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Research Article

Screening of Efficient Monocrotophos Degrading Bacterial Isolates from Paddy Field Soil of Sivagangai District, Tamil Nadu, India

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Abstract

Background and Objective: The soil samples were collected from the pesticides contaminated sites of different agricultural farms in Sivagangai district, Tamilnadu, India. The present study has been worked microbial and enzymatic for the degradation of monocrotophos (MCP) in soil of Agricultural farms. Bacterial transformations have been the main focus of our study on monocrotophos pesticide degradation. **Methodology:** Physico-chemical properties of monocrotophos (MCP) contaminated soil samples were analyzed. Enrichment techniques were used to isolate the monocrotophos degrading microbes. Preliminary screenings of monocrotophos degrading bacterial isolates were analyzed, such as screening for phosphatase activity, detection of ammonia, enzyme esterase detection. Effects of different concentrations of MCP in degradation process were measured. The degradation experiment was carried out by inoculating the MSM medium with different concentration of MCP. The MCP degradation by these isolates and their equimolar mixture were confirmed by using analytical tools, fourier transform infrared spectroscopy (FTIR) for 15th, 30th and 45th day. **Results:** Site 1, 4 and 8 have the higher amount of macro and micro nutrient levels. Totally 20 different bacterial strains were isolated using mineral salt medium containing MCP as a carbon and phosphorus source by enrichment method. About 25% of isolates have the ability to produce phosphatase activity and 35% of Isolates have both ammonia and esterase enzyme production. Based on the MCP tolerance, exhibited in gradient plate assay the isolates BAGN005, BKGN007 and BVGN010 were identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Pseudomonas stutzeri*, respectively. The results of this study demonstrated that BAGN005, BKGN007 and BVGN010 and mixed culture were hydrolase the peaks at 2649.19 and 2115.72 cm^{-1} which indicates MCP degradation and selected for further study as efficient candidates for bioaugmentation of MCP contaminated soil environments. **Conclusion:** In this study, it reports on monocrotophos degrading bacterial Isolates that have been proven as the promising agent for bioremediation of pesticide polluted environments. The indigenous strains *Bacillus subtilis* (BAGN005), *Bacillus licheniformis* (BKGN007) and *Pseudomonas stutzeri* (BVGN010) were quite efficient in degrading the MCP. It is the first study on MCP degrading capabilities of bacterial species *Bacillus tequilensis*, as far as our knowledge goes.

Key words: Soil, pesticide, bacterial isolate, biodegradation, FTIR analysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Organophosphorus pesticides are widely used in India for protection of agricultural yields. Excessive levels of organophosphorus pesticides can result in degradation of soil quality, reduction of crop yield and inferior agricultural products, consequently posing significant hazards to ecosystem health, humans and animals. Monocrotophos [dimethyl-(E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate] is an organophosphorus, nonspecific systemic insecticide and acaricide used to control common mites, ticks and spiders by contact and stomach action. Its water-soluble nature helps it penetrate quickly into plant tissue¹. Herbicides and pesticides cause serious damage to life present in water, lack access to safe drinking water excess use of fertilizers. Excess phosphorous results in the eutrophication. Among the pesticides 98% were classified as acutely toxic for fishes and crustaceans². Effect of contamination may result in the loss of biodiversity and functioning of soil like nutrient cycling, weathering, filtering etc. Heavy metals also inhibit microbial activity³. Microbial degradation depends not only on the presence of microbes with the appropriate degradative enzymes, but also on a wide range of environmental parameter. Microorganisms have the ability to interact, both chemically and physically with substances leading to structural changes or complete degradation of the target molecule.

Soil enzyme activity is the direct expression of the soil biological community to presence of available pollutants. Soil enzymes are present in two forms, intracellular (within bacteria and fungi) or extracellular (enzymes immobilized onto soil particles). These enzymes carried out reactions that transform pesticides by oxidation, reduction or hydrolysis, producing metabolites more water-soluble and less toxic than the original compounds⁴.

It highlights the participation of main groups of microorganisms (bacteria, actinomycetes and fungi), which are involved in pesticide biodegradation during the composting processes. Soil enzymes have been reported as useful soil quality indicators due to their relationship to soil biology. There are three main soil enzymes. Such as dehydrogenases, ureases and β -glucosidases, they must be monitored in composting of soil polluted by organophosphates and organochlorines pesticides⁵.

Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. The promising technology which utilizes the ability of microorganisms to remove pollution from the environment eco-friendly, economical and versatile is bioremediation⁶.

The available methods (physical or chemical) are either incomplete or costly. Bioremediation provides a potential tool for restoration of contaminated environment. Bioremediation contribute an eco-friendly, economical and efficient method for detoxification of pesticides^{7,8}.

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

MATERIALS AND METHODS

Physico-chemical properties of the soil: The samples were air dried at room temperature, crushed and sieved through a 2 mm mesh prior to analysis. Particle size was determined by Hydrometer method⁹, while soil pH was determined in a 1:1 soil to water suspension using a pH meter. Organic carbon was determined by Wet oxidation method¹⁰ while total nitrogen was done by, Macro-kjeldahl method¹¹ and available phosphorus by Bray-P.1 method¹². Exchangeable cations (Ca, Mg, Na and K) were extracted with 1N ammonium acetate ($C_2H_7NO_2$). The Ca and Mg were determined by EDTA titration method, while Na and K were determined by Flame photometry¹³. Exchangeable acidity was determined by Titration method¹⁴ while Effective Cation Exchange Capacity (ECEC) was by summation.

Enrichment technique for isolation of monocrotophos degrading microbes: The soil samples were collected from the pesticides contaminated sites of various paddy fields in Sivagangai district, Tamil Nadu, India, which is having a history of repeated pesticide applications. Bacterial isolates degrading monocrotophos were obtained by enrichment culture in the enrichment medium, free of P sources and contains: $NaNO_3$: 2 g, KCl: 0.5 g, $MgSO_4 \cdot 7H_2O$: 0.5 g, glucose: 10 g, $FeCl_3$: 10 mg, $BaCl_2$: 0.2 g, $CaCl_2$: 0.05 g, distilled water: 1 L and 100 mg L^{-1} of monocrotophos at pH 6.8. Monocrotophos contaminated soil (5 g) was used to inoculate 100 mL of enrichment medium amended with 500 mg L^{-1} MCP and kept for incubation at $28 \pm 2^\circ C$ for 7 days rotatory shaker. Ten milliliters of the enriched samples were transferred to sterile 90 mL medium, to repeat the enrichment procedure to get better degrading strains. Bacterial colonies were isolated by pour plate technique on nutrient agar plates. Isolated bacterial cultures were maintained on agar slopes of the mineral salt medium containing monocrotophos¹.

Morphological identification: Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on nutrient agar and stored at 4°C. Identification of the different bacterial isolates was carried out by the routine bacteriological methods^{15,16}.

Preliminary screening of monocrotophos degrading bacterial isolates

Screening for phosphatase activity: The isolated pure strains were screened for extracellular phosphatase production using hydroxyapatite (Soil extract agar) as a screening medium¹⁷. The pure cultures were streaked at the center of the sterile Hydroxyapatite plates and the plates were incubated at 37°C for 24 h. The observation was made to see the phosphate solubilization zone around the colony. For further study, positive and better zone formed cultures were taken.

Detection of ammonia: The complete mineralization of monocrotophos was further verified by detection of ammonia. The mineral salt medium agar plates containing monocrotophos (500 mg L⁻¹) and phenol red indicator were prepared and inoculated with the culture. The plates were incubated for 8 days at 30°C. After incubation period the plates were observed for the color change from yellow to pink of the medium. The appearance of pink color indicates the production of ammonia. Ammonium sulphate was eliminated from the medium to ensure that the change to alkaline condition indicated by pink color of the medium was not due to the ammonium ions of the ammonium sulphate¹⁸.

Enzyme esterase detection: For the detection of enzyme esterase, the isolates were spot inoculated on nutrient agar and MCP agar plates containing 1% tributyrin in water emulsion, respectively and the plates were incubated at 30°C for 48 h to observe esterase activity. To ensure that the isolates were not lipolytic, they were spot inoculated on nutrient and MCP agar plates containing 10% (v/v) groundnut oil in water emulsion and the plates were incubated for 48 h at 30°C¹⁹.

Effect of different concentrations of MCP in degradation process: To determine the efficiency of MCP concentrations on degradation by the bacterial isolate. The degradation experiment was carried out by inoculating the MSM medium with different concentration of MCP (100, 250, 500, 1000, 1500 and 2000 ppm) with 1% inoculum that was then incubated for 72 h at 30°C under static condition. Samples were observed after 72 h of incubation period and their zone of diameter was measured^{20,21}.

Gene sequencing: The bacterial strains showing promising results in the above said methods were selected and confirmed by 16S rRNA sequencing at MacroGen Inc. Seoul, South Korea. Alignments of 16S rRNA gene sequence of these isolates were done with BLAST search of the National Centre for Biotechnology Information (NCBI). For long term preservation, the bacterial isolates were stored in 40% glycerol at -70°C²⁰.

Analytical methods

Bacterial sample preparation for FTIR: Bacterial isolates degrading monocrotophos were obtained by liquid culture in the enrichment medium, free of P sources and contains: NaNO₃: 2 g, KCl: 0.5 g, MgSO₄·7H₂O: 0.5 g, glucose: 10 g, FeCl₃: 10 mg, BaCl₂: 0.2 g, CaCl₂: 0.05 g, distilled water, 1 L and 500 mg L⁻¹ of monocrotophos at pH 6.8. Isolated bacterial samples were used to inoculate 100 mL of MSM medium amended with 500 mg L⁻¹ MCP and kept for incubation at 28±2°C for 15th, 30th and 45th day, rotatory shaker.

FTIR analysis: Infrared spectra of the parent compound (MCP) and sample after bacterial degradation were recorded at room temperature (25°C) in the frequency range of 4000-400 cm⁻¹ with a fourier transform infrared (FTIR) spectrophotometer (Bruker model, TENSOR-27, German, with OPUS-6.5 version software for Windows) with a helium neon laser lamp as a source of infrared radiation. Aqueous samples (15th, 30th and 45th day) from MCP degradation flasks were extracted with ethyl acetate and solvent was evaporated using a rotary vacuum evaporator. The contents were re-dissolved in acetone. A drop of this sample in acetone was placed in between two sodium chloride discs, after cleaning with ethyl acetate. The background spectrum for acetone was corrected from the sample spectrum¹.

RESULTS AND DISCUSSION

The physicochemical properties of the MCP contaminated soil samples collected from 9 different agricultural forms were analyzed (Table 1). The site 1, 4 and 8 have very high quantities of macro and micro nutrient levels. The pH ranged from moderately acidic to alkaline (5.3-8.4). Four samples were neutral and moderately alkaline (6.6-7.3), three soil samples were acidic (4-5) and two soil samples were moderately alkaline (7.9-8.4). The neutral to alkaline pH may be attributed to the reaction of applied fertilizer material with soil colloids, which resulted in the reaction of basic cation on the exchangeable complex of the soil²².

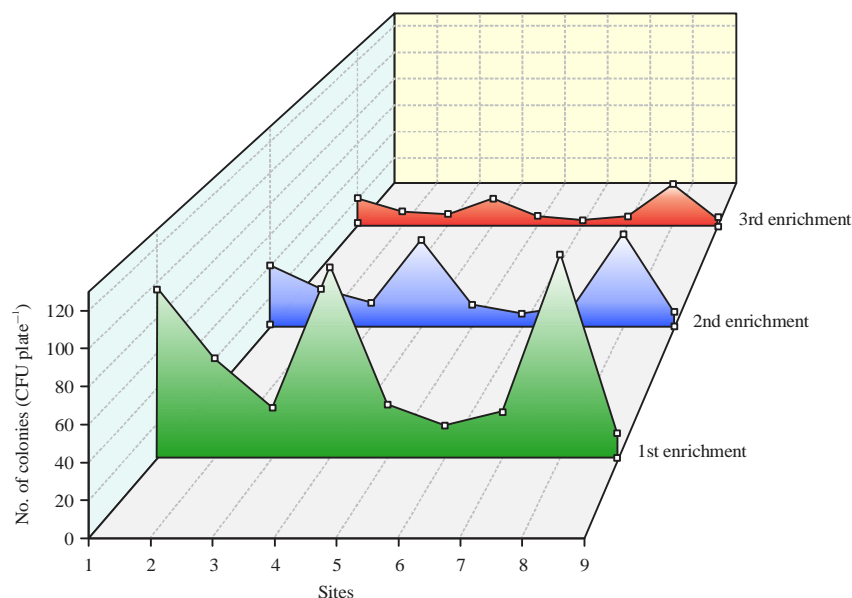


Fig. 1: Enrichment technique for isolation of MCP degrading microbes

Table 1: Physio-chemical properties of collected soil samples

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

EC: Electrical conductivity

The electrical conductivity varied from 0.02-2.3 dS m⁻¹, with a mean value of 0.25 dS m⁻¹. Available nitrogen status varied from 12-61 mg kg⁻¹ with an average value of 47 mg kg⁻¹. Low nitrogen status in the soils could be due to low amount of organic carbon in the soil. The available phosphorus content varied from 5-31.3 kg ha⁻¹ with an average of 12.92 kg ha⁻¹. Available Potassium status varied from 7-15 kg ha⁻¹ with an average of 11 kg ha⁻¹. Site 1, 4 and 8 have the higher amount of macro and micro nutrient levels.

Various factors may limit the rate of pesticide degradation including lack of essential nutrients. Positive effects of carbon and energy more usable compounds in the bio mix on microbial activity and MCP degradation have been widely demonstrated²³. Bio-stimulation can improve the degradation of several pesticides in soil. In the investigated soil samples which was a necessary nutrient for bacterial biodegradative

activities was agreement with the previous studies by Tortella *et al.*²⁴. Similar results were reported by Coppola *et al.*²⁵.

In the present study it was observed that there was a significant increase in MCP degrading microorganisms in the 1st week of enrichment. The total bacterial counts were range from 14.2-115.6 CFU plate⁻¹. All the bacterial isolates were screened for their ability to growth of mineral salt medium amended with 500 mg⁻¹ of monocrotophos (Fig. 1).

The highest average growth rates were observed for site 1, 4 and 8 soil samples. The maximum numbers of colonies were in the first enrichment methods which were recorded as 115.6, 108.7 and 96.2. The poor growth rates were recorded in site 9 by the first enrichment method which is 14.2 CFU plate⁻¹. All the 115 bacterial cultures were categorized under 20 different bacterial groups based on the various morphological and biochemical screening methods.

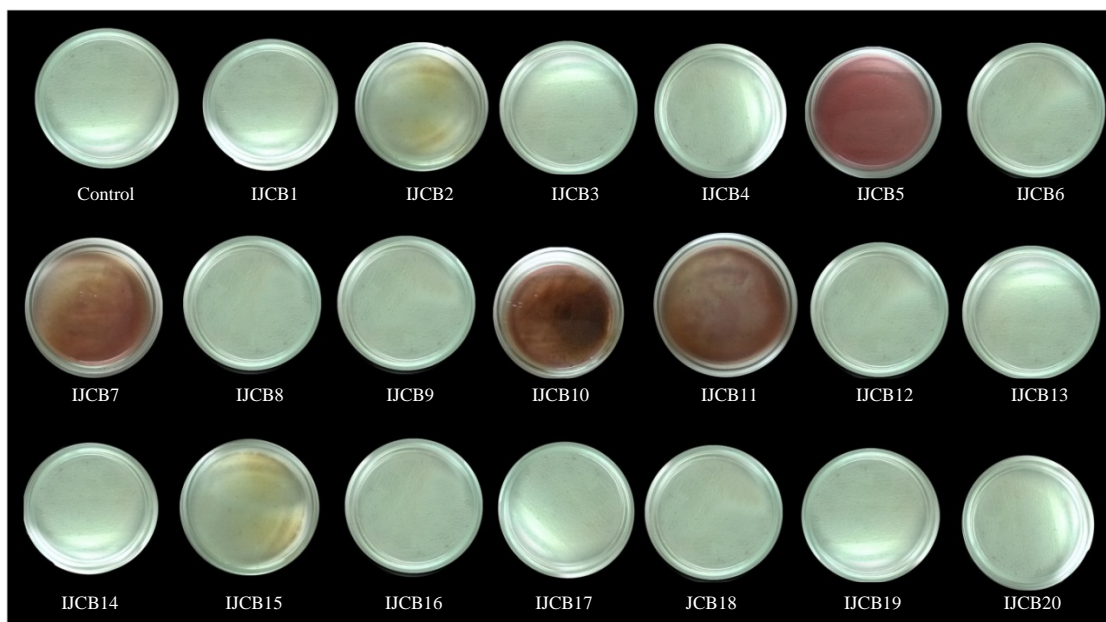


Fig. 2: Detection of ammonia

Both Gram negative and Gram positive bacteria were obtained but Gram negative bacterial strains were dominant. In similar studies Naphade *et al.*²⁶ isolated five morphologically distinguishable pesticide tolerating bacterial colonies.

These results suggests that differences were observed in total bacterial counts and the MCP degrading bacteria mostly grow in MCP contaminated soil and these bacteria were grown in higher concentration of NPK (Nitrogen phosphorus and potassium) compounds¹. Rangaswamy and Venkateswaralu²⁷ isolated a MCP degrading *Bacillus* sp. from previously treated soil. Megharaj *et al.*²⁸ isolated MCP degrading algae from the contaminated soil. In this study it was observed that the majority of the cultivable bacterial strains belong to the *Bacillus* sp., *Pseudomonas* sp. and *Enterobacter* sp., most frequently reported¹⁵. All of the pure bacterial isolates were sequentially subcultures on to mineral salt medium¹⁶. Similarly, several species of bacteria were isolated from different environment which degrade organophosphorus compound in laboratory cultures and in soil²².

All the 20 isolated bacterial strains were subjected to phosphate solubilizing activity on hydroxyapatite medium. The highest zone observed for BAGN005, BKGNO07, BVGN010 and BAGN011. Better zone obtained from the plate assay revealed that the presence of phosphate solubilized in isolated culture. Phosphatase is one of the important enzymes produced from several microorganisms.

In this study pesticide contaminated soil samples were chosen to isolate the phosphate solubilizer since the soil had deposition of phosphate. The bacterial strains isolated from agricultural soil contaminated with pesticides had phosphate solubilizing capacity by producing extra cellular phosphatase. BSGP015, BKGNO02 had the moderate production of phosphatase. From the isolated samples, better zone by the bacterial strains were considered based on solubilization efficiency test¹⁷.

In the present study, when the 20 bacterial isolates were inoculated on medium containing MCP, change in color of the medium was seen, indicating production of ammonia as an end product. Figure 2 shows the result of ammonia production, such as, BAGN005, BKGNO07, BVGN010, BAGN011 had the highest production of ammonia.

The result indicates that five isolates are capable of utilizing MCP as a nutrient source and production of phosphate. Thus, soil is an excellent source for unknown microorganisms and it has been studied that *Bacillus* genus is most frequently isolated from soil²⁹. Since MCP is known to be toxic, ability of the organisms to breakdown MCP was further analyzed (Table 2).

These bacterial species had been implicated in Phosphatase production by different researchers^{1,14,30}. BSGNO15 had the moderate production of ammonia. It had been seen that the initial pH of the medium (pH 6.8) was changed to alkaline (pH 7.8-8.0) due to the formation of ammonia¹⁸. This caused a change in the color of the medium

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 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	-	-	-

from yellow to pink. Such color change was not observed in the control medium. The plates containing monocrotophos had demonstrated the production of urease and release of ammonia, as there was a change in color of an indicator, phenol red from yellow to pink.

Methylamine, phosphates, ammonia, volatile fatty acids, carbon dioxide and one unidentified compound were reported as the intermediates of MCP degradation by bacterial cultures³⁰. In addition, Bhadhade *et al.*³¹ reported that ammonium sulphate was eliminated from the medium to ensure that the change to alkaline condition indicated by pink color of the medium was not due to the ammonium ions of the ammonium sulphate³¹.

A clear zone was observed around the colonies on monocrotophos agar plate containing 1% (v/v) tributyrin which indicated that produced the enzyme esterase. The highest clearance zone was observed for BAGN005, BKGN007 and BVGN010. No zone of clearance was observed around the colonies on nutrient and monocrotophos agar plates containing groundnut oil, indicated that the strain were not lipolytic activity¹⁹.

Barathidasan and Reetha¹⁹ reported that the *Pseudomonas stutzeri* were found to produce methylamine as the metabolite formed by the enzyme esterase, which could be an amidase capable of selecting amides as substrates, since esterase sometimes attack amide linkages. Such metabolite has been reported in the degradation of many organophosphorus pesticides, such as

dicrotophos, which is first demethylated to MCP and then is further degraded with the formation of methylamine³².

By the method described earlier by Harish *et al.*³³ and Bhadhade *et al.*³¹, the degradation was studied at different MCP concentrations³³. The result obtained shows that the degradation process was decreased gradually as the MCP concentration increased. Figure 3 shows the maximum degradation of 95.83% was obtained within 72 h of incubation at 1000 ppm MCP concentration. This has also been reported by Acharya *et al.*²¹.

Growth and degradation kinetic studies proved that able to grow in minimal salt medium containing 1000 ppm monocrotophos as the only carbon source. However, the degradation was above 85% in all the MCP concentration tested with a range of 500 ppm to 1500 ppm (Fig. 4).

These isolates have strong MCP degrading capabilities. Studies by many authors^{21,31,33} showed their pesticide degradability, it was shown that the excess of available phosphorus stimulate the growth of pesticide degrading organisms. Among the 7 bacteria under study, the highest degradation is observed at 1000 ppm by BVGN010. It is the first study on MCP degrading capabilities of bacterial species BSGP006 *Bacillus tequilensis* as far as our knowledge goes. The FTIR spectroscopy gives structural changes of the MCP. Following the chemical screening, the molecular structure extracted from the strains of BAGN005, BKGN007 and BVGN010 were confirmed by FTIR spectroscopy. The IR spectra of the molecule and products are shown in Fig. 5-8.

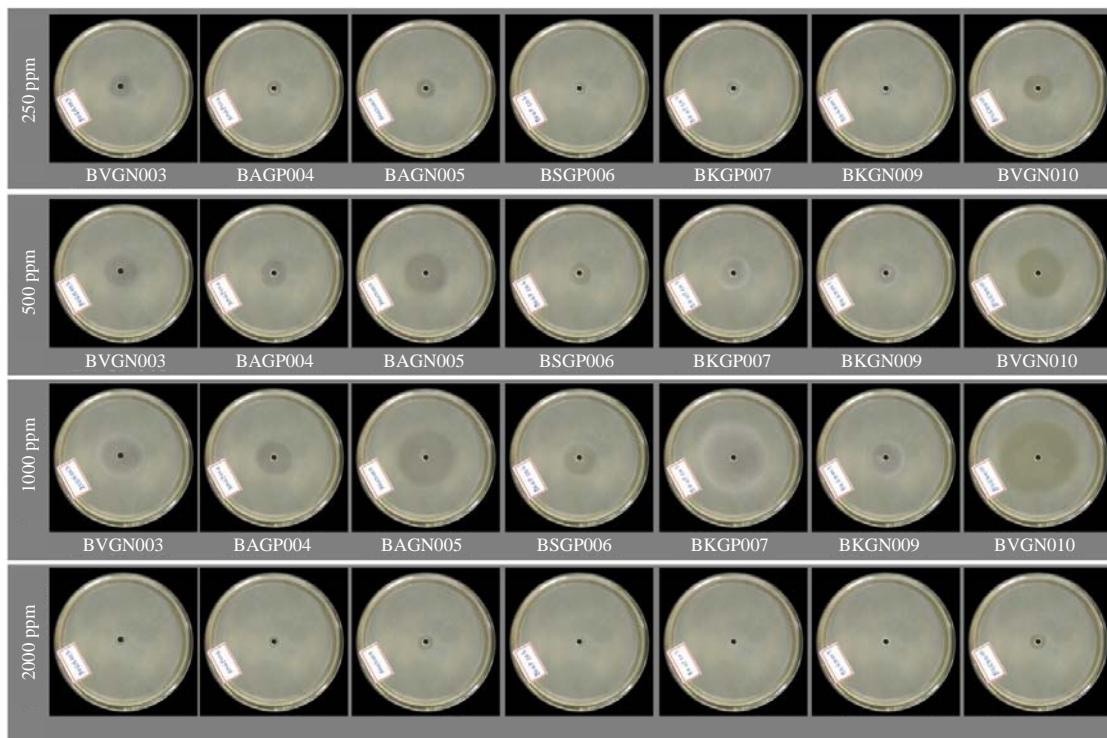


Fig. 3: Bacterial isolates at different concentration

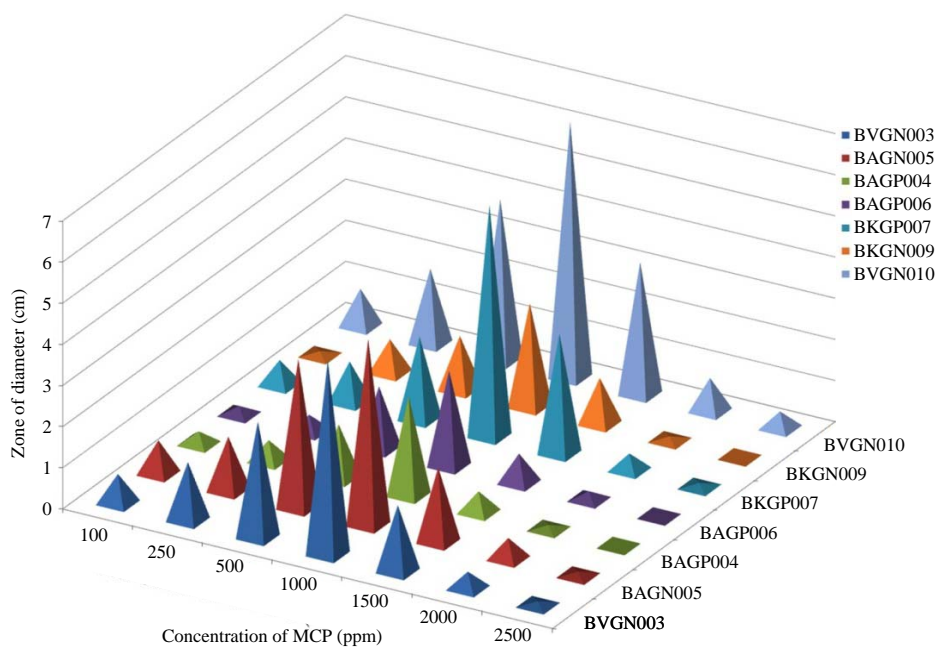


Fig. 4: Different concentration of MCP degradation process

The IR spectroscopic analysis gave further insights into the chemical structure of the polymer and reflects the monomeric units. In this study, the functional groups of the

polymer MCP was confirmed as C=O groups by FTIR spectroscopy. The results obtained by these were exactly similar to that of other researchers³⁴⁻³⁶.

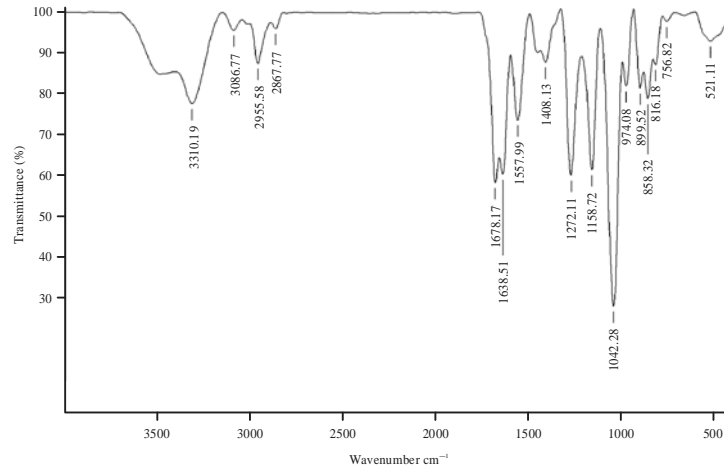


Fig. 5: FT-IR spectrum of the control sample

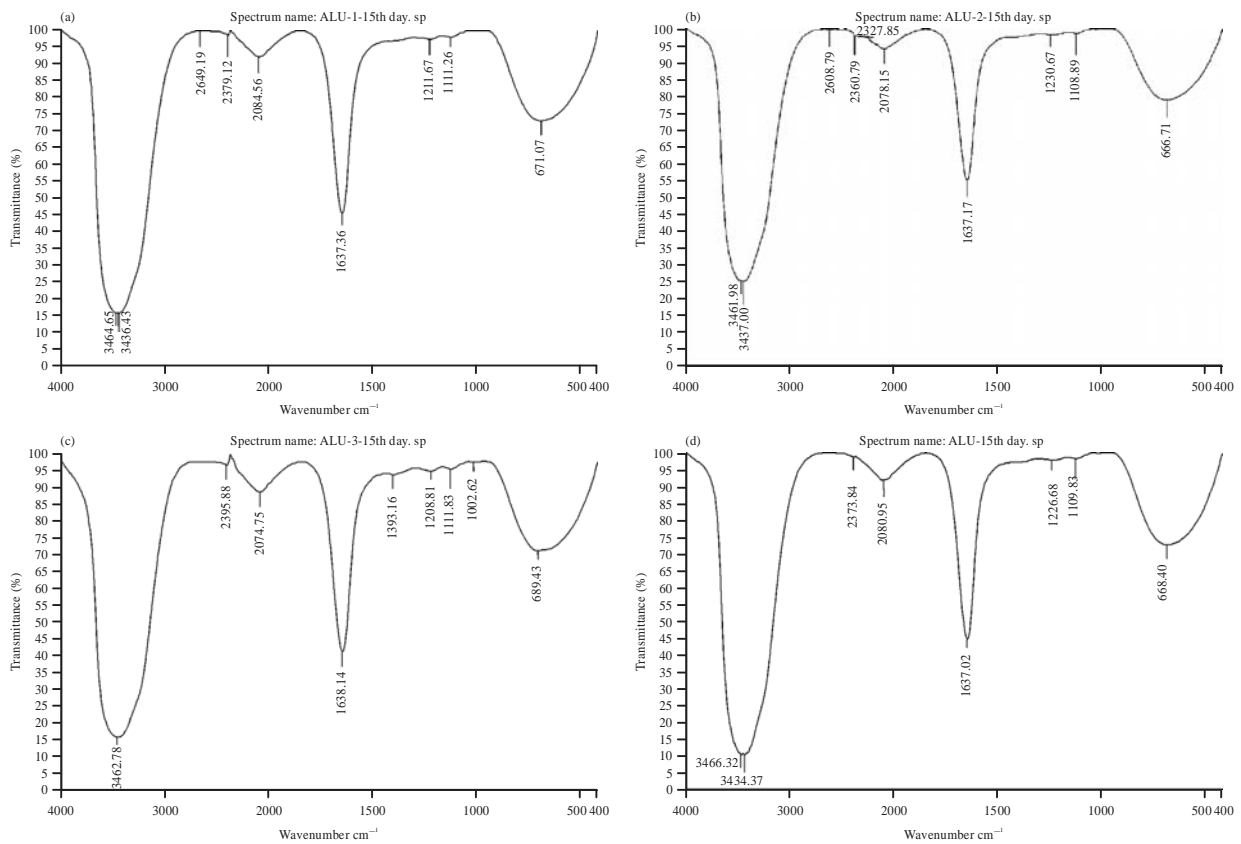


Fig.6(a-d): FT-IR spectrum of extracted 15th day from *Pseudomonas stutzeri* (BAGN005), *Bacillus licheniformis* (BKGP007), *Bacillus subtilis* (BVGN010), *Consortium* (Mixed culture)

The Peaks at 2649.19 and 2115.72 cm^{-1} which indicates the characteristic for vinyl bond were completely reduced in the spectrum after degradation of 15th day of incubation (Fig. 6). The characteristics of inorganic phosphates were noted at a peak of 1121.00 cm^{-1} . The characteristic for aliphatic amines shows at the Peaks of 1114.17 and

1115.77 cm^{-1} . There is some difference between the spectra indicating degradation of MCP and some new peaks were observed at 1002.65, 1055.84 and 1015 cm^{-1} (Fig. 8). About 78 cm^{-1} which were characteristics for NH bond C=O bond and the deformation mode of the N-H bond, respectively.

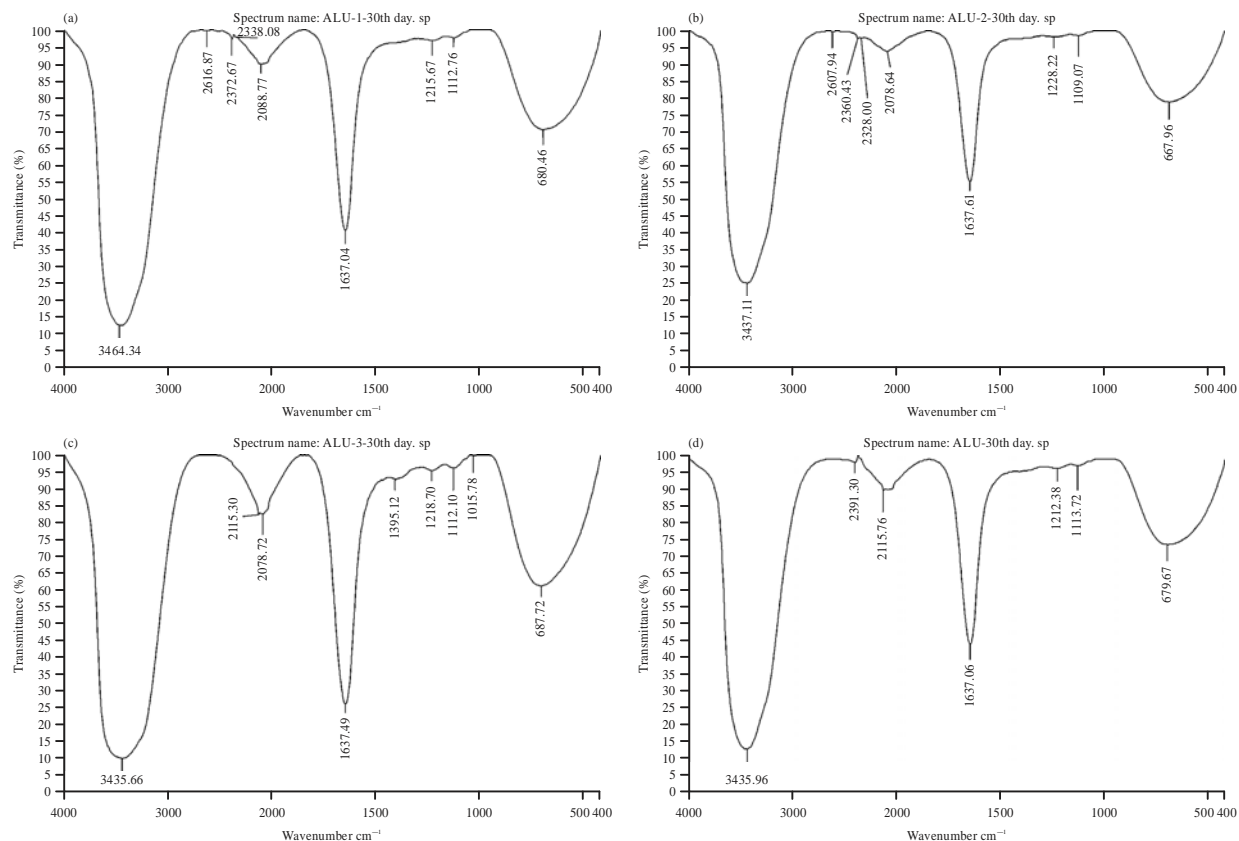


Fig. 7(a-d): FT-IR spectrum of extracted 30th day from *Pseudomonas stutzeri* (BAGN005), *Bacillus licheniformis* (BKG007), *Bacillus subtilis* (BVG010), *Consortium* (Mixed culture)

The adsorption bands obtained were consistent with the report of Bhalerao and Puranik¹. These results were similar to recent findings. These clearly indicate the hydrolytic cleavages of MCP by bacteria. Microbial degradation of MCP has been shown to occur by attack on the aliphatic or light aromatic fractions of the pesticide. Although, some studies have reported their removal at high rates under optimal conditions, high molecular weight aromatics, resins and asphaltenes are generally considered to be recalcitrant or exhibit only low rates of biodegradation³⁷.

The results indicate that in the 15th, 30th and 45th days of incubation gives better MCP indicated mineralization. The residues might get dissipated due to combined impact of abiotic process as well phytoremediation effect. Therefore, higher reduction of MCP has been observed in the 30th day sample. Thus MCP effected complete degradation of parent compound while only traces remain in the media inoculated with *Pseudomonas stutzeri*, *Bacillus licheniformis* and *Bacillus subtilis* as Consortium.

Inoculations with all the species of bacteria after 45 days were compared to control samples. Interestingly, these three

intermediate compounds of bacterial degradation of MCP such as 3-hydroxy-N-methylbutyramide, dimethyl phosphate and methyl phosphate were observed, subsequently indicating that the metabolites were further utilized completely by the bacteria³⁸. The results of the present study suggest that bacterial metabolism of organophosphate insecticides like monocrotophos is highly likely in soil environments only when such chemicals are encountered by the nontoxic levels.

Eight potential phosphate solubilizing bacterial isolates such as BPG001 (*Enterobacter cloacae*), BKG002 (*Pseudomonas stutzeri*), BVGN003 (*Enterobacter cloacae*), BAG004 (*Bacillus paralicheniformis*), BAGN005 (*Pseudomonas stutzeri*), BSG006 (*Bacillus tequilensis*), BKG007 (*Bacillus licheniformis*) and BVGN010 (*Bacillus subtilis*) of field-grown rice were identified based on their 16S rRNA gene sequence analysis, confirmed by MacroGen Inc. Seoul, South Korea. Alignments of 16S rRNA gene sequence from the isolates were done with BLAST search of the National Centre for Biotechnology Information (NCBI). For long term preservation, the bacterial isolates were stored in 40% glycerol at -70°C³⁹.

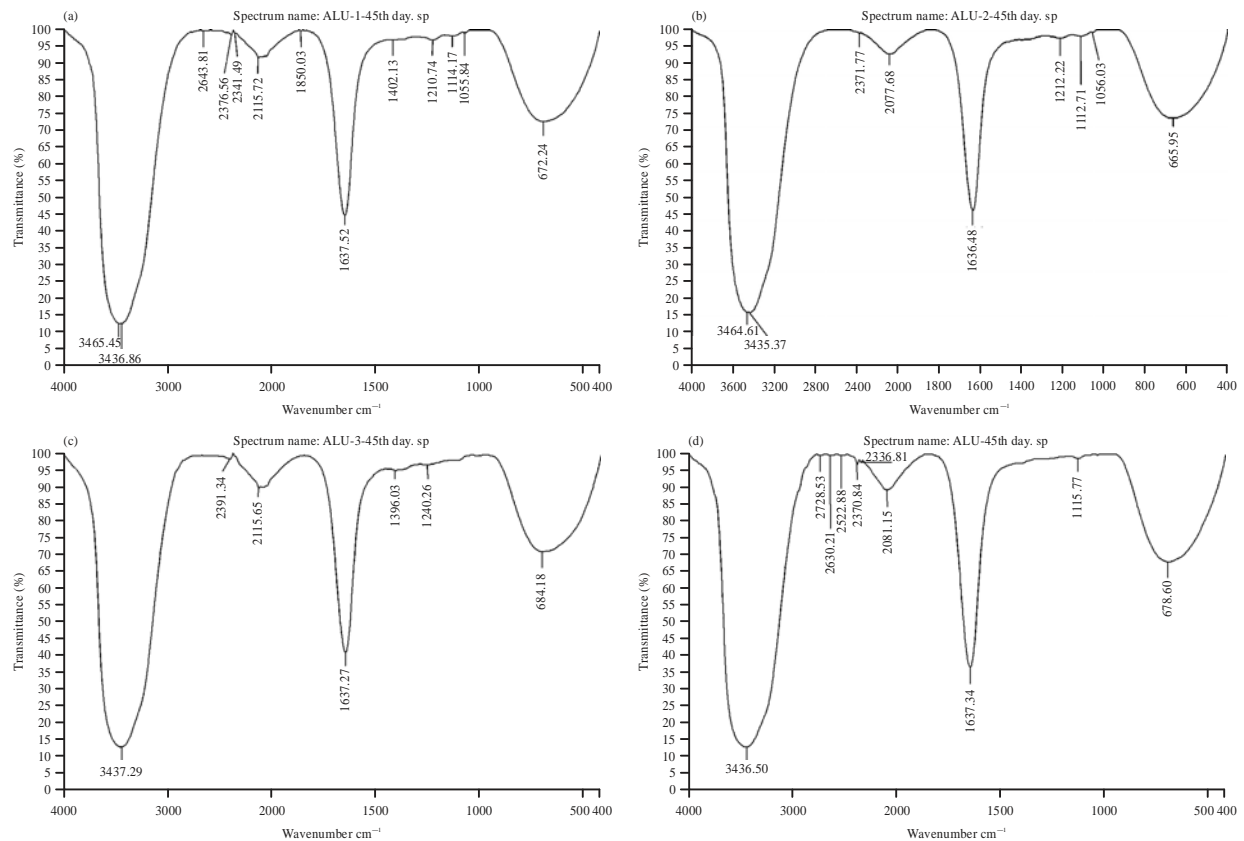


Fig.8(a-d): FT-IR spectrum of extracted 45th day from *Pseudomonas stutzeri* (BAGN005), *Bacillus licheniformis* (BKGP007), *Bacillus subtilis* (BVGN010), *Consortium* (Mixed culture)

CONCLUSION

It is to be concluded that bacterial isolates from the pesticide contaminated soil samples can be exploited in the biodegradation of monocrotophos and bioremediation of the environment. In present study, 7 MCP degrading organism have been isolated from contaminated agricultural sites that have been proven as the promising agent for bioremediation of pesticide polluted environments. Therefore, novel microorganism should be intensively screened for bioremediation and stimulating the plant growth.

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