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Evaluation of Actinomycetes secondary metabolites for Larvicidal activity against Culex quinquefasciatus

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Abstract: *Six actinomycetes samples, including Nocardiopsis alba, Streptomyces euisocasilis, Streptomyces dot, Streptomyces mutabilis, R1 and R2 strains, were employed in our investigation. These secondary metabolites of actinobacteria may be used to create novel, secure C. quinquefasciatus control products. After being inoculated into a 500 ml conical flask with 200 ml of starch casein broth, the pure Actinobacteria isolates were shaker-incubated for 7 days at 28 ± 2 °C. Every secondary metabolite produced by Actinobacteria was found to be harmful to Culex quinquefasciatus larvae. All of the isolates demonstrated larvicidal efficacy against the larvae of the Culex quinquefasciatus mosquito.*

Keywords: Actinomycetes, metabolites, Larvicidal activity

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INTRODUCTION

Actinomycetes are Gram-positive, unicellular bacteria that are members of the Actinomycetales Order. This group's members are extensively dispersed across nature and can be found in many different types of environments worldwide. Greek terms "mykes/mukes" (meaning fungi) and "atkis" (meaning ray) are the source of the name Actinomycetes. It has been demonstrated that they produce the same disease as fungus and share certain traits with them, such as mycelial development. Actinomycetes are not only extensively spread but also adaptable in terms of feeding and have the ability to produce a variety of spores. Due to these traits, the group has been able to successfully compete with nearby creatures. Although some of the members of this group are pathogenic (e.g., Actinomyces israelii causes actinomycosis), they are also
important soil organisms that break down a variety of resistant organic compounds and produce biologically active compounds that find application in the pharmaceutical and insecticidal industries. The majority of naturally occurring secondary metabolites have been shown to have strong potential antagonistic action. One such organism is the actinomycetes, which are distinguished by their capacity to generate significant secondary metabolites. Among these, the genus Streptomyces is the most prolific producer of antibiotics, making up around 80% of the natural compounds derived from actinomycetes that have been documented (Jensen et al., 2005). Despite the fact that thousands of antibiotics have been identified, it is believed that these only make up a small portion of the repertory of bioactive substances that Streptomyces species can create (Watve et al., 2001).

There are more mosquitoes, significant vector groups, and diseases they can spread (WHO 2014a). Dengue, yellow fever, chikungunya, and the Zika virus are all caused by Aedes aegypti. West Nile virus, dengue, and chikungunya are caused by Aedes albopictus. Lymphatic filariasis is caused by Culex quinquefasciatus. Encephalitis in Japan is caused by Culex species. Malaria caused by Anopheles. By biting and sucking blood, these vectors and the diseases they spread cause severe losses to people and their animals, as well as indirect harm from the spread of vector-borne illnesses. In the early stages of vector control, inorganic compounds were employed; but, as organic chemical compounds gained greater traction and had faster-acting effects, they gradually supplanted inorganic compounds. Therefore, the primary goals of this work are to determine the larvicidal activity of the secondary metabolites and to distinguish them from specific Actinomycetes.

SUPPLEMENTS AND METHODS Examples

Six actinomycetes samples were employed in our investigation, including R1 and R2 strains of Streptomyces mutabilis, Streptomyces euisocasilis, Streptomyces alba, and Dot from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. Casein agar with starch Using Starch Casein Agar (SCA), actinomycetes can be found. Setting up the medium: In 1000 millilitres of distilled water, suspend 34.38 grammes of nutritional agar. Bring the mixture to a boil and thoroughly dissolve the medium. Autoclave at 15 Ibs pressure (121˚C) for 15 minutes to sterilise. Blend thoroughly and transfer to sterile Petri dishes.

To prepare broth or liquid media, suspend all materials (except from agar) in 1000 millilitres of distilled water. Bring the mixture to a boil and thoroughly dissolve the medium. Autoclave at 15 Ibs pressure (121˚C) for 15 minutes to sterilise. Mix thoroughly and transfer to a conical flask.

Getting pure culture ready

After being streaked onto the suitable culture slants, a loop containing all of the bacteria was cultured at 28±2ºC for seven days.

Extracellular biometabolite extraction from actinobacteria

After being inoculated into a 500 ml conical flask with 200 ml of starch casein broth, the pure actinobacteria isolates were shaker-incubated for 7 days at 28 ± 2 °C. The supernatant from the cell-free culture was removed, dissolved in an equal volume of ethyl acetate, and thoroughly mixed for one hour in a separating funnel. After being taken out and properly dried, the solvent extract was diluted in DMSO and employed in the larvicidal bioassay.

Gathering eggs and caring for larvae

Using a 'O'-style brush, the eggs of C. quinquefasciatus were removed from the National Centre for Disease Control field station located in Mettupalayam, Tamil Nadu, India. Once in the lab, the eggs were moved to enamel trays measuring 18 by 13 by 4 centimetres and holding 500 millilitres of water

in preparation for hatching. The mosquito larvae were fed a 3:1 ratio of yeast to purebred dog biscuits. The larvae were fed continuously until they reached the pupal stage.
upkeep of adults and pupae

Using a dipper, the pupae were removed from the culture trays and placed into 12 x 12 cm plastic containers with 500 mL of water. A 90×90×90-cm mosquito cage held the plastic jars until the adults emerged. Larvae of mosquitoes were kept at 27+2 °C, 75–85% relative humidity, and 14:10 light/dark hours.
Strong actinobacteria's larvicidal impact on Culex quinquefasciatus

After initial screening, the actinobacteria with strong larvicidal properties was chosen for additional investigation. The third instar of the Culex quinquefasciatus larvae was utilised for bioassay experiments once they were collected. Three replicates of twenty larvae each were used to expose a total of sixty larvae. The experiments were carried out at room temperature (28 ± 2
°C) for 24, 48, and 72 hours. Tap water that has been dechlorinated was used as the control. Each

20 larvae was kept in 20 millilitres of water for the bioassay experiments. Different actinobacterial secondary metabolite extract concentrations (500, 250, and 125 µl) were applied. After exposure for 24, 48, and 72 hours, the number of dead larvae was counted, and the average of three replicates was used to calculate the % mortality.

The formula for corrected mortality is: Observed mortality - Observed mortality / 100.- Manage death by 100
The formula for percentage mortality is: number of dead larvae / total larvae × 100.
Outcomes
Distinguishing Secondary Metabolites
After being inoculated into a 500 ml conical flask with 200 ml of starch casein broth, the pure Actinobacteria isolates were shaker-incubated for 7 days at 28 ± 2 °C.

Figure 1- Sub cultures of Actinomycetes

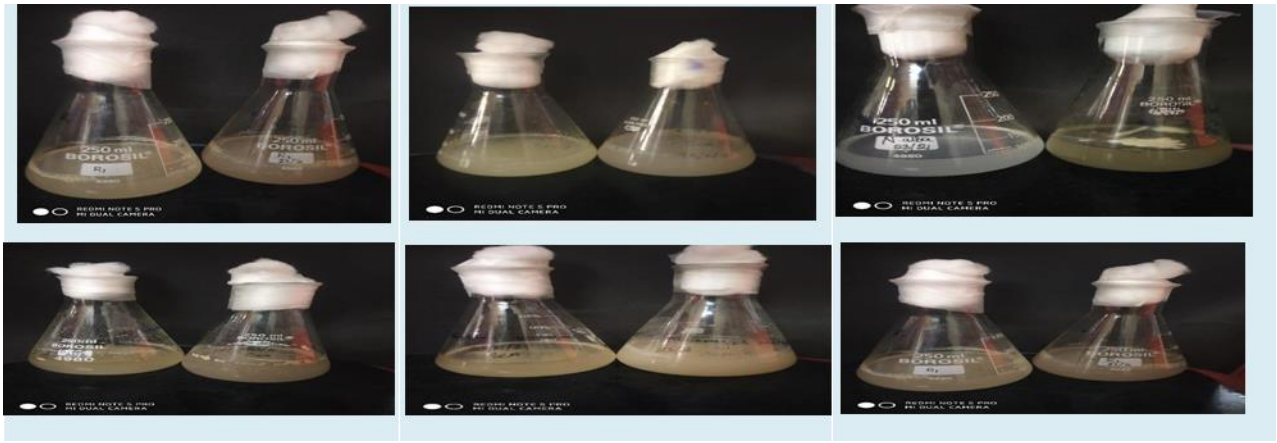
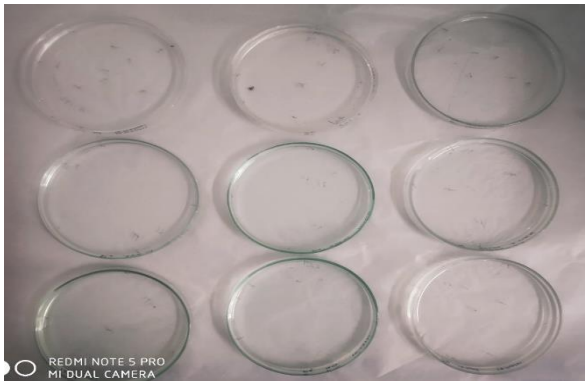


Figure 2- Broth culture Actinomycetes

Figure-3: A-broth cultures at shaker ; B- Separation funnel

The supernatant from the cell-free culture filtrates was removed, dissolved in an equal volume of ethyl acetate, and thoroughly mixed in a separating funnel for one hour. For the larvicidal bioassay, the solvent extract was taken out, dried appropriately, diluted in DMSO, and used (Figure 3).Larvicidal behaviour Six Actinobacterial secondary metabolites in total were employed in this investigation to evaluate the larvicidal activity. All of the isolates exhibited larvicidal efficacy against the larvae of the Culex quinquefasciatus mosquito (Figure 4). Every secondary metabolite produced by Actinobacteria was found to be harmful to Culex quinquefasciatus larvae. The results of this investigation demonstrated some Actinobacteria's larvicidal properties at different concentrations and exposure times.After 24 hours, 48 hours, and 72 hours, the number of living larvae was counted, and the percentage of mortality was computed.Within twenty-four hours, 500 microliters of Nocardiopsis alba exhibited the highest percentage of inhibition and 125 microliters of Streptomyces mutabilis the lowest percentage of death. 48 hours later, 500 µl of Streptomyces euisocasili showed the highest percentage of inhibition and 125 µl of R1 showed the lowest percentage of death. 500 µl of Nocardiopsis alba showed the highest percentage of inhibition in 72 hours, whereas 125 µl of R2 at 24 hours displayed the lowest percentage of death. Overall, 500 µl of Nocardiopsis alba at 72 hours showed the highest percentage of inhibition while 125 µl of Streptomyces mutabilis at 24 hours showed the lowest percentage of mortality.

The highest percentage of death was observed at 500 µl concentrations of secondary metabolites produced by Actinomycetes.



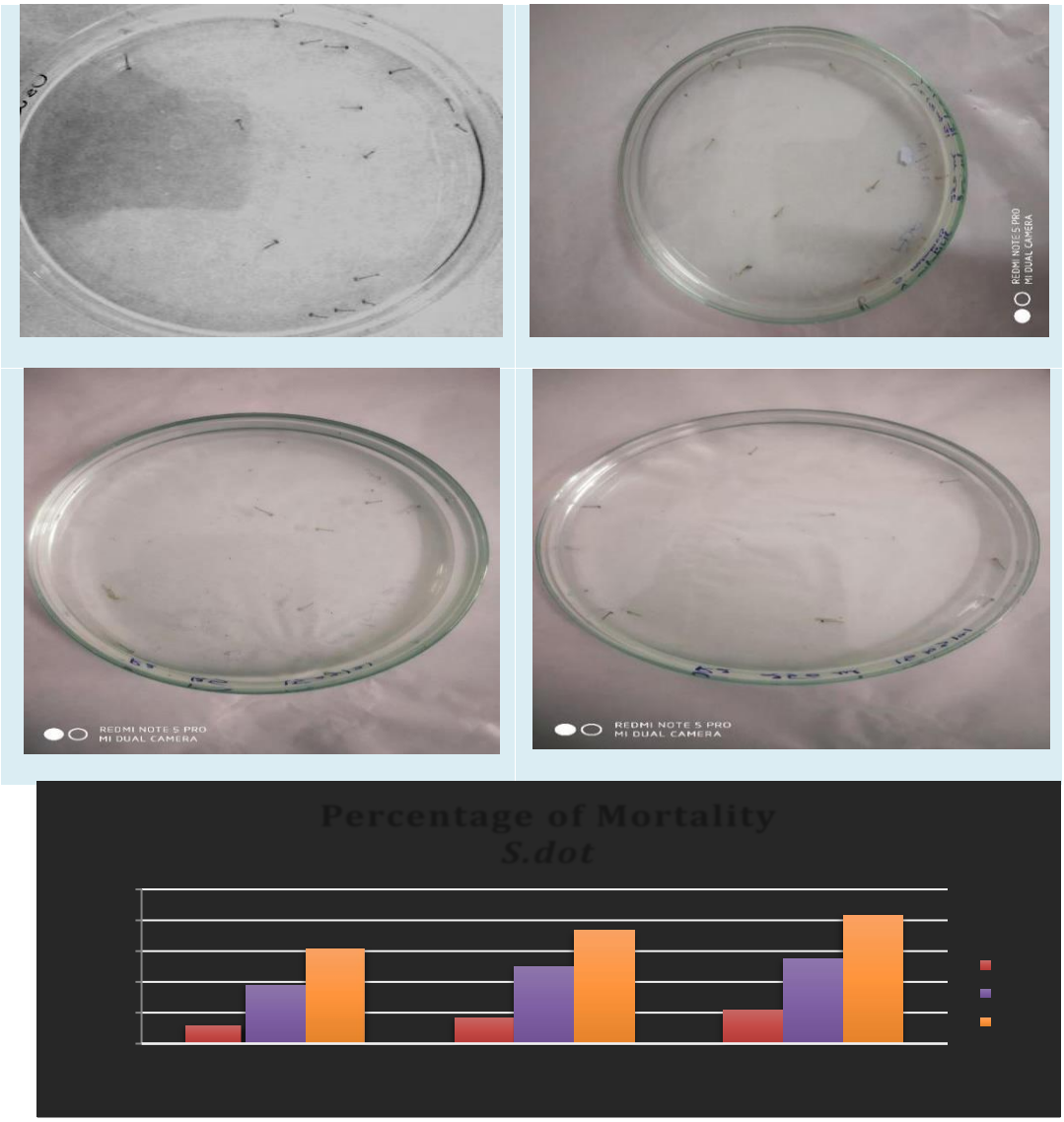


Figure-4: Setup for Larvicidal Activity of Actinomycetes

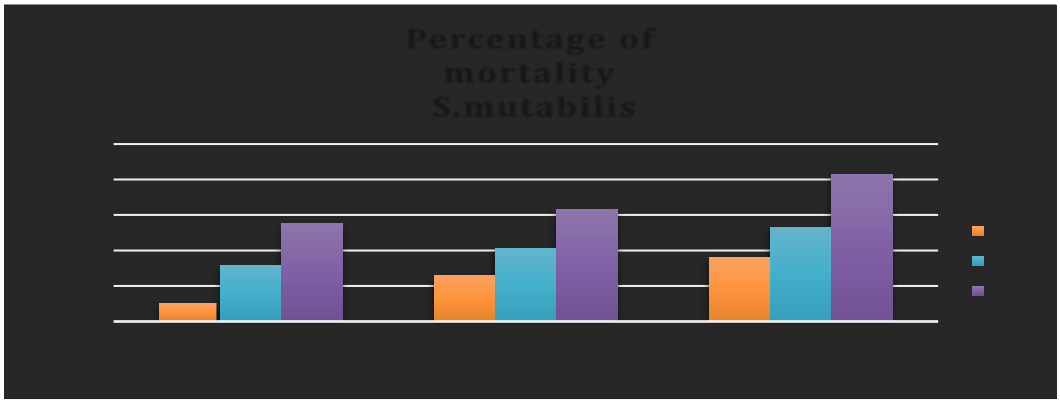


Figure-5: Percentage of Mortality S.mutabilis

Figure-6: Percentage of Mortality R2

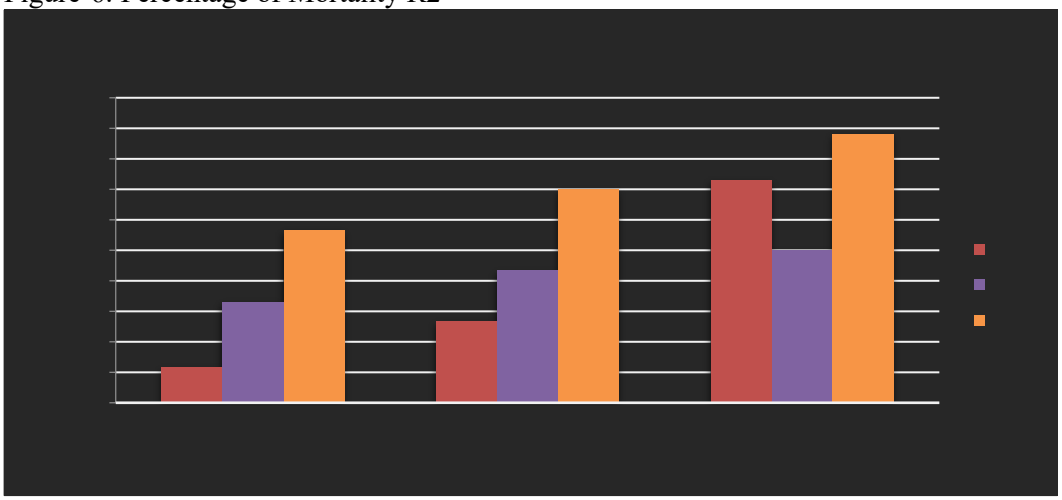


Figure-7: Percentage of Mortality S.dot

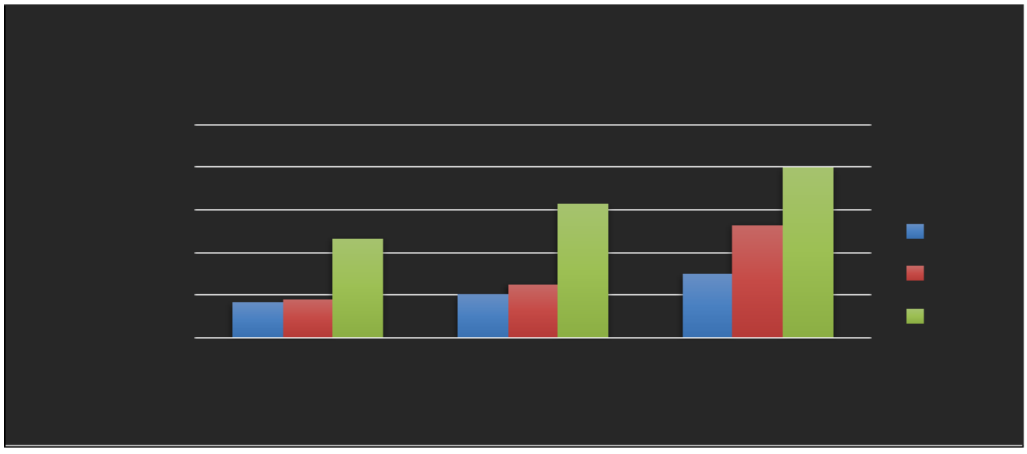


Figure-8: Percentage of Mortality N. alba

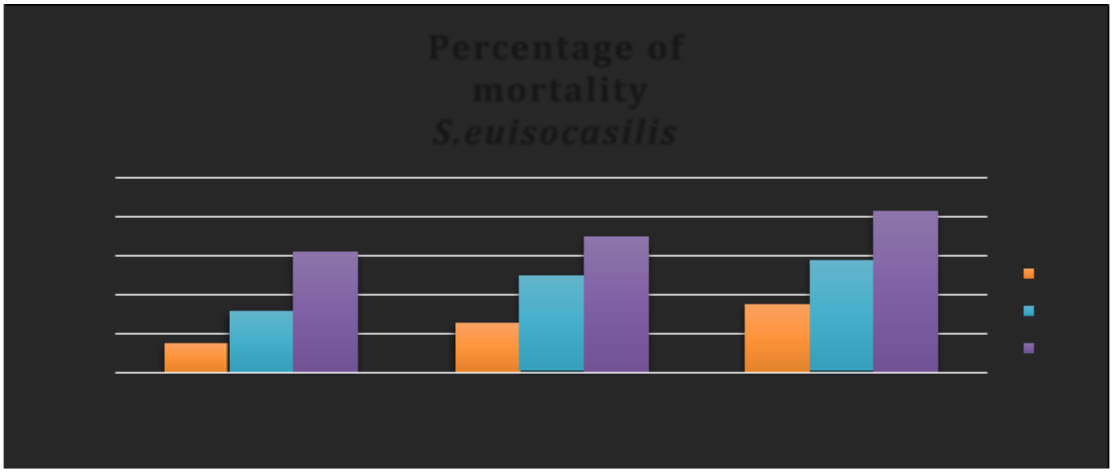


Figure-9: Percentage of Mortality R1

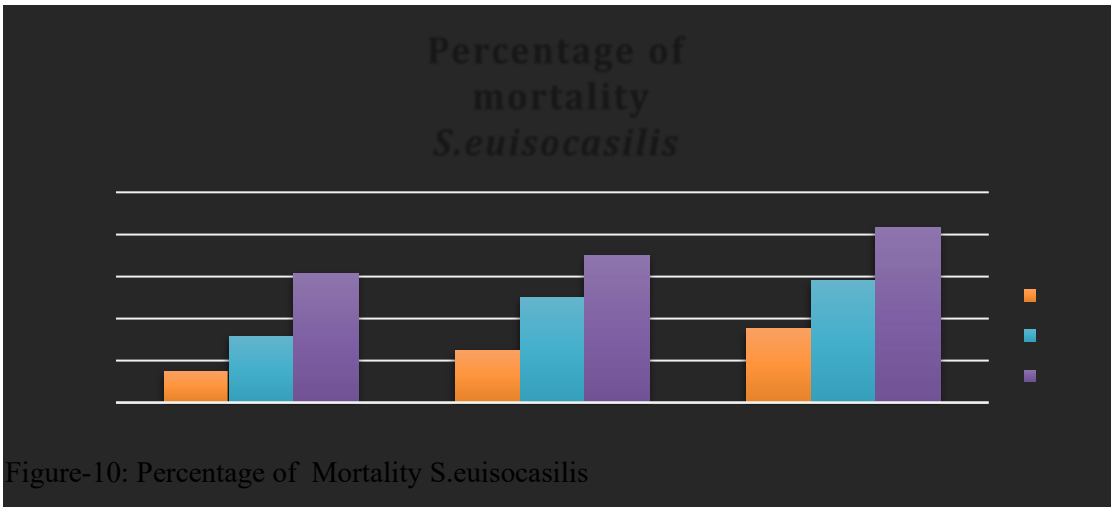
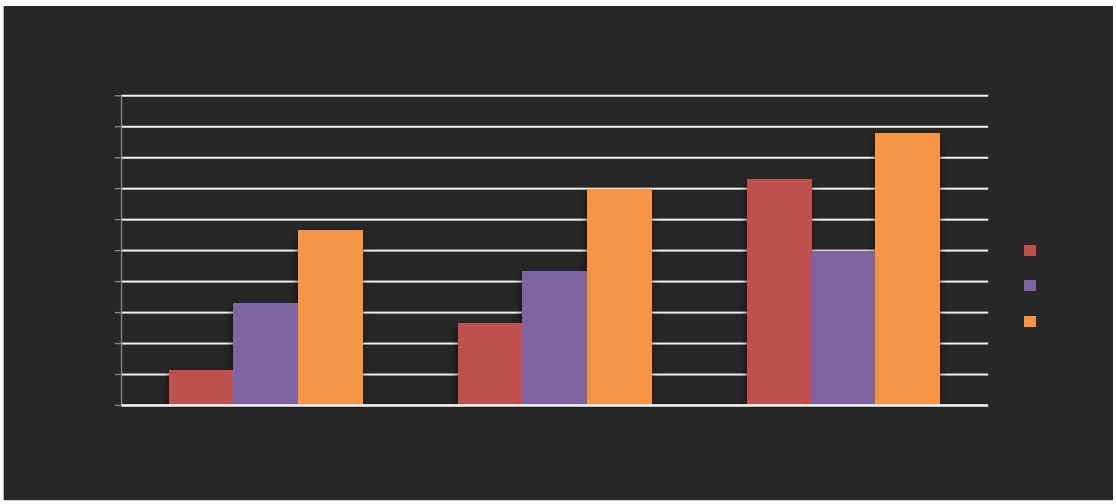


Figure-10: Percentage of Mortality S.euisocasilis

Table 1 :Percentage of Mortality for 24 hrs ,48 hrs, 72 hrs



Duration	Concentration	Percentage of Mortality(%)					
		R1	R2	*S.dot*	*N. alba*	*S.mutabilis*	*S.euisocasilis*
24 hr	125	16.6	16.66	11.66	11.6	10	15
	250	20	15	16.66	26.6	26	25
	500	30	23.33	21.66	73	36	35
48 hr	125	18	35	38	33	31.6	31.6
	250	25	41	50	43.3	41	50
	500	53	45	55	50	53	58
72 hr	125	46.6	45	61.6	56.6	55	61.6
	250	63	53.3	73.3	70	63.3	70
	500	80	63.33	83.3	88	83	83.3

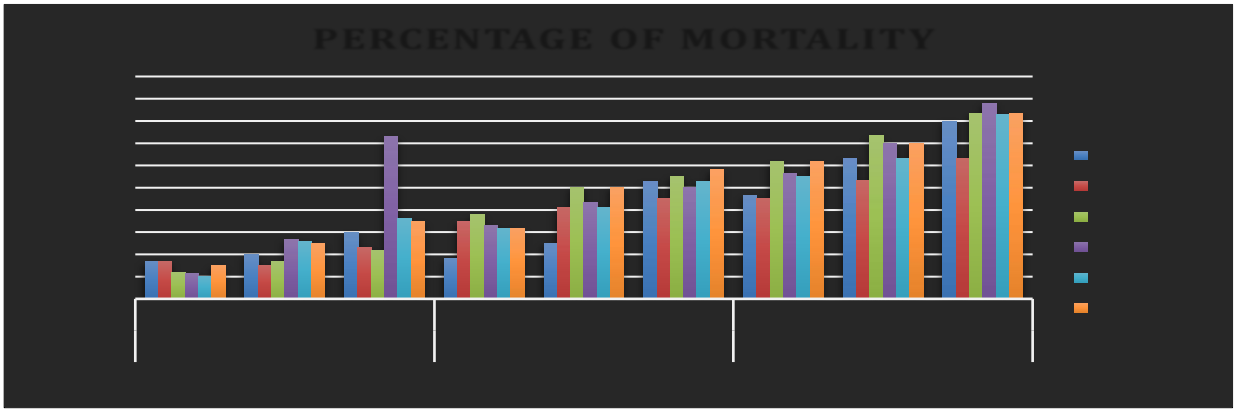


Figure-11: Percentage of Mortality for 24hrs, 48hrs 72 hrs

Discussion: The findings of this study are consistent with those of Balakrishnan et al. (2016), who recovered 30 actinobacterial isolates—including colonies of white, grey, brown, pink, and yellow color—from soil samples from the Parangipettai mangrove ecosystem. Of these, twenty-one isolates exhibited larvicidal activity against Ae. aegypti and An. stephensi mosquito larvae; five isolates, S. fungicidicus, S. griseus, S. albus, S. rochei, and S. alboflavus, in particular, were more toxic to the late third instar larvae of Ae. aegypti and An. stephensi in particular. The results of
this investigation showed that certain marine actinobacteria have larvicidal and pupicidal properties at low concentrations and brief exposure times. The extracellular metabolites that actinomycetes exude into the culture media contain the active chemicals of the fungus (Barakate et al., 2002). When their growth slows down or stops, actinomycetes synthesise the secondary metabolites (Doull and Vining 1990; Sanchez and Demain 2002). Actinomycetes' secondary metabolites have been extensively researched for their antibacterial qualities (Rana and Salam 2014; Phongsopitanun et al., 2014). Investigating the acute and long-term toxicities of actinomycetes on various vector mosquitoes has piqued the interest of academics in recent years. Actinomycetes have been shown in numerous studies to be harmful to various species of mosquitoes. Thirty actinomycetes that were isolated from soil samples from the Muthupet mangrove forest in the Tiruvarur District were screened against Anopheles mosquito larvae by Vijayakumar et al. (2010). When they screened for larvicidal activity using the culture filtrate, they discovered that 23 isolates had larvicidal activity, of which 2 isolates were significantly effective.

Researchers in India have looked at the actinomycete metabolites' ability to repel insects, such as mosquitoes. The metabolites of actinomycetes have been suggested as viable substitutes for synthetic pesticides by Mishra et al. (1987). The isolation of actinomycetes' anti-mosquito active substances has been documented by Smokvina et al. (1990). The ovicidal, larvicidal, and adulticidal properties of fungi and actinomycetes against Cx. quinquefasciatus, An. stephensi, and Ae. aegypti have been investigated by Vijayan and Balaraman (1991).

Thirty actinobacteria were recovered from the Muthupet mangrove ecosystem by Dhanasekaran et al in 2010. Strong larvicidal activity was demonstrated by four isolates from the genera Streptomyces, Streptosporangium, and Micropolyspora against Anopheles larvae.

Eight possible actinomycete isolates were identified from a total of 283 pure isolates taken from soil samples collected from the Nilgiris and Kalakkad Mundanthurai Tiger Reserve in the Tirunelveli District, according to research conducted in 2016 by M. Gabriel Paulraj et al. The active isolates in this investigation demonstrated species-specific activity against various mosquito
species, which was a significant finding. The study revealed that CFR-16, which was extracted from the soil of Coonoor forest in Nilgiris, was the most potent isolation against Ae. aegypti, An. stephensi, and Cx. quinquefasciatus out of the eight active isolates.
In their 2014 study, Balakrishnan et al. investigated the production of silver nanoparticles using an aqueous extract from the leaves of A. marina, which is a possible source of larvicidal action against diseases spread by mosquitoes.

OVERVIEW AND RESULTS
Six actinomycetes samples were employed in our investigation, including R1 and R2 strains of Streptomyces mutabilis, Streptomyces euisocasilis, Streptomyces alba, and Dot from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. After being inoculated into a 500 ml conical flask with 200 ml of starch casein broth, the pure Actinobacteria isolates were cultured for 7 days at 28 ± 2 °C in a shaker incubator. The supernatant from the cell-free culture filtrates was removed, dissolved in an equal volume of ethyl acetate, and thoroughly mixed in a separating funnel for one hour. After being taken out and properly dried, the solvent extract was diluted in DMSO and employed in the larvicidal bioassay.
Six Actinobacterial secondary metabolites in total were employed in this investigation to evaluate the larvicidal activity. All of the isolates demonstrated larvicidal efficacy against the larvae of the Culex quinquefasciatus mosquito. Every secondary metabolite produced by Actinobacteria was found to be harmful to Culex quinquefasciatus larvae. The results of this investigation demonstrated some Actinobacteria's larvicidal properties at different concentrations and exposure times.
After 24 hours, 48 hours, and 72 hours, the number of living larvae was counted, and the percentage of mortality was computed. After 24 hours, 125 µl of Streptomyces mutabilis exhibited the lowest percentage of mortality, whereas 500 µl of Nocardiopsis alba showed the highest percentage of inhibition.
These secondary metabolites of actinobacteria may be used to create novel, secure C. quinquefasciatus control products. These compounds obtained from Actinobacteria could be a good substitute for synthetic insecticides in managing C. quinquefasciatus populations in the field because they are naturally occurring pesticides.

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