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STUDY AND IDENTIFICATION OF POTENT DEGRADER OF CHLORPYRIFOS PESTICIDE

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ABSTRACT

Microbial degradation of chemicals in the environment is an important route for removal of harmful compounds. These compounds range from plastics through organic chemicals (both industrial chemicals used in large quantities and trace chemicals such as pesticides) to organometallics such as methylmercury. The largest volume of pesticides produced and sold comprises the chlorinated hydrocarbons. These compounds composed of hydrogen, chlorine and carbon atoms.

Microbial degradation of pesticides such as 2, 4,-D, Alachlor, Amitrole, Chlorsulfuron, DDT etc applied to soil is the principle mechanism which prevents the accumulation of these chemicals in the environment. Here in this paper is maily based on identifying the the organisms that can degrade cholorpyrifos. This is done using techniques mainly by colony counting method**.** Microorganisms are also grown on BTB plates which indicate the color change and it's mainly a PH indicator. Then degradation studies is performed .From this degradation studies the best organism utilizing cholrpyrifos as carbon source is identified and then Thinlayer chromatography is performed. As per the experimental results it is found that SD 31 and CD 31 are the one with good degradation values and utilizes CPP efficiently as the carbon source

INTRODUCTION

In soil, chlorpyriphos may remain biologically active for periods ranging from days to months. Dosage rates, soil type, soil moisture and organic matter content, temperature and insecticide formulation are among the factors which influence biological persistence. Chemical persistence is equally variable, with initial half lives ranging from less than a week to a month or more. It is moderately persistent in nature as its residues were detected in soil even after 3 months and hence causes potential environmental hazards.

It is speculated that the bioaccumulation ability of chlorpyriphos and other organophosphorus pesticides in living tissues may spell a potential environmental risk to marine organisms and humans as well.

Insecticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation.

Microorganisms play an important role in degrading synthetic chemicals in soil. They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. The metabolism of chlorpyriphos by microorganisms in soil has been reported with 3, 5, 6-trichloro-2-pyridinol (TCP) as the primary breakdown product. Use of pesticide degrading microbial systems for bioremediation, thus, receives attention because of its cost effectiveness and ecofriendly nature.

2. MATERIALS AND METHODS

2.1 SOIL SAMPLING

For the laboratory experiment, five soil samples were taken with the help of sterilized spatula from fields of sunflower from University of Agricultural Sciences, Dharwad, Karnataka, where exclusively chlorpyriphos was applied to control various insect pests.

2.2 ENRICHMENT OF SOIL SAMPLES

The soil samples collected from different locations were mixed thoroughly and filled into soil columns and the native microflora in the soil were enriched by weekly addition of the synthetic solution of chlorpyriphos (25 ppm and 50ppm) for about eight weeks. Soil was crushed and plant debris and rocks were manually removed. From this about 1 gram of loose fine soil was taken and mixed with 9ml of sterilized water and shook thoroughly. This stock solution was supplemented with 25ppm and 50ppm of Chlorpyripos and incubated in a shaker.

2.3 ISOLATION OF MICROORGANISMS CAPABLE OF DEGRADING CHLORPYRIPHOS

After a week, one ml from the stock solution was inoculated to the same fresh medium. This was repeated 5-6 times for selective enrichment of pesticide degrading bacteria. Finally, it was serially diluted and pour plated on to solid media. The master plates were allowed to incubate and were then maintained at cool temperatures for preservation of the microorganisms.

For isolating bacteria, mineral salts medium with chlorpyriphos as the carbon source (25 ppm and 50ppm) was used. For fungi, Saborose Dextrose and Czapek Dox medium with chlorpyriphos as the carbon source (25 ppm and 50ppm) was used.

2.4 SCREENING AND SELECTION OF MICROORGANISMS THAT CAN DEGRADE CHOLORPYRIFOS

After incubation, the representative microorganisms growing on the plates were purified following the four-way streaking method. The bacterial isolates were tested for their ability to grow on chlorpyriphos by inoculating to the mineral salts medium containing chlorpyriphos (25 ppm) with and without agar. The fungal strains capable of growth at this concentration of chlorpyriphos were selected and inoculated to Subaraud Dextrose and Czapek Dox media of 25ppm and then inoculated to higher concentration of chlorpyriphos (50 ppm). Finally, the strains with the tolerance to the highest chlorpyriphos concentration were selected and used for further studies.

2.5 COLOUR CHANGE BY MICROBIAL ISOLATES ON CHLORPYRIPHOS MEDIUM CONTAINING BTB INDICATOR

All five stock soil solutions were spotted on separate chlorpyriphos medium containing BTB indicator and incubated for 3 days at 30°C. The colour change produced by the bacterial and fungal isolates on the medium was observed

2.6 DEGRADATION STUDIES

One ml sample was diluted to 10 ml and three ml of Al (OH)3 suspension and one ml H2O2 were added and stirred for a minute.

b) Titration

The prepared sample was directly titrated with AgNO3 (0.0141 N) in presence of 1 ml of K2CrO4 indicator solution in the pH range 7.0 to10.0, until a pinkish yellow colour end point was obtained.

$$\text{Chloride released mg/l} = \frac{(X-Y) \times N \times 35.450}{\text{ml of sample}}$$

Where,

X= ml of sample

Y = ml titration for blank

N = Normality of AgNO3

3. RESULTS

Experimental growth of fungi is shown in the figures and compared the initial growth colonies of fungi with few days of incubated colonies of fungi. This mainly indicates the rapid multiplication of fungi in the presence of cholrpyrofos and utilising it as a carbon source.

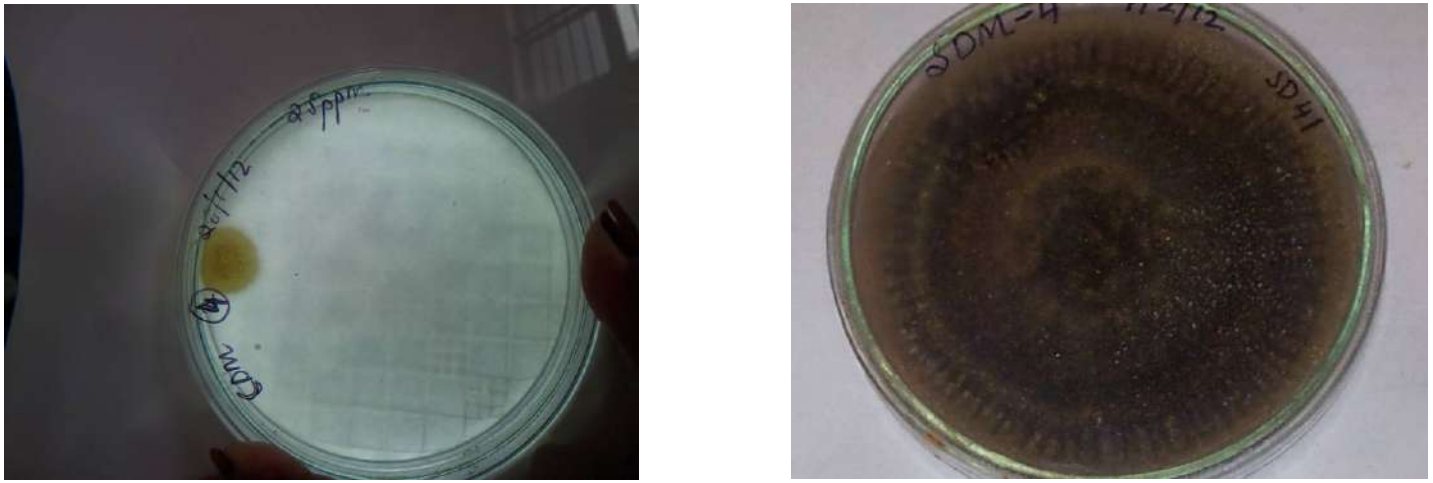

Figure1:- Fig a Showing initial growth of microorganisms and fig b showing rapid multiplication of microorganisms on SD 41 plate after few days of incubation

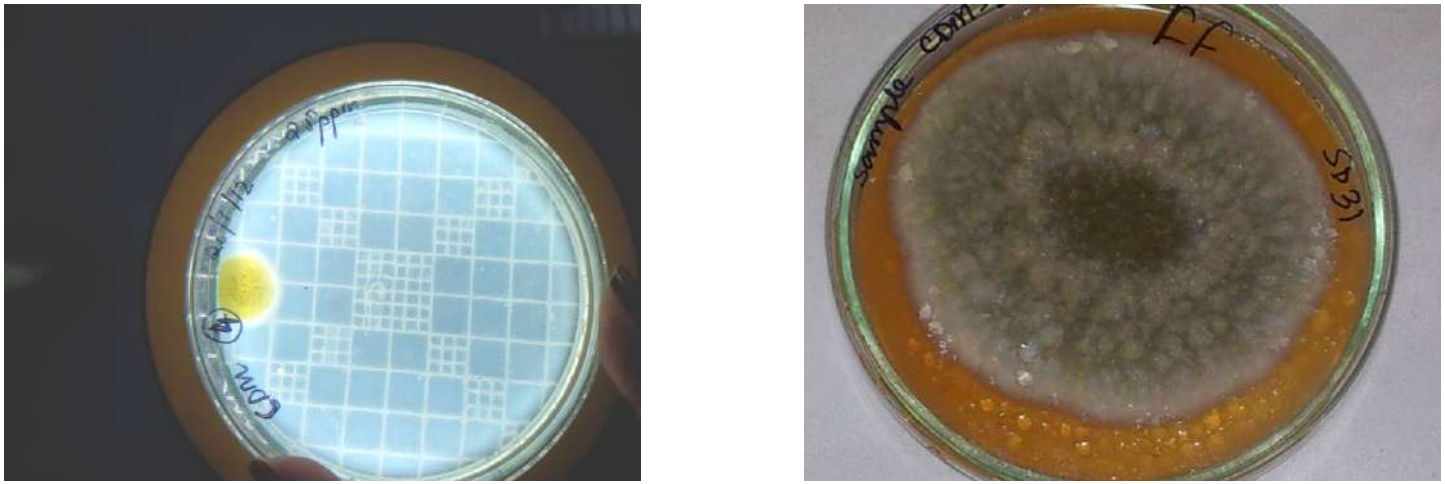

Fig 2:-figure a Showing initial growth of microorganisms and fig b showing rapid multiplication of microorganisms on SD 31 plate after few days of incubation

3.1 MICROSCOPIC VEIW BY STAINING

Microscopic view of the plates is mainly performed to study the detailed structure and identify to which type of genus it belongs to based on the observation

Figure3:- figure showing the detailed microscopic view of the SD31 plate

Figure 4:-showing the detailed microscopic view of the SD21 plate

3.2 GROWTH OF MICROORGANISMS on 50ppm AND CONFIRMATION TEST Whether the organisms are using CCP or not as their carbon source

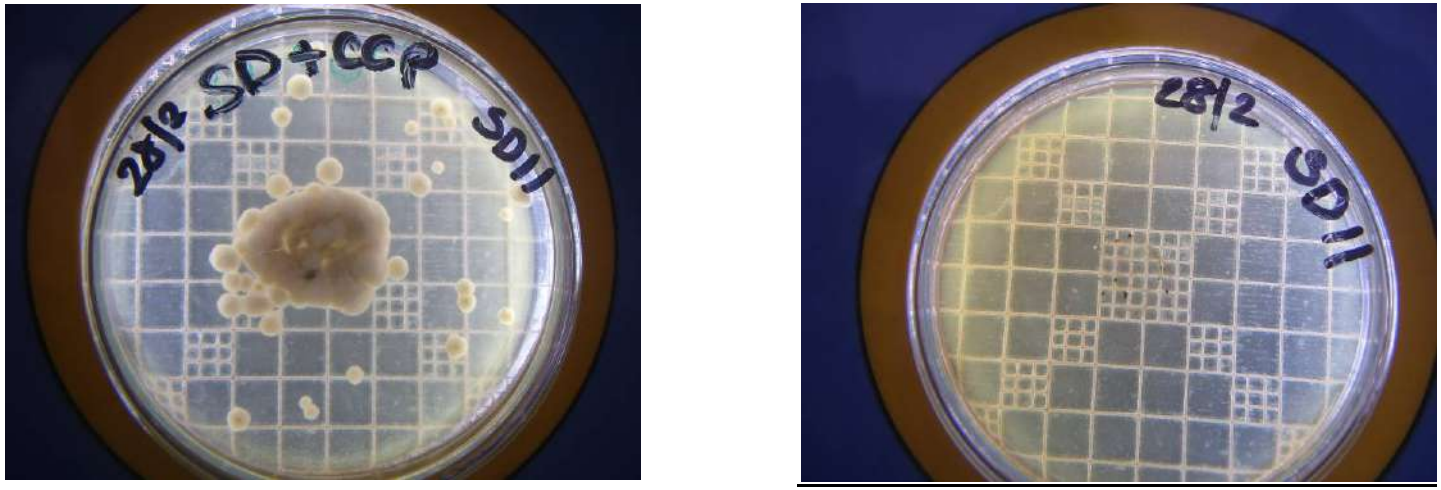

Figure 5: Figure showing the rapid growth of micro organisms in presence of CCP and fig b showing the less growth in micro organisms without CCP on SD11 plate. This indicates that Organisms uses CCP as the carbon source for their growth

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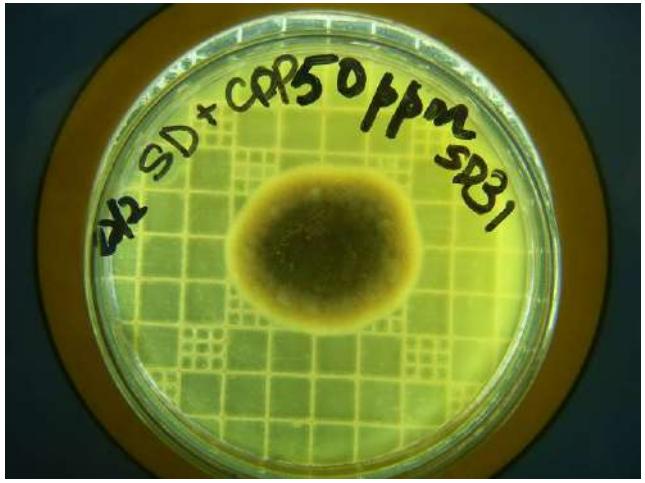

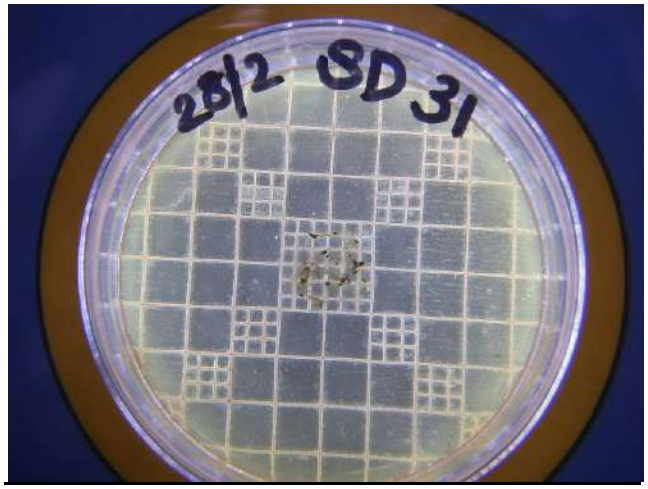

Day	Control	Control (shaker)	SD11	SD31	SD41	SD42
5	2	2.8	5.5	5.2	2.0	6.3
6	2.5		6.0	6.0	5.3	6.7
7	3.0		7.0	7.0	5.2	9.1
8	6.5		7.4	8.1	5.6	9.3

Day	Control	Control (shaker)	SD11	SD31	SD41	SD42
1	2.0	2.8	2.3	7.1	2.3	4.8
2	2.5		6.5	7.2	5.0	6.7
3	6.0		6.8	7.3	5.2	9.4
4	6.3		7.3	8.3	5.7	9.8

Pesticides constitute the key control strategy for pest management and have been making significant contribution towards improving crop yields. Currently, among the various groups of pesticides that are being used world over, organophosphates form a major and most widely used group, accounting for more than 36 per cent of the total world market. Quinalphos, monocrotophos, chlorpyriphos, malathion, parathion are some of the widely used organophosphorus pesticides. Amongst these, chlorpyriphos dominates. It is a synaptic poison having broadspectrum insecticidal activity and is used to control insects attacking corn, cotton, citrus, fruits, nut crops, potato, beets, pulses *etc*. Chlorpyriphos is widely distributed and has been reported in bottled drinking water, leading cold drink brands and vegetables as well.

In order to study the applicability of these efficient strains in the degradation of chlorpyriphos in industrial effluents, we plan to attempt was made to test the bioremediation ability of one of the isolates. Immobilization technique is a very important biotechnological method in waste water treatment. The storage stability and repeated usability of the immobilized microorganisms is well known. Cells of isolate can be immobilized in calcium alginate beads and tested for chlorpyriphos degradation. Cent per cent degradation was achieved by the immobilized strain within 120 hrs. In the second charge, the rate of degradation was further enhanced. According to our experimental study it was found that SD 31 and CD 31 are the efficient degraders and can be used for further agricultural practices if it is developed.

3.3 Thin Layer Chromatography

After intervals of one day 1ml of the growth sample was transferred to a centrifuge tube and spun at 10,000 rpm for 10 minutes. The metabolites in the supernatant were extracted with an equal amount of chloroform, this is added to extract them from the pellet and they are pooled.

The upper aqueous phase was discarded and to the remaining chloroform phase, a pinch of NaSO4 was added to absorb any water present. It was spun at 8000rpm for 8minutes allowing the NaSO4 to be pelleted.

The clear upper layer was concentrated by air flushing. The residue was redissolved in 200 µl acetone and spotted on TLC plate (aluminium plate coated with silica). The standard chloropyriphos were also spotted and chromatpgraphed and Rf vales are calculated using this standard. The plated was chromatogrammed using a solvent system of hexane and ethyl actetate (7:3). A small beaker was filled with a small amount of the solvent mixture and covered with a glass plate. One the space within the beaker was saturated with the gas phase, the plate is placed in the beaker and lid is replaced. The plate was observed under UV light, the spots were marked and Rf values calculated and Rf value was found to be 0.35.

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