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Research Article

CHARACTERIZATION AND EVALUATION OF *Streptomyces griseus* FOR ITS POTENTIAL ACTIVITY AND CONTROL OF MOSQUITO LARVAE

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Abstract

Mosquito are the hazardous to human life in all over the world. A major treat achieving successful mosquito control to overcome insecticide resistance to environment. This study was evaluated that larvicidal activity of *Streptomyces* isolated from soil sample in Thiruvannamalai Tamilnadu. Confirmatory identification of *Streptomyces* sp. was based on biochemical characterization and microscopic examination of the organism. The isolated *Streptomyces* was evaluated for its larvicidal activity against mosquito larva because *Streptomyces griseus* produced Chitinase enzymes that are used as potential activity against mosquito larvae. Bioassay methods are employing three dilutions of the *Streptomyces griseus* culture. The confirmatory isolated organisms that are same as *Streptomyces griseus.* 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*. A mortality rate more than 20 % was noted after 60 minutes, increased to 92 %, and then increased exposure time for all dilutions of *Streptomyces griseus* used. This proved that *Streptomyces* is an effective bio- larvicide used to reduce or eradicate mosquito larva.

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1. Introduction

Diseases that are spread by mosquito vectors, such as viruses, bacteria, and parasites are known as mosquito-borne diseases. Although not having the disease themselves, they can spread it. Nearly, more million people get a mosquito transmitted diseases every year resulting in higher than one billon death. 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 organic materials. It is a novel that method for obtaining a bio-insecticide from *Streptomyces griseus*. Many tropical fevers are spread by *Culex quinquefasciatus* mosquitoes. They are present in tropical and subtropical areas. Streptomyces griseus are marine origin that are being frequently documented. Therefore, chitinase - producing Streptomyces from the soil marine have the capacity to block the production of chitin in insects.

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

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Encephalitis Virus (SLEV), West Nile Virus (WNV) and Eastern Equine Encephalitis Virus (EEEV) (Wilke *et al.,* 2015). According to WHO, malaria is the world's most wide spread disease, significantly diseases spread by vectors. These diseases have been spread in more than 200 countries with correctly more than 4 billion people lived in endemic areas. More than eight hundred thousand (8,00,000) fatalities and 300 million cases of the diseases record annually (Breman *et al.,* 2001).

Streptomyces, a natural product, is one of the new efforts that must be made in order to control mosquitoes in a way that is more effective, efficient, and safe for the environment. *Streptomyces griseus* can be isolated from a variety of habitats, including marine soil, mangrove lands, and sea sponges soil, mud soil, river bank soil, dirt in the forest soil. *Streptomyces* has been found in the mud soil and highly polluted soil, as well as secondary metabolites produced by microorganisms have a long history of being trusted sources of pharmaceuticals. Aerobic, filamentous, non-motile, Gram-positive bacteria such as the *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. A genus consists of commercially and microorganisms with a significant economic impact that can produce. The several *Streptomyces* strains obtained from the soil of the Philippines have yield antibiotic Erythromycin and Riboflavin. The well-known *Streptomyces griseus* are species of bacteria in genus *Streptomyces* commonly found in soil and few strains are also present in deep sea sediments. It is a Gram positive bacterium that containing high amount of GC (Guanine - Cytosine) content has the greatest diversity of species in nature, and it is also the most abundant varieties primarily come from soil. 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. Almost all S*treptomyces* species are produced antibiotics, as has been demonstrated. Regarding the economy and health, there have been numerous in-depth studies. Global producer of antibiotics was *Streptomyces.* Soils are thoroughly screened for the *Streptomyces* species in 1940. *Streptomyces griseus* a member of the Actinobacteria, has larvicidal activity against mosquito *Anopheles* larva. Chemical-based, non-chemical-based, and biological control techniques are all used in vector control. The present study was under taken to study larvicidal activity from *Streptomyces griseus* of Potential activity for eco-friendly mosquito control against mosquito larvae.

2. Materials and Method

Sample Collection

Three soil samples were collected from Thiruvannamalai district, 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 of bacteria. 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 mosquitoes. Mosquito larva were observed until many larva 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 larva was 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 soil sample, *Streptomyces griseus* was isolated by Pour plate technique in Nutrient 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 Nutrient broth and incubated at 37 ºC for 24 hours. The colonies were sub-cultured onto

ISP-4 Media plates and incubate at 37 ºC for 24 hours to obtain the pure culture of *Streptomyces griseus*. Series of tests which further employed to identify *Streptomyces griseus* in after the formation of colonies with smooth leathery, look like skin and hard. Further, the *Streptomyces griseus* was examined by Gram staining technique and Biochemical tests.

Bioassay method

The *Streptomyces griseus* were isolated and selected and tested against larva of mosquito. The culture of *Streptomyces griseus* from slant bottles 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 larvae were transferred into each of the disposable cups were kept at 25 ºC - 30 ºC for 6 hours. At intervals of 30 minutes, each cup was observed for the larval presence and larval mortality rate was calculated.

3. Results and Discussion

Biochemical and morphological characteristics of *Streptomyces griseus* result are shown in Table - 1. Indole test showed positive result with Red colour ring formation. Methyl red test, Voges Proskauer test, Citrate utilization test and Triple sugar iron test showed Negative results. Motility produced Positive result. The characteristics and isolated organisms shown in Table - 2 the result for bioassay of 10^{-1} diluent of *Streptomyces griseus* against mosquito larvae. Mortality rate was found to be increase as the incubation time also increases. Nearly, 60 % was recorded after 150 minutes and the 96 % 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 larvae. The mortality rate of 60 % was recorded after 180 minutes, 80 % mortality rate was recorded after 240 minutes. The Table - 4 results showed the bioassay for 10^{-5} diluents of *Streptomyces griseus* against mosquito larvae. The mortality rate of 68 % was recorded after 210 minutes, after which a mortality rate of 84 % was recorded after 240 minutes.

Mosquitoes are a major and disease spreader that causes significant health risk to people in society. Several chemical pesticides have been developed for the control of mosquitoes in recent years. 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. 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 GC content are used as secondary metabolites and contain chitinase enzymes that are used biocontrol substances against mosquito larvae. The biological traits demonstrated that the organisms are motile.

Several diluents shown in the bioassay for the control of mosquito larvae exhibited various degrees of efficiency, with result 78 % 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 larvae of the mosquito. This was reported by Abdul Wahab (2018). 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*. 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	Biochemical Test	Result
1.	Indole test	Positive
2.	Methyl Red test	Negative
3.	Vogues Proskauer test	Negative
4.	Citrate Utilization test	Negative
5.	Triple Sugar Iron test	Negative
6.	Motility test	Positive

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

Minutes	0	30	60	90	120	150	180	210	240
Number of larva	26	23	19	16	13	11	9	6	2
Control	26	26	26	26	26	26	26	26	26
Mortality	0	3	7	10	13	15	17	20	24
Mortality rate (%)	0	12	28	40	52	60	68	80	96

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

Minutes	0	30	60	90	120	150	180	210	240
Number of larva	26	23	22	20	18	15	11	8	6
Control	26	26	26	26	26	26	26	26	26
Mortality	0	3	4	6	8	11	15	18	20
Mortality rate (%)	0	12	16	24	32	44	60	72	80

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

Minutes	0	30	60	90	120	150	180	210	240
Number of larva	26	24	23	22	22	20	17	9	5
Control	26	26	26	26	26	26	26	26	26
Mortality	0	2	3	4	4	6	9	17	21
Mortality rate (%)	0	8	12	16	16	24	36	68	84

4. Conclusion

Streptomyces griseus that are present in soil that has worked to be a good larvicidal agent against mosquito larvae in the laboratory. The result showed in this study clearly demonstrated that the efficiency of *Streptomyces griseus* are controlling mosquito larva 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. Hence, chemical pesticides are harmful to human and environmental life. The use of *Streptomyces griseus* as biocontrol agent against mosquito larva that used as eco- friendly and does not affect ozone layer.

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