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RESEARCH ARTICLE

REVIEW ARTICLE MICROBIAL DEGRADATION OF CHLORPYRIFOS

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ABSTRACT

Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is an organophosphorus compound using among the world to control insects and mites on a number of crops. Chlorpyrifos is one of the world's most widely used organophosphorus pesticides in agriculture. Chlorpyrifos is having high soil absorption co-efficient, but low water solubility. Microbial degradation is depends of the selection of microbial strains. Biodegradation of organophosphates involves activities of phosphatase, esterase, hydrolase and oxygenase enzymes. But the hydrolysis of chlorpyrifos is an important process in the degradation of organophosphorus insecticides, usually resulting in an increase in the number of polar metabolites and a reduction in acute toxicity. Two mechanisms of chlorpyrifos hydrolysis may occur due to pH effects. Recently, research activity in this area has shown that a diverse range of microorganisms is responsible for chlorpyrifos degradation. This article therefore aims at giving an overview of the present status of research in biodegradation of chlorpyrifos**.**

INTRODUCTION

Pesticides are chemical compounds which covers a wide range of compounds insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators (Aktar *et al.*, 2009). Organophosphorus compounds, which are a group of highly toxic agricultural chemicals widely used for crop production, have generated a number of ecological problems such as air pollution, water pollution and dislocating biogeochemical cycles(Zeinat *et.*, 2008, Horne *et.*, al 2002, Cisar and Snyder, 2000).

Chlorpyrifos, a pesticide that can easily enter the human food chain has more victims to its credit than carcinogenic air pollutants such as polycyclic aromatic hydrocarbons (PAHS). A study conducted by researchers from US based Columbia University found a strong link between prenatal exposure to chlorpyrifos and low birth weight and smaller head size of infants.

Several studies correlate the smaller size of the head with lower Intelligence quotient (IQ) and poor functioning. The researchers studied 263 pregnant females, who lived in areas exposed to almost the same level of pollution. The researchers used blood plasma levels to estimate the amount of chlorpyrifos. The use of chlorpyrifos has been vastly restricted in US and some European countries, even for agricultural purposes. However, it is still widely used in developing countries like India, where in the year 2000, it was the fourth highest consumed pesticide after monocrotophos, acephate and endosulfan. (Ansaruddin, P.A. 2003)

Chlorpyrifos is a non-systemic insecticides used in agricultural activities to control insects and pests. Chlorpyrifos is used both for agricultural pest control and in households as a termiticide. Commercially, it is available in commercial scale in various formulations under the trade names such as Lorsban, Pyrinex, Spannit, Tricel, Dursban, Piridane, Silrifos, and Talon to name a few. Chlorpyrifos interferes with the normal functioning of the central nervous system, including the brain. It works basically the same way in humans as it does in insects. In fact, chlorpyrifos (Dursban) belongs to a group of chemicals known as organophosphates, which were first developed by the Germans in the 1930s and were later used to kill people in concentration camps during World War-II. More recently, members of the Japanese cult Aum Shinriko used a related organophosphate compound Sarin in trying to exterminate commuters on Tokyo metro. The US government is investigating whether substantial use of organophosphates to control desert pests during the Gulf War caused the neurological ailment known as the Gulf War Syndrome. (David, C. 2000) Chlorpyrifos shows a wide spectrum of biological activity and is used to control range and forage insect pests as well as soil dwelling grubs, rootworms, borers and subterranean termites. It is available in a variety of formulations, such as granules, wettable powder, dustable powder and emulsifiable concentrate. (Swati & Singh 2002)

Extensive use of chlorpyrifos contaminates air, ground water, rivers, lakes, rainwater and fog water. The contamination has been found up to about 24 kilometres from the site of application. Symptoms of acute poisoning include headache, nausea, muscle twitching and convulsions and in some extreme cases even death. Human birth defects have also been associated with exposure to chlorpyrifos and its products. It also affects the male reproductive system. Chlorpyrifos is toxic to a variety of beneficial arthropods including bees, ladybird beetles and parasitic wasps. It kills fishes at concentrations as low as a few parts per trillion. Birds are also susceptible with effects ranging from reduced weight of nestlings, deformities and death. In plants there have been reports of delayed seedling emergence, fruit deformities and

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abnormal cell division upon prolonged exposure to chlorpyrifos. (NCAP, 2000)

Unlike in the case of other organophosphates, however, there have been no reports of enhanced degradation of chlorpyrifos since its first use in 1965 until, recently when Singh et al. 2003, isolated six chlorpyrifos degrading bacteria from an Australian soil showing enhanced degradation of chlorpyrifos. Yang et al. 2005 isolated *Alcaligenes faecalis* DSP3, which is capable of degrading chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCP). Although the microbial degradation of chlorpyrifos has been investigated, the existing papers lack information on the genetic and enzymatic aspects involved in its degradation.

This study reviews therefore, aims at throwing a chronological light on the research efforts undertaken worldwide to isolate, characterization and identification of chlorpyrifos degrading bacteria to investigate their degradation potential of chlorpyrifos.

Microbiological transformation of chlorpyrifos and its metabolites

In general, microorganisms demonstrate considerable capacity for the metabolism of many pesticides. Although they are capable of catalyzing similar metabolic reactions as mammals and plants, they possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds. There are two general types of reactions which are used in degradation of these materials: catabolism and cometabolism or incidental metabolism. Catabolism results from degradation in which microorganisms degrade or partially degrade the target compounds (xenobiotics) and gain energy or nutrients from this process which contributes to cell growth and development of the microorganisms. Incidental metabolism or cometabolism results when microorganisms gain neither energy nor nutrients for themselves; only involve these degradative processes due to their inherent metabolic activities (Racke, 1993).

Studies conducted in soil have generally reported significantly longer dissipation half–lives under sterilized versus natural conditions and led to the conclusion that microbial activities are important in the degradation of chlorpyrifos in soil (Miles et al. 1984). Evidence from soil degradation studies indicates that cleavage and mineralization of the heterocyclic ring occurs in soil due to the activities of microorganisms (Racke & Coats 1990). However, the singularly most important microbial role in the chlorpyrifos degradation pathway may be the further metabolism and mineralization of 3, 5, 6-trichloro-2-pyrinidinol (TCP) and 3, 5, 6-trichloro-2-methoxypyridine (TMP) metabolites (Racke 1993).

Microbial enzymes have been shown to hydrolyze chlorpyrifos under controlled conditions. Munnecke and his co-worker in 1975 first reported the ability of parathion hydrolase, an organophosphorus ester-hydrolyzing enzyme isolated from a mixed microbial culture, to hydrolyze chlorpyrifos.

The hydrolysis of chlorpyrifos is an important process in the degradation of organophosphorus insecticides, usually resulting in an increase in the number of polar metabolites and a reduction in acute toxicity. Two mechanisms of chlorpyrifos hydrolysis may occur due to pH effects: neutral hydrolysis and alkaline hydrolysis. Neutral hydrolysis involves nucleophilic attack of water at the ethoxy carbon, hydrolyzing chlorpyrifos to deethyl chlorpyrifos and ethanol. Alkaline hydrolysis of chlorpyrifos involves in the phosphorus atom which is attacked by the nucleophilic hydroxide.

Jones and Hastings (1981) reported the metabolism of 50-ppm chlorpyrifos in cultures of several forest fungi (*Trichoderma harzianum, Penicillium vermiculatum, and Mucor sp.).* After 28 days, chlorpyrifos and its metabolite TCP were present in all cultures at levels of 2-5 % and 1-14% of applied, respectively. Ivashina (1986) studied chlorpyrifos degradation by several microbial cultures maintained in liquid media containing 10 ppm chlorpyrifos. Dissipation was more rapid in a sucrose-supplemented media containing *Trichoderma sp*. and glucose supplemented media containing Bacillus sp. than in control media containing no microorganisms. Chlorpyrifos disappeared from the microbial cultures in a linear fashion over a 2-week period. Lal and Lal (1987) observed some degree of degradation by the yeast *Saccharomyces cervisiae*. Only half the initial chlorpyrifos was recovered 12 h after the cultures were inoculated with 1-10 ppm. The possible metabolism by two lactic acid bacteria (*Lactobacillus bulgaricus and Streptococcus thermophilus)* was reported by Shaker et al. (1988). The synthetic culture medium, in which the bacteria were grown initially contained 7.4 ppm, but displayed a 72-83% loss in chlorpyrifos after 96 h. Havens and Rase (1991) circulated a 0.25 % aqueous (EC) solution of chlorpyrifos through a packed column containing immobilized parathion hydrolase enzyme obtained from Pseudomonas diminuta. Approximately 25 % of the initial dose was degraded after 3 h of constant recirculation through the column. Strains of *Aspergillus flavus and Aspergillus niger* isolated from agricultural soil with previous history of chlorpyrifos use were, also reported to biomineralise chlorpyrifos in liquid culture medium (Swati & Singh 2002). Yu et al. (2006) isolated and characterized a fungal strain capable of degrading chlorpyrifos.18S rDNA gene analysis revealed that they showed that the fungal strain had a high level of homology (99%) to those from other *Verticillium species.* They found that the degradation of chlorpyrifos in by the fungal strain in mineral salt medium increased almost linearly with increasing concentrations of chlorpyrifos (r^2 =0.9999), suggesting that the degradation is subjected to pseudo-first order kinetics. With the first order kinetic function, the DT_{50} of chlorpyrifos at concentrations of 1, 10, and 100 mg/l, were calculated to be 2.03, 2.93, and 3.49 days, respectively. In the controls the hydrolysis percentages of chlorpyrifos were found to be less than 5%. They further used the cell free extracts of the strain to detoxify chlorpyrifos in vegetables and reported that the cell free extracts of the fungus can used for en hanced degradation in vegetables.

Some evidence also indicates that, the metabolites of chlorpyrifos are also degraded and mineralized by soil microorganisms. Several researchers have noted the extensive mineralization of TCP and TMP to carbon dioxide in soil. Racke et al. (1988) reported that approximately 65-85 % of TCP applied (5ppm) to several soils was mineralized within 14 days. The initially accelerating rate of mineralization observed in these soils was indicative of microbial enzyme induction or adaptation. Racke and Robbins (1991) probed a suite of soils for evidence of the presence of TCP-catabolizing microorganisms. Of the 25 soils investigated, only two

displayed significant degradation of TCP within 21 days of inoculation into mineral salt medium containing 5-ppm TCP as the sole carbon source. Isolation a pure culture of bacteria capable of using 3, 5, 6-trichloro-2-pyridinol (TCP) as the sole source of carbon and energy under aerobic conditions was reported for the first time by Feng and his co-workers in 1998. The bacterium was identified as a *Pseudomonas sp.* and designated as ATCC 700113. The TCP degradation yielded CO_2, chloride and some unidentified polar metabolites. They further reported that the degradation of the parent compound, TCP, by the *Pseudomonas sp.* appeared to involve a reductive de-chlorination mechanism.

Microbial degradation of chlorpyrifos

Soil microorganisms play a significant role in degradation of chlorpyrifos and its metabolites. (Thiegs, 1964) first investigated chlorpyrifos microbial transformation rates in natural and autoclaved sandy loam soil. He observed that both non-autoclaved and autoclaved soil contained TCP, a common chlorpyrifos hydrolysis product, but a relatively lower chlorpyrifos degradation rate in the autoclaved soil.

Subsequently, (Miles *et al*., 1979) reported similar results from studies comparing autoclaved and natural mineral and organic soils. Chlorpyrifos half-lives in natural and sterile mineral soil were less than 1 and 17 weeks, respectively; and 2.5 and more than 24 weeks in natural and sterile organic soil, respectively.
(Getzin 1981a) presented additional results which demonstrated that microorganisms enhance chlorpyrifos and TCP biodegradation in natural soil. Half-lives in sterile soils were approximately 1.7 to 2.7-fold longer than in natural soils. In addition, TCP accumulated in the sterile soil but degraded quite rapidly in the natural soil. This information indicates the importance of microbial involvement in further degrading this primary metabolite (Getzin, 1981a).

Reports by (Miles *et al.,* 1983) have shown that remarkable differences can exist in chlorpyrifos persistence in sterile natural mineral (sandy loam) and organic (muck) soils when held at three different temperature and four moisture levels for a period of 24 weeks.

The half-lives of chlorpyrifos in muck and sterile muck were 6 weeks and 24 weeks respectively, and the results from sandy loam were quite similar (Miles *et al.,* 1983). Chlorpyrifos was least stable in air-dry soil (Miles *et al.,* 1984).

The rate of disappearance of chlorpyrifos increased with temperature increased from 3°C to 28°C. The authors postulated that this was correlated with the increasing microbial activities and the higher microbe populations (Miles *et al.,* 1983).

There are reports indicating less involvement of microbes in chlorpyrifos degradation. (Jones and Hasting, 1981) reported no significant difference in chlorpyrifos degradation rates between sterile and natural forest soils. (Yoshioka *et al.,* 1991) found that there was no significant difference in the degradation rate between sterile and non sterile soil containing high initial concentrations (100 ppm) of chlorpyrifos, but differences were found in the degradation rate between sterile and non sterile soil containing lower initial concentrations (50 ppm).

Chlorpyrifos, like many other organophosphorus insecticides, is strong cholinesterase inhibitors (Matsumura, 1985; Chambers and Levis, 1992). It also affects soil microbial activities (Pozo *et al*., 1995) reported that a soil aerobic di nitrogen-fixing bacterial population was initially suppressed by the addition of chlorpyrifos. However, the bacterial populations were able to recover 14 days later. Soil fungi, nitrifying bacteria, and denitrifying bacteria were not affected by the addition of chlorpyrifos (Pozo *et al*., 1995).

Racke *et al.,* 1994) studied chlorpyrifos degradation in soil applied at termiticidal application rate. They found the half-lives of chlorpyrifos ranged from 175 to1576 days at an initial concentration of 1000 µg g-1. One important reason for the extra long chlorpyrifos residual life was a possible chlorpyrifos inhibitory effect on the soil microbial population at high concentrations (Racke *et al.,* 1994).

(Cink and Coats, 1993) reported a relatively low mineralization rate of chlorpyrifos at higher concentrations in an urban Iowa soil. Soil moisture level and chlorpyrifos concentration are two major factors that dramatically influence the extent of chlorpyrifos mineralization. For example, a 17% mineralization rate was observed at 10 ppm, whereas only a 0.67 and 0.30% mineralization rates occurred at 500 and 1000 ppm respectively, at the same moisture level (0.30 bar).

(Somasundaram *et al.,* 1989) suggested that TCP may have the capability of inhibiting microbial degradation of chlorpyrifos in soil because they found chlorpyrifos degradation half-life increased in soil treated with TCP before the application of chlorpyrifos.

The possibility of enhanced microbial chlorpyrifos degradation had been studied by (Racke, 1988, Coats 1990 and Racke *et al.,* 1990). Microbial populations adapted for rapid degradation of isofenphos did not change the rate of chlorpyrifos degradation. In laboratory studies, an *Arthrobacter sp*. isolated from soils with a history of isophenphos use rapidly metabolized isophenphos in pure culture but did not metabolize or co metabolize chlorpyrifos (Racke and Coats, 1988). Repeated treatments of soils with chlorpyrifos in the laboratory have not resulted in accelerated rates of chlorpyrifos degradation, and the annual field application of chlorpyrifos for 2-4 years did not alter the half-life of chlorpyrifos in the field (Racke *et al.,* 1990).

(Sethunathan and Pathak, 1972) reported rapid diazinon biodegradation in rice paddy water after repeated diazinon application, but not in the case of chlorpyrifos. (Lal *et al.,* 1987) studied chlorpyrifos degradation in the culture of the ciliate protozoan *Tetrahymena performs.* They found no significant chlorpyrifos metabolism by this protozoan, although they demonstrated its ability to metabolize DDT to DDD and DDE.

On the other hand, there are also reports showing that enhanced microbial chlorpyrifos degradation can occur, however, in some of these reports there is a lack of sufficient evidence. Direct microbial metabolism of chlorpyrifos in the laboratory was first reported by the (Hirakoso, 1969). However, twenty-seven different bacterial species *(Pseudomonas spp., Bacillus spp.,* and others) failed to metabolize chlorpyrifos in laboratory cultures grown in peptone and glucose peptone media (Hirakoso, 1969).

Significant levels of chlorpyrifos degradation have been reported by (Jones and Hasting, 1981) which were attributed to several species of forest soil fungi (*Trichoderma harzianum*, *Penicillium multicolor*, *Penicillium vermiculatum*, *Mucorsp.*). But chlorpyrifos loss in the control groups over the same time period was also significant. Both microbial factors and abiotic factors such as the loss through evaporation were possible, as concluded by (Jones and Hasting, 1981). One common yeast species (*Saccharomyces cerevisiae*) degraded chlorpyrifos rapidly in the medium, but the details of metabolism mechanism or metabolites were not provided (Lal and Lal, 1987).The wood-rotting fungus *Phanerochaete chrysosporium* was able to mineralize chlorpyrifos (27.5%) during 18-days incubation in nitrogen-limited cultures (Bumpus *et al.,* 1993).Their results clearly demonstrated that the chlorinated pyridinyl ring of ^{14} C-chlorpyrifos may undergo ring cleavage during biodegradation by *P. chrysosporium*. While only the wood-rotting fungus *Phanerochaete chrysosporium*, isolated from soil, has been unequivocally shown to have the capability of chlorpyrifos degradation in the laboratory, there have been several reports indicating microbial degradation of TCP and TMP (Racke *et al.,* 1988, Racke and Robbins, 1991 and Feng *et al.,* 1997a, b). Approximately 65-85% of the TCP applied to several soils was mineralized within 14 days (Racke *et al.,* 1988). Racke and Robbins (1991) conducted research on the degradation of TCP in 25 different soils, and found that the addition of glucose accelerated chlorpyrifos degradat-on in the soils. Two of 25 soils containing microbial populations had the capability of utilizing TCP as a sole carbon source in mixed culture. Cultures isolated from soil that had 45.1% TCP mineralization were able to rapidly degrade TCP after a lag of about 7 days.

However, there was little TCP degradation in the culture inoculated with the sterilized soil (Racke and Robbins, 1991). TCP was mineralized by immobilized *Pseudomonas sp*. strain M285 (Feng *et al.,* 1997b). Overall, 75% of the mineralization rate was achieved by using this technique. Recently, they reported that they had successfully isolated a *Pseudomonas sp*. that is capable of mineralizing TCP to $^{14}C_2$, chloride, and unidentified polar metabolites (Feng *et al.,* 1997a). The addition of carbon sources such as glucose, maleic acid, and succinic acid stimulated this mineralization process.

CONCLUSION

Isolation and characterization of chlorpyrifos degrading bacteria is crucial for enhancing our understanding of the variety of mechanisms. Microbiological transformation of chlorpyrifos and its metabolites was observed. The bio-degradative pathways relating to their enhanced degradation in the environment. Chlorpyrifos has now been shown to undergo enhanced biodegradation by bacterial and fungal species. Bioremediation technologies are in the process of development for this toxic compound and related nerve agents using organophosphorus hydrolase enzyme.

Future Perspective

Future ,studies on to examine the potential for degradation of chlorpyrifos pesticide by the bacteria isolated from different cultivated soil and finding the optimum conditions of bacteria and extraction of pesticide analysis by using this methods TLC, GC and HPLC.

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References

1. Ansaruddin, P.A. and Vijayalakshmi, K., 2003 The womb is not safe anymore. Indegenous Agriculture
2. David, C. (eds) 2000 Dursban is everywhere. In: DURSBAN CASE STUDY: The pesticide theEPA has finally banned. http://www.tompaine.com/Archive/scontent/3233.html.cited 09 Jun,2000
3. Degrading bacterium. Appl Environ Microbiol 69(9): 5198-5206.
4. Feng, Y. Minard, R.D. And Bollag, J.M. 1998. Photolytic and Microbial degradation of 3, 5, 6-trichloro-2-pyridinol. Environ Toxicol Chem 17(5): 814-819.
5. Guha, A., Kumari, B., Bora, T.C., and Roy, M.K. 1997. Possible involvement of plasmids in degradation of Malathion and chlorpyrifos by Micrococcus sp. Folia Microbiol 42(6): 574-576.
6. Havens, P.L., and Rase, H.F. 1991. Detoxification of organophosphorus pesticide solutions In: Tedder, D.W., Pohland, F.G. (eds) Emerging technologies in Hazardous waste management II. ACS, Washington, DC: 261-281.
7. Huang, X., Lee, L.S., and Nakatsu, C. 2000. Impact of animal waste lagoon effluents on chlorpyrifos degradation in soils. Environ Toxicol Chem 19(12): 2864-2870.
8. Ivashina, S.A. 1986 Interaction of Dursban with soil micro-organisms, Agrokhimia 8: 75-76
9. Jones, A.S., Hastings, F.L. 1981 Soil microbe studies. In: Hastings F.L., Coster J.E. (eds) Field and laboratory evaluations of insecticides for southern pine beetle control.USDA. Southern Forest Experiment Station, Forest Service, SE 21:13-14
10. Jones, A.S., Hastings, F.L. 1981 Soil microbe studies. In: Hastings F.L., Coster J.E. (eds) Field and laboratory evaluations of insecticides for southern pine beetle control.USDA. Southern Forest Experiment Station, Forest Service, SE 21:13-14
11. Lal, S. and Lal, R. 1987. Bioaccumulation, metabolism, and effects of DDT, fenitrothion, and chlorpyrifos on Saccharomyces cerevisae. Arch Environ Contam Toxicol 16: 753-757.
    Ivashina, S.A. 1986 Interaction of Dursban with soil micro-organisms, Agrokhimia 8: 75-76
12. Lal, S. and Lal, R. 1987. Bioaccumulation, metabolism, and effects of DDT, fenitrothion, and chlorpyrifos on Saccharomyces cerevisae. Arch Environ Contam Toxicol 16: 753-757.
13. Mallick, K., Bharati, K., Banerji, A., Shakil, N.A., and Sethunathan, N. 1999. Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bull Environ Contam Toxical 62:40-54
14. Mallick, K., Bharati, K., Banerji, A., Shakil, N.A., and Sethunathan, N. 1999. Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bull Environ Contam Toxical 62:40-54
15. Miles, J.R.W., Harris, C.R., and Tu. C.M. 1984. Influence of moisture on the persistence of chlorpyrifos and

chlorfenvinphos in sterile and natural mineral and organic soils. J Environ Sci Hlth B14:237-243

16. Miles, J.R.W., Harris, C.R., and Tu. C.M. 1984. Influence of moisture on the persistence of chlorpyrifos and chlorfenvinphos in sterile and natural mineral and organic soils. J Environ Sci Hlth B14:237-243
17. Mishra, D., S. Bhuyan, T.K., Adhya. , and Sethunathan. N. 1992 Accelerated degradation of methyl parathion, parathion and fenitrothion by suspensions from methyl parathion and p-nitrophenol treated soils. Soil Biol Biochem 24: 1035-1042.
18. Mishra, D., S. Bhuyan, T.K., Adhya. , and Sethunathan. N. 1992 Accelerated degradation of methyl parathion, parathion and fenitrothion by suspensions from methyl parathion and p-nitrophenol treated soils. Soil Biol Biochem 24: 1035-1042.
19. Munnecke, D.M. and Hsieh, D.P.H. 1975 Development of microbial systems for the disposal of concentrated pesticide suspensions. Med Facul Landbouww Rijksuniv Gent 40: 1237-1247.
20. Munnecke, D.M. and Hsieh, D.P.H. 1975 Development of microbial systems for the disposal of concentrated pesticide suspensions. Med Facul Landbouww Rijksuniv Gent 40: 1237-1247.
21. Racke, K.D., Coats, J.R., and Titus, K.R., 1988. Degradation of chlorpyrifos and its hydrolysis product, 3, 5, 6- trichloro-2-pyridinol, in soil. J Environ Sci Hlth B (23): 527-539.
22. Sethunathan, N., and Yoshida, T. 1973. A *Flavobacterium sp*. that degrades diazinon and parathion. Can J Microbiol 19: 873-875.

Sethunathan, N., Pathak, M.D. 1972. Increased biological

hydrolysis of diazinon after repeated application in rice paddies J agric Food Chem 20:586-589.

23. Sethunathan,N.1971Biodegradation of diazinon in paddy fields as a cause of its inefficiency for controlling brown planthoppers in rice fields. PANS 17:18-19
24. Shaker, N., Abo-Donia, S., El-Shaheed, Y.A., and Ismail. 1988. Effect of lactic acid bacteria and heat treatments on pesticides contaminated milk. Egypt J Diary Sci 16: 309-317.
25. Singh, B.K., Walker, A., Morgan, J.A.W., and Wright, D.J. 2003 Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-
26. Swati and Singh, D.K. 2002 Utilization of chlorpyrifos by Aspergillus niger and A. flavus as carbon and phosphorus source. 17th World Congress of Soil Science, 14 - 21 Aug 2001
27. Yang, C. Liu, N., Guo, X., and Qiao, C. 2006 Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS Microbiol Lett 265:118-125.
28. Yang, L., Zhao, Y.H., Zhang, BX., Yang, C.H., and Zhang, X. 2005 Isolation and characterization of a chlorpyrifos and 3, 5, 6- trichloro-2-pyridinol degrading bacterium. FEMS Microbiol Lett 251: 67-73.
29. Yu Y.L., Fang, H., Wang, X., Wu X.M., Shan, M and Yu, J.Q. 2006 Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation DOI 10. 1007/s/0532-005-9020-z

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