

Inhibition effect of cypermethrin insecticide on soil fungal and bacterial species

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ABSTRACT: Insecticides are commonly used in integrated weed management programs in agricultural plantations. Their usage not only controls the weed populations but also affects microbial populations especially bacteria and fungi in soil, and hence modify soil biochemical and biological processes critical for ecosystem functioning. This study determined the effect of inorganic insecticide (Cypermethrin) on soil fungal and bacterial populations. Soil samples were contaminated with Cypermethrin 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 ($90.67 \pm 0.06 \times 10^{-6}$ cfu/g) as well as fungal count ($5.11 \pm 0.01 \times 10^{-6}$ cfu/g) than the Cypermethrin treated soil samples ($22.33 \pm 0.02 \times 10^{-6}$ and $4.34 \pm 0.03 \times 10^{-6}$ cfu/g) for bacterial and fungal counts respectively, which significantly lower organic residues decomposition to release soil nutrients in soils. The isolated bacteria species from the soil samples were identified as *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus lentus*, *Bacillus megatarium* and *Bacillus femulus* while the isolated fungi were identified as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium chlamidosporium* and *Fusarium solain*. The *in vitro* anti-bacterial activity of the insecticide shows considerable level of inhibition against all the test organisms exhibiting its highest activity at 2.0 ml against *Bacillus megatarium* (29.16 ± 0.14 mm) and the least against *Staphylococcus aureus* (13.50 ± 0.03 mm). Cypermethrin caused significant inhibition of fungal growth at Day 3, recorded the highest % inhibition fungal growth (56.14%) against *Fusarium chlamidosporium* and least % inhibition (16.12%) was recorded against *Fusarium solain*. This study suggested that insecticide applications in agricultural plantations significantly reduced the mineralization rate as well as growth and development of both bacterial and fungal species in soil.

Keywords: Bacteria, cypermethrin, fungi, insecticide, microbes, soil.

INTRODUCTION

In many developing nations, current agricultural methods follow unsustainable practices which have resulted in a huge amount of toxic effluents being emitted directly or indirectly into the soil, 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 have adversely affected beneficial soil (micro) biota (Radhika and Kannahi, 2019). Cypermethrin is a synthetic pesticide

usually used in agriculture, forestry, horticulture and urban regions to control insects and pests of cotton, fruits and vegetables such as caterpillars, aphids and grasshoppers (Kumar and Omkar, 2018). The biological and chemical properties of soil are very important in the biogeochemical cycles of nutrients, enzymes produced by soil microorganisms being responsible with biochemical transformations. Assessment of the effect of insecticides such as Cypermethrin on the functionality of an ecosystem

can be achieved based on metabolic activity and microbial biomass determinations (Chen *et al.*, 2020).

Agricultural expansion and indiscriminate use of insecticides over the years has often led to a negative effect on the soil ecosystem causing heavy population damage, toxicity and soil pollution. The insecticides applied to the agricultural field are expected to only be toxic to the target organisms, biodegradable and eco-friendly to some extent. But unfortunately, most of the insecticides are non-specific and kill organisms that are harmless and very useful to the various ecosystems. Chemical control methods associated with the use of chemicals should be considered only as a complementary. In selecting a pesticide and the appropriate formulation, consideration should be given to its biological effectiveness (including residual activity where appropriate) against the pest concerned, the susceptibility of the target organism, the methods of application, its safety to humans, and its toxicity to non-target organisms (WHO, 2018; Rodrigues *et al.*, 2021).

Cypermethrin are one of the most prominent Pyrethroids used as insecticides (Lukaszewicz *et al.*, 2018). Cypermethrin is a potential toxic pollutant that directly threatens the aquatic ecosystems and environment. Like other insecticides, the widespread use of Cypermethrin has been associated with adverse effects on non-target species. Consistent with its lipophilic nature, Cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain (Gulhan *et al.*, 2017; Aldemir *et al.*, 2019; Kumar *et al.*, 2009a,b). The half-life (the amount of time required for half of what was originally applied to break down or move away from the test site) for cypermethrin in a typical fertile soil is between 4 and 12 days (WHO, 1989; Kaur and Balomajumders, 2020).

This study investigates the effect of Cypermethrin at field recommended dosage and how it adversely affects the abundance of fungi and bacteria present in the soil and identify some microorganisms that are tolerable to this insecticide toxicity. This will help in ecological sound strategy for sustaining soil health and advancing food security without degrading soil biodiversity. In soils, microbes play a pivotal role in cycling nutrients essential for life (Bergmann *et al.*, 2018). This will also help in reducing Cypermethrin poisoning because of its long persistence in soil and currently there was no available antidote for Cypermethrin poisoning (Liang *et al.*, 2023).

MATERIALS AND METHODS

This research work was conducted at Usmanu Danfodiyo Fadama Teaching and Research Farm and Microbiology Laboratory of Usmanu Danfodiyo University Campus, Sokoto State, located on latitude 13°03'44" to 13°08'46"N and longitude 5°14'02" to 5°18'01"E. The State falls between Sudan savanna agro ecological zone of north-

western Nigeria, at altitude of about 315 m 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. Annual rainfall amount ranging from 390 to 790 mm (NIMET, 2022).

Soil samples were collected 2 weeks after treatment application from the field (Cypermethrin and control plots) at a depth of 0-10 cm. The samples were collected and placed in an ice pack container and transported immediately to the Microbiology Laboratory for analysis.

Preparation of culture media for fungi

The culture media used was prepared in sterilized material according to M38-A2 protocol. Sabouraud dextrose Agar (7.8 grams) and antibiotic (streptomycin) was suspended in 200 ml of distilled water, heated with frequent agitation on a hot plate and boiled for one minute to completely dissolve the medium, autoclaved at 121°C for 15 minutes, and cooled to 45-50°C. The media was then dispensed into petri dishes and allowed to solidify for some hours.

Nutrient Agar powder (5.6 grams) was suspended in 200 ml of distilled water, mixed and heated with frequent agitation on a hot plate until dissolved, it was then sterilized by autoclaving at 121°C for 15 minutes and cooled to 45°C. The media was then dispensed into petri dishes and allowed to solidify for some minutes.

Isolation and identification of bacterial isolates

A series of test tubes was prepared for the isolation of bacteria and fungi. 9 ml of sterile distilled water was put into each of the test tube, one gram of the soil sample was added to give a dilution of 10^{-1} . The contents were shaken properly and 1 ml of the solution was added to the next test tube containing 9 ml of distilled water to make a concentration of 10^{-2} . The serial dilution was made up to 10^{-6} dilution for the soil sample. 0.1 ml 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 was obtained according to biological standards (Cheesbrough, 2010).

Gram staining

A well-prepared smear of bacterial isolate was made on sterile microscope slide using a heat flamed wire loop and

air dried. This procedure is done according to the method described by Smith and Hussey (2005). The air-dried smear on the microscope was heated over a gentle flame. The microscope slides were then covered with crystal violet stains (primary stains) for about 1 minute and then was rinsed with a gentle jet of flowing water. The smear was then treated with a few drops of gram's iodine (Lugol's iodine) and allowed to act for 1 minute. The slides were rinsed off with distilled water and decolorized in absolute ethyl alcohol for about 30 seconds. After this, 2-3 drops of safranin stain were added and allowed to stay for 1 minute. The stain was then rinsed off with distilled water and the slide air dried. The slides after drying was examined using low power with the aid of oil immersion and viewed under microscope using x100 objective lens. At the end of it all, gram positive bacterial isolates will appear purple, while gram negative isolates will appear pinkish (Cheesbrough, 2010). Biochemical tests such as catalase, citrate utilization, urease, oxidase and indole tests were all carried out on the isolates sub-cultured on nutrient agar plates (Cheesbrough, 2010).

Isolation and identification of fungal isolates

After serial dilution, Sabouraud Dextrose Agar plates was set with 0.1 ml aliquot from the 10^{-6} dilution of soil sample using the spread plate technique. The plate was then incubated at room temperature for 48 hours. The plates were examined for fungal growth after every 24 hours for 5 days. Individual colonies were then sub cultured in Sabouraud Dextrose Agar until pure cultures were isolated.

Determination of the antimicrobial activity of cypermethrin on bacterial isolates: Assay of antimicrobial activity

Agar well diffusion method was used to evaluate the antimicrobial activity of the insecticides. The subcultured bacteria isolates were smeared on a sterile petri plate containing nutrient agar using a swab stick. The plates were kept on a flat bench for 30 minutes to solidify. Four wells (4 mm) deep were made on the agar using a sterile 6 mm diameter cork-borer. Then 0.5 ml, 1.0 ml and 2.0 ml of the insecticides were pipetted into the wells using a micro pipette, 0.5 ml 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°C for 24 hours. The zone diameter of inhibition was recorded.

Determination of the effects of insecticides *in vitro* on radial growth of fungal isolates

The screening for resistance was done by subjecting

purified fungi to different concentrations of Cypermethrin incorporated into the growth medium. The molten SDA was prepared using the manufacturer's guideline and was modified with three concentrations (2.5 ml, 3.5 ml and 5.0 ml) and aseptically inoculated at the center of the plate with each of the fungi isolates. The control treatment was investigated without Cypermethrin application. Each of these treatments was labeled and set up in duplicate. The resistance of each of the fungus to each treatment was determined by measuring the colony extension of each fungus on each of the plate by drawing two perpendicular lines which meets at a right angle at the center of the plate. The plates are then incubated at $27\pm 2^\circ\text{C}$ for 7 days. On each day, the diameter of the extension was measured using a meter rule. Resistance of each fungus to the Cypermethrin was recorded as percentage (%) radial growth inhibition and calculated using the formula in equation 1;

$$\text{The percentage (\%) inhibition} = \frac{R_1 - R_2}{R_1} \times 100 \dots\dots\dots 1$$

Where R_1 is growth in the control and R_2 is the growth in the treatment

Data obtained was 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

Microbial enumeration of insecticide contaminated soil samples

The microbial population of the soil samples as shown in Table 1, soil samples had substantial amount of microbial growth with the control (untreated soil sample) having the highest bacterial ($90.67 \pm 0.06 \times 10^{-6}$ cfu/g) and fungal load ($5.11 \pm 0.01 \times 10^{-6}$ cfu/g) as compared to the control.

Micro and macroscopic identities and characteristics of fungal isolates from soil samples

The macroscopic and microscopic characteristics of the fungal isolates is contained in Table 2. Four isolates were obtained in total. One of the isolates was observed to form colonies with rapid growth, downy to powdery, initially white turning yellow, becomes deep brown to yet black on plate which was further revealed by the aid of a light microscope to be made up of non-branched conidiospores with bulb and thick septal hyphae with conidiospores in chain and hence identified using truly classical method as *Aspergillus niger*. While the other isolate was observed to form colonies with rapid growth

Table 1. Microbial count of insecticide contaminated soil samples.

Sample code	Bacteria ($\times 10^{-6}$ cfu/g)	Fungi ($\times 10^{-6}$ cfu/g)
Control	90.67 \pm 0.06 ^b	5.11 \pm 0.01 ^b
Cypermethrin	22.33 \pm 0.02 ^a	4.34 \pm 0.03 ^a

Values are in \pm mean S.E. (S.E = Standard error of Mean). Values between experimental treatment group within a column bearing the same superscript are not significantly different at the 5% level ($p < 0.05$), cfu/g: colony forming unit per gram of soil.

Table 2. Microscopic and macroscopic identities, and 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	<i>Fusarium solani</i>
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	<i>Aspergillus niger</i>
Rapid growth rate, wooly and pinkish red and achraceous brown	Abundant aerial mycellium	<i>Fusarium chlamidosporium</i>
Rapid growth, downy to powdery, and yellow green	Conidiospore is non-branched with bulb and with oval greenish conidia	<i>Aspergillus flavus</i>

Table 3. Zones of inhibition cypermethrin on the test isolates.

Isolates	Diameter zone of inhibition (mm)			
	Ciprofloxacin (0.5 mg/ml)	Cypermethrin		
		0.5 ml	1.0 ml	2.0 ml
<i>Bacillus cereus</i>	20.10 \pm 0.23 ^b	7.14 \pm 0.10 ^{ab}	9.21 \pm 0.10 ^a	17.78 \pm 1.03 ^b
<i>Bacillus pumilus</i>	22.04 \pm 0.24 ^c	5.34 \pm 0.05 ^a	12.11 \pm 0.03 ^b	22.10 \pm 0.03 ^c
<i>Bacillus lentus</i>	21.24 \pm 0.11 ^c	15.19 \pm 0.01 ^c	16.21 \pm 0.02 ^c	21.11 \pm 0.23 ^c
<i>Bacillus megatarium</i>	23.05 \pm 0.18 ^d	17.42 \pm 0.04 ^c	22.31 \pm 0.05 ^d	29.16 \pm 0.14 ^d
<i>Bacillus femulus</i>	18.61 \pm 0.11 ^a	9.38 \pm 0.15 ^b	13.31 \pm 0.01 ^b	16.48 \pm 0.11 ^b
<i>Staphylococcus aureus</i>	22.15 \pm 0.10 ^c	4.31 \pm 0.21 ^a	7.22 \pm 0.40 ^a	13.50 \pm 0.03 ^a

Values are in \pm mean S.E. (S.E = Standard error of Mean). Values between experimental treatment group within a column bearing the same superscript are not significantly different at the 5% level ($p < 0.05$).

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 to be *Fusarium chlamidosporium*. Another isolate appeared rapidly growing with downy to powdery, and yellow green colonies. It was further identified to be *Aspergillus flavus* and another one was observed to form white colony and powdery with rapid growth which was further revealed by the aid of a light microscope to be made up of small vesicle of head spore becoming multinucleated and conidiospores are moderately curved septae and hence identified to be *Fusarium solani*.

Antibacterial activities of insecticides on test isolates

The insecticides in this study exhibited an increasing zone

of inhibition in a dose (concentration) dependent manner against all the test isolates (Table 3). Cypermethrin recorded a high diameter zones of inhibition against *Bacillus megatarium*, *Bacillus pumilus*, and *Bacillus lentus* at 2.0 ml with zones of inhibition of 29.16 \pm 0.14 mm, 22.10 \pm 0.03 mm and 21.11 \pm 0.23 mm, respectively. Distilled water served as the negative control with no diameter zones of inhibition on the test bacteria.

Percentage (%) inhibition of Cypermethrin insecticide concentrations in diameter for fungal isolates

The results of this study as shown in Table 4 expresses that at different insecticide concentration, they exhibited high potency in inhibiting mycelial growth of the fungal isolates. Also, the result shows that there was a decrease

Table 4. Effects of cypermethrin on the growth and resistance of fungi isolates.

Fungal isolate	Days	Cypermethrin		
		2.5ml	3.5ml	5.0ml
<i>Aspergillus niger</i>	Day 3	1.06	13.29	18.61
	Day 4	2.07	3.79	8.96
	Day 5	2.03	4.32	9.90
	Day 6	0.50	1.00	3.00
	Day 7	0.10	0.25	0.33
<i>Aspergillus flavus</i>	Day 3	7.45	34.57	50.00
	Day 4	8.96	28.62	39.66
	Day 5	9.65	27.16	45.68
	Day 6	3.76	24.06	36.84
	Day 7	0.10	22.00	31.25
<i>Fusarium chlamidosporium</i>	Day 3	9.21	36.12	56.14
	Day 4	8.41	33.91	49.21
	Day 5	8.88	28.65	44.24
	Day 6	5.26	26.76	39.61
	Day 7	1.82	23.14	34.73
<i>Fusarium solan</i>	Day 3	0.89	9.87	16.12
	Day 4	1.35	5.79	10.67
	Day 5	1.11	4.86	8.25
	Day 6	0.21	1.21	2.54
	Day 7	0.10	0.67	1.12

in the percentage (%) inhibition of the fungi growth by the insecticide as the day proceeds. The insecticide (Cypermethrin) exhibits the highest percentage inhibition (56.14%) at 5.0 ml on day 3 against *Fusarium chlamidosporium*, and the least percentage inhibition activity against fungal growth was recorded by *Fusarium solan*. Fungal species showed different degree of sensitivity. In all the fungal isolates, the fungi toxicity effect of Cypermethrin on the growth and development of fungal species, therefore, ranked in the order of *Fusarium chlamidosporium* > *Aspergillus flavus* > *Aspergillus niger* > *Fusarium solan*

DISCUSSION

Microbial enumeration of colony from soil sample

In the present investigation, the microbial population of the two soil portions (Cypermethrin and control) obtained after contamination with the insecticides were highly populated with microbes. This may be due to the fact that microorganisms are ubiquitous (Rinnan *et al.*, 2020). More so, the contaminated soil sample when plated on the appropriate medium to assess the tolerant bacteria isolate

count and tolerant fungi count indicated a higher bacteria population than the fungal community count. This implies that there were active bacteria degraders/tolerant of the insecticides in the soil samples. This result is in agreement with the report of Sujadi *et al.* (2019) who also recorded high bacteria population especially in oil agar medium when determining the biodegradation of glyphosphate using *Rhodococcus erythropolis*. It is worthy of note that soil portion treated with Cypermethrin had significant lower microbial growth population than the untreated soil samples. This could be due to the presence of organisms with unique traits in degrading Cypermethrin better. This result was within the range and is in agreement with the study of Abioye *et al.* (2017) who determined the biodegradation of insecticides in soil with organic waste amendment for 84 days.

Characteristics and identity of microbial isolates

In the present study, the biochemical characteristics and identity of the bacteria isolates obtained from the Cypermethrin contaminated soil portions were rod and coccus shaped. All the isolates were positive for Gram's staining, catalase, and glucose sugar test, and negative for

indole and gas test. The isolates were *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus lentus*, *Bacillus megatarium*, and *Bacillus femulus*. These results are in agreement with previous studies (Porwal *et al.*, 2009; Logan and Vos 2015) that have identified similar isolates as good potential insecticide degraders/tolerant and based on similar biochemical testing procedures. The present study, revealed likewise the microscopic and macroscopic characteristics and identity of fungal isolates obtained from Cypermethrin contaminated soil portions. The fungal isolates possessed structures which include hyphae, mycelium, conidiophores, conidia blastophores, septae and were suspected to be *Aspergillus flavus*, *Aspergillus niger*, *Fusarium chlamidosporium* and *Fusarium solain*. This result agrees with the report of Al-Hawash *et al.* (2018) who identified most of these species to be active degraders/tolerance of most agriculturally used insecticides and herbicides.

Microbial-toxicity effect of Cypermethrin on bacterial and fungal population growth

The *in vitro* anti-bacterial activity of cypermethrin shows the insecticide exhibited considerable level of inhibition against all the test organisms with the highest activity exhibited by cypermethrin at 2.0 ml was recorded against *Bacillus megatarium* (29.16±0.14 mm) followed by *Bacillus pumilus* (22.10±0.03 mm) and the least against *Staphylococcus aureus* (13.50±0.03 mm) (Table 3). This result corroborates the report of Maqbul *et al.* (2022) who attributed the activity of insecticides against some pathogenic organisms to bactericidal potency. The results of the percentage inhibition also prove the efficacy of the insecticides over fungal isolates used in this study. Comparing the results from each insectides at Day 3 at 5.0 ml of the insecticides against each fungal isolates, Cypermethrin recorded (56.14%) against *Fusarium chlamidosporium*, and the least percentage inhibition was recorded against *Fusarium solain*. The result shows that there was a significant decrease in the percentage inhibition of the fungal growth by the insecticides as the days proceeds, and percentage inhibition increases with increasing concentration of the insecticide though the resistance zone of inhibition varied from one fungus to another. Earlier, Zain *et al.* (2018) showed that significant increase of fungal growth inhibition was observed with increasing insecticide concentration indicating a positive correlation between growth inhibition and treatment rates. In this study, growth inhibition was pronounced on *Fusarium chlamidosporium* than all fungi screened at almost all the concentrations of the insecticide treatments.

Conclusion

In conclusion, the contaminated soil sample recorded a

higher bacteria count than the fungal count. The isolated bacteria specie from the contaminated soil samples were of the *Bacillus species* and *Staphylococcus aureus*, while the isolated fungi were of the *Aspergillus* and *Fusarium* species. The present study also revealed that the insecticides exhibited significant antimicrobial activity against the test isolates and the insecticides had a higher fungal inhibition than bacteria, and the extent of inhibition depended on type of insecticide, and concentration applied. The antibacterial and the percentage (%) inhibition activity of the insecticides showed a considerable cytotoxic activity against all the test organisms. This study suggested that insecticide applications in agricultural plantations induced transient effects on the growth and development of both the bacterial and fungal community in soil. The antimicrobial potential of the insecticides revealed in this study can significantly lower the bacterial and fungal diversity which further affect organic residue decomposition and nutrients mineralization in soils. Insecticide application to the soil should take into consideration of their effect on the numerous beneficial soil organisms. Insecticide should therefore be screened to know their biocidal effect on soil organisms before their application in order not to affect functional diversity and soil quality both physical, chemical and biological properties.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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