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DEGRADATION STUDIES OF METHYL PARATHION BY AN INDIGENOUS SOIL BACTERIAL ISOLATE

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ABSTRACT

The farming community profoundly uses different types of pesticides, to protect their crops from variety of insects. Methyl parathion (MP) is an Organophosphorous pesticide, which is used worldwide and as a consequence its excessive consumption causes pollution of groundwater, surface water and soil. The concerned pesticide is highly toxic and its residues persist in the environment. The present study was thus aimed to isolate an indigenous soil bacterium by enrichment technique capable of degrading the concerned pesticide. A total 10 strains were isolated from the soil, of these, only one strain was found to be potential and was used for further studies. The potential strain was able to degrade methyl parathion to para- nitro phenol which is the first hydrolysis product of methyl parathion. The degradation of MP was studied through spectrophotometer and Gas Chromatography.

Key words : Methyl parathion, Bioremediation, Spectrophotometer, Gas Chromatography.

Introduction

The term pesticides cover a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. There has been a steady growth in pesticide production in India since 1952, the time when first pesticide industry was established. Chemical fertilizers and pesticides have been making a remarkable contribution in the agricultural sector towards improving the crop yield per hectare since better harvest requires rigorous cultivation, irrigation, and use of chemicals and fertilizers to protect the plants from pests and plant diseases. (Doxtader and Croissant, 1992). They do provide a protective covering to the crops from insects and pests but, are also responsible for the contamination of soils and water bodies, due to leaching and runoff to the nearby water reservoir (David *et al*., 1993; Liu *et al*., 2001; Shroff, 2000 and Mahiuddin *et al*., 2014). Their extensive use exerts intense and fatal effects on wildlife populations and on humans as well. Nearly 500,000 illness and 20,000 deaths can be ascribed annually to the use of chemical pesticides. Cases of acute pesticide poisoning account for significant morbidity and mortality rate worldwide (Mahiuddin *et al*., 2014). Due to the environmental concern associated with the gathering of pesticides in food chain, it is prerequisite to develop safe, convenient and economically feasible methods for pesticide detoxification (Pimental, 1983). Several conventional remediation techniques used in the past, have met with serious opposition due to their costly expenses and most importantly, most of the time they do not destroy the contaminating compound but rather transfer it from one environment to another place (Hurst *et al*., 1997). Bioremediation has virtually emerged during recent past as most ideal, alternative, environment friendly and ecologically sound technology for removing pollutants from the environment, restoring the polluted sites, and preventing further contamination (Roe *et al*., 1998).

Organophosphorous pesticides such as methyl parathion (MP) and methamidophos are extremely hazardous compounds. These compounds are biodegradable in nature but, some of their traces or residues do exists in nature which are highly toxic that ultimately affects human and animal's life cycle. Since

MP is highly toxic, its degradation has been studied by many researchers using different microorganisms. Munnecke *et al*. (1974) isolated a mixed microbial culture which was able to grow on parathion. The strain had the capability to degrade 50 mg of parathion per liter per hour. Choudhary *et al*. (1988) isolated two mixed bacterial cultures by soil enrichment technique, the isolated strain was capable of utilizing MP as a sole source of carbon. Pino *et al.* (2011) isolated a selected microbial consortium capable of degrading MP and PNP from the contaminated soil site. In culture, the consortium was able to degrade 150 mg/L of MP and PNP in 120 hrs, but after adding glucose or peptone to the culture media, the time of degradation decreased to 24 hrs. In soil, the consortium was able to degrade 150mg/L of MP in 120 hours at different depths and also managed to decrease the toxicity. Hindumathy *et al*. (2013) reported the effect of pesticide-chlorpyrifos on soil microbial flora and studied the pesticide's degradation by strains isolated from the contaminated soil. Gang *et al*. (2014) reported the influence of kaolinite and goethite on microbial degradation of MP and observed that biodegradation was improved by kaolinite and was depressed by goethite.

In view of the above and the literature survey findings, the present study was taken up with the objective to isolate indigenous microorganisms and exploit their potential in degrading organophosphorous pesticide-methyl parathion.

Materials and Methods

Soil Sample collection : Soil samples were collected from nearby agricultural fields, which had the history of pesticide application of at least 10 years. Samples were drawn from a depth of 5-10 cm to minimize air contamination. Media components used were of analytical grade obtained from Sigma-Aldrich (USA). Isolation of methyl parathion degrading strain(s) was done through enrichment technique.

Determination of minimum inhibitory concentration (MIC) : All the isolated colonies of pure cultures were checked for their ability to tolerate maximum concentration of MP through the bore well method. In this method, series of Nutrient Agar Plates were prepared, after solidification of plates, well was bored in the plates, and then various concentrations MP (50 ppm, 100 ppm,150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm and 400 ppm) were added in the wells and then the plates were kept for incubation for 24 h. After incubation the plates were observed for the zone of inhibition.

Effect of carbon/nitrogen/phosphorous supplementation on *in vitro* MP utilization : To determine the possibilities of utilization of MP as carbon / nitrogen / phosphorous source by the test bacterium, appropriate modifications were made to the minimal media (MM).After incubation, the samples were withdrawn at various time intervals and analyzed through spectrophotometer by measuring absorbance at 540nm.

Degradation studies of Methyl parathion : The degradation of MP was studied with the help of spectrophotometer and Gas Chromatography. The isolated strain was inoculated in 100ml MM with 10ppm concentration of MP, the flasks was incubated at 120 r.p.m at 35^{O}C for 7 days. After 7 days of incubation, absorption spectra were taken with the help of UV-visible spectrophotometer in the UV-range (200-400nm).

Results and Discussion

Microorganisms play a very important role in the degradation of toxic chemicals which get released into the environment as a result of anthropogenic activities. Conventionally, primary methods available for disposal of hazardous wastes are incineration, land filling and use of different chemicals, but these methods are very expensive and often create new environmental problems. Many researchers in the past have suggested, that biodegradation is an attractive option for the detoxification of these products, since it utilizes natural processes and offers the potential for less costly treatment. However, biodegradation rates usually are low because the compound being destroyed is toxic or recalcitrant, which causes the growth to be slow as well. Several decomposition products may be produced as thorough biodegradation often requires a consortium of organisms to metabolize the resulting products. Considerable

Table 1 : Recovery of bacterial strains from different concentration of MP.

Strains	Concentration of MP*			
	25	50	75	100
C1	+	+	+	+
C2	+	+	+	+
C3	+	+	+	+
C4	+	+	-	-
C5	+	+	-	-
C6	+	+	+	+
C7	+	+	+	-
C8	+	+	+	+
C9	+	-	+	-
C10	+	+	+	+

* Mg/100 ml/100 gm soil Incubations–15 days at 35^{0}C ± 1^{0}C.

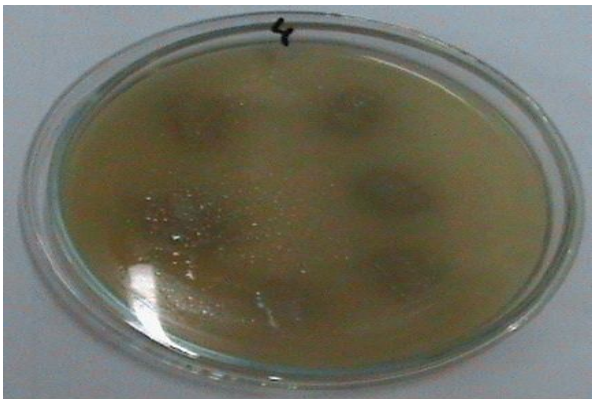


Fig. 1 : Six isolates *viz.* C1, C2, C3, C6, C8 and C10 with pesticide degrading ability (Pick and patch technique).

Fig. 2 : Bore Well Method: Minimum inhibitory concentration of MP (350 mg/l).

research has been directed towards the development of alternative processes for biodegradation of pesticides. Among these, Rosenberg *et al.* (1979) isolated two *Pseudomonas sp.* that were able to hydrolyze a number of organophosphorous compounds including parathion, and use the ionic cleavage products as a sole source of carbon. Ahmed *et al.* (2009), who reported the time course of 15 days for MP degradation from *Pseudomonas* sp. Kim *et al.* (2002), investigated the degradation of coumaphos using a recombinant strain of *E.coli* containing the opd gene for OPH. Significantly higher degradation rates were obtained compared to those obtained with the microbial consortium naturally present in coumaphos dip waste. Thus, present investigation was undertaken with the objective to isolate indigenous microorganism capable of degradation.

Soil samples supplemented with different concentrations of MP were incubated for 15 days at 35 ±1°C. A total 10 isolates were obtained from the soil samples supplemented with MP (Table 1). Out of these, six isolates *viz.*, C1, C2, C3, C6, C8 and C10 which appeared in higher concentration were further screened for their ability to tolerate maximum concentration of MP

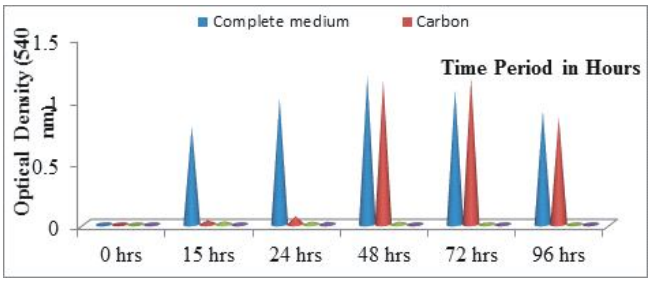


Fig. 3 : Effect of carbon/nitrogen/phosphorous sources on *in vitro* MP utilization by test strain.

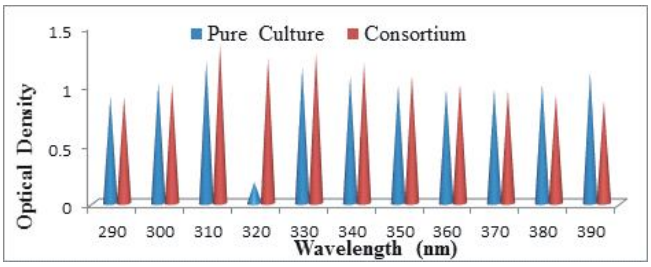
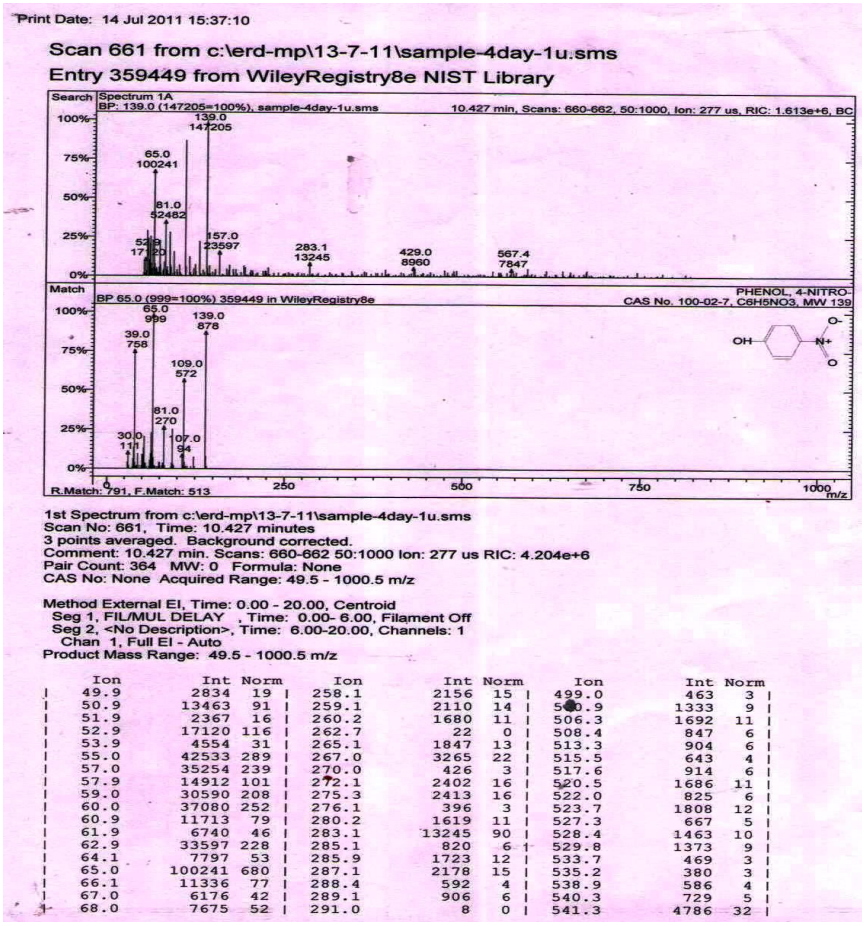


Fig. 4 : *In vitro* degradation of MP by the test strain and consortium.

(Fig. 1). Thus, C1 was most potent isolate, finally selected for further evaluation. Minimum Inhibitory concentration of MP was determined by bore well technique. The test strain C1 grew well in all the concentrations ie.100-400ppm concentrations of MP. It was noticed that, with increase in concentrations of MP, the activity declined significantly and at 350ppm it was unable to utilize it. This concentration of MP was therefore found to be inhibitory for the bacterial growth. Thus, the MIC of the strain C1 for MP was 350ppm or 350mg/Lit. (Fig. 2). Microorganisms require both macro and micro nutrients for their metabolic and developmental activities. There are also several reports of utilization of pesticides as sole source of carbon, nitrogen and phosphorous (Sogorb and Vilanova, 2002; Colosio *et al.*, 2009; Hemmert and Redinbo, 2010; Yang *et al.*, 2005). Therefore, the present investigation was carried out to find out whether MP was utilized as nutrients by the test bacterial strain. It was observed that the test bacterium showed rapid degradation of MP in complete medium (Fig. 3), which was followed by carbon deficit medium. It was also recorded that the test strain C1 grew well in complete medium, but in the carbon deficit medium, the strain required an initial period of long lag phase to get acclimatized to the substrate and after 20 hours, utilization of MP started and increased gradually.

For the degradation studies, it is obvious from the Fig. 4 and Table 2 that absorbance varied significantly at different wavelength. Maximum absorbance *i.e.* 1.2018 and 1.3409 were observed at 310 nm in case of both pure culture and consortium, respectively. It was gradually



Ion	Int	Norm	Ion	Int	Norm	Ion	Int	Norm
49.9	2834	19	258.1	2156	15	499.0	463	3
50.9	13463	91	259.1	2110	14	500.9	1333	9
51.9	2367	16	260.2	1680	11	506.3	1692	11
52.9	17120	116	262.7	22	0	508.4	847	6
53.9	4554	31	265.1	1847	13	513.3	904	6
55.0	42533	289	267.0	3265	22	515.5	643	4
57.0	35254	239	270.0	426	3	517.6	914	6
57.9	14912	101	272.1	2402	16	520.5	1686	11
59.0	30590	208	275.3	2413	16	522.0	825	6
60.0	37080	252	276.1	396	3	523.7	1808	12
60.9	11713	79	280.2	1619	11	527.3	667	5
61.9	6740	46	283.1	13245	90	528.4	1463	10
62.9	33597	228	285.1	820	6	529.8	1373	9
64.1	7797	53	285.9	1723	12	533.7	469	3
65.0	100241	680	287.1	2178	15	535.2	380	3
66.1	11336	77	288.4	592	4	538.9	586	4
67.0	6176	42	289.1	906	6	540.3	729	5
68.0	7675	52	291.0	8	0	541.3	4786	32

Fig. 5 : MP degrades through the hydrolysis pathway by the formation of para-nitro phenol (PNP).

Table 2 : Absorption spectra of strain C1 and the consortium in the UV range (290-400).

Wavelength (nm)	Absorbance at different wavelength	
	Pure Culture	Consortium
290	0.9014	0.9011
300	1.0150	1.0010
310	1.2018	1.3409
320	0.1795	1.2294
330	1.1477	1.2699
340	1.0681	1.189
350	0.9951	1.0857

decreased with increased in wavelength. Spectra recorded at 310 wavelengths, is reported as the absorption maxima of para nitrophenol (PNP)–the hydrolysis product of MP (Ghosh *et al.*, 2010). Thus, it clearly indicates that the isolated strain C1 degrades MP by the formation of PNP since maximum absorbance was observed at 310nm. The sample was checked by gas chromatography after proper extraction (Keprasertsup *et al.*, 2001). The chromatograph showed the presence of PNP (Fig. 5). Thus the isolated strain C1 degraded MP by forming PNP (first hydrolysis product of MP). Several earlier workers have also reported similar results (Ghosh *et al.*, 2010; Oga, 2003 and Griza *et al.*, 2008). Labana *et al.* (2005) also found that the *Arthrobacter, Bacillus* & *Pseudomonas* spp showed maximum absorbance at 320 wavelength which gradually decreases beyond this. Verma *et al.* (2014) reported the pesticide relevance and their microbial degradation in soil.

Conclusion

We have isolated total 10 strains from pesticide contaminated soil. Out of these, only one strain C1 was found to be potent MP degrader. The strain C1 was finally selected for further analysis. The spectrophotometric determination of, both strain C1 and consortium showed maximum absorbance at 310 nm wavelength. The strain C1 and consortium degraded MP by the formation of PNP (first hydrolysis product of MP).

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