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In Vitro Isolation of Microorganism For Bioremediation of Monocrotophos Pesticide

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Abstract *-Biodegradation and bioremediation both are same processes which are based on the metabolism of pesticide and insecticide by microorganism . Microorganism play an important role for saving our environment by degrading xenobiotic compounds , chemical wastes , which are toxic either in their native form or modified to be toxic. Isolation of microbial strain able to degrade chemical compounds was started usually from polluted sources, such as soil .Almost 30% of the precious agricultural output of India is lost owing to pest infestation .In India , pesticide consumption for protecting crops is about 3% of the total word consumption . Monocrotophos (MCP) an organophosphorus insecticide is widely used to control insects on crops. Persistance of monocrotophos (MCP) in environment and its toxic effect on biota necessitate its removal. In the present study soil fungus and bacteria capable of utilizing or breaking down MCP was isolated from soil sample isolated from a site contaminated by the same pesticide, various soil samples from agricultural and industrial areas .The isolated microbes were inoculated into the medium with 0.5 % MCP. Three fungal and two bacterial species were found to show tolerance action against 0.5 % MCP and of which one was identified as Aspergillus sp and one of the bacterium as Pseudomonas aeruginosa .These microorganism show bioremediation in environment .*

Keywords *– Bioremediation ;xenobiotic : Monocrotophos ;Organophosphorus pesticide*

I. INTRODUCTION

2015 is" International year of soils".Soil conservation is very essential since "if we do not produce we donot eat".Pesticide management is very important as far as soil quality is concerned. Pesticides constitute the key control strategy for crop ,pest and disease managemnt. Continous application of these pesticides to the soil and aquatic system resulted in health hazards and environmental pollution which has triggered much public concern .The wide spread use of these pesticides over the years has resulted in problems caused by their interaction with the biological system in the environment . Notwithstanding the hazards ,the pesticides will continue to be an indispensable tool for the managemnt of pest in the years to come as there is no suitable alternative to totally replace them.Considering the toxic effect of thee pesticides it is essential to remove them from the environment employing suitable remediation measures .Bioremediation exploiting microbial technology is one of the recent technique for environmental cleanup .in the process heterotrophic microorganisms break down hazardous compounds to obtain carbon and energy.

Currently among the various groups of pesticides ,organophosphates form the major , accounting for more than 36% of the total world market (Kanekar et.al.2004).Among the insecticides monocrotophos ,quinalphos and chlorpyrifos top the list of organophosphorous insecticides

In this experiment we use monocrotophos (MCP) pesticide which is an organophosphorous compound.

Organophosphorous compounds alone make up for 70 % of the pesticides used world wide. The global problem of pest resistance , resurgence and pesticide residues in crop and soil associated with the excessive use of pesticides necessitate employing a variety of detoxification methods.MCP used in agricultural operation persists as soil residue and seeps into ground water .Natural degradation of MCP take place over a period of 12-16 days and process could be expedited through bioremediation(S.Sam Manohar Das&S.Anitha,2007).

It is moderately persistant in nature as its residues were detected in soil even after 3 months (Chapman et al.1984). Extensive use of MCP contaminates air ground water , rivers , lakes rain water and fog.Contamination has been found upto about 20 kilometres from site of application

.considerable residues of MCP were found in tomatoes (Aysal et al. 1999) ,cotton seeds , potatoes and oil seed (Bhatnagar and Gupta 1998; Gupta et al.2001).It is speculated that the bioaccumulation ability of MCP in living tissues may spell a potential environmental risk to marine organism and human as well (Serrano et al;1997;Tilak et al ;2004)

When organophosphates are released into the environment their fate is decided by various environmental conditions and microbial degradation is the key factor for the disappearance of these pesticides .since they posses the unique ability to completely mineralise many aliphatic, aromatic and heterocyclic compounds

The present programme has been designed to isolate microorganism capable of degradating monocrotophos (MCP)

II .MATERIALS AND METHODS

1) Soil sample collection

Soil samples of 500 g was collected from agricultural fields of khumari , chattisgarh (where MCP is used),industrial areas following soil collection methods.

2) Pesticide collection

monocrotophos 36 % sl (HILCRON) was brought from Hindustan insecticides LTD

3) Isolation and identification of Microorganisms

The stock solution is prepared by dissolving 1g of soil sample in 9 ml of distilled water.This gives a dilution factor of 1:10.The solution was allowed to stand for 30 seconds.After all the soil debris has settled down,the supernatant was the serial diluted to dilution factor of 1:1000.This was inoculated into nutrient broth for bacteria and czapek dox broth for fungus containing different concentration of MCP(0.3,0.5 , 0.7 %). incubate these broth in suitable condition as per needed .After 3 days of inoculation the bacterial and fungal strains were plated on to a NAM and Czapek dox plate respectively containing a 0.5 % concentration of MCP. NAM plate was incubated for 1 day at 37C and czapek dox plate were incubated for 4 days at 25C. After these incubation periods one fungal and two bacterial strains were observed on plate,of which one was identified as Aspergillus sp. By lactophenol cotton blue staining method.One of the bacteria was identified as Pseudomonas aeruginosa using citrate and catalase test.The other bacteria was gram negative rod shaped bacteria.

III. RESULTS AND DISCUSSION

In present work we have isolated two bacteria and three fungal species which show tolerancy against 0.5 % MCP.

The microscopic characteristics of isolated fungus 1 and 2 was aseptate mycelium and spores have spines.

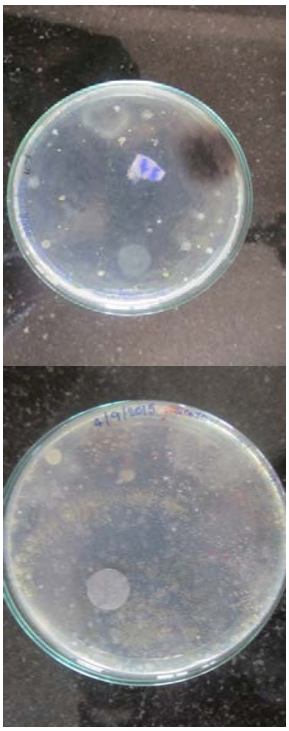
Fungal culture prepared by the slide culture method at 25°C using czapek dox agar and stained with lactophenol cotton blue . The vesicle (enlarged structure at the end of the conidiophore) and phialides (cells that produce conidia) are visible (Larone .D.H ,1995).This helped in identification of the fungal species as *Aspergillus sp.*

Fungus 3 Colonies are growing in shades of green, consisting of a dense felt of conidiophores giving a brush-like appearance .Thus it may be *Penicillium* sp.

Of the two bacterias one formed a blue colour colony while the other gave a golden yellow colour .

Microbial degradation of MCP by *Flavobacterium* sp was reported in1973 (by yoshida and sethunathan).since the golden yellow coloured species resembles that of *Flavobacterium* ie. rod shaped and gram negative ,we expect the bacterium to be of *flavobacterium* sp.

The blue colour colony is a gram negative ,coccobacillus bacterium.it give positive citrate test and positive catalase test.Thus it helped in identifying the bacterium as *Pseudomonas aeruginosa* . *Pseudomonas aeruginosa* can



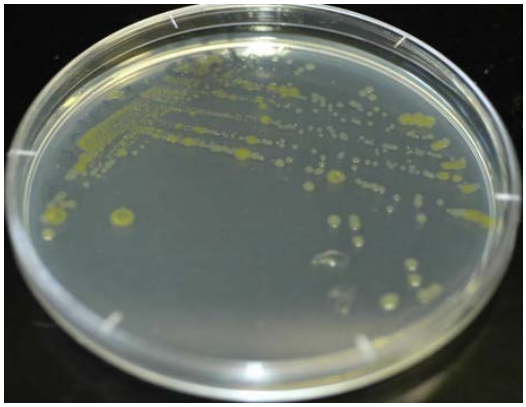


Fig 1: Plates of Czapek Dox & NAM showing *Aspergillus* sp.,fungus2, fungus3and *pseudomonas aeruginosa* ,bacteria 2

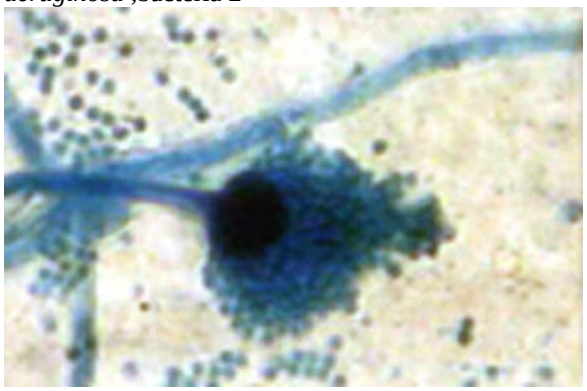


Fig 2 : Lactophenol cotton blue staining of *Aspergillus* sp.

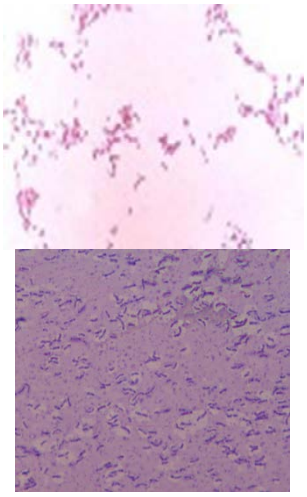


Fig 3: microscopic image of Gram staining of *Pseudomonas aeruginosa* and bacteria 2

utilise monocrotophos as a phosphorous source and not as a carbon source(sing and sing ,2003).These two bacterial and one fungal species thus can be used in bioremediation process to remediate the monocrotophos pesticide .further work can be proceeded using various soil samples collected from various part of country and isolating more microorganisms. Then can test the bioremediation rates and methods of those isolated microbes on increasing concentration of MCP.

IV .CONCLUSION

Three fungal and two bacterial species were found to show tolerance action against 0.5 % MCP and of which two was identified as of *Aspergillus sp* and one of the bacterium as *Pseudomonas aeruginosa*. These microorganism show bioremediation in environment .

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