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Effect of chemical and biological pesticides on soil microbial population, enzymatic activities and physicochemical parameters as indicators of soil fertility, soil health and food safety

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Abstract

In agricultural fields, insect pests are regularly controlled with pesticides in an effort to boost crop yields and alleviate food scarcity. Along with non-target microbial floral and soil physicochemical characteristics, these herbicides also impact the activity and abundance of useful soil microbial communities. Significant ecological repercussions follow from this. Assessing the impact of chemical and biological pesticides on microbial population, enzymatic activities and physicochemical parameters as indicators of soil fertility with a view to promoting soil health and food safety was the aim of this investigation. Soil samples treated with chemical and biological pesticide were evaluated. Microbial counts were carried out by soil dilution plate technique. The enzymatic activities, soil respiration and physicochemical parameters of the soil were done using standard procedures. Microbial population of treated and untreated soil samples ranged from 2.15 ± 0.81 × 10^3 to 3.40 ± 0.20 × 10^6 CFU/mL, enzymatic activities (0.00 ± 0.00 to 0.16 ± 0.00 µg), soil respiration (10.15 ± 1.07 to 17.00 ± 0.90 mg/kg), and physiochemical parameters (0.10 ± 0.00 to 177.28 ± 2.00 mg/kg). The application of biological pesticides; tobacco (*Nicotiana tabacum),* titonia *(T.diversifolia),* and neems *(Azadirachta indica)* extracts singly or in combination and most importantly in combination with 10% chemical pesticides when compare to application of 100% chemical pesticides provided optimal microbial population, enzymatic activities and physiochemical parameters which are indictors of excellent soil fertility. This study has revealed the need for farmers to consider the use of the combination of biological pesticides and 10% percentage of chemical pesticides in order to reduce the deleterious effect of 100% use of chemical pesticides and promote soil fertility and health for better food quality and safety.

Keywords: Pesticides, Microbial population, Physiochemical parameters, Soil fertility, Food safety.

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INTRODUCTION

The world population is projected to grow by 70 million per annum in the year 2050 which could lead to increased demand for food production by 70% (FAO, 2019). The availability of agricultural land is limited. Therefore, there is need for the use of pesticides which support optimal growth of crops reducing food insecurity. Pesticides, either biological or chemical play's significant role in food production and food security of a nation. This is particularly important in countries that face food shortage due to incidence of crop pest and diseases. Moreover, long term food shortage could lead to hunger, malnutrition, unemployment, poverty, anxiety and depression. The goal to end hunger, food insecurity, and all forms of malnutrition by 2030 has been made, it is proven that this will not happen unless an extraordinary event takes place (FAO, 2021). If recent movements continue, the number of people affected by hunger would exceed 840 million by that year. Approximately 418 million of the world's undernourished people live in Asia, about one-third in Africa, about 282 million, and 60 million in Latin America and the Caribbean (FAO *et al.,* 2021). Agricultural practices could contribute to alleviation of some of the listed challenges especially with increasing relevance of biological pesticides. The implementation of biochemical application as a non-toxic, environmentally friendly method of pest management has become necessary due to the negative effects associated with chemical pesticides (FAO, 2019). Biopesticides are a potent substitute for chemical pesticides that have the following advantages: high efficacy, target specificity, low chemical residue, and reduced environmental concerns (Ruiu, 2018). Studies have demonstrated that pesticides always affect the microorganisms in the soil. In addition to the type and concentration of the pesticides, environmental factors such as chemistry, biology, and physical state additionally influence how pesticides affect soil microorganisms (Sethi *et al.,* 2013). Biological pesticides, sometimes referred to as bio-pesticides, are specific kinds of insecticides made from naturally occurring substances like bacteria, plants, animals, and minerals. "Neem *(Azadirachta indica*) extract is a bio pesticide which is cheap and environmentally friendly." The primary active component of neem extract, azadirachtin, is poisonous to insects and possesses antifeedant properties. According to Song *et al.* (2018), they are structurally analogous and active chemicals. *T. diversifolia*, a shrub belonging to the Asteraceae family, is commonly seen bordering farms. Utilizing its biomass as a soil supplement helps to preserve and enhance the soil's biological, chemical, and physical characteristics while also making more nutrients available to the soil (Hafifah *et al.,* 2016). Solid waste from tobacco (Nicotiana tabacum) is categorized as agro-industrial waste. Its root exudate might raise the amount of nutrients that are available in the soil. (Yang *et al.,* 2019).

Dehydrogenases occur intracellularly in all living microbial cells and are linked to microbial respiratory processes. The activity in the soil system is an indicator of overall microbial activity of the soils as well as changes in the microbial population in response to variation in soil conditions which have serious implications for nutrient cycling with micro-organisms. The indiscriminate use of pesticides disturbs the soil environment as well as the flora and fauna of the soil, which results in the loss of soil fertility (McLuckie *et al.,* 2020). Enzyme activity such as phosphatase, dehydrogenase enzyme activities in the soil environment are considered to be major contributors to overall soil microbial activity and more recently, to soil quality and safety (Zhang *et al.,* 2016). Microorganisms in the soil are essential to the upkeep of ecological processes, crop productivity, and soil health. It is noteworthy that research studies have been carried out to investigate the chemical properties, microbial activity and biomass of soil amended with chemical pesticides and aqeous neem leaf extract (Alice *et al.,* 2016, Balazs, 2020 and Esimbekova *et al.,* 2022). But not too many studies have been carried out on chemical pesticides in combination with biochemical pesticides such as tobacco, neems and titonia. Thus, the purpose of this study was to analyze the effects of commonly used chemical and biological pesticides on soil microbial populations, enzymatic activities, and physiochemical parameters as indicators of soil fertility, quality, and safety.

MATERIALS AND METHODS

Treatment of soil with chemical and biological pesticides

Tobacco (*Nicotiana tabacum)*, titonia *(T.diversifolia), and* neems *(Azadirachta indica)* were used as biological pesticides while glyphosate are used as chemical pesticide. Each biological pesticide extract was dissolved in water and inoculated on the surface of the soil. The chemical pesticide, a non-selective systemic foliar applied herbicide was also inoculated on the soil surface.

Soil sampling

Soil samples treated with both biological and chemical pesticides were gathered at the study farm (Institute of Agricultural research and Training moor plantation, Ibadan, Nigeria) in polythene bags from the surface layer between 0 and 15 cm, with untreated soil samples serving as the control. After being collected, the soil samples were allowed to air dry, sieved using a 2 mm mesh, and then stored at 4 ˚C until needed.

Determination of microbial counts

The total soil microbial counts were determined by soil dilution plate technique according to the method described by Adesina and Adelasoye (2014) and Stanley (2015) using nutrient agar (Hi-Media, India) for bacterial and PDA agar (Hi-Media, Mumbai, India) for fungi. Phosphorous solubilizers, potassium solubilizers and nitrogen fixers was determined by method described by Tan *et al.* (2014). Pikovskaya (Hi-Media, Mumbai India) for phosphorous solubilizers Aleksandrov broth (Hi-Media, Mumbai India) for potassium solubilizers and Jenson (Hi-Media, Mumbai India) for Nitrogen fixers. The agar plates for bacteria were incubated for 24–48 h at 37 ˚C and 3–4 days at room temperature for fungi.
Determination of the Soil Enzymatic Activity

Soil urease activity

Urease activity was measured using the method that Tabatabai and Bremner (1972) outlined. A 50 ml volumetric flask containing one gram of soil sample was filled with 0.20 ml toluene and nine ml THAM buffer. The flasks were then swirled for a few minutes. One milliliter of a 0.2M urea solution was added to the flask and given another whirl. After that, the flask was sealed and kept at 37˚ C in an incubator. About 35 ml of Ag_2SO_4 solution was added and filtered after two hours. Using a Kjeldahl distillation apparatus, 20 ml of an aliquot was obtained and placed in a 100 ml distillation flask. The amount of NH_4-N released was found to be 0.2 g MgO. The unit of measurement for urease activity was µg NH_4-N released gram-1 of compost hour-1.

Dehydrogenase activity

Samples of soil were gathered and soaked in a polythene bag. A 20 g soil sample was incubated for 24 hours at 30˚C with 0.2 g $CaCO_3$ and 2 ml 1% diphenyltetrazolium chloride. After the incubation period, 25 milliliters of methanol were used to extract the soil sample. Triphenyl tetrazolium chloride is reduced to triphenyl tetrazoilum formazan, which is red in color, by the H+ ions produced by the microbial action. By detecting the intensity of red hue at 485 nm, dehydrogenase activity was found. It was then quantified as µg of TPF released/g soil/hour. (Tate and Terry, 1980).

Amylase activity

“The amylase activity of soil was determined in adaptation to the procedures described by Roberge (Roberge, 1978)” with modification. Starch was taken as substrate and incubated at 4hous at 37°C. “Amylase activity was computed using the formular CxV/dwt x sw x t Where; C is the measured concentration of glucose (pg ml'1) in the sample minus measured concentration of glucose (pg ml'1) in the blank. V is the volume of the suspension in ml, sw is the weight of soil taken (1 g), dwt is the weight of lg oven dry soil and t is the incubation time in hours”.

Phosphatase activity

Phosphatase activity of soil sample was determined by following the procedure of Eivasi and Tabatabai (1977). The soil samples' phosphatase activity was reported as µg paranitrophenyl solution. The process used to assess the acid phosphatase activity of the soil samples was also used to determine the alkaline phosphatase activity, with the exception of adjusting the pH of the modified universal buffer to 11.

Measurement of soil respiration

Microbial soil respiration was measured in the form of CO_2 evolved during incubation by the method of Andreson *et al*. (1990).

Determination of the physicochemical parameters of the soil

Organic carbon was determined according to the method of Nelson and Sommers (1996). The pH (in water, 1:2.5 w/v) soil samples of the soil samples was determined using standard procedures. Total N/C was obtained according to procedure of Bremner (1965). Macro- and microelement analyses were determined spectrophotometrically in samples digested by a mixture of HNO_3 and $HClO_4$ (Kacar, 1995).

Data analysis

Data obtained were presented as mean ± SD and subjected to analysis of variance (ANOVA).

Turkey's post hoc test was used to determine the p-value for the differences observed between test samples and control. Value of $p<0.05$ was considered to be statistically significant.

RESULTS

The microbial population growth in the soil sample on the various media was significantly different ($p<0.05$) among the biopesticides applied (Table 1). The bacterial growth was optimum when 100% chemical pesticide was applied, while application of tobacco only caused reduction in bacterial population the most. For fungal growth, it was the application of 10% chemical pesticides that did not have any impact while the application of combination of titanium, neem and 10% chemical pesticide increased fungal population to the maximum. The soil treated with combination of titonia, neem and 10% chemical pesticides did not have significant impact on the population of potassium solubilizers (7.20±0.40cfu/ml) but increased the population of phosphate solubilizer (5.85±0.11cfu/ml). The highest population growth (5.85±0.11cfu/ml) was observed in phosphate solubilizers with the application of combination of titonia + neem + 10% chemical pesticide while the population reduced to the minimal with the application of tobacco only (3.00±0.17cfu/mL).

Table 1: Effect of biological and chemical pesticides on microbial population growth.

Pesticides	Bacterial ($X10^6$) cfu/mL	Fungi ($X10^5$) cfu/mL	Potassium solubilizers ($X10^3$) cfu/mL	Nitrogen fixers ($X10^3$) cfu/mL	Phosphate solubilizers cfu/mL ($X10^3$)
Titonia + neem + 10% chemical pesticide	2.55 ± 0.18^{b}	4.50 ± 0.08^{a}	7.20 ± 0.40^{a}	4.50 ± 1.04^{b}	5.85 ± 0.11^{a}
100% chemical pesticide	3.40 ± 0.20^{a}	4.00 ± 0.10^{a}	5.50 ± 0.20^{c}	5.50 ± 0.30^{a}	4.65 ± 0.31^{b}
10% chemical pesticide	2.80 ± 0.09^{b}	2.50 ± 0.00^{c}	6.95 ± 0.58^{bc}	4.95 ± 0.28^{b}	5.10 ± 0.00^{a}
Titonia + neem	1.65 ± 0.52^{c}	4.50 ± 0.40^{a}	4.65 ± 0.13^{c}	4.95 ± 0.70^{b}	4.10 ± 0.39^{b}
Tobacco only	1.00 ± 0.16^{c}	3.50 ± 0.12^{b}	2.80 ± 0.10^{d}	2.15 ± 0.81^{c}	3.00 ± 0.17^{c}
Tobacco + 10% chemical pesticides	2.10 ± 0.24^{b}	3.50 ± 0.31^{b}	6.70 ± 0.82^{bc}	4.20 ± 0.00^{b}	3.85 ± 0.52^{c}
Control	2.95 ± 0.00^{b}	2.50 ± 0.06^{c}	7.95 ± 0.37^{a}	5.60 ± 0.20^{a}	4.65 ± 0.03^{b}

Table 1 displays the results as means ± standard deviation. The Duncan multiple range test rating of the post hoc test is shown by the superscripts. While the means with the same superscript were similar, those with different superscripts differed dramatically from one another down the column.

The applied biopesticides were significantly different ($P<0.05$) in their urease, dehydrogenase, amylase and phosphatase activities when comparatively studied (Table 2). The urease activity measured from the soil sample ranged from 0.01-0.0 μGnh_4-N/g soil/hour across the biopesticides used. The soil sample treated with 10% chemical pesticide and tobacco only had 0.03μGnh_4-N/g soil/hour of urease activity. The control experiment yielded the highest dehydrogenase activity (0.16 µg of TPF released/g soil/hour) but not significantly different from treatment (tobacco + 10% chemical pesticides) while no dehydrogenase activity was observed from the soil sample treated with 100% chemical pesticide and tobacco only. Amylase activity was highest in the soil sample treated with tobacco only (0.08±0.02Au/ml) while 100% chemical pesticide yielded no amylase activity at all. The two biopesticides with the optimum phosphatase activity were titinia+neem+10%chemical pesticide and titonia+neem with 0.05 µg of PNP released/g soil/hour, but no phosphates activity was observed in tobacco+10% chemical pesticide treated soil sample and the control experiment. Potassium concentration and sodium were maximum when titonia + neems were used as biopesticide. The control experiment however yielded the highest observed CEC level which was not significantly different from treatment (titonia + neems + 10% chemical pesticides). The entire parameters above were compared among the treatments and it was found that they all had significantly different ($p<0.05$) effects on the soil properties measured above. Titonia +neems treated soil yielded the highest recorded AvP (Average phosphorus). Hundred percent (100%) chemical pesticides yielded the highest organic carbon (1.34±0.00) follow by titonia + neems + 10% chemical pesticides (1.33±0.00). Titonia + neems + 10% chemical pesticides also yielded highest (Table 5). Total nitrogen (0.18±0.00) and optimum Fe was optimal when the soil samples were treated with

titonia and neems while for cu, it was the application of titonia neem and 10% chemical pesticides (2.25±0.45).

Table 2: The effect of pesticide on enzymatic activities as indicator of soil fertility

Sample code	Urease (µgNH_4-N/g soil/hour)	Dehydrogenase (µg of TPF released/g soil/hour)	Amylase (Au/ml)	Phosphatase (µg of PNP released/g soil/hour)
Titonia + neem + 10% chemical pesticide	0.01±0.00^{b}	0.04±0.00^{b}	0.05±0.00^{b}	0.05±0.00^{a}
100% chemical pesticide	0.01±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{d}	0.02±0.00^{b}
10% chemical pesticide	0.03±0.01^{a}	0.03±0.00^{b}	0.04±0.00^{c}	0.01±0.00^{c}
Titonia + neem	0.01±0.00^{b}	0.04±0.01^{b}	0.05±0.00^{b}	0.05±0.00^{a}
Tobacco only	0.03±0.00^{a}	0.00±0.00^{b}	0.08±0.02^{a}	0.01±0.00^{c}
Tobacco + 10% chemical pesticides	0.01±0.00^{b}	0.15±0.03^{a}	0.01±0.00^{d}	0.00±0.00^{d}
Control	0.01±0.00^{b}	0.16±0.00^{a}	0.03±0.00^{c}	0.00±0.00^{d}

Table 2 displays the results as means ± standard deviation. The Duncan Multiple Range Test rating of the post hoc test is shown by the superscripts. While the means with the same superscript were similar, those with different superscripts differed dramatically from one another down the column. The observed rate of soil respiration was significantly different ($p<0.05$) across the biopesticides studied (Table iii). The treatment with 100% chemical pesticide had the least soil respiration (10.15 mg/kg soil/hour) while tobacco only and tobacco + 10% chemical pesticides had the highest (17.00 mg/kg soil/hour)

Table 3: The effect of chemical and biological pesticides on soil respiration

Sample code	Soil respiration (mg/kg soil/hour)
Titonia + neem + 10% chemical pesticide	14.95±2.21^{b}
100% chemical pesticide	10.15±1.07^{c}
10% chemical pesticide	14.20±0.25^{b}
Titonia + neem	14.10±1.30^{b}
Tobacco only	17.00±0.90^{a}
Tobacco + 10% chemical pesticides	17.00±0.32^{a}
Control	14.90±0.11^{b}

Table 3 displays the results as means ± standard deviation. The Duncan Multiple Range Test rating of the post hoc test is shown by the superscripts. While the means with the same superscript were similar, those with different superscripts differed dramatically from one another down the column. The pH. measured from the soil samples after treatment with the various pesticides (chemical and biopesticides) ranged from 5.78-6.28. The pH was higher in the soil treated with tobacco only, titonia and neem, tobacco and 10% chemical pesticide, titonia, neem and 10% chemical pesticides when compare to control (Table 4a).

Table 4: Physicochemical parameters of the soil samples after the application of pesticide

S/N	pH	% Salt	% Clay	% Slit	Ca cmo/ Kg	Mg cmo/k g	K cmo/K g	Na cmo/K g	H+	CEC
Ti + N + 10% CP	6.20± 0.42^{a}	67.84±2 .00^{bc}	21.30±1 .80^{a}	10.86± 0.50^{d}	7.31± 0.40^{a}	1.40± 0.26^{c}	0.31± 0.49^{c}	0.16± 0.00^{c}	0.12± 0.00^{a}	9.30± 0.50^{a}
100% CP	5.78± 0.20^{b}	72.08±1 .07^{a}	18.00±1 .80^{b}	9.92± 1.06^{e}	4.42± 0.09^{b}	1.89± 2.00^{b}	0.40± 0.03^{c}	0.26± 0.10^{b}	0.12± 0.00^{a}	7.09± 0.04^{b}
10% CP	5.66± 0.50^{b}	71.46±0 .00^{a}	14.35±0 .39^{d}	14.19± 2.00^{a}	4.07± 0.00^{b}	1.91± 0.60^{c}	0.30± 0.00^{c}	0.12± 0.00^{c}	0.13± 0.00^{a}	6.52± 0.03^{c}
Ti + N	6.20± 0.30^{a}	69.77±4 .00^{b}	16.50±2 .83^{c}	13.73± 0.43^{b}	4.58± 0.00^{b}	1.40± 0.71^{c}	0.58± 0.01^{b}	0.36± 0.00^{a}	0.11± 0.00^{a}	7.03± 0.65^{b}
To only	6.20± 0.00^{a}	66.92±2 .40^{c}	20.86±1 .30^{a}	12.22± 0.21^{c}	2.75± 0.02^{d}	2.33± 0.00^{b}	0.56± 0.00^{b}	0.25± 0.00^{b}	0.10± 0.00^{a}	5.98± 0.00^{c}
To + 10% CP	5.91± 0.60^{b}	68.78±0 .30^{b}	19.95±0 .48^{b}	11.27± .0.80^{cd}	3.69± 0.00^{c}	4.24± 0.00^{a}	0.26± 0.00^{c}	0.30± 0.00^{a}	0.11± 0.00^{a}	8.61± 0.80^{ab}
Control	5.78± 0.04^{b}	70.72±0 .64^{a}	18.95±1 .00^{b}	10.33± 0.51^{d}	5.63± 0.04^{ab}	2.95± 0.01^{b}	0.73± 0.11^{a}	0.25± 0.00^{b}	0.12± 0.00^{a}	9.68± 0.00^{a}

Table 4 displays the results as means ± standard deviation. The Duncan Multiple Range Test rating of the post hoc test is shown by the superscripts. While the means with the same superscript were similar, those with different superscripts differed dramatically from one another down the column.

Table 5: Physicochemical parameters of the soil samples after treated with pesticides

S/N	Mn mg/Kg	Av. P	% T.N	% Org.C	B mg/kg	S mg/kg	Cu mg/Kg	Fe mg/kg	Zn mg/kg	EC Ms /cm
Ti + N + 10% CP	49.06± 1.60^{d}	31.90± 0.40^{a}	0.18± 0.02^{a}	1.33± 0.01^{a}	0.38± 0.00^{a}	8.48± 0.08^{b}	2.25± 0.45^{a}	151.47± 3.07^{b}	1.48± 0.50^{c}	73.63± 1.00^{b}
100% CP	108.92 ±0.93^{a}	21.15± 0.10^{b}	0.16± 0.00^{a}	1.34± 0.00^{a}	0.42± 0.00^{a}	7.43± 0.40^{c}	1.88± 0.09^{b}	137.01± 4.00^{c}	1.84± 0.71^{b}	60.87± 0.30^{c}
10% CP	57.92± 0.80^{cd}	13.53± 0.02^{c}	0.09± 0.00^{a}	1.22± 0.00^{a}	0.06± 0.00^{b}	5.15± 0.00^{d}	1.32± 0.10^{c}	102.19± 0.63^{cd}	2.89± 0.00^{a}	80.64± 1.03^{a}
Ti + N	55.16± 2.00^{cd}	34.14± 0.66^{a}	0.07± 0.00^{a}	1.14± 0.00^{a}	0.18± 0.00^{a}	5.84± 0.00^{d}	0.96± 0.07^{c}	177.28± 2.00^{a}	2.09± 0.20^{ab}	63.62± 0.50^{c}
To only	80.30± 0.61^{b}	14.62± 1.04^{c}	0.08± 0.00^{a}	0.85± 0.00^{b}	0.22± 0.00^{a}	11.9± 1.20^{a}	1.63± 0.02^{b}	152.02± 2.60^{b}	1.71± 0.00^{b}	51.14± 0.90^{d}
To + 10% CP	93.43± 0.87^{b}	19.27± 0.91^{b}	0.17± 0.00^{a}	1.16± 0.00^{a}	0.28± 0.00^{a}	8.41± 0.55^{b}	2.25± 0.01^{a}	84.50± 1.20^{d}	1.84± 0.00^{b}	62.66± 1.00^{c}
Control	77.26± 0.51^{c}	22.70± 0.12^{b}	0.13± 0.00^{a}	1.06± 0.00^{b}	0.29± 0.00^{a}	5.93± 0.15^{d}	1.59± 0.00^{bc}	152.20± 3.00^{b}	2.19± 0.04^{a}	55.28± 0.16^{d}

Table 5 displays the results as means ± standard deviation. The Duncan multiple range test rating of the post hoc test is shown by the superscripts. While the means with the same superscript were similar, those with different superscripts differed dramatically from one another down the column.

DISCUSSION

In our study, the result in table 1 indicated that most of the pesticides could greatly affect the population growth of soil microorganism. The increase in bacterial population observed in soil treated with 100% chemical pesticides (glyphosate) could be as a result of ability of most microorganism to degrade chemical pesticides making use of it as carbon source. Similar trends were reported by Balazs *et al.,* (2020) who reported abundant increase in microbial diversity and phyla both in main and rhizophere soil after treatment of soil with organochlorine pesticide lindane and also Khmelevtsova *et al.,* (2023) who reported increase in microbial population in genera like *Streptomyces, pseudomonas*, *rhodoplane* and *kaistobacter* after treatment of soil with S-ethydiprophythiocarbamate and altrazine pesticides. The application of tobacco to soil causes reduction in bacterial growth. This may be due to their ability to suppress microbial biomass when added to soil (Adediran *et al.,* 2004). In fungal population growth, it was observed that the application of 10% chemical pesticides does not have any impact on the fungal population while the application of combination of titanium, neem and 10% chemical pesticide increases fungal population to the maximum. This could be attributed to the component of neem which enhanced the growth of soil microorganism. It has been established that some protein or carbohydrate residues in neem are beneficial to the microorganisms. The highest population growth (5.85±0.11cfu/ml) was observed in phosphate solubilizers with the application of combination of titonia + neem + 10% chemical pesticide. Application of tobacco only and 100% chemical pesticides cause reduction in potassium solubilizers. This is in agreement with the findings of Taiwo and Oso, 1997 who reported significant reduction in microbial population and mineralization activities with the application of atrazine, npyrethrin and mixture of metobromuron and metolachor and the report of Ezaka *et al.,* (2020) who reported reduced potassium solubilizers with the treatment of glyphosate with high concentration of 14.4mg/ml. In all the biopesticides applied, tobacco only had been observed to cause drastic reduction in all the microbial population except fungi on which it has moderate effect on (3.50±0.12). This could be as a result of the nicotine content of tobacco which has been established to be toxic to most soil heterotrophs. Adediran *et al.*, (2003) however, found that adding tobacco waste to soil reduced the amount of microbial biomass, and they hypothesized that this could have been due to the nicotine in the trash. It was also noted that the use of chemical pesticides results in an increase in the number of bacteria, but the use

of all biological pesticides reduces the population of bacteria.

The interactions between soil and various environmental elements (such as acid rain, heavy metals, pesticides, and other industrial chemicals) that have an impact on the activities of soil enzymes determine their function and activity. Pesticide active components are specifically designed to disrupt the target organism's processes (enzymatic reaction, mitochondrial respiratory chain, macromolecules biosythensis, etc.). However, because of complexity and diversity of molecular processes the side impact of pesticide for a non-target species may harm their enzyme system and balance of important biomolecules (McLuckie *et al.,* 2020). The significance of the urease enzymes in the nitrogen cycle and the amylase enzyme's role in the soil carbon cycle led to their selection. Phosphatase was selected due to its significance in the phosphorus cycle, while dehydrogenase was chosen for the assessment of its total microbial activity. Urea is hydrolyzed by urease to produce carbon dioxide and ammonia, which microorganisms and plants can absorb (Bremner and Mulvanay, 1978). In table 2, the application of tobacco and tobacco with 10%chemical pesticides yielded the highest urease activity (0.03±0.00 and 0.03±0.01 μgNH_4-N/g). The high organic carbon content of tobacco, which in turn promotes the metabolic activity of the soil microbial population, may be the cause of the increased urease activity in soil when tobacco is the only ingredient applied. Lui *et al.* (2022) previously reported on this, observing that applying 100% organic residue led to the maximum rise in urease activity. All living microbial cells include intracellular dehydrogenases, which are connected to the respiratory functions of microbes. It was found that soil treated with 100% chemical pesticides showed no dehydrogenase activity. This was in line with the results of Lui *et al.* (2022). Amylase is a starch hydrolysing enzyme. It is known to be constituted by α-amylase and β-amylase. This enzyme is widely distributed in plants and soils so it plays a significant role in the breakdown of starch. In this study, the soil with the application of 100% chemical pesticides did not have any amylase activities and this could be due to the pesticide induced changes in starch degrading enzyme because of the pesticide's toxicity and also because these pesticides are degraded by soil microbial enzymes and in so doing could incorporate the intermediates or products into their biomass which could be inhibitory to amylase oxidizing microorganisms. Amylase activities was observed to be highest in soil with tobacco application (0.08±0.02 Au/ml). This was in conformity with the work of Mingov *et al.,* (2019) who reported enzymatic inhibition with the use of pesticide and the work of Esimbekova *et al.* (2022) who reported that the commercial pesticide fenvalerate which is a commercial chemical pesticide formulation were toxic and inhibited activity of amylase, esterase, glutathione reductase and other enzymes. It was observed that pesticides application with the optimum phosphatase activity are titonia+neem+10%chemical pesticide and titonia+neem with 0.05 µg of PNP released/g soil/hour each but no phosphates activity was observed in tobacco+10% chemical pesticide treated soil sample and the control experiment. The organic content of many biopesticides, particularly neem, may be the cause of their highest phosphatase activities. Moreover, the additional organic material may contain intra- and extracellular enzymes and may encourage soil microbial activity. It might possibly result from azadirachtin's impact on soil microbes, which would then break down and release phosphates from the deceased microbial biomass.

Table 3 shows the effect of chemical and biological pesticides on soil respiration. Soil respiration (carbondioxide evolution) is the flux of carbondioxide (CO_2) emitted by soil to the atmosphere.it is a major component of the global carbon cycle (Hursh *et al.,* 2017). It is an essential indicator of soil health and it is related to the quantity of the soil organic carbon in the upper soil and litter layers (Numa *et al.,* 2021). The observed rate of soil respiration was significantly different ($p<0.05$) across the pesticides (chemical and biological pesticides) studied. The application of tobacco and tobacco with 10% chemical pesticides yielded the highest soil respiration (17.00 mg/kg soil/hour) probably due to the ability of tobacco to supports the growth of soil heterotrophs. Hundred percentage (100%) chemical pesticide had the least soil respiration due to the inhibitory effect of chemical pesticides on soil respiration. This result is consistent with studies published by Sweta *et al.,* (2023) which found that *imidacloprid* and *dimethoate* had a diminishing effect on the evolution of carbon dioxide from treated soil samples, and by Karpun *et al.,* (2021) which also found that applying *Chlorpyrifos*, *dimethoate, phosalone,* and *kresoxim-methyl* causes a decrease in soil respiration.

The physiochemical characteristics of the soil samples following pesticide application are displayed in Table 4. Following application of several pesticides (chemical and

biopesticides), the pH of the soil varied from 5.66 to 6.20. The activity of numerous important microorganisms and the availability of nutrients are both impacted by pH. Since high alkalinity is bad for microorganisms, all of the samples were determined to be non-alkaline and within the typical range for healthy growth.

The pH was higher in the soil treated with tobacco only, titonia and neem, tobacco and 10% chemical pesticide, titonia, neem and 10% chemical pesticides when compare to control. For calcium, it was titonia + neems + 10% chemical pesticides that yielded the highest while potassium concentration, and sodium were maximum when titonia + neems was used as biopesticide. Alice *et al.,* 2016 also observed increase in calcium, sodium and potassium concentration when titonia and neems were added as soil amendment. Application of tobacco and 10% chemical pesticides was observed to have the highest magnesium and application of titonia and neem was observed to have increase potassium level compare to other pesticides. This is in agreement with the finding of Hafifah *et al.,* (2016) who reported in his findings that potassium (K) increased from 2.59 cmol/kg to 8.34 cmol/kg. The increase in exchangeable K on plot treatments with *Titonia diversifolia* was probably due to a great release of this nutrient by the decomposing residues that contained large amounts of K. it was reported that *T. diversifolia* was superior in soil Kolawole *et al*. (2014) also reported that soil exchangeable K was found to increase with mulch rate up to 20 t/ha. The control experiment (9.68±0.00) however yielded the highest observed CEC level followed by titonia and neem and 10% chemical pesticide (9.30±0.50), and as well as application of tobacco and 10% chemical pesticides (8.61±0.80). These observed trends could be due to significant organic matter which provide stable soil aggregate condition and prevented eroding of valuable nutrients and this also explained the trend of significant difference for effective cation exchange capacity (CEC).

In Table 5, the entire parameters above were compared among the treatments and it was found that they all had significantly different ($p<0.05$) effects on the soil properties measured above. Titonia +neems treated soil yielded the highest Av.P (average phosphorus). This could be due to the increase of soil ph observed in the soil treated with titonia as it has been established that phosphate becomes more accessible by plant as pH rises (Habi *et al*., 2018). Hafifah *et al,* (2016) reported that the highest increased of P was found in treatment with *T.diversifolia* and the lowest in control. Compared to the initial soil quality, the soil available P increased from 0, 37 mg/kg to 64, 24 mg/kg. The high organic carbon recorded in application of 100% chemical pesticides could be because chemical pesticides are a good source of carbon and some microorganisms make use of it as carbon source of energy. Similar finding was reported by Baboo *et al.* (2013) that from day 7 to day 28, the TOC increased gradually. In soils with high organic carbon concentrations, certain pesticides degrade more quickly, most likely due to increased microbial activity. Furthermore, the presence of organic carbon greatly influences the fate of these pesticides by promoting their breakdown in soil. The maximum total nitrogen (TN) (0.18±0.02) was obtained by applying titonia, neem, and 10% chemical insecticides. This result is consistent with research by Aboyeji *et al.,* (2020), which found that applying *T. diversifolia* green manure enhanced the amount of organic N content available in the soil, as opposed to using chemical pesticides, and that this had a favorable impact on vegetative metrics. The Fe value was at its peak when the optimum Fe was observed when the soil samples were treated with titonia and neems (177.28±2.00).

CONCLUSION

In spite of the well-acknowledged role of microflora in soil ecosystem services, the assessment of soil fertility based only on the physicochemical characteristics of the soil has been consistently carried out with little research done on the microbial population and enzymatic activities. The use of pesticides to combat diseases and pests resulted in soil contamination and a sharp rise in the dangerous residues present in many agricultural products, endangering the ecosystem of the soil. In this present study, application of most of the biological pesticides singly and in combination with 10% chemical pesticides increased microbial population, enzymatic activities and micro and macronutrients when compared to the chemical pesticide. Increased microbial population, enzymatic activities and physiochemical parameters are good indicators of soil fertility. This studied also recommend the need for farmers to consider the judicious use of biological pesticides such as tobacco, neems and titonia in combination with 10% chemical pesticides. This integrated approach of the application of biological and chemical methods to battle diseases and pests may be a best solution to the problems associated with relying solely on chemical pesticides. The method has a potential of promoting good soil health,

increased soil fertility, food quality and safety. Future studies should be focus on the metagenomics and metatranscriptomics to analyse the microbial community structure and functional genes which will give insight into how pesticides affect microbial diversity and function.

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Author contribution

Author AAB conceived and designed the research work. AAB, EE and OAO performed the experiment. AAT and AS analyzed the data. The original manuscript was prepared by AAB while TLB and AAT revised the manuscript. All authors read and approved the final manuscript.

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The authors have no conflict of interest to declare.

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