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Mosquito larvicidal potential of marine *Streptomyces griseus* against *Aedes aegypti*

V Vedha[1*], Abdulbari A Alfaris[2], Narayan D Totewad[3], P Saranraj[4], K Gayathri[4]
[1] Department of Microbiology, Madras Christian College, East Tambaram, Chennai, Tamil Nadu, India
[2] Department of Surgery and Obstetrician, College of Veterinary Medicine, Basrah University, Basrah, Iraq
[3] Department of Microbiology, B.K. Birla College of Arts, Science and Commerce, Kalyan, Maharashtra, India
[4] Department of Microbiology, Sacred Heart College, Tirupattur, Tamil Nadu, India

Abstract
Mosquitoes are linked to the spread of infections to people and other vertebrates. Mosquitoes causes diseases like dengue, filariasis, and malaria. Zoonotic arboviruses transmitted by mosquitoes such viruses are saint Louis Encephalitis Virus, West Nile Virus and Eastern Equine Encephalitis Virus. Chemical insecticides used in the environment to control Mosquitoes bring high major issues like biomagnifications of Toxins through Food chain. Avoiding these toxins, many scientists have developed Biopesticide from the Biological sources. The present study was aimed to evaluate the Larvicidal activity of *Streptomyces griseus* isolated from the Marine soil sample in Besant Nagar Sea Shore, Chennai, Tamil Nadu, India. The *Streptomyces* sp. was isolated and identified as *Streptomyces griseus* based on the Microscopic examination, Plating on Culture medium and Biochemical tests. The identified *Streptomyces griseus* was evaluated for its Larvicidal activity against Mosquito larva of *Aedes aegypti*. The *Streptomyces griseus* was prepared in various dilutions *viz*., 10^{-1}, 10^{-3} and 10^{-5} and used for the Larvicidal bioassay studies. The outcome of bioassay results that shown a range in the high value of effectiveness that depending on the level of exposure to the *Streptomyces griseus*. The Mortality rate was found to be increase as the incubation time also increases, and then increased exposure time for all dilutions of *Streptomyces griseus*. In conclusion, it was observed that the *Streptomyces griseus* is an effective bio-larvicide used to reduce or eradicate Mosquito larva and preventing Vector borne diseases.

Keywords: *Streptomyces griseus*, Insect vector, Mosquito, *Aedes aegypti*, Larvicidal activity and Mortality rate

Introduction
Mosquitoes are one of the major vectors in India, they are responsible for transmitting a number of Vector borne diseases (Wilke *et al*., 2015) [1]. Vector borne diseases like Encephalitis, Malaria, Chikungunya, Dengue, West Nile Virus, and Yellow Fever cause significant damage to the economic, commercial and labor outputs (Breman *et al*., 2001) [2]. *Streptomyces griseus* is a Gram positive, Aerobic, Non-motile Filamentous Actinobacteria. It has been found in variety of habitats, including marine soil, mangrove lands, and sea sponges soil, mud soil, river bank soil, dirt in the forest soil. *Streptomyces griseus* are known for their mycelia growth and contain high amount of Guanine - Cytosine (GC) concentration with an average 72.2 % inside of them and containing Chitinase compounds that are potential activity against Mosquito larvae (Brown *et al*., 2002) [3]. Several *Streptomyces* strains obtained from the soil have yield Antibiotics such as Erythromycin and Riboflavin (Bruhl, 2020) [4]. Since 20th in the past century, the genus *Streptomyces griseus* has being extremely incapable of producing the effectiveness of antibiotics in treating human disease particularly, illnesses that affect plants and also affect crops. *Streptomyces griseus* has a Larvicidal activity against Mosquito *Aedes aegypti* larvae (Bravo *et al*., 2007) [5]. Chemical-based, non-chemical-based, and biological control techniques are all used in vector control (Cuervo Parra *et al*., 2016) [6]. The present study was aimed to study the Larvicidal activity of *Streptomyces griseus* against *Aedes aegypti* larvae for analyzing its potential activity for eco-friendly Mosquito control (Abi and Rahini, 2023) [7].

Materials and Method
Collection of Marine Soil Samples
Three different Soil samples were collected from the Besant Nagar Sea Shore, Chennai, Tamil Nadu, India. The soil samples were collected aseptically from top of 6 cm depth. The soil samples were placed in plastic bags and labeled approximately. The soil samples were transported to the laboratory and stored at Room temperature for the isolation and identification of Actinobacteria.

Collection of *Aedes aegypti* Mosquito Larvae
Rain water were collected using wide mouth container during Rainy time. The container was kept outside for 15 days. This was to give to lay Eggs by the *Aedes aegypti* Mosquitoes. Mosquito larvae were observed until many larvae were observed in the container on the 8th or 9th Day. These larvae were allowed to breed in their natural environment except during screening period which lasted for 8 days. Then, the larvae were harvested using sieve and kept on moistened cotton wool to prevent them from dying before use.

Isolation and Culturing of *Streptomyces griseus* from Soil
From the collected Marine soil samples, *Streptomyces griseus* was isolated by Pour plate technique in Kenknight and Munaier's agar medium. The sample added plates were

incubated at 37 ºC for 24 hours. After incubation, the Colony morphology of Actinobacteria were observed and the isolated colonies were inoculated into Kenknight and Munaier's broth and incubated at 37 ºC for 24 hours. The colonies were sub-cultured onto Kenknight and Munaier's agar media plates and incubated at 37 ºC for 24 hours to obtain the pure culture of *Streptomyces griseus*. Initially, the *Streptomyces griseus* was examined by Microscopic examination technique and Plating in the Culture medium. After that, series of Biochemical tests were further employed to identify *Streptomyces griseus.*

Bioassay method for Larvicidal activity analysis

The *Streptomyces griseus* were tested against Larvae of *Aedes aegypti* mosquito. The culture of *Streptomyces griseus* from the Agar slant were picked using a sterile wire loop and diluted five folds 10^{-1} to 10^{-5} in the sterile distilled water. Five ml in each of the cultures in the first, third and fifth test tubes were added to three disposable cups containing 50 ml of sterile distilled water, providing each cup with different dilution factors. Twenty six *Aedes aegypti* larvae were transferred into each of the disposable cups were kept at Room temperature for 6 hours. At intervals of 30 minutes, each cup was observed for the *Aedes aegypti* larval presence and larval mortality rate was calculated by using Abbot's formula (Abbot, 1925) [8].

Results and Discussion

Mosquitoes are a major and disease spreader that causes significant health risks. Several chemical pesticides have been developed for the control of Mosquitoes in recent years (Ben Dov, 2014) [9]. Most of the insecticides that are made by synthetic as well as chemical discovered to be harmful to both human health and the environment, as well as a wide variety of insects. So, these biological methods that cause less harm to human health with relation to the diversity of insects, and environment (Etim, 2019) [10]. The success of *Streptomyces griseus* as a larvicide to prevent the mosquito larva that produced excellent result. The fundamental traits of the bacteria *Streptomyces griseus*, having a rod form and being Gram positive and contain high amount of G-C content are used as Secondary metabolites and contain Chitinase enzymes that are used Biocontrol substances against Mosquito larvae (El Khawagh *et al*., 2011) [11].

The isolated *Streptomyces* sp. was identified as *Streptomyces griseus* based Microscopic examination (Gram positive motile Actinobacteria), Plating on Culture medium and Biochemical tests. The characteristics of the identified *Streptomyces griseus* was furnished in the Table – 1. The effect of *Streptomyces griseus* on *Aedes aegypti* Larvae was studied in the present research and the findings were given in Table – 2 to Table – 4. The *Streptomyces griseus* was prepared in various dilutions *viz*., 10^{-1}, 10^{-3} and 10^{-5} and used for the Larvicidal bioassay studies. The result for Larvicidal bioassay of 10^{-1} diluent of *Streptomyces griseus* against *Aedes aegypti* larvae was given in Table - 2. Mortality rate was found to be increase as the incubation time also increases. Nearly, 66 % was recorded after 150 minutes and the 93 % mortality rate was recorded after 240 minutes. The Table – 3 showed Bioassay of mosquito larva for the 10^{-3} diluents of *Streptomyces griseus* on Mosquito *Aedes aegypti* larvae. The mortality rate of 62 % was recorded after 180 minutes, 81 % mortality rate was recorded after 240 minutes. The Table - 4 results showed the Larvicidal bioassay for 10^{-5} diluents of *Streptomyces griseus* against Mosquito *Aedes aegypti* larvae. The mortality rate of 54 % was recorded after 210 minutes, after which a mortality rate of 66 % was recorded after 240 minutes. In comparison, 10^{-1} diluent of *Streptomyces griseus* exhibited more Larvicidal activity against *Aedes aegypti* larvae followed by 10^{-3} diluent and 10^{-5} diluent.

Several diluents of the Actinobacteria *Streptomyces griseus* showed the Bioassay for the control of Mosquito larvae *Aedes aegypti* and exhibited various degrees of efficiency with result of high Mortality rate in 240 minutes. This could be related to the organisms capacity to create a toxins, the main insecticidal ingredient generated during vegetative of *Streptomyces griseus* to control the growth of Mosquito larvae *Aedes aegypti* (Abdul Wahab, 2018) [12]. After 30 minutes, little mortality was seen in period of incubation which could be to the larvae's exposure period as well as the variety of creatures found in the container. The majority of the larvae were dying after 30 minutes of incubation, Extremely little mortality was seen in all of the dilution. The majority of the larvae, where death occurs after 240 minutes, and mortality is high. Over 100 % rate was observed in all of the diluents of *Streptomyces griseus*. This may also be related to the point at which the larvae were exposed, as well the growth in cell population in the media. It can be connected to the rise in the quantity of creatures that the insect consumed larvae, which harms to gut of larvae.

Table 1: Characteristics of *Streptomyces griseus*

S. No	Test	Result
1	Gram staining	Gram positive
2	Motility test	Positive
3	Colony colour	Yellow
4	Catalase test	Positive
5	Oxidase test	Negative
6	Indole test	Negative
7	Methyl Red test	Positive
8	Vogues Proskauer test	Negative
9	Citrate Utilization test	Negative
10	Urease test	Negative
11	Starch hydrolysis test	Positive
12	Casein hydrolysis test	Positive
13	Gelatin hydrolysis test	Negative

Table 2: Bioassay of 10^{-1} diluents of *Streptomyces griseus* culture against *Aedes aegypti* Mosquito larvae

Larvicidal Bioassay	Time Duration (Minutes)								
	0	30	60	90	120	150	180	210	240
Number of larvae	26	23	19	16	13	11	9	6	2
Control	26	26	26	26	26	26	26	26	26
Mortality	0	4	8	11	14	17	18	22	24
Mortality rate (%)	0	16	31	43	54	66	70	85	93

Table 3: Bioassay of 10^{-3} diluents of *Streptomyces griseus* culture against *Aedes aegypti* Mosquito larvae

Larvicidal Bioassay	Time Duration (Minutes)								
	0	30	60	90	120	150	180	210	240
Number of larvae	26	23	22	20	18	15	11	8	6
Control	26	26	26	26	26	26	26	26	26
Mortality	0	4	5	7	9	12	16	19	21
Mortality rate (%)	0	16	20	27	35	47	62	73	81

Table 4: Bioassay of 10^{-5} diluents of *Streptomyces griseus* culture against *Aedes aegypti* Mosquito larvae

Larvicidal Bioassay	Time Duration (Minutes)								
	0	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Number of larvae	26	24	23	22	22	20	17	9	5
Control	26	26	26	26	26	26	26	26	26
Mortality	0	3	4	5	6	7	10	14	17
Mortality rate (%)	0	12	16	20	24	27	39	54	66

Conclusion

In conclusion, *Streptomyces griseus* was prevalent in the Maine soil, possess Mosquito larvicidal potential against *Aedes aegypti* Larvae and worked to be a good Larvicidal agent. The result of the present research clearly demonstrated that the efficiency of *Streptomyces griseus* are controlling Mosquito *Aedes aegypti* Larvae because it contains Chitinase enzymes that used for controlling of Mosquito larvae. So, the isolated *Streptomyces griseus* that can be used as biolarvicides, effectively to control mosquito larva instead of chemical pesticides. Chemical pesticides are harmful to human and environmental life. The use of *Streptomyces griseus* as biocontrol agent against *Aedes aegypti* Larvae that used as eco-friendly and does not affect the environment.

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