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Profenofos Degradation Potential of *Bacillus cereus* and *Aneurinibacillus migulanus* Isolated from Paddy Crop Field Soil

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The indiscriminate application of pesticides to increase crop production could have adverse impact on the biota. Profenofos is an organophosphate pesticide used to kill flying, crawling, chewing and sucking insects in paddy cultivation. The present research was designed to isolate the bacteria prevalent in profenofos exposed paddy crop field soil and to evaluate their potential to degrade profenofos in soil under laboratory conditions. *Bacillus cereus* and *Aneurinibacillus migulanus* were the dominant bacteria. Among them, *Aneurinibacillus migulanus* accelerated the degradation of profenofos within 36 hour when compared to the control. 1,3- dimethyl benzene and 4-phenyl but-3-ene-lyne were obtained as degraded products of profenofos in control, which were not detected in soil exposed to *Bacillus cereus* and *Aneurinibacillus migulanus*. On the other hand, n-propyl benzene was obtained as degraded metabolite in soil exposed bacteria, which were not detected in the control. Isopropyl benzene was detected in soil exposed to *Bacillus cereus* and 1,2,3-4-tetra methyl benzene was detected in soil exposed to *Aneurinibacillus migulanus* as byproducts of profenofos degradation when compared to the control. Thus, these bacteria could play a vital role in degrading profenofos in soil.

Keywords: Organophosphate; Profenofos; *Bacillus cereus*; *Aneurinibacillus migulanus*; Biodegradation.

Pesticides are widely studied as environmental contaminants because of their extensive use in the control of pests affecting agricultural crops, homes, and gardens. Because of their chemical characteristics, they represent a type of pollutant that shows variable persistence and biochemical and photochemical degradation[1]. Some studies show that, less than 1% of the total quantity of pesticides used in agriculture reaches its target. The remainder contaminates soil and other environmental compartments, air, and surface and groundwater. The fact that they are not biodegradable, together with their continued use, makes them a significant problem and a critical issue, with potentially damaging and unforeseen consequences for the future [2,3,4,5]. Other important aspects to be considered are the products of pesticide transformation (TP). There is great interest in studies on the formation of pesticide sub-products in the environment, since they can present a greater risk to the ecosystem than the original pesticides[6,7,8,9]. On the other hand, some pesticide TP may present lower toxicity than the

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original substances from which they are formed[10]. Thus, the results of pesticide use have not been completely elucidated because most studies are focused principally on the primary residues and not on their transformation products [11].

Some pesticides are considered to be persistent organic pollutants (POP) in the environment[12]. These POP possess long half-lives and can accumulate in the environment and in organisms, being transferred throughout the food chain until they reach human beings[13]. On the other hand, many pesticides can be degraded. The degradation processes generate a large number of sub-products in low concentrations that are considered to be beneficial for the systems for treatment and disinfection of crops, soils and groundwater, but hinder chemical analysis. Leaching of the applied pesticide may pollute the surface/ ground water, ultimately resulting in adverse effects on the biological systems[14]. The present study aims to utilise autochthonous bacteria persisting in profenofos contaminated paddy crop soil to degrade profenofos.

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

Treatment	RT	Area	Remaining pesticide (ppm)
ControlSoil + 25000 ppm profenofos	14.249	78300000000	101.03
Test soil + 25000 ppm profenofos+ 1ml *Bacillus cereus*	14.256	94110000000	121.432
Test soil + 25000 ppm profenofos+ 1ml *Aneurinibacillus migulanus*.	14.265	33370000000	43.058

RT - Retention time

Table 2. Metabolites of Profenofos degradation by bacteria in soil detected by GC-MS

Treatment	R.T	Residues obtained
Control (soil + 25000 ppm Profenofos)	3.905	Ethyl benzene
	4.924	1,3- dimethyl benzene
	6.457	1,2,4 tri methyl benzene
	7.989	4 ethyl 1,2-dimethyl benzene
	10.219	4-phenyl but-3-ene-lyne
	14.239	4-bromo-2 chloro phenol
Test (Soil + 25000 ppm Profenofos + *Bacillus cereus*)	4.617	Isopropyl benzene
	4.897	n- propyl benzene
	5.105	1-ethyl-2-methyl benzene
	5.797	1,2,4-trimethyl benzene
	6.427	1,2,3 tri methyl benzene
	6.733	4-ethyl-1, 2-dimethyl benzene
	7.445	2- Isopropyl benzaldehyde
	7.971	4 ethyl 1,2-dimethyl benzene
	14.254	4-bromo-2 chloro phenol
Test(Soil + 25000 ppm Profenofos + *Aneurinibacillus migulanus*)	3.907	Ethyl benzene
	4.899	n-propyl benzene
	5.800	1,2,3 tri methyl benzene
	6.736	4 ethyl 1,2-dimethyl benzene
	7.451	1,2,3-4-tetra methyl benzene
	14.236	4-bromo-2 chloro phenol

RT-Retention time

MATERIALS AND METHODS

Bioremediation of Profenofos in soil inoculated with *Bacillus cereus* and *Aneurinibacillus migulanus.*

Bacillus cereus was subcultured in autoclaved nutrient broth for 48 hours at 30 ºC in a rotatory shaker at 150 rpm. After 48 hours, 1ml of *Bacillus cereus* broth culture (100 µl: 45 X 10^{15} cfu/ ml) was incubated into 50 g of sterile paddy crop field soil in 250 ml cotton plugged conical flask containing 25,000 ppm profenofos in triplicates . 20 ml of autoclaved minimal salt medium was added to maintain 60 % humidity. Simultaneously, 25000 ppm of profenofos containing sterile soil were maintained as test control and 20 ml of autoclaved double distilled water was added to maintain 60 % humidity. The conical flasks were kept at 30 ºC for 36 hours. Same procedure was followed to examine degradation of profenofos by *Aneurinibacillus migulanus* (100 µl: 67 x 10^{15}cfu/ ml).

GC-MS analysis of degraded products of profenofos

After 36 hours of incubation, the samples were subjected to GC-MS analysis. The control and the treatment containing Profenofos were extracted for GCMS analysis based on the method of Malghani *et al.*,[15] 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 vaccum evaporator (Buchi R-210, Surkzer) and cleaned up in silica gel column (1:3 cm dimeter x 243 cm length) . The pesticide extract were eluted with n-hexane

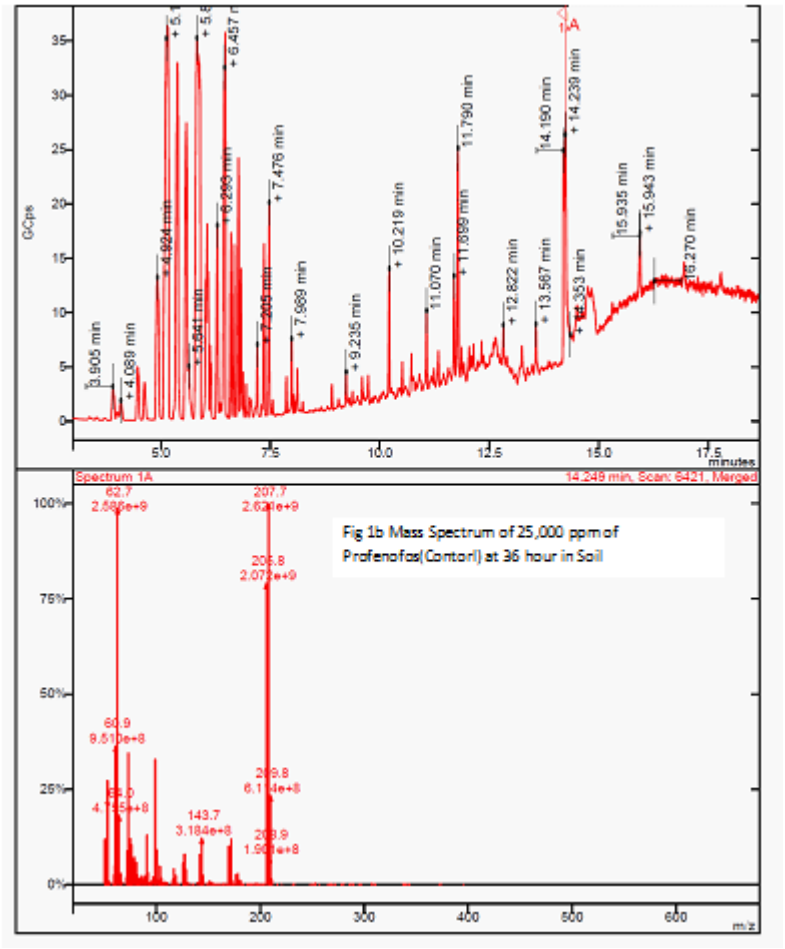

Fig. 1a. Gas chromatogram of 25,000 ppm of Profenfos (control) at 36 hour in soil

collected in a glass vial and subjected to gas chromatograph- Mass spectrometer (GC-MS) analysis.

Instrumental Analyses

The qualitative and quantitative determination of Profenofos and Lambdacyhalothrin were performed by GC-MS (45 X GC - 44, Bruker) equipped with auto injector (8410). The analyses separation was performed in a 60 m x 0.25 mm I.D x 0.25 µm film thickness BR 5ms 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 150 ºC at 10 ºC / minutes, to 250 ºC at 5 ºC/ minutes, to 280 ºC at 2 ºC / minutes, finally to 320 ºC at 5 ºC/ minutes and hold for 10 minutes. One 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 ionisation (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. The metabolic peaks were identified using documented data from National Institute of Standards and Technology (NIST) Library database.

RESULTS

It is evident from table 1 that *Aneurinibacillus migulanus* accelerated the degradation of profenofos (43.058 ppm) than *Bacillus cereus* (121.432 ppm) and control (101.03 ppm).

Metabolites of Profenofos (25000 ppm) degradation by *Bacillus cereus* and

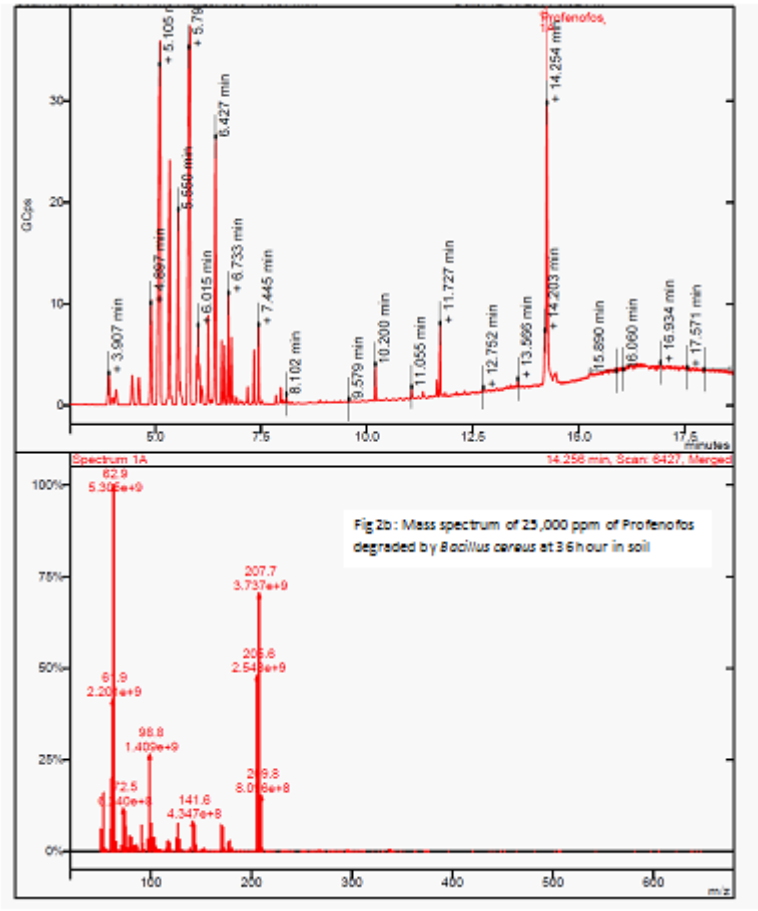

Fig. 2a. Gas chromatogram of 25,000 ppm of Profenofos degraded by Bacillus cereus at 36 hour in soil

Aneurinibacillus migulanus in soil is presented in Table 2. On addition of Profenofos (25000 ppm) to the soil for a period of 36 hours, the metabolites detected through GCMS were Ethyl benzene, 1,3-dimethyl benzene, 1,2,4 tri methyl benzene, 4 ethyl 1,2-dimethyl benzene, 4-phenyl but-3-ene-lyne and 4-bromo-2 chloro phenol (fig 1a,1b). On inoculation with *Bacillus cereus* in 25,000 ppm of profenofos added soil, the resultant metabolites were Isopropyl benzene, n- propyl benzene, 1-ethyl-2-methyl benzene, 1,2,4-trimethyl benzene, 1,2,3 Tri methyl benzene, 4-ethyl-1, 2-dimethyl benzene, 2- isopropyl benzaldehyde, 4 ethyl 1,2-dimethyl benzene and 4-bromo-2 chloro phenol (fig 2a,2b).

Comparatively, Ethyl benzene, 1,3-dimethyl benzene and 4- phenyl but-3-ene-lyne detected in the control were not detected in *Bacillus cereus* inoculated soil containing profenofos . In addition, n- propyl benzene was formed. On inoculation of *Aneuinibacillus migulanus* with profenofos added soil, the resultant metabolites were Ethyl benzene, n-propyl benzene, 1,2,3 tri methyl benzene, 4 ethyl 1,2-dimethyl benzene, 1,2,3-4-tetra methyl benzene and 4-bromo-2 chloro phenol (fig 3a,3b). The degraded metabolites were similar to the control, except the absence of 1,3 dimethyl benzene and 4- phenyl but-3- ene- lyne.

DISCUSSION

The formation of 4-bromo- 2- chloro phenol (BCP) as metabolic intermediate of profenofos degradation observed in this study in consistent with the findings of Malghani *et al*., [15] who have also reported that on inoculation of *Pseudomonas aeruginosa* (isolated from soil

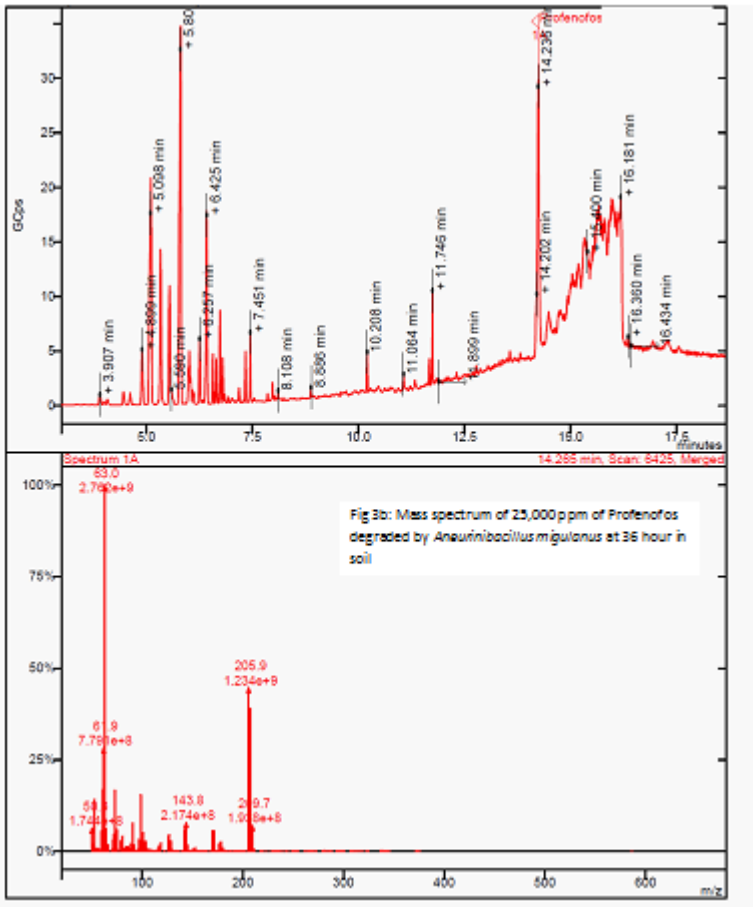

Fig. 3a. Gas chromatogram of 25,000 ppm of Profenfos degraded by Aneurinibacillus migulanus at 36 hour in soil

exposed to profenofos for a long period) in soil treated with 200 µg / g profenofos resulted in the formation of 4-bromo- 2- chlorophenol (BCP) as one of the metabolite and have reasoned out that it could be due to breaking of ester bond linkage of the parent compound by the bacterium. Further, they have stated that the degradation occurred due to a hydrolysis mechanism. In addition, they have also stated that BCP was also mineralised by *Pseudomonas aeruginosa*. The present result also agrees with that of Jabeen *et al* .,[16] who have reported that bacterial consortium (PABC) containing of *Achromobacter xyloroxidam, Pseudomonas aeruginosa*, *Bacillus sp*., *Citrobacter kosesi* efficiently degraded profenofos when compared to pure isolates. They have also observed that 4- bromo -2- chlorophenol (BCP) further metabolised to simpler products.

Abass *et al*.,[17] have observed that *in vitro* biotransformation studies of profenofos resulted in the formation of desthiopropylprofenofos and hydroxyprofenofos as metabolites. Co – metabolite degradation of organophosphate pesticide by bacteria have been reported by Horne *et al*., [18] and Richard *et al*.,[19]. The utilisation of carbon and phosphorus of organophosphates as a source for the growth of bacteria have been demonstrated by Subhas and Dileep[20]. As evinced in this study, previously we (Anitha Palanimanickam and Umamaheswari Sepperumal [21]) have observed that *Bacillus cereus* and *Aneurinibacillus migulanus*, degraded profenofos to benzoic acid and 4- bromo-2- chlorophenol and phosphorothioic acid, O,O,S-triethyl ester in MSM after 10 days of incubation individually.

CONCLUSION

The results obtained in the present investigation permit us to conclude that Profenofos degradation could be mediated by bacteria prevalent in profenofos exposed soil. The mineralisation of the pesticide residues would reduce the pollution load in paddy crop field soil**.**

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REFERENCES

1. Bandala, E.R.., Gelover, S., Leal, MT., Bulnes, CA., Jimenez A., Estrada, C.A., Solar photocatalytic degradation of Aldrin. *Catal. Today*., 2007; **76:** 189-199.
2. Kapustka, L.A., Williams, B.A., Fairbrother, A. Evaluating risk predictions at population and community levels in pesticide registration - hypotheses to be tested. *Environ. Toxicol Chem.*, 1996; **15(4)**: 427-31.
3. Bandala, E.R., Octaviano, J.A., Albiter, V., Torres, L.G., Wickramanayake, G.B., Hinchee, R.E., Solar photocatalytic degradation of Aldrin. *Catal. Today,* 2002; **76 (2-4)**: 189-199.
4. Acero, J.L., Benitez, F.J., Real, F.J., Gonzales, M.J., Hazard, M. Chlorination of organo phosphorus pesticides in natural waters. *J. Hazard Mater*, 2008; **153(1-2)**: 320-328.
5. Veiga, M.M., Silva, D.M., Veiga, L.B.E., Pesticide pollution in water systems in a small rural community in Southeast Brazil. Cademos de saiide publica Ministerio da Saude, Fundagao Oswaldo Cruz. *Escola Nacional de Sau.de Publica,* 2006; **22(11):** 2391-2399.
6. Nawab, A., Aleem, A., Malik, A. Determination of organochlorine pesticides in agricultural soil with special reference to y-HCH degradation by Pseudomonas strains. *Biores. Technol*., 2003; **88(1)**, 41-46.
7. Sinclair, C.J., Boxall A.B., Assessing the ecotoxicity of pesticide transformation products. *Environ. Sci. Technol.* 2003; **37(20)**, 4617-4625.
8. Pozo, O., Pitarch, E., Sancho, J.V., Hernandez, F. Determination of the herbicide 4- chloro-2-methylphenoxyacetic acid and its main metabolite, 4-chloro-2-methylphenol in water and soil by liquid chromatography Electrospray tandem mass spectrometry. *J. Chromatogr. A.* 2001; **923(1-2)**, 75-85.
9. Sabik, H., Jeannot, R., Rondeau, B. Multiresidue methods using solid-phase extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground and surface waters. *J. Chromatogr. A*. 2000; **885(1-20),** 217-236.
10. Borga, K., Gabrielsen, G.W., Shaare, J.U. Biomagnification of organochlorines along a Barents Sea food chain. *Environ. Pollut.* 2001; **113(2)**, 187-198.
11. Strandberg, B., Strandberg, L., Bergqvist, P., Falandysz, J., Rappe, C. Concentrations and

biomagnification of 17 chlordane compounds and other organochlorines in harbor porpoise (Phocoena phocoeria) and herring from the southern Baltic Sea. *Chemosphere*, 1998; **37(9-12)**, 2513-2523.

12. Bavcon, M., Trebese, P., Zupandc-Kralj, L. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionization detector. *Chemosphere*, 2002; **50**: 595-601.
13. Kodaka, R., Sugano, T., Katagi, T., Takimoto, Y. Clay-catalyzed nitration of a carbamate fungicide diethofencarb. *J. Agric. Food, Chem.* 2003; **51 (26)**, 7730-7737.
14. Kulkarni, A.R., Soppimath, K.S., Dave, A.M., Mehta, M.H., Aminabhavi, T.M. Solubility study of hazardous pesticide (chlorpyrifos) by gas chromatography. *J. Hazard. Mater. A* . 2000; **80**,9-13.
15. Malghani, S., Chatterjee, N., Hu, X., Zejiao, L. Isolation and characterization of profenofos degrading bacterium. *J. Environmental Sciences*. 2009; **21**, 1591-1597.
16. Jabeen, H., Iqbal, S., Anwar, S., Parales, R.E. Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. *International Biodeterioration and Biodegradation*. 2015; **100,** 89-97.
17. Abass, K., Petri, R., Jorma, J., Olavi, P. *In vitro* metabolism and interaction of profenofos by human, mouse and rat liver preparations. *Pesticide Biochemistry and Physiology*., 2007; **87(3)**, 238-247.
18. Horne, I., Sutherland, T.D., Harcourtt, R.L., Russell, R.J., Oakeshott, J.G. Identification of an opd (Organophosphate Degradation) gene in an *Agrobacterium* isolate. *Applied Environmental Microbiology*. 2002; **68(7)**, 3371-3376.
19. Richard, D.R., Irina, K., Ashok, M., Wilferd, C. Biodegradation of organophosphorus pesticides using surface-expressed organophosphorus hydrolase . *Nature Biotechnology*. 1997; **15**, 984-987.
20. Subhas, D., Dileep, K.S. Utilization of monocrotophos as phosphorus source by *Pseudomonas aeruginosa* F10B and *Clavibacter michiganense subsp. Insidiosum* SBL11. *Canadian Journal of Microbiolgy*. 2006; **52(2)**, 157-168.
21. Anitha, P., Umamaheswari, S. Prevalence of pesticide degrading bacteria in Paddy crop field. *Der Pharmacia Lettre*, 2016; **8(1)**: 281-295.