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A STUDY ON SCREENING OF MONOCROTOPHOS PESTICIDE DEGRADING BACTERIA ISOLATED FROM AGRICULTURAL SOIL

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ABSTRACT

Soil samples were taken from pesticide-contaminated areas of several farms in the Bhopal area of MP, India. In this study, monocrotophos (MCP) in agricultural soil was degraded using microbial and enzymatic methods. Our research on the degradation of monocrotophos pesticides focused primarily on bacterial transformation. Methodology: The physicochemical properties of soil tests defiled with monocrotophos (MCP) were researched. Monocrotophos-corrupting microorganisms were detached utilizing a fixation approach. Primer screenings of monocrotophos debasing bacterial secludes, like phosphates action, alkali identification, and catalyst esterase discovery, were done. The impacts of different MCP focuses on the corruption interaction were examined. The corruption try was completed by immunizing various centralizations of MCP into MSM media. For the fifteenth, 30th and 45th days, the MCP corruption by these disengages and their equimolar blend was approved utilizing scientific strategies, Fourier change infrared spectroscopy (FTIR). Locales 1, 4, and 8 have the most elevated levels of large scale and miniature supplements. A fixation approach utilizing a mineral salt medium containing MCP as a carbon and phosphorus source yielded an aggregate of 20 distinct bacterial strains. Phosphate movement is delivered by around 25% of segregates and alkali and esterase chemical combination is created by around 35% of separates. Segregates BAGN005, BKGN007, and BVGN010 were distinguished as Bacillus captions, Bacillus lichen froth, and Pseudomonas faltering, individually, in light of the MCP obstruction identified in the slope plate examine. The consequences of this study show that BAGN005, BKGN007, BVGN010, and blended societies show hydrolysis tops at 2649.19 and 2115.72 cm-1 and show MCP debasement, further concentrate as a legitimate contender for MCP bioaugmentation-sullied soil climate. This study centers around monocrotophos-debasing bacterial disconnects that have been demonstrated to be promising specialists for pesticide bioremediation. Bacillus captions (BAGN005), Bacillus lichen (BKGN007), and Pseudomonas Stutter (BVGN010) were all nearby strains and were exceptionally compelling in corrupting MCP. Apparently, this is the main investigation of the capacity of the bacterium Bacillustequilensis to debase MCP.

Keywords: Screening of Monocrotophos, Agricultural, Soil, Pesticide Degrading.

1. INTRODUCTION

Soil samples were taken from pesticide-contaminated areas of several farms in the Bhopal area of MP, India. In this study, monocrotophos (MCP) in agricultural soil was degraded using microbial and enzymatic methods. Our research on the degradation of monocrotophos pesticides focused primarily on bacterial transformation.

India relies heavily on organ phosphorus pesticides to safeguard agricultural outputs, and these pesticides have widespread applications in the country's physical and chemical soil properties. Soil degradation, lower crop yields, and subpar agricultural quality are all possible outcomes of using too much organic phosphorus-based pesticides, which poses serious risks to ecosystem, human, and animal health. The Organ phosphorus, Nonspecific Systemic Insecticide and Acaricide Monocrotophos [Diethyl (E) 1Methyl2 (Methylcarbamoyl) Vinyl Phosphate] is effective against common mites, ticks, and spiders through contact and Stomach Action. To put it simply, eutrophication is caused by a phosphorus flood. It has been determined that 98% of pesticides are toxic to fish and crustaceans as classified. Tarnishing can have a negative impact on biodiversity and soil health. By means of nutrient transport, retention, filtration, and so on. Heavy metals slow the growth of microbes and other organisms in three ways. In addition to microorganisms and the right kind of tertiary catalysts, the ability to degrade organic matter also depends on other ecological constraints. Synthetic and natural microbial associations can result in structural modifications or complete corruption of target particles. As soon as contaminates are present, soil catalysts act to immediately articulate organic networks in the soil. Both intracellular

(from bacteria and fungi) and extracellular (from plants) enzymes are present in soil (enzymes immobilised on soil particles). These enzymes catalyse the oxidation, reduction, and hydrolysis reactions necessary to transform pesticides into metabolites that are more hygroscopic and less toxic than the parent compound 4. This highlights the significance of bacteria, actinomycetes, and fungi in the biodegradation of pesticides during composting.

Enzymes in the soil have been cited as a biologically relevant indicator of soil quality. Three primary enzymes can be found in soil. Dehydration, wrinkles, and -glycosidase levels, for instance, should be tracked when composting soil contaminated with organ phosphorus or organ chlorine pesticides 5. As a result of chemical and physical interactions, microorganisms can alter the structure of substances or degrade them entirely. Bioremediation is an exciting technology because it uses microorganisms to clean up polluted areas in a sustainable, cost-effective, and adaptable manner. All of the current physical and chemical approaches fall short in some way or are prohibitively expensive. One possible method of cleaning up a polluted area is through bioremediation. Natural, low-cost, and effective, bioremediation approaches to pesticide detoxification are now available 7,8. This research set out to identify and characterise bacteria in soil capable of degrading Mcp. This research discusses the biological and molecular characteristics of several Mcp-degrading bacteria, outlining recent developments in Mcp biodegradation.

2. MATERIALS AND METHODS

2.1.The soil's physical-chemical properties

Prior to analysis, the material was air dried at ambient temperature, ground and sieved with a 2 mm sieve. Particle size was determined using hydrometer method 9 and soil pH was determined using a 1: 1 pH meter. Soil vs. aqueous solution. Wet oxidation method 10 was used to measure organic carbon, Macrokjeldahl method 11 was used to measure total nitrogen, and Bray P.1 method 12 was used to measure available phosphorus. Exchangeable captions (Ca, Mg, Na, and K) ($C_2H_7NO_2$) were extracted using 1N ammonium acetate. The Ca and Mg concentrations were determined using the EDTA titration method and the Na and K concentrations were determined using the flame luminosity method13. Titration method 14 was used to measure the exchangeable acidity and the sum was used to determine the effective caption exchange volume (ECEC).

2.2 Monocrotophos-degrading bacteria are isolated using an enrichment approach.

Soil tests were gathered from pesticide-polluted areas of different paddy fields in the Shivagangai district of MP, India, which has a long history of pesticide use. Bacterial confines corrupting monochromotophos got enhanced in improvement medium containing no P source and containing the accompanying parts: $NaNO_3$: 2 g, Kcl: 0.5 g, DDL4.7H_2O: 0.5 g, Glucose: 10 g, $FeCl_3$: 10 mg, $BaCl_2$: 0.2 g, $CaCl_2$: 0.05 g, refined water: 1 l and 100 mg L - Monochrometophos (5 g) defiled soil ought to be vaccinated with 500 mg L - 11MCP. It was placed in 100 ml enriched medium cultured for 7 days. 282 ° C rotary shaker. To obtain a more degradable strain, a 10 ml concentrated sample was transferred to 90 ml sterile medium and the concentration procedure was repeated. Bacterial colonies were isolated on nutrient agar plates using infusion plate technology. Isolated bacterial cultures were maintained on agar slopes in mineral salt medium containing monocrotophos 1.

2.2. Identification by morphology

The beating was used to purify the established bacterial colonies. The isolated strains were maintained at 4 ° C and on nutrient agar medium. Various bacterial isolates have been identified using standard bacteriological methods 15, 16.

2.3. Monocrotophos-degrading bacterial isolates undergo preliminary testing.

2.3.1. Screening for phosphates activity

Utilizing hydroxyapatite (soil-separated agar) as the screening medium, segregated unadulterated secludes were evaluated for extracellular phosphate production17. Unadulterated societies were streaked into the focal point of sterile hydroxyapatite plates and brooded at 37 ° C for 24 hours. Culture produced in a positive and better zone was taken for further research.

2.3.2. Detection of ammonia

Utilizing hydroxyapatite (soil-separated agar) as the screening medium, segregated unadulterated secludes were evaluated for extracellular phosphate production17. Unadulterated societies were streaked into the focal point of sterile

hydroxyapatite plates and brooded at 37 ° C for 24 hours.

2.3.3. Enzyme esterase detection

Limits were spot-inoculated on supplement agar and MCP agar plates containing the first Eder emulsion in water for detection of propellant esterase, and the plates were afflicted at 30 ° C. for 48 hours to induce esterase development watches. Separates were spotted on supplements containing 10% (v / v) nut oil liquid emulsion and MCP agar plates and incubated at 30 ° C19 for 48 hours to confirm that they were not biodegradable.

2.3.4. Varied MCP concentrations have different effects on the breakdown process.

Determine the effectiveness of MCP concentration for degradation of bacterial isolates. Degradation experiments inoculate MSM medium with different concentrations of MCP (100, 250, 500, 1000, 1500, and 2000 ppm) using 1% inoculation material and incubate at 30 ° C for 72 hours under static conditions. It was done by doing. After 72 hours of incubation, the samples were inspected and their diameter range was measured 20, 21.

3. ANALYTICAL TECHNIQUES

3.1.FTIR sample preparation for bacteria

Determine the effectiveness of MCP concentration for degradation of bacterial isolates. Degradation experiments inoculate MSM medium with different concentrations of MCP (100, 250, 500, 1000, 1500, and 2000 ppm) using 1% inoculation material and incubate at 30 ° C for 72 hours under static conditions. It was done by doing. After 72 hours of incubation, the samples were inspected and their diameter range was measured 20, 21.

4. FTIR ANALYSIS

Utilizing a Fourier change infrared (FTIR) spectrophotometer (Bruker model, TENSOR27, German, involving OPUS6.5 form programming for Windows) and a helium-neon laser light as an infrared wellspring of discharge compound (MCP) Aqueous examples (15, 30, 45th day) was separated. The dissolvable was vanished utilizing a revolving vacuum evaporator. The items were redissolved in CH3)2CO. Subsequent to washing with ethyl acetic acid derivation, a drop of this example in CH3)2CO was set between two sodium chloride circles. The foundation range of CH3)2CO was fitted utilizing test range 1.

5. RESULTS AND DISCUSSION

We examined the physicochemical properties of MCP-debased soil tests from nine unique horticultural structures (Table 1). The degrees of macronutrients and micronutrients at Sites 1, 4 and 8 are exceptionally high. The pH went from feebly acidic to pitifully soluble (5.38.4). Four soil tests were impartial and reasonably soluble (6.67.3), three were acidic (45) and two were genuinely antacid (6.67.3). (7.98.4). The adjustment of pH from impartial to antacid is because of the response of treated compost material with soil colloids, bringing about a fundamental response to the dirt trade complex22.

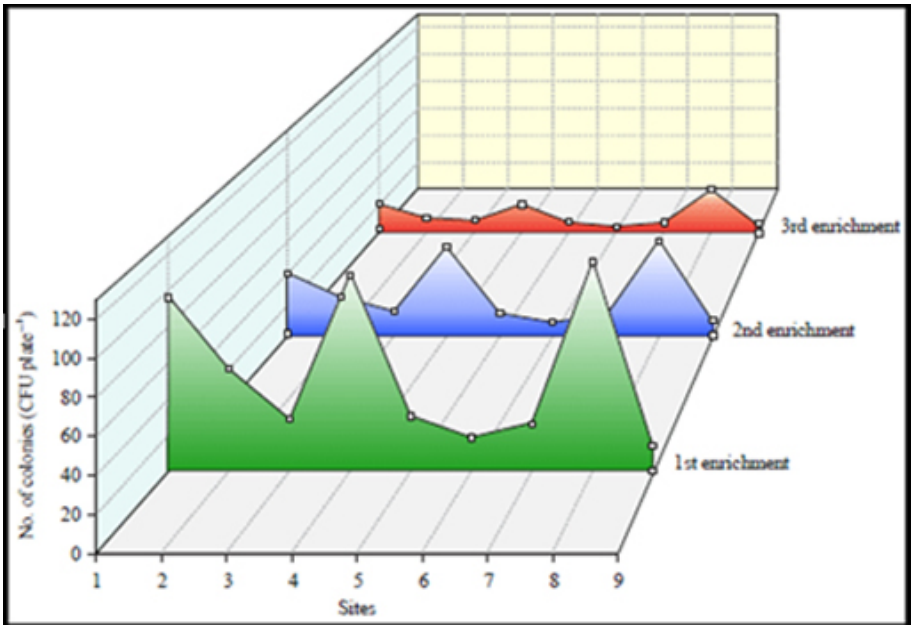

Figure: 2 Isolation of MCP-degrading bacteria using an enrichment approach.

Soil samples	EC	pH	N (kg ha⁻¹)	P (kg ha⁻¹)	K (kg ha⁻¹)	Zn (kg ha⁻¹)	Cu (kg ha⁻¹)
Site1	0.60	7.70	59	31.30	15	12.5	50.0
Site2	0.10	7.74	51	7.00	11	10.0	12.0
Site3	0.09	7.80	48	9.00	8	5.4	9.0
Site4	0.40	8.10	57	19.00	15	12.5	40.0
Site5	0.02	6.80	31	5.00	10	12.5	15.0
Site6	0.30	5.90	53	11.00	9	3.5	12.0
Site7	0.12	5.30	12	6.00	7	1.5	21.0
Site8	0.60	8.12	61	17.00	14	12.5	42.0
Site9	0.10	5.90	51	11.00	10	3.5	17.0
Mean	0.25	7.04	47	12.92	11	8.21	24.2
Range	0.12-2.3	6.9-8.3	21-140	7.5-25	62.5-90	79.012	64.026

Table: 1 Physical and chemical parameters of soil samples gathered.

5.1. EC: Conductivity of electricity

When the average value was 0.25dSm-1, the electrical conductivity was 0.022.3dSm-1. The amount of nitrogen available ranged from 1261 mgkg-1 and averaged 47 mgkg-1. Low nitrogen conditions in soil can be caused by a lack of soil organic carbon. The available amount of phosphorus ranged from 5 to 31.3 kg ha-1 and averaged 12.92 kg ha-1. Potassium availability ranged from 7 to 15 kg ha-1 and averaged 11 kg ha-1. Macronutrient and micronutrient levels are high at sites 1, 4, and 8.

Degradation of pesticides can be slowed down by a variety of conditions, including lack of necessary nutrients. Microbial activity and MCP degradation have been shown to be positively affected by carbon and more energetic chemicals in the biomix23. Some pesticides can be improved by biological stimulation of the soil. The presence of nutrients required for the bacterial biodegradation activity ofin the analyzed soil samples was consistent with previous studies by Tortilla et al.24. Coppola et al. 25 reports similar results.

In the current study, the number of MCP-degrading bacteria increased significantly during the first week of enrichment. The total number of plates ranged from 14.2 to 115.6 CFU per plate. The ability of all bacterial isolates to grow in mineral salt medium supplemented with 500 mg-1 monocrotophos was tested (Figure 1). Soil samples at Sites 1, 4, and 8 showed the fastest compound annual growth rates. In the initial concentration method, the number of colonies was the highest at 115.6, 108.7, and 96.2, respectively. The first enrichment method, 14.2 cfu plate-1, revealed an inadequate growth rate at site 9. Based on multiple morphological and biochemical screening methods, 115 bacterial cultures were divided into 20 separate bacterial groups.

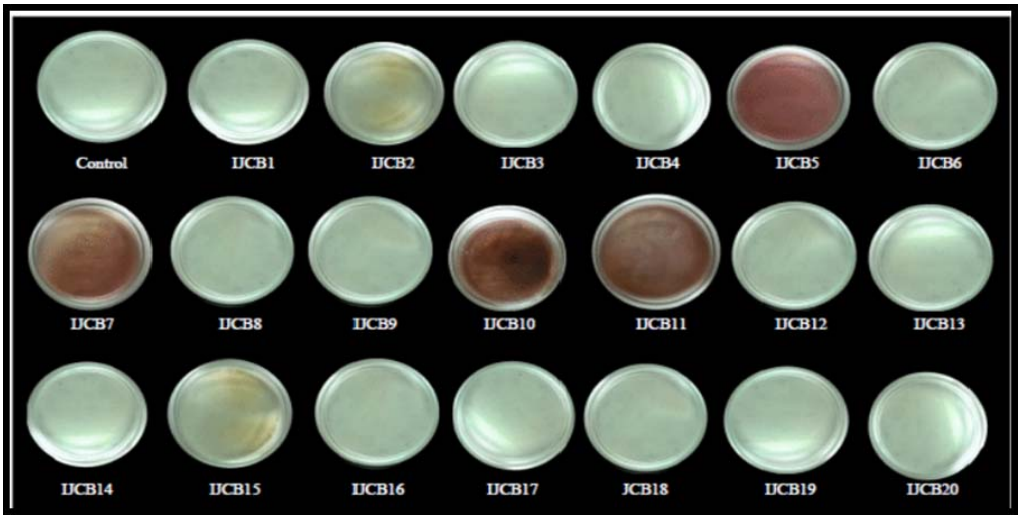

Figure: 3 Ammonia detection.

Gram-negative and Gram-positive microorganisms were found, yet Gram-negative microbes were more normal. In an identical review, Nap hade et al. 26 tracked down five morphologically unmistakable pesticide-safe bacterial states. These outcomes demonstrate

that distinctions in all out-plate numbers were distinguished and that MCP-corrupting microbes filled essentially in MCP-defiled soil and in high convergences of NPK compounds (nitrogen, phosphorus, potassium)1. From previously treated soil, Rangaswamy and Venkateswaralu27 have identified Bacillus spp. That degrades MCP. MCP-degrading algae were identified in soil contaminated with Megharajetal.28. The majority of the bacterial strains that could be cultivated in this study were in the most commonly reported genus Bacillus, Pseudomonas. And Enterobacter sp.15. All pure bacterial isolates were sequentially subculture in mineral salt medium 16. Similarly, microorganisms in different environments have been identified that degrade organ phosphorus compounds in laboratory cultures and soil22. The five isolates were found to be able to use MCP as a food source to produce phosphate. The results show that soil is a rich source of undiscovered microorganisms, with the genus Bacillus being most commonly isolated from soil29. Since MCP is known to be harmful, the ability of organisms to degrade MCP has been further studied (Table 2). Various researchers have associated these types of bacteria with phosphates production 1,14,30. Ammonia emissions were moderate with BSGN015. By the synthesis of ammonia 18, the initial pH (pH 6.8) of the medium was adjusted to alkaline (pH 7.88.0).

Micro-organisms	Screening for phosphatase activity presence of phosphate solubilizing zone	Detection of ammonia color change to yellow to pink	Detection of enzyme esterase clear zone around the colony
BPGP001	-	-	-
BKGN002	-	++	-
BVGN003	-	-	+
BAGP004	-	-	-
BAGN005	+++	+++	+++
BSGP006	-	-	-
BKGP007	+++	+++	+++
BPGP008	-	-	-
BKGN009	-	-	+
BVGN010	+++	+++	+++
BAGN011	+++	+++	-
BAGN012	-	-	-
BSGP013	-	+	-
BKGP014	-	-	-
BSGP015	++	++	+
BSGN016	-	-	-
BPGN017	-	-	-
BPGN018	-	-	-
BVGP019	-	-	+
BVGN020	-	-	-

Table: 2 Monocrotophos-degrading bacterial isolates were tested.

Therefore, the tint of the medium changed from yellow to pink. No such variety change was seen on the control medium. The age of inconvenience and the arrival of alkali were exhibited by the variety shift of the pointer phenol red from yellow to pink on the monocrotophos plate.

Methylamine, phosphates, smelling salts, unstable unsaturated fats, carbon dioxide, and unidentified mixtures have been recognized as intermediates in MCP corruption by bacterial culture30. Furthermore, Bhadhade et al. 31 observed that ammonium sulfate was taken out from the medium so the soluble state demonstrated by the pink tint of the medium was not brought about by the ammonium particles of ammonium sulphate31.

6. CONCLUSION

It is resolved that bacterial disconnects from pesticide-tainted soil tests can be utilized for monocrotophos biodegradation and ecological bioremediation1. Seven MCP degrading organisms were isolated from contaminated agricultural areas in this investigation, and they were found to be a promising agent for pesticide bioremediation. As a result, novel microorganisms should be thoroughly evaluated for bioremediation and plant growth stimulation.

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