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ISOLATION OF CHLORPYRIFOS DEGRADING BACTERIA FROM CONTAMINATED SOIL AND EVALUATION OF THEIR BIOREMEDIATION POTENTIAL

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Abstract:
Extensive use of pesticides in the agricultural fields leads to a direct harmful and toxic effect on environment by causing pollution of soil and water which ultimately harms human beings. In modern agriculture, large numbers of pesticides are being used to improve crop production which can negatively affect the plant growth and development.Present study focuses on the screening and isolation of the potential pesticide degrading microorganisms from different soil samples which can be used for Bioremediation of pesticide polluted Soil so that the toxic effect of these pesticides could be minimized on plant growth. In present study, six Pesticide (Chlorpyrifos) degrading bacteria were isolated from pesticide polluted soil samples. Biochemical studies were performed for identification of the bacteria. Isolates were screened for Pesticide tolerance by growing the bacterial isolates in media supplemented with Chlorpyrifos. A pot assay was also conducted to check the efficacy of the two best isolates for pesticide degradation and growth of *Capsicum annuum* plant. The pesticide degradation by these two isolates was confirmed using gas chromatography technique.

Keywords: toxic, Pesticide, bacteria, Screening, Chlorpyrifos, *Capsicum annuum*, Bioremediation

Introduction:

One of the major concerns of today in the world and especially in the country like India is the environmental pollution. This environmental pollution can be caused by different means including industries, household waste, one of which is the excessive use Pesticides. Chlorpyrifos is a type of organophosphate pesticide which kills the insect by attacking on its nervous system[1].It was introduced in 1965 by Dow Chemical Company India.Chlorpyrifos majorly works on variety of insects, including cutworms, flies,fire ants, lice corn rootworms, cockroaches, grubs, flea beetles, termites etc.Chlorpyrifos is used as an insecticide not only on vegetable crops and grain, cotton, fruit, nutsbut also onornamental plantsand lawns extensively [2].Excessive use of Chlorpyrifos pollutes the soil as well as water system thereby causing a harmful effect on the environment and ecosystem. Other than the use, the manufacturing process of this pesticide also generates waste[3].The soil and groundwater present in close vicinity to agricultural fields gets severely contaminated because of the organophosphorous pesticides [4]. Hence there is a need to clear up such organophosphorous pesticides by safe and effective means.Bioremediation is one of the very effective tools used to clean up environmental pollutions caused by the harmful substances such as metals and pesticides etc. Bioremediation of soil involves the removal of pollutant from soil by using degradation capability of different microbial sources such as bacteria, Soil etc. Degradation of organophosphorus pesticides present in soil can be achieved by use of certain naturally occurring microorganisms. Till date, many researchers have reported an efficient degradation of Chlorpyrifos present in the contaminated soil. Ifediegwu, M.C. et al. have worked onthe isolation and identification of Chlorpyrifos degrading bacteria such as *Pseudomonas, Klebsiella*etc. from Agricultural Soil [5].Yadav et al. gave a report on Malathion and Dichlorvos degradation efficiency ofdifferent bacterial isolates [6].Bioremediation potential of *Pseudomonas aeruginosa*was investigated byFulekar et al.[7].Many of the researchers have also identified some fungal strains capable of degradation of pesticides[8,9].The present research study throws some light on potential of bacterial strains responsible to degrade the pesticide chlorpyrifos. The pesticide tolerance of bacterial species was checked. The bacteria were characterized by Biochemical identification. The pesticide degradation capability of selected isolates was also analyzed by gas chromatography.

Materials and Methods:

2.1Collection of Soil Sample:6 different Soil samples from the agricultural fields present in the area nearby Raigad district were collected. Soil samples collected had the previous history of pesticide usage in past few years. Soil samples were stored at cool and dry place.

2.2Screening and Isolation of pesticide degrading microorganisms:Isolation of the pesticide degrading microorganisms from soil was done by performing serial dilution of the soil sample and further spreading the dilutions on the plates containing the medium with 100ppm of commercially available Chlorpyrifos. The Medium

used for isolation was nutrient medium according to Pankaj et al.[10].The plates were incubated at Room temperature for 24 hours. Microorganisms growing on pesticide containing media were further maintained on NA agar slants containing 100ppm Chlorpyrifos and stored at 4^0C. The isolates were further characterized by performing certain biochemical tests such as Glucose, Lactose, Maltose, Citrate, Nitrate etc. Gram's staining was also performed to know the Gram's nature of the isolates.

2.3Pesticide tolerance of the Isolates:Pesticide tolerance of the isolates was determined by growing the isolates on Nutrient agar supplemented with increasing concentrations of Chlorpyrifos(500ppm – 6500ppm). The Isolates Showing best results were further selected for pot assay.

2.4Pot assay on*Capsicum annuum*:Pot assay was performed on *Capsicum annuum* by growinggerminated plantletsin the Soil contaminated with 50ppm, 100ppm and 150ppm of chlorpyrifos in presence of Isolates. The parameters checked for the plant growth were Height of the plant and Leaf size. Controls were also maintained for Soil and Soil + pesticide without isolates.

2.5Pesticide degradation analysis by Gas Chromatography(GC):Soil mixed with 150ppm of chlorpyrifos and the selected isolates was incubated at Room temperature for 20 days. The Control was maintained for only soil and soil with pesticide. The extraction of chlorpyrifos from soil was done by adding Chloroform to 5g of soil sample. The extract was filtered through Whatmann filter paper and used as a Sample for GC analysis.

Results and Discussion:

3.Total six bacterial cultures were isolated from six different soil samples used. The isolated cultures are shown in the figure given below (Figure 1).

The Biochemical characterization and Gram's nature of the isolates is tabulated below in Table 1. Three of the Isolates were found to be Gram Positive whereas three were found to be Gram negative. Gram negative nature of the isolates was confirmed by growing them on MacConkey's agar plates.

The isolated cultures were further tested for their pesticide tolerance and showed difference in the tolerance level of Chlorpyrifos. Out of six, two Gram positive isolatesnamely S1-5 and S1-6 were found to tolerate the Chlorpyrifosup to 6500ppm concentration which indicates their pesticide degradation capability.

Pot assay was performed to check the effect of Isolates on the growth of *Capsicum annuum* plants grown in pesticide contaminated soil. There was significant increase in the height of plant and Size of leaves when plants were grown in pesticide contaminated soil in presence of isolates S1-5 and S1-6 separately. The data shown in Figure 2(a) and 2(b) indicate that the isolates were able to effectively degrade the pesticide which further improved the growth of *Capsicum annuum*plant.

GC spectrum data further confirmed the degradation of chlorpyrifos by isolates S1-5 and S1-6.GC spectrum of Soil with Chlorpyrifos isshown in the figure 3(a). Figure 3(b) and 3(c)shows the GC spectra of chlorpyrifos extracted from soil inoculated with S1-5 and S1-6. The comparison of GC spectra with control indicated81.25% and 96.3% reduction inpeak area of Chlorpyrifos at retention time 19.67 minutes in soil samples with 150ppm chlorpyrifos inoculated with S1-5 and S1-6 respectively. The data indicates that both S1-5 and S1-6 are efficient in degradation of chlorpyirfos from soil.

Table 1: Biochemical characterization of the isolates'+': Positive, '-' Negative

Isolates	Gram's nature	Glucose	Lactose	Maltose	Citrate	Nitrate
SC-1	Gram positive cocci	+	_	_	_	_
S1-5	Gram postivie cocci	+	+	_	_	+
S1-6	Gram positive rods	+	+	_	_	_
S1-2	Gram negative cocobacilli	+	_	_	_	_
S3-5	Gram negative rods	+	_	_	_	_
S2-3	Gram negative rods	+	_	_	_	+

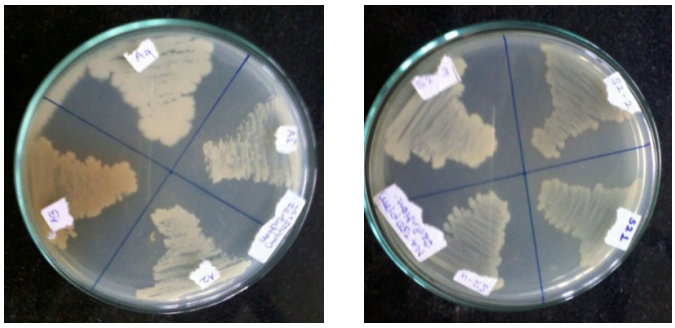
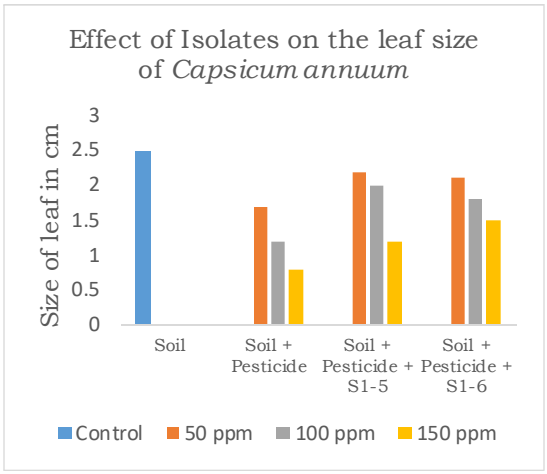
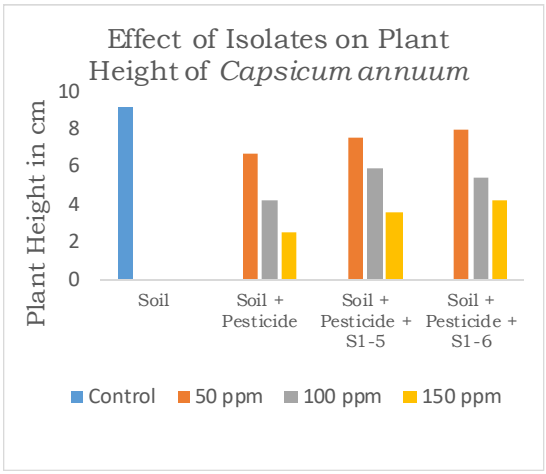


Figure 1: Isolation of pesticide degrading organisms on NA plates congaing 100ppm of Chlorpyrifos

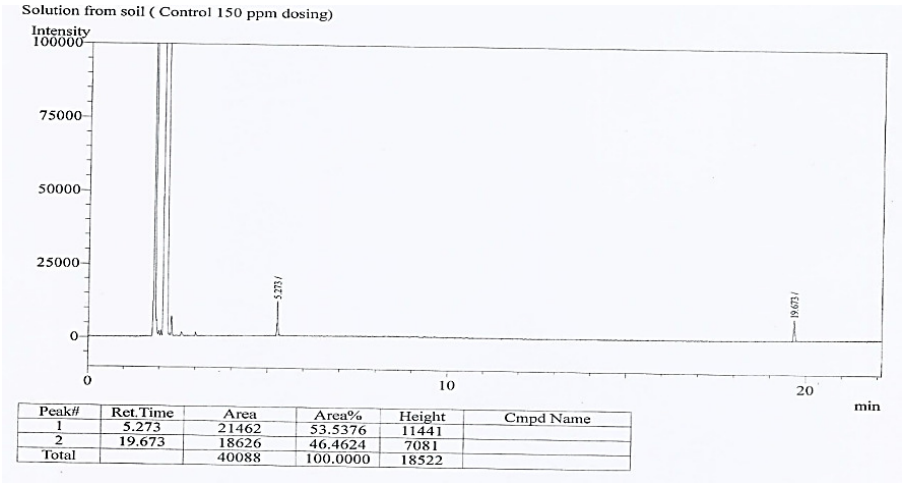


2(a)



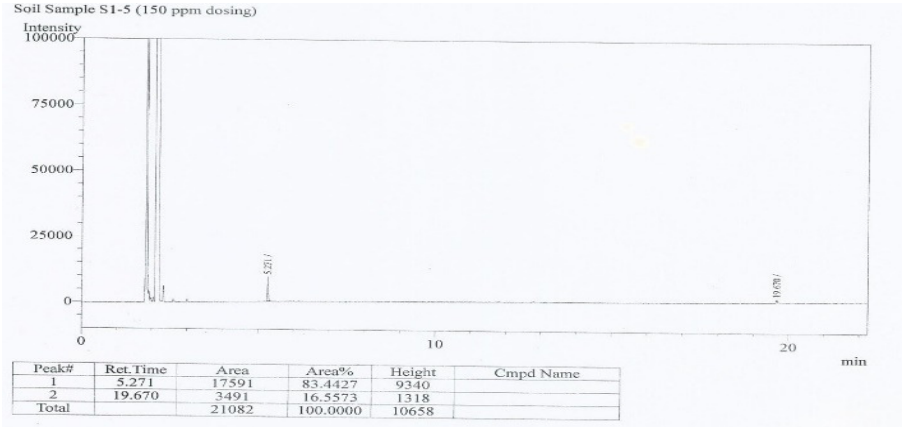
2(b)

Figure 2:2(a) Effect of Isolates on the leaf size, 2(b)Effect of Isolates on the Plant Height of *Capsicum annuum*



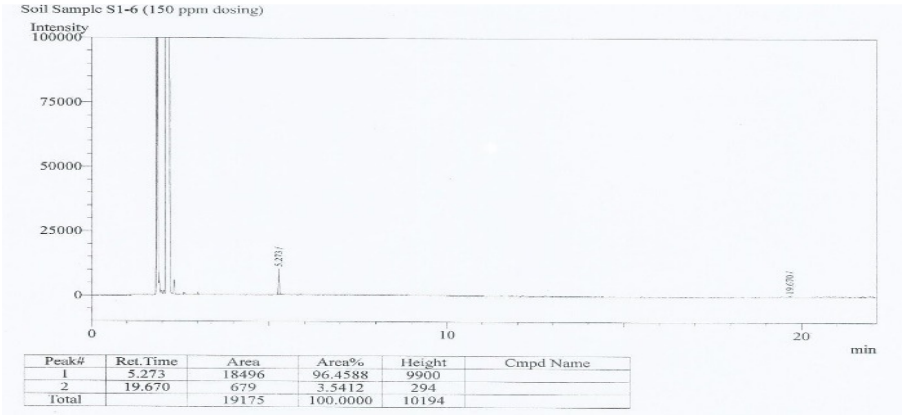
Peak#	Ret.Time	Area	Area%	Height	Cmpd Name
1	5.273	21462	53.5376	11441	
2	19.673	18626	46.4624	7081	
Total		40088	100.0000	18522	

3 (a)



Peak#	Ret.Time	Area	Area%	Height	Cmpd Name
1	5.271	17591	83.4427	9340	
2	19.670	3491	16.5573	1318	
Total		21082	100.0000	10658	

3 (b)



Peak#	Ret.Time	Area	Area%	Height	Cmpd Name
1	5.273	18496	96.4588	9900	
2	19.670	679	3.5412	294	
Total		19175	100.0000	10194	

3(c)

Figure 3:3(a) **-** GC spectra of Chlorpyrifos extracted from control Soil with chlorpyrifos, 3(b) –Soil inoculated with S1-5 and 3(c)Soil inoculated withS1-6

Conclusion:

4.S1-5 and S1-6, the Gram positive isolates were found to degrade the organophosporous pesticide, chlorpyrifos. The isolates also improved the growth of *Capsicum annuum* when grown in chlorpyrifos contaminated soil which indicates that the isolated bacterial strains can be used for bioremediation purpose by carrying out further field tests.

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