Impact of Lambda Cyhalothrin on Bacterial and Fungal Species in Soil

Garba Aliyu Abubakar[1], **Akinsuyi Faizah**[1]

1 - Department of Soil Science and Land Resources Management, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding Author: Garba Aliyu Abubakar, e-mail: garba.aliyu@udusok.edu.ng

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Abstract

In agricultural plantations, insecticides are frequently employed in integrated pest management. Their use modifies the biochemical and biological processes in the soil that are essential to the health of ecosystems. This study determined the inhibition effect of Lambda cyhalothrin insecticide on soil fungal and bacterial species. Soil samples were contaminated with insecticides for 14 days, and the microbial count, isolation, identification, and antimicrobial analysis were carried out using standard methods. The control (uncontaminated soil sample) recorded the highest bacterial count ($90.67\pm0.06\times10^{-6}$ cfu/g) as well as highest fungal count ($5.11\pm0.01\times10^{-6}$ cfu/g) as compared to the Lambda cyhalothrin treated soil samples which recorded ($13.32\pm0.14\times10^{-6}$ cfu/g) and ($2.31\pm0.42\times10^{-6}$ cfu/g) as the bacterial and fungal load count respectively. The bacteria species from the contaminated soil samples were identified as *Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Bacillus lentus, Bacillus megaterium,* and *Bacillus femulus* while the isolated fungi were *Aspergillus flavus, Aspergillus niger, Fusarium chlamidosporium* and *Fusarium solain*. The *in vitro* antibacterial activity of the insecticides shows a considerable level of inhibition. Lambda cyhalothrin at 2.0ml had the highest activity against *Bacillus megaterium* (24.15±0.22 mm) and the least against *Bacillus femulus* (14.96±0.07mm). Lambda cyhalothrin shows significant inhibition of fungal growth. 5.0ml of the insecticide on Day 3, recorded the highest % inhibition of fungal growth, with Lambda cyhalothrin recording (58.61%) against *Fusarium chlamidosporium* and least % inhibition was recorded against *Fusarium solain* with Lambda cyhalothrin (33.98%). The bacterial and fungal communities in the soil exhibits changes in growth and development as a result of insecticide applications.

Keywords: insecticides, lambda cyhalothrin, microbial inhibition, bacillus species

Introduction

Current agricultural practices in many developing countries use unsustainable practices, which led to the direct or indirect release of massive amounts of hazardous effluents into the land, air, and water. (Aislabie and Deslippe, 2018). Currently, various agrochemicals (i.e., pesticides, herbicides, fungicides, insecticides, chemical fertilizers, etc)

are being used non-judiciously which has adversely affected beneficial soil microbial communities (Radhika and Kannahi, 2019). Pesticide use in agriculture is thought to be highest, accounting for up to 85% of the world's total production (Lykogianni et al., 2021).

Lambda cyhalothrin is a synthetic pyrethroid and non-systematic insecticide used to control insect pests of many different crops in fibre, cereals and vegetables to control insect pests such as aphids, Colorado beetle and thrips (Martinez et al., 2018; Srinivasa et al., 2022). The US EPA has classified it as a moderately hazardous class D carcinogen and it changes the soil microbial composition (He et al., 2022; Laborde et al., 2023).

However, indiscriminate use of these chemicals could pose a risk to the environment and have an immediate impact on agricultural productivity and soil microorganisms (Sodhozai et al., 2024).

Most soil microbes such as fungi, bacteria, archaea, protozoa, etc which are beneficial in performing fundamental functions such as nutrient cycling, crop residue decomposition, and stimulating plant growth (Gupta et al., 2022). The biological and chemical properties of soil are very important in the biogeochemical cycles of nutrients, and enzymes produced by soil Microorganisms are responsible for biochemical transformations (Neemisha and Sharma, 2022). Agricultural expansion and indiscriminate use of insecticides over the years have often led to a negative effect on the soil ecosystem causing heavy population damage, toxicity and soil pollution, which affect the nutrient transformation, that leads to loss of fertility in soil and crop quality (Prashar and Shah, 2016). The insecticides applied to the agricultural field are expected to only be toxic to the target organisms, biodegradable and eco-friendly to some extent. Unfortunately, most insecticides are non-specific and kill organisms that are harmless and very useful to the various ecosystems. Due to the potential negative effects of pesticide compounds on soil microorganisms, the fate of pesticides in the soil environment with regard to pest management strategy, non-target organism exposure, and off-site mobility has become a matter of environmental concern, which consequently might impact soil fertility (Schuster and Schroder, 1990; Ajaugo et al., 2003).

The use of Lambda cyhalothrin in rice fields can alter the non-targets organisms such as cyanobacteria (*Calothrix sp*) that fix nitrogen which resulted to gradual decline in the growth and reduction in dry weight biomass (Gupta and Baruah, 2015). High rate of lambda-cyhalothrin was also observed to effects a significant fraction of the macroinvertebrate groups (Hill et al., 2018). Due to the extensive use of lambda-cyhalothrin, residues have been identified in storm and irrigation runoff water, particularly in the sediments that are connected with these bodies of water. These residues have been shown to be hazardous to aquatic life, including fish and amphipods (Tu et al., 2016; Xu et al., 2016). It was also observed that application of Lamda cyhalothrin can significantly altered the soil communities such as collembolans, enchytraeids, Isopods and millipedes (Farmer et al., 1995; Rieff et al., 2020). Overuse of pesticides integrated into agricultural methods (such as conventional and no-tillage tillage) may alter soil functions, harm soil biota, and have an adverse effect on ecosystems (Pramanik et al., 2019). Application of Lamda cyhalothrin at three times the recommended dose significantly lower the growth of *Bacillus subtilis* up to 76.8% when compared to the control group due its cellular toxicity towards bacteria (Sodhozai et al., 2024).

The purpose of the study was to determine the effect of the application of Lambda cyhalothrin at field recommended dosage and how it adversely affects the abundance of Fungi and Bacteria present in the soil. In addition, the potential effect of Lambda cyhalothrin on the biochemical and microbiological properties was carried out to determine the microbial hydrolytic activity as well as to identify some microorganisms that are tolerable to this insecticide toxicity. This will contribute to a sound ecological approach that maintains soil health and increases food security without compromising soil biodiversity.

Material and Methods

This research was carried out at Fadama Teaching and Research Farm and Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto State, located on Latitude 13°03′44″N and Longitude 5°14′02″E. The State falls between Sudan savanna agroecological zone of northwestern Nigeria, at an altitude of about 315m above sea level. It has an annual average temperature of 30.6 ° C with maximum daytime temperatures for most of the year generally under 40 °C and annual rainfall amounts ranging from 390 mm to 790mm (Ekoh, 2020). A total of 30 soil sub-samples were collected systematically at a depth of 0-10cm from different soil sampling spots with an intervals of (30cm) from one sampling point to another to make a representative sample (composite sample), 2 weeks after Lambda cyhalothrin was applied on the field. The samples were collected using soil auger, packed into sterile polythene bags placed in an ice pack container and transported immediately to the Microbiology Laboratory for analysis.

Nutrient Agar (NA) powder (5.6 grams) was suspended in 200ml of distilled water and heated with frequent agitation on a hot plate until dissolved and sterilized using autoclave at 121°C for 15 minutes and allowed to cooled. Sabouraud Dextrose Agar (7.8 grams) and streptomycin antibiotic was also suspended in 200ml of distilled water, heated on a hot plate and boiled to completely dissolve the medium, autoclaved at 121 °C for 15 minutes, and cooled to 45-50 °C.

The serial dilution was made up to 10^{-6} dilutions for the soil sample. 0.1ml of the 10^{-6} dilution was cultured on the nutrient agar plates using the spread plate technique. The plates were then incubated upside down at 37°C for 24 hours. Individual colonies were then sub-cultured in the prepared culture media until pure cultures are isolated and then stored and maintained in prepared culture media slants. Identification of bacteria isolates was based on colony morphology, cultural characteristics, gram staining properties, microscopy and biochemical tests (oxidase, indole, catalase, citrate utilization, and urease test). Results were obtained according to biological standards (Cheesbrough, 2010) Gram staining and biochemical test (catalase, indole, oxidase, urease, and citrate test) were also performed based on the procedure describe (Cheesbrough, 2010).

Microbial resistance antimicrobial activity of the Insecticides. The subcultured bacteria isolates were smeared on a sterile petri plate 0.5ml, 1.0ml and 2.0ml of the insecticides were pipetted into the wells using a micropipette, 0.5ml of ciprofloxacin solution was used as a positive control. The plates were allowed to stand on a flat bench for 30 minutes to allow diffusion of the insecticides into the agar before it was incubated at 37^{o}c for 24hrs. The zone diameter of inhibition was recorded. For fungi also three concentrations of Lambda Cyhalothrin (2.5ml, 3.5ml, 5.0ml and control) each replicated three times. The resistance of

was determined by measuring the colony extension on each of the plate by drawing two perpendicular lines which meet at a right angle at the center of the plate. The plates were incubated at 27±2ºC for 7 days. Resistance of each fungus to the Lambda cyhalothrin was recorded as a percentage (%) radial growth inhibition and calculated using the formula below;

$$The\ percentage\ (\%)\ inhibition = \frac{R1 - R2}{R1}\ X\ 100$$

- where R1 is growth in the control and R2 is the growth in the treatment.

Data obtained were subjected to descriptive statistics and ANOVA, mean separation was done using Least Significant Difference (LSD) at P<0.05 Statistical Package for Social Sciences (SPSS 22.0).

Results and Discussion

The microbial population of the soil samples is shown in Fig. 1. The control soil samples (untreated soil sample) had the highest bacterial ($90.67\pm0.06\times10^{-6}$ cfu/g) and fungal load ($5.11\pm0.01\times10^{-6}$ cfu/g) as compared to Lambda cyhalothrin treated soil samples which recorded ($13.32\pm0.14\times10^{-6}$ cfu/g) and ($2.31\pm0.42\times10^{-6}$ cfu/g) as the bacterial and fungal load count respectively. The lower count recorded can drastically lower the potential yield of field crops (Georgieva and Karadzhova, 2020).

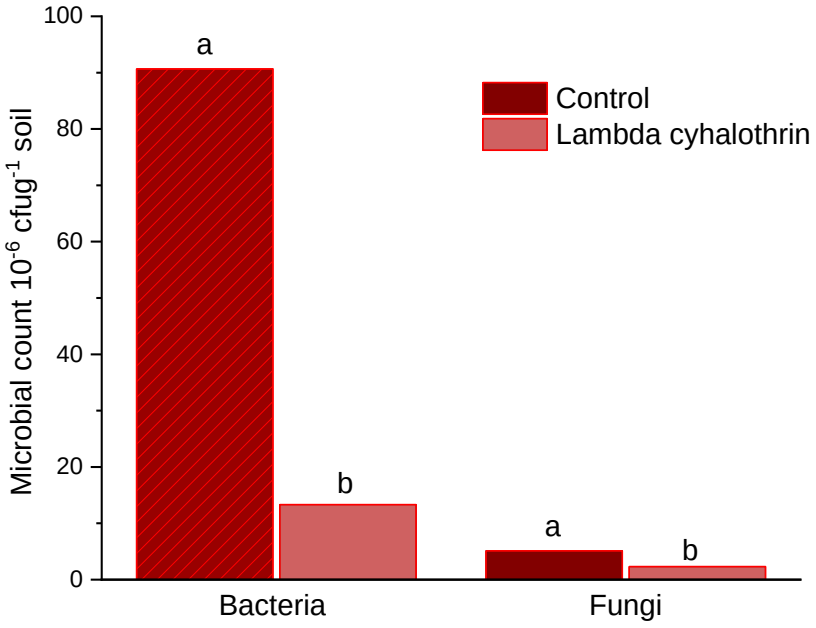


Figure. 1. *Microbial Count of Insecticide Contaminated Soil Samples.*

Biochemical characteristics and test results of the bacteria isolates obtained from the control and Lambda cyhalothrin contaminated soil samples. All the isolates were positive for Gram's staining, glucose and catalase while negative results were recorded for indole, gas and

methyl red (except isolate MGL2 which was positive) (Table 1.). Some isolates (WCL2(2), MGL1, MCC2 and MC2L) were cocci in shape, while the rest isolated were rod in shape. Some of the isolates (WCC2, GCC(1), WCL2, GCC2 and WMCL2) were negative for both lactose and sucrose sugar test while quiet a number (WCL2(2), MGL1, MCC2 and MC2L) recorded negative for motility and spore formation test to mention but a few of the tests. Furthermore, the identified bacteria from the Lambda cyhalothrin contaminated soil samples were identified as: *Staphylococcus aureus, Bacillus megaterium, Bacillus lentus, Bacillus femulus,* and *Bacillus cereus* respectively. These Bacillus species are the potential degraders and increase the phosphorous availability in soils (Fatima *et al.,* 2022).

Table. 1. *Biochemical Characteristics of Bacteria Isolates from insecticide contaminated soil samples.*

Isolate Code	Gram' Stain	Shape	Glc	Lac	Suc	Cat	SH	MR	VP	In	H_2S	Gas	Mot	SF	Cog	Ur	Cit	Identified Bacteria
GCC(1)	+	rod	+	-	-	+	+	-	+	-	+	-	+	+	NA	-	-	*Bacillus cereus*
GCC2	+	rod	+	-	-	+	-	-	+	-	-	-	+	+	NA	-	+	*Bacillus pumilus*
MCC2	+	cocci	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	*Staphylococcus aureus*
MCC(C)	+	rod	+	+	+	+	+	-	+	-	-		+	+	NA	+	-	*Bacillus lentus*
WCC2	+	rod	+	-	-	+	+	-	+	-	+	-	+	+	NA	-	-	*Bacillus cereus*
MGL1	+	cocci	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	*Staphylococcus aureus*
MGL2	+	rod	+	+	+	+	-	+	-	-	+	-	+	+	NA	+	+	*Bacillus megaterium*
WCL2	+	rod	+	+	+	+	+	-	+	-	-	-	+	+	NA	-	-	*Bacillus lentus*
WCL2(2)	+	cocci	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	*Staphylococcus aureus*
WCL2	+	rod	+	-	-	+	-	-	+	-	-	-	+	+	NA	-	+	*Bacillus femulus*
WMCL2	+	rod	+	-		+	+	-	+	-	+	-	+	+	NA	-	-	*Bacillus cereus*
MCC(C)	+	rod	+	+	+	+	+	-	+	-	-		+	+	NA	+	-	*Bacillus lentus*

Key: +: positive outcome; **-:** negative outcome, **Glc:** Glucose, **Lac:** Lactose, **Suc:** sucrose, **Cat:** Catalase, **SH:** Starch hydrolysis, **MR:** Methyl red, **VP:** Voges Prausker's test, **In:** Indole, **H_2S:** Hydrogen sulphide, **Mot:** Motility test, **SF:** Spore Formation, **Cog:** Coagulase test, **Ur:** Urease, **Cit:** Citrate test, **NA:** No Activity

Characteristics of the Fungal Isolates

Four isolates were identified. One of the isolates from sample was observed to form a white colony and powdery with rapid growth which was further revealed by the aid of a light microscope to be made up of small vesicles of head spore becoming multinucleated and conidiospores are moderately curved septae and hence identified to be *Fusarium* solain. Colonies with rapid growth, downy to powdery, initially white turning yellow, becomes deep brown to black on a plate which was further revealed by the aid of a light microscope to be

made up of non-branched conidiosphores with bulb and thick septal hyphae with conidiosphores in a chain and hence identified as *Aspergillus niger*. While the other isolate was observed to form colonies with rapid growth rate, wooly and pinkish red and achraceous brown which was further revealed by the aid of a light microscope to be made up of abundant aerial mycellium and hence identified as *Fusarium chlamidosporium.* Isolate appeared rapidly growing with downy to powdery, and yellow-green colonies. It was further identified to be *Aspergillus flavus* (Table 2.). This outcome is consistent with the findings of Al-Hawash *et al.,* (2018), who found that the majority of these species actively degrade or have tolerance to the majority of pesticides and herbicides used in agriculture.

Table. 2. *Characteristics of Fungal Isolates from insecticide contaminated soil samples.*

Morphology on Plate	Microscopy	Identified fungus
White colony and powdery with rapid growth	Small vesicle of head spore becoming multinucleated and conidiospores are moderately curved septae	*Fusarium solain*
Rapid growth, downy to powdery, initially white turning yellow, becomes deep brown to yet black	Non-branched conidiospores with bulb and thick septal hyphae with conidiospores in chain	*Aspergillus niger*
Rapid growth rate, wooly and pinkish red and achraceous brown	Abundant aerial mycellium	*Fusarium chlamidosporium*
Rapid growth, downy to powdery, and yellow green	Conidiosphore is non-branched with bulb and with oval greenish conidia	*Aspergillus flavus*

Percentage (%) inhibition of lambda cyhalothrin insecticide concentrations in diameter for bacterial isolates. Lambda exhibited an increasing zone of inhibition in a dose (concentration) dependent manner against all the test isolates (Table 3). Lambda cyhalothrin at 2.0ml, also recorded better zone of inhibition of (24.2±0.22 mm, 22.5±0.12mm and 22.1±0.61mm) against *Bacillus megaterium*, *Staphylococcus aureus* and *Bacillus lentus*, respectively. Distilled water served as the negative control with no diameter zones of inhibition on the test bacteria. The anti-bacterial activity of Lambda cyhalothrin shows the insecticide exhibited a considerable level of inhibition against all the test organisms with the highest activity exhibited. A relative trend was recorded for Lambda cyhalothrin which at the same concentration had the highest activity against *Bacillus megaterium* (24.15±0.22mm) and least against *Bacillus femulus* (14.96±0.07mm).

Table. 3. *Zones of Inhibition Lambda cyhalothrin on the test isolates.*

Diameter zone of inhibition (mm)				
Isolates	Ciprofloxacin	Lambda cyhalothrin		
	0.5ml	0.5ml	1.0ml	2.0ml
Bacillus cereus	20.10±0.23 [b]	9.39±0.11[c]	12.2±0.11[c]	19.8±0.02 [c]
Bacillus pumilus	22.04±0.24 [b]	3.16±0.10 [e]	8.31±0.10 [d]	16.5±0.15 [d]
Bacillus lentus	21.24±0.11 [b]	18.2±0.11 [a]	20.1±0.11 [a]	22.1±0.61 [b]
Bacillus megaterium	23.05±0.18 [a]	15.1±0.23 [b]	17.4±0.23 [b]	24.2±0.22 [a]
Bacillus femulus	18.61±0.11 [c]	7.20±0.11 [d]	12.2±0.11 [c]	14.9±0.07 [e]
Staphylococcus aureus	22.15±0.10 [b]	11.6±0.10 [c]	17.6±0.10 [b]	22.5±0.12 [b]

Values between experimental treatment group within a column bearing the same superscript are not significantly different at the 5% level (P<0.05).

Table. 4. *Effects of Lambda cyhalothrin on the Growth and Resistance of Fungi Isolates.*

Fungal isolate	Days	Lambda cyhalothrin		
		2.5ml	3.5ml	5.0ml
Aspergillus niger	Day 3	2.13	22.87	34.57
	Day 4	3.79	11.03	26.55
	Day 5	7.61	19.54	35.28
	Day 6	1.75	11.28	23.56
	Day 7	0.50	3.50	11.25
Aspergillus flavus	Day 3	4.89	25.21	41.92
	Day 4	7.22	23.19	35.61
	Day 5	8.56	22.18	34.71
	Day 6	2.48	18.42	29.66
	Day 7	0.00	13.61	18.56
Fusarium chlamidosporium	Day 3	11.32	41.15	58.61
	Day 4	12.11	37.68	55.84
	Day 5	9.47	34.94	49.72
	Day 6	8.62	29.56	43.64
	Day 7	4.21	22.84	35.81
Fusarium solain	Day 3	1.98	21.64	33.98
	Day 4	2.12	10.32	24.65
	Day 5	1.36	16.21	22.98
	Day 6	1.19	9.04	16.61
	Day 7	0.15	5.24	15.21

Lambda cyhatlothrin at different concentrations exhibited high potency in inhibiting mycelial growth of the fungal isolates. Also, the result below shows that there was a significant decrease in the percentage (%) inhibition of fungi growth by the insecticides as the

day proceeded (Table 4). Highest percentage inhibition (58.61%) at 5.0ml on day 3 and least percentage inhibition activity against fungal growth was recorded by Fusarium solain against the isolate. Fungal species showed different degree of sensitivity to different insecticides. This reduction of fungal colonies can adversely affect the pepper yield from 29 % to 9 % in the field (Georgieva and Karadzhova, 2020). In all the fungal isolates, the fungi toxicity effect of insecticide treatments on the growth and development of fungal species, therefore, ranked in the order of Fusarium chlamidosporium. > Aspergillus flavus > Aspergillus niger > Fusarium solain. In other studies, (Wilson et al., 2019), Lambda cyhalothrin was strongly associated with antifungal activity (Wilson et al., 2019). The result shows that there was a significant decrease in the % inhibition of the fungal growth by the insecticides as the days proceeded, and % inhibition increases with increasing concentration of the insecticide though the resistance zone of inhibition varied from one fungus to another. (Zain et al., 2018) showed a significant increase of fungal growth inhibition was observed with increasing insecticide concentration indicating a positive correlation between growth inhibition and treatment rates. Fusarium chlamidosporium showed a greater degree of growth inhibition than all other fungi evaluated at nearly all insecticide treatment dosages.

Conclusions

Lambda cyhalothrin induces 148% decrease in bacterial and population and 75.5% decrease in fungal population as compared to control. These results showed the stimulatory effect of the lamba cyhalothrin on microbiota. This inhibitory effect was observed on isolated bacteria and fungal species from the contaminated soil samples *Bacillus species* and *Staphylococcus aureus, Aspergillus* and, *Fusarium* species. The present study also revealed that higher doses of lamda cyhalothrin exhibited significant inhibitory effect against the microbiota, with . highest % inhibition (58.61%) against *Fusarium chlamidosporium* (*F. chlamidosporium*) and the extent of inhibition depended on the concentration applied. Conclusively the, study suggested that applications of Lambda cyhalothrin in agricultural plantations induced transient effects on the growth and development of both bacterial and fungal communities in soil. When applying insecticides to the soil, care should be taken to minimise their impact on the many beneficial soil organisms. Insecticides should therefore be screened to know their biocidal effect on soil organisms before their application so that damage to the soil ecosystem can be prevented. Further studies should clarify the pesticides' mode of action.

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