International Journal of Engineering, Science and Mathematics
Vol. 7 Issue 3, March 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Efficiency of the Probiotic Bacteria in Degradation Of The Pesticide Chlorpyrifospresent In Agricultural Soil

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ABSTRACT

Pesticides are a large and varied group of substances that are specifically designed to kill organisms including of weeds, insects,and the indiscriminate use of pesticides in agricultural field resulted into contamination of soil environment leading to toxicity.India is an agriculture based country and maximum portion of its economy is dependent on agriculture. The promotion of highyielding varieties of crops has led to large scale use of chemicals as pesticides. Chlorpyrifos, is one of the most commonly andwidely used commercial organophosphate pesticide which considers as one of the most frequently used chlorinatedorganophosphorus pesticides. It is a broad-spectrum, moderately toxic pesticide that has been widely used in the prevention ofboth agricultural pests and urban public health pests. It was introduced in 1965 by Dow Chemical Company India. It has largeblights on public health and environment resulting from its long residual period in soil and water. The microbial action in theenvironment causes the natural degradation of the pesticides which might convert parent compounds to intermediates orcomparatively less toxic compounds. The adaptability of microorganisms during bioremediation releases certain enzymes, whichmetabolizes wide spectrum of anthropogenic chemicals. Chlorpyrifos is a broad-spectrum, moderately toxic pesticide that hasbeen widely used in the prevention of agricultural pests. Using Probiotic bacteria to control environmental troubles has been anongoing campaign for decades. Probiotics are defined as live microorganisms, which when administered in adequate amounts,confer a health benefit on the host. Health benefits have mainly been demonstrated for specific probiotic strains of the followinggenera: Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Bacillus,Escherichia coli .The present study can be utilised for the development of effective bioremediation process for pesticidecontaminated soil. Soil sample was collected having history of chlorpyrifos from four different soil sample (Rice, wheat,maizeand vegetable) samples were serially diluted up to 10-7 dilution. The diluted soil sample are inoculated on NA medium by usingspread plate technique under aseptic conditions and incubated at 37°C temperature for optimum growth. Six chlorpyrifospesticide utilizing Probiotic bacteria were isolated and identified through cultural and biochemical tests. Afterwards, two selectedprobiotic strains were cultured onto NA medium supplemented with chlorpyrifos at five concentrations (0, 50, 100, 150, 200 µgml-1) for 12 days. Their growth is analysed by specectrophotometric method. The result showed that Probiotic bacteria fore.g.Bacillus sp. shows maximum growth. The results of this research indicated that the isolated bacteria Bacillus sp. can be used fordegradation of Chlorpyrifos contaminated soil.

Key words: Isolation, Chlorpyrifos, Containminated, Agricultural Soil.

INTRODUCTION

Pesticides are organic chemical purposely presumably intendedfor increasing agricultural yield, soil productivity, productsquality, minimizing losses of agricultural products caused bycroppeet and to control the insect vectors for prevention of theoutbreak of human and animal epidemics. Increased use of thepesticide and herbicides in agriculture has also beenimplemented for food storage. Recently, over 500 compoundsare registered and used worldwide as pesticides or metabolitesof pesticides.After World War II the use of pesticide in agriculture field hasprogressively increased leading to increased world foodproduction. Amongst the South Asian countries of the totalpesticide consumption, India is largest of pesticide consumercountry which account for 3% of the world consumption forcrop protection. The most commonly used pesticides in Indiainclude organophosphates, organochlorins, neonicotiniodsetc.As per definition of ideal pesticide, a pesticide must be lethalto the targeted pests, but not to non-target species, includingman but unfortunately, this is not so, hence the controversy ofuse and abuse of pesticides has come into the light. However,due to their unplanned and indiscriminate use, only 10% ofapplied pesticides reach the target organism and the remaininghigh percentage of it is deposited on non-target areas such assoil, water, sediments and causes serious environmental. pollution. Similarly, they also causes impacts onto non targetorganism such as wild life, besides affecting public health. Thereis now overwhelming evidence of some of thesechemicals that cause unwanted side effects to the environmentand do pose potential risk humans and other life forms(Jeyaratnam,1985; Igbedioh, 1991; Forget, 1993). Accordingto the ICMR bulletin report about 1 million deaths and chronicillnesses are reported per year due pesticide poisoning worldwide.Because of the potential hazards to the nature andhumans many of the pollutants which are toxic in nature weresubsequently banned. Although their use has been discontinuedfrom long time, these chemicals accumulate in soils andsediments where they can enter the food chain directly or percolate down to the water bodies. It is important to developnovel processes for the control and treatment of this type ofpollution, namely by taking part of the catalytic potential ofbiological systems that can be used to reduce pesticidecontamination.

Chlorpyrifos, is one of the most commonly and widely usedcommercial organophosphate pesticide which considers as oneof the most frequently used chlorinated organophosphoruspesticides (Maya *et al*., 2011). Reduction in the abundance ofmicrobial communities in soil has been observed aftercontaminations with chlorpyrifos (Chu *et al*., 2008). Also,chlorpyrifos bounds to soil constituents that may be introducedinto rivers by surface runoff from agricultural lands (Wu andLaird, 2004). The microbial action in the environment causesthe natural degradation of the pesticides which might convertparent compounds to intermediates or comparatively less toxiccompounds. The adaptability of microorganisms duringbioremediation releases certain enzymes, which metabolizeswide spectrum of anthropogenic chemicals. Many bacteria thatare able to degrade organophosphate pesticides have beenisolated from soil around the world, which could utilizeChlorpyrifos as the only source of carbon and phosphorousYang *et al*. (2006). The first step in dealing with pollutioncaused by Chlorpyrifos is the isolation and screening ofmicrobial species that can degrade the chlorpyrifos effectively,and identify Chlorpyrifos utilizing microbes from agriculturalsoil using an enrichment culture technique and determiningthe growth response of bacteria in minimal salt mediumsupplemented with chlorpyrifos.

MATERIAL AND METHODS

Pesticide used: Commercial grade pesticide Chlorpyrifos with500 mg/mlconcentration was obtained.

Media preparation: Nutrient Agar medium used for isolationof bacteria contain the following(g/l) (pH7.2- 7.4, peptone5g,beef extract 3g , sodium chloride 5g , agar 15g , D.W1000ml)and potato Dextrose Agar media used for isolation of fungicontain the following (g/l):(pH 5.4, potato 200g ,dextrose 20g ,agar 20 g , D.W 1000ml) Urea agar, Nutrient agar, SimonCitrate agar, MacConkey agar, and Nutrient broth were alsoused during the isolation and identification of Chlorpyrifosdegrading bacteria.

Sample collection: Agricultural soil were taken from differentarea of Patna, Bihar, which have past few year of history ofchlorpyrifos used in pest control were selected for this study.Samples were collected randomly from three rice fields andthree other crops fields from 12 - 15cm depth of the field andstored aseptically for further analysis.

Isolation of chlorpyrifos degrading bacteria: For isolation of microbes from different soil samples serialdilution were carried out by dissolving 0.1g of each soilsamples in 9.9ml of normal saline solution. For isolation ofbacteria 1ml of soil suspension of different samples werespread over the pre sterilized petriplates containing nutrientagar media at dilution 10-5,10-6 and 10-7 and culture plateswere incubated at 370 C for 24h. Chlorpyrifos utilizingmicrobes were isolated from soil samples by the enrichmentculture technique nutrient culture medium, using chlorpyrifosas the sole source of carbon as described by Zhu *et al*., (2010).This was done by sub-culturing those pure culture of bacteriaand fungi on pesticides containing NA media and PDA mediaplates and incubated at 370C for bacterial plates and 260C forfungal plates for 24-48h.

Characterisation and identification of isolates: The bacterial isolates grown on Chlorpyrifosagar wassubjected to physiological and biochemical tests. The testscarried out include: Gram staining, catalase test, citrateutilization, oxidase test, indole production, motility, sugarfermentation, methyl-red test, nitrate reduction, starchhydrolysis, Voges-Proskeur test and hydrogen sulphideproduction. Identification was based on recommendations ofBuchanna and Gibbons 1984). , and then using Bergey'sManual of Determinative Bacteriology for confirmation.

Growth kinetic detection in presence of chlorpyrifos: Growth curve experiments were performed with differentdoses of chlorpyrifos in order to determine the optimumconcentration of chlorpyrifos that stimulates the growth ofisolates in liquid medium at different concentration(i.e200µg,250µg) of chlorpyrifos at interval of 2,4,8,12,and14days using spectrophotometer at 600nm by taking O.D(optical density), (Alexeander 1999).

Optimization at different physical parameters: And then, the optimization of those bacterial isolates whichshows better growth response in different concentration ofchlorpyrifos have been done to determine the degrading abilityof isolates to degrade chlorpyrifos pesticide at different pH,(2,4,7,9,11) and temperature (4,6,25,37,40 and 600C).

RESULTS

Isolation of Microorganism from soil sample: An average number of bacterial colonies were 6.2 X 10 6

CFU/gm in soil sample. Nine bacterial strains are isolatedfrom soil sample. The bacterial isolates grown on

Chlorpyrifos agar was subjected to physiological andbiochemical tests. The results of morphological,

cultural andbiochemical tests carried out are shown in Table 1, 2 and 3.

Microbial Characterisation: On the basis of the various biochemical tests performed thefour bacterialstrains identified (Table-2) isolated were furtheridentified In the non-contaminated soil, bacterial populationare higher. In contrast, in pesticide-contaminated soil,population are greatly suppressed. The bacterial population ingeneral is not able to survive and multiply well in the presenceof pesticide.

Table 1. Morphological characteristics of the Isolates

Sr No	Colony Design at ion	Total No of Colonies	Types of Colonies	Colony No	Colony Characteristic				Gram Reaction	Shape
					Color	Margin	Elevation	Opacity		
1	P1	5	1	1	Creamy	Irregular	Flat	Opaque	+ve	Rod
2	P2	10	2	1	Creamy	Irregular	Flat	Opaque	+ve	Rod
				2	Creamy	Irregular	Flat	Translucent	+ve	Coccus
3	P3	8	2	1	Creamy	Irregular	Flat	Opaque	+ve	Rod
				2	White	Irregular	Flat	Opaque	+ve	Coccus
4	P4	10	1	1	Creamy	Regular	Flat	Opaque	+ve	Rod
5	P5	11	2	1	White	Irregular	Flat	Opaque	+ve	Coccus
				2	Creamy	Irregular	Flat	Opaque	+ve	Rod
6	P6	8	2	1	Creamy	Regular	Raised	Translucent	+ve	Rod
				2	Creamy	Irregular	Flat	Opaque	+ve	Coccus

Table 2. Result of biochemical test of four selected bacterial strains

Sr. No.	Biochemical Tests	Selected Bacterial Isolates			
		P1	P2	P5	P6
1	Amylase	-	-	+	-
2	Casein Hydrolysis	-	-	-	-
3	Catalase	+	-	-	-
4	Gelatin Hydrolysis	+	+	+	+
5	Nitrate Reduction	-	-	+	-
6	Citrate Utilization	+	+	+	+
7	Indole Production	-	+	-	-
8	MR	-	+	+	+
9	VP	-	-	-	-
10	Urease	-	-	-	-
11	Dextrose	+	+	+	+

Table 3. Identification of the all bacterial isolates

Sr. No.	Strains	Identified as
1	P1	Bacillus sp.
2	P2	Staphylococcus sp.
3	P5	Micrococcus sp.
4	P6	Micrococcus sp.

It has been reported that one of the primary metabolites of (3,5,6-trichloro-2-pyridinol) possesses antibacterial propertiesYong *et al*. (2011). A significant decline in bacterialpopulations observed in the present study could be attributed tothe generation of such antibacterial metabolites. Similarobservations were reportedregarding the utilisation ofChlorpyriphos as a carbon source by bacteria isolated using anenrichment procedure Yang *et al*. (2006).

Conclusion

Chlorpyriphos has a harmful effect on soil microorganisms andtheir biodiversity, as well as enzymatic activity. The microbialand biochemical soil indices identified in the study providednecessary information about soil quality and fertility. Thecalculation of Colony Forming Unit (CFU) of soil confirms thefact that the use of this fungicide in contaminating dosescreates a risk to living organisms. These findings suggest thatuse ofChlorpyriphos designed for the control of diseases incrops and vegetables should be used carefully and according tothe manufacturer's recommendations. Uncontrolled dosesdistort the homeostasis of soil, which can have a strong impacton plant growth and yield.

REFERENCES

1. Alexander, M. 1999. Biodegradation and bioremediation. (ed.2M. Alexander). San Diego: Academic Press of chlorpyrifos alone and in combination with chlorothalonil andtheir effects on soil microbial populations. *J. Environ. Sci.*20, 464–469.
2. Buchanna, R.E., Gibbons, N.E. Bergey's manual ofdeterminative bacteriology. 8th ed. Baltimore: Williams and Wilkins; 1984

3. Chu, X.Q., Fang, H., Pan, X.D., Wang, X., Shan, M., Feng, B.,Yu, Y.L., 2008. Degradation
4. Jeyaratnam J. 1985. Health problems of pesticide usage in thethird world. *Br. J. Ind. Med.* 42:505-506.
5. Maya, K., Singh, R.S., Upadhyay, S.N., Dubey, S.K. 2011.Kinetic analysis reveals bacterialefficacy for biodegradation of chlorpyrifos and its hydrolyzingmetabolite TCP. Process Biochemistry, 46: 2130–2136.
6. Wu, J., Laird, D.A. 2004. Interactions of chlorpyrifos withcolloidal materials in aqueous systems. *J. Environ. Qual.*33, 1765–1770.
7. Yang, C., Liu, N.; Guo, X. and Qiao, C. 2006. Cloning of mpdgene from a Chlorpyrifos degrading bacterium and use ofthis strain in bioremediation of contaminated soil. *FEMSMicrobiology Letter* 265: 118-125.
8. Yong, X., Raza W., Yu G., Ran W., and Shen Q.“Optimization of the production of poly-γ-glutamic acid by Bacillus amyloliquefaciens C1 in solid-statefermentation using dairy manure compost and monosodium glutamate production residues as basicsubstrates”, *Bioresour Technol.,* Vol 102, pp. 7548-7554, 2011