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Degradation of Monocrotophos in Sandy Loam Soil by *Aspergillus* sp.

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P A P E R I N F O

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The present study premeditated to explore and compare the potential of *Aspergillus* sp. to degrade monocrotophos in soil. Two different strains of *Aspergillus* sp. viz *Aspergillus niger* JQ660373 and *Aspergillus flavus*, were tested under anaerobic conditions for their monocrotophos (150 µg Kg^{-1}) degrading ability in sandy loam soil for a period of 30 days. The water-holding capacity was maintained at 60% and samples were incubated at 25 ± 4 °C. After regular time interval of 5 days, the samples were collected and estimated for residual monocrotophos concentration. Residual monocrotophos was extracted with ethyl acetate and estimated by spectrophotometric method at 254nm. The degradation of monocrotophos in soil was observed to be rapid and followed first order kinetics. A 99% of applied pesticide was degraded within 30 days of incubation. It was found that *Aspergillus niger* was more efficient for the degradation of monocrotophos than *Aspergillus flavus*. The half life of monocrotophos for *Aspergillus niger* and *Aspergillus flavus* was found to be 7.35 and 9.23 days, respectively. Degradation process was assessed by HPTLC and FTIR analysis. These revealed reduction in the peak of standard monocrotophos as a measure of hydrolytic cleavage of vinyl bond with the formation of inorganic phosphates and release of carbon dioxide and ammonia. It could be concluded from the study that these two fungal strains had the ability to degrade monocrotophos in soil.

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INTRODUCTION

Soil is a complex matrix containing different component viz. chemical, physical and biological. They undergo constant changes due to environmental factors and anthropogenic management [1-3]. Therefore, it is best known as the sink of pesticide residues.

Monocrotophos (MCP) is a broad-spectrum organophosphorus (OP) insecticide and acaricide, developed by Ciba-Geigy (now Novartis) and was first registered in 1965. MCP is widely used for agricultural and household purposes; it works systemically as multipurpose agrochemicals pesticides [4]. It is characterized by a P–O–C linkage and amide bond and is a perilous chemical especially for conditions of use in developing countries [5]. It has been barred due to its acute toxicity against beneficial and non-target insects such as honey bees [6, 7], fish [8] birds and mammals [9].

However, its usage for the control of major insect pests in agriculture has been continued in developing countries like India, primarily due to lack of alternative replacements [10]. Use of temporary suspension of pyrethroid insecticide resulted in the development of high resistance to pests [11]. This ultimately led to reliance on cheap insecticides such as MCP for control of pests in agriculture. It is surprising to know that MCP had a lion's share (one-third) of total sales of pesticides in 1996 alone in India.

Despite of its indiscriminative and wide usage, only limited number of studies were undertaken to find its impact on agroecosystem under tropical conditions. Although it has short-life on different agricultural crops [12-16] and in soil [17] but frequent exposure to pests developed moderate insecticide resistance in them [18]. But still nervous system of different pests as well as humans and soil ecology is adversely affected by this perilous chemical. In view of all these facts degradation

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of MCP from soil in the ambient environment is very necessary.

Microorganisms play a very major role in the degradation. Bioremediation has emerged as a slow but viable method due to the time lag that requires weeks to months and complexity of microbial mechanisms for degradation of organic pollutants [19]. Low levels of MCP residues in agroecosystem are rapidly degraded by chemical and biological means [20-22].

The use of bioremediation technology is typically less expensive than the traditional physical-chemical methods as this offers the potential to treat contaminated soil and groundwater on site without the need for excavation [23, 24]. Hence, bioremediation requires little energy input and preserves the soil structure [25].

Reduced impact on the natural ecosystems is perchance the most attractive feature of bioremediation, which should be more acceptable to the public [26]. MCP degradation is extensively studied in liquid culture medium by means of different bacteria [5, 27-30], algae [31], fungi [32-34] and extracellular fungal enzymes [35]. However, the biodegradation of MCP in soil remained a mystery for a long time.

Although, Gundi and Reddy [36] had reported, the degradation of MCP is naturally occurring in soil samples. Jia et al., [30] had also reported the degradation of MCP in fluovo aquic soil using *Paracoccus* sp. Owing to these studies, the present study is designed to analyse the potential of two different fungal strains viz *Aspergillus niger* and *Aspergillus flavus* for the degradation of MCP in sandy loam soil of Rajasthan under controlled conditions.

MATERIALS AND METHODS

Chemicals

MCP of analytical grade (99.5% purity) was procured from Sigma and its stock solution of 1 mg ml^{-1} was prepared in ethanol. All the other chemicals employed in the present study were of analytical grade and purchased from Himedia and Rankem, India.

Microbial culture

Two different fungi viz *Aspergillus niger* JQ660373 and *Aspergillus flavus* were used for the study of MCP degradation in soil. These were previously tested for their MCP degrading ability in liquid media by different workers [33, 34]. Spore suspension of each of these fungal strains was made up to 1×10^8 spores ml^{-1} in 0.85% saline.

Soil sampling

Soil samples were collected from eight different agricultural fields previously treated with OP pesticides. Soil samples (1 Kg each) were collected in different polyethylene bags from within 10 km circumference of Banasthali Campus. These different soil samples were dried at 40°C for 1 week. Dried sampled were meshed through 2mm mesh and mixed in equal proportion. This mixed sample named as SS_{mix}. The samples were stored at 4°C for further use.

Physico-chemical attributes of sampled soil

Physico-chemical properties of soil were analyzed using standard methods. Type of particles in soil, organic carbon percentage, exchangeable cations and pH were as following-

Particle size: Clay- 8.9%, silt- 5.3%, sand- 85.8%, Texture class-Loamy sand

Organic Carbon- 0.33%, Exchangeable Cations: Ca-7.5 m.e/100gm soil, Mg-2.00 m.e/100gm soil, Na-0.65 m.e/100gm soil, K-0.039 m.e/100gm soil, Soil reaction (pH) - 7.88.

Soil samples were sterilized at 200°C for 24 hours in a hot air oven to inhibit the growth of microorganism. Thereafter the samples were cooled down to room temperature for further use.

Experimental setup

Experiment was set in triplicates. A 50 g of soil sample was weighed by using physical balance. Each of this soil was put in 250 ml Erylmener flask. A 1 ml ($1 x10^8$ spores per ml) spore suspension of each isolate was inoculated in 50 g of sterilized soil containing 150 µg kg^{-1} MCP concentration and incubated at temperature 24±4 °C under static culture conditions for 30 days. The water holding capacity of soil was maintained at 60%. The flasks were incubated in dark to rule out the possibility of photo degradation. Effective antibiotics, streptomycin and penicillin (30 mg Kg^{-1}), were added in the sterile soil to avoid any bacterial contamination. Control sample containing sterile soil + MCP (150 µg Kg^{-1}) was prepared simultaneously. At regular time interval of 5 days each flask was removed and residual MCP was extracted and calculated.

Extraction of residual MCP

Residual MCP was extracted from soil with equal amount of ethyl acetate (1ml ethyl acetate for 1g soil). The solvent fraction was pooled. The solvent was allowed to evaporate and residues were dissolved in 2 ml ethyl acetate. Clean up of residual MCP was done by using florosil column and cyclo hexane: ethyl acetate (1:1 v/v) as solvent system. Again the purified fractions were collected and solvent evaporated to dryness and residue was re-dissolved in minimum amount of ethyl acetate. Purified samples of MCP were stored at -20ºC and used for further quantification by spectrophotometer. The results were confirmed by HPTLC and FTIR spectra. Residual MCP was quantified by spectrophotometer at wavelength of 254

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