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Evaluation of cypermethrin insecticides on the growth of some selected soil bacteria isolated from Makurdi, Middle Belt, Nigeria

Ebah Esther Eneyi*, Ehoda Christian Ochofie, Emmanuel Olumuyiwa Onifade and Ikala Ruth Ohie

Department of Microbiology, College of Sciences, Federal University of Agriculture Makurdi, Benue State, Nigeria.

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In the present investigation, a total of 30 soil samples were collected from five different sites in Makurdi metropolis to investigate the effect of cypermethrin on some soil bacteria. Physicochemical properties of the soil samples from various sites were determined using standard procedures. Some selected soil bacteria such as *Escherichia coli, Klebsiella* spp*., Bacillus* spp*., Staphylococcus* spp. and *Proteus* spp. were isolated by the use of serial dilution and pour plating method. The percentage prevalence of Isolates shows that a total of 10 bacteria were isolated with samples from high level accounting for 50% of the isolates, North bank 30% and Wurukum samples 20%. *Klebsiella* spp. accounted for 30% of the isolates, *Escherichia coli* 10%, *Staphylococcus* and *Bacillus* spp. accounted for 20%. The resistance pattern of the isolates at different concentration (1-3%) of cypermethrin showed that all the isolates could grow in the presence of cypermethrin at 1% except *Bacillus* spp. However at 2%, only *Klebsiella* spp*.* grew; *Staphylococcus* spp., *Escherichia coli* and *Bacillus* spp. were inhibited and therefore showed no growth. These findings revealed that cypermethrin had effects on the soil bacteria at higher concentration inhibiting their growth but tolerated at lower concentration. Therefore, indiscriminate use of insecticides should be avoided and more eco-friendly means of pest control should be employed to reduce the negative impact of synthetic pesticides on beneficial soil organisms.

Key words: Cypermethrin, resistance, pesticide, biomass, eco-friendly.

INTRODUCTION

Soil is the uppermost layer of the earth; it is naturally occurring physical covering of the earth's crust. The earth crust includes solids, liquids and gases. So soil is made up of the combination of geological parent materials, glaciers among others (Azhar et al., 2013). Diverse species of bacteria, archaea, fungi, insects, annelid and some invertebrates as well as plants and algae inhabit the soils. Thus, soils are the foundations of all terrestrial ecosystems and harbor vast microbial diversity. According to the previous findings, the total weight mass of organisms below temperate grassland can exceed 45 tons per hectare, equaling or exceeding above ground biomass out of which bacteria are present in great numbers with archae 10-fold less (Ritz et al., 2003). An

*Corresponding author. E-mail: ebahesther23@gmail.com.

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estimated number of bacteria per gram of soil range from 2000 to 18000; while fungi on the other hand contribute to a great extent the largest portion of the population of soil microbiota (Azhar et al., 2013; Aislabie and Deslippe, 2013).

Microorganisms have ability to grow in the presence of many pesticides. Thus, they play quite a number of roles in the soil. Nonetheless, haphazard application of pesticides often leads to environmental hazard to soil microbiota as well as physico-chemical properties of the soil (Pal et al*.,* 2006). The use of pesticides is no longer new in controlling pest, especially insect pest in agriculture. The use of these pesticides in agricultural soils usually has adverse effects on soil microbiota metabolic activities. This also influences the biological activities especially biochemical reaction of the soil flora and fauna under the influence of the pesticides (Madakka and Rngaswamy, 2009; Baxter and Cummings, 2008; Li et al., 2008; Mahia et al., 2008). So, low concentration of these chemical (pesticides) can still affect the soil microbiota. The effects of insecticides can decrease the soil microbes quantitative by altering the soil biochemical activity, and qualitative by decreasing the microbial population in the soil (Cycon et al., 2006, 2010; Vig et al., 2008).

Soil flora and fauna is difficult to determine because many variables such as habitat, soil structure, organic and inorganic composition, texture, pH and temperature should be taken into consideration despite the effect of the soil pesticide or insecticide on the soil (Monkeidje et al., 2002; Beulke et al., 2004). With regards to the application of pesticides in agricultural soil, there is tendency for transformation in biological and non-biological processes whereby one or more products are eventually formed. These transformation products are carried out by different mechanisms physical, chemical and biological agents in which microbes have an important role to play. Even though mechanisms of pesticide degradation in soil may be either abiotic or biotic in nature; degradation by living organisms (biotic) has received much attention (Hafez and Thiemann, 2003).

Dated back to the1980s, cypermethrin had been used in agricultural set up. It is a pesticide which has brought solution in controlling many pests affecting plants. Cypermethrin is a synthetic pesticide usually used in agriculture, forestry, horticulture and urban regions to control insects and pests of cotton, fruit and vegetables (Akbar et al., 2015; Tallur et al., 2008). It has been so useful in the control of pests in stores, warehouses, industrial buildings, termites in houses, laboratories, treatment of ectoparasitic infestations of cattle and other livestock, food processing plants, to kill cockroaches, fleas and insects on cotton and lettuce. The chemical name of cypermethrin is [Cyano-(3-phenoxyphenyl) methyl] 3-(2, 2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate and its molecular weight is 416.30 g/mol (EPA, 1992). Cypermethrin has poisonous effects at low concentrations of about 10 µg/L in an aquatic habitat (Vinodhini and Narayanan, 2008). Therefore, physico-chemical properties of soil are based on the environment of the soil which varies from 14.6 to 76.2 days (half-life) (USDA, 1995). Studies have shown that due to the non-polar nature and low water solubility of cypermethrin, it is usually adsorbed on to soil surface (Liu et al., 2005).

Cypermethrin has effects on vertebrates and invertebrates nervous system by producing hyper excitable state and damaging the voltage dependent sodium channels. the degrading microorganisms for Cypermethrin (CY) were *Pseudomonas aeruginosa*, *Klebsiella* spp*., Bacillus subtilis, Rhodococcus* spp., *Micrococcus* spp*., Serratia* spp. arebacteria, while fungi consist of *Aspergillus terreua, Monilochaetes and Fusarium, Alcaligenes* spp*.* (Deng et al., 2015).

Further investigation revealed the kinetic parameters of the degradation of CY by *Aspergillus terreua* under the effects of different factors. The particular strain could degrade CY validly over a range of temperatures (25-35°C) and pH (6.0-8.0); so, pesticide-degrading bacteria had advantages in the environment due to the favorable temperature and pH scale (Deng et al., 2015). This research work evaluates the effect of cypermethrin on the growth of some selected bacterial species of agricultural importance isolated from soil.

MATERIALS AND METHODS

Study area

This research work was confined to Makurdi, the capital city of Benue state. Makurdi is located on Latitude 7°44°N and Longitude 8°35°E in the tropical guinea savanna flood plain of River Benue, North central Nigeria. It has a population of about 500,797 heads as at 2007. The town has an annual rainfall of 1090mm with dry and rainy seasons with temperature ranging between 27°C to 35°C, a subtropical humid climate (Abah, 2012).

Sample collection

Soil samples were collected from 5 different farm sites in five areas in Makurdi metropolis. The area includes High level, North bank, Wurukum, Modern market and Wadata. Six samples each were collected randomly from each site in those areas making a total of 30 samples. The samples were collected using a sterile carpel (spatula) and packed into sterile polythene bags and transported to the Biological Science Laboratory of the Federal University of Agriculture Makurdi for investigation. The history of pesticide application on these soils was unknown.

Preparation of culture media

The culture media used were prepared in sterilized material according to protocols and to the manufacturer's instruction. Nutrient Agar Medium (28.0 grams was suspended in 1000 ml purified or distilled water, it was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min and Cooled to 45-50°C, it was mixed well before it was poured into sterile Petri plates); Eosin Methyline

Blue Agar (37.5 grams of the medium was suspended in 1 L of purified water, autoclaved at 121°C for 15 min and also Cooled to 45-50°C before it was gently poured into the Petri dishes, MacCkonkey Agar medium (49.53 g of the dehydrated medium was suspended in 1 L of purified or distilled water, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min and cooled to 45-50°C, mixed well before it was poured into sterile Petri plates) and Mannitol Salt agar (111.025 g of the medium was suspended in 1 L of distilled water, autoclaved at 121°C for 15-20 min, and cooled to 45-50°C before it was poured into petri dishes).

Isolation and identification of bacteria isolates

One gram of the composite soil sample was suspended in 9 ml sterile water and shaken for 5 min, 1 ml of the soil suspension was serially diluted (till 10^{-7}). Each elution was plated into already prepared Nutrient Agar Media (NA), Eosin Methylene Blue Media, MacConkey agar, Mannitol salt Agar and incubated at 27°C for 24-72 h.

Individual colonies were then sub cultured in Nutrient Agar plates and the other prepared culture media until pure cultures were isolated and then stored and maintained in nutrient agar 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

A well prepared smear of each 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 then heat fixed over a gentle flame. The microscope slides were then covered with crystal violet stains (primary stains) for about 1 min and then washed 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 min. It serves as mordant to a dye-iodine complex in the cytoplasm of cells. The slides were again washed with a gentle jet of water and decolorized in absolute ethyl alcohol for about 30 s. After these, 2-3 drops of safranin stain was added and allowed to stay for 1 min. The stain was then washed in a gentle jet of water and the slide air dried. The slides after drying were examined using low power and then finally with oil immersion (x100) lens of the microscope. At the end of it all, gram positive bacterial isolates stained and appeared purple, while gram negative isolates appeared pinkish (Cheesbrough, 2010).

Biochemical test

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. Biochemical tests were all carried out on the isolates subcultured on nutrient agar plates.

Catalase test: A loop full of each single colony of the culture test isolates were picked with a sterile wire loop and emulsified in a drop of normal saline on a clean slide and 1-2 drops of hydrogen peroxide was added to each smear on the slides. The presence of air bubbles (effervescence) indicates negative results (Cheesbrough, 2010).

Indole test: The test organism was inoculated into 3 ml of sterile peptone water in a bijou bottle and inoculated at 31°C for 48 h. Indole production was tested by the addition of 0.5 ml kovac's reagent to broth culture and allowed to stand for 30 s. Appearance of bright pink color at the top layer of the broth indicates a positive result, while yellow color at the top of the broth indicates a negative result (Cheesbrough, 2010).

Oxidase test: A piece of filter paper was placed on a clean petri dish and three drops of freshly prepared oxidase reagent was added. Using a piece of sterile stick, a colony of the test organism was collected and smeared on the filter paper. The appearance of blue-purple color within 10 s confirms the organism to be *Pseudomonas aeruginosa* (Cheesbrough, 2010).

Urease test: The broth medium is inoculated with a loop full of pure culture of the test organism. The surface of the agar slants is streaked with the test organism. The cup of the test tube is left loosely and the tube inoculated at 35°C in ambient air for 18-24 h. A positive test result is indicated if the color of the slant changed from light orange to magenta, while no color change indicates a negative test (Cheesbrough, 2010).

Citrate test: The medium was inoculated with the aid of a wire loop by streaking the slant medium back and forth with a light inoculum picked from the center of a well isolated colony. The medium was then incubated aerobically at 35 - 37°C for 18 - 24 h and observed for color change from green to intense blue along the slant, while no growth and no color change indicates a negative result (Cheesbrough, 2010).

Determination of the effect of cypermethrin on bacteria isolates

In the determination of the effect of cypermethrin on bacteria isolates, nutrient agar medium was used. Nutrient agar media were prepared with and without the addition of cypermethrin. A control was obtained by inoculating bacteria isolates aseptically on nutrient agar medium without cypermethrin by streaking, while the effect on bacterial isolates was determined by streaking each isolate on nutrient agar plates containing cypermethrin at different concentrations (1-3%). After inoculation, plates were incubated at room temperature for 24 h. Cypermethrin resistant and susceptible isolates at the different concentrations were identified by their growth as compared with control. The minimum inhibitory concentration of each isolate was identified and recorded (Cheesbrough, 2010).

Soil analysis

Physicochemical properties of the soil such as pH, soil temperature, total organic matter and organic carbon were determined. Soil pH was determined using pH meter method. Soil temperature was determined by inserting the thermometer inside the soil on field and reading taken as soon as the thermometer became stable after about 3-5 min. Organic matter and organic carbon was determined using loss of ignition method; 10 g of sieved soil sample was transferred into an ashing vessel and placed in a drying oven set at 105°C. It was allowed to dry 4 h, removed and cooled in a dry atmosphere. The dried soil sample was measured to the nearest 0.01 g. The ashing bottle with soil was placed into a muffle furnace and temperature brought to 400°C and ashed in the furnace for 4 h. The vessel was removed from the furnace and allowed to cool. Soil was weighed again to the nearest 0.01 g. Percentage of organic matter is given as:

W_1-W_2/W_1

Table 1. Physicochemical parameters of soil.

Sample	pH	Temperature (°C)	Organic matter (%)	Organic carbon (%)
High level	5.1	20.0	11.90	6.5
Wurukum	4.9	24.0	11.95	6.95
North bank	4.7	28.0	12.0	6.95
Mordern market	5.0	24	14.3	12.35
Wadata	4.7	24	12.9	7.45

Table 2. Total viable count of bacteria.

Locations	Total viable count, TVC ($X10^5$)
High Level	0.000 ± 0.000^d
Wurukum	234.850 ± 0.015^b
North bank	0.000 ± 0.000^d
Mordern market	267.375 ± 0.125^a
Wadata	227.480 ± 0.020^c
P-Value	<0.01

Table 3. Total coliform count of bacteria.

Locations	Total coliform count, TCC ($\times10^5$)
High Level	5.510 ± 0.010^b
Wurukum	1.510 ± 0.010^c
North bank	0.000 ± 0.000^d
Mordern market	1.535 ± 0.035^c
Wadata	20.650 ± 0.150^a
P-value	<0.01

Where;
W_1 = Weight of soil at 105°C
W_2 = Weight of soil at 400°C
% of Organic Carbon = % Organic matter × 0.58 (FAO, 2008).

Statistical analysis

Statistical analysis was carried out using version 20.00 of the SPSS software. Descriptive statistical tools such as the mean and standard deviation were computed alongside the t- test at P≤0.05 (Pallant, 2007).

RESULTS AND DISCUSSION

From the present study Table 1 shows the physicochemical parameters of soil samples collected from different sample sites. Samples from Wadata had the highest organic matter content (12.90%), slightly followed by modern market (14.30%), Northbank (12.00%), Wurukum (11.95%) and High level (11.90%). The soil samples had pH values ranging from 4.7 to 5.1. They were all acidic in nature. Modern market samples had the highest organic carbon content (12.35%) followed by Wadata (7.45%), Wurukum, and North bank samples had 6.95% each; while High level had the least (6.5%). The temperature ranged from 20 - 28°C across the five locations.

The total viable count (counts from nutrient agar plates) shows that samples from Modern Market had the highest count with about $267.375\pm0.125^a \times10^5$, followed by Wurukum with $234.850\pm0.015^b \times10^5$ and Wadata with $227.480\pm0.020^c \times 10^5$. Samples from High level and North Bank however showed no growth. Means on the same column with different superscript are statistically significant ($p<0.05$) as shown in Table 2. The variation is shown in the result across the five locations.

The total coliform count of samples as presented in Table 3 shows that samples from Wadata had the highest coliform count (20.650 ± 0.150^a) $\times10^5$ followed by samples from High with a count of (5.510 ± 0.010^b) $\times10^5$. Samples from Wurukum and Modern Market had counts of (1.535 ± 0.035^c) $\times 10^5$ each. Means on the same column

Table 4. Cultural, morphological and biochemical characteristics of isolates.

S/N	Colony color	Colony shape	Morphology	Gram reaction	Catalase test	Citrate test	Urease test	Indole test	Oxidase test	Organism identified
1	Cream	Circular	Cocci	+	+	+	-	-	-	*Staphylococcus* spp.
2	Green metalic sheen	Circular	Rod	-	+	-	-	+	-	*Escherichia coli*
3	White	Irregular	Rod	+	+	+	-	-	-	*Bacillus* spp.
4	Mucoid pink	Irregular	Rod	-	+	+	+	-	-	*Klebsiella* spp.
5	Pale	Circular	Rod	-	+	+	+	-	-	*Proteus* spp.

+ = Positive; - = Negative.

Table 5. Percentage prevalence of bacteria isolates.

Locations	Bacteria				
	***Klebsiella* spp.**	***Escherichia coli***	***Staphylococcus* spp.**	***Proteus* spp.**	***Bacillus* spp.**
High level	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
Wurukum	10.00±0.00	0.00±0.00	0.00±0.00	10.00±0.00	0.00±0.00
North bank	10.00±0.00	0.00±0.00	10.00±0.00	0.00±0.00	10.00±0.00
Mordern market	Not encountered	Not encountered	Not encountered	Not encountered	Not encountered
Wadata	Not encountered	Not encountered	Not encountered	Not encountered	Not encountered

Table 6. Susceptibility test of isolates at different concentrations of cypermethrin.

Isolates	1%	2%	3%
Klebsiella spp.	+	+	-
Escherichia coli	+	-	-
Staphylococcus spp.	+	-	-
Bacillus spp.	-	-	-

+ = Presence of growth (resistant); - = Absence of growth (susceptible).

with different superscript are statistically significant ($p<0.05$).

Table 4 shows the cultural, morphological and biochemical characteristics of isolates. Isolates were identified based on colony color, shape, morphology, Gram's reaction, biochemical test such as catalase, citrate, indole, oxidase and urease. *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Klebsiella* spp. and *Proteus* spp. were all isolated and identified.

In Table 5, the percentage prevalence of isolates shows that a total of 10 bacteria were isolated with samples from High level, accounting for 50% of the isolates, North bank 30% and Wurukum samples 20%. *Klebsiella* spp. accounted for 30% of the isolates, *E. coli* 10%, *Staphylococcus* and *Bacillus* spp. all accounting for 20% respectively.

Finally, the sensitivity of isolates to cypermethrin at different concentration is presented in Table 6. From the result, *Klebsiella* spp. was resistant to cypermethrin at 1 and 2%, but susceptible at 3%. *E. coli* and *Staphylococcus* spp. were both resistant at 1% but susceptible at both 2 and 3%. However, *Bacillus* spp. was susceptible at 1%. The physicochemical parameters of the soils revealed that samples from the different sites had pH range of 4.7 to 5.1, organic matter content ranging from 6.95 to 12.9, organic carbon ranging from 6.5 to 14.3 and temperature ranging from 20 to 28°C.

Studies have shown that the physicochemical parameters of soil affect the activities of microorganisms (Ashim et al., 2008). Soil properties like organic matter, clay content, pH, temperature, affect the degradation of pesticides in soil. It is pertinent to study their effects on soil microorganisms which are the chief degraders of pesticides. Gold et al. (1996) reported that soil pH and

clay content greatly affected the persistence of bifenthrin, chlorpyriphos, cypermethrin, and fenvelerate under field conditions. Microbial activity is stimulated by increase in temperature and some ecological groups tend to dominate within certain temperature ranges. The maximum growth and activity of microorganisms in soil occurs at 25-35°C (Alexander, 1977) and pesticide degradation is optimal at mesophilic temperature range of around 25-45°C (Topp et al., 1997). Soil organic matter process enhances microbial activity by cometabolism (Perucci et al., 2000).

The total viable count of bacteria in various sites reveal that samples from Modern market had the highest count with 267.5×105±10.6×105 followed by samples from Wurukum with 235×105±7.1×10^5. Wadata samples had counts of 227.5×105±3.5×10^5. Samples from North bank and High level had no growth. Total coliform count shows that samples from Wadata had counts of 20.5 ×10^5±0.71 × 10^5; samples from High level had counts of 5.5×10^5 ± 0.71×10^5 while samples from Wurukum and Modern market area had counts of 1.5×105± 0.71×10^5. Samples from North bank however showed no growth.

In this study, percentage prevalence of isolates showed that about 50% of the isolates were from High level samples, while Wurukum and North bank samples had 20 and 30% isolates respectively. Other isolates from Wadata and Modern market got contaminated and therefore not suitable for use. The bacteria isolates includes *Klebsiella* spp.*, E. coli, Staphylococcus* spp.*, Bacillus* spp. and *Proteus* spp*.* However, *Pseudomonas* was not isolated from the soil. Similar bacteria were used by Radhika and Kannaiah (2014) to test their ability to remediate cypermethrin.

The result of this investigation showed that four different bacteria genera showed different resistant capacities to various doses of the commercial insecticide cypermethrin. At lower concentration of the insecticide, it was observed that almost all the bacteria isolates were resistant and could grow. However at higher concentrations of the insecticides there was a marked inhibition in the growth of the isolates as compared to the control test. These results are similar to that reported by Radhika and Kannaiah (2014).

Murugessan et al. (2009) observed that at 0.3% concentrations of cypermethrin, *Corynebacterium* and *Bacillus* spp. could grow and degrade cypermethrin, but at higher concentration, they were inhibited. The growth of *Pseudomonas aeruginosa*, *Klebsiella* spp. and *E. coli* were reported to grow at 1% concentration of cypermethrin. In this research, the effects of cypermethrin insecticides at concentrations ranging from 1-3% were carried out. So, the result showed that *E. coli* and *Staphylococcus* spp. were able to grow at 1% concentration of cypermethrin, *Klebsiella* spp. was able to grow to 2% of the concentration of the insecticide but was inhibited at 3%. *Bacillus* spp. however was not able to grow at 1% concentration of the insecticide. This finding corroborates with that of Al-Rasslany et al. (2010) who observed that microbial population suffered significant decrease after 7 days of cypermethrin exposure.

Conclusion

From this study, some selected group of bacteria (*Klebsiella* spp.*, E. coli, Staphylococcus* spp.and *Bacillus* spp.) were isolated and it was observed that at lower concentration of cypermethrin (1%), some soil bacterial isolates (*Klebsiella* spp.*, E. coli,* and *Staphylococcus* spp.) were resistant to it and could tolerate their presence and grow, being able to degrade it. However, cypermethrin had a negative impact on the growth of the selected soil bacteria (*Staphylococcus* spp.*, Klebsiella* spp., *E.coli,* and *Bacillus* spp.) at higher concentrations. *Klebsiella* spp however, was found to be more resistant to it and could grow and degrade cypermethrin at 2% concentration. Therefore, soil microorganisms play key roles in the environment which include decomposition processes, biochemical cycling, energy transfer through trophic levels and numerous microbes-microbes interactions, microbes-plant and microbes-animal interactions. It is recommended therefore that, the use of insecticides should be controlled, as higher concentration of insecticides such as cypermethrin is toxic to soil microbes. It is also recommended that, all insecticides should be treated in the soil and their bactericidal and bacterostatic effect be tested prior to approval for commercial sales and labeling by the Environmental Protection Agency (EPA), *in-vivo* experiments should be carried out on field where cypermethrin has been applied according to the manufacture's specification and their effect on microbial load ascertained over a given period. In addition to this, government and non-governmental agencies should place ban on the use of chemical substances used as insecticides which are hazardous to the environment and health of humans in the long run due to bioaccumulation. Also, strict compliance should be ensured by both producers and distributors, and defaulters be punished by the law enforcement agencies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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