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Isolation and Screening of Pesticide Tolerating Bacteria from Agricultural soil

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ABSTRACT

In the current study, pesticide tolerating bacteria were isolated and screened from agricultural soil. The soil sample was collected from a grape farm located at Lokhandewadi in Nashik district, Maharashtra. Samples were collected aseptically followed by isolation and identification of bacterial strains using serial dilution technique followed by Spread plate technique for isolation. Results revealed that 25 bacterial strains were isolated from the soil sample wherein 5 isolates were found to be pesticide tolerant at 50 ppm concentration that were identified to be of *Bacillus spp*. and *Micrococci spp*. Thereafter, screening of pesticide tolerating bacteria was performed for a widely used insecticide.

Keywords: Agriculture, Pesticides, Bacteria.

1. INTRODUCTION

Agriculture is the foremost occupation of India [1]. The vegetables are the most fundamental part of Indian agriculture and nutritional well-being [24]. Growth of various crops and their usage in food chain is possible due to agriculture [1]. Soil is the major resource of plant life. Therefore, quality of crop and food system is directly linked with the quality of farm soil and thereby human health [3]. It is estimated that globally more than 1.85 million of toxic chemicals are released in the air and water [9]. In order to maintain agricultural production, large number of pesticides have been used [8]. The international agricultural sector is the chief user of pesticides, consuming over 4 million tons per annum [9].

Pesticides have played a vital part in agriculture by protecting crops and livestock from yield reductions [3]. These are the chemical compounds used to kill or manage any pests at tolerable level [2]. According to the World Health Organization, as per the proposed classification of pesticides by hazard, the active components of pesticides are classified as extremely hazardous class 1A, highly hazardous class 1B, and moderately hazardous class 2 and slightly hazardous class 3 active components (WHO 2004). Recurrently used pesticides include herbicides, fungicides, insecticides, and rodenticides [9]. Extreme use of pesticide leads to accumulation of large amounts of pesticide residue in the soil affecting non-target species like humans, flora and fauna through the food chain [4].

In the current study, we have worked with a widely used insecticide Emamectin Benzoate (4"-epi-methylamino-4"-deoxyavermectin B1). Emamectin benzoate (MK-0244) is a novel semi-synthetic derivative of the natural product Abamectin in the Avermectin family of 16-membered macro-cyclic lactones. Emamectin benzoate is highly powerful to a broad spectrum of lepidopterous pests [15]. The bacterial strains were isolated from a grape farm in Lokhandewadi, Nashik. These isolates were screened using Emamectin benzoate to determine the tolerance of the pesticide at 50ppm concentration. Hence, bacterial strains were isolated and screened successfully against the insecticide Emamectin Benzoate.

2. RESEARCH METHODOLOGY

Collection of soil sample:

Soil sample was gathered from a farmland near Lokhandewadi in Nashik, Maharashtra. It was collected from a grape farm where the soil was contaminated with pesticide. Samples were collected using standard method (Methods in Applied Soil Microbiology and Biochemistry) using sterile polythene bags and were transferred aseptically to the laboratory.

Isolation of bacterial strains from soil:

1 gram of soil sample was inoculated aseptically in 9 ml sterile distilled water tubes and serial dilutions were carried out from 10^-1 to 10^-6 concentration (Methods of Applied Microbiology and Biochemistry). 0.1 ml of diluted sample from 10^-4, 10^-5 and 10^-6 respectively were inoculated on sterile nutrient agar plates aseptically using spread plate technique for bacterial isolation. These plates were incubated at room temperature for 72 hours, observations were noted after every 24 hours [23]. The well isolated colonies were picked and inoculated on a sterile nutrient agar plate and preserved as a microbial library.

Screening of potential organisms:

There were 25 isolates found from soil in Grape farm. 100 ml sterile pesticide stock and 135ml Sterile Nutrient broth was prepared. The media used for screening of potential organism contained 50ppm concentration of pesticide stock that is 15 ml pesticide stock in 135 ml sterile nutrient broth. All the bacterial isolates found were then inoculated into this media and were kept at room temperature on a rotary shaker at 120 rpm for 3 to 4 days. The isolates that tolerated pesticide at this concentration were further identified using biochemical tests, gram character, microscopy and morphology. The Pesticide tolerance potential was recognised at O.D. 0.5 initially and then the Optical Density of the isolates were noted after incubation using Visible Spectrophotometry [24].

3. RESULT AND DISCUSSION

3.1 ISOLATION OF BACTERIA FROM PESTICIDE CONTAMINATED SOIL:

25 bacterial strains were isolated from Lokhandewadi (LW) grape farm. The observation of isolated colonies from agricultural soil after every 24 hours is as follows:

TABLE NO. 1- COLONY COUNT

Time	Colony count		
	10^(−4)	10^(−5)	10^(−6)
24 hours	9	-	-
48 hours	18	2	-
72 hours	21	3	1

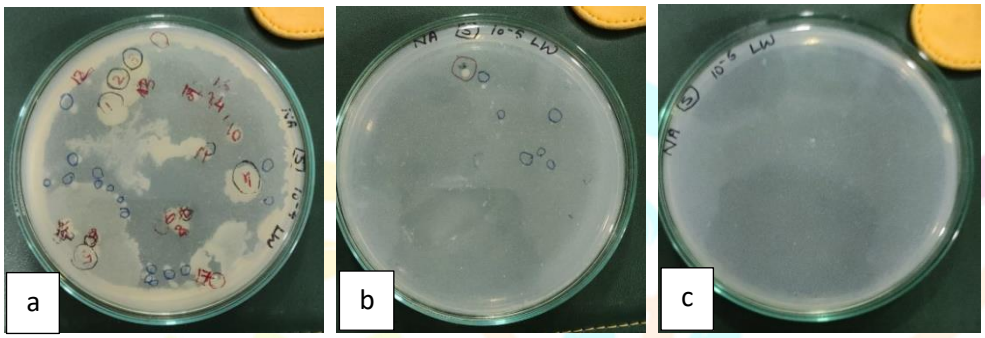

Fig. a) Isolated colony of 10^-4 concentration b) Isolated colony of 10^-5 concentration c) Isolated colony of 10^-6 concentration

3.2 SCREENING OF PESTICIDE TOLERATING BACTERIA**:**

Among 25 isolates isolated from agricultural soil contaminated with pesticide, 5 isolates showed tolerance against the pesticide. The biochemical properties of these 5 isolates in shown in the table below. The isolates identified using these tests were found to be of *Bacillus spp.* and *Micrococcus spp*

TABLE NO 2- PESTICIDE TOLERANCE POTENTIAL.

Isolate No	Initial O.D.	Optical Density after incubation	Pesticide Tolerance Potential Indication
1	0.5	2.02	++++
2		1.23	+++
3		1.64	++++
4		1.02	++
5		1.82	++++

TABLE NO. 3- IDENTIFICATION OF ISOLATES UPTO GENUS LEVEL USING BIOCHEMICAL TEST.

Isolate	Indole	Methyl Red	Voges-Proskauer	Citrate Utilization	Oxidase	Catalase	Organism
1	Negative	Positive	Positive	Negative	Positive	Positive	*Micrococcus spp.*
2	Negative	Positive	Positive	Negative	Positive	Positive	*Micrococcus spp.*
3	Negative	Positive	Positive	Negative	Positive	Positive	*Micrococcus spp.*
4	Negative	Negative	Positive	Positive	Positive	Positive	*Bacillus spp.*
5	Negative	Negative	Positive	Positive	Positive	Positive	*Bacillus spp.*

4. CONCLUSION

The bacterial strains were successfully isolated from agricultural soil collected from grape farm. Results obtained in this work indicate the role of bacteria in tolerating pesticide at 50 ppm concentration. The isolates that had the ability to tolerate pesticide at 50 ppm were gram positive strains *Micrococcus spp.* and *Bacillus spp*. Hence, pesticide tolerating bacteria were successfully isolated and screened.

FUTURE PERSPECTIVE

1. Molecular characterization of bacterial strains using various techniques including gas chromatography, DNA extraction, PCR analysis and Genome sequencing can be done.
2. 16S r RNA sequencing of bacterial strain isolated from agricultural soil is possible.
3. We can compare tolerance of bacteria at different concentration of pesticide and prepare statistical data to determine the highest level of tolerance of bacteria in pesticide.
4. Determination of Bioremediation potential of bacteria at different pesticide concentrations and use of those bacteria for bioremediation of pesticides in agricultural soil.
5. Antibiotic sensitivity assays can be carried out using different antibiotics.
6. Effect of environment on growth of bacteria can be studied.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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