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Original Research Paper

Life Science

AN ANALYSIS STUDY ON MONOCROTOPHOS PESTICIDE DEGRADING BY SELECTED BACTERIAL ISOLATES

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ABSTRACT During the past two decades, global pesticide usage has grown significantly. This leads to the frequent presence in groundwater and surface waters of pesticide residues and process products. Soil samples have been obtained from locations of various agricultural fields affected by pesticides. The research has been microbial and enzymatic in soil of agricultural fields for the breakdown of monocrotophos (MCP). These isolates and their equimolar combination verified the MCP's degradation by utilizing analytic techniques, fourier FTIR, 15th, 30th and 45thday infrarote spectroscopy. The macro and micronutrient levels of locations 1, 4 and 8 are greater. Total of 20 bacterial strains using MCP as a source of carbon and phosphorus were identified by enrichment technique using mineral salt medium. The findings of this research show that hydroses of the peaks of 2649.19 and 2115.72 cmG1 were BAGN005, BKGN 007, and BVGN 010, which shows MCP's degradation and were chosen as efficient candidates for the bio-augmentation of soil pollutants from MCP. In this research, monocrotophos are reported as a potential bioremediating agent for contaminated settings that degrade bacterial isolates. In the degradation of MCP, indigenous strains Bacillus subtilis, Bacillus licheniforms, BKGN007 and Pseudomonas stutzeri, BVGN 010. This is the first research on MCP degradation of Bacillus tequilensis bacterial species, as far as our knowledge is concerned.

KEYWORDS : Pesticide, Monocrotophos pesticide, Degrading, Bacterial isolates, Bacillus

INTRODUCTION:

The use of various chemical contributions will be easily linked to present agriculture. In order to make maximum use of agricultural output and to meet needs for larger food stocks of the rapidly increasing human population, alternative classes of pesticides are employed to manage different types of pesticides. Only to the target organism must an ideal pesticide be toxic and recyclable and should not seep into groundwater. This is not unfortunately the situation at all and the widespread usage of pesticides in modern farming is worrying.

Microbial, chemical and photographic deterioration in the environment may destroy the pesticides. However, the primary determinant of organophosphral destiny is microbial breakdown in the environment and frequently the main mechanism of pesticide degradation in soils(Singh et al., 2004;Zrostlikova et al., 2003).

Several investigators examined bacteria's ability to breakdown MCP (Singh et al., 2009). The breakdown of organophosphate compounds is induced by all these enzymes.

The most significant and efficient method of removing these chemicals from the environment is the biological breakdown of pesticides. Substances which lead to structural alterations or total destruction of the target molecule are able to interact, both chemically and physically (Raymond et al., 2001; De Schrijver and De Mot, 1999; Wiren-Lehr et al., 2002).

Biodegradation and application for the bioremediation of soils polluted with Enterobacter strain B-14 chlorpyriphos was described (Singh et al., 2004). Degradation of chlorpyriphes by Arthrobacter species in mineral salts media was reported by Mallick et al. (1999). Two soil fungus, Trichodermaviridae and Aspergillusniger, have been reported to have been destroyed successfully (Mukherjee and Gopal, 1996). Recently the detoxification of this herbicide is reported to be effective (Mukherjee et al., 2004).

Objectives:

- To isolate soil bacteria that can degrade MCP
- To determine their potential effectiveness in the Soil
- To study describes recent advances in biodegradation of MCP by addressing the biology and molecular characterization of some MCP degrading bacteria
- To study the microbial culture has great potential utility for the bioremediation of agricultural soil contaminated with organophosphorus pesticides

MATERIAL AND METHODS:

Physico-chemical Properties Of The Soil:

The materials were air dried, crushed and sifted using a 2 mm screen before examination at room temperature. Hydrometer was used for determining particle size while soil pH was measured using 1:1 water suspension soil by use of a pH meter. Particle size Wet oxidation technique was used to quantify organic carbon whereas total nitrogen, macro-kjeldahl method and phosphorus availability were produced by Bray-P.1 method.

Chemicals And Reagents:

In advancement contemplates, the specialized compound (virtue 75.07 percent) of monocrotophoswas utilized and bought from United Phosphorus Ltd, Gujarat , India. All substance and reagent composition, and techniques for media preparation were utilized in this study.

Collection Of Soil Samples

Soil tests from 8–10 spots from each field was gathered arbitrarily from seven examining locales (ranchers' fields) from various destinations, and completely blended to set one up composite example. They are gathering tests from the 15 cm upper soil layer. The example evacuates plant material and different flotsam and jetsam by hand, and the soil is sieved utilizing a 4 mm work. Tests are taken to the lab and put away at 4 ° C until investigation is performed. At the hour of experimentation, tests are brought to room temperature, and enacted before further use.

Soil Analysis

The entirety of the investigations is completed in copies. The soil tests gathered was examined for different boundaries like pH, dampness content, soil surface, all out nitrogen, natural carbon, phosphorus, replaceable potassium , calcium , magnesium, and sodium.

Isolation And Screening Of Monocrotophos Degrading Microorganism

10 g soil test was suspended in 30 ml of sterile BMM that contains 50 g/ml of monocrotophos in each 250 ml Erlenmeyer carafe. All the cups are hatched with a revolving shaker (200 rpm) at 37 ° C for 10 days. One flagon was evacuated every day during experimentation to check the monocrotophos spectrophotometric degradation (Double Beam UV-VIS, UV 570 4SS). Following ten days of incubation the supernatants are weakened in arrangement up to 10-9. Spread 100 l of 10-7 weakened soil suspension inoculum over BMM-agar plate enhanced with monocrotophos (50g/ml) and brooded at 37oC for 24hrs. It was choose the BMM-monocrotophos plates which demonstrated the presence of bacterial colonies for additional investigation. To acquire their unadulterated societies, the bacterial colonies that showed up on the plate BMM-monocrotophoswas evacuated aseptically and reselected on plate BMM-monocrotophos. Colonies were checked, and CFU/ml was resolved for each culture. As demonstrated by the most elevated CFU/ml, the moderately effective degraders of monocrotophos were chosen dependent on their most elevated development.

The CFU/ml was calculated by using the following formula:

$$\text{CFU/ml} = \frac{\text{No. of colonies x Reciprocal of dilution factor}}{\text{Volume of culture taken for spreading}}$$

Residues Analysis by HPLC

The degradation capacity of the chose disconnects is confirmed by HPLC (Shimadzu LC-10 ATVP Binary Gradient System , System Controller SCL-10 AVP – Model, UV-VIS Detector – SPD–10 AV vp-Model, Liquid Chromatograph LC–10 ATVP-Model) Study 20 was seek after the common convention for investigation of HPLCs. L of the methanol water (70:30, v/v) is infused into a C18 segment HPLC framework with molecule measurement of 5 m. The stream rate is kept at 1ml, min-1. The concentration of monocrotophoswas determined by contrasting pinnacle zones of test chromatography and standard chromatogram top regions (Bishnoi et al., 2005).

$$\text{Concentration of monocrotophos in sample (µg/ml)} = \frac{\text{Peak area of chromatogram of sample}}{\text{Peak area of chromatogram of standard Monocrotophos}} \times \text{Concentration of standard Monocrotophos}$$

The percent degradation of monocrotophoswas assessed by subtracting leftover monocrotophos in experiments from the respective controls

$$\text{\% Monocrotophos degradation} = \frac{\text{Concentration of Monocrotophos in control} - \text{Concentration of Monocrotophos in experimental}}{\text{Concentration of Monocrotophos in control}}$$

RESULTS:

The physicochemical characteristics of soil samples contaminated with MCP were analysed from nine distinct farm types (Table 1). There were four neutral and moderately alkaline soil samples (6.6-7.3), three acidic soil samples (4-5) and two moderately alkaline soil samples (7.9-8.4). The neutral pH of the fertilizer material applied to soil colloids may be ascribed to the response of the basic cation to the exchangeable compound of the soil.

Table 1: Physio-chemical Properties Of Collected Soil Samples

Soil samples	EC	pH	N (kg haG^{1})	P (kg haG^{1})	K (kg haG^{1})	Zn (kg haG^{1})	Cu (kg haG^{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

EC: Electrical Conductivity

The conductivity ranged with a mean value of 0.25 dSmG1 from 0.02 to 2.3 dSmG1. The available status of nitrogen ranged between 12-61 mg kgG1 and an average of 47 mg kgG1. A low level of organic carbon in the soils may be caused by low levels of soil nitrogen. The accessible content of phosphorus ranged from 5-31,3 kg haG1 to 12,92 kg haG1 on average. The status of Potassium available ranged between 7-15 kg haG1, averaging 11 kg haG1. The macro and micronutrient levels of locations 1, 4 and 8 are greater. In the current research, the number of MCP bacteria in the 1st week of enrichment was significantly increased. The total number of bactéries ranged from CFU plateG1 14.2 to 115.6. The capacities of mineral salt medium modified by 500 mg G1 monocrotophos have been tested in all bacterial isolates (Figure 1).

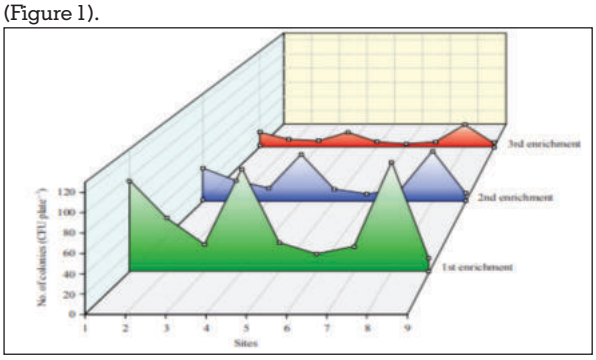

Figure 1: Monocrotophosenhanced Microbe Degrading By Isolation Technique

The first enrichment method, 14.2 CFU plateG1, indicated the poor growth rates at site 9. Based on the various morphological and biochemical screening methods, all 115 cultures were divided into 20 different body types. The phosphate solubilizing activity on the hydroxyapatite medium was present in all 20 isolated strains of bacteria. BAGN005, BKGN007, BVGN010 and BAGN011 are the highest area. Better zone obtained from the platform test revealed a solubilization in isolated cultivation of phosphates. In this study, the phosphate solubilizer was isolated by contaminated soil samples from pesticides since the soil was exposed to phosphate. Phosphate solubilizing capacity by producing extra cellular phasphatase exists in the bacterial strains isolated from agricultural soil contaminated with pesticides. Based on a solubilization effectiveness test, a better zone was assessed by the bacterial strains from the isolated samples. In this study, the medium containing MCP was given a color change, which indicated that the 20 bacterial isolates were inoculated to the medium and that ammonia was produced as an end product. Figure 2 depicts the ammonia production results such as the highest ammonium production BAGN005, BKGN007, BVGN010, BAGN011.

The result shows that the MCP as nutrient source and phosphate production are capable of being used by five isolates. Soil is therefore an excellent sources of unknown microorganisms, and soil is most often isolated from soil29. The genus Bacillus is studied. The ability of organisms to split MCP has been analyzed further since MCP is known to be toxic (Table 2).

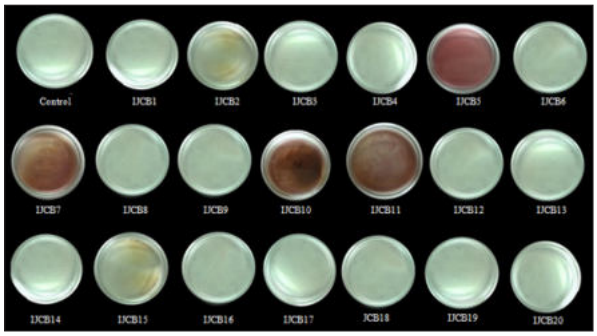

Figure 2: Detection Of Ammonia

Table2: Screening Of Monocrotophos Degrading Bacterial Isolates

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

These bacterial species were involved by different researchers in the production of phosphatase. Moderate ammonia production was performed by BSGN015. The medium's original pH (pH 6.8) had been repaired by the formation of ammonia18 into alkaline (pH 7.8-8.0). The color of the medium changed from yellow to rosé. In the control medium, no such color changes were observed. The monocrotophos container plates had shown that urease was produced and ammonia was released, because a change in phenol red color from yellow to pink was observed.

Kinetic studies of growth and degradation have shown that the only carbon source is the low salt medium containing 1000 ppm of monocrotophos. In the entire MCP concentration tested from 500 ppm to 1500 ppm the degradation was above 85 percent (Figure 4).

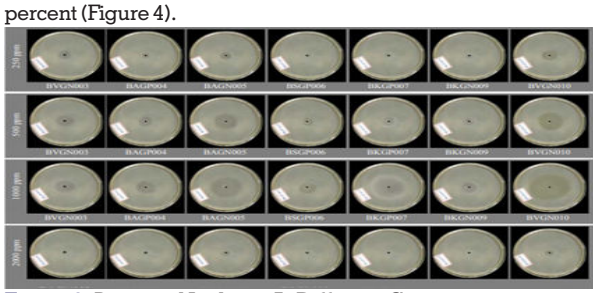

Figure 3: Bacterial Isolates At Different Concentration

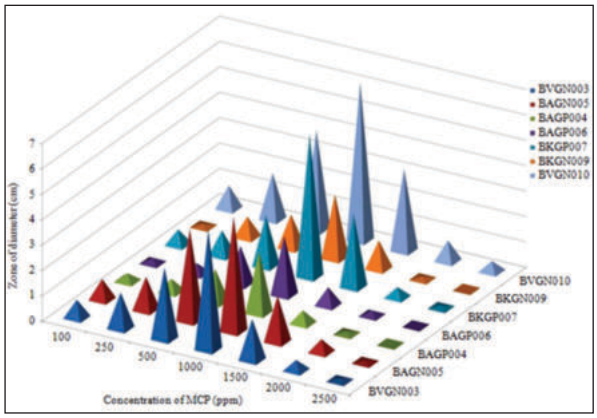

Figure 4: Different Concentration Of MCP Degradation Process

A clear area of 1% (v/v) tributyrin around the colonies was observed on the monocrotophos agar plate, indicating that the enzyme esterase was produced For BAGN005, BKGN007 and BVGN010, the highest clearance zone was observed. Nutrient and monocrotophos agar plates with groundnut oil showed no clearance zone around colonies that did not have lipolytic activity. The structural changes in the MCP are achieved by FTIR spectroscopy. BAGN005, BKGN007 and BVGN010. Figure 5-8 illustrates the IR spectrum of the molecule and its products.

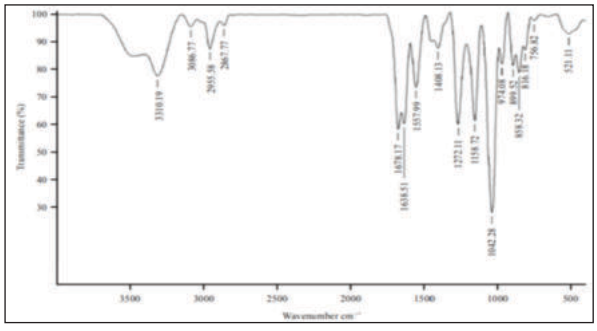

Figure 5: Control Sample Through FT-IR Spectrum

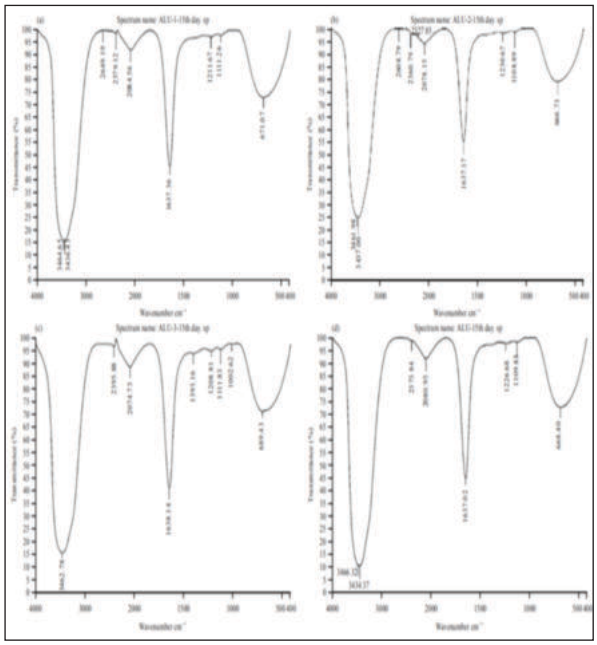

Figure 6(a-d): Extracted 15th day FT-IR spectrum from Pseudomonas stutzeri (BAGN005), Bacillus licheniforms (BKGP007), Bacillussubtilis (BVGN010), Consortium (Mixed culture)

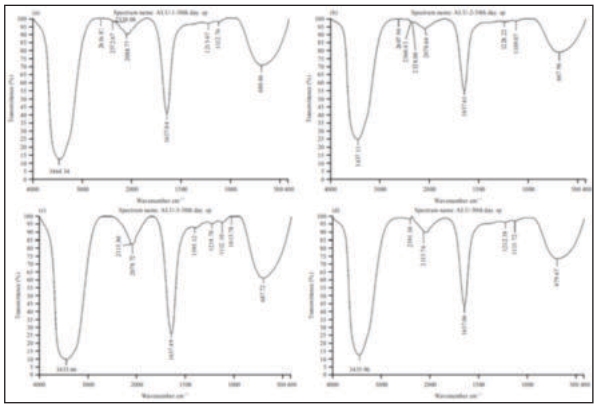

Figure 7(a-d): FT-IR spectrum of extracted 30th day from Pseudomonas stutzeri (BAGN005), Bacillus licheniforms (BKGP007), Bacillussubtilis (BVGN010), Consortium (Mixed culture)

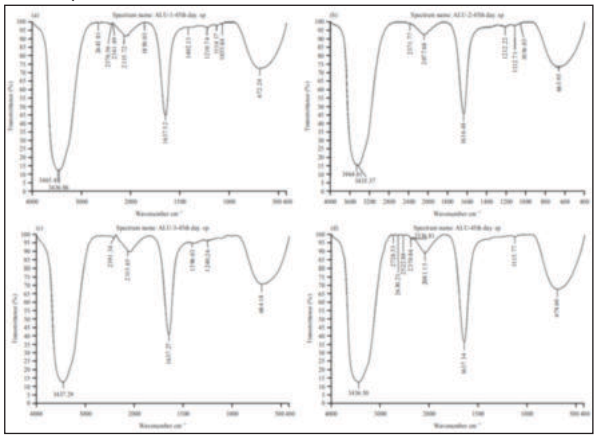

Figure 8(a-d): FT-IR spectrum of extracted 45th day from Pseudomonas stutzeri (BAGN005), Bacillus licheniforms (BKGP007), Bacillussubtilis (BVGN010), Consortium (Mixed culture)

DISCUSSION:

The rate of degradation, including lack of essential nutrients, may be restricted by several factors. More useful compounds in the bio-mix were widely shown to have positive effects of carbon and energy in the microbial activity and MCD (Williams, &Cofie, 2015). The degradation of several soil pesticides could improve by bio-stimulation. The presence of a required nutrient for bacterial biodegradative activities in the examined soil samples was consistent with earlier research by Tortella et al (2010). (2010). Coppola et al. found similar findings (2007). (2007). Both Gram negative and Gram positive bacteria were collected but Gram negative bacterial strains were prevalent. Naphade et al. (2012) identified five morphologically distinct pesticide tolerant bacterial colonies in a comparable study. These findings indicate that variations in total bacterial counts were detected, and that MCP degrading bacteria grew primarily in MCP contaminated soil and in greater concentrations of NPK (nitrogen, phosphorous, and potassium) compounds.

The MCP isolating Bacillus sp., isolated from previously treated soil, was Rangaswamy and Venkateswaralu. Megharaj and colleagues isolated MCC algae from polluted soil (1987). Bacillus sp., Pseudomonas sp., and Enterobacter sp. were found to be the most often reported cultivable bacteria strains in the research. Sequentially subcultures into the mineral salt medium were all pure bacterial isolates. Several bacterial species have also been isolated from various environments, which degrade the compound of organophosphorus in laboratory and soil cultures.

CONCLUSION:

It should be concluded that the biodegradation and bioremediation of monocrotophos from the pesticide contaminated soil samples may be exploited. In this report, seven MCP degrading organisms have been isolated as the promising agent for the bioremediation of pesticide contaminated environments from contaminated agricultural sites. Therefore a new microorganism for bioremediation and plant growth stimulation should be intensively screened.

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