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Fish Gut Endophyte: A Source of Organic Insecticide

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Abstract: ***Mosquito-borne diseases such as dengue, Zika, and chikungunya constitute a significant global public health concern, particularly in tropical and subtropical regions. Conventional vector control methods that rely heavily on chemical insecticides have resulted in environmental degradation and have facilitated the emergence of insecticide-resistant mosquito populations, thereby diminishing the efficacy of these interventions. The investigation suggests that the intestinal bacteria of guppy fish (Poecilia reticulata) have the larvicidal potential to control Aedes aegypti. The mosquito larvae suffered fatal damage when exposed to the extract isolated bacteria Lysinibacillus pakistanensis J20M5LARS by high mortality rate. The research demonstrates an environmentally responsible biological mosquito control system which operates as an organic replacement for chemical insecticides in pest management.***

Keywords: Guppy, Lysinibacillus pakistanensis, eco friendly insecticide

I. INTRODUCTION

Stagnant water areas serve as the main risk locations for the health threats caused by Malaria and other mosquito transmitted diseases. Standard insecticide controls cause both environmental destruction while making mosquito populations resistant to insecticides.

Vector-borne diseases pose a significant global health burden, particularly in tropical subtropical regions, where they disproportionately affect impoverished communities. Aedes aegypti is the primary vector responsible for transmitting arboviruses such as dengue, Zika, and chikungunya (Katak *et al*., 2021).These mosquitoes have adapted well to urban environments, exhibit strong human-biting preferences, and have contributed to recent outbreaks worldwide (Roy et al., 2019). Dengue alone is estimated to cause 95–114 million infections annually, with millions requiring hospitalization (WHO, 2016).

Poecilia reticulata, commonly known as the guppy, is a small freshwater fish native to northeast South America. Known for its vibrant colors (Pratama, 2018; Tobungan, 2019) and elaborate tail patterns, the guppy is popular in home aquariums around the world . It is a hardy species that can adapt to a range of environmental conditions, making it ideal for both beginner and experienced aquarists. Guppies are also livebearers, meaning they give birth to free-swimming young rather than laying eggs, and they reproduce rapidly under suitable conditions. Guppy could be a useful animal model for age-related changes in physiological functions (Imai *et al*.,2022).

The implementation of guppies serves as an environmentally responsible biological control solution for the problem. The guppies can effectively reduce mosquito populations, thereby minimizing the risk of disease transmission. Guppy fish could be used as an appropriate, effective and acceptable biological method to control mosquito breeding. Additional studies using larvivorous fish for vector control have been evaluated in the past with varying degrees of success, although no cluster randomized controlled studies had been completed. By studying the interactions between guppies and mosquito larvae, we can determine the biological factors that contribute to larval mortality.

II. METHODOLOGY

A. Sample Collection

Studies based on intestinal bacterial activity on Poecilia reticulata involved the collection of guppy fishes from Kanjirapuzha, Palakkad and its preservation in controlled aquatic conditions.

B. Isolation of Fish intestinal bacteria

Guppy was collected and surface sterilized by kept it in 70% ethanol. Then it was kept in sterile distilled water. Then the intestine of the guppy was removed by using a sterile blade and the intestine was grinded by using mortar and pestle. This was directly inoculated onto sterilized nutrient agar plates by spread plate method and streak plate method. Incubate the plates at 37°C for 24 hrs. After incubation observed the results (Kayath *et al*., 2019).

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C. Characterization of bacteria

The isolated bacteria undergoes various techniques like Gram staining and other biochemical tests. Motility test is also done.Through the process that included genomic DNA extraction then 16S rRNA gene amplification and PCR product sequencing followed by BLAST analysis.

D. Extraction of Secondary metabolites from bacteria

The bacteria is isolated in sterile nutrient broth and incubated for 10 days. After incubation the broth was centrifuged at 5000 rpm for 10 minutes. After that supernatant and pellets were collected.

E. Bioassay for larvicidal activity

Supernatant and pellets were collected in a test tube and mosquito larva were added. Then incubate the tubes for which shows rapid larvicidal activity (Kayath *et al*., 2019).

III. RESULTS

A. Sample Collection and Isolation of gut endophyte

Guppies were collected. Bacteria were isolated from the intestine of guppies and the colonies were subculture on nutrient agar media by spread plate and streak method.White circular colonies were observed.



Fig 2: Dissected intestine of Guppy

B. Characterization of Bacteria

The isolated endophytic bacteria was Gram positive motile bacteria with the following biochemical characteristics:

Table 1: Biochemical tests

Sl. no	Biochemical Test	Results
1	Indole Test	Negative
2	MR Test	Negative
3	VP Test	Negative
4	Citrate Utilization Test	Positive
5	Catalase Test	Positive

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6	Oxidase Test	Positive
7	Urease Test	Positive
8	TSI Test	Positive
9	Carbohydrate Fermentation Test	
9.a	Lactose	Acid with no gas
9.b	Maltose	Acid with no gas
9.c	Sucrose	Acid with no gas
9.d	Glucose	Acid with no gas
9.e	Fructose	Acid with no gas

C. Extraction of Secondary metabolites from bacteria

The larvae added to the test tube containing the Supernatant killed within 6-7 hours. It has high larvicidal activity.

D. Bioassay for larvicidal activity

Bioassay for determining the larvicidal activity of bacteria were done through micro dilution method.The MIC values indicate the lowest concentration of the bacteria which kills the mosquito larva. The larva in test tube with 1:500 dilution killed with in 19 hrs and the larva kept in the test tube 1:250 killed within 43 hrs.

IV. DISCUSSION

The *Poecilia reticulata* (guppy fish) potential to be used as a sustainable biotic control for the larvae of Aedes aegypti is highlighted in this study providing an eco-friendly alternative for the use of chemical insecticides which pollute the environments and cause resistance of mosquitos to chemical insecticides. Guppies showed efficient larva predation while strains of bacteria isolated from their intestine in particular Lysinibacillus pakistanensis, showed strong larvicidal properties that implied a dual mechanism to control mosquitoes. However, despite the potential of guppy introduction, more field studies are needed in order to estimate large-scale prospects and possible ecological impact on native species. Further studies should be carried out to extract active bacterial compounds, perform field trials and determine guppy interaction in their natural environment to make them viable in the long-term vector control strategy.

V. CONCLUSION

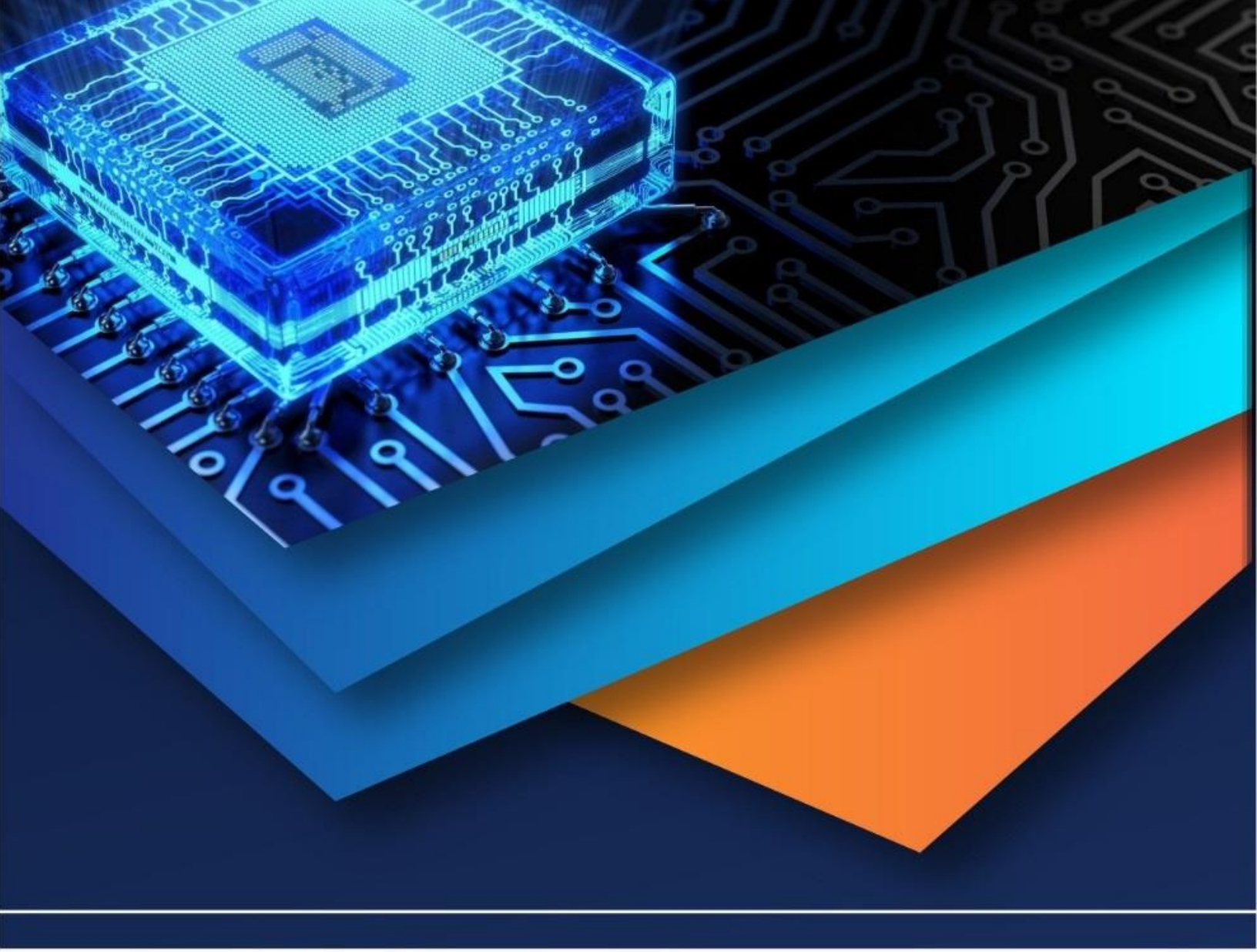
This research investigated the potential of guppy fish (*Poecilia reticulata*) in controlling the mosquito larvae especially Aedes aegypti and as an effective biological control agent. The research proved that guppies feed on mosquito larvae actively and bring down their population in an efficient manner without the use of chemical insecticides. Moreover, there is successful isolation of bacterial strains with the property of larvicidality from the guppy's intestine. Biochemical and morphological characterization of these bacteria discovered them as natural mosquito control agents, a further justification of the role of guppies in curbing the transfer of vector borne diseases.

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