A Project- Report on

"Biocontrol Potential of *Bacillus thuringiensis* Isolated from Soil against Mosquito Larva"

Submitted by

Mr. Avdhut Mohan Patil

Submitted to

VIVEKANAND COLLEGE, KOLHAPUR,

DEPARTMENT OF BIOTECHNOLOGY.

FOR PARTIAL FULFILMENT OF BACHLOR OF SCIENCE IN BIOTCHNOLOGY

THIS YEAR – 2022-23

UNDER THE GUIDENCE OF,

MRS. S. S. PATIL

(ASSISTANT PROFESSOR BIOTECH ENTIRE)

Department of Biotechnology (Entire), Vivekanand College, Kolhapur.

"Education for Knowledge, Science and Culture"

- Dr Bapuji Salunkhe

Vivekanand College (Autonomous), Kolhapur

Department of Biotechnology

Certificate

This is certified that Mr. / ~~Ms.~~ Avdhut Mohan Patil

Roll No 9329 **has satisfactorily completed his/~~her~~ Project work on the topic** Bioconmol potentional of Bacillus thuringiensis, **as a part of syllabus prescribed by Board of Studies, Department of Biotechnology, Vivekanand College (Autonomous), Kolhapur for B.Sc. III (Entire) Biotechnology and this report represents his/her bonafied work in year 20**22 **- 20**23**.**

Date: 28/4/23

Place: Kolhapur

VIVEKANAND COLLEGE ESTD JUNE 1964 KOLHAPUR-416 003

Project Guide 26/4/23

Examiner 02/05/2023

Head 28/4/23

Head
Department of Biotechnology (Entire)
Vivekanand College, Kolhapur (Autonomous)

DECLRATION

I hereby declare that the project work entitled "**Biocontrol Potential of *Bacillus thuringiensis* Isolated from Soil against Mosquito Larva**" submitted to Vivekanand College, Kolhapur for the award of the degree of "Bachelor of Science in Biotechnology" is the result of bonafied work carried out by me under the guidance of **Asst. Professor-Biotech entire Mrs. S. S. Patil.**

I further declare that the results presented here have not been the basis for the reward of any other degree.

Place: Kolhapur

Date: 28 | 4 | 23

Mr. Avdhut Mohan Patil

Department of Biotechnology (Entire), Vivekanand College, Kolhapur.

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Place: Kolhapur

Date: 28/4/23

Mr. Avdhut Mohan Patil

Department of Biotechnology (Entire), Vivekanand College, Kolhapur.

INDEX

AIM AND OBJECTIVES

1) Isolation

   Isolation of *Bacillus thuringiensis* (Bt) from soil.

2) Confirmation/ Characterization of the Isolated Bacteria.

   To perform following confirmatory tests

   a) Indole Test
   b) Catalase Test
   c) Methyl Red Test (M.R.)
   d) Vogues-Proskauer Test (V.P)
   e) Motility Test
   f) Gram Staining

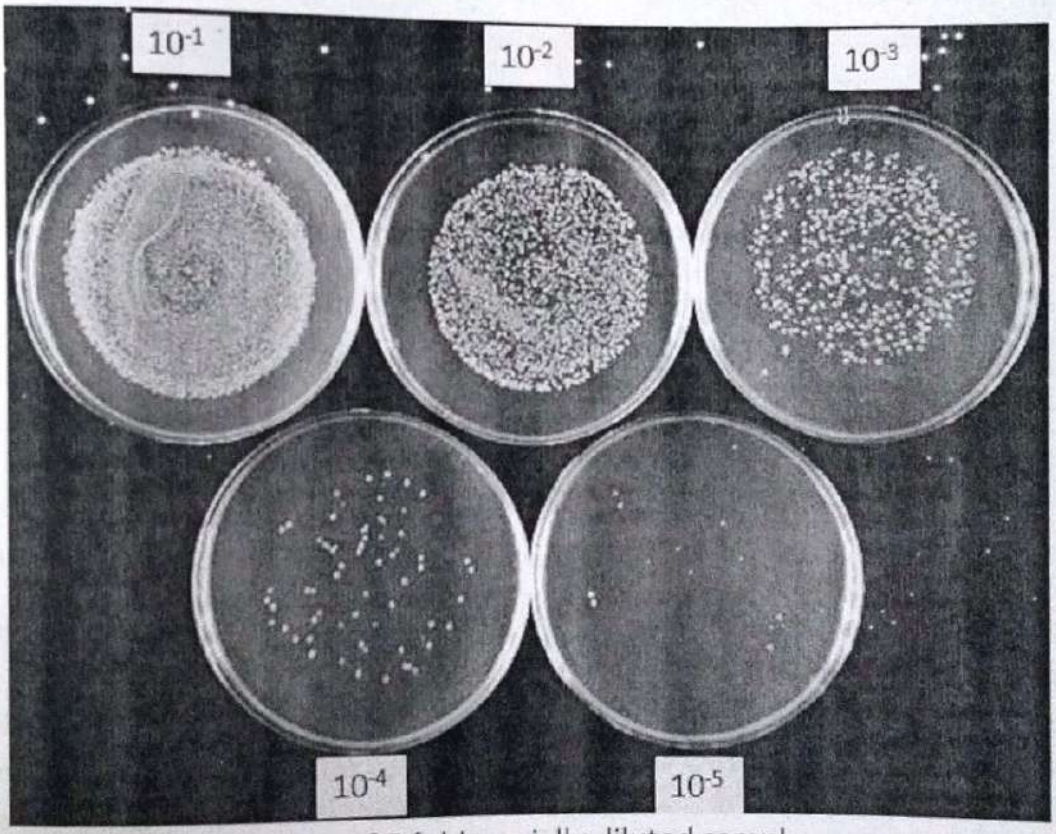
3) Biocontrol Potential

   a) To evaluate the efficacy of Bt strains isolated from soil against mosquito larvae in laboratory bioassays.
   b) To compare the efficacy of Bt to that of chemical insecticides commonly used in mosquito control programs.

sterilization and then poured into sterile petri dishes (about 20 ml per plate) under aseptic conditions. The plates were allowed to solidify and incubated at 37°C for 24 hours and the sterility of the medium was checked.

Isolation of *Bacillus thuringiensis* from Soil-

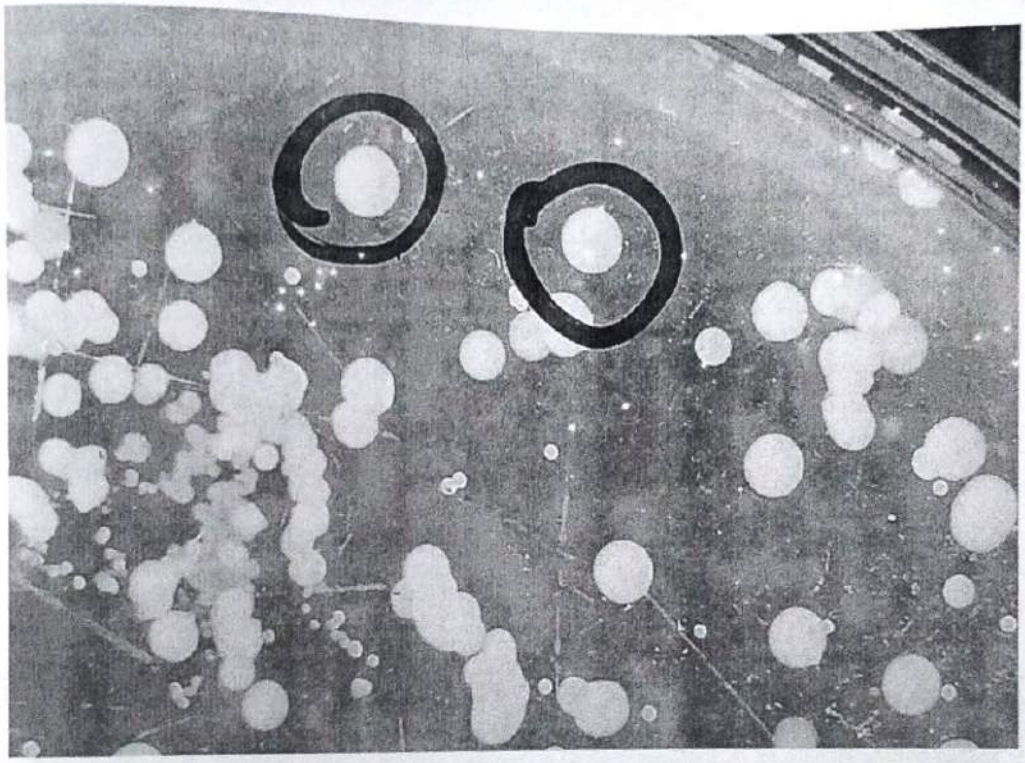
- Five grams (5 g) of soil sample was weighed and added to 100 ml of distilled water.
- The samples were heated on a hot air oven for 10 minutes to eliminate all bacteria incapable of producing endospores.
- Since it is known that *Bacillus thuringiensis* produces spores, it will be safe to assume that if it was present in the soil, it would be in our heated sample.
- The samples were then diluted 5 fold to eliminate all humic materials within the samples and to reduce the overall colony forming units within each sample.



Result of 5 folds serially diluted sample.

Culturing of *Bacillus thuringiensis*-

- The diluted samples were cultured on nutrient agar plates for 24 hours at 37°C in to order to give the spores chance to germinate on media with adequate nutrients and optimal temperature.
- The media, however, offers favourable growth for a wide range of bacteria as well as Bacillus thuringiensis.
- The colonies were subcultured onto Luria-Bertani plates and incubated at 37°C for 24 hours, so as to obtain pure cultures of *B. thuringiensis*.
- Series of tests which include gram staining and biochemical tests were further employed to identify *Bacillus thuringiensis* after the formation of colonies with smooth round shape and earthy odour.

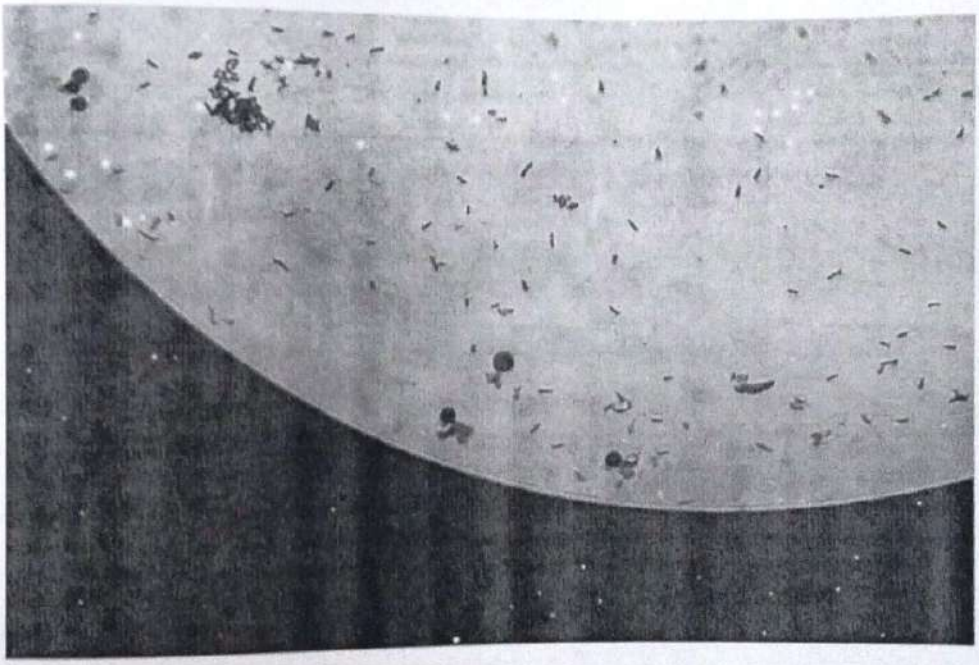


Colonies selected (for streaking on LB media palte from 4th fold of NA media plate.

The colonies were subcultured onto Luria-Bertani and incubated at 37°C for 24 hours, so as to obtain pure cultures of *B. thuringiensis*. Series of tests which include gram staining and biochemical tests were further employed to identify *Bacillus thuringiensis* after the formation of colonies with smooth round shape and earthy odour.

Gram Staining Techniques

- A smear of colonies isolated after the identification was made on clean glass slides using a sterile wire loop.
- They were air dried and fixed. The smears were flooded with crystal violet for about 60 seconds and were washed with tap water.
- They were then tipped off with iodine for 30 seconds and then washed with tap water. They were decolourized with acetone and washed off with tap water.
- The fixed smears were counterstained with safranin and allowed for 60 seconds and then washed off with tap water and allow to air dry.
- Oil immersion was added to the stained slides and viewed under a microscope using x100 objective for the morphological characteristics of the isolates.



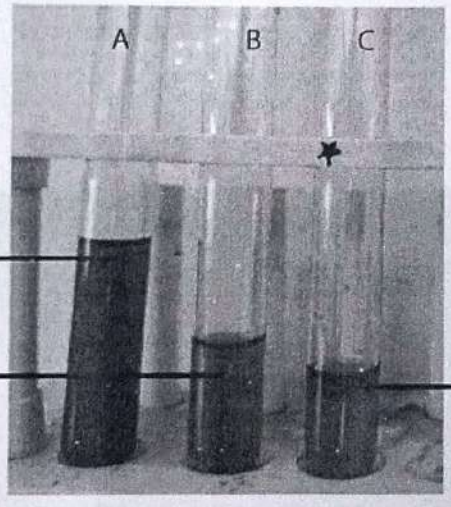
Gram positive, rod shaped bacteria.

RESULTS

Results of confirmatory tests-

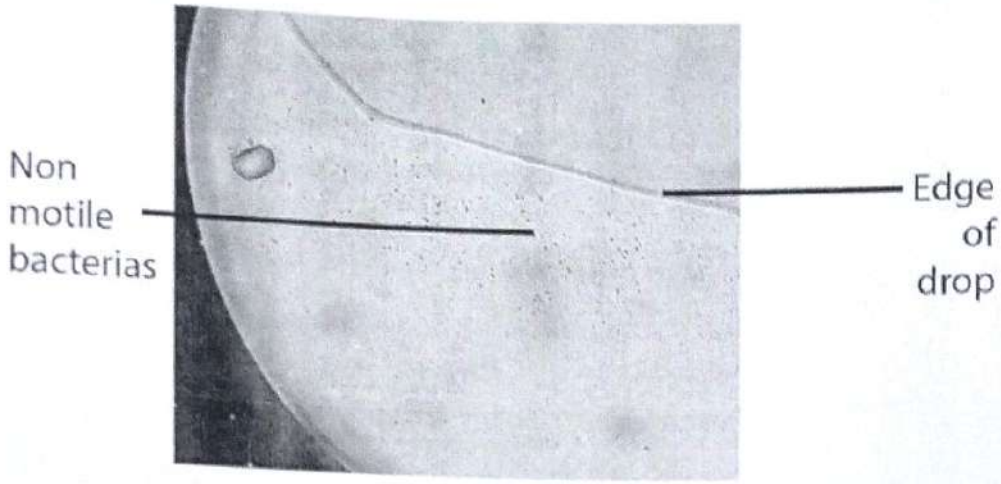
Test	+ve Result	-ve Result
Indole Test	✓	X
Catalase Test	✓	X
Methyl Red Test (M.R.)	✓	X
Vogues-Proskauer Test (V.P)	✓	X
Motility	X	Non Motile
Gram Staining	Gram positive	X

Visual results of performed tests-

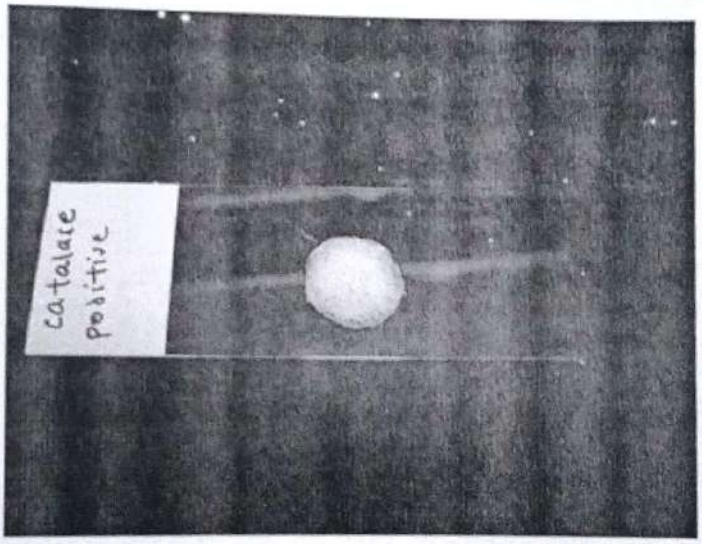


A. Methyl Red Test (M.R.)
B. Vogues-Proskauer Test (V.P)
C. Indole Test

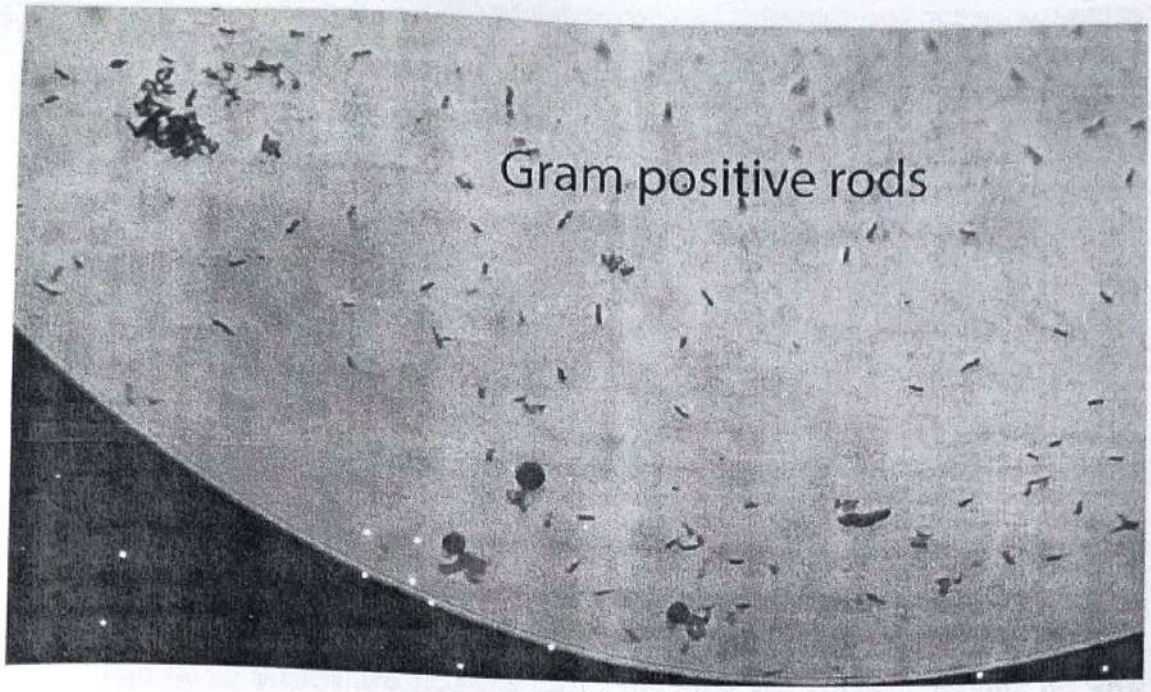
D. Motility-



E. Catalase Test



F. Gram Staining



Result of Study of Biocontrol Potential

There were 10 larvae in each glass (3 glasses). After 21 the results were observed, they are as follows-

Glass with concentration	Observation at 0 hour	Observation at 21th hour
1 Fold diluted	10 larva alive	0 larva alive
2 Folds diluted	10 larva alive	2 larva alive
3 Folds diluted	10 larva alive	6 larva alive

CONCLUSION

Bacillus thuringiensis naturally found in the soil has proved to be a good larvicidal agent against mosquito larvae in the laboratory. The organism and it product can be further studied to search for novel compounds that can be used in the control of mosquito-borne diseases.