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Degradation of Profenofos in Soil inoculated with *Bacillus cereus* and *Aneurinibacillus migulanus*

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Abstract: The unplanned application of pesticide in paddy crop field to enhance paddy production could cause adverse impact on the environment. The present study was aimed to isolate the bacteria prevalent in profenofos applied soil to determine their profenofos degrading ability and its assay of the degraded metabolites. The dominant bacteria isolated from paddy crop field soil were *Bacillus cereus* and *Aneurinibacillus migulanus*. These bacteria were spiked with profenofos. After 36 hours of incubation in soil the extract were analysed by GCMS. Profenofos degradation was enhanced by *Aneurinibacillus migulanus* (99.45 % of profenfos degraded) compared to *Bacillus cereus* (98.01 %) and control (88.46%). Thus *Aneurinibacillus migulanus* could be used as bioagents to degrade profenofos.

Keywords: Profenofos, *Bacillus cereus*, *Aneurinibacillus migulanus*, GC-MS, Biodegradation.

Introduction

Persistence of pesticide residues in crop field soil is of environmental concern. Though several research works are in progress to solve this issue, viable, cost effective and ecofriendly protocols is the need of the hour. The persistence of organisms in pesticide contaminated soil implies that these organisms would have developed certain mechanism to metabolize these residues. With this perspective, this study was undertaken to trace the bacteria persist in profenofos applied paddy crop field soil and to assess their potential to degrade profenofos.

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Material and Methods

Sample Collection:

Profenofos applied paddy crop field soil were collected from Kelekalkandar kottai village, Tiruchirappalli district, Tamilnadu, India, in sterile autoclaved screw capped glass bottles, and kept at 4°C until processing. Further Research work was carried out in PG Research Department of Zoology, Periyar EVR College, Tiruchirappalli district, Tamil nadu, India.

Isolation of pesticide resistant bacteria from paddy crop field soil:

1 g of profenofos applied paddy crop field soil was aseptically inoculated in 99 ml of sterile minimal salt media (MSM) into cotton plugged flasks in triplicates. Conical flasks were kept under continuous shaking at room temperature for one week. Minimal salt media containing the following salts: $CaCl_2$ – 0.002 g, $MgCl_2$ – 0.02 g, K_2HPO_4 – 0.1 g, KH_2PO_4– 0.1 g, NH_4NO_3 - 0.1 g and $FeCl_3$ trace amount in distilled water (pH 7.2 – 7.4) up to 1 L were used for inoculation of soil sample. Total heterotrophic bacteria were isolated and identified following Bergey's manual of Determinative Biology [1]. The dominant bacteria *Bacillus cereus* and *Aneurinibacillus migulanus* were selected for pesticide degradation studies.

Profenofos in soil inoculated with Bacillus cereus and Aneurinibacillus migulanus.

Bacillus cereus was subcultured in autoclaved nutrient broth for 48 hours at 30ºC in a rotatory shaker at 150 rpm. After 48 hours, 1ml (100 µl: 45 X 10^{15} cfu/ ml) of *Bacillus cereus* broth culture was incubated into 50 g of sterile paddy crop field soil in 250 ml of cotton plugged conical flask containing 5000 ppm profenofos for 36 hours in triplicates . 20 ml of autoclaved minimal salt medium was added to maintain 60 % of humidity. Control (without *Bacillus cereus*) was maintained simultaneously. Similar procedure was followed for *Aneurinibacillus migulanus* (100 µl: 67 X 10^{15} cfu/ ml).

GC-MS analysis of degraded metabolites of Profenofos

After 36 hours of incubation the samples were subjected to GC-MS analysis. The control and Profenofos treated soil were extracted for GCMS analysis based on the method of Malghani *et al*., (2009) [2] with minor modifications. The pesticides in the control and treatment **were** extracted using organic solvent extraction three times with acetone and hexane (1:1) mixture, then the extract was concentrated using rotary vacuum evaporator (Buchi R-210, Surkzer) and cleaned up in silica gel column. The pesticide extract were eluted with n-hexane collected in a glass vial and subjected to Gas chromatograph- Mass Spectrometer (GC-MS) analysis.

Instrumental Analysis

The qualitative and quantitative determination of Profenofos was performed by GC- MS (45 X GC

44, Bruker) equipped with auto injector (8410). The separation analysis was performed in a 60 mm x 0.25 mm I.D x 0.25 µm film thickness BR 5 ms column (Made in USA) and helium was used as a carrier gas at a flow rate of 1 ml / min. The column temperature was programmed as 70 ºC to 150ºC at 10 ºC / min, to 250 ºC at 5 ºC/ min, to 280 ºC at 2 ºC / min, finally to 320 ºC at 5ºC/ min and hold for 10 minutes. 1 µl of the extract was injected into the injection port (at 280 ºC) using auto injector. The mass spectrometer was operated in scan mode and the ion source temperature was kept at 250 ºC.

The electron ionization (EI) unit was operated at 70 eV and at an emission current of 60 µA. Full scan data was obtained in a mass range of m/ z 50-650. Scanning interval and sample rate were 0.5 and 0.28, respectively.

Results

Bacteria were isolated from paddy crop field soil exposed to Profenofos. The 16S rRNA gene sequencing was performed and identified as *Bacillus cereus* (Gene Bank Accession Number: KY293394) and *Aneurinibacillus migulanus* (Gene Bank Accession Number: KY293393).

Residual quantification analysis of Profenofos by gas chromatography due to bacterial activity is presented in table 1. Relative to control (88.46 %), *Bacillus cereus* and *Aneurinibacillus migulanus* elicited 98.01 % and 99.45 %, respectively. These results indicate that *Bacillus cereus* and *Aneurinibacillus migulanus* triggered the complete degradation of profenofos.

Table 1: GCMS depicting result showing the biodegradation of Profenofos by Bacillus cereus and Aneurinibacillus migulanus after 36 hours of exposure.

Treatment	RT	Area	Pesticide Residual (ppm)	% of degraded pesticide
Control Soil+ 5000 ppm profenofos	14.218	447000000000	576.774	88.46
Test soil+ 5000 ppm profenofos + 1ml *Bacillus cereus*	14.249	77060000000	99.432	98.01
Test soil+ 5000 ppm profenofos 1ml *Aneurinibacillus migulanus.*	14.256	21200000000	27.354	99.45

In the control soil, apart from profenofos the various metabolites obtained were n-propyl benzene, 1-ethyl-2-methyl benzene, Isopropyl benzene, 1,2,3-trimethyl benzene, O-Butyl O, O-diethyl phosphorothioate and 4-bromo-2-chloro phenol (Fig 1,1a, table 2). Profenofos residue was persistence in Bacillus cereus inoculated soil were 1-ethyl-2-methyl-benzene and 4-bromo-2-chlorophenol (Fig 2, 2a).

Fig :1 Gas Chromatogram of 5000 ppm of Profenofos (control) at 36 hour in soil

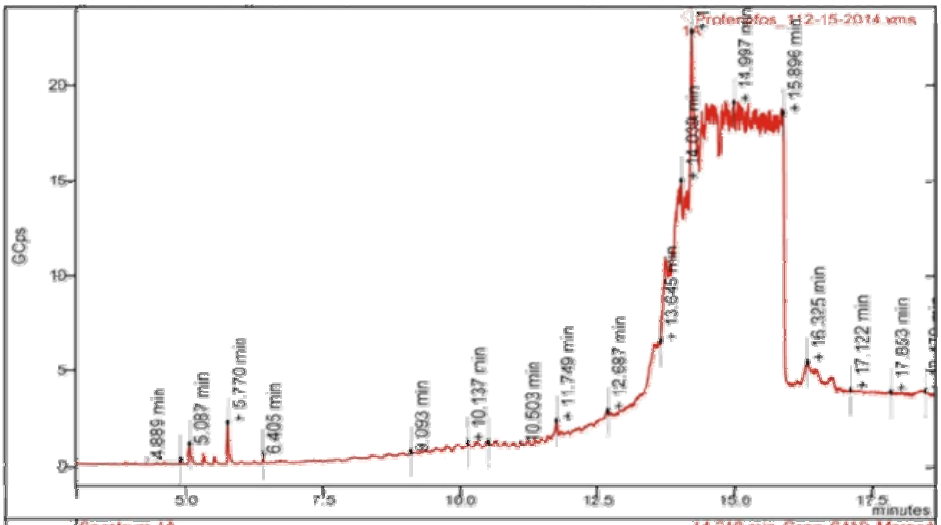

Fig :1a Mass Spectrum of 5000 ppm of Profenofos (control) at 36 hour in soil

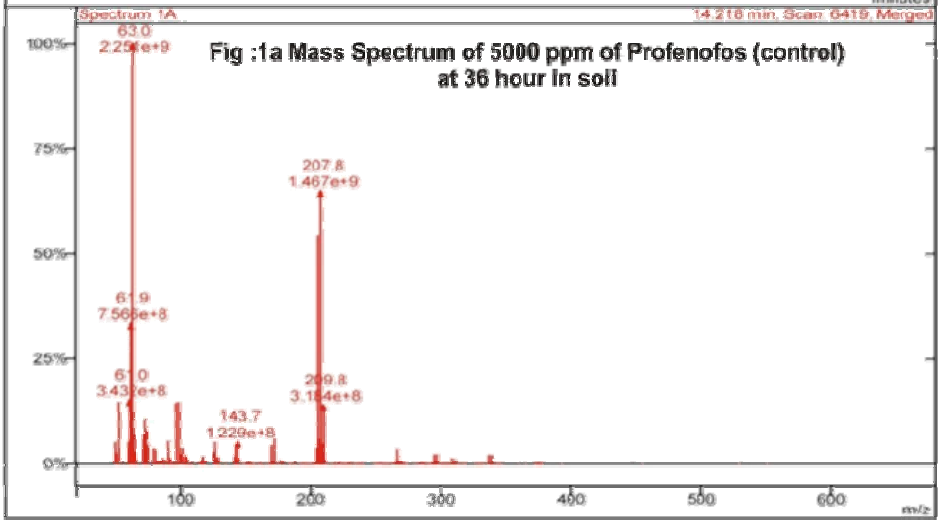

Fig: 2 Gas chromatogram of 5000 ppm of Profenofos degraded by *Bacillus cereus* at 36 hour in soil

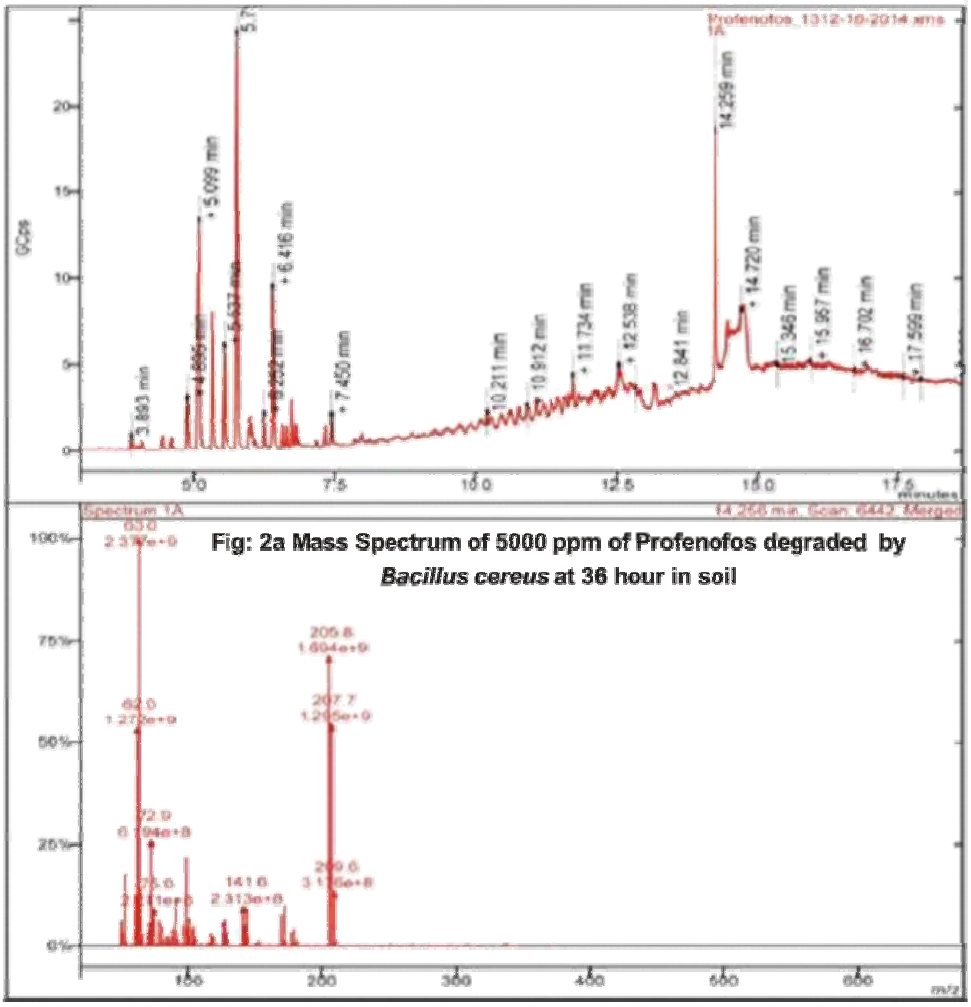

Fig: 2a Mass Spectrum of 5000 ppm of Profenofos degraded by *Bacillus cereus* at 36 hour in soil

Inoculation of 5000 ppm of profenofos with *Aneurinibacillus migulanus* degraded profenofos into Ethyl benzene, n-propyl benzene, 1-ethyl-3-methyl benzene, isopropyl benzene, 1,2,3 trimethyl benzene, 2-ethyl,1,3- dimethyl benzene, O-Butyl O,O diethyl phosphorothioate and 4-bromo-2-chloro phenol (Fig 3,3a, table 2).

Fig: 3 Gas chromatogram of 5000 ppm of Profenofos degraded by *Aneurinibacillus migulanus* at 36 hour in soil

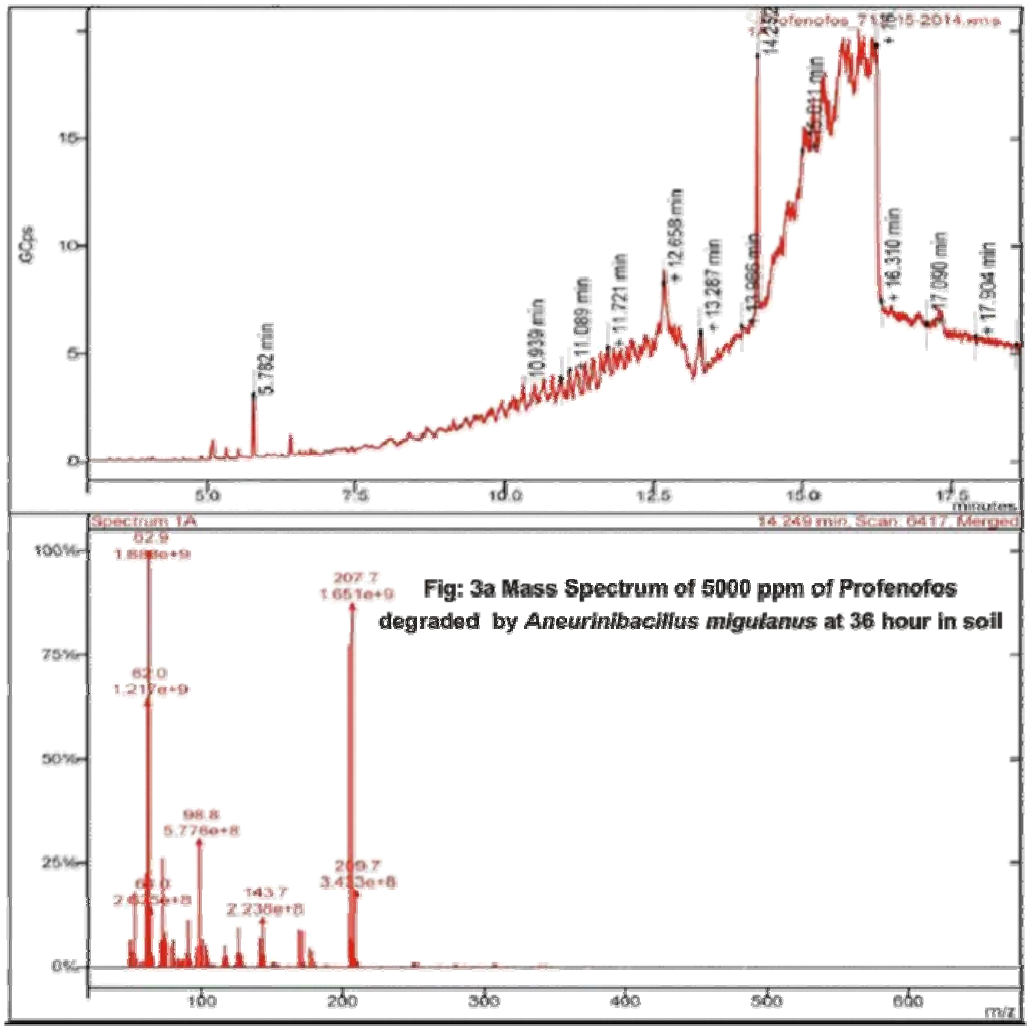

Fig: 3a Mass Spectrum of 5000 ppm of Profenofos degraded by *Aneurinibacillus migulanus* at 36 hour in soil

Table 2: Biodegraded metabolites of Profenofos in soil detected by GC-MS

Treatment	R.T	Residues obtained
Control (soil + 5000 ppm Profenofos)	4.889	n-Propylbenzene
	5.087	1-ethyl-2-methyl benzene
	5.770	Isopropyl benzene
	6.405	1,2,3-trimethyl benzene
	11.749	O-Butyl O, O- diethyl phosphorothioate
	14.218	4-Bromo-2-chloro-phenol
Test (Soil + 5000 ppm Profenofos + *Bacillus cereus*)	5.782	1-ethyl-2-methyl- benzene
	14.249	4-Bromo-2-chloro-phenol
Test (Soil + 5000 ppm Profenofos + *Aneurinibacillus migulanus*)	3.893	Ethyl benzene
	4.895	n-Propyl benzene
	5.098	1-ethyl-3-methyl benzene
	5.332	Isopropyl benzene
	6.010	1,2,3-Trimethyl benzene
	6.418	Isopropyl benzene
	6.731	2-ethyl, 1, 3-dimethyl benzene
	7.450	2-ethyl, 1, 3-dimethyl benzene
	11.734	O-Butyl O,O-diethyl phosphorothioate
	14.256	4-Bromo-2-chloro-phenol

Discussion

Malghani et al., [2] have demonstrated that the degradation of profenofos by *Pseudomonas putida* and *Burkholderia gladioli* enhanced on prolonged exposure (5 days: 70%; 25 days: 96.06 and 99.37% respectively). Furthermore, they have stated that the rate of degradation of profenofos varied among different species of bacteria. According to our present study 98.01 % and 99.45 % profenofos degradation was achieved within 3 days using *Bacillus cereus* and *Aneurinibacillus migulanus*, respectively.

As evinced in this study, Hina Jabeen et al., [3] have demonstrated that 93.39 % of profenofos was degraded and revealed that 4- bromo – 2- chlorophenol was the major metabolite which was subsequently metabolized to simpler compounds. Sumana Siripattanakul-Ratpukdi et al., [4] opined that integration of bioaugmentation and biostimulation techniques could trigger the bioremediation of profenofos effectively.

The acceleration of profenofos degradation by *Aneurinibacillus migulanus* observed in this study is in good accord with our previous findings that compared to *Bacillus cereus*, *Aneurinibacillus migulanus* enhanced the degradation of Lambda cyhalothrin [5].Furthermore, the resultant metabolite of profenofos degradation by bacteria in soil observed in this study lies in parallel to the bacterial mediated degradative metabolites of profenofos assayed in MSM in our previous study [6].

Conflict of Interest

The authors declare no conflict of interest

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