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ORIGINAL RESEARCH ARTICLE

The Effects of Pesticide Application on Soil Microbiota and Weed Dynamics in Cowpea Cropping Systems

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ABSTRACT
Soil is a critical habitat for diverse microorganisms and plants, pivotal in nutrient cycling, organic matter decomposition, and overall ecosystem productivity. However, agricultural practices often involve the use of pesticides to manage pests and increase crop yield, which can significantly impact soil biodiversity and health. Pesticides such as dimethoate (an organophosphate insecticide) and lambda-cyhalothrin (a synthetic pyrethroid) are commonly used in farming to control insects and pests that threaten crops like cowpea (*Vigna unguiculata*). This study investigates the impact of Dimethoate (A), Lambda-Cyhalothrin (B), and their combination (A+B) on soil microbial and weed populations. Soil samples were sourced from BIODEC Katsina and cowpea seeds were obtained from IITA, Kano office, Nigeria. Microbial enumeration and identification were performed using standard pour plating and biochemical techniques. Results showed pesticide concentrations influenced microbial populations, with A+B at 40 mL/L yielding the highest bacterial (39.75 CFU/g) and fungal (34 CFU/g) counts. Dimethoate alone at 50 mL/L resulted in the lowest bacterial (7.25 CFU/g) and fungal (8 CFU/g) counts. Synergistic effects were observed with A+B, promoting microbial proliferation and weed growth at moderate concentrations. Correlation analysis revealed a strong negative relationship between Dimethoate and microbial populations ($r = -0.921$, $p < 0.001$) and a positive correlation for Lambda-Cyhalothrin ($r = 0.731$, $p < 0.01$). The findings highlight the potential of pesticide combinations to alter soil microbial dynamics and weed populations. Judicious and selective use of pesticides to target pests while preserving beneficial soil organisms is recommended.

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Cowpea, Pesticides, Soil, Bacteria, Fungi and Weeds

INTRODUCTION
Cowpea (*Vigna unguiculata L. WALP)* (Fabaceae) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Tazerouni *et al.,* 2019). Cowpea was introduced from Africa to many regions in the world approximately 2000 to 3500 years ago.

It is a widely adapted legume grown worldwide (Xiong *et al.,* 2016). Recently, it has become an essential crop in many countries in tropical Africa, Asia, and South America (Mahalakshmi *et al.*, 2007).

Microbes, such as bacteria and fungi, can be used as bio-fertilizers to increase nutrient bioavailability and improve soil structure (Rashid *et al.*, 2016). Bacterial and fungal inoculate an organic amendment can exploit, translocate, mineralize, and mobilize soil reserves. They can also increase organic matter or fix atmospheric nitrogen (Riaz *et al.*, 2020). Bacteria and fungi promote nutrient bioavailability through N fixation, P, K, and Fe mobilization, and organic acids and siderophores production. They also produce organo-polysaccharides and proteins that help promote soil aggregate stability (Fasim and Uziar, 2019). Fungi and biological N-fixing bacteria can contribute significantly to the total N demand of cowpeas and other legumes (Rashid *et al.,* 2016).

It is essential to manage weeds in agriculture as they can significantly impact the quality and quantity of crops (Petit *et al.*, 2011). Farmers typically employ manual or chemical weed control methods (Abbas *et al.*, 2018). In cowpea production, manual weed control is commonly used, involving weeding twice with a hand hoe at 2 and 4-5 weeks after planting to maintain a clean field (Omoigui *et al.*, 2020). Delaying weed control can result in a substantial decrease in yield. Chemical weed control entails using herbicides, which should be selected based on the predominant weed species and herbicide availability. However, herbicide application is not advised in areas where leaves are consumed (Davis and Frisvold, 2017). In semi-arid regions, Striga gesnerioides and Alectra species pose significant threats as parasitic weeds affecting cowpeas (Ilunga 2014).

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Pesticides were originally introduced to improve crop yields and protect them from pests. However, these highly toxic chemicals affect targeted organisms and harm non-target organisms in the environment (Khan *et al.*, 2023). Pesticides inhibit seed germination and enzymatic activity of the plant, leading to reduced growth, photosynthesis rates, and yields. Overusing pesticides can result in residues in plants, seeds, fruits, and soil where it's grown (Tallapragada and Lather, 2022). The accumulation of pesticides in the soil disrupts soil microorganisms, enzymes, and other physiochemical parameters, ultimately affecting soil fertility (Baweja *et al.*, 2020). This study aimed to explore the effects of pesticide application on soil microbiota and weed dynamics in cowpea cropping systems.

MATERIALS AND METHODS

Sample Collection

Soil samples were gathered at the Bioresources Development Centre in Katsina, Nigeria, using a soil auger at 15-20cm depths and placed into sterile polyethylene bags. Cowpea seeds (*Vigna unguiculata*) variety (573-1-1) were sourced from the International Institute of Tropical Agriculture (IITA) Kano office in Kano, Nigeria. The pesticides lambda-cyhalothrin and dimethoate were procured from a pesticide retailer in Katsina Market. Weed species were collected and authenticated at the herbarium unit of the Biological Sciences Department, Umaru Musa Yar'adua University Katsina. The species and Voucher numbers are as follows: *Striga hermonthica* (UMYUH 31), *Phyllanthus amarus* (UMYUH 2512), and *Cyperus rotundus* (UMYUH 2332)

Determination of Biological Components of the Soil.

Pour plating technique was used to enumerate heterotrophic bacteria and fungi. The samples were ten-fold serially diluted (10^{-10}) and inoculated onto plate count agar (PCA) for bacteria and acidified potato dextrose agar (PDA) for fungi containing Streptomycin (1mg/100 ml) (for fungi) using 0.1 ml of the sample. The inoculated plate count agar Petri dishes were incubated at 37°C for 24 hours, while the potato dextrose agar plates were incubated at room temperature 28 degrees Celsius for 3 days. The observed colonies were counted using a colony counter and expressed as colony-forming units per gram (cfu/g), according to Ameh *et al.* (2017). The number of bacterial or fungal cells in a soil sample was calculated using the following equations:

- No. of bacterial cells / 1gm moist soil =

$$\frac{\text{number of colonies} \times \text{inverted dilution}}{\text{Weight of moist soil}}$$

- No. of fungal cells / 1gm moist soil =

$$\frac{\text{number of colonies} \times \text{inverted dilution}}{\text{Weight of moist soil}}$$

Identification and Characterisation of Microbial Isolates

The characterisation of bacterial and fungal isolates was conducted by analyzing their cultural and biochemical properties alongside their macroscopic and microscopic features. This process was carried out following the methods outlined by Cheesbrough (2006).

Visual assessment was employed in weed assessment, which involves observing and evaluating weed infestations visually, noting species, density, and coverage. (Liebman and Zimdahl, 2018).

RESULT AND DISCUSSION

Soil treated with the combinations of Dimethoate and Lambda-Cyhalothrin (A+B) at 40mL/L concentration shows the highest bacterial count (39.75). Dimethoate (A) at 50mL/L concentration shows the lowest bacterial count (7.25) compared with the control. In all the runs of Dimethoate and Lambda-Cyhalothrin (A+B), Lambda-Cyhalothrin (B) shows the highest manifestation of bacterial count in the soil when compared to Dimethoate (A) (Figure 1).

The combination of Dimethoate and Lambda-Cyhalothrin shows synergistic effects, which is accepted by the study of Bunemann *et al.* (2006), which said Negative pesticide impacts are more prevalent than significant ones, with few confirmed effects on soil organisms. as shown in (Figure 1). Research by Isha *et al.* (2022) has demonstrated that Dimethoate can adversely affect soil bacteria and fungi. The substance is known to decrease the population of these microorganisms in the soil and can even lead to the demise of certain bacterial species (Isha *et al.*, 2022). According to studies by Cyocon *et al.* (2010), the detrimental effects of dimethoate and lambda-cyhalothrin can also affect non-target soil microorganisms, turnover rate, nutrients, microbial community structure, and soil quality, which supports this study. Equally study by Van Scoy and associates (2016) shows that Numerous organisms have been discovered to be negatively impacted by dimethoate pesticides. This research follows the Jayaraj *et al.* (2023) study, in which 27 bacterial species were isolated from 10 soil samples taken from an agricultural field. The ability of these microorganisms to resist pesticides was investigated, and only three types of bacteria were found to endure. According to Ahmed *et al.* (2022), some microorganisms may be adversely affected by pesticides like dimethoate, indicating that some bacteria may resist these substances. According to Martikainen *et al.* (1998), dimethoate decreased soil microarthropod populations, with the decrease being greater in the upper soil layer than the lower soil layer. Karpun *et al.*, (2021). The amount of soil microbial respiration was reduced as a result of the pesticides being applied in commercial quantities. Contrary to this study, Anwar *et al.* (2023) indicated that certain bacteria can withstand the effects of Lambda-Cyhalothrin at lower and moderate concentrations. Lambda-Cyhalothrin can support the growth of some soil

bacteria, making them useful for soil remediation (Abdulsalam *et al*., 2023). This research is in line with Omotayo & Okoro's (2022) report, which said Lambda-Cyhalothrin supported the growth of bacteria in a given soil. Although Lambda-Cyhalothrin negatively affects soil bacteria it equally supports the growth of certain ones (Sodhozai *et al*., 2024).

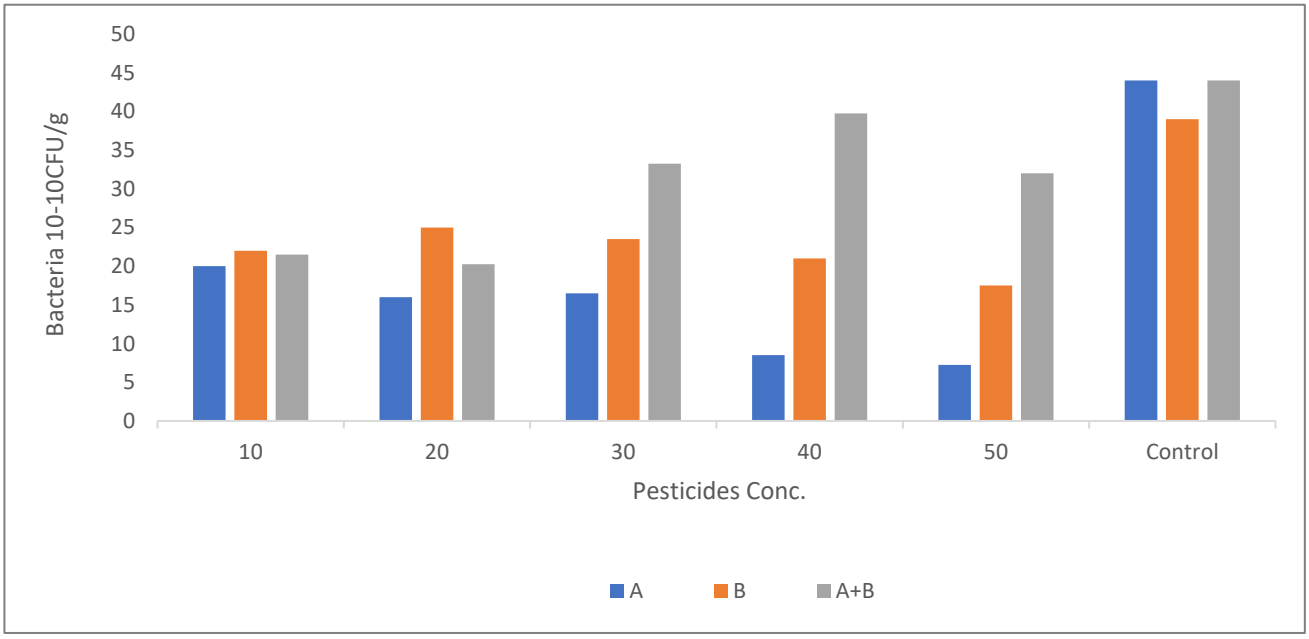

Figure 1: Showing Soil Bacteria 10-^{10} CFU/g,

The growth of the high population of Bacteria was expressed by the combination of Dimethoate and Lambda-Cyhalothrin (A+B) at 30mL/L. 40mL/L and 50mL/L could be due to Lambda-Cyhalothrin (B), which manifested in the result (Kaur *et al.,* 2021). Compared to the control, lambda-cyhalothrin generally increased the population of bacteria in the contaminated soils at all dose rates administered (Omotayo and Okoro, 2022).

Table 1: Mean Bacterial Colony Counts of Pesticides Contaminated Soil at Different Concentrations of (A) Dimethoate Pesticides

Different Concentrations	Mean Bacterial Colony Counts (CFU/g)
10 ml/L	20×10^{10}
20 ml/L	16×10^{10}
30 ml/L	17×10^{10}
40 ml/L	9×10^{10}
50 ml/L	7×10^{10}
Control	44×10^{10}

Table 2: Mean Bacterial Colony Counts of Pesticides Contaminated Soil at Different Concentrations of (B) Lambda-Cyhalothrin Pesticides

Different Concentrations	Mean Bacterial Colony Counts (CFU/g)
10 ml/L	22×10^{10}
20 ml/L	25×10^{10}
30 ml/L	24×10^{10}
40 ml/L	21×10^{10}
50 ml/L	18×10^{10}
Control	39×10^{10}

Table 3: Mean Bacterial Colony Counts of Pesticides Contaminated Soil at Different Concentrations of the (A+B) Combination of Dimethoate and Lambda-Cyhalothrin Pesticides

Different Concentrations	Mean Bacterial Colony Counts CFU/g
10 ml/L	22×10^{10}
20 ml/L	20×10^{10}
30 ml/L	33×10^{10}
40 ml/L	40×10^{10}
50 ml/L	32×10^{10}
Control	44×10^{10}

Table 4: Gram Stain and Biochemical Profile of Bacterial Isolates

Test	*Bacillus thuringiensis*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
Gram Staining	Gram-positive rods	Gram-positive rods	Gram-negative rods	Gram-positive rods
Catalase	Positive	Positive	Positive	Positive
Starch Hydrolysis	Positive	Positive	Negative	Positive
Methyl Red Test	Variable (some strains positive	Positive	Negative	Negative
Motility	Positive	Positive	Positive	Positive
Casein Hydrolysis	Positive	Positive	Negative	Positive
Blood Hemolysis Test	β-Hemolysis	β-Hemolysis	β-Hemolysis	γ-Hemolysis

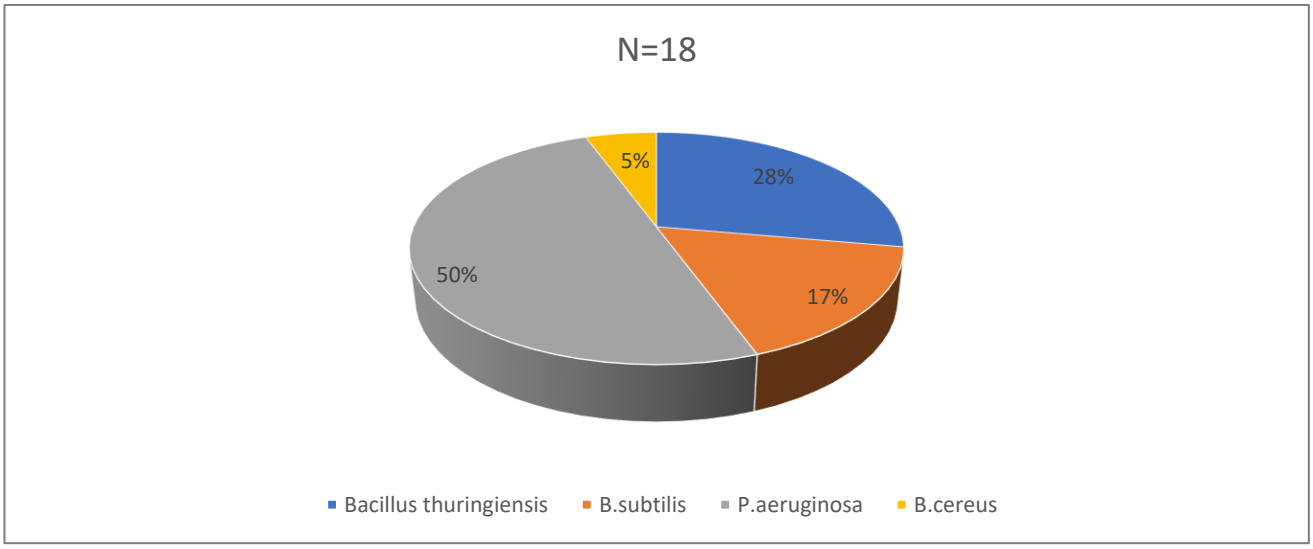

Figure 2: showing the distribution of bacteria obtained from soil

Fungi

Soil treated with the combinations of Dimethoate and Lambda-Cyhalothrin (A+B) at 30mL/L concentration shows the highest fungal count (34), while Dimethoate (A) at 50mL/L concentration shows the least fungal count (8). The result further shows that Dimethoate and Lambda-Cyhalothrin (A+B) at increasing concentrations (10mL/L to 50mL/L) encourage fungal count when compared to Dimethoate (A) and Lambda-Cyhalothrin (B) as shown in (Figure 2).

The application of pesticides was accompanied by dramatic shifts in the alfa diversity of the microbial community (Al-Haifi *et al.,* 2006). The growth of the high fungi population is expressed by the combination of Dimethoate and Lambda-Cyhalothrin (A+B) at 20mL/L. 30mL/L. 40mL/L and 50mL/L could be due to the presence of Lambda-cyhalothrin in the combination. However, research by Bunemann *et al.* (2006) shows that Negative pesticide impacts are more prevalent than significant ones, with few confirmed effects on soil organisms' fungi inclusive. In another study by Vig *et al.* (2008) based on pesticides and the overall amount of soil fungi examined, none of the pesticides employed had any negative effects on the fungi organisms. Following dimethoate treatment, there were notable changes in Azotobacter levels. Scientific studies indicate that pesticides can impede the function of soil microbes, which play a crucial role in decomposing organic matter and releasing nutrients for plant uptake (Kalia & Gosal, 2011). The potential long-term consequences of this phenomenon can significantly impact soil health and hamper plant growth and development (Shahid, 2022). Pesticides such as dimethoate and lambda-cyhalothrin at the recommended field dose demonstrated a modest harmful effect on entomopathogenic fungi, indicating the possibility of their compatible use (Tkaczuk *et al.*. 2015).

However, according to Ondrackova *et al.* (2019), the mycelial development of Purpureocillium and Akanthomyces strains was not significantly affected by pesticides, and the mycelial growth of Beauveria strains was (22.6–30%) suppressed by the active component's tau-fluvalinate, pirimicarb, and acetamiprid. Pesticides accelerated the mycelial growth of Cordyceps strains compared to controls. With twice the prescribed rates having larger inhibitory effects compared to the control soil, both pesticides considerably decreased the number of microorganisms. It is possible to conclude that pesticide use hurts soil microorganisms crucial to soil fertility (Sodhozai *et al.*, 2024). Ilusanya *et al.* (2020), in their report, emphasize to maintain the sustainability of the food supply and the environment, efforts should be made to employ alternative, non-chemical methods.

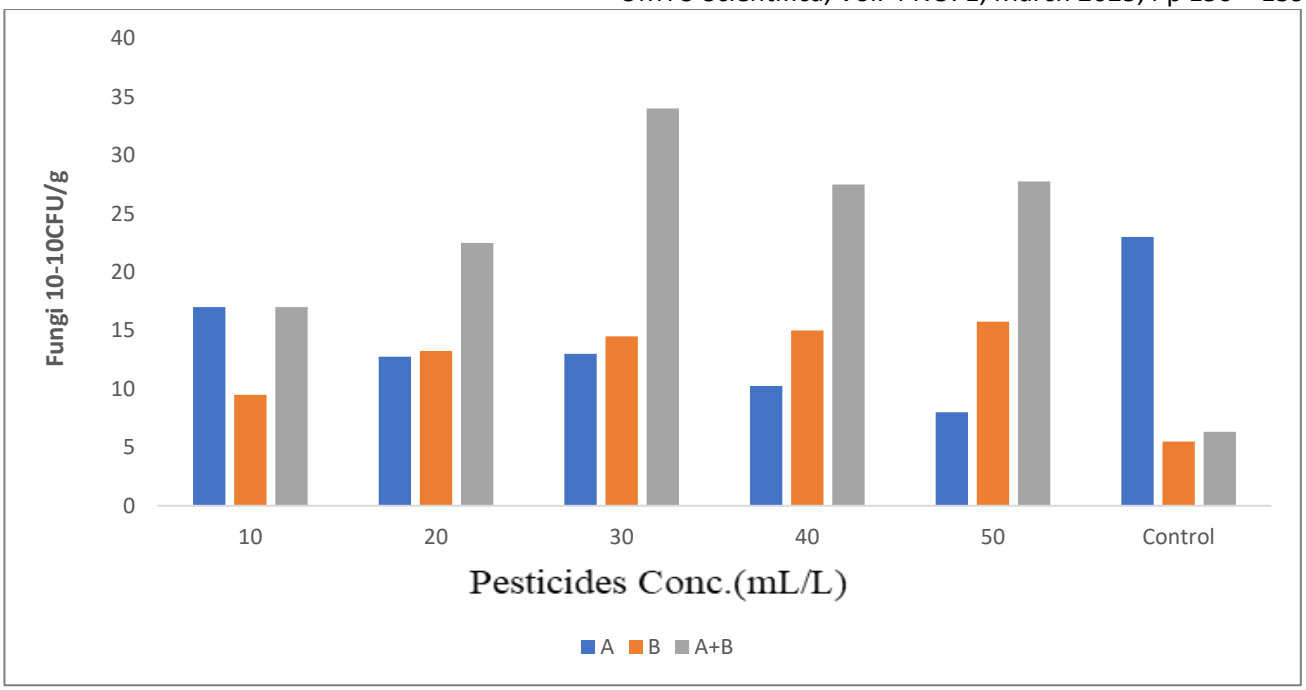

Figure 3: Showing Soil Fungi 10-^{10} CFU/g

Table 5: Mean Fungal Colony Counts of Pesticides Contaminated Soil at Different Concentrations of (A) Dimethoate Pesticides

Different Concentrations	Mean Fungal Colony Counts CFU/g
10 ml/L	17×10^{10}
20 ml/L	13×10^{10}
30 ml/L	13×10^{10}
40 ml/L	10×10^{10}
50 ml/L	8×10^{10}
Control	23×10^{10}

Table 6: Mean Fungal Colony Counts of Pesticides Contaminated Soil at Different Concentrations of (B) Lambda-Cyhalothrin Pesticides

Different Conc	Mean Fungal Colony Counts CFU/g
10 ml/L	10×10^{10}
20 ml/L	13×10^{10}
30 ml/L	15×10^{10}
40 ml/L	15×10^{10}
50 ml/L	16×10^{10}
Control	6×10^{10}

Table 7: Mean Fungal Colony Counts of Pesticides Contaminated Soil at Different Concentrations of the (A+B) Combination of Dimethoate and Lambda-Cyhalothrin Pesticides

Different Concentrations	Mean Fungal Colony Counts CFU/g
10 ml/L	17×10^{10}
20 ml/L	23×10^{10}
30 ml/L	34×10^{10}
40 ml/L	28×10^{10}
50 ml/L	28×10^{10}
Control	6×10^{10}

Table 8: Macroscopic and Microscopic Structures of Fungi from Pesticide Contaminated Soil

Fungus	Macroscopic Features	Microscopic Features
Aspergillus niger	Colonies are fast-growing, black on the upper side and pale to white on the reverse side. They are powdery or granular.	Hyphae are septate and hyaline. Conidiophores are long and smooth with a bulbous vesicle at the tip. Conidia are spherical, black, and arranged in radiating chains.
Aspergillus flavus	Colonies are fast-growing, yellow to green on the upper side and pale to yellow on the reverse. Their texture can be velvety.	Hyphae are septate and hyaline. Conidiophores are rough and bear a vesicle with phialides. Conidia are spherical, yellow to green, and form in chains.
Fusarium oxysporum	Colonies are cottony or woolly, initially white, later developing pink, purple, or violet pigmentation on the reverse side.	Hyphae are septate. Microconidia are oval, single-celled, and borne on monophialides. Macroconidia are sickle-shaped (falcate), multicellular, and tapered at both ends. Chlamydospores (thick-walled resting spores) may also be present.

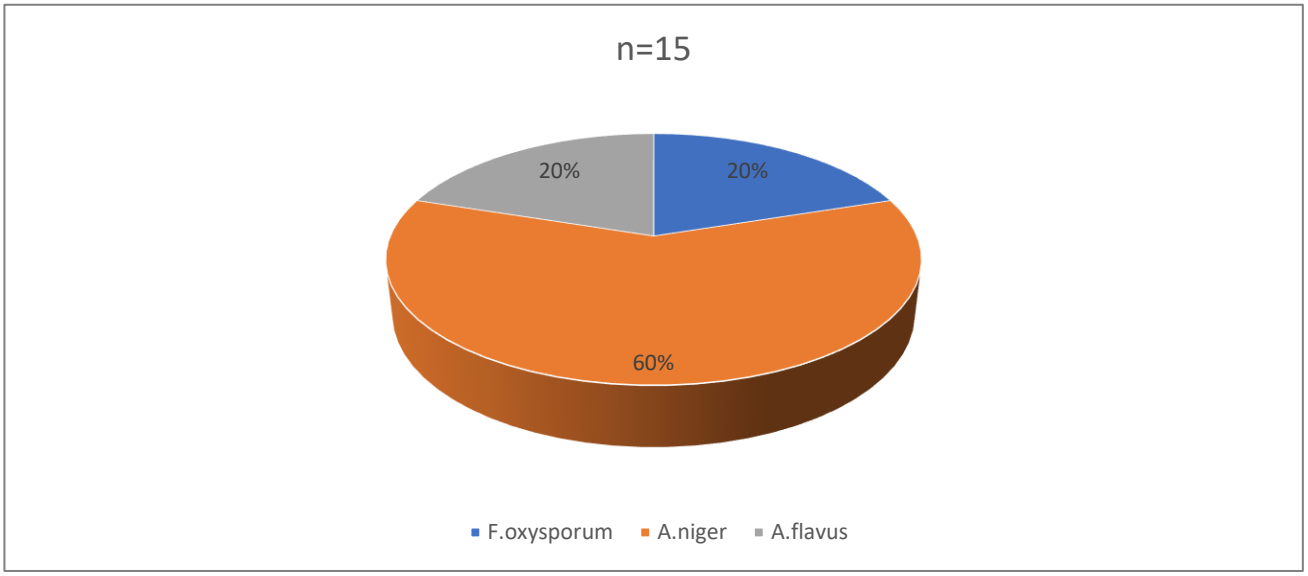

Figure 4: showing the distribution of fungal Isolates obtained from soil

Weeds

The impact of pesticides on weed growth in soil has been studied, and the results suggest that the combination of Dimethoate and Lambda-Cyhalothrin (A+B) has a significant effect on weed populations, which may be attributed to the synergistic effects of the pesticide mixture on weed growth. Soil treated with the combinations of Dimethoate and Lambda-Cyhalothrin (A+B) at 10mL/L concentration shows the highest Weeds number (2.75), while Dimethoate (A) at 50mL/L and Lambda-Cyhalothrin (B) at (20mL/L) concentration show the least number of weeds (1.5) compared to control as shown in (Figure 3).

A combination of Dimethoate and Lambda Cyhalothrin has been shown to promote weed growth in the soil by eliminating helpful plants and insects that control weed proliferation. According to Kaur *et al.* (2014), individual pesticide-resistant plants are typically uncommon in a regular population. However, chemicals used excessively without discretion can eliminate the normal susceptible population (Koch and Ashford, 2006). This then gives the resistant individuals an advantage, and they continue to multiply without competition. Eventually, over generations, they become the dominant portion of the population (Gill and Greg, 2014).

In their study, Hanley and Whiting (2005), which was in line with this research, found that while the application of pesticides did not affect germination, exposure to either one (dimethoate) or both pesticides reduced the growth of seedlings in four different species studied. The results suggest that A+B applications can stimulate weed growth, leading to increased weed populations, while high concentrations of the combination of dimethoate and Lambda-Cyhalothrin (A+B) can negatively impact weed growth and development.

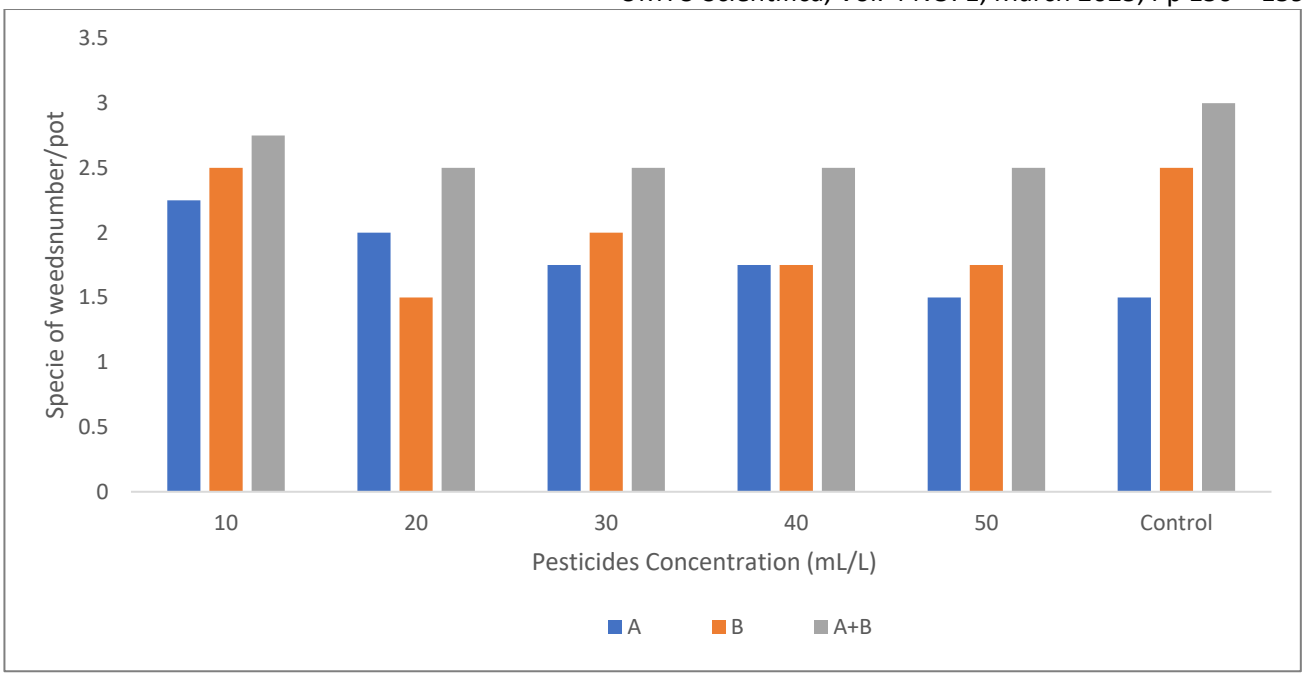

Figure 5: Showing Soil Weeds number per pot

Table 9: Mean Weed Species Number of Pesticides Contaminated Soil at Different Concentrations of (A) Dimethoate Pesticides

Different Concentrations	Mean Weeds Specie Number ± S.D.
10 ml/L	2±0.43
20 ml/L	2±1.00
30 ml/L	2±0.43
40 ml/L	2±0.43
50 ml/L	2±1.00
Control	2±0.43

Table 10: Mean Weed Species Number of Pesticides Contaminated Soil at Different Concentrations of (B) Lambda-Cyhalothrin Pesticides

Different Concentrations	Mean Weeds Specie Number ± S.D. CFU/g
10 ml/L	3± 1.00
20 ml/L	2± 1.00
30 ml/L	2± 0.00
40 ml/L	2± 0.43
50 ml/L	2± 0.43
Control	3± 0.10

Table 11: Mean Weed Species Number of Pesticides Contaminated Soil at Different Concentrations of (A+B) Dimethoate and Lambda-Cyhalothrin Pesticides

Different Concentrations	Mean Weeds Specie Number ± S.D. CFU/g
10 ml/L	3±0.43
20 ml/L	3±1.00
30 ml/L	3±1.00
40 ml/L	3±1.00
50 ml/L	3±1.00
Control	3±1.00

Table 12: Regression Analysis between pesticide concentration and microbial population changes.

		CONC	CONTROL	A	B	A_B
Pearson Correlation	CONC	1.000	-.076	-.921	.030	.694
	CONTROL	-.076	1.000	.249	.731	.114
	A	-.921	.249	1.000	.198	-.588
	B	.030	.731	.198	1.000	.251
	A+B	.694	.114	-.588	.251	1.000
Sig. (1-tailed)	CONC	.	.417	.000	.467	.013
	CONTROL	.417	.	.244	.008	.377
	A	.000	.244	.	.291	.037
	B	.467	.008	.291	.	.242
	A+B	.013	.377	.037	.242	.

KEY: A: Dimethoate Pesticide, **B**: Lambda-Cyhalothrin Pesticide, **A+B**: Combination (Dimethoate and Lambda-Cyhalothrin Pesticide).

Table 12 summarizes correlation coefficients and significance levels between soil fungi and bacteria. A strong negative correlation ($r = -0.921$, $p < 0.001$) exists, suggesting that as the pesticides (A) increase, the microorganism population decreases significantly. This indicates an inhibitory action as a result of the pesticides applied. A moderate positive correlation ($r = 0.731$, $p < 0.01$) between Concentration and pesticides (B) suggests a dependent interaction between this pesticide and microorganisms in the soil. This relationship exists as a result of the pesticide applied. The correlation between Concentration and A+B is moderately strong and positive ($r = 0.694$, $p = 0.013$). This could indicate that the combined influence of pesticides A and B positively impacts the soil microbial population. Significant correlations ($p < 0.05$) are observed between Concentrations A, B, and A+B, with microbial population. The observed correlations provide insights into how pesticide treatments interact to influence microbial populations. For example, strong negative correlations between microbes and pesticide (A) represent antagonistic interactions between fungal and bacterial species (Crowther *et al.,* 2019). Similarly, the positive association between microbes and pesticide (B) reflects microbial proliferation in response to specific pesticide treatments (Fierer *et al*., 2009). The positive correlation between microbe and pesticide (A+B) suggests that the interaction of A and B variables may enhance microbial growth or activity, aligning with Wardle et al (2004) findings.

CONCLUSION

This study highlights the significant impact of dimethoate, lambda-cyhalothrin, and their combination on soil microbial populations and weed dynamics in contaminated soils. The findings demonstrate that these pesticides, while effective in pest control, can disrupt soil microbial diversity and influence weed proliferation. Combined pesticide application showed potential synergistic effects, leading to more pronounced changes in soil health and ecosystem balance.

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