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Persistence and biodegradation of monocrotophos using soil microbes

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ABSTRACT

The laboratory experiments were performed for determining the natural and induced degradation of organophosphate pesticide (monocrotophos (MCP)) in aqueous medium. The rate of degradation of MCP was analyzed by the determination of residual concentration using UV–Vis spectrophotometer and HPLC. The persistence experiment showed that MCP and its metabolites persist till 120 d in aqueous medium. The degradation pathway has also been proposed on the basis of identification of formed metabolites by gas chromatography/mass spectrophotometry. For biodegradation studies, interesting micro-organisms which are capable of degrading MCP in aqueous stream were isolated from the different contaminated soils of Malwa region of Punjab, India. The isolated microbes were inoculated into minimal media with MCP for 17 d. The result revealed that about 68% of MCP has been successfully degraded in 17 d with isolated microbes and no metabolite has been observed during the biodegradation.

Keywords: Monocrotophos; Persistence; Biodegradation; Micro-organisms; Metabolite

1. Introduction

Since the green evolution, the changes in agriculture practices have resulted in severe increase in pesticides usage worldwide. The widespread use of the pesticides has resulted in the presence of the pesticides and their residues in various environmental matrices [1]. Pesticide residues reach the aquatic environment through direct run-off, leaching, carelessly disposal of empty containers, equipment washing, etc. Pesticide contamination of surface has been well-documented worldwide [2,3], and the toxicity effects of organophosphate pesticides (OPs) were also well recognized by several workers [4–6]. Therefore, these compounds are potential hazards to human health as well as ecosystems by surface and ground water contamination and constitute a major issue of concern.

Monocrotophos (MCP) (dimethyl [(E)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate) is most popular and widely used OP pesticide owing to its low cost and high efficiency in controlling pests mainly on cotton crop, rice, and sugarcane and active against variety of insects in India. MCP is a highly toxic, broad spectrum, fast-acting cholinesterase inhibiting OP insecticide with both systemic and residual contact actions. The Environmental Protection Agency classified MCP as a class I (highly) toxic compound. However, it is continuously used for the control of major pests in agriculture in developing countries like India primarily due to lack of alternative replacements [7]. The total reported national production of MCP in

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India was 5,118 metric tonnes in 2007–2008 [7]. The sorption and degradation of MCP in soils have been reported well [8,9].

Due to the large scale use, persistence and toxicity of MCP, there is a need for analyzing the persistence in water and finding the new and unexploited organisms capable of degrading MCP not only at the laboratory scale but also in the environment. Thus, to enhance the degradation of MCP, the strategy adopted is to employ the micro-organisms which are already the members of natural soil community for degradation. The microbial breakdown of pesticides as carbon and nitrogen sources is an important means of biodegradation and bioremediation. There are only few studies in literature related to persistence and enhanced biodegradation of MCP [10–13]. Earlier work done by our group includes degradation of atrazine by Acinetobacter genus by our group [14], monitoring of atrazine by a new fluorometric assay [15], biotransformation of diuron by bacterial strain Micrococcus [16], and enhanced biodegradation of endosulfan by biosurfactant [17]. The present paper deals with the studies on persistence and induced biodegradation of MCP by microbial population isolated from selected soils.

2. Experimental

2.1. Materials

Commercial samples of technical grade: MCP (72%) was obtained from Crops Chemical Limited, Kotkupura, Punjab (India), and used for present study. All other chemicals are of analytical grade. The most important physiochemical properties of MCP are listed in Table 1.

Three cultivated soils of Malwa region of Punjab, India, were selected and collected from the surface layer of soil (1–15 cm) to isolate microbes from it. These soil samples showed a wide variation in total organic matter and clay and silt content (CSAS) varying from 1.32 to 2.11% and 5.45 to 20.45%, respectively. The electrical conductivity varies from 44.3 to 124.2 $\mu\Omega^{-1}\,cm^{-1}$, whereas less variation was found in bulk density (1.219–1.388%) and pH (7.35–8.84) of the soils.

2.2. Methods

2.2.1. Persistence in the aqueous stream

Experiments in duplicate were preformed in 500 mL dark colored reagent bottle containing 20 ppm of MCP. The bottles were capped and kept in the dark. Aliquots of the solutions were taken at 0, 1, 2, 4, 8, 15, 40, 60, 80, 100, and 120 d and the amount of pesticide remaining was determined. The samples were analyzed by UV–Vis spectrophotometer and HPLC. The metabolites formed during natural degradation were identified by gas chromatography/mass spectrophotometry (GC/MS) technique.

2.2.2. Biodegradation studies

2.2.2.1. Media and culture conditions. Minimal media with the following constituency was used for growth of bacterial cultures at 30°C. It contains 1.6 g disodium hydrogen phosphate, 0.2 g potassium dihydrogen orthophosphate, 1 g ammonium sulfate, 0.2 g magnesium sulfate, 0.01 g ferrous sulfate, 0.02 g calcium chloride, and 0.1 g sodium chloride in 1 L of the solution.

2.2.2.2. Isolation and characterization of bacteria. Serial dilution plating technique was used to obtain different isolates (about 10 strains) from contaminated soils which were screened for their ability to grow on MCP and three strains showed better growth. Minimal

Table 1
Properties of the pesticide used in the present study

Common name	Monocrotophos
Pesticide group	Organophosphate
IUPAC name	Dimethyl [(E)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate
Molecular formula	$C_7H_{14}NO_5P$
Molecular weight	223.2
Activity	Aliphatic organophosphate insecticide and acaricide
Solubility in water	10,00,000 mg L^{-1}
log K_{ow}	−0.22
Vapor pressure	0.29 MPa
Toxicity class	*I*
Acute oral LD50 for rats	14 mg kg^{-1}

media with 10–40 ppm of MCP was spiked as sole carbon source for primary screening. On the basis of MCP tolerance capacity, from the three strains, the only potential strain designated as M1(S) was chosen for further studies on the basis of its growth at 20 ppm MCP.

2.2.2.3. Identification of strains. The organism designated as M1(S) was identified as *Pseudomonas synxantha* by 16sRNA sequencing method. It is deposited in NCBI GenBank with Accession Number JQ406550. Primers used for identification of microbes in this method are 27F (5′ AGAGTTTGATCCTGGCTCAG 3′) and 1492R (3′ TACGGYTACCTTGTTACGAC 5′).

2.2.3. Instruments

The spectra were taken with UV–Vis spectrophotometer (Shimadzu 1650). HPLC analysis was performed on Dionex 3680 instrument equipped with a UV–Vis detector and C-18 column. The mobile phase was a mixture of acetonitrile and water (20:80, v/v). The injection volume was 10 μL, the eluent was delivered at a rate of 1.4 mL min^{-1}, and the wavelength of detection was 210 nm.

Metabolites of MCP were identified with a gas chromatograph interfaced with a mass selective detector. The samples for GC/MS analysis were prepared by extraction of sample with ethyl acetate. The extracts were dried with anhydrous sodium sulfate overnight. The finished sample was concentrated under reduced pressure to 1 mL and then analyzed by GC/MS. The GC (Polaris Q Thermo Electron Corporation) is equipped with a DB-1MS capillary column. The operating conditions were as follows: argon (carrier gas) flow, 30 mL min^{-1}; hydrogen flow, 30 mL min^{-1}; air flow, 300 mL min^{-1}; injector temperature, 220°C; column temperature, 210°C; and detector temperature, 230°C. The injected volume and scan time was 2 L and 0.2 s, respectively. Chromatographic data were acquired by recording the full scan mass spectra in the range m/z 50–500. The identification of products was done by interpretation of mass spectra and fragmentation pattern corresponding to identify chromatographic peaks.

3. Results and discussion

3.1. Persistence of MCP

MCP is an organophosphate pesticide, extensively used in India. The laboratory experiments were carried out for 120 d to study the persistence of MCP in water. The amount of MCP present was measured by recording absorbance at λ_{max} 216 nm at different intervals. Fig. 1 shows that the peak at 216 nm either diminished or shifted to different wavelength during the 120 d study. The absorption spectra obtained after 120 d show the presence (70.9%) of MCP. The results were further supported by the HPLC analysis. HPLC studies also confirm the presence of MCP after 120 d and showed the formation of the new peaks. The new peaks at different retention times were reflect the formation of metabolites during natural degradation of MCP.

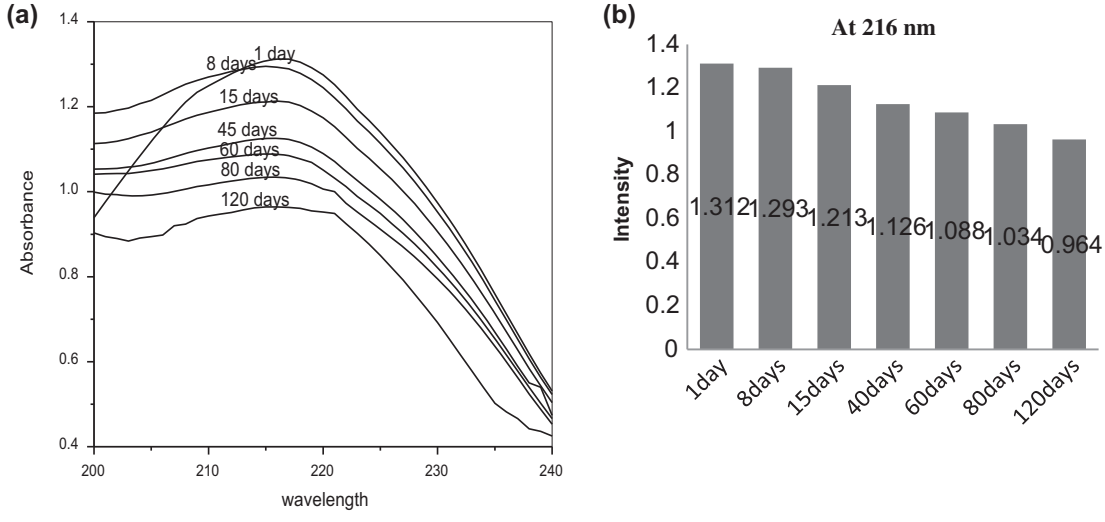

Fig. 1. (a) Time dependent UV–Vis spectra. (b) Intensities of naturally degraded samples of MCP at different time intervals.

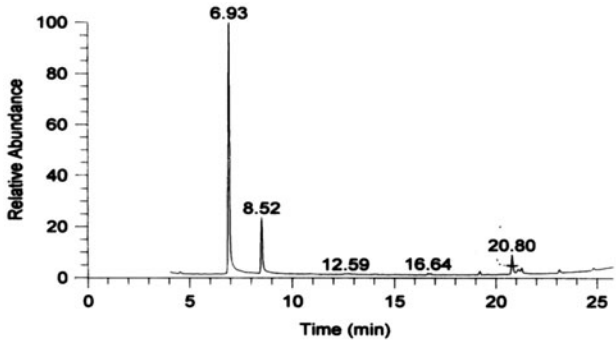

Fig. 2. Gas chromatogram of sample of MCP after 120 d.

3.2. Identification of metabolites

Hyphenated GC/MS technique was employed to assess the transformed products formed after 120 d. The compounds were separated by GC as per their retention time and were further detected by mass spectrometry. The intermediates were identified by interpretation of their fragmentation pattern in the mass spectra. Four intermediates with retention time 6.93, 8.52, 16.64, and 20.80 min were identified as transformed products of MCP by GC/MS (Fig. 2). The mass spectral peaks of identified transformed product A are 209, 171, 149, 139, 127, 110; of B are 248, 207, 115, 110; of C are 177, 111, 82; and of D are 207, 192, 147, 136, 105, 73. On the basis of identified metabolite, the degradation pathway has been proposed (Fig. 3).

Table 2
Percentage degradation of MCP using different isolates in the present study

Bacterial isolate	Percentage degradation
Pseudomonas synxantha	67.8
Bacillus subtilis	16.58
Salmonella enterica	6.67

The proposed pathway of the formation of the transformed products can be rationalized by cleavage of C–O bond and hydrolysis of the compound. The product B [N-methylacetoacetamide] has also been reported by Gundi and Ready [9] during hydrolysis of MCP in soils. N-methylacetoacetamide is less toxic to animals in comparison to MCP [18]. The fate of metabolites is not exactly known but these metabolites appear to be mineralized to H_2O, CO_2, NH_3, etc. in the environment.

The experimental studies of the persistence of MCP in the aqueous stream confirmed the presence of MCP and its metabolites in natural water even after 120 d. This highly persistence nature of MCP and its metabolite encouraged us to investigate the induced microbial degradation of MCP to explore the avenue of degradation of pesticides in the soil itself before its further leaching to the ground water and runoff in rivers. Earlier reported laboratory studies show that the MCP persisted for 20 d with half life of 9–11 d in soils

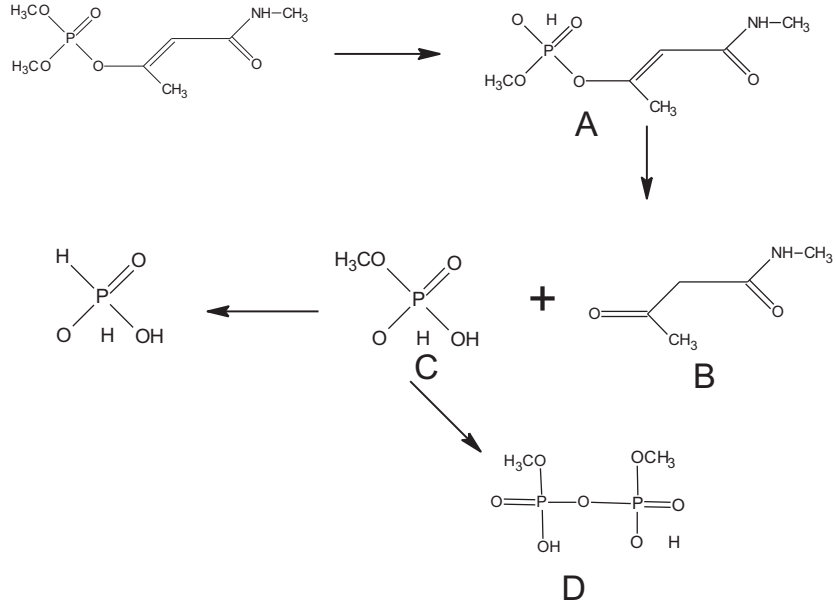

Fig. 3. Proposed degradation pathway for natural degradation of MCP in water.

collected from cotton and ground fields [9]. The reason of less persistence of MCP in soils is due to microbial degradation in soils.

3.3. Biodegradation studies

The biodegradation studies were carried out with three different strains, i.e. *P. synxantha*, *Bacillus subtilis*, and *Salmonella enterica*. Twenty parts per million MCP was spiked in inoculated minimal media by these microbes for 17 d. After 17 d, the amount of MCP was monitored by UV–Vis spectrophotometer and HPLC. The UV measurements at λ_{max} 216 nm at different time periods were recorded. From these three species, *P. synxantha* has shown maximum biodegradation efficiency (Table 2). To confirm the efficiency of *P. synxantha*, degraded sample was further analyzed by HPLC technique. Results confirmed that the peak area observed in control at retention time 5.148 min gradually decreased in biodegraded experiments as shown in Fig. 4. From the standard graph, concentration of MCP remained in degraded product was 32.2%. The microbial degradation of MCP by bacteria was also studied by Rangaswamy and Venkateswaralu [19], which also showed the susceptibility of MCP for bacterial degradation. The promising results were also shown by fungal isolate (*A. Oryzae* ARIFCC 1054) for degradation of MCP [12]. These results suggest that

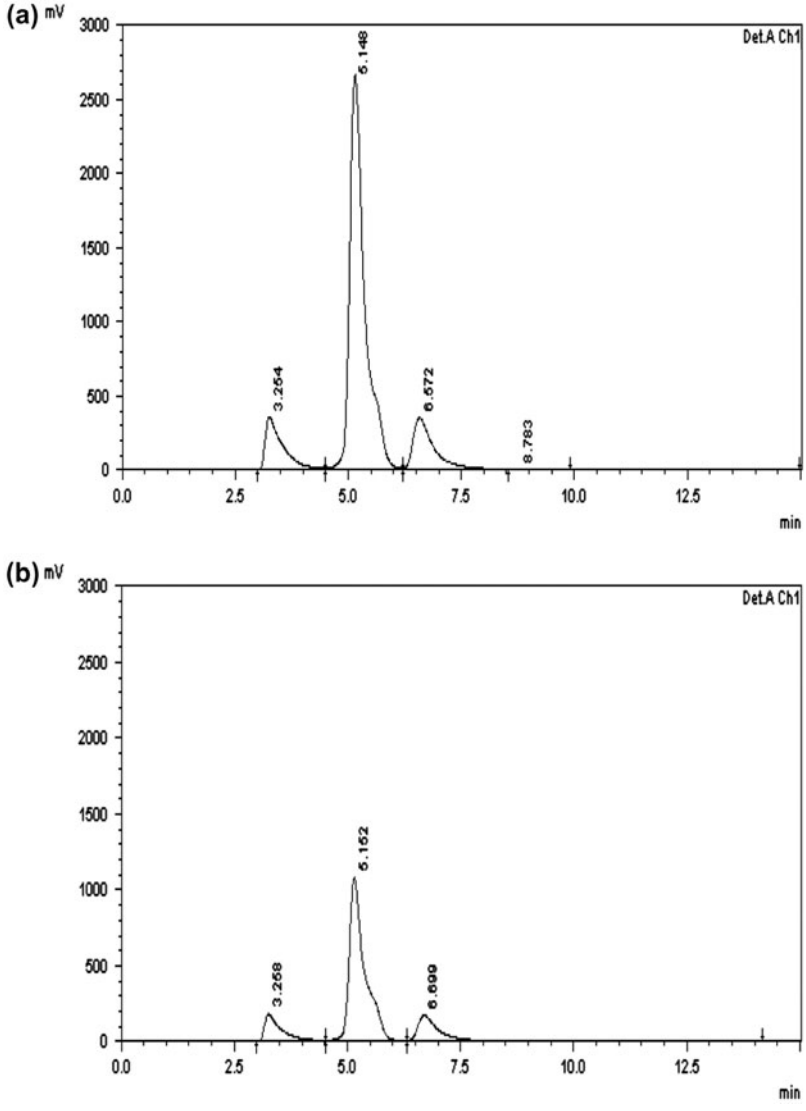

Fig. 4. HPLC chromatographs of biodegraded samples of MCP with M1 (a) control and (b) after 17 d.

the soil bacteria could be used for treatment of aqueous stream.

4. Concluding remarks

The experimental studies of the persistence of MCP in the aqueous stream confirmed the presence of MCP and its metabolites in natural water even after 120 d. Four intermediates were identified as degradation products of MCP by GC/MS studies. On the basis of identified metabolite, the degradation pathway has been proposed. The dramatic enhancement of the toxic MCP degradation (in 17 d only) was observed in the presence of micro-organisms isolated from contaminated soil. *P. synxantha* has maximum biodegradation efficiency. It can concluded that this study will be a helpful in finding out new degradation pathways and new remediation strategies for removal of MCP from contaminated waters. This process is also ecofriendly and environmentally acceptable.

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