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Research Article

Biodegradation of Chlorpyrifos Using Staphylococcus sp., Isolated From the Rhizospere of *Calotropis gigantea* r.Br.

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Abstract: The present study explained about the effect of chlorpyrifos pesticide on rhizospheric soil from the plant of Calotrophis gigatica has been investigated. The current investigation was carried out the objectives such as biodegradation of chlorpyrifos by Staphylococcus sp. isolated from the soil previously exposed to chlorpyrifos. Subsequently to study the toxicity of different bioremediated media using L. reticulates as a test animal after different days of incubation. Staphylococcus species is a native soil bacterium growing on the cholrpyrifos mixed in the broth medium. To investigate whether the chlorpyrifos has been degraded by the bacteria both control (broth + chlorpyrifos) and experimental medium (broth + chlorpyrifos + Staphylococcus bacteria) were exposed to L. reticultes after 24, 48, 72 and 96hrs of incubation. When the experimental fish L. reticulates fed with control broth medium there was a heavy mortality within the short period of time in 12ppm concentration. While the fishes survived when fed with broth containing chlorpyrifos incubated with bacterium for 96hrs. From the present study confirmed that the isolated chlorpyrifos degrading bacterium could be used successfully for the removal of the pesticides in agriculture their use and toxicity it is recommended that research or biodegradation particularly on bioremediation sites particularly pesticides contaminated areas in agricultural field.
Keywords: Pesticides, Chlorpyrifos, Bioremediation, Bacterium.

INTRODUCTION

Organophosphorus pesticides are widely used in agriculture to control major insect pests. These compounds have been implicated in several nerve and muscular diseases in human beings, organophosphorus compound poisoning is a worldwide health problem with around 3 million poisoning and 20,0000 deaths annually[1, 31]]. Many of the organophosphate and organo chlorine pesticides are photosensitive described by [2]. These are degraded by ultraviolet energy (UV) of the chlorinated hydrocarbon pesticides [3]. Several environmental fate processes, including sorption, hydrolysis, volatilization, transport and accumulation of bound residues, are coupled with degradation [3] each of these processes may respond differently to environmental conditions, thus making comprehension of factors controlling degradation challenging [4] and biological removal of chemo-pollutants becomes the method of choice wit the help of a variety of xenobiotic compounds including pesticides for their growth and mineralize and detoxify them [5].

Microbial degradation of organophosphate pesticides is of particular interest because of the high mammalian toxicity and for the remediation of organo phosphorus pesticides [6]. Chlorpyrifos is an insecticide that has been one of the pesticides most used worldwide since 1965. Currently, over 850 registered chlorpyrifos products are on the market. Chlorpyrifos is being used globally as an agriculture based pesticide [7]. Chlorpyrifos is a widely used organophosphate insecticide [8]. Recent advances in bioremediation technology have introduced more effective and efficient methods for handling the pesticide [9]. In most of the studies chlorpyrifos has been reported to be degraded by soil bacteria, such as Escherichia coli [10], Arthrobacter sp. [11]. Agro bacterium sp. [6] and Enterobacter asburiae [12]. The present investigation was carried out the objectives such as biodegradation of chlorpyrifos by Staphylococcus sp. isolated from the soil previously exposed to chlorpyrifos. Then to study the toxicity of different bioremediated media using L. reticulates as a test animal after different days of incubation.

MATERIALS AND METHODS

Collection of microbial sample from the pesticide contaminated site

Soil samples from the rhizosphere of Calotropis gigentea R. Br sprayed with chlorpyrifos was collected for screening the presence of soil bacteria. The soil samples were collected after 36 h of the application of

pesticide. Chlorpyrifos is a liquid formulation and was applied at the base of the plant. The soil samples were collected from the region of pesticide application. Soil samples were carefully removed to the laboratory in sterilized container to avoid other microbial contamination.

Isolation, identification and screening of the isolates

The collected soil samples were serially diluted with distilled water and agar plates were inoculated with different dilutions of bacterial sample. After 24 h a bacterial strain was observed in the culture with spherical contour. The isolated strain was identified using the Bergy's manual (Vol. I and II). The isolates were screened for their efficiency in the bioremediation of chlorpyrifos. For screening their ability to tolerate the pesticide medium, the isolated microbe was inoculated on the medium containing different concentration (10, 20 and 50 µl) of chlorpyrifos. The bacterium that tolerates maximum concentration of chlorpyrifos was selected and subcultured for further studies.

Preparation of different broth media

To study the bioremediation of chlorpyrifos different broth media were prepared with nutrient broth. (i) Medium with nutrient broth and 1% chlorpyrifos (ii) Medium with nutrient broth and 1% chlorpyrifos inoculated with isolated bacterial strain. The above mentioned cultures were maintained in the room temperature. After 24, 48, 72 and 96 h of incubation the broth medium were analysed for the degradation of chlorpyrifos by Staphylococcus sp.

Test for the remediation of chlorpyrifos

To check whether the chlorpyrifos detoxified or not different concentration of the broth from the two different cultures were poured into 1 litre of water and ten guppies (Lepistes reticulates) were introduced into each container containing the medium. The mortality rate of L. reticualtes recorded after different hours of exposure.

RESULT

The results of the present study were presented in tables 1 and 2. In response to 2 ppm chlorpyrifos the mortality of 2.4 ± 0.55, 4.4 ± 0.55, 6.4± 0.55 and 8.4 ± 0.55 was recorded after 24, 48, 72 and 96 hr exposure respectively. The corresponding value for the medium containing chlorpyrifos inoculated with the bacterium (24 hr incubation) is 2 ± 0.70, 3.8 ± 0.84, 5.8 ± 0.84 and 7.8 ± 0.84 which is 7.14 percent decreased from the control value. When L. reticulates exposed to 2 ppm chlorpyrifos inoculated with bacterium (48 hr incubation) the mortality was noted 1.6 ± 0.55 after 24 hr, 3.6 ± 0.90 in 48 hr, 5.6 ± 0.55 in 72 hr (12.5 percent decreased over control) and 7.8 ± 0.84 in 96 hr (table 5). While L. reticulates exposed to 2 ppm chlorpyrifos (bioremediated for 72 hr) the mortality is 0.8 ± 0.45 in 48 hr, 2.4 ± 0.55 in 72 hr and 4 ± 0.70 in 96 hr exposure. The corresponding value for 96 hr bioremediated medium is 0.4 ± 0.55, 1.4 ± 0.55 and 2.6 ± 0.90 respectively which is 69.04 percent decreased from the control value.

In response to 4 ppm chlorpyrifos the mortality of 2.6 ± 0.55, 4.6 ± 0.55, 6.6 ± 0.55 and 8.6 ± 0.55 was recorded after 24, 48, 72 and 96 hr exposure respectively. The corresponding value for the medium containing chlorpyrifos inoculated with the bacterium (24 hr incubation) is 2.4 ± 0.55, 4.4 ± 0.90, 6.2 ± 0.84 and 8.2 ± 0.84 which is 4.66 percent decreased from the control value. Similarly, when L. reticulates exposed to 4 ppm chlorpyrifos inoculated with bacterium (48 hr incubation) the observed mortality was 0.6 ± 0.9 after 24 hr, 3 ± 1.0 in 48 hr, 5.2 ± 1.01. It was 21.21% and 7.8 ± 0.84 in 96 hr decreased than control.

Consecutively, When L. reticulates exposed to 4 ppm chlorpyrifos (bioremediated for 72 hr) the mortality is 1.6 ± 0.44 in 48 hr, 3.6 ± 0.55 in 72 hr and 5.4 ± 0.55 in 96 hr exposure and 37.20% decreased from the control value. The corresponding value for 96 hr bioremediated medium is 1.2 ± 0.45, 3.2 ± 0.45 and 5.2 ± 0.45 respectively.

In response to 6 ppm chlorpyrifos the mortality of 2.8 ± 0.45, 4.8 ± 0.45, 6.8 ± 0.45 and 8.8 ± 0.45 was recorded after 24, 48, 72 and 96 hr exposure respectively. The corresponding value for the medium containing chlorpyrifos inoculated with the bacterium (24 hr incubation) is 2.6 ± 0.55, 4.6 ± 0.55, 6.6 ± 0.55 and 8.6 ± 0.55. Which were 2.28 percent decreased from the control value. When, L. reticulates exposed to 6 ppm chlorpyrifos inoculated with bacterium (48 hr incubation) the mortality is 2.2 ± 0.45 after 24 hr, 4.2 ± 1.09 in 6.2 ± 1.09 (8.82 percent decreased over control) and 8.6 ± 1.14 in 96 hr. In L. reticulates exposed to 6 ppm chlorpyrifos (bioremediated for 72 hr) the mortality is 1.2 ± 0.45 in 48 hr, 3 ± 0.70 in 72 hr and 5 ± 0.70 in 96 hr exposure and 43.18 percent decreased from the control value. The corresponding value for 96 hr bioremediated medium is 1.4 ± 0.55, 2.8 ± 0.45 and 4.8 ± 0.85 respectively.

In response to 8 ppm chlorpyrifos the mortality of 3 ± 0.70, 5.2 ± 0.83, 7.2 ± 0.83 and 9.2 ± 0.83 was recorded after 24, 48, 72 and 96 hr exposure respectively. The corresponding value for the medium containing chlorpyrifos inoculated with the bacterium (24 hr incubation) is 2.8 ± 0.84, 5.0 ± 1.0, 7.0 ± 1 and 8.8 ± 1.09 which is 4.35 percent decreased from the control value. In L. reticulates exposed to 8ppm chlorpyrifos inoculated with bacterium (48 hr incubation) the mortality is 2.6 ± 0.90 after 24 hr 4.6 ± 1.14 in 48 hr, 6.8 ± 1.09 (5.56 percent decreased over control) and 8.8 ±1.09 in 96 hr (table 16). When L. reticulates exposed to 8 ppm chlorpyrifos (bioremediated for 72 hr) the

mortality is 2.2 ± 0.45 in 48 hr, 4.6 ± 0.55 in 72 hr and 6.6 ± 0.55 in 96 hr exposure and 28.27 percent decreased from the control value. The corresponding value for 96 hr bioremediated medium is 2 ± 0.70, 4 ± 0.70 and 6 ± 0.70 respectively. In response to 10 ppm chlorpyrifos the mortality of 3.4 ± 0.55, 5.4 ± 0.55, 7.4 ± 0.5 and 9.4 ± 0.55 was recorded after 24, 48, 72 and 96 hr exposure respectively. The corresponding value for the medium containing chlorpyrifos inoculated with the bacterium (24 hr incubation) is 2.8 ± 0.84, 5.0 ± 1.0, 7.0 ± 1.0 and 9.0 ± 1.0 which is 4.26 percent decreased from the control value (Table- 2).

Table 1: Mortality of L. reticulates fingerlings in water containing 2,4and 6 ppm concentrations of Chlorpyrifos enriched nutrient broth bioremediated at 24 and 48hrs

	Hours of observations of							
Replicates	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**
	No. of dead							
24 at **2ppm**	1.2 ± 0.8	2±0.7	2.8± 0.8	3.8 ± 0.8	4.8 ± 0.8	5.8 ± 0.8	6.8 ± 0.8	**7.8 ± 0.8**
24 hrs at **4ppm**	1.4 ± 0.6	2.4±0.5	3.4± 0.9	4.4 ± 0.9	5.2 ± 0.8	6.2 ± 0.8	7.2 ± 0.8	**8.2 ± 0.8**
24h 6 ppm	1.6±0.55	2.6±0.55	3.6±0.55	4.6±0.55	5.6±0.55	6.6±0.55	7.6±0.55	**8.6±0.55**
24h 8ppm	1.6±1.15	2.8±0.84	3.8±0.83	5.0±1.0	6.0±1.0	7.0±1.0	8.0±1.0	**8.8±1.09**
48hrs at 2ppm	0.6 ± 0.5	2.8 ±0.5	3.6±0.9	4.8 ±0.8	5.6 ± 0.5	5.6 ± 0.5	6.6 ± 0.9	**7.8 ± 0.8**
48hrs at 4ppm	0.6±0.9	2 ± 0.7	3 ± 1	4 ± 1	5.2 ± 1.1	6.4 ± 1.5	7.8 ± 0.8	**0.6±0.9**
48h8ppm	1.4±0.90	2.6±0.90	3.8±1.09	4.6±1.14	5.8±1.09	6.8±1.09	7.8±1.09	**8.8±1.09**

Table-2: Mortality of L. reticulates fingerlings in water containing 2, 4 and 6 ppm concentrations of Chlorpyrifos enriched nutrient broth bioremediated at 72 and 96hrs

Replicates	**Hours of observation**							
	12	**24**	**36**	**48**	**60**	**72**	**84**	**96**
	No. of dead							
72 hrs 2ppm	-	-	-	0.8 ± 0.5	1.8 ± 0.5	2.4± 0.4	3 ± 0.7	4 ± 0.7
72h 4ppm	-	-	0.4±0.55	1.6±0.55	2.6±0.55	3.6±0.55	4.4±0.55	5.4±0.55
72 hs6ppm	-	-	-	1.2±0.45	2.2±0.45	3 ±0.70	4 ±0.70	5 ±0.70
72hr8ppm	-	-	0.8±0.45	2.2±0.45	3.2±0.45	4.6±0.55	5.6±0.55	6.6±0.55
96h 2ppm	-	-	-	0.4±0.55	1 ±0.70	1.4±0.55	1.6±0.55	2.6±0.90
96h 4ppm	-	-	0.4±0.55	1.2±0.45	2.2±0.45	3.2±0.45	4.2±0.45	5.2±0.45
96hrs 6ppm	-	-	0.6±0.55	1.4±0.55	2.2±0.45	2.8±0.45	3.4±0.55	4.8±0.84
96h 8ppm	-	-	0.6±0.55	2 ± 0.70	3 ±0.70	4 ± 0.70	5 ± 0.70	6 ± 0.70

DISCUSSION

Bioremediation which involves the use of microorganisms to detoxify and degrade pollutants has received increased attention as an effective biotechnological approach to clean up polluted environments [6]. Previously, [13 ; 7] discussed about the several chemicals had been successfully removed from soil and aquatic environments using degrading microorganisms. In the present investigation, Staphylococcus sp. was utilized for remediating chlorpyrifos. Based on the findings of this work, the bacterial strain used in this study was more effective in the remediation of chlorpyrifos this kind of similar observation made by several researchers [14; 12; 15; 16]. When L. reticulates was exposed to the medium containing chlorpyrifos, maximum mortality was recorded within a short period with a mortality rate of 9.8 ± 0.55 in 12 ppm whereas the corresponding mortality rate recorded for L. reticulates exposed to broth containing Staphylococcus sp. is 8.4 ± 0.55 after 96 hr incubation. Which is 14.29 percent decreased from the control medium similar findings also been postulated by [18].

The bacteria Staphylococcus sp. has a high potential for chlorpyrifos degradation. Staphylococcus sp. are known to metabolize a broad range of organic compounds and therefore an ideal choice as the bacteria to be used for degradative biotechnologies [19; 20]. In fact, an extraordinary range of catabolic pathways in a single species such as P. ceparia utilizes more than 100 different substances as the only carbon, nitrogen or sulfur source [21]. Later, [22] studied the degradation of chlorpyrifos by Flavobacterium and Arthrobacter sp. and reported that the strains were able to use chlorpyrifos as a source of carbon. However due to the absence of the standard metabolites for chlorpyrifos in the samples tested they were not able to characterize and quantify the biodegraded products of chlorpyrifos. This kind of similar result also been studied[7].

Similarly in this study the inoculated Staphylococcus sp. could utilize the chlorpyrifos as a sole of carbon and phosphate [23]. Therefore the pesticide medium inoculated with the bacterium was less toxic to the L. reticulates when compared to the control medium containing chlorpyrifos alone [24].

The higher nutrient availability and larger microbial population of cowdung slurry and soil pesticide mixture was found to affect bioremediation of pesticides under controlled environmental conditions[7]. This is in agreement with the finding that animal-derived lagoon effluents are a good source of inorganic nutrients and organic matter and they have an impact on degradation and transport of soil applied pesticides [25; 26; 27]. The adaptability of microorganisms during bioremediation releases enzymes, which metabolizes wide spectrum of anthropogenic chemicals [28; 29]. The bacteria used in this study could survive in the medium containing chlorpyrifos either due to tolerance to the pesticide or due to their ability to degrade it [17]. This is an agreement with the studies done by Singh *et al.;* [30] isolated fungal strains from the groundnut fields in Rajasthan and found that A. niger and A. flavus utilized chlorpyrifos as a sole source of carbon and phosphorus. They reported that when A. flavus was provided with 200 mg Kg^{-1} of chlorpyrifos it utilized about 96.2% of chlorpyrifos as nitrogen and phosphorus source within 24 hrs of incubation, various authors have also reported that chlorpyrifos is degraded metabolically in liquid medium by a Flavobacterium sp. and also by an E. coli clone with an opd gene [22].

CONCLUSION

Bioremediation is a one of the process which involves the degradation of the unwanted pollution and other pesticides by various microbes. Several chemicals have been successfully removed from the soil and aquatic environment using degrading organisms. These specific microorganisms detoxify or degrade the pesticide metabolically and co-metabolically. The present research work as been highlighted the bacterial species of Staphylococcus it's a native bacterium growing on the chlorpyrifos contaminated soil was utilized to degrade the chlorpyrifos mixed in the broth medium.

REFERENCES

1. Karalliedde L, Senanayake N; Organophosphorus insecticide poisoning. J. Int. Fed Clin. Chem, 1999; 11: 4 – 9.
2. Martens R; Degradation of endosulfan by soil microorganisms. Appl. Environ. Microbiol, 1976; 6: 853 – 858.
3. Li CF, Bradley RL; Degradation of chlorinated hydrocarbon pesticides in link and Butter coil by Ultraviolet Energy. J. Dairy Science, 1968; 52: 1.
4. Cupples MA, Sims KG; Factors controlling degradation of pesticides in soil. Pestic Sci., 1999; 55: 566 – 614.
5. Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnik SS; Biodegradation of organophosphorus pesticides. Proc. Ind. Nat. Sci. Acad, 2004; B70: 57 – 70.
6. Horne I, Setherland TD, Harcort RL, Russel RJ, Oakeshott TG; Identification of N OPD (Organophosphate degrading) gene in an Agro bacterium isolate. App. Environ. Microbial., 2002; 68 (10): 3371 – 3376.
7. ChoC.H.M, Mulchandani A, Shem W; Bacterial cell surface display of organophosphorus hydrolase for selective screening of improved hydrolysis of organophosphate nerve agent. Appl. Enviro. Microbiol, 2002; 68: 2026 – 2030.
8. Bhagobaty RK., Mallick A; Utilization of Chlorpyrifos as a sole source of carbon by bacteria isolated from waste water irrigated agricultural soils in an industrial area of Western Uttar Pradesh. Ind. Res. J. Microbial., 2008; 3 (5): 293 – 307.
9. Fulekar MH; 2005(a). Bioremediation technologies for environment technologies for environment. Ind. J. for Environ. Prot., 25(4): 358 – 364.
10. Kumar S, Mukerji KG, Pal R; Molecular aspects of pesticide degradation by microorganisms. Crit. Rev. Microbiol, 1996; 22: 1 – 26.
11. Mallick BK, Banerji A, Shakil NA, Sethunathan NN; Bacterial degradation of chlorpyrifos in pure culture and in soil. Bull. Environ. Contam. Toxicol, 1999; 62: 48 – 55.
12. Greulich K, P flugmacher; Uptake and effect on detoxification enzymes of cypermethrin in embryos and tadpoles of amphibians. Arch. Environ. Contam. Toxicol, 2004; 47: 489 - 495.
13. Barles RW, Daughton GC, Hseih DPH; Accelerated parathion degradation soil inoculated with accumulated bacteria under field conditions. Arch. Environ. Contam. Toxicol, 1979; 8: 647-660.
14. Guha A, Kumari B, Roy MK; Possible involvement of plasmict in degradation of malathion and chlorpyrifos by Micrococcus sp. Folia. Microbiol, 1997; 42: 574 – 576.
15. Jilani S, Khan AM; Isolation characterization and growth response of pesticide degrading bacteria. J. Biol. Sci, 2004; 4(1): 15 – 20.
16. Bhagobaty RK., Joshi SR, Mallick A; Microbial degradation of Organo phosphorus pesticide: Chlorpyrifos (Mini – Review). The internet Journal of Microbiology, 2007; 4 (1): 33 – 40.
17. Geetha M, Fulekar MH; Bioremediation of pesticides in surface soil treatment unit using microbial consortia. Afr. J. Environ. Sci. and Technol., 2008; 2(2): 36 – 45.
18. Beck AJ, Jones KC; Kinetic constrain on the in situ remediation of soils contaminated with organic chemicals. Environ. Sci. Poll. Res., 1995; 2: 244-252.
19. Beck AJ, Jones KC; Kinetic constrain on the in situ remediation of soils contaminated with organic

chemicals. Environ. Sci. Poll. Res., 1995; 2: 244-252.

20. Singh BK, Walker A, Morgan JA, Wright DJ; Biodegradation of chlorpyrifos by enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol, 2004; 70: 4855–4863.
21. Dagley S; Biochemistry of aromatic hydrocarbons degradation is Pseudomonas. In: The Bacteria, 1986; 10.
22. Mallick K, Bharati K, Banerji A, Shakil NA, Sethunathan N; Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bull Environ Contam Toxicol, 1999; 62: 48-54.
23. Racke KD, Coats JR; Resistance of Chlorpyrifos to enhanced biodegradation in soil. J. Agric. Food Chem, 1990; 38: 1430 – 1436.
24. Digark MK., Ozcelik S, Celik S; Degradation of ethion and methylation by some microorganisms. 35th IUPAC congress. Istanbal, 1995; 14: 19 – 84.
25. Huang XL, Linda S, Nakatsu, C; Impact of animal waste lagoon effluents on chlorpyrifos degradation in soils. J. of Environ. Toxical. Shem, 2000; 9: 2864 – 2870.
26. Yu YL, Fang H, Wang X, Wu XM, Shan M; Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation, 2006; 17: 487-494.
27. Jilani S, Khan AM; Biodegradation of cypermethrin by Pseudomonas in a batch activated sludge process. Int. J. Environ. Sci. Tech, 2006; 3(4): 371 – 380.
28. Parekh NR, Walker A, Roberts SJ, Weleh S; Rapid degradation of triazinone herbicide metamitron by a Rhodococcus sp. Isolated from treated soil. Appl. Bacteriol, 1994; 77: 467 – 475.
29. Fulekar MH; 2005 (b). Environmental biotechnology. Oxford and IBH publishing house, New Delhi, India.
30. Singh BK, Walker A, Morgan JA, Wright DJ; Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl Environ Microbiol 2003; 69: 5198–5206.
31. Ajaz M, Jabeen N, Akhtar S, Rasool SA; Chloropyrifos resistant bacteria from Pakistani soils, isolation, identification, resistance profile and growth kinetics. Pak. J. Bot., 2005; 37: 381-388.