *et al.*; Madhu *et al.*; Bayen) [10-16]. Nowadays governments and private organizations are looking forward to find new insecticidal agents from natural resources without any negative impacts (Gandhi *et al.*; Liu *et al.*; Cecilia *et al.*; Reegan *et al.*) [17-19, 9]. Microbes are important natural resources to find some active insecticidal molecules to control mosquitoes. Microbial compounds are good and natural alternatives to synthetic insecticides.
Actinomycetes are a group of bacteria responsible for producing large number of biologically active secondary metabolites (Ikeda *et al.*; Ganesan *et al.*; Valan Arasu *et al.*) [20-22], which are

very useful to control insect pests. Actinomycetes are Gram positive, aerobic, filamentous bacteria containing 55% of GC in their DNA (Ganesan *et al*.; Saravanakumar *et al*.) [21, 23]. More than 75-80% of the important secondary metabolites are isolated from *Streptomyces* sps. (Valan Arasu *et al*.) [24]. *Streptomyces enissocaesilis* isolated from the soil from Western Ghats in the present study was analyzed for its larvicidal, ovicidal and repellent activity against *Cx. quinquefasciatus*.

2. Materials and methods

2.1 Sample collection and isolation

Soil sample was collected from different places of Venganayakkan region of Western Ghats of Tamil Nadu, India. Collected sample was transported aseptically to the laboratory using sterile plastic containers. Isolation of *Streptomyces enissocaesilis* (S12-17) was performed by serial dilution using spread plate technique (Valanarasu *et al*.) [24]. The plates were incubated at 28 °C for 10-14 days.

2.2 Extraction of secondary metabolites

Streptomyces enissocaesilis (S12-17) was inoculated in Erlenmeyer flask containing 15 liters of ISP-2 (International Streptomyces Project No.2) medium and incubated at 28 °C for 10-14 days. The culture broth was separated by centrifuging at 10000 rpm for 15 min; the culture broth was mixed in equal volume of ethyl acetate and shaken vigorously and the pellet was soaked in the methanol. The solvent was separated by using separating funnel and it was concentrated by using vacuum rotary evaporator at 50 °C. The concentrated extract was transferred to a clean sterile vial and stored at -20 °C (Saravanakumar *et al*.) [23].

2.3 Biochemical and cultural characterization

Biochemical characterization was done using biochemical kit (KB014 HiAcinetobacter Identification Kit) according to the manufacturer's procedure. Cultural characterization of isolate S12-17 was performed using Bergey's manual of Systematic Bacteriology (Locci) [25]. Pure isolate was observed after 10 days of incubation at 28 °C. Various parameters such as pH, NaCl, temperature, media and growth duration were studied for the optimal growth of the isolate S12-17.

2.4 Antibiotic susceptibility test

Susceptibility of the isolate S12-17 was evaluated by disc diffusion method by using previously described method of Yao [26]. The isolate S12-17 was grown on slants of ISP-2 medium at 28 °C for 2-4 days. Spores of the active cultures were seeded into 5 ml sterile water and used within 1 hour. (Waksmans) [27]. Totally 37 antibiotics were used for the present study. The loaded and readymade antibiotics discs were placed on top of the solidified ISP-2 medium and left for 30 min at room temperature for diffusion. The plates were incubated at 28 °C for 2-3 days and zones of inhibition were recorded in millimeters.

2.5 Insect rearing

The larvae of *Cx. quinquefasciatus* were obtained from Entomology Research Institute, Loyola College, Chennai. Mosquito larvae were free from pathogens, insecticides or repellents and fed with dog biscuits and Brewer's yeast (3:2). Rearing conditions for mosquitoes were 75–85% relative humidity, 27±2 °C temperature and a photoperiod of 14±0.5 h. The F1 generation larvae were used for the experiment (Reegan *et al*.) [28].

2.6 Larvicidal bioassay

Larvicidal bioassay of the isolate S12-17 was evaluated by previously defined method of WHO [29], with slight modification. Test concentrations viz., 62.5,125, 250 and 500 ppm were prepared using DMSO (dimethyl sulfoxide) (249 ml water and 1ml DMSO) and five replicates were maintained for each concentration. Totally 20 third instar larvae of *Cx. quinquefasciatus* were used in each replication. DMSO and water served as control. After 24 h of exposure period the dead larvae were observed and percent mortality was calculated for each concentration using the following formula (a). Corrections for mortality were done using Abbot's formula [30], when control mortality was below 5% (b).

a. Percentage of Mortality

$$\frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \times 100$$

$$\frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \times 100$$

b. Corrected percentage of mortality

$$1 - \frac{n \text{ in T after treatment} \times 100}{n \text{ in C after treatment}} \times 100$$

Where n is the number of larvae, T is the number of treated larvae and C is the number of larvae in control. The corrected percentage mortality value for each concentration was considered to estimate LC_{50} and LC_{90} values using US EPAprobit analysis software (version 1.5).

2.7 Ovicidal Bioassay

Ovicidal bioassay was carried out by previously described method of Elango *et al*. [31], with slight modification. Twenty freshly laid eggs of *Cx. quinquefasciatus* were treated separately with the extracts at 62.5, 125, 250, and 500ppm concentrations. DMSO in water and water alone served as controls. Five replicates were maintained for each concentration. The ovicidal activity was assessed up to 120 h post treatment and thereafter control and treated eggs were observed under the microscope and photographed using stereo zooming microscope (Wild M7S TYP 308700, Switzerland). The non-hatched eggs with unopened opercula were counted in each treatment, and the percent mortality was calculated using the following formula and analysed in Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, USA:

a. Percentage of egg Mortality

$$\frac{\text{No. of unhatched eggs}}{\text{Total No. of eggs}} \times 100$$

2.8 Repellent Bioassay

Repellent bioassay was carried out using 2 to 6 days old adults. Hundred laboratory reared blood-starved adult female *Cx. quinquefasciatus* mosquitoes were introduced into separate laboratory cages (45 × 45 × 40 cm). The experiment was conducted at nighttime; the forearms of a human subject were washed, thoroughly rinsed, and allowed to dry before the application of the extract at 5, 2.5, and 1mg/cm2

concentrations. The *Streptomyces enissocaesilis* (S12-17) extracts were applied on the right upper forearm and remaining regions were covered with gloves. The arm was left undisturbed. The left arm served as control. N-N Diethyl benzamide (12%, w/w) was used as negative control. The mosquito bites were observed for three full minutes of every fifteen minutes. Protection time and confirmed bite were obtained. The experiment was replicated five times in separate cages, and different volunteer was used to test the repellency of the extract. The protection time of extract was calculated using previously described methods (Fradin and Day; Venkatachalam) [4, 32].

2.9 Effect of active extract on non-target organisms

Both the extract was studied for toxicity against non-target organisms by previously described method of Maheswaran and Ignacimuthu [33] against *P.reticulata* (predatory fish) was collected from pond of Fishery Research Institute, Chetpet, and Chennai, India. Ten replications were maintained individually. The fishes were exposed to different test concentrations of 62.5, 125, 250, and 500 ppm for extract and 0.5. 1.0, 1.5 and 2.0 ppm for compound. Azadirachtin and temephos were used as positive control with ten replicates along with ten controls. Moribund and abnormalities were observed upto 24 h exposure. After treatment the fishes were transferred to normal water and the post treatment effect was observed continuously for 15 days. The LC_{50} and LC_{90} values were obtained by probit analysis. Suitability index (SI) or fish safety factor (PSF) was calculated for each SI/PSF:

$$\frac{LC_{50} \text{ of non-target organism}}{LC_{50} \text{ of target vector species}}$$

2.10 DNA isolation and Molecular identification

The genomic DNA of active isolate *Streptomyces enissocaesilis* (S12-17) was extracted using the HipurA *Streptomyces* DNA purification kit-MB 527-50 pr (Himedia), according to the manufacturer's guidelines (http://himedialabs.com/TD/MB527.pdf). The following primers (27F-5'AGAGTTTGATCMTGGCTCAG3' and 1492R- 5' TACGGYTACCTTGTTACGACTT 3') were used to amplify the 16S ribosomal sequence from genomic DNA in thermal cycler (ep gradient eppendorf). The PCR cycle conditions were as follows: initial denaturation for 3 min at 94 °C, 35 cycles of denaturation 1 min at 94 °C, annealing at 54 °C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 7 min and finally held at 4°C. The PCR products were confirmed with 1% agarose gel electrophoresis stained by ethidium bromide (Farris) [34]. The confirmed PCR product was given for sequencing. It was carried out using dideoxy chain–termination method in applied Biosystems automated sequencer by Synergy Scientific Services.

2.11 Phylogenetic tree and species identification

The sequence of *Streptomyces enissocaesilis* (S12-17) was identified and compared to the reference species of actinomycetes closely related to the genomes in the database, using NCBI BLAST tool (http:www.ncbi.nlm.nih.gov/BLAST). Phylogenetic tree of isolate S12-17 was constructed using neighbor-joining method using MEGA4 software. The sequence was submitted to the GenBank, NCBI, USA (Saltau) [35].

3. Results

3.1 Isolation and characterization of *Streptomyces enissocaesilis*

The *Streptomyces enissocaesilis* S12-17 was isolated from Western Ghats of Tamil Nadu, India, and subcultured on AIA (Actinomycetes isolation agar) medium. ISP-2 (International *Streptomyces* Project No.2) medium was found to be very good base for the production of isolate *Streptomyces enissocaesilis* S12-17 with larvicidal properties. The isolate S12-17 was Gram positive and filamentous. Morphological characterizations are summarized in Table 1. Growth was inhibited at 70°C and 10% (100 grams/litre) NaCl. The temperature for growth of S12-4 ranged from 20-60°C and pH ranged from 6-12. Biochemical analysis was positive to acetoin, malonate, ONPG, mannitol, xylose, rhamnose and citrate.

3.2 PCR amplification and Molecular identification

The genomic DNA of the isolate S12-17 (*Streptomyces enissocaesilis*) (Accession No. KT827105.1) was runon 1% agarose gel stained with ethidium bromide. PCR product was sequenced and submitted to Genbank. The isolate S12-17 was 89% homologous to *Streptomyces enissocaesilis* strain NRRL B-16365 16S ribosomal RNA gene partial sequence (NR_115668.1). This similarity was also corroborated by phylogenetic tree constructed using MEGA4 software (Fig. 1).

3.3 Antibiotic susceptibility

Streptomyces enissocaesilis (S12-17) was susceptible to antibiotics such as,cephaloridine, amikacin, gentamycin, vancomycin, streptomycin, ketoconazole and doxycycline among the 37 antibiotics used (Table 2).

3.4 Larvicidal and ovicidal activity

The *S. enissocaesilis* isolate S12-17 presented good Larvicidal activity against *Cx. quinquefasciatus*. Table 2 shows the effective lethal concentrations of both extract (LC_{50} and LC_{90}) against mosquito larvae. The ethyl acetate and methanol extracts of isolate S12-17 showed 90% and 80%activity respectively against *Cx. quinquefasciatus* at 500 ppm concentration (Table 3). After 24 h treatment the larvae showed restless movement followed by death, whereas normal movement was observed in control. The ovicidal activity of extracts of isolate *Streptomyces enissocaesilis* S12-17 against *Cx. quinquefasciatus*is given in Fig 2. The ethyl acetate and methanol extracts of *Streptomyces enissocaesilis* were highly toxic to the eggs of *Cx. quinquefasciatus* with 100% and 89% activity respectively at 500ppm concentration. Hatchability of the egg was normal in solvent and water control. Ovicidal activity was observed as partially hatched eggs and dead larvae.

3.5 Repellent activity

The total protection time for both extracts of isolate S12-17 against *Cx. quinquefasciatus* was recorded by using standard skin repellent methods and the results are given in Table 4. The repellency of the extract depended on the concentration. In general both the extracts gave good repellent activity but ethyl acetate extract was better. The lowest concentration of ethyl acetate and methanol extracts gave 30.4min and 21.4min at 1mg/cm^2. But, the negative control N-N Diethyl benzamide 12% gave 115min at 1mg/cm2 concentration.

3.6 Toxicity of extracts against non-target organisms

The ethyl acetate and methanol extract was checked for its toxicity against a non-target organism *Poecilia reticulata*. The results are presented in table 5. *P. reticulata* showed least susceptibility with LC_{50} and LC_{90} values of 1009.26 and 3281.79ppm respectively. Suitability Index/Predatory Safety

Factor (SI/PSF) showed that the ethylacetate and methanol extract from *S. enissocaesilis* did not harm *poecilia reticulata* at tested concentrations (Table 6). Persistence and movement activity of *P. reticulata* was normal as in control during the exposure period to various doses of methanol extract.

Table 1: Morphological characterization of the active isolate of *Streptomyces enissocaesilis* (S12-17)

Medium	Characters					
	Aerial mycelium	**Substrate mycelium**	**Soluble pigment**	**Colony margin**	**Growth**	**Gram stain**
ISP-2	Grey	White	-	filaments	+++	+
ISP-4	Grey	White	-	filaments	+++	+
ISP-6	White	Yellow	-	filaments	+++	+
ISP-7	-	-	-	-	-	-
YPG	Grey	White	-	filaments	+++	+
MNGA	Grey	Grey	-	filaments	+++	+
M3	Grey	White	-	filaments	+++	+
AIA	Grey	White	-	filaments	+++	+
SCA	Grey	Grey	-	filaments	+++	+
BENNET	Grey	Grey	-	filaments	+++	+

Growth; -: no soluble pigment, Gram stain; +: positive; -: negative

Table 2: Antibiotic testing (in mm) against *Streptomyces enissocaesilis* S12-17

S. No.	Antibiotics	Zone of inhibition
1.	Cephaloridine (30mcg)	14
2.	Clavulanic acid (30mcg)	R
3.	Rifamycin (30mcg)	R
4.	cephalothin(30mcg)	R
5.	Ticarcittin (75mcg)	R
6.	Oxacillin (1mcg)	R
7.	Penicillin (10units)	R
8.	Amikacin (30mcg)	33
9.	Cefotaxime (30mcg)	R
10.	Gentamycin (10mcg)	22
11.	Ampicillin (25mcg)	R
12.	Norfloxacin (10mcg)	R
13.	Imipenem (10mcg)	R
14.	Vancomycin (30mcg)	31
15.	Polymyxin (300 units)	R
16.	Streptomycin (30mcg)	20
17.	Trimoxazole (25mcg)	R
18.	Ketoconazole (30mcg)	8
19.	Fluconazole (30mcg)	R
20.	Nalidixic acid (10mcg)	R
21.	Carbenicillin (100mcg)	R
22.	Spectinomycin	R
23.	Tetracyclie (10mcg)	R
24.	Aureomycin	R
25.	Actidione (30mcg)	R
26.	Neomycin (30mcg)	R
27.	Kanamycin (30mcg)	R
28.	Amoxicillin (30mcg)	R
29.	Erythromycin (15mcg)	R
30.	Ciprofloxacin (5mcg)	R
31.	Doxycycline (30mcg)	19
32	Nystatin (50mcg)	R
33.	Menicycline (30mcg)	R
34.	Linezoid (15mcg)	R
35.	Azithromycin (15mcg)	R
36.	Clarithromycin (15mcg)	R
37.	Cotromoxazole (25mcg)	R

R-Resistant

Table 3: Lethal concentration (in ppm) of isolate *Streptomyces enissocaesilis* (S12-17) extracts against the larvae of *Cx. quinquefasciatus.*

Extracts	Mosquito species	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)	95% Confidence limit		intercept±SE	slope±SE	χ^2
			LL	UL		LL	UL			
Cx. quinquefasciatus	Ethyl acetate	207.18	173.79	249.65	635.21	476.61	993.62	1.1±0.77	2.6±0.3	3.6*
	Methanol	282.30	234.98	52.53	894.13	637.44	1556.73	1.2±0.8	2.5±0.3	3.9*
	Azadirachtin	1.29	1.15	1.43	2.54	2.22	3.04	4.3 ± 0.4	4.5 ±0.1	0.7*
	Temephos	1.72	1.52	1.94	4.0	3.46	4.81	3.5 ± 0.3	4.1 ±0.1	4.0*

LC_{50} lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, LL lower limit (95% confidence limit), and UL upper limit (95% confidence limit).
$*P \leq 0.05$, level of significance of chi-square values.

Table 4: Complete protection time of the two solvent extracts of isolate *Streptomyces enissocaesilis* S12-17 against *Cx. quinquefasciatus.*

Extract	Concentration mg/cm2	Complete protection time (min)	
		Control	Treated
Ethyl Acetate	1	1.36±0.05	30.4±1.1
	2.5	1.14±0.05	62±0.5
	5	2±0.5	127.2±0.8
Methanol	1	1.14±0.1	21.4±1.6
	2.5	1.8±0.3	42.8±0.8
	5	1.14±0.1	80.6±0.8
N-N Diethyl benzamide 12%	1	0.49±0.1	115±1
	2.5	1±0.1	184.8±0.8
	5	1±0.6	298.4±0.8

Table 5: Toxicity of Ethylaceate and Methanol extract of *Streptomyces enissocaesilis* S12-17 against non-target organisms.

Treatment	*Poecilia reticulata*	
	LC_{50}	LC_{90}
Ethylacetate	1009.26	3281.79
Methanol	1009.26	3281.79
Azadirachtin	2.34	3.56
Temephos	1.74	2.35

Table 6: SI/PSF of non-target organisms with respect to the larval stages of *Cx. quinquefasciatus* exposed to active extract.

Treatment	Predatory species	*Cx.quinquefasciatus*
Ethylacetate	*Poecilia reticulata*	4.87
Methanol	*Poecilia reticulata*	3.57
Azadirachtin	*Poecilia reticulata*	1.81
Temephos	*Poecilia reticulata*	1.01

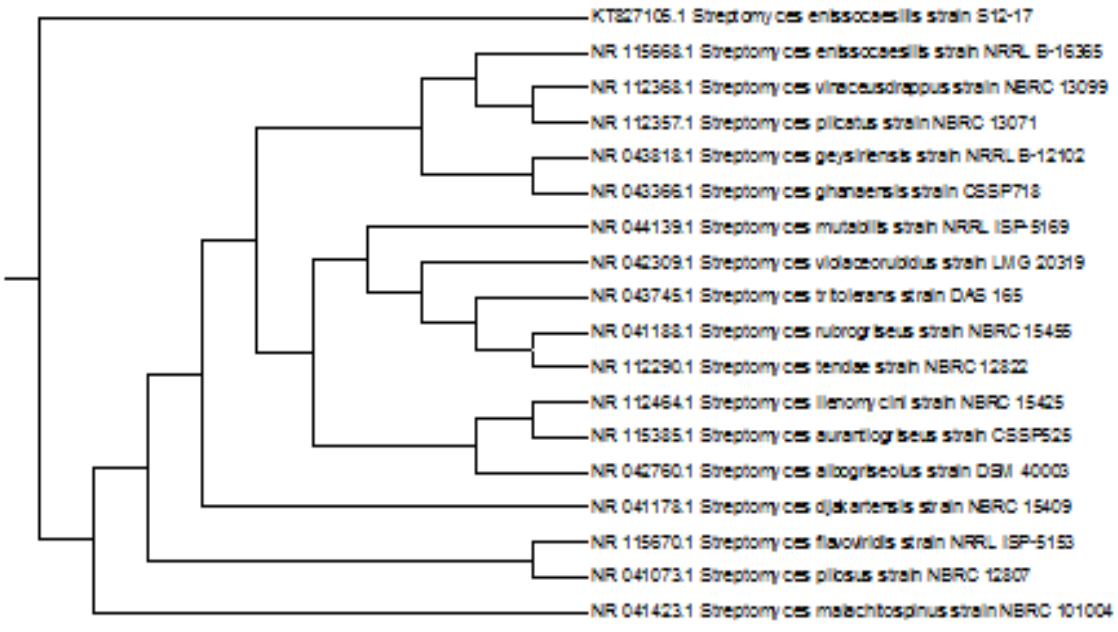

Fig 1: Phylogenetic tree indicating the taxonomic position of *Streptomyces enissocaesilis* isolate S12-17.

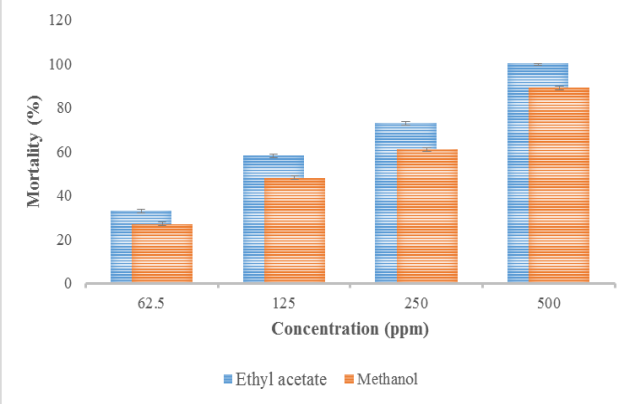

Fig 2: Percent ovicidal activity of *Streptomyces enissocaesilis* isolate S12-17Ethylacetate and methanol extracts against *Cx. quinquefasciatus*.

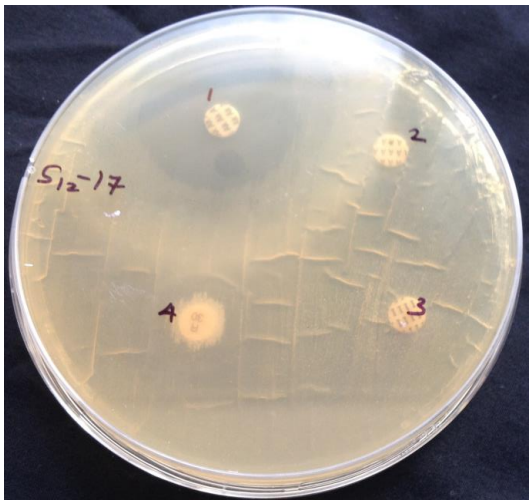

Fig 3: Antibiotic susceptibility of *Streptomyces enissocaesilis* isolate S12-17resistant to the Amikacin (30mcg).

4. Discussion

Vector borne diseases are major problem and provide the biggest threat to the humans and the environment. Use of chemical insecticides like pyrethroids, organochlorines, organophosphates and carbamate is increasing every year. Mosquitoes have developed resistance due to increasing use of synthetic pesticides (Reegan *et al*.; Madhu *et al*.; Bayen) [28, 15, 6]. Insecticides from natural source have potential and are alternative to synthetic chemicals. These insecticides are ecofriendly.

In the present study *S. enissocaesilis* isolate S12-17 presented good mosquitocidal activity against *Cx. quinquefasciatus.* The ethyl acetate and methanol extracts of isolate S12-17 showed good larvicidal activity with LC_{50} and LC_{90} values of 207.18, 635.21 ppm and 282.30, 894.13ppm against *Cx. quinquefasciatus*. These results were comparable to that of Saurav *et al*. [36] who isolated *Streptomyces* VITSVK5 sp from marine soil sample; the butanol extract presented 100% and 99% larval mortality against *Cx. quinquefasciatus* and *An. stephensi*. Anwar *et al*. [37] isolated actinomycetes from various sites in salt range, Kalar Kahar, Pakistan. Methanolic extracts of the isolates SA-10BC, SA-9K, SA-9L had 100% larval mortality, SA -94c, SA-53 and SA-10B exhibited 80% larval mortality and isolate SA-10n showed 60%, larval mortality against *Cx. quinquefasciatus* at 5mg/ml concentration. Deepika *et al*. [38] isolated *Streptomyces sp*. VITDDK from soil samples collected north of Chennai, Tamil Nadu, India. The ethyl acetate extract showed 100% larvicidal activity against *Cx. quinquefasciatus* and *An. subpictus* at 1000ppm concentration. Three actinomycetes were isolated from soil samples collected from island of Nicobar; among them *Streptomyces gedanensis* (LK-3) had good larvicidal activity against *Cx. gelidus* and *Cx. quinquefasciatus* with LC_{50} values of 108.08 and 146.24 respectively at 500 ppm (Karthik *et al.*) [39]. These results and their study related to mosquito controls are substantiating our results.

Ovicidal activity of ethylacetate and methanol extracts of *Streptomyces enissocaesilis* isolate (S12-17) was 100% and 89% respectively against *Cx. quinquefasciatus* at 500 ppm concentration (Fig 2). Repellent activity of isolate S12-17 was dependent on the concentration of the extract. Ethyl acetate and methanol extracts showed 127.2 and 80.6 min at 5mg/cm^2 concentration against *Cx. quinquefasciatus*. The actinomycetes isolated from marine sediment LK-1 and LK-3 had 100% ovicidal activity against *C. gelidus* and *C. tritaeniorhynchus* at 1000 ppm respectively, 240 min protection was recorded in extract from LK-2 and LK-3 at 1,000 ppm against mosquito bites of *Cx. tritaeniorhynchus* and *Cx. gelidus*, respectively (Karthik *et al.*) [39]. These results corraborate our results. Both the extracts were harmless to tested non-target organism *P. Reticulata*.

In antibiotic sensitivity study, seven antibiotics were detrimental to the growth of of isolate S12-17 and highest zone of inhibition was recorded for amikacin at 30mcg/disc (Fig 3). These results were comparable to that of Rajput *et al*. [40] who had isolated *Streptomyces sp*. from Kotumsar caves of India. Polymyxin resistant species were 85.71%; 57.14% species were resistant against gentamycin; 42.85% were resistant against streptomycin, tetracycline, kanamycin and 28.57% were resistant against erythromycin, chloramphenicol and neomycin. *Streptomyces sp*. isolated from actinomycetoma cases of human and donkeys were susceptible to novobiocin, gentamycin and doxycycline (Hamid) [41]. Valanarasu *et al*. [22] had isolated *Streptomyces sp*. and it was highly susceptible to ciprofloxacin, gentamicin and cephaloridine. The isolated strain was taxonomically very close to the *S. enissocaesilis* (89% similarity). Sequence was confirmed by BLAST and phylogenetic analysis; the sequence was submitted to the GenBank (Accession No. KT827105.1).

5. Conclusion

These results of the present study clearly show that the ethylacetate and methanol extracts from *S. enissocaesilis* isolate S12-17 exhibit good larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus*. The extracts was tested for toxicity against non-target organism and found to be nontoxic. These extracts from isolate S12-17 can be probed further to find some alternative to chemical insecticides.

6. Conflict of interests

The authors have no conflict of interest

7. Acknowledgements

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