

Degradation of Lambdacyhalothrin in Soil Inoculated with *Bacillus cereus* and *Aneurinibacillus migulanus*

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Indiscriminate application of pesticides in paddy crop field soil to enhance paddy production could cause adverse impact on the both soil fertility and bacterial communities. The present study aimed to isolate bacteria prevalent in Lambdacyhalothrin exposed soil to determine their Lambdacyhalothrin degrading ability and assay the degraded metabolites. The observations registered in this study revealed that the dominant bacteria isolated from paddy crop field soil were *Bacillus cereus* and *Aneurinibacillus migulanus*. These bacteria were inoculated in soil spiked with Lambdacyhalothrin. After 36 hours of incubation, the extract were analysed by GCMS. Lambdacyhalothrin degradation was accelerated by *Aneurinibacillus migulanus* (100 % of pesticide degraded compared to *Bacillus cereus* 94.09 % and control 55.2 %). Thus *Aneurinibacillus migulanus* could be the used as bioagents to degrade lambdacyhalothrin.

Keywords: Lambdacyhalothrin, *Bacillus cereus*, *Aneurinibacillus migulanus*, GCMS, Biodegradation.

The application of pesticides is a pivotal strategy to control pest. In Tiruchirappalli district of Tamil Nadu, India, commonly applied pesticide in paddy cultivation is Lambda cyhalothrin. The persistence of Lambdacyhalothrin and its degradative products in paddy crop field soil could deteriorate the soil quality. Hence, protocols have to be evolved to eliminate these residues from the soil. In this study, we have aimed to tap the potential of autochthonous bacteria inhabiting the paddy crop field soil to degrade lambdacyhalothrin.

MATERIAL AND METHODS

Isolation of pesticide resistant bacteria from paddy crop field soil

1 gm of Lambdacyhalothrin exposed paddy crop field soil were aseptically inoculated

in 100 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: CaCl₂ – 0.002 g , MgCl₂ – 0.02 g, K₂ HPO₄ – 0.1 g, KH₂PO₄– 0.1 g, NH₄NO₃ - 0.1 g and FeCl₃ trace amount in distilled water (pH 7.2 – 7.4) upto 1 L were used for inoculation of soil sample. Total heterotrophic bacteria were isolated and identified following Bergeys manual of Determinative Biology (Sneath, *et al.*, 1994). The dominant bacteria *Bacillus cereus* and *Aneurinibacillus migulanus* were selected for pesticide resistant studies.

Lambdacyhalothrin in soil inoculated with *Bacillus cereus* and *Aneurinibacillus migulanus*

Bacillus cereus was subcultured in autoclaved nutrient broth for 48 hours at 30 °C in a rotatory shaker. After 48 hours, 1ml (45 x 10¹⁵ cfu/ ml) of *Bacillus cereus* broth was inoculated in 50 g of sterile paddy crop field soil in 250 ml of cotton plugged conical flask containing

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500 ppm Lambdacyhalothrin 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* (29×10^{15} cfu/ml).

GC-MS analysis of degraded metabolites of Lambdacyhalothrin

After 36 hours of incubation, the samples were subjected to GC-MS analysis. The control and the Lambdacyhalothrin treated soil were extracted for GCMS analysis based on the method of Malghani *et al.*, (2009) 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 with silica gel column (1.3 cm diameter x 243 cm length). The pesticide extract were eluted with n-hexane collected in a glass vial and subjected to gas chromatograph- Mass spectrometer (GC-MS) analysis.

Instrumental Analyses

The qualitative and quantitative determination of Lambdacyhalothrin was performed by GC-MS (45 X GC - 44, Bruker) equipped with auto injector (8410). The analyses separation was performed in a 60 mm x 0.25 mm I.D x 0.25 μ m film thickness BR 5 ms column (made in USA) and Helium was used as a carrier

Table 1. GCMS result showing the biodegradation of Lambdacyhalothrin by *Bacillus cereus* and *Aneurinibacillus migulanus* after 36 hours of exposure.

Treatments	RT	Area	Remaining pesticide (ppm)	% of Pesticide degraded
ControlSoil+ 500 ppm Lambdacyhalothrin	20.487	23940000000	223.765	55.2
Test soil+ 500 ppm Lambdacyhalothrin+ 1ml (45×10^{15} cfu/ ml) <i>Bacillus cereus</i>	20.353	3160000000	29.536	94.09
Test soil+ 500 ppm Lambdacyhalothrin 1ml (29×10^{15} cfu/ ml) <i>Aneurinibacillus migulanus</i>	-	-	-	100%

RT – Retention time
-No Peak

Table 2. Biodegraded metabolites of Lambdacyhalothrin in soil detected by GC-MS on inoculation with bacteria

Treatment	R.T	Degraded metabolites
Control (soil + 500 ppm Lambdacyhalothrin)	6.918	Isopropylbenzene
	9.462	2-ethyl-1,3-dimethyl benzene
	10.454	1,2,3,4-tetramethyl-5-methylene 1,3- cyclopentadiene
	14.532	4-methyl benzoic acid pentafluoro phenyl ester
	16.094	1-methyl-4-(1-methylethyl)- cyclohexane
	20.487	Lamdacyhalothrin
Test (soil + 500 ppm Lambdacyhalothrin + <i>Bacillus cereus</i>)	20.353	Lamdacyhalothrin
Test(Soil + 500 ppm + lamdacyhalothrin + <i>Aneurinibacillus migulanus</i>)	6.539	Isopropylbenzene
	7.626	1,2,4-trimethyl benzene
	8.584	1,2,4-tris(methylene) cyclohexane
	9.182	1-ethyl-2,4-dimethyl-benzene
	20.518	methyl ester 1,4-benzenedicarboxylic acid
	21.972	3-Chloro propanoic acid,

R.T – Retention Time

gas at a flow rate of 1 ml / min. The column temperature was programmed as 70 °C to 150 °C at 10 °C / min, to 250 °C at 5 °C/ min, to 280 °C at 2 °C / min, finally to 320 °C at 5°C/ min and hold for 10 minutes. 1 µl of the extract was injected into the injection port (at 280 °C) using auto injector. The mass spectrometer was operated in scan mode and the ion source temperature was kept at 250 °C.

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.

RESULTS

Bacteria was isolated from paddy crop field soil exposed to lambdacyhalothrin. 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 Lambdacyhalothrin by gas chromatography due to bacterial activity is presented in table 1. Relative to the control (55.2 %), *Bacillus cereus* and *Aneurinibacillus migulanus* accelerated the degradation of Lambdacyhalothrin by 94.09 % and 100 %, respectively. These results indicate that *Bacillus cereus* and *Aneurinibacillus migulanus* play a vital role in accelerating the degradation of Lambdacyhalothrin.

In the control soil, apart from Lambdacyhalothrin the various metabolites obtained were Isopropyl benzene, 2-ethyl-1,3-dimethyl benzene, 1,2,3,4-tetra methyl-5-methylene 1,3-cyclopentadiene, 4- methyl benzoic acid pentafluoro phenyl ester, 1 methyl-4- (1-methyl ethyl)- cyclohexane (Fig

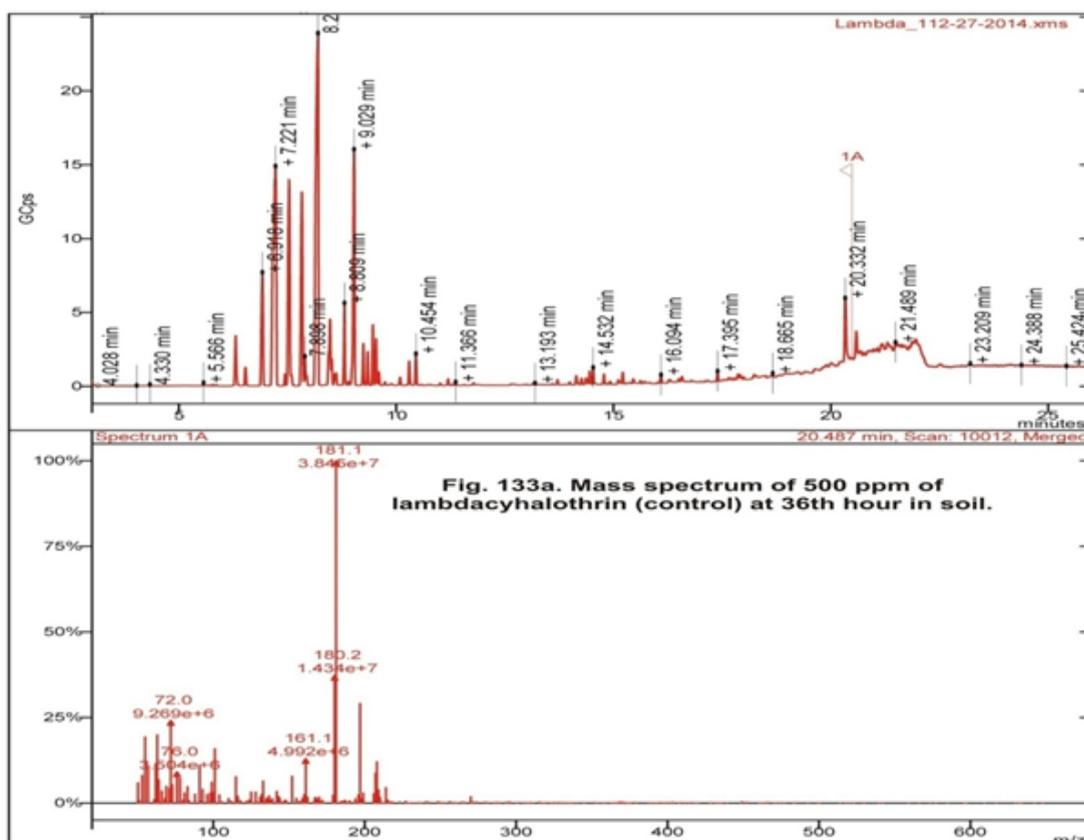


Fig. 1. Gas chromatogram of 500 ppm of lambdacyhalothrin (control) at 36th hour in soil

1, table 2).Lambdacyhalothrin residue also was persistence in *Bacillus cereus* inoculated soil containing 500 ppm Lambdacyhalothrin. In addition, only Lambdacyhalothrin persisted and no other metabolites were present (fig 2, table 2).*Aneurinibacillus migulanus* resulted in the formation of Isopropyl benzene, 1,2,4-Trimethyl benzene, 1,2,4- tris (methylene) cyclohexane, 1- Ethyl-2,4-dimethyl benzene, methyl ester 1,4-Benzene dicarboxylic acid and 3-Chloro propanic acid (Fig 3, table 2).

DISCUSSION

As evinced in this study, Geeta *et al.*, (2014) have demonstrated that bacteria accelerated the degradation of pesticides in soil (arbandazim: *Bacillus sp.*, *Exiguabacterium*, *Achromobacter*;imidacloprid: *Achromobacter*; *Microbacterium*, *Pseudomonas sp.* and α and β – endosulfan :, *Xanthomonas sp.*, *Microbacterium sp.*, *Bacillus sp.*). They have

also observed dose – dependent degradation of Carbendazim by *Exiguobacterium*. In addition, they have reported that comparatively, consortium of bacteria enhanced the degradation of pesticides.

The results of the present finding are in corroboration with the work done by Agarry *et al.*, (2013) who have demonstrated that bacterial consortium (*Proteus vulgaris*, *Vibrio sp.*, *Serratia sp.*, and *Acinetobacter sp.*) isolated from agricultural farm soil from Ladike Akintola University of Technology, Nigeria were able to grow in nutrient medium containing Dichlorvos as the only carbon source. Further, they have observed that the percentage removal of Dichlorvos pesticide from the soil was relatively higher in soil amended with NH_4NO_3 (75%) and KH_2PO_4 (85%), respectively.

Bacillus mediated degradation of Lambdacyhalothrin observed in this study is consistent with the findings of Osama el Gialanielsaid *et al.*, (2010) who have compared the endosulfan degradation potential of mutant

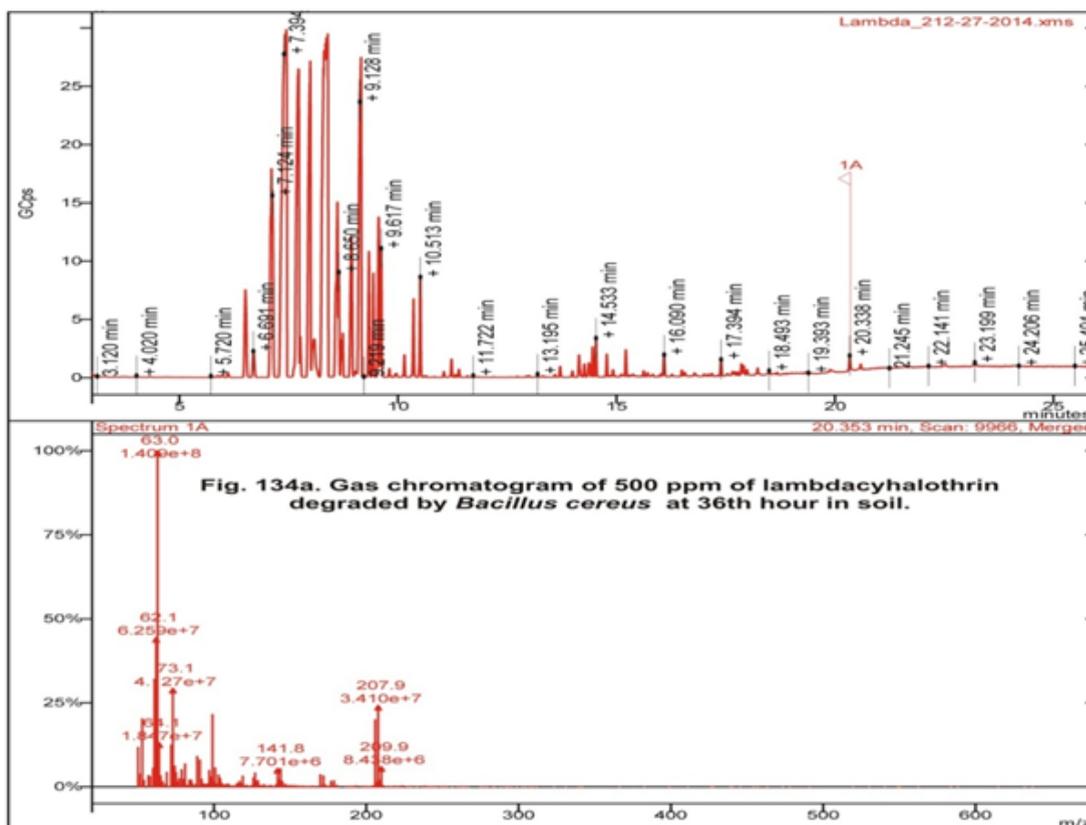


Fig. 2. Gas chromatogram of 500 ppm of lambdacyhalothrin degraded by *Bacillus cereus* at 36th hour in soil

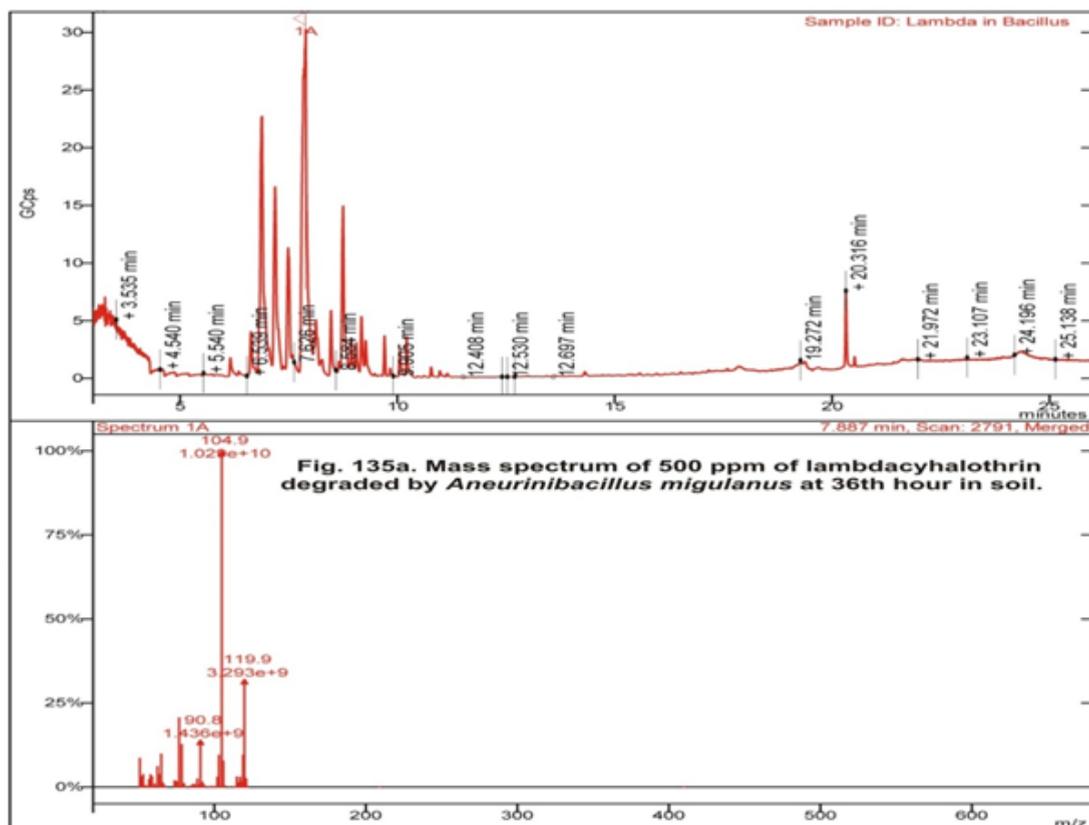


Fig. 3. Gas chromatogram of 500 ppm of lambda-cyhalothrin degraded by *Aneurinibacillus migulanus* at 36th hour in soil

strains of *Bacillus sp.* (isolated through consecutive exposure to elevated concentrations of endosulfan under carbon free media) and wild type strain and have confirmed through GLC (Gas liquid Chromatography) that wild type of bacteria (from stock culture) caused 83 % reduction in half lives of both \pm and 2 endosulfans. Enhanced degradation of Lambda-cyhalothrin by *Aneurinibacillus migulanus* is well supported by Abdullah *et al.*, (2016) who have also evinced acceleration of degradation of Lambda-cyhalothrin by *Pseudomonas putida*.

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