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MYCOREMEDIATION OF MONOCROTOPHOS

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ABSTRACT

The persistence of Monocrotophos (MCP) an extensively used Organophosphorous pesticide in environment and its toxic effect on biota necessitate its removal. A soil fungus capable of utilizing or breaking down MCP was isolated from a site contaminated by the same pesticide. The fungus *Aspergillus* sp. was inoculated into the medium with 0.5% MCP. After 8 days of incubation the biodegradation of MCP was analyzed using GC-MS .The results of GC-MS revealed that the MCP got degraded into volatile fatty acids like Behenic acid,Stearic acid,Palmitic acid and other intermediate metabolites which were not found in the control medium. The finding of this study highlights the potential of *Aspergillus* sp. in the breakdown of MCP.

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KEY WORDS

Monocrotophos, Bioremediation, *Aspergillus* sp., Volatile fatty acids.

INTRODUCTION

In India almost 30 percent of agricultural output is lost through pest infestation and pesticide consumption for crop protection accounts for about 3 percent of the total world consumption [1]. It is estimated that four million tons of pesticides are applied to crops annually world-wide for pest control, but less than one percent of the total applied pesticides reach the target pests[2] .Organophophorous compounds alone make up for 70 percent of the pesticides used world wide. The global problems of pest resistence, resurgence and pesticide residues in crop and soil associated with the excessive use of pesticides necessitate employing a variety of detoxification methods.

Monocrotophos (MCP), an organophophorous insecticide used in agricultural operations persists as soil residue and seeps into ground water. It is a systemic pesticide harmful to human beings, affecting eyes and the CNS [3].Since it is toxic, it must be degraded completely or at least detoxified, so that ground water or water sources do not get contaminated with residual MCP. Natural degradation of MCP takesplace over a period of 12-16 days[4] and the process could be expediated through bioremediation .The bioremediation of MCP has been already tried using *Bacillus* sp. [5] ; several species of algae and by the soil fungus, *Penicillium corylophilum*[6]. Two microbial isolates, *Pseudomonas aeruginosa* and *Clavibacter michigenese* utilize MCP as the sole source of phosphorous[7].

Among the several microbes capable of degrading MCP, fungal isolates are highly effective. This study is carried out to understand how MCP is degraded by a soil fungus, *Aspergillus* sp. isolated from soil previously contaminated with the same pesticide.

MATERIALS AND METHODS

(i)Collection of soil samples:

Soil samples (about 250 g) were collected in 500 ml sterile polythene bags from snake guard (*Trichosanthes cucumerina* L.) crop field, where MCP alone was used during that plantation season.The collected soil samples were brought to the laboratory.

***(ii)Isolation , Identification and Screening of fungus*:**

The collected soil samples were washed with distilled water and allowed to stand still for 30 minutes. After all the soil debris has settled down, the supernatant was decanted into a sterile test tube and serially diluted. Dilutions below 10^{-6} were plated in Potato Dextrose Agar (PDA) medium. After 6-7 days of incubation a number of fungal strains were observed on the plate. The most prominent fungus was selected and identified as *Aspergillus* sp. The isolated fungus was inoculated into plates containing PDA admixed with different concentrations of MCP (0.3, 0.5, 0.7 and 1 %) to screen the ability of *Aspergillus* sp.to tolerate the pesticide contaminated medium.

(iii)Bioremediation Assay:

To study the bioremediation of MCP using *Aspergillus* sp., two different culture media were prepared in triplicate – medium

containing PDA and 0.5 percent (w/w) of MCP and medium containing PDA, 0.5 percent (w/w) of MCP inoculated with the spores of *Aspergillus* sp. The plates were then maintained for about 8 days at 28^{o} C in an incubator.

(iv)Preparation of sample for GC- MS analysis:

An aliquot of 15 g of the culture medium (both control and experimental) was mixed with 50 ml of ethyl acetate in a polytron for 2 minutes. Then 50 g of anhydrous sodium sulfate was slowly added with agitation. The mixture was filtered through a Whatman No. 1 filter paper and the liquid was suspended in a separating funnel. This process was repeated again with another 10 ml of the same solvent. The extract was condensed in an evaporator and redissolved in 10 ml of cyclohexane to obtain the sample ready to be injected into the gas chromatograph unit.

RESULTS

Based on the GC analysis, the maximum area with high peak abundance was observed for peaks 6 (RT. 15.55 min) and 7 (RT. 17.10 min) in control culture medium containing 0.5 % of monocrotophos (Fig.1), whereas in the experimental set, the maximum area with high peak abundance was observed for peaks 2 and 4 with retention timings of 15.48 and 17.19 min (Fig.2).

Mass spectral analysis for control medium revealed the presence of 1, 2 Epoxy -3 propyl acetate, Methyl propyl acetate, Laevulinic acid, Isobutysone and Isocyanic acid in the first peak with the retention time of 5.43 min, and for peak 2 (retention time, 8.1 min) includes Isotetreadecane, 3-butane-2-01, Iso de decane and ethyl propyl ketone.Possible compounds identified in peak 3 (retention time of 10.6min) includes Isotetradecane, n-Nonadecane, Isohexadecane and n-Tridecane.

Based on the mass spectral analysis, the possible compounds identified in experimental culture medium containing 0.5% monocrotophos inoculated with fungus was different when compared with control medium. The possible breakdown compounds observed for peak 1 (retention time 1.492 min) includes Carynic acid, Hexadecoic and Enantic acid and the compounds recorded for peak 4(retention time of 17.2 min) was n-hexa decoic acid, n-tetra decylglycid, n-octadecanoic acid and Penta decyclic acid.

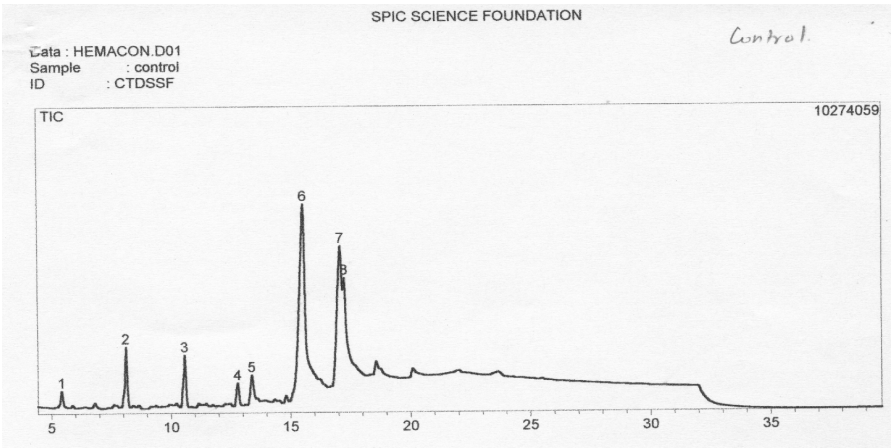

Fig .1.
Gas chromatographic pattern of monocrotophos in control culture media

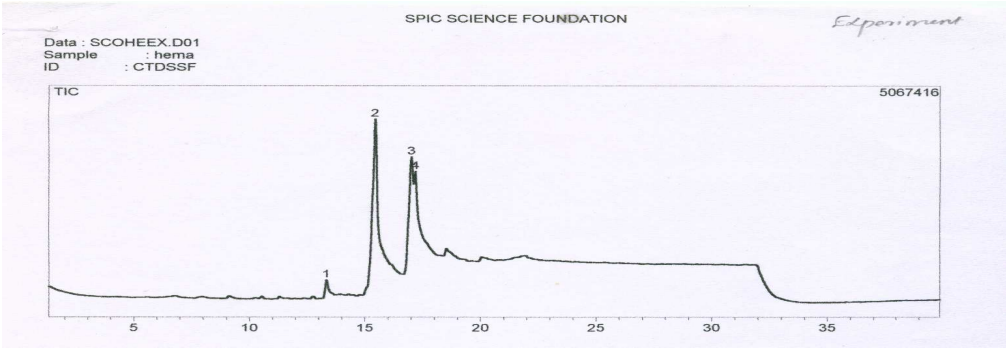

Fig. 2.
Gas chromatographic pattern of monocrotophos in culture media inoculated **with *Aspergillus* sp.**

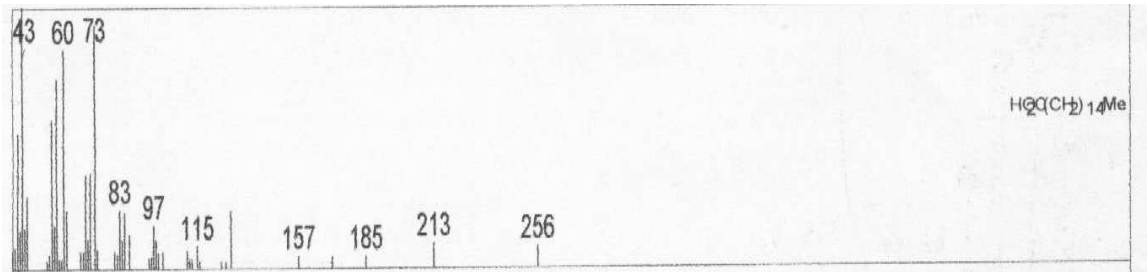

Fig .3.
Mass spectrum of palmitic acid

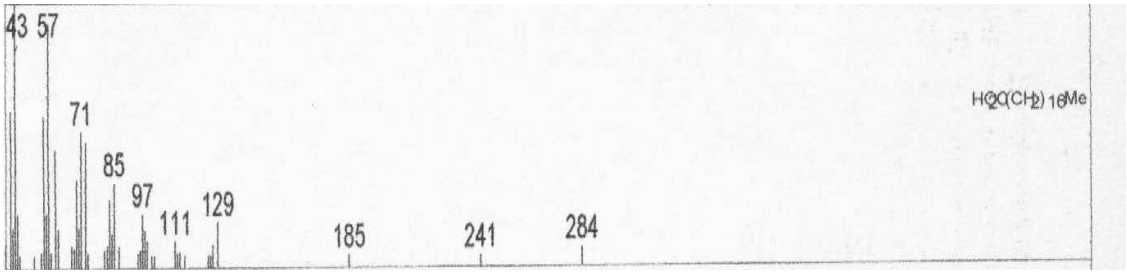

Fig.4.
Mass spectrum of stearic acid

DISCUSSION

The fungus used in the present investigation was isolated from the area which is contaminated with monocrotophos. So the fungus isolated from this site is expected to possess the natural ability to detoxify MCP.Possible compounds present in the control and experimental

medium after 8 days of incubation were different. The number of peaks recorded for control is 8 and experimental 4. This is due to the degradation in the medium inoculated with *Aspergillus* sp. (Fig.1 and 2).The retention time recorded for control and experimental medium is also different.

The results of this investigation reveal that the fungus, *Aspergillus* sp. breaksdown the MCP into different non toxic volatile fatty acids (VFA). Based on the mass spectral analysis the possible breakdown compounds of MCP includes unknown metabolites, VFA such as carycnic acid,n-nonoic acid,enantic acid ,stearic acid and palmictic acid having a retention time of 15.492 min. The above compounds were not recorded from the mass spectrum of control medium.

The volatile fatty acids thus formed seem to be the products of enzymatic synthesis in the fungus. The main breakdown intermediate metabolic products of MCP degradation by plants and animals include Vinyl and monomethyl phosphates[8]. Degradation or detoxification of organophosphorous pesticide by the action of microorganisms is generally through the hydrolysis of P-O alkyl and P-O aryl bonds.Unidentified metabolites in the MS analysis are possibly products of hydrolysis endorsing the views of earlier authors who suggested that the initial degradation of MCP in soils occurred through hydrolytic reactions carried out by microbes to form di methyl and n-methyl compounds[9].Similarly in the present study such functional groups were observed with a retention time of 17.02 min, the possible compounds being 3-methyl -1-hexanol and 2 methyl-5-octanol.

Removal of the N –methyl groups is through the formation of relatively stable N-hydroxy-methyl intermediates followed by the elimination of formaldehyde. The removal or modification of the N- substitutents can result in an increase, a decrease or little change in toxicity [10]. The degradation of monocrotophos in soils is mainly through hydrolysis with the formation of N-methylacetoacetamide[4]. Metabolic reactions such as N- demethylation, O- demethylation, hydroxylation of N- methyl groups and cleavage of the phosphate- crotanamide linkage occur during the metabolism of monocrotophos by microbial cultures and in soils with the formation of O-desmethylmonocrotophos, monomethyl phosphate, dimethyl phosphate, N-methyacetoacetamide and N-methyl butyramide.

Most of the organophosphorous compounds are degraded to some extent by various hydrolases of microbes. These hydrolases attack the phosphorous ester bond or the anhydride bond as well as an ester or aryl bond in the leaving group. The disruption of any of these bonds to form a diester or a monocarboxylic acid is probably one of the most important mechanisms of inactivation and detoxification [11].

Degradation of monocrotophos in soil is through hydrolysis with the formation of N-methylaceto acetamide[4]. The breakdown compounds of MCP are mainly hydrophilic compounds and probably the significant one is dimethyl phosphate[12]. The biodegradation of MCP by the fungus ,*Penicillium corylophilum* is through the formation of a thermo labile extra cellular oxidizing enzyme active over a wide range of pH[6].

Fungal utilization of organophosphate pesticides and their degradation by *Aspergillus flavus* and *A.sydowii* in soil have been very clearly established[13].The fungal genera, *Aspergillus* and *Penecillium* are often involved in the bioconversion of triazines.Certain fungi have also been reported to bring out the methylation of Pentachrophenol and arsenic derivatives.

From this study, it has been observed that the soil fungus *Aspergillus* sp. is highly effective in

the degradation of MCP .Considering the demand on organophophorous pesticides in agriculture, the use of bioremediating fungi in toxicity alleviation is recommended. Further research on biodegradation, particularly on bioremediation of sites contaminated with such hazardous pesticides will be needed for effective toxicity alleviation.

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