

BIODEGRADATION OF CARBOFURAN AND PARAQUAT BY INDIGENOUS SOIL MICROORGANISMS

ABSTRACT

Aim: The study was aimed at biodegrading Carbofuran and Paraquat using active microbes present in a farmyard soil at the Federal University of Petroleum Resources (FUPRE), Ugbomoro Community, Delta State.

Study design: Microcosms were set-up in triplicates; sampling for pesticides' loss and microbial counts were done bi-weekly.

Place and duration of study: Study was done in Environmental Management and Toxicology Department, FUPRE and Chemistry Department, University of Benin between June 2019 and July 2019.

Methodology: Carbofuran and Paraquat were applied to farmyard soil containing active microbial population at recommended and four times the recommended doses. A sterile control was equally setup. Biodegradation was monitored with the High Performance Liquid Chromatography (HPLC). Microbial counts were monitored using standard methods.

Results: Carbofuran decreased from 108.6 ± 0.69 $\mu\text{g}/\text{kg}$ (day 0) to 39.2 ± 3.8 $\mu\text{g}/\text{kg}$ (day 28) and 301.4 ± 1.29 $\mu\text{g}/\text{kg}$ (day 0) to 241.4 ± 2.83 $\mu\text{g}/\text{kg}$ (day 28) for recommended and four times recommended rates, respectively. Also, there were complete removal of Paraquat in treatments with the recommended rate while it decreased from 268.3 ± 5.21 $\mu\text{g}/\text{kg}$ (day 0) to 144.4 ± 2.38 $\mu\text{g}/\text{kg}$ (day 28) at four times the recommended rate. In the abiotic control, were little losses of both pesticides. Total heterotrophic bacterial, fungal and actinomycetes counts increased (day 14 to 21) in contaminated soils. Significant differences in degradation with respect to pesticides treatment and time were observed.

Conclusion: The microorganisms grew at different concentrations reducing both Carbofuran and Paraquat in contaminated soils. Their presence and high numbers confirmed that they are ubiquitous, diverse and can adjust to any harsh environment. Increased microbial counts showed that they grew in presence of the chemicals and degraded them. Thus, this indigenous microbial population can be used for the clean-up of these pesticides pollution in farms to improve such environment.

Keywords: Carbofuran, Paraquat, Indigenous microorganisms, High Performance Liquid Chromatography.

1. INTRODUCTION

Application of pesticides on plants is meant to protect plants against several sets of pests.

Although, these chemicals are applied in low concentrations, once in the soil they can change the biochemical proprieties of that soil and also have impact on soil microorganisms. The impacts of pesticides on soil microorganisms consist of reduction in microbial counts, alterations in biochemical activity, quantitative and qualitative decrease of the microbial community [1]. Still, determining the influence of pesticides on microbial communities in soil is a challenge because many variables like habitat, soil structure, organic and inorganic composition, texture, pH and temperature should be taken into consideration [2].

The breakdown of pesticides by organisms depends on some physical and chemical environmental factors which include but are not limited to; temperature, moisture and soil pH. Breakdown is also dependent on constituents of the pesticides (which may include; its hydrophilicity, degree of solubilization), microbial population and diversity and biochemical reactions. The breakdown of pesticides by these physical and chemical environmental factors is a resultant effect of physico-chemical alterations or changes of the pesticides by processes which includes; photolysis, hydrolysis, oxidation and reduction. Also, there may be the challenge of bioavailability of these pesticides due to partitioning which results in attachment or adherence of the pesticide compounds to soil and soil colloids still in its original chemical form or structure [3].

Nevertheless, the main way of detoxifying pesticides is through biological means powered by enzymes (enzymatic reactions/changes) present in plants and microorganisms [4,5,6]. The issue of waste (liquid and solid) generation as a result of huge amounts (millions of tons) of pesticides utilized on a yearly basis is a major concern globally. Moreover, the indiscriminate application of pesticides on soil and even water leads to pollution which in turn affects the food chains. Among the main consequences of soil pollution, is the loss of fertility, which directly or indirectly allow the survival of the flora and fauna, given the tight interrelationships among the different elements, which constitute the ecosystems [3].

Biodegradation is total breakdown of an organic substance into its inorganic constituents by microorganisms. Biodegradation of pesticides is controlled by the bioavailability of the pesticide to a pesticide-degrading microorganism and the activeness of the microorganism. In the microbial world, bacteria, fungi and actinomycetes are the main transformers and pesticide-degrading organisms [7]. Fungi alter or modify pesticides and other non-biological (xenobiotics) substances biologically by causing slight modifications

in the structure of the substance, thereby detoxifying it. The biologically restructured (biotransformed) pesticide is discharged into the environment, where it is accessible or bioavailable to bacteria for further breakdown [8].

Nonetheless, the key way of neutralizing pesticides is via natural means driven by enzymes (enzymatic responses/modifications) existing in plants and microorganisms [4,9,10,11,12]. Their growth competences in these pesticides contaminated ecosystems will nurture the break and elimination of these toxicants from such systems, hence this study. Furthermore, knowledge of what a community is composed of and the way it is structured is central to many ecological and environmental studies such as chemical and environmental engineering, soil microbiology, biodegradation, bioremediation and marine microbiology.

2. MATERIAL AND METHODS

2.1 Pesticides characterization

The pesticides (Carbofuran and Paraquat) were characterized using a gas chromatography- mass spectroscopy (GC-MS) [13]. The different compounds were identified after preparing and digesting samples using the gas chromatography-mass spectrophotometer Model 7820 (Agilent instruments, USA) based on mass to charge ratio.

2.2 Pesticides degradation

The modified methods of Naqvi, Kanhar, Shar, Hussain & Ahmed [14] and Madella & Kadiyala [15] were adopted. The treatments were done in triplicates at two different concentrations of the pesticides with an abiotic control. One (1) kg of farmyard soil was weighed into each pot, spiked with pesticides at recommended and four times the recommended application rates. The set up was left for 28 days. The experimental conditions were as follow;

- | | | | | | | | |
|----|---|----------|------|---|-----------|----------|----------|
| T1 | –sterile | farmyard | soil | + | pesticide | (abiotic | control) |
| T2 | – farmyard soil + recommended concentration of the pesticide | | | | | | |
| T3 | – farmyard soil + four times the recommended concentration of the pesticide | | | | | | |

Pesticide residue in soil was determined using High Performance Liquid Chromatography (HPLC) weekly [16].

2.3 Microbiological analysis

The population count of microorganisms was carried out by traditional viable cell counts weekly. One (1) gram of each soil sample was suspended in 9 ml of sterile distilled water. Serial dilution was done aseptically under laminar flow. Aliquots (0.1ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Rose-Bengal chloramphenicol agar was used for the enumeration of fungi [17]. Plate count agar (PCA) was used for the enumeration of heterotrophic bacteria [18]. Actinomycetes were enumerated using starch-casein agar [19] and individual colonies were recorded as colony forming units (CFU).

3. RESULTS AND DISCUSSION

3.1 Pesticides

The pesticides used in this study were characterized using a Gas Chromatography-Mass Spectrophotometer (GC-MS) and the results were based on mass to charge ratio. Paraquat contained 2- amino- 1- propanol, 1-propanol, 2-amino, 4,4'- bipyridine, 3, 3'-bipyridine, paraquat dichloride, bromo-benzene, neopentane and dimethyl- diazene. Carbofuran contained over thirty (30) different compounds including tetradecane, oxalic acid, allyl pentadecyl ester, 2-aminononadecane, 2,6- pyrazinediamine, n-nonadecanol-1, 1-ecosanol, nonadecane, isobutyl nitrite, heptanonitrile, carbofuran and others as seen in Table 1.

Table 1: Constituent compounds of pesticides used during the investigation

Pesticide		
S/N	Paraquat	Carbofuran
1	2- amino- 1- propanol	Oxalic acid, allyl pentadecyl ester
2	Benzene	Oxalic acid, allyl tridecyl ester

3	4,4'- bipyridine	Tetradecane
4	3, 3'-bipyridine	O-decyl- hydroxylamine
5	Paraquat dichloride	Propanoic acid
6	Bromo – benzene	Oxalic acid, allyl decyl ester
7	Neopentane	2-aminononadecane
8	Dimethyl – diazene	2,6- pyrazinediamine
9		6-methylheptyl vinyl ether
10		2,4,6,8-tetramethyl-1-undecene
11		1-cyclohexylnonene
12		1, 1,5-pentadecanediol
13		n-nonadecanol-1
14		1-ecosanol
15		2,3-dihydro-2,2-dimethyl-7-benzofuranol
16		Carbofuran
17		Nonadecane
18		2,6,10,14-tetramethyl heptadecane
19		Oxalic acid, allyl hexadecyl ester

Pesticide

S/N	Paraquat	Carbofuran
20		2-methyl-1-nitro – propane

21	O-(2-methylpropyl) - hydroxylamine,
22	Oxalic acid, allyl heptyl ester
23	1-isociano – butane
24	Oxalic acid, allyl hexyl ester
25	Heptanonitrile
26	1-nitro – heptane
27	2-methyl-1-nitro- propane
28	2,2-dimethyl-butane
29	Isobutyl nitrite
30	2,2,4-trimethyl-3-pentanone
31	2-methyl-, 2-propenyl ester
32	2,3-dihydro-2,2-dimethyl-7-benzofuranol
33	Oxalic acid, allyl pentadecyl ester
34	1,3-propanediol
35	Tetradecane
36	Oxalic acid, allyl nonyl ester
37	Oxalic acid, butyl propyl ester

3.2 Pesticides degradation by soil microorganisms

Soil microorganisms' ability to breakdown Carbofuran and Paraquat in soil after their application at recommended and four times recommended rates were assessed using HPLC. From the study, there were decreases in Carbofuran from $108.6 \pm 0.69 \mu\text{g/kg}$ (day 0)

to $39.2 \pm 3.8 \mu\text{g/kg}$ (day 28) at the recommended rate while it reduced from $301.4 \pm 1.29 \mu\text{g/kg}$ (day 0) to $241.4 \pm 2.83 \mu\text{g/kg}$ (day 28) at four times recommended rate as seen in Fig.1.

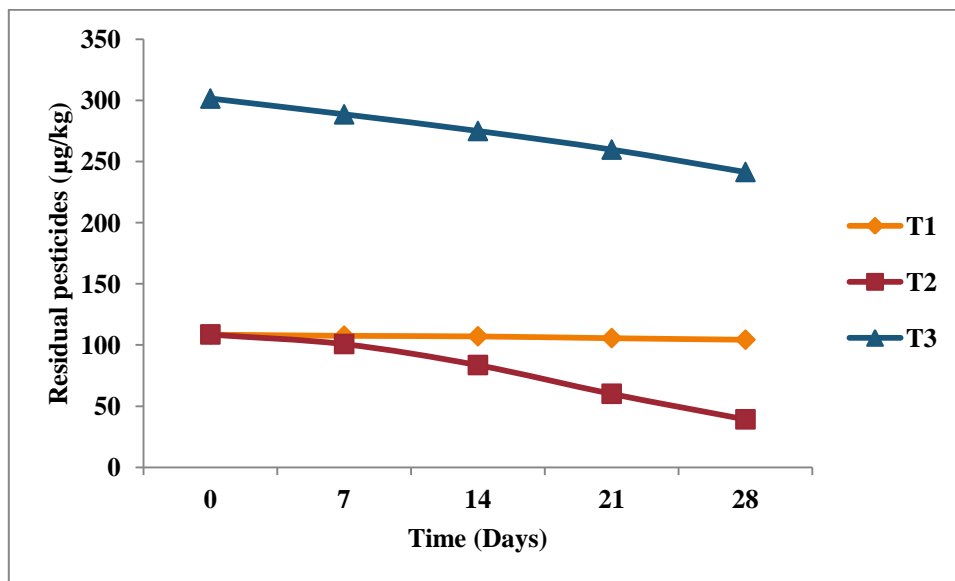


Fig.1. Biodegradation of Carbofuran

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

There were complete removal of Paraquat in treatments with the recommended rate while it decreased from $268.3 \pm 5.21 \mu\text{g/kg}$ (day 0) to $144.4 \pm 2.38 \mu\text{g/kg}$ (day 28) at four times the recommended rate (Fig.2). There were little losses of both pesticides in the abiotic control. Also, there were significant differences in degradation with respect to pesticides treatment and time.

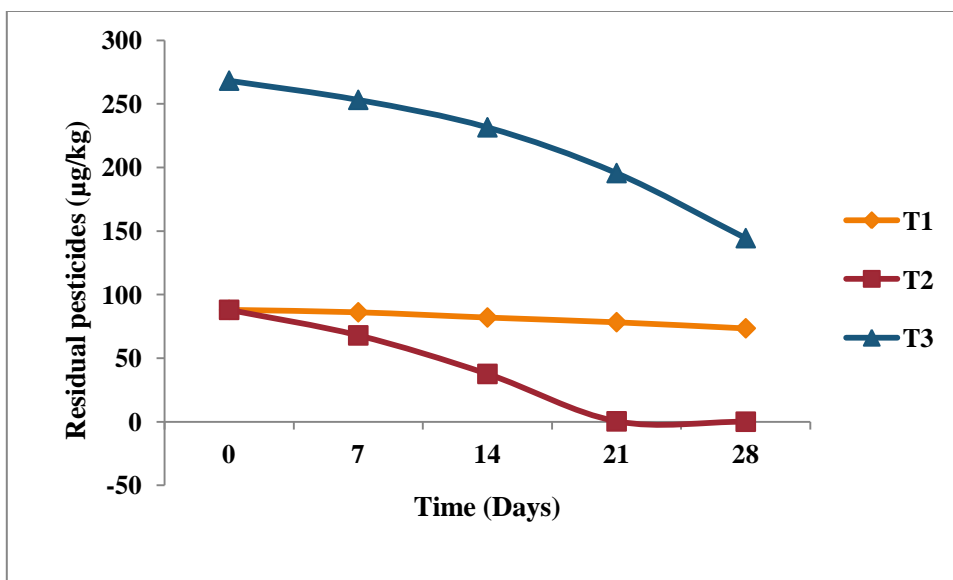


Fig.2. Biodegradation of Paraquat

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

The high performance liquid chromatography (HPLC) results showed the ability of topsoil microorganisms to reduce the pesticides under study. Soil microorganisms completely degraded Paraquat (99.7%) at recommended field rate within the study period while at four times the recommended rate only 46.3% was degraded. Carbofuran treated soil at recommended rate had 64.3% degradation and 19.9% at four times the recommended rate. In the abiotic control soil, 17.1% and 4% losses were recorded for Paraquat and Carbofuran, respectively. These results were in corroboration with the findings of Naqvi *et al.* [14]. They recorded significant losses during pesticides degradation studies by soil microorganisms using the HPLC with only a little loss from the sterile controls.

According to Nisha *et al.* [20], soil isolates capable of carrying out some form of Carbofuran and Paraquat degradation have been reported; such bacterial species included *Bacillus*,

Pseudomonas, *Flavobacterium*, *Arthrobacter* and *Sphingomonas*. Higher degradation was obtained at recommended proportions of Carbofuran and Paraquat with degradation faster in set-ups containing Paraquat. These soil microorganisms have the capacity to make use of Carbofuran and Paraquat as source of energy to grow in so doing degrade them. The difference in degradation by soil microbes could be traceable to carbon and nitrogen atoms in each pesticide [16] in addition to their toxicities [21]. These test microorganisms will be competent in reduction of Carbofuran and Paraquat hazards of contaminated area.

3.3 Microbial counts

The microbial dynamics during the degradation of Carbofuran and Paraquat in soil after their application at recommended and four times recommended rates were assessed using the traditional plate count method. Total heterotrophic bacterial (THB) counts in Carbofuran treated soil is shown in Fig.3. There was a decrease in total heterotrophic bacterial counts in treatments from $245.3 \pm 6.03 \times 10^4$ CFU/g (day 1) to $127 \pm 7.21 \times 10^4$ CFU/g (day 7) and increases from $159.7 \pm 6.43 \times 10^4$ CFU/g (day 14) to $200.3 \pm 3.45 \times 10^4$ CFU/g (day 21) in the recommended rate and a decrease at day 7 ($73.0 \pm 6.08 \times 10^4$ CFU/g), thereafter, increases from $112.0 \pm 7.55 \times 10^4$ CFU/g (day 14) to $158.3 \pm 7.51 \times 10^4$ CFU/g (day 21) in four times recommended rate microcosms.

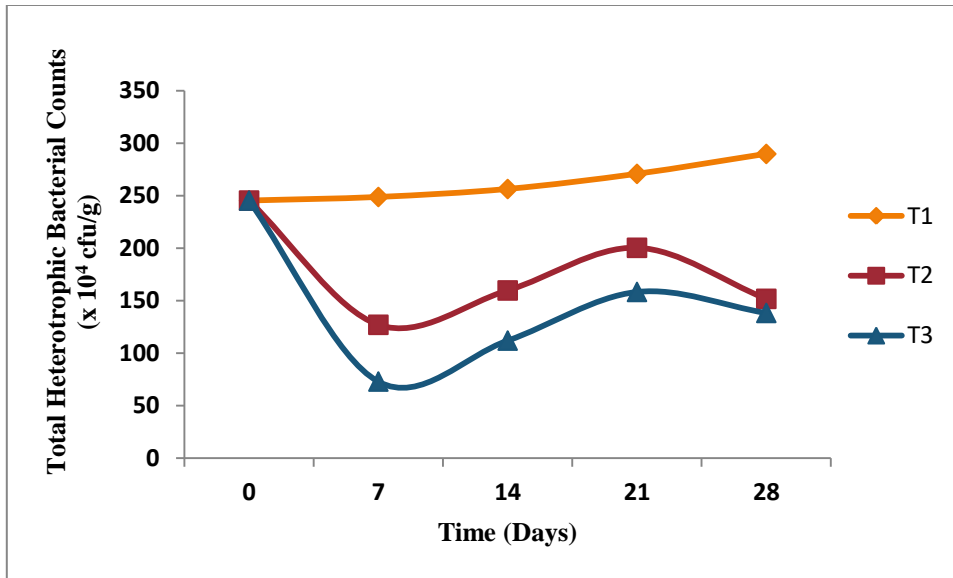


Fig.3. Total heterotrophic bacterial counts in Carbofuran

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

Also, the THB counts increased from $199.7 \pm 6.43 \times 10^4$ CFU/g (day 14) to $240.3 \pm 4.93 \times 10^4$ CFU/g (day 21); $152.0 \pm 7.60 \times 10^4$ CFU/g (day 14) to $222 \pm 7.55 \times 10^4$ CFU/g (day in treatments with Paraquat at recommended and four times recommended rates, respectively as shown in Fig.4. Counts increased throughout the study for the control soils from $245.3 \pm 6.03 \times 10^4$ to $289.6 \pm 2.31 \times 10^4$ CFU/g.

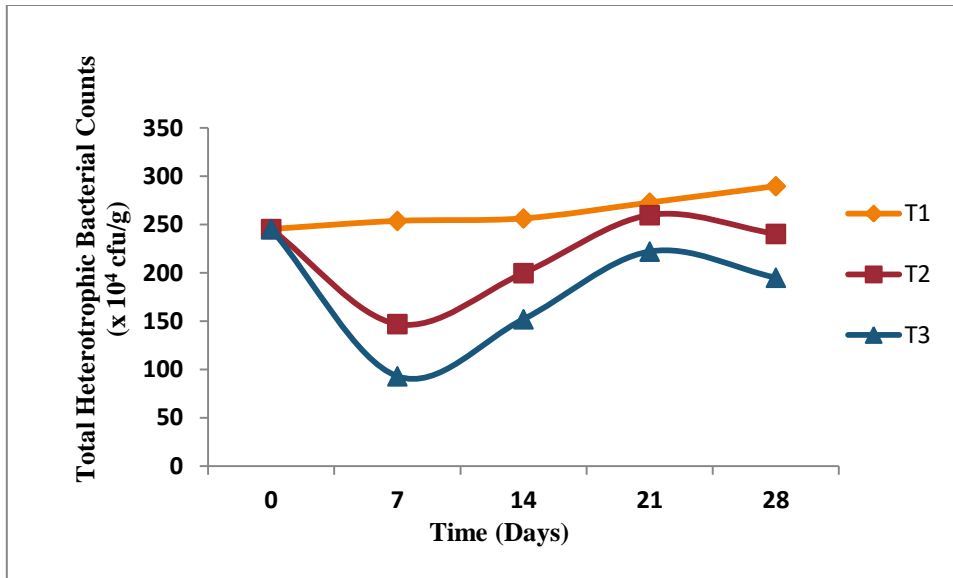


Fig.4. Total heterotrophic bacterial counts for Paraquat

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

The fungal counts in both treatments and control soils increased throughout the study after the decrease observed at day 7. Figure 5 shows increases in Carbofuran treated soils were from $65.0 \pm 6.24 \times 10^3$ CFU/g (day 14) to $82.7 \pm 3.06 \times 10^3$ CFU/g (day 21) and $52.6 \pm 2.06 \times 10^3$ CFU/g to $64.7 \pm 3.51 \times 10^3$ CFU/g at recommended and four times the recommended rates, respectively.

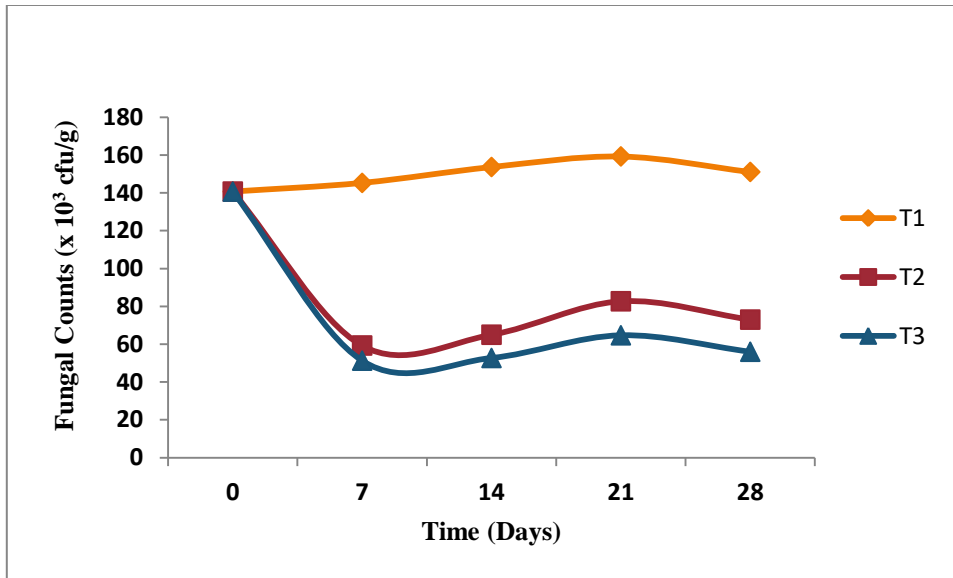


Fig.5. Fungal counts for Carbofuran

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

Moreso, the counts increased from $108.3 \pm 1.53 \times 10^3$ CFU/g to $126 \pm 6.00 \times 10^3$ CFU/g in treatment at recommended rate and $96.3 \pm 5.53 \times 10^3$ CFU/g to $108 \pm 3.00 \times 10^3$ CFU/g at four times the recommended rates in Paraquat polluted soils (Fig.6). Fungal counts increased from $140.7 \pm 1.53 \times 10^3$ CFU/g to $176 \pm 1.05 \times 10^3$ CFU/g in the control soils during the study.

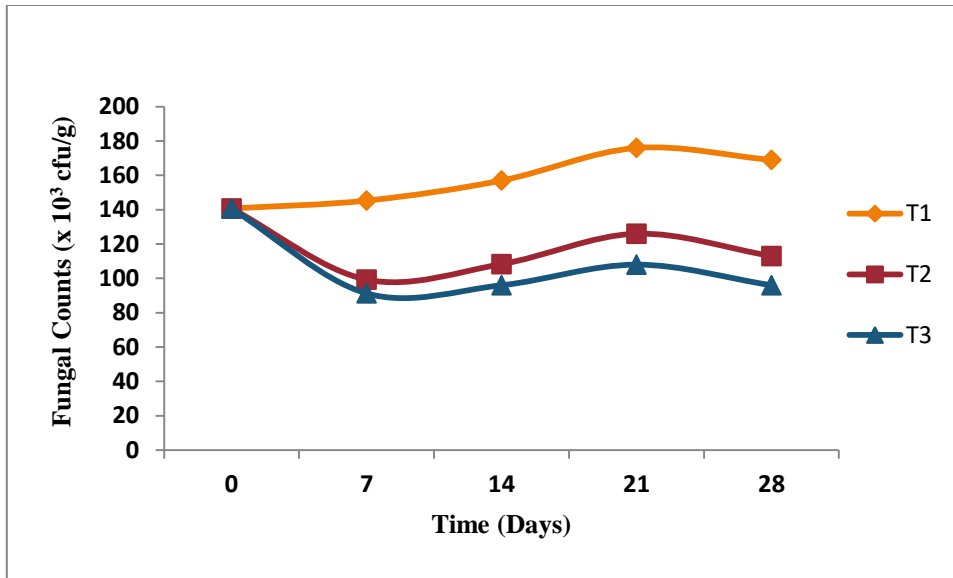


Fig.6. Fungal counts for Paraquat

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

Furthermore, the number of actinomycetes increased from day 14 to day 21 during the study for all treatments. Actinomycetes increased from $131.7 \pm 0.58 \times 10^2$ CFU/g to $149.3 \pm 9.71 \times 10^2$ CFU/g at recommended rate and $91.3 \pm 2.52 \times 10^2$ CFU/g to $132.3 \pm 5.86 \times 10^2$ CFU/g at four times the recommended rate in Carbofuran treatments (Fig.7).

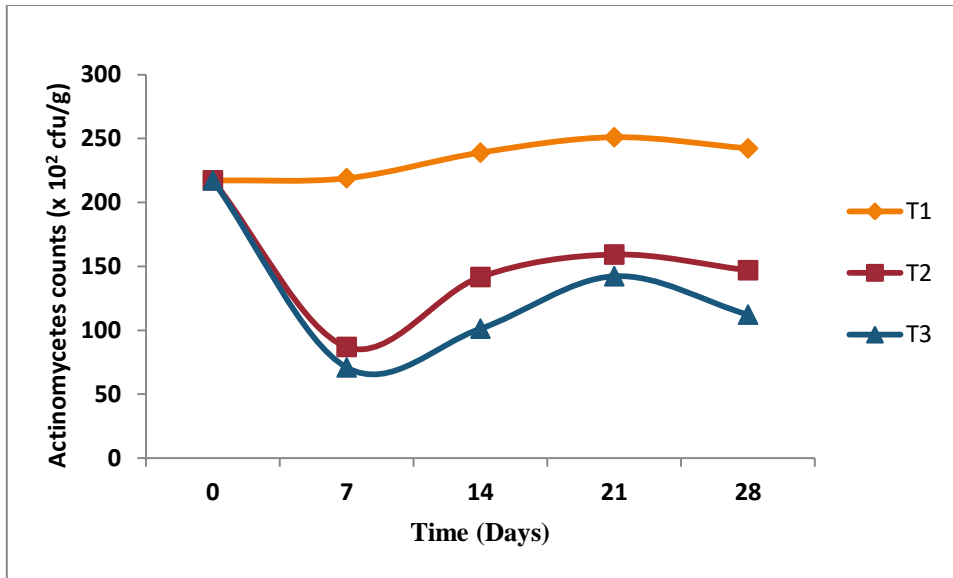


Fig.7. Actinomycetes counts for Carbofuran

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

Their counts equally increased from $170.0 \pm 10.44 \times 10^2$ CFU/g to $215.3 \pm 7.09 \times 10^2$ CFU/g; $141.3 \pm 2.52 \times 10^2$ CFU/g to $182.3 \pm 5.86 \times 10^2$ CFU/g at recommended rate and four times the recommended rates, respectively, for Paraquat treatments (Fig.8). Increases were from $217.0 \pm 6.08 \times 10^2$ CFU/g to $248.7 \pm 8.14 \times 10^2$ CFU/g in control pots.

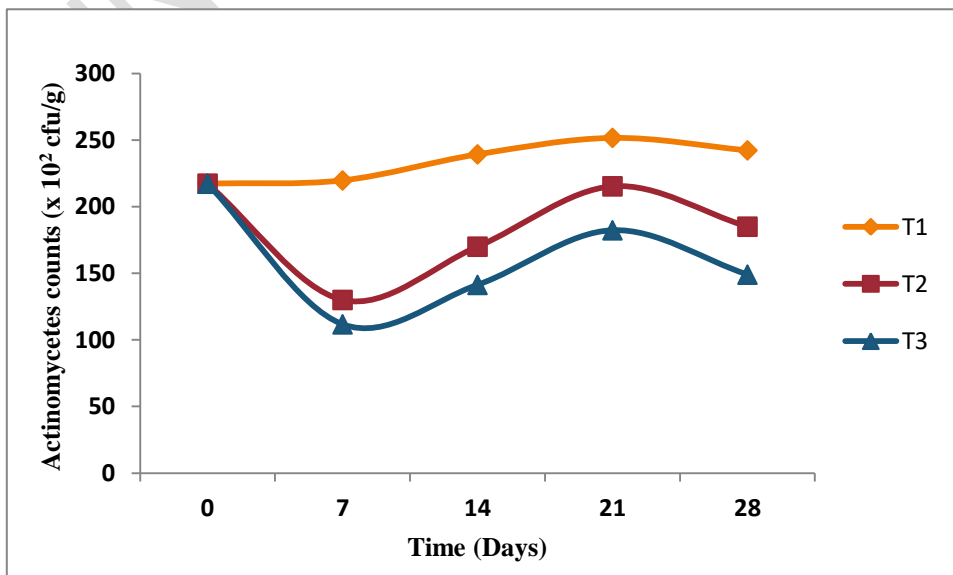


Fig.8. Actinomycetes counts for Paraquat

Key:

T1- abiotic control (sterile soil + pesticide)

T2 – farmyard soil + pesticide at recommended rate

T3 - farmyard soil + four times pesticide at recommended rate

However, statistical analysis using the two way ANOVA revealed significant differences ($P = 0.05$) in all microbial counts with regard to treatments and time (days).

The observed differences in counts in the polluted soil showed they were less than those in control soil in conformation with the findings of Baboo *et al.* [22]. They equally observed increases in microbial counts (total heterotrophic bacteria, fungi and actinomycetes) in control. The initial drop in their numbers could be traced to the toxic result of pesticides on exposure.

Also, the study revealed a general increase in total heterotrophic bacteria than other microbial counts in the different treatments, this corroborates with discoveries of Chen *et al.* [23]. Also, they detected that the usage of Chlorpyrifos impacted the fungal community dynamics in a quick and lasting way but only affected bacterial community temporarily; the versatility of bacterial genes to adapt and degrade xenobiotics may well be accountable for corresponding increases.

The presence and high numbers of these microorganisms confirms that they are ubiquitous, diverse and in addition, can adjust to any harsh environment [24,25,26]. Increased microbial (total heterotrophic bacteria, fungi, actinomycetes) counts showed that they grew in presence of the chemicals and degraded them. Lastly, the increased microbial population in this study is attributed to the ability of the isolates to catabolize fractions of pesticides and incorporate them into their biomass, hence, their activities could be suitable for clean-up of such polluted sites [27]. There are numerous reports about microorganisms' abilities to

catabolize different types and fractions of complex compounds integrating them into their biomass, in so doing, restore the environment to its original state [28,29,30,31,32,33,34,35].

4. CONCLUSION

Within this stopgap/short term study, these outcomes indicated that single applications of these pesticide activities at recommended dose pose little or no threat to soil microbial biodiversity and functions. However in practice, pesticides are applied multiple times under various environmental conditions, and commercial formulations contain a range of additional compounds that are not disclosed. The soil used in this study was collected from a pesticide free environment. Therefore, it is possible that either pesticide-tolerant organisms were present or these organisms developed the abilities. Despite being largely consistent with previous studies, our results must be inferred with restraint and additional work is crucial to effusively understand the impending influences of pesticides on soil microbial populations.

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