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Research article

Effect of Paraquat on Soil Microorganisms

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Abstract
Pesticides are widely used in agriculture for pest control but inadvertently affect soil microbial communities. This study determined the effect of Paraquat on soil microorganisms. A microcosm study was conducted to investigate the effect of the pesticide on soil microorganisms, over a 7-day period. Five different treatments were set up: T1 (control); T2, 10kg of soil + 50 ml of pesticide, T3; 10kg of soil + 100ml of pesticide, T4; 10kg of soil + 150 ml of pesticide, T5; 10kg of soil + 200 ml of pesticide. Microbial population was determined by standard plate count. Bacterial and fungal isolates were characterised based on their cultural characteristics and biochemical tests. The results showed that the bacterial and fungal population in treated soil, decreased significantly ($p<0.05$) at higher concentrations of pesticide (T4 and T5). The bacteria *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp. and *Arthrobacter* sp., and the fungi *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp., were identified as the pesticide degrader/utilizers. This study suggests that the exposure to pesticides negatively impacts the survival and proliferation of soil bacteria and fungi. The use of pesticides should be avoided or in unavoidable cases reduced to the minimum to protect soil microorganisms.

Keywords: Agriculture, microbial communities, paraquat, pesticide-utilising microorganisms

Introduction

Increase in human population has placed higher demand for food. This has resulted in significant advanced in agricultural technology including the use of chemicals, to optimize crop quality and yield (Zhu *et al.*, 2004). The activities of crop pests have posed a daunting challenge to food safety and food security, and have limited the quality of productive capacity of soil as a strategic agricultural input (Araujo *et al.*, 2003). This trend has resulted in the application of chemical compounds known as pesticides that can deter, repel, and kill agricultural pests.

According to Sukukl and Spiteller (2001), a pesticide is a substance or mixture of substances intended for preventing, destroying, repelling, or lessening the damage of any pest. The pest can be insects, plant pathogens, mollusks, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread diseases, and are seen as a nuisance. The most commonly used pesticides are classified as fungicides, insecticides, rodenticide and herbicides. The ability to exterminate weeds and other agricultural pests make pesticides highly essential since these actions increase the yield and growth of plants.

From an environmental health standpoint, an ideal pesticide should be toxic only to the target organism, biodegradable and undesirable residues should not affect non-target surfaces (Vischetti *et al.*, 2000). However, preponderance of pesticides in use are not target specific and do not reach the target organisms, thereby leaving residues in the environment (Dennis *et al.*, 2018). The persistence of these pesticides is influenced by various factors. It was reported by Perucci *et al.* (2000) and Dennis *et al*. (2018) that the soil properties, components of pesticides and the climate influences the persistence of pesticides, and that these factors powerfully

interrelate with one another. Owing to its recalcitrant nature, pesticides often cause adverse effects to even non-target organisms, which is a cause for concern (Olurominiyi and Emily, 2011). If pesticides persist for longer periods, there may be an increased absorption and accumulation of toxic chemicals by plants, thus making the consumption of such contaminated plant hazardous (Perucci *et al.,* 2000).

Due to the toxicity of pesticides, there have been growing concerns over their effect on beneficial soil microorganisms (Haney and Senseman, 2000). Microorganisms are core components of soil, where they play indispensable role as nutrient recyclers in biogeochemical cycles and nutrient fixation, and invariably optimize soil structure and function and affect its fertility (Adam and Duncan, 2001). For example, the maintenance of soil fertility is aided by nitrifying bacteria. Elemental nitrogen is required by plants in large quantities. The activities of the nitrifying bacteria present in the soil make nitrogen available through nitrification processes but in the form of nitrate ions (Perucci *et al*., 2000; Adam and Duncan, 2001; Ahmed and Holmstrom, 2014; Oyedeji and Immanuel, 2023, Oyedeji *et al*., 2024). The application of pesticides may result in damage to the soil microbiota, especially the important ones such as nitrogen-fixing bacteria, the nitrifying bacteria, siderophore-producing bacteria, phosphate solubilizing bacteria, mycorrhiza, plant growth promoting and other microbial groups that contributes to soil health and overall functionality (Baxter and Cummings, 2008). Pesticides affect soil microorganism even at low concentrations, depending the chemical properties, biochemical interaction and soil conditions (Subhani *et al.,* 2000; Cycon *et al.,* 2010).

Again, a major portion of pesticides from agriculture applications may amass in soil and further accumulate with indiscriminate and repeated use of pesticides, though subject to a variety of transport, adsorption/desorption and degradation processes (Andreu and Pico, 2004). Pesticides and their degradation products, interact with the soil microbiota, altering microbial diversity, biochemical reactions and soil enzymatic activities (Dennis *et al.,* 2018). Any perturbation of soil microbial population and diversity, may eventually alter soil ecosystem and bring about loss of soil fertility.

The direct application of pesticides to plants result to run-off which introduces pesticides into the soil. Once pesticides reach the soil, they are either stuck within the pore spaces or immobilized on the soil surface, particularly soil organic matter and humus, because of their large surface area (Haney and Senseman, 2000; Araujo *et al.,* 2003). Animals accumulate pesticides mostly through feeding. Pesticide transport and transfer to human upon eating food treated with pesticides or grown in soil treated with pesticides, has been reported in several studies. The study by Band *et al.* (2011) elucidates the accumulation of pesticides and their impacts on the tissues and organs of biological systems. In a related study by Ezemonye *et al.* (2010), varying concentrations and the impact of some selected groups of pesticides in Warri, Delta state, Nigeria were recounted. Similarly, Adeyemi *et al.* (2011) recounted the occurrence and concentration of the residues of organochlorines in fish samples harvested in a Lagoon in Lagos. The follow-up study conducted by Ikpesu and Ariyo (2013) gave an account of food crops containing high concentrations of lindane. This resulted to the eventual ban of Lindane in food preservation. The hazardous impacts of pesticides on the environment and microbial community are scarcely known by farmers in developing societies, despite an increase in their application (Ikpesu and Ariyo, 2013).

Paraquat (methyl viologen) is a chlorinated hydrocarbon insecticides used in farmlands and household for the control of weeds and grasses. Several studies have reported on the disruptive nature of Paraquat on soil microbiota, the impact on the nutritional quality of crop, ecological consequences of application in area where it had been used on a large scale (Dennis *et al*., 2018). It is therefore important to be able to estimate the impact of Paraquat on soil microorganisms, as it affects local farming communities. This study aimed to assess the effect of Paraquat, a common herbicide, on soil microorganisms.

Materials and Methods

Sources of Chemicals/Reagents

Analytical-grade reagents were procured and used in this study. Paraquat was purchased from A2Z Chemicals Ltd, a local chemical vendor residing in Yenagoa.

Soil Sampling
The organisms used in this study were isolated from farmland, in Toru-Orua Community, Sagbama Local Government Areas, Bayelsa State, Nigeria. Topsoil (0-15cm) samples around the root of plants were collected using a sterile soil auger and were stored in sterile plastic bags, and taken to the laboratory for analysis.

Experimental Design
The experimental design involved five different treatment groups.
Treatment 1: This served as a control group. The soil samples in group 1 were not polluted by the pesticides. Each soil samples weighed 10kg.
Treatment 2: 10kg of soil + 50ml of pesticide
Treatment 3: 10kg of soil + 100ml of pesticide
Treatment 4: 10kg of soil + 150 ml of pesticide
Treatment 5: 10kg of soil + 200 ml of pesticide

The soil was exposed to the pesticides for 7 days. During this period, enumeration of soil microbial pollution was carried out to determine the impact of the pesticides on the microbial pollution on day 1, 2, 3, 5 and 7 (Araujo *et al*., 2003; Dennis *et al*., 2018).

Microbial Isolation and Identification
Pour plate method was used for the isolation of microorganisms from soil the samples. The colonies were randomly selected and picked off with a sterile wire loop. The colonies were subcultured on fresh nutrient agar plates by streaking colonies on the agar surface using the three-loop method. The plates were inverted and incubated at room temperature (28±2^{o} C), to obtain pure isolates (Aneja, 2003).

Enumeration of Total Heterotrophic Bacteria
Spread plate method was used to determine the total heterotrophic bacterial count (THBC). A ten-fold dilution of soil sample suspension (1:10) ranging from 10^{-1} to 10^{-6} was made. 1ml from 10^{-5} and 10^{-6} dilution was used to inoculate nutrient agar plates and spread uniformly with a flame-sterilized bent glass rod. Plates were incubated at room temperature for 24 hours. The mean count of microorganisms from triplicate plates was obtained and expressed in CFU/g (Aneja, 2003).

Enumeration of Total Fungi
The fungal isolates were isolated using Potatoe Dextrose Agar (PDA). 1ml of the ten-fold serial dilution of 10^{-3} of the soil sample was inoculated on sterile PDA and spread uniformly with a flame-sterilized bent glass rod. The inoculated PDA plates were incubated at room temperature for 5 days (Aneja, 2003).

Pesticide Degrading/Tolerant Bacteria
Bushnell Haas medium was used to determine the ability of the soil bacteria to tolerate or degrade the pesticide. 10ml of Bushnell Hans broth was modified with 1ml of the pesticide. Thereafter, 0.1ml of the bacterial inoculum (broth) was introduced into the medium. The medium was incubated for 7 days to determine the viability of the bacterial test isolates (Aneja, 2003). Insert a reference

Identification of Bacterial and Fungal Isolates
Bacterial isolates were identified on the based on their Gram reaction and biochemical profiles such as oxidase, catalase, indole, coagulase, citrate and Kligler's iron agar, with reference to Aneja (2003). Macroscopic and microscopic approaches were adopted for identification of fungi with reference to Salvamani and Nawawi (2014).

Results
Microbial Population in Soil
Table 1 shows bacterial population in treated and unpolluted soil samples. In treatment T2 bacterial population decreased from 2.49 x 10^3 CFU/g on day 1 to 2.25 x 10^3 CFU/g on day 7; in T3, it decreased from 2.5 x 10^3 CFU/g to 2.18 x 10^3 CFU/g; in T4, it decreased from 2.4 x 10^3 CFU/g to 1.96 x 10^3 CFU/g and in T5, it decreased from 2.43 x 10^3 CFU/g to 1.83 x 10^3 CFU/g. In the control (unpolluted soil), the population varied from 2.24 x 10^3 CFU/g to 2.33 x 10^3 CFU/g.

Table 1: Response of soil bacteria to pesticide

Treatments	Day-1	THBC (CFU/g)		Day-7
		Day-3	Day-5	
T1 (Control)	2240 ± 11.2[a]	2330 ± 9.45[a]	2450 ± 24.2[a]	2330 ± 11.5[a]
T2	2490 ± 21.0[a]	2320 ± 15.0[a]	2280 ± 21.5[a]	2250 ± 17.0[a]
T3	2500 ± 13.0[a]	2500 ± 13.0[b]	2250 ± 8.88[c]	2180 ± 7.37[ab]
T4	2400 ± 8.02[a]	2140 ± 26.0[b]	2040 ± 21.3[c]	1960 ± 9.84[ab]
T5	2430 ± 11.6[a]	1930 ± 8.5[b]	1840 ± 3.05[c]	1830 ± 10.5[ab]

Table 2: Response of soil fungi to pesticide

Treatments	Day-1	TFC (CFU/g)		Day-7
		Day-3	Day-5	
T1 (Control)	160 ± 5.50[a]	120 ± 0.00[a]	80 ± 3.51[a]	150 ± 2.00[a]
T2	150 ± 0.00[a]	110 ± 1.15[b]	100 ± 1.52[c]	70 ± 1.15[ab]
T3	150 ± 3.00[a]	130 ± 1.52[a]	120 ± 0.00[a]	100 ± 2.00[a]
T4	140 ± 3.00[a]	130 ± 1.52[b]	100 ± 0.00[c]	80 ± 2.00[ab]
T5	140 ± 1.15[a]	120 ± 0.57[a]	110 ± 1.52[b]	80 ± 4.00[ab]

Table 3: Pesticide utilizing bacteria

Isolate	Growth in modified Bushnell Haas media
Pseudomonas sp.	+
Micrococcus sp.	+
Bacillus sp.	+
Staphylococcus sp.	+
Arthrobacter sp.	+

Table 4: Pesticide utilizing fungi

Fungi	Growth in modified Bushnell Haas media
Penicillium sp	+
Aspergillus sp.	+
Trichoderma sp	+
Fusarium sp.	+

Table 2 shows fungal population in treated and unpolluted soil samples. In treatment T2, fungal population decreased from 1.5 x 10^2 CFU/g on day 1 to 7.0 x 10^2 CFU/g on day 7; in T3, it decreased from 1.5 x 10^2 CFU/g to 1.0 x 10^2 CFU/g; in T4, it decreased from 1.4 x 10^2 CFU/g to 8.0 x 10^1 CFU/g; and in T5, it decreased from 1.4 x 10^2 CFU/g to 8.0 x 10^1 CFU/g. In the control (unpolluted soil), the population varied from 1.6 x 10^2 CFU/g to 1.5 x 10^2 CFU/g.

Pesticides Utilising Microorganisms

Isolates capable of growing at different Paraquat concentrations are shown in Table 3 below. Five (5) bacterial isolates were recovered from the Bushnell-Haas medium, and were identified as: *Arthrobacter* sp., *Bacillus* sp.., *Micrococcus* sp., *Pseudomonas* sp. *and Staphylococcus* sp. The pesticide-utilizing fungi detected include *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp. (Table 4).

Discussion

Bacterial and fungal population decreased in the pesticide treated soil, relative to the control. This study suggests that the exposure of soil microorganisms to pesticides negatively impacts their proliferation and survival. The results obtained from Treatment 1 indicate slight variations in the population of soil bacteria on the different days of exposure. Nevertheless, the analysis of variance (ANOVA) indicates there is no significant statistical difference in the population of the soil bacteria during the different days of exposure to the pesticide. This suggests that the population of soil bacteria in unpolluted soils is fairly stable. In relation to the response of the soil bacteria in Treatment 2, it was observed that there was a light decline in the population of the soil bacteria from 2490 ± 21.0 at day 1 to 2250 ± 17.0 at day 7. The analysis of variance indicated that there were no significant statistical difference in the population of soil bacteria over the days of exposure. However, at higher concentrations of the pesticides (Treatments 3 – 5) the analysis of variance suggests that there was significant statistical difference in the population of soil bacteria at the different days of exposure.

In this study, it was observed that the population of the soil bacteria declined across the different days of exposure, even at low pesticides concentrations. This is similar to the findings of Cycon *et al*. (2006) who reported that even if the pesticides are used at low concentrations, they affect chemical and biological properties of the soil microorganisms. In contrast, Zhang *et al.,* (2008) reported an increase in the number of gram-negative bacteria on application of the insecticide, cypermethrin, which may have acted as a nutrient for the growth of some them. The utilization of pesticide by some bacteria may also be responsible for the varied response observed in this study. According to the study of Haney and Senseman, (2000), the effects of pesticides on microorganisms vary depending on the chemical dosage, the properties of the soil and various environmental factors.

This study also investigated the response of the soil fungi to pesticide. Similar to the response of the soil bacteria to pesticide, the control treatment did not show significant statistical difference ($p<0.5$). Likewise, treatment 2 recorded no significant statistical difference. Nevertheless, Treatments 4 and 5 showed that the difference in the population of the soil fungi had significantly decreased ($p<0.05$). In comparison to the response of the bacteria, the soil fungi showed greater degree of response to the pesticide exposure. In the study of Matus *et al.* (2023) it was suggested that soil fungi have a greater ability to resist the application of pesticides.

Isolates capable of growing at different Paraquat concentrations or tolerant to the pesticides include five (5) bacterial isolates (*Arthrobacter* sp., *Bacillus* sp.., *Micrococcus* sp., *Pseudomonas* sp. *and Staphylococcus* sp.) and four (4) fungal species (*Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp.). The results obtained is in harmony with the report of Baxter and Cummings, (2008) that expressly affirmed that the application of pesticides may result in damage to the soil microbiota, especially the important ones such as nitrogen-fixing bacteria, the nitrifying bacteria, siderophore-producing bacteria, phosphate solubilizing bacteria, mycorrhizal, plant growth promoting and other microbial groups that contributes that contributes to soil fertility. Nevertheless, the effect of pesticides on soil microorganisms and their activity depends upon the type of pesticides used, quantities, and soil conditions (Subhani *et al.,* 2000; Singh and Mandal 2008; Cycon *et al.,* 2010).

Certain bacteria and fungi are able to utilize pesticide as a source of carbon. Thus, they are able to survive and proliferate in pesticide polluted environments. In this study, soil bacteria and fungi were tolerant to pesticides, which is taken an indication of their ability to utilize pesticide as carbon source. The study of Wanguyun and Geraldi (2019) reported that the decomposition of pesticides by certain groups of microorganisms begin a few days after exposure. Alternatively, the secondary microbial population producing induce enzymes usually breakdown pesticides while they undergo a prolonged adaptation time. In a related study, Hicks (2013) reported that pesticides can be utilized and degraded by some bacterial genera such as; *Bacillus,* Actinomycetes, *Mycoplana, Agrobacterium, Arthrobacter, Corynebacterium, Pseudomonas, Arthrobacter, Flavobacterium, Nocardia* and *Trichoderma* (Hicks, 2013). However, some groups of microorganisms may be indifferent to the application of pesticides. Matus *et al.* (2023) reported that the majority of herbicides do not have a drastic negative impact on the soil

fungal population, at least at the recommended levels. The fungi belong to the group of microorganisms that, after an initial sensible response to the presence of pesticides in the soil, rapidly establish normal metabolism, even enabling them to increase in number, particularly in the case of fungicide and insecticide application (Perucci *et al.*, 2000; Dennis *et al*., 2018).

Conclusion

This study demonstrated that the exposure of microorganisms to pesticide depletes the soil microbial population. The effect of the pesticide on the soil microorganisms was observed to be dependent on both the duration of exposure and the concentration of the pesticide pollutant. Some bacteria and fungi were able to utilize and grow in pesticide polluted medium. These organisms were identified as *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp., and *Arthrobacter* sp., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp. They can be explored for degradation of pesticides.

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