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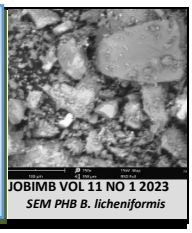

Isolation, Characterization and Screening of Potential Lambda-Cyhalothrin-degrading Bacteria from Cultivated Soil in Moro, Kwara State, Nigeria

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ABSTRACT

The indiscriminate use of Lambda Cyhalothrin (LC), one of the commonly used synthetic pyrethroids (SPs) insecticides in agriculture, has led to the contamination of different environments with potentially serious impacts on the health of humans and animals. This study investigated the presence of LC-degrading bacteria in cultivated soils. Bacteria were isolated from agricultural soil on mineral salt medium (MSM) using an enrichment technique. They were characterized and identified. Their growth in different concentrations (50 mg/L, 75 mg/L, 100 mg/L, and 125 mg/L) of LC was studied, Potential to degrade LC was assessed by the growth of the bacteria in a mineral salts medium containing LC as the sole carbon source over 14 days. Five bacteria able to grow in MSM with LC as the sole source of carbon and on nutrient agar enriched with 100mg/L of LC were isolated. Their growth (turbidity and viable counts of the bacterial cells) increased from the third day till the sixth day, after which it declined till the 14th day. The Lambda Cyhalothrin-degrading bacterial isolates (LCDB) were tentatively identified as *Bacillus* species, *Klebsiella* species, *Pseudomonas* species, and *Lysinibacillus*species. These results indicated that these bacterial isolates are potentially able to degrade LC and can be useful for the remediation of SPs-contaminated agricultural soils.

INTRODUCTION

Globally, synthetic pyrethroids (SPs) are the most commonly used insecticides for agriculture and home applications. They are chemical analogs of the natural pyrethrins derived from the *Chrysanthemumcinerariaefolium* flower [1, 2]. They belong to the fourth group of insecticides according to the WHO classification and include 42 substances [3]. One of the most widely used SPs in agriculture is Lambda-cyhalothrin (LC) with the chemical name: (R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]2,2dimethylcyclopropane carboxylate with the molecular weight of 449.8 g/mol and 0.005 mg/L solubility in water. LC is used to control aphids, Coleopterous and Lepidopterous pests in cotton, cereals, hops, potatoes, vegetables, and flowers in agriculture and in domestic use to control cockroaches, mosquitoes, ticks, and flies [4, 5]. It belongs to the type II pyrethroids which have the cyano group and cause chloreoathetosis and salivation [6]. They are characterized with high insecticidal activity and have been widely used against insect pests [7]. The indiscriminate and excessive use of LC has posed a great threat to human health and to the ecosystems [8]. SPs primarily enter the human body through skin contact and through through inhalation and ingestion in food or water. Therefore, there is an urgent need for effective strategies to remove LC from the environment.

Biodegradation involves the use of living microorganisms to detoxify and degrade hazardous materials and is generally considered to be an effective and safe way to remove contaminants from the environment. Several bacteria and fungi strains that could degrade SPs in situ or under experimental conditions have been reported [9]. In addition, various SP-degrading strains, the enzymes involved and molecular modification of these enzymes have been compared extensively

[10]. Microbial degradation of SPs is affected by various abiotic and biotic factors such as humidity, salts and pH, as well as by the bioavailability of the molecule to degrading microorganisms. Therefore, this study aims to isolate, identify and screen for potential lambda cyhalothrin degrading bacteria in the cultivated soils in Moro, Kwara State, Nigeria.

MATERIALS AND METHODS

Study Area

The study involved three farms in Moro Local Government Area: Elemere (8.6856917°N, 4.514993°E), Malete (8.710625°N 4.455877°E) and Manso (8.710355°N 4.465551°E) that had histories of repeated Lambda-cyhalothrin insecticide use.

Chemicals

The technical grade lambda-cyhalothrin: Karate 5EC (emulsifiable concentrate) was purchased from an agrochemical retailer in Malete market, Kwara State, Nigeria. All chemicals used in this study were of analytical grade and were purchased from the Central Research and Diagnostic Laboratory, Ilorin, Kwara State, Nigeria.

Soil Sample Collection and Preparation

The soil samples were taken in December, 2019. Ten random locations were chosen for sampling. One kilogram of soil was taken from 0-15cm depth at each portion of the farm and composited into one sample. The composite sample was taken into sterile plastic bags and transported to the laboratory [11].Visible plant debris and fauna were removed from the collected soil samples by hand picking and the soils were gently sieved (<2mm fraction) and stored in sealed polythene bags at 4 ºC to preserve the moisture for biological activity before analysis [12, 13].

Isolation of Bacterial Isolates

Isolation of bacteria was carried out using enrichment techniques with Mineral salt medium (MSM). Five grams of the pesticide-contaminated soil sample was inoculated into the 100 ml of MSM medium supplemented with 20 mg/L of lambda-cyhalothrin (LC) as the sole source of carbon and energy and then incubated on a shaker at 160 rpm at 37 ºC for 7 days. At the end of 7 days, the culture medium was transferred into a fresh MSM supplemented with 20 mg/L of LC.

From the enriched medium, 5 ml of the broth culture was inoculated into 50 ml of freshly prepared sterile MSM enriched with 20 mg/L of LC. The medium was incubated on a shaker at 160 rpm at 37 ºC for another seven days. This procedure was repeated three times with increasing concentrations of LC up to 50 mg/L to exclude the LC- tolerant bacteria from growing. The bacterial isolates were grown on nutrient agar supplemented with LC several times until pure cultures are obtained.

Growth of Bacterial Isolatesin Different Concentrations of LC

Bacterial isolates from the enrichment phase were selected. A suspension of each isolate was prepared and standardized to 0.5 McFarland, then 5 ml aliquots of the suspension were introduced into flasks containing 50 ml of MSM supplemented with different concentrations of the LC (50 mg/L, 75 mg/L, 100 mg/L, and 125 mg/L). The controls were prepared with an equal volume of MSM containing each of the bacterial cultures but without LC. Incubation was carried out at 37 ºC for seven days on a rotary shaker operating at 160 rpm and growth of the isolated monitored in terms of optical density (turbidities) over seven days [14].

Screening for Potential LC-Degrading bacterial isolates

The bacterial isolates that could withstand 100 mg/L of LC were selected for the biodegradation of LC. Aliquots of the standardized suspension of the selected isolates were inoculated into flasks of mineral salt medium containing 100 mg/L of LC. The flasks were incubated at 37 ºC for 14 days on a rotary shaker operating at 150 rpm, and their growth was monitored using optical density (turbidities) determined at intervals of 0, 3, 6, 12, and 14 days with a spectrophotometer (UV/Vis 721 (D): Axiom Medical Ltd.UK) at 620nm [14].

The total viable bacterial counts were also determined at intervals of 0, 3, 6, 12, and 14 days. This was done by inoculating 0.1ml of the culture from each of the MSM onto nutrient agar plates using sterile glass spreaders. The plates were incubated at 37 ºC for 24 hours, after which the bacterial colonies that developed were counted and expressed as viable bacteria count [15].

Characterization and Identification of the Potential LC-Degrading Bacterial Isolates

Pure cultures of the isolates were prepared, and the isolates were then characterized based on morphological and biochemical properties [16, 17]. They were identified according to [18] and the [19].

RESULTS

Ten (10) bacterial isolates capable of growth in LC-enriched MSM were obtained (**Table 1**). They all showed good growth in up to 100 mg/L of LC. The bacteria were tentatively identified as *Bacillus* sp. I, *Bacillus* sp. II, *Bacillus* sp. III, *Bacillus* sp. IV,*Klebsiella* sp. I, *Klebsiella* sp. II, *Lysinibacillus* sp. I,*Lysinibacillus* sp. II,*Pseudomonas* sp. Iand *Pseudomonas* sp. II. Five of the isolates, which had very heavy growth at 100 mg/L of LC were partially identified (**Table 2**) and then screened for the potential to degrade LC. The isolates showed steady growth up to day 6 (optical density and total viable bacterial counts increased), after which there was a gradual decline (**Figs. 1** and **2**). LCDB2 produced the best growth (highest turbidity and total viable bacterial counts) while LCDB 3 produced the lowest growth (lowest turbidity and total viable bacterial counts).

Table 1. Growth of the bacterial isolates in different concentration of LC.

Isolates	Growth in Different Lambda Cyhalothrin (mg/L)			
	50	75	100	125
LCDB1	++	+++	++++	+
LCDB2	++	+++	++++	+
LCDB3	++	+++	++++	+
LCDB4	++	+++	++++	+
LCDB5	++	+++	++++	+
LCDB6	++	+++	++++	+
LCDB7	++	+++	++++	+
LCDB8	++	+++	++++	+
LCDB9	++	+++	++++	+
LCDB10	++	+++	++++	+

Key: + = light turbidity ++ = moderate turbidity +++ = heavy turbidity
++++ = very heavy turbidity LCDB= Lambda Cyhalothrin –Degrading Bacteria

Table 2. Biochemical characteristics of potential LC –degrading bacteria.

Characteristics	LCDB1	LCDB2	LCDB3	LCDB4	LCDB5
Gram reaction	+	+	-	-	+
Cell Form	Rod	Rod	Rod	Rod	Rod
Spore Formation	+	+	-	-	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	-	+
Urease	+	+	+	+	-
Citrate Utilization	-	-	+	+	-
Indole	-	-	+	-	-
Methyl Red	-	-	+	-	-
Voges Proskaeur	-	-	-	+	+
Hydrogen Sulphide Production	-	-	-	-	-
Starch Hydrolysis	+	+	-	+	-
Glucose Fermentation	+	+	+	+	-
Lactose Fermentation	-	-	+	+	-
Sucrose Fermentation	+	-	+	+	-
Probable Isolates	*Bacillus* sp. I	*Bacillus* sp. II	*Pseudomonas* sp. I	*Klebsiella* sp. I	*Lysinibacillus* sp. I

+ = positive reaction - = negative reaction

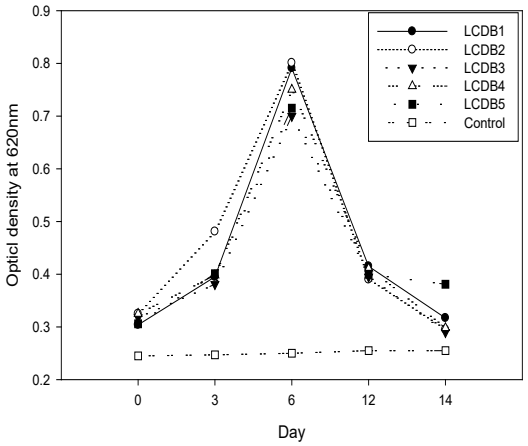

Figure 1: Growth of bacterial strain capable of degrading LC

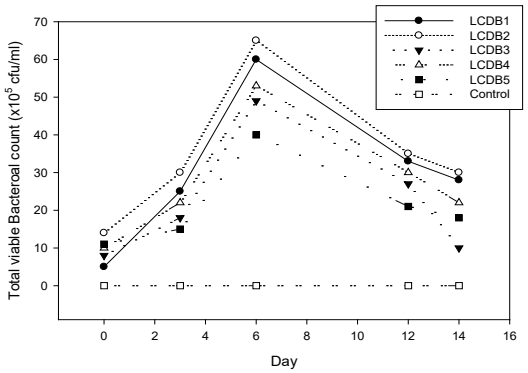

Figure 2: The total viable bacterial counts of the five degraders
Key: LCDB= Lambda Cyhalothrin –Degrading Bacteria

DISCUSSION

The luxuriant growth of the ten bacteria isolates in the 100 mg/L concentration of LC better than the other concentrations seems to align with the findings of Onuorah [20], who reported that bacterial isolates are capable of completely degrading 100 mg/L of LC better than the other concentrations. The increase in turbidity and total viable bacterial counts indicated growth of the organisms. The declining growth (reduction in the turbidities and total viable bacterial counts) after the sixth day of growth in the MSM may be due to the reduction in the concentration of substrate (pesticide) which was the only source of carbon for the isolates. It may also be because of the production of metabolites due to the degradation of the substrate, which may be unfavourable for their growth. Since there was no other carbon source, the organisms must be utilizing the pesticides for growth and degradation of LC was taking place. The order of degradation bythe five isolates is LCDB2>LCDB1>LCDB4> LCDB5>LCDB3.

The statusof *Bacillus* sp. I*, Bacillus* sp. II, *Pseudomonas* sp. I, *Klebsiella* sp. I and *Lysinibacillus*sp. I isolated in this study as potential Lambda cyhalothrin-degrading bacteria is further strengthened by their proliferation in MSM with LC as their sole source of carbon. *Bacillus* species have been reported severally for their potentials to degrade SPs. *Bacillus cereus* ZH-3 has been used to degrade fenprothrin [21, 22]. Chen [23] also reported that lambda-cyhalothrin was effectively degraded by *Bacillus thuringiensis* Strain ZS-19. Tang [24] reported that a *Bacillus licheniformis* strain can degrade SPs successfully while a coculture of *Bacillus licheniformis* and *Aspergillus oryzae*M-4 has been used to degrade cypermethrin efficiently. This result is in line with the findings of Farooq [25], who reported that *Bacillus aryabhattai*and *Bacillus circulans*degraded LC in 72 hours.

Pseudomonas oleovorans is involved in the degradation of Lambda cyhalothrin pesticide [26] while *Pseudomonas fulva* strain P31 degraded D-phenothrin [27]. Thatheyus [26] had also reported that a *Klebsiella* spp efficiently removed lambda-cyhalothrin. Tang [28] demonstrated the efficiency of *Klebsiella pneumoniae* BPBA052 in the degradation of SPs and their metabolites. A co-culture of *Acinetobacter junii*LH-1-1 and *Klebsiella pneumoniae* BPBA052 was used to degrade deltamethrin [29]. Anjos [30] also recognized that *Lysinibacillusxylanilyticus*was able to degrade synthetic pyrethroids while Onuorah [20], had indicated that *Pseudomonas putida, Lysinibacillus macrolides and Klebsiella pneumoniae* were able to degrade lambda-cyhalothrin and dichlorvos. Recently, an esterase enzyme EstGS1 from a halophilic actinobacteria *Glycomycessalinus* effectively hydrolyzed deltamethrin, lambda-cyhalothrin, fenvalerate and permethrin in four hours [9].

Microbial degradation of pesticides by soil bacteria is a crucial means of removing the pesticides from the environment, thereby preventing environmental pollution. In this study, *Bacillus* sp. II had the highest growth in the LC- mineral salts medium, followed by *Bacillus* sp. I, *Klebsiella* sp. I and *Lysinibacillus* sp. I while *Pseudomonas* sp. I had the least growth. The results of this study showed that bacteria with the potentials to degrade the insecticide lambda cyhalothrin are abundant in the soil. The 100 mg/L concentration which the isolates were able to tolerate and degrade is higher than the recommended dosage for application of the pesticide. This gives the assurance that using the insecticide at concentrations lower than 100 mg/L is not likely to result in the persistence of the pesticide in the soil or cause pollution of soil as the pesticide will be easily degraded by soil bacteria into harmless and environment-friendly products.

CONCLUSION

Bacteria isolated from pesticide-contaminated agricultural soils in this study showed ability to degrade the SP insecticide lambda-cyhalothrin. Five of the LCDB isolates are promising candidates

for LC biodegradation and may be used for the bioremediation of synthetic pyrethroids insecticide- contaminated soils.

REFERENCES

1. Ensley, S.M. Pyrethrins and pyrethroids. In Veterinary toxicology (pp. 515-520). 2018. Academic Press.
2. Gammon DW, Liu Z, Chandrasekaran A, El-Naggar SF, Kuryshev YA and Jackson S. Pyrethroid neurotoxicity studies with bifenthrin indicate a mixed type I/II mode of action. Pest Manag Sci. 2019;75:1190–1197.
3. World Health Organization (WHO). Pesticide evaluation scheme, vector ecology and management. Vol. 3. World Health Organization;Geneva, Switzerland. 2016.
4. Gu BG, Wang HM, Chen WL, Cai DJ and Shan ZJ. Risk assessment of lambda cyhalothrin on aquatic organisms in paddy field in China. Regul Toxicol Pharma. 2017;4(8):69–74.
5. He LM, Troiano J, Wang A and Goh K. Environmental chemistry, ecotoxicity, and fate of lambda-cyhalothrin. Rev Environ Contam Toxicol. 2018;19(5):71–91.
6. Xu H, Li W, Schilmiller AL, Eekelen HV and deVos RCH. Pyrethric acid of natural pyrethrin insecticide: Complete pathway elucidation and reconstitution in *Nicotiana benthamiana*. New Phytol. 2019;223:751–765.
7. Hao X, Zhang X, Duan B, Huo S, Lin W, Xia X, and Liu K. Screening and Genome Sequencing Of Deltamethrin-Degrading Bacterium ZJ6. CurrMicrobiol. 2018;75(11):1468-1476.
8. Kumar TT and Jahangir HS. Biodegradation of lambda cyhalothrin by *Rhodococcus erythropolis*. Life Sci Inform. 2018;4(5):192-200.
9. Liu Y, Tang S, Wang X, Wang X, Tang X, Wu Q, Huang Z and Ding J. A novel thermostable and salt-tolerant carboxylesterase involved in the initial aerobic degradation pathway for Pyrethroids in *Glycomyces salinus*. J Hazard Mater. 2023;451:131128. doi:10.1016/j.jhazmat.2023-131128.
10. Fang Y, Xu W, Zhang W, Guang C and Mu W. Microbial elimination of pyrethroids: Specific strains and involved enzymes. Appl Microbiol Biot. 2022;106(21):6915-6932.
11. Moghaddam NS, Zakaria MP, Omar D, Sijam K and Khakvar R. Effects of imidacloprid on the biodiversity of soil microbes in selected soils of Malaysia. Environ Sci Dev. 2011;**4**:7-10.
12. Estefan G, Sommer R and Ryan J. Method of Soil, Plant and Water Analysis:A Manual for the West Asia and North Africa region. *Third edition,* International Centre for Agricultural Research in Dry Areas. 2013.
13. Wang BB, Wang CL, Liu WX, Liu X, Hou J, Teng Y, Luo YM and Christie P. Biosurfactant-producing microorganism *Pseudomonas* sp SB assists the phytoremediation of DDT-contaminated soil by two grass species. Chemosphere. 2017;182:137-142.
14. Parte SG, Kharat AS and Mohekar AD. Isolation and characterization of dichlorovos degrading bacterial strain *Pseudomonas stutzerismk.* Res J Life Sci Bioinform Pharma Chem Sci. 2017;2(5):282-288.
15. Mohamed MS. Degradation of methomyl by the novel bacterial strain *Stenotrophomonas maltophilia* M I. Electron J Biotechnol. 2009;12 (4):1-6.
16. Shahzad F, Shafee M, Abbas F, Babar S, Tariq M and Ahmad Z. Isolation and biochemical characterization of Rhizobium meliloti from root nodules of Alfalfa (medico sativa). J Anim Plant Sci. 2012;22(2):522-524.
17. Arora PK and Bae HL. Bacterial degradation of chlorophenols and their derivatives. Microb Cell Fact. 2014;13(1):1-17.
18. Bergey DH. Bergey's Manual of Systematic Bacteriology. Firmicutes, Vol. 3. Springer-Verlag, New York, 2009;pp 1–1476.
19. Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood, WA, Krieg NR and Phillips GB. Manual of Methods for General Bacteriology. Eds:1994;(Pp 524) Published by ASM. ISBN-10-0914826298.
20. Onuorah S, Ngwu V and Nwankwo J. Lambda cyhalothrin and dichlorvos pesticides degradation potentials of bacteria isolated from agricultural soil in Enugu, Enugu State, Nigeria. Int J Adv Technol Sci. 2021;2(1):1-15.
21. Liu J, Huang WW, Han HT, She CC and Zhong GH. Characterization of cell free extracts from fenpropathrin-degrading strain *Bacillus cereus* ZH-3 and its potential for bioremediation of pyrethroid-contaminated soils. Sci Total Environ. 2015;523:50–58.
22. Zhang H, Zhang YM, Hou ZG, Wang XM, Wang J, Lu ZB, Zhao XF, Sun FJ and Pan HY Biodegradation potential of deltamethrin by the *Bacillus cereus* strain Y1 in both culture and contaminated Soil. Int J Biodeterior Biodeg. 2016;106:53–59.
23. Chen SH, Deng YY, Chang CQ, Lee J, Cheng YY, Cui ZN, Zhou JA, He F, Hu MY and Zhang LH. Pathway and kinetics of cyhalothrin biodegradation by *Bacillus thuringiensis* strain ZS-19. Sci Rep.2015;5:8784-8791.
24. Tang J, Liu B, Shi Y, Zeng CY, Chen TT, Zeng L and Zhang Q. Isolation and identification of fenvalerate-degrading potential of *Bacillus licheniformis* CY-012. Biotechnol Biotech. Equip. 2018;32(3):574–582.
25. Farooq H, Khalid M and Hashmi I. Bioremediation of synthetic pyrethroid by hydrolases of *Bacillus aryabhattai* and *Bacillus circulans* derived from indigenous soil. J Environ Treat Technol. 2022;10(3):187-194.
26. Thatheyus AJ, Alexander D and Selvam G. Synthetic Pyrethroids: Toxicity and Biodegradation. Appl Ecol Environ Sci. 2013;1(3):33-36.
27. Yang J, Feng Y, Zhan H, Liu J, Yang F, Zhang K, Zhang L and Chen S. Characterization of a pyrethroid-degrading *Pseudomonas fulva* strain P31 and biochemical degradation pathway of d-Phenothrin. Front Microbiol. 2018;9:1003. doi:10.3389/fmicb.2018.01003.
28. Tang J, Hu Q, Liu B, Lei D, Chen TT, Sun Q, Zeng CY and Zhang Q. Efficient biodegradation of 3-phenoxybenzoic acid and pyrethroid pesticides by the novel strain *Klebsiella pneumoniae* BPBA052. Can J Microbiol. 2019;65(11):795–804.
29. Tang J, Hu Q, Lei D, Wu M, Zeng C and Zhang Q. Characterization of deltamethrin degradation and metabolic pathway by co-culture of *Acinetobacter junii* LH-1-1 and *Klebsiella pneumoniae* BPBA052. Appl Microbiol Biotech. Exp. 2020;10:106-117.
30. Anjos CS, Birolli WG and Porto ALM. Biodegradation of the Pyrethroid pesticide esfenvalerate by a bacterial consortium isolated from Brazilian Savannah. J Braz Chem Soc. 2020;31(8):1654-1660.