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Microbial Degradation of Methyl Parathion by a Soil Bacterial Isolate and Consortium

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Abstract: *Pesticide consumption causes, pollution of groundwater, surface water and soil.Its use is increasing at an alarming rate, for increasingthe agricultural yield. Their extensive use exerts profound and deleterious effects on wildlife populations and humans. Methyl parathion(MP) is one such pesticide which is widely used throughout the world to protect the crops from chewing and sucking insects such as aphids, boll weevils and mites, this pesticide is highly toxic and its residues remain in the environment .The present study was thus aimed to isolate a soil bacteria by enrichment technique capable of degrading the concerned pesticide thereby reducing its toxicity by utilizing it as a source of carbon and energy .A total 10 strains were isolated from the soil, of these ,only one strain was found to be potential , which was identified as* ***Achromobactersp C1*** *andwas used for further studies. The degradation of MP was studied spectrophotometrically by the test strain C1 and consortium and it was found that they both, degraded MP by the formation para nitro phenol(PNP).*

Keywords: *methyl parathion, microbial degradation,spectrophotometer.*

1. INTRODUCTION

Pesticides are a large and varied group of substances that are specifically designed to kill biological organisms including rodents, weeds and insects. Fungicides, herbicides, insecticides, nematicides, rodenticides, fumigants, disinfectants, wood preservatives and antifoliantsare examples of pesticides(Puget sound water quality authority-PSWQA, 1991).The use of pesticides has become a vital ingredient of the modern agricultural system. It's a belief that,without their use increase in agricultural production, would be practically impossible.It is estimated that a four millions tons of pesticides are applied to the world crops annually for pest control, but less than 1% of the total applied pesticides usually get to the target pests[1,2]. Moreover, their extensive utilization may result into their accumulation in the agricultural produce(vegetables, fruits etc.) . Though they play a very important role in agriculture, there are many health hazards associated with their application, primarily farm workers have special risks. Nevertheless for majority of the population, the principal vector is through ingestion of food contaminated with various pesticides, also degradation of water quality by pesticide runoff has two principal human health impacts, the first being the consumption of sea foods that are contaminated with pesticides and second by direct consumption of contaminated water.

Organophosphorous insecticides such as methyl parathion(MP) and methamidophos are used extensively throughout the world , It is used to control chewing and sucking insects such as aphids, boll weevils, and mites in a wide variety of crops, including cereals, fruits, vines, vegetables, ornamentals, cotton and field crops. It is a broad spectrum non- systemic pesticide that kills pests by stomach poisoning, it is an acaricide, and has some fumigant action as well. It acts by inhibiting acetylcholine esterase, an important enzyme in the nervous system. On exposure to MP, the enzyme is unable to function, causing accumulation of acetylcholine, which interferes with the transmission of the nerve impulse at the nerve endings.Since the cost of the pesticide is comparatively low and its effect is high, it is the most preferred chemical by many [3].In human beings MP poisoning causes the following symptoms–general weakness, headache, excessive sweating, salivation, nausea, vomiting, diarrhea, abdominal cramps and tremors. In very severe cases respiratory failures may lead to death[4]. Consequently this has led to serious concerns for the development of safe and convenient

strategies to deal with its widespread dispersal in the atmosphere in a cost effective way [5]. Several conventional techniques are used for the detoxification of such toxic compounds viz; incineration, land-filling, excavation but due to operational difficulties, treatment expenditure, the above mentioned methods have met serious opposition. Degradation with the help of microbial flora is generally considered to be safe and effective technique for detoxification of such chemicals in a cost effective way. Many amazing characteristics of microbes such as their small size, ubiquitous distribution, high specificity, surface area, potentially rapid growth rate and unrivaled enzymatic and nutritional adaptability make them, as one of the most important recycling agents of pesticides in nature. Biodegradation is nothing but a metabolic process that involves the complete breakdown of an organic compound .In the context of environmentalsciences; it is defined as the use of biological agents to eliminate hazardous substances from the environment [6]. The present study thus deals with the isolation of a bacterial isolate and aconsortium that can be used efficiently to degrade the target pesticide (MP). The overall goal of our work is to develop technology for biodegradation of hazardous substances at significant higher rates than currently are obtained with native organisms. MP was selected because its biodegradation has been well characterized and because of its persistence in the environment [7-9]. Extensive survey of literatures has given an impression that most of the studies relatedto microbial biodegradation in pure culture wascarried out in laboratory [10]. Investigations related to the role of consortium *in vitro* degradation are very less, while it is very important because natural micro flora compete significantly in microbial active environment, typical of a composting system [11,12]. Therefore, the present investigation was carried out to assess the rate of degradation of MP by the test bacterium and consortium with the help of spectrophotometer.

2. METHODS

1. Collection of soil samples:

Soil samples were collected from the fields which had the history of pesticide application of at least 10 years. After suitable survey, the soil samples were collected from the agricultural field at, Jabalpur (MP). Samples were taken randomly from a depth of 5-10 cm to minimize air contamination. The soilsamples were taken by means of sterilized spatulas and collected in sterile polythene bags. The soil samples were then brought to the laboratory and maintained at 4^0C.

2. Isolation of methyl parathion degrading strain: Enrichment Technique:

Enrichment technique was used for the isolation of MP degrading strain.1 gm of soil sample was taken and added to 50 ml of minimal media (MM) which had the following composition K_2HPO_4-5.8 g/l, KH_2PO_4 -4.5g/l,$(NH_4)_2$ SO_4 -2 g/l,$MgCl_2$ -0.16 g/l, $CaCl_2$- 20 mg, $NaMoO_4$ -2mg, $FeSO_4$ –1mg, $MnCl_2$ -1mg with 1 gm glucose/lit with 5ppm MP (final concentration) Selected colonies were transferred on series of nutrient agar plates to obtain the axenic culture. Only one strain(C1)was found to be potential MP degrader and thus was selected for future studies.The test strain's detailed biochemical test was done in the laboratory.It was found that the strain belonged to the genus *Achromobacter*.

3. Invitro degradation of MP by the test bacterium and consortium (Natural flora):

The degradation of MP was studied spectrophotometrically.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,absorptionspectra were taken with the help of UV-visible spectrophotometer in the UV-range (200-400nm) [13,4].

3. RESULT AND DISCUSSION

Soil microorganism plays an important role in successful operation of MP degradation [14]. Currently, the primary methods available for disposal of hazardous wastes are incineration, land filling and chemical treatment, but these methods are costly and often create new environmental problems. Many researchers in the past have suggested, that biodegradation of benign products is an attractive option for degradation of Organophosphorous(Ops) pesticides, since it utilizes natural processes and offers the potential for less costly treatment[15-17]. However, biodegradation rates usually are low because the compound being destroyed is toxic or recalcitrant, which causes the

growth to be slow as well. Numerous decomposition products may be produced so that complete biodegradation often requires a consortium of organisms to metabolize the resulting products. Considerable research has been directed toward the development of alternative processes for biodegradation of Ops. Among these, Kim *et al.*[18] 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 coumaphosdip waste.

Total 10 strains were isolated from the soil samples, out of these one strain was found to potent. It was found that the strainbelonged to the genus *Achromobacter*.It is obvious from the Fig.1.& Table1that absorbance varied significantly at different wavelength. Maximum absorbance i.e. 1.2018 and 1.3409 were observed at 310 nm incase of both pure culture and consortium respectively. It was gradually decreased with increased in wavelength. Spectrarecordedat 310 wavelength, is reported as the absorption maxima of para nitrophenol (PNP)–the hydrolysis product of MP [13]. 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 [19]. It showed the presence of PNP (Fig 2). Thus the isolated strain C1 degraded MP by the formation of PNP(hydrolysis product of MP). Several earlier workers have also reported similar results[13,20 and 21].Labana*et al.* [22] also found that the *Arthrobacter, Bacillus & Pseudomonas* showed maximum absorbance at 320 wavelength which gradually decreases beyond this. Zhongli [4] isolated a MP degrading strain M6 and reported a absorption peak at 310nm which is due to appeared, end product, PNP. Ghosh*et al.* [13] have also reported similar observation with *Pseudomonas* sp. & *Fransicella*sp.which were able to degrade MP with the formation of PNP.Verma *et al*[23] reported the pesticide relevance and their microbial degradation in soil.Kavita Rani *et al*[2] reported the bioremediation and biodegradation of pesticide from contaminated soil and water.

4. 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.It was found that the strain C1 belonged to the genus *Achromobacter*. 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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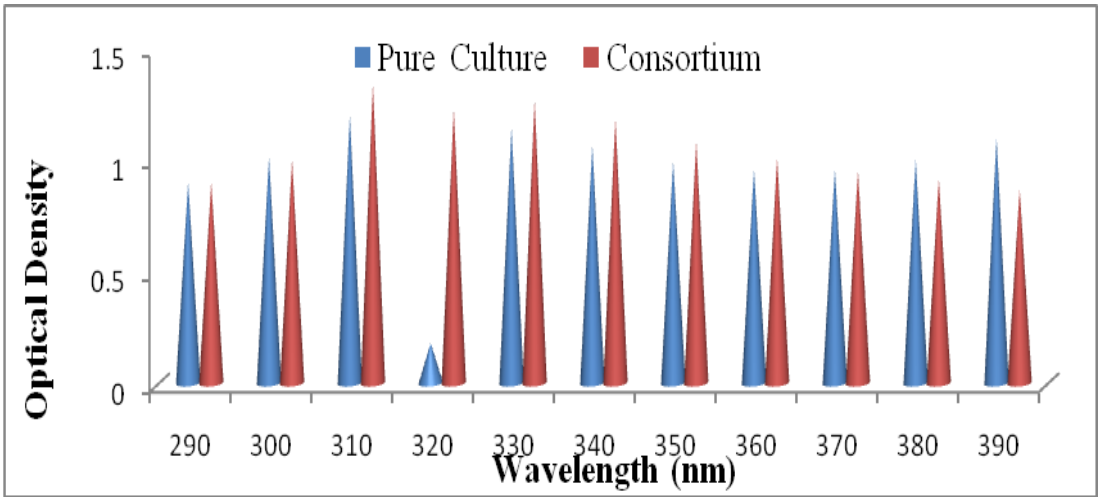
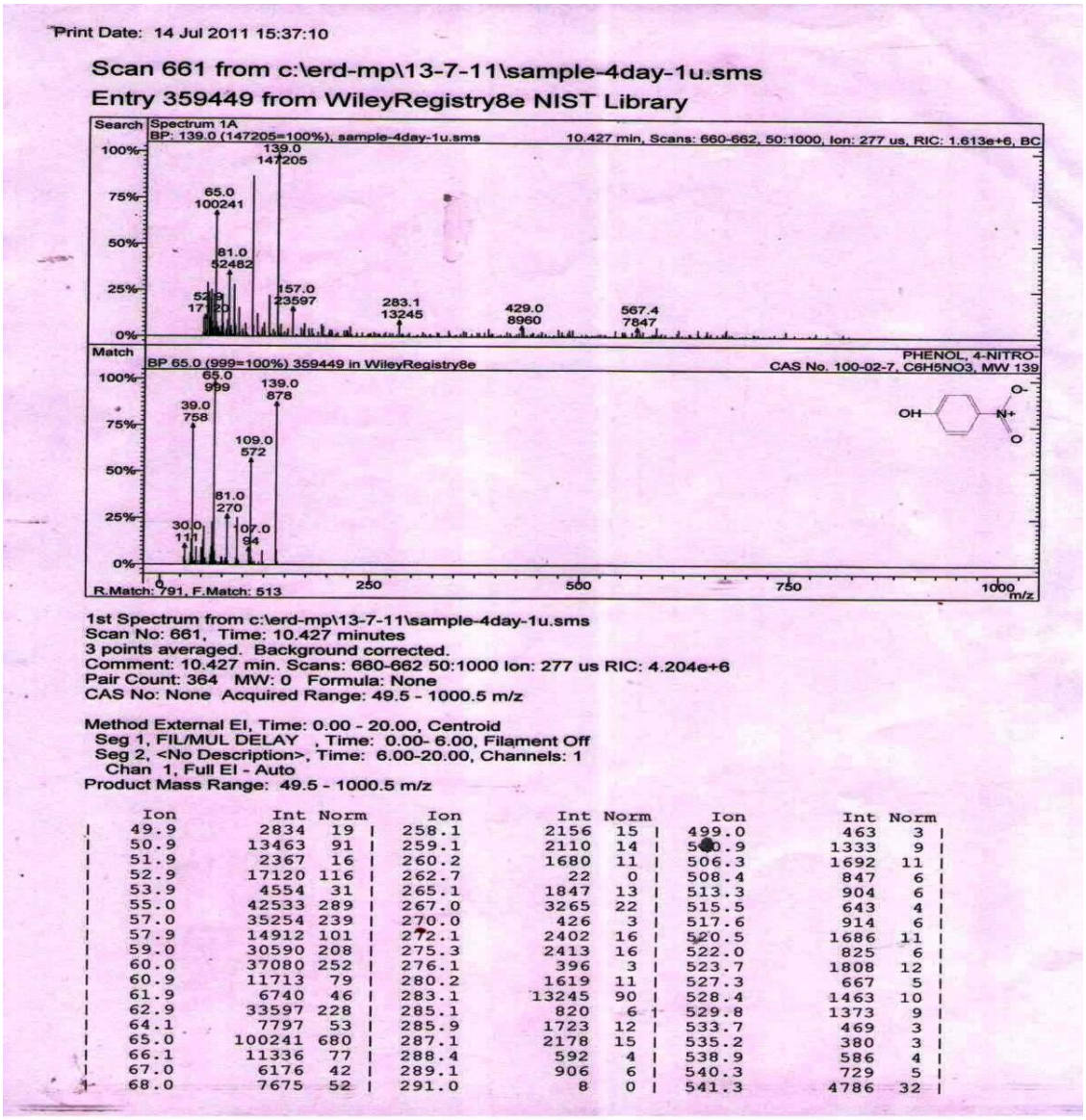


Fig.1. *In vitro* degradation of MP by the test strain and consortium



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 2. *MP degrades through the hydrolysis pathway by the formation of para-nitro phenol(PNP)*

Table1. *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

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