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BIODEGRADATION OF CHLORPYRIFOS BY *PSEUDOMONAS DESMOLYTICUM* NCIM 2112

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ABSTRACT

Chlorpyrifos is an organophosphorus pesticide and widely used in the agricultural field to control beetles, whiteflies, bugs, and flies like insects. This investigation describes the biodegradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112. The biodegradation is influenced by other carbon and nitrogen sources and indicates that glucose and maltose are effective at 0.5% concentration and $NaNO_3$ and yeast extract at 0.05%. The pH and temperature optima for degradation was found to be 7.0 and at 30^0C respectively. *Pseudomonas desmolyticum* NCIM 2112 degrades chlorpyrifos into non toxic metabolites like 2-pyridinol and thiophosphate.

KEYWORDS: **Pesticide, Chlorpyrifos, *Pseudomonas,* Biodegradation.**

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INTRODUCTION

Pesticides are used to control the damages caused by the pest to commercially important crops in agriculture field. Among the pesticide use chlorpyrifos is at the top list of organophosphorus pesticides in the Indian market. Chlorpyrifos is an organophosphorus insecticide and it gets reported as toxic chemical as it show mutagenic, carcinogenic and tumorogenic effects on organisms[1]. Aerial application of chlorpyriphos is a common method of application against surface feeding insects of cotton, rice, mustard etc.[2, 3]. Pesticides and their additives also show toxic effect on the growth of soil bacteria and interrupts with the degradation of organic matter[4]. Chlorpyrifos accumulates at the top soil and influence the biochemical properties of soil microorganisms. The growth of symbiotic nitrogen fixing bacteria is also getting hampered due to the excessive use of chlorpyrifos in the agriculture field was reported[5]. Chlorpyrifos shows a marked adverse effect on soil enzyme activity which get considered as an index of soil organic matter degradation[6].In soil environment microbes are responsible for the degradation of pesticides. The diversity of resident microbes on phyllosphere and in rhizosphere gets destabilized as the pesticides reach these areas[7]. Only specific microorganisms which are tolerant to pesticides may remain and further multiply[8]. Microorganisms are important in maintaining soil fertility and are also important agents which detoxify pesticides in soil. Additions of pesticides affect the microbial components of an ecological niche and thus a simultaneous effect is observed on biotransformation reaction occurring in soil[9]. Biodegradation is the only way to minimize such problem concerned with pesticide toxicity in agricultural land. Bacteria have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. So by studying effect of different physico chemical parameters on the degradation effective biodegradation strategy can be employed. *Pseudomonas desmolyticum* NCIM 2112 shows an immense capability of biodegradation of xenobiotic compound. So use of this bacterial strain to degrade the pesticide like chlorpyrifos will be helpful to make ecotoxic free agriculture practices. This investigation deals with biodegradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112.

MATERIALS AND METHODS

Pesticide used

A pesticide used in this present study is chlorpyrifos was purchased from the local market of Kolhapur, (M.S.), India.

Growth of Bacteria

P.desmolyticum NCIM 2112 was grown in a mineral based medium containing 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05%, $KH_2PO_4$0.05%, $MgSO_4$, 0.05% KCl, 0.0001% $FeSO_4$, 0.05% yeast extract, glucose 1.0 % and pH 7.00 at 28^0C under aerobic conditions on rotary shaker at 120 rpm.

Degradation of chlorpyrifos

To study the degradation of chlorpyrifos, *P.desmolyticum* NCIM 2112 was grown in the mineral based medium as mentioned above, but without glucose and with 10 mg L^{-1} concentration of chlorpyrifos as the sole sources of carbon and nitrogen. The flasks were incubated on rotary shaker at 120 rpm at 30^0C. The content of the flasks was checked by taking 5 ml of culture drawn from mineral based medium and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and the supernatant was analyzed by UV-visible spectrophotometer (Cyberlab UV 100) to determine the chlorpyrifos degradation by *P.desmolyticum* NCIM 2112. Degradation study was conducted for every 2 days interval up to 6 days. The percent degradation of compound was determined using the formula, Percent degradation = Ab – Aa /Ab X 100, where Ab is absorbance of compound before degradation and Aa is absorbance at the same wavelength after degradation.

Effect of additional carbon sources on degradation of chlorpyrifos by *Pseudomonas desmolyticum* ***NCIM 2112***

For the determination of effective carbon source concentration of chlorpyrifos degradation carbon sources like glucose, lactose, maltose and Raffinose at different concentrations like 0.5, 1.0, 1.5 and 2.0% were tested.

Effect of additional nitrogen sources on degradation of chlorpyrifos by *Pseudomonas desmolyticum NCIM 2112*

Similarly, effect of various nitrogen sources for the degradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112 was studied. Nitrogen sources like peptone, yeast extract, $NaNO_3$ and malt extract at different concentrations like 0.05, 0.1, 0.15 and 0.2 % were tested.

Effect of pH and temperature on degradation of chlorpyrifos by *Pseudomonas desmolyticum NCIM 2112*

The effect of pH on degradation performance of *Pseudomonas desmolyticum* NCIM 2112 was investigated by adjusting pH values of the medium to 3, 5, 7, and 9 using 0.1 N HCl and 0.1 N NaOH. Similarly, the effect of temperature on the degradation of chlorpyrifos was tested by incubating the medium at different temperature values such as 4^0C, 20^0C, 30^0C, and 40^0C for about 6 days

Extraction of the metabolites

After 6 days of incubation the broth (100 ml) was centrifuged at 10000 x g for 15 min. The supernatant obtained was used to extract metabolites with ethyl acetate (1:1). The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary evaporator. The obtained residue was dissolved in small volume of HPLC grade methanol and used for GCMS analysis.

GCMS analysis

The extract was also analyzed by QP2010 gas chromatography coupled with mass spectroscopy (Shimadzu).GCMS analysis were performed as per the method previously described[10]. Gas chromatography was performed in temperature programming mode with a DB 530-m fused silica capillary column (0.25 mm,i.d × 0.25µm film thickness) attached to a mass spectrophotometer. Samples were injected into a split mode temperature program of 180^0C for 1.5 min, 260^0C for 20 min, at the rate of 10^0C/min. Injector temperature was 260^0C and detector temperature was 280^0C. Nitrogen was used as carrier gas. The compounds were identified on the basis of mass spectra and were compared using National Institute of Standards and Technology (NIST) library.

Statistical analysis

All the experiments were carried out in triplicate. Analysis of the variants was carried out on all data at $P< 0.05$ using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

RESULTS AND DISCUSSION

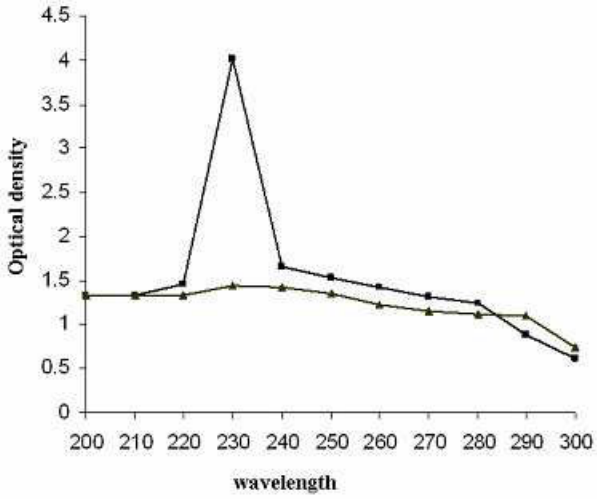
Due to excessive use of pesticides for the agriculture purposes over the years rise many problems of biological systems in the environment. By considering the toxicity of the compound it is essential to remove them from the environment. Biological removal of the pesticide is the easiest way as the microorganisms can use such hazardous compound and convert them into non toxic metabolites. In the present study ability of *P.desmolyticum* NCIM 2112 to degrade chlorpyrifos was determined. The result indicated that various carbon and nitrogen sources influence the biodegradation of chlorpyrifos. Study of pH and temperature on degradation performance shows that physicochemical factor contributes for the biodegradation. *P.desmolyticum* NCIM 2112 can degrade cholrpyrifos and convert into non toxic metabolites.

UV-VIS analysis of Chlorpyrifos degradation

UV- visible spectral analysis of cell free broth at 200-400 nm wavelengths was carried out to

confirm the degradation of chlorpyrifos. The wavelength maxima of chlorpyrifos were found to be 230nm. As shown in (Figure 1) there was a change in the absorbance spectra of chlorpyrifos before and after degradation. The degradation was up to 98%.

Figure 1
UV-visible spectra of chlorpyrifos anddegraded product.



UV-visible spectra of chlorpyrifos (—■—) UV-visible spectra of chlorpyrifos after degradation (—▲—)

At every 2, 4, and 6 days of incubation the percent degradation was calculated, as summarized in Table 1. The physicochemical parameters such as pH, temperature and shaking condition were found to affect the degradation.

Table 1
Percent degradation of chlorpyrifos after 2, 4, and 6 days of incubation with P.desmolyticum NCIM 2112.

	Before incubation	After 2 days of incubation	After 4 days of incubation	After 6 days of incubation
Wavelength maxima	230	230	230	230
Percent Degradation	0%	58.7±0.333%	63.52±0.333 %	98%±0.333

-Values are mean of ±SEM of three experiments

Effect of additional carbon sources on degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112

The degradation study shows that the efficiency of *Pseudomonas desmolyticum* NCIM 2112 in presence of glucose and maltose at 0.5% of concentration is better as compared to lactose and Raffinose. The effective degradation was found to be 98% in the presence of glucose and 95% in presence of maltose respectively. In the presence of lactose and raffinose the degradation was found to be 85% and 80% as shown in (Figure 2).

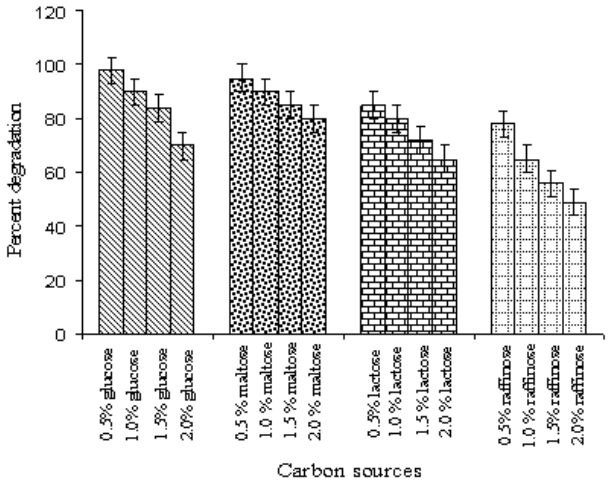


Figure 2
Effect of different percentage of carbon sources on degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112

Effect of additional nitrogen sources on degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112

Similarly nitrogen sources such as $NaNO_3$ and yeast extract are found to be effective in the biodegradation process at 0.05% concentration. Nitrogen sources at 0.05% concentrations were found to be effective for chlorpyifos degradation. The degradation was found to be 98% in the presence of $NaNO_3$ and yeast extract respectively. In presence of peptone it is 60% and in presence malt extract it is found to be 50% respectively (Figure 3).

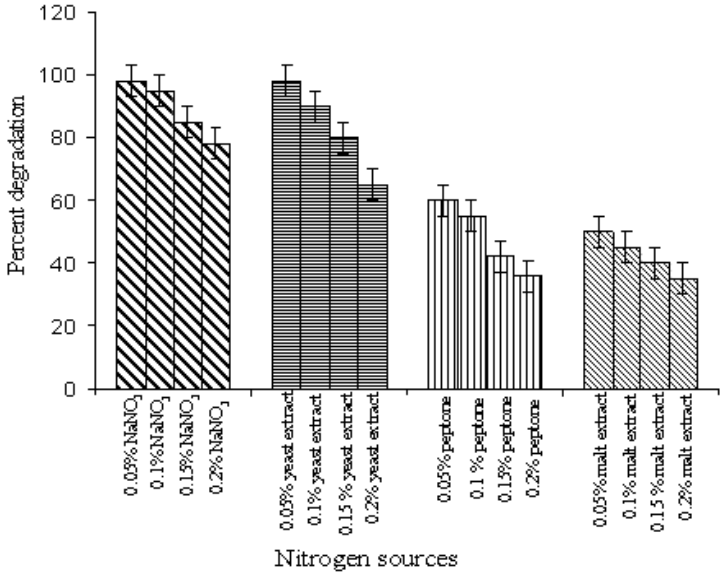
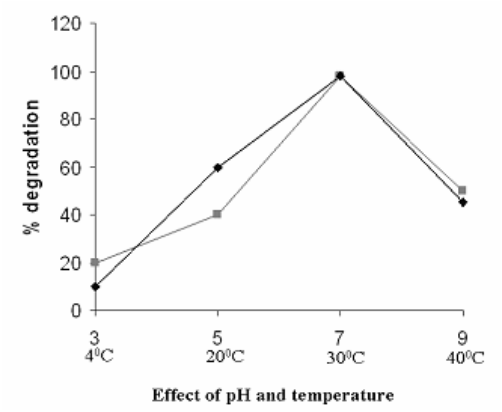


Figure 3
Effect of different percentage of nitrogen sources on degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112

Effect of pH and temperature on degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112

pH and temperature optima for chlorpyrifos degradation by *Pseudomonas desmolyticum* NCIM 2112 was found to be 7 and 30^{0}C respectively as shown in (Figure 4). At both of these optimum parameters chlorpyrifos degradation was found to be 98%.Lowest degradation was observed at pH 3 and at 4^{0}C.

Figure 4
Effect of different pH and temperature on the degradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112.



Effect of pH and temperature on degradation of chlorpyrifos pH range (—■—), temperature range (—◆—).

Proposed degradation pathway

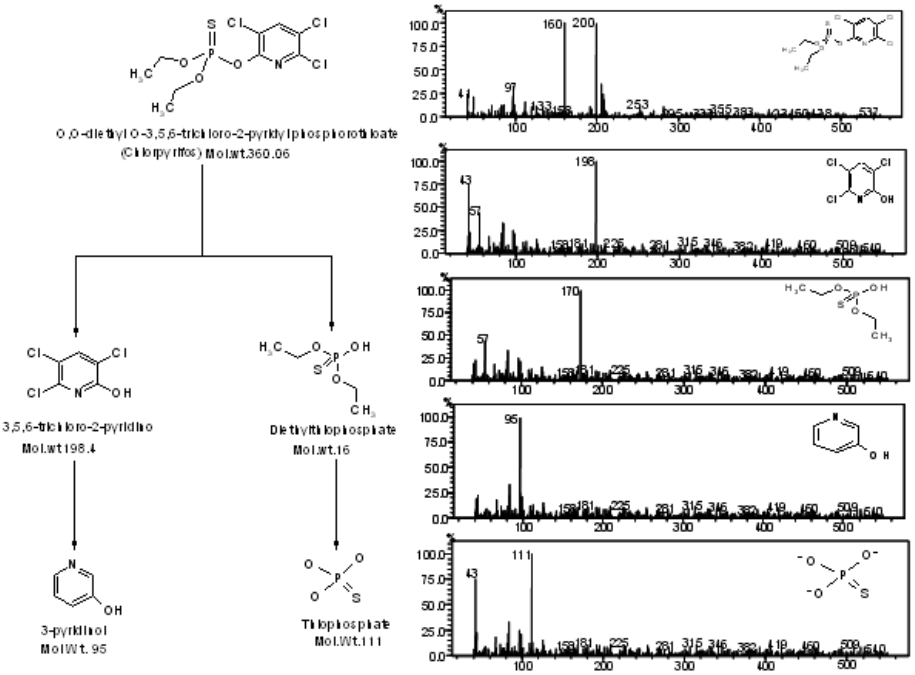


Figure. 5
***Proposed degradation pathway of chlorpyrifos by Pseudomonas desmolyticum* NCIM 2112**

Upon GCMS analysis chlorpyrifos shows retention time of 9.558 minutes. As compared with the NIST library database the retention time of chlorpyifos was matched with respect to mass/charge ratio v/s relative intensity. The results obtained after degradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112 obtained different metabolites as compared to the studies previously described. The results obtained from GCMS analysis clearly show the formation of 2-pyridinol and thiophosphate from cholrpyrifos degradation by *Pseudomonas desmolyticum* NCIM 2112 as shown in (Figure 5).

DISCUSSION

Bioremediation technique receives attention towards the cleaning of pesticide polluted agriculture land because of their cost effectiveness and ecofriendly nature. Degradation of chlorpyrifos by *Enterobacter* strain B-14 in contaminated soil was previously get studied[11] and it was getting concluded that after degradation the metabolite obtained were utilized by *Enterobacter* for growth and energy. Similarly by using mineral salt medium biodegradation of chlorpyrifos by *Arthrobacter* species have been reported[12]. Involvement of plasmids for the biodegradation of chlorpyrifos like pesticides was previously reported[13]. The phytotoxic effects of pesticide can be counteracted by inoculation with promising degrading bacteria both individually and in combination[14].The insecticide additive toxicity with respect to soil parameters was previously studied, and it was getting concluded that insecticide additive is responsible for decreasing soil fertility and by means of using promising degrading bacteria bioremediation of soil can be possible[15].

CONCLUSION

The results of current research work shows that the bacteria *Pseudomonas desmolyticum* NCIM 2112 degrade chlorpyrifos and convert it into non toxic metabolites like 2-pyridinol and thiophosphate. There are many reports of chlorpyrifos degradation by using a mixed culture of bacteria are get reported, but by using bacteria like *Pseudomonas desmolyticum* NCIM 2112 complete biodegradation of chlorpyrifos is first time get reported here. The obtained results indicates that by using effective degradation strategies i.e. by determining effective physicochemical parameters and using the bacteria *Pseudomonas desmolyticum* NCIM 2112 chlorpyrifos degradation in contaminated water and soil is possible.

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