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Effect of Chemical Synthetic Pesticides on Plant-Growth Promoting- Bacteria Isolated from Tansian University Agricultural Farm Umunya, Anambra State

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ABSTRACT

This study investigated how synthetic chemical pesticides, specifically cyhalothrin and cypermethrin, affect beneficial soil microorganisms. These microorganisms, known as plant growth-promoting bacteria (PGPB), are crucial for enhancing plant growth and health. However, the widespread use of chemical pesticides contributes to various environmental issues. Soil samples were collected under sterile conditions from an agricultural farm at Tansian University in Umunya, Anambra State. A soil auger was used to take samples from a depth of 0-20cm at several points. The samples were placed in new polyethylene bags and immediately taken to the laboratory for analysis. To test the pesticides' effects, 200 grams of soil were placed into five separate plastic bowls. Four of the bowls were treated with different concentrations of the pesticides (5%, 10%, 15%, and 20%) and thoroughly mixed. The fifth bowl served as an untreated control. The bacterial populations were then carefully analyzed using standard microbiological methods. The total heterotrophic bacteria count (THBC) ranged from 2×103 to 8×103 colony-forming units per gram (CFU/g). The highest microbial count (8×103 CFU/g) was found in the control sample, which had no pesticide. The bacteria identified from the soil included Streptomyces sp., Klebsiella sp., Enterobacter spp., Serratia spp., Proteus spp., Pseudomonas spp., and Bacillus spp. Among these, Bacillus spp., Serratia spp., and Streptomyces spp. were the most abundant. The results clearly showed that the presence of cyhalothrin and cypermethrin led to a reduction in the number of soil microorganisms compared to the untreated soil. These findings suggest the need to limit the use of conventional pesticides and promote the use of biopesticides and other sustainable agricultural practices.

Keywords: Beneficial bacteria, Synthetic pesticides, Plant Growth Promoting Bacteria, Farm soil, Sustainable agriculture.

Introduction

Plant Growth-Promoting Bacteria (PGPB) are a group of beneficial microorganisms that inhabit the rhizosphere, the zone surrounding plant roots (Gray and Smith, 2005; Awari, 2023). These bacteria play a crucial role in improving plant health and productivity through various mechanisms, including enhancing nutrient availability, producing growth-regulating substances, and protecting against pathogens (Dimkpa et al., 2009; Grover et al., 2011; Glick, 2012). Modern agriculture relies on diverse practices, such as tillage, irrigation, fertilization, and pest control, to maximize crop yields and land productivity (Egurefa et al., 2024; Awari et al., 2023). The interaction between these farming techniques and PGPB is a key area of study for developing sustainable and efficient crop production systems. PGPB are valued for their ability to

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enhance agricultural output by directly stimulating plant growth and indirectly improving the soil environment through increased nutrient supply and pathogen suppression. As a "green technology," PGPB are being promoted as a sustainable alternative to chemical fertilizers, which can improve soil health. However, as noted by Awari et al. (2020), natural resources are facing rapid degradation due to various human and industrial activities. These activities contribute to environmental issues such as groundwater contamination (Agu et al., 2014; Agu et al., 2015), reduced soil quality (Agu et al., 2013; Awari et al., 2023; Okafor et al., 2016; Orji et al., 2014), and a decline in biodiversity.

Soil microorganisms are vital to the health of agroecosystems, as they are involved in nutrient cycling, organic matter decomposition, nitrogen and phosphorus fixation, and plant protection. Since soil biodiversity is highly sensitive to farming practices, changes in the soil microbiome serve as an important indicator of soil quality (Awari et al., 2023).

This study's objective was to analyze the effect of synthetic pesticides, specifically cyhalothrin and cypermethrin, on the PGPB population in farm soil at Tansian University, Umunya, Anambra State.

Materials and Methods

Study Area

Soil samples were collected from agricultural farmland at Tansian University, Umunya, Anambra State. The site is situated at approximately N6° 12.1782 latitude and E6° 53.4305 longitude.

Sample Collection

On June 24, 2024, soil samples were collected from ten different points using a soil auger to a depth of 0-20 cm. The samples were handled aseptically, placed into new polyethylene bags, and immediately transported to the microbiology laboratory for analysis.

Sample Preparation

The impact of the pesticides on PGPB was evaluated using five plastic bowls. Each bowl contained 200 grams of soil. Four of the bowls were treated with different concentrations of a synthetic pesticide mixture (5%, 10%, 15%, and 20%) and homogenized. The fifth bowl was left untreated to serve as a control. The bacterial counts were subsequently analyzed at regular intervals using standard laboratory procedures.

Serial Dilution

Serial dilutions were performed to reduce the microbial load of the soil suspensions up to a 10-5 dilution. For each of the five samples, one gram of soil was mixed with 9 ml of sterile saline solution and shaken vigorously to create a uniform suspension.

Procedure for Serial Dilution:

- 1. **Preparation:** All necessary materials, including the soil suspension, sterile diluents, pipettes, and a series of test tubes, were prepared.
- 2. **Initial Dilution:** A measured volume of the soil suspension was transferred into the first test tube containing a known volume of diluent.
- 3. **Subsequent Dilutions:** A portion of the liquid from the first diluted tube was transferred to the next tube with a new diluent. This step was repeated to achieve the desired dilution series.
- 4. **Mixing:** Each new dilution was thoroughly mixed to ensure an even distribution of microorganisms.

5. **Recording:** The volumes and dilution factors for each step were carefully documented.

Bacterial Isolation

Procedure:

- **Plating:** A small volume (100 μl) of the final diluted suspension was spread onto various agar plates, including nutrient agar, eosin methylene blue agar, and MacConkey agar.
- Incubation: The plates were incubated at 37°C for 24 hours to allow bacterial colonies to form.
- **Colonial Identification:** Initial characterization of the isolated colonies was performed by visually examining their appearance, size, shape, color, and other morphological features on the plates.

Identification of Organisms

The isolates were further identified based on their cultural characteristics and biochemical reactions.

Gram Staining:

This method was used to classify bacteria as either Gram-positive or Gram-negative based on their cell wall structure. The procedure involved staining the cells with crystal violet and iodine, followed by a decolorizing agent, and finally, a counterstain (safranin) to make Gram-negative cells visible.

Biochemical Tests:

A series of tests were conducted to determine the metabolic and enzymatic activities of the isolates.

- Catalase Test: This test checks for the presence of the enzyme catalase. A positive result (rapid bubbling) occurs
 when hydrogen peroxide is added to a bacterial sample, indicating the enzyme is breaking down H2O2 into water
 and oxygen.
- Oxidase Test: This test identifies bacteria that possess the enzyme cytochrome c oxidase. A dark purple or blue color on a filter paper strip after adding the oxidase reagent indicates a positive result.
- Nitrate Reduction Test: This test determines if bacteria can reduce nitrate (NO3-) to nitrite (NO2-) or other
 nitrogen compounds. A red color after adding reagents indicates a positive result for nitrite. If no color change
 occurs, the addition of zinc powder helps to confirm if the nitrate was unreduced (negative) or fully reduced to
 other compounds (positive).
- **Methyl Red Test:** This test identifies bacteria that produce stable acids from glucose fermentation, which lowers the pH. A red color after adding the methyl red indicator indicates a positive result.
- **Voges-Proskauer (V.P.) Test:** This test detects the production of acetoin from glucose fermentation. A positive result is indicated by the formation of a red color after adding alpha-naphthol and potassium hydroxide.
- **Citrate Utilization Test:** This test determines if bacteria can use citrate as a sole carbon source. A positive result is indicated by a color change from green to blue on a Simmons citrate agar slant, signifying an increase in pH.
- Sugar Fermentation Tests (SFT): These tests assess a bacterium's ability to ferment specific sugars (glucose, lactose, and mannitol). A positive result is indicated by a color change from red to yellow in the broth, showing acid production. Gas production is noted by bubbles in a Durham tube.

Results and Discussion

Results

Table 4.1 shows the total heterotrophic bacterial counts (THBC) for soil samples treated with varying concentrations of cyhalothrin and cypermethrin and the untreated control. The THBC was highest in the control sample (8×103 CFU/g) and progressively decreased with increasing pesticide concentration: 7×103 at 5%, 4.0×103 at 10%, 3.2×103 at 15%, and 2×103 at 20%.

Table 2 highlights the presence of specific bacterial isolates. Streptomyces spp., Staphylococcus spp., and Bacillus spp. were found in all samples. The biochemical tests outlined in Table 3 confirmed the identification of several bacteria, including Streptomyces spp., Klebsiella spp., Enterobacter spp., Serratia spp., Proteus spp., Pseudomonas spp., Staphylococcus spp., and Bacillus spp.

Table 4.1: Microbial Count of Bacterial Isolates

 Parameter Tested 	2.	Control	3.	200gsoil 5.		200gsoil	7.	200gsoil	9.	200gsoil	
	Tested			4.	+5%CHCP	6.	+10%CHCP	8.	+15%CHCP	10.	+20%CHCP
11.	THBC (CFU/g)		12. $8x10^3$		13. $7x10^3$		14. 4.0×10^3		15. 3.2×10^3		16. 2x10 ³
17.	Total Coliform (CFU/g)		18. 6.5x10 ³		19. 4x10 ³		20. 2.6x10 ³		21. 2.5x10 ³		22. 2.5x10 ³
23.	Feacal coliform (CFU/g)		24. 5x10 ³		25. 3.5x10 ³		26. 2.0x10 ³		27. 3x10 ³		28. 1x10 ³
29.	E. coli (CFU/g)		30. $4x10^3$		31. 3x10 ³		32. 2.5×10^3		33. 1.5×10^3		34. 2x10 ³

KEY: THBC= Total heterotrophic bacterial count, CFU/g= colony-forming unit per gram.

CHCP: Cyhalothrin and cypermethrin.

Table 4.2: Presence of the Bacteria Isolates

Isolate	Control	200gsoil	200g soil	200g soil	200gsoil	
		+5%CHCP	+10%CHCP	+15%CHCP	+20%CHCP	
Streptomyces spp.	\checkmark	$\sqrt{}$	\checkmark	√ √		
Klebsiella spp.	$\sqrt{}$	\checkmark	\checkmark	√ 0		
Enterobacter spp.	$\sqrt{}$	0	0	0 0		
Serratia spp.	\checkmark	0	0	√ √		

Bacillus spp.	$\sqrt{}$	\checkmark	\checkmark	$\sqrt{}$	$\sqrt{}$
Protues spp.	$\sqrt{}$	0	0	0	0
Pseudomonas spp.	\checkmark	0	0	\checkmark	$\sqrt{}$
Sta gh : spp.	$\sqrt{}$	\checkmark	\checkmark	$\sqrt{}$	$\sqrt{}$

KEY

 $\sqrt{}$ - PRESENT

0 - ABSENT

CHCP: Cyhalothrin and Cypermethrin

Table 4.3: Biochemical Identification of the Bacteria

Colonial Characteristics	Grams Morphology	Catalase	Oxidase	NRT	MR	Citrate	V.P	Glucose	Lactose	Mannitol	PROBABLE ORGANISM
Smooth and shiny colonies with green pigments	Gram negative small short single rods	+	+	+	+	+	-	+	-	+	Pseudomonas spp.
Smooth moist shiny colonies	Gram negative small short single rods	+	-	+	+	-	-	+	+	+	Escherichia coli
Moist and mucoid raised creamy colonies	Gram negative large rods in short chains	+	-	+	-	+	+	+	+	+	Klebsiella spp.
Moist and mucoid raised creamy colonies	Gram Positive rods	+	-	+	-	+	-	+	+	-	Bacillus spp.
Creamy and moist colonies	Gram negative segmented rods	+	-	+	+	-	+	+	+	+	Staphylococcus spp.
Moist, smooth, shiny and mucoid colonies	Gram negative rods in short pairs	+	-	+	-	+	+	+	+	+	Enterobacter spp.

Colonial Characteristics	Grams Morphology	Catalase	Oxidase	NRT	MR	Citrate	V.P	Glucose	Lactose	Mannitol	PROBABLE ORGANISM
Smooth, shiny and moist colonies	Gram negative rods in pairs	+	-	+	-	+	+	+	-	+	Serratia spp.
Smooth and shiny colonies	Gram negative straight rods	+	-	+	-	+	-	+	-	+	Proteus spp.
Dry, chalky and pigmented colonies	Gram Positive mycelium	+	-	+	-	+	-	+	-	+	Streptomyces spp.

KEY: (+) = Positive, (-) = Negative, (AG) = Acid and Gas, (A) = Acid, (G) = Gas

DISCUSSION

The findings demonstrate a clear relationship between the concentration of synthetic chemical pesticides and the number of soil microorganisms. The results from the bacterial counts showed a progressive decrease in microbial population as the pesticide concentration increased. The control sample, which was not treated with pesticides, had the highest total heterotrophic bacterial count (THBC) at 8×103 CFU/g. In contrast, the sample with the highest pesticide concentration (20%) had the lowest count, at 2×103 CFU/g. The counts for the 5%, 10%, and 15% concentrations were 7×103 , 5.5×103 , and 4×103 CFU/g, respectively. This decline in microbial load following pesticide application is consistent with findings from other studies, such as that by Awari et al. (2020), which also observed a systematic reduction in microbial counts.

Similar trends were seen in the analysis of specific microbial groups. For example, the total coliform, fecal coliform, and *E. coli* counts all decreased as the pesticide concentration increased, further indicating the detrimental effect of the chemicals on the microbial community. The study also identified several bacterial isolates in the soil, including *Proteus sp.*, *Enterobacter cloacae*, *Klebsiella sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Streptomyces lydicus*, and *Serratia sp.*, with *Bacillus sp.*, *Streptomyces sp.*, and *Staph. sp.* being the most prevalent. This prevalence of *Bacillus sp.* in both treated and untreated soils may be due to its ability to degrade pesticide components, as suggested by Kumar et al. (2014). The control sample generally had a greater diversity of isolates compared to the pesticide-treated samples.

Characterization and biochemical tests, such as the Nitrate Reduction Test (NRT), were performed to identify the bacteria. Many of the identified organisms were positive for NRT, which is consistent with the role of PGPB in reducing nitrate and enhancing nitrogen availability for plants, thereby supporting increased biomass and yield.

Conclusion

In conclusion, this study confirms that cyhalothrin and cypermethrin pesticides have a significant negative impact on soil microorganisms, including PGPB, resulting in a reduction of microbial counts. These findings highlight the importance of adopting sustainable agricultural practices, such as replacing conventional pesticides with biopesticides. The use of chemical pesticides not only negatively affects soil ecosystems but also poses risks to crops, wildlife, and human health.

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