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Effects of an organophosphate (glyphosate) and a quaternary ammonium (paraquat) herbicides formulation on soils' culturable bacterial and fungal populations

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Abstract

Herbicides play significant roles in weed management and contribute immensely to increase in productivity in agronomy system. However, its continuous application could have some dire effect on non-target soil microbiota. Therefore, this study assessed the impact of glyphosate and paraquat herbicides on soil culturable bacterial and fungal population. The loamy composite soil samples of forest reserve, Owena, Ondo State, Nigeria were used for the experiment. Each of the herbicide formulation was applied at the concentration rates ranged from half of the field recommended rate (0.5FR), the recommended rate (FR), two, four, and eight times the recommended rate (2, 4 and 8) FR respectively. The treatments were replicated thrice and arranged in complete randomized design, while the untreated soil samples serve as control. The standard pour plate technique was used for the enumeration of bacterial and fungal colonies after 5, 10, 15, 20, 25, 30 and 35 days of exposure. The findings showed that the glyphosate pesticides formulation applied at concentration range of 0.5 to 2FR significant stimulate bacterial populations of the soil samples while the fungal populations was not affected at the same concentration. However, the treatments at higher doses (4FR and 8FR) significantly reduced the number of bacterial and fungal counts of the soil samples. For paraquat treated soils, the treatment rate below the double recommended field rate did not have any significant effect ($p > 0.05$) on both bacterial and fungal populations. While the increases in inhibitory effect were observed with corresponding increases in paraquat application rates in the soil samples.

Keywords: Glyphosate, paraquat, bioindicator, bacteria, fungi.

Introduction

The integrity of soil system relies on its efficiency in sustaining plants and animals productivity; enhance air and water quality, and support human health and habitation[1]. However, the soil system had been exposed to rapid deterioration and progressively losing its functional worth due to poor management especially in developing part of the world[2]. Protection of soil quality under exhaustive land use and fast economic development has been identified as one of the major challenge for sustainable resource use and management in the developing world[3]. These could have dire impacts on food production as a result of pollution and deterioration of soil quality[4,5]. Among the major threats to soil quality destabilization is the practices adopted for agricultural system.

In recent time, the rapid increases in human population has necessitated the evolution of improve agronomic practices that could matched the growing demand for food and other agricultural products. Therefore, effective weed management and control mechanisms had been one of the solutions that had immense contribution towards the successes recorded in agriculture. This is due to its capability of reducing the inter-specific competition for needed resources between the crop and weed and thereby able to boost agro-productivity. Though, manual weeding is one of the most environmental friendly approach to weed control but its practices is tedious and highly labour intensive[7]. On the other hand, the adoption of herbicides for weed control has been seen as the most convenient, economical and effective way of weed management. Because it reduces the number of personnel needed on farm, allow farm to be planted with minimum soil disturbance and allow earlier planting period. However, if herbicides are not applied appropriately as recommended, it could have direct or indirect effect on soil non-target organisms.

Glyphosate is an organophosphate pesticide of phosphonomethyl derivative class of amino acid glycine and one of the most widely used herbicide worldwide. It is non-selective in action with direct systemic effect against a wide range of herbaceous plants[7]. The application of glyphosate is not only limited to the control of weeds in agricultural land but also used to manage weeds and unwanted grasses in urban, pastures, forestry and aquatics environment. Though, it is considered as relatively safe pesticide compound in the environment due to its rapid inactivation in soil. However, there had been many concerns about its potential effects which could results from its high affinity to absorb and adsorb to the soil system[8].

Paraquat is a quaternary ammonium, fast acting, very effective and non-selective contact herbicide. It is used to control broad-leaved weeds and grasses. Like glyphosate, it application is not limited to farm land but also used in plantations and fruit orchards as well as general weed control in urban area[9]. It exerts its herbicide toxicity through its redox potential[10]. Based on chemical characteristics paraquat is a polar organic compound and could adsorb to clay and soil organic matter easily. Though, the adsorption of these pesticides to soil components could lessen its toxicity and prevent their further spread in the environmental. However, their potential effects on soil biological sentinel is of great concerns as these microbes are the major engine room for the biotransformation of major important nutrients in the soils.

There had been major concern about the status of soil quality due to the possible impacts of anthropogenic activities which could impact on ecosystem services[11]. Therefore, there is a need for regular monitoring of soil quality through the use of sensitive and measurable indicators. The selection of organism as bioindicator species for ecotoxicological studies is determined by ecological features and ecological niches of such organisms. However, for an organism to be qualified as a good indicator of environmental monitoring it should possess the features such as sensitivity, ease of assay and identification as well as ease of analysis[12]. The information gather from bioindicator are used to detect changes in the natural environment, monitor the effect of pollution and monitor the progress of environmental cleanup.

Soil microorganisms play some key role in soil quality status as they are the engine room for mineralization of organic matters and biotransformation of mineral elements[13,14]. They also help in improving the soil texture, nutrient and crop productivity. Some groups of bacteria have the capability to secret polysaccharides and other organic glue which could enhances the structure of soil system. Fungi that involve in symbiotic relationship with plant usually produce sticky polysaccharide-peptide complex (glomalin) which possess cementing properties and helps to hold the soil particles together[15]. They are being regarded as reliable indicator of soil health due to their direct contact with soil components and roles in soil sustainability. Likewise, their short generation time, make them to respond rapidly even at low pollutant level. The soil biological components such as soil organic matter, soil microbial biomass, total bacteria and total fungi have been reported to be the key and sensitive indicator of soil health[16]. Likewise, it has been confirmed that soil microbes showed the observable changes in term of numbers in the presence of pollutants[17].

The effects of pesticides on soil microorganisms had been reported to vary depending on the properties as related to chemical components and structure, soil factors and other environmental factors[18]. The continuous and extensive use of pesticides has being linked to a rapid decline in the quality of soil and consequently impacts the diversity of soil flora and fauna[19,20]. Reports have shown that the soil quality can be evaluated in view of the counts of bacterial and fungal population[18,21]. Therefore this study assess the impact of an organophosphate (glyphosate) and a quaternary ammonium (paraquat) herbicides formulation on soils' culturable bacterial and fungal populations.

Materials and methods

Study Area: The study set-up and experimentation were carried out in Microbiology Laboratory of Kwara State University, Malete, Nigeria.

Soil sample collection: Surface soils (0-15cm) layer of the loamy sand soils of Forest Reserve area, Ondo State, Nigeria were collected with soil auger. The soil sub-samples were bulked, crumbled and thoroughly mixed together. The soil composite sample was then taken to the laboratory for further processing and treatment.

Determination of moisture content of the soil sample: The moisture content of the soil sample was determined by using oven drying method of analysis as described by Kramarenko *et al*. with slight modification[22]. About 10g of the composite sample were weighed into the glass beaker and initial weight was recorded. The weighed beaker-soil sample was then oven drying at 70^{o}C for 24h. The beaker-soil sample was repeatedly weighed in order to obtain the final stable weight. The moisture content was expressed in percentage of water content in dried weight of soil as follows:

$$\text{Moisture content} = \frac{\text{Weight of moist soil} - \text{Weight of dried soil}}{\text{Weight of dried soil}} \text{ x } 100$$

Determination of water holding capacity of the soil sample: The Water holding capacity (WHC) was determined by using the method described by Sujatha *et al*. with slight modification[23]. Three grams (3g) of soil sample were weighed on a piece of initially weighed Whatman filter paper. The weighed samples were oven-drying at 70^{o}C for 24h. The weight of 24h oven-dried soil samples on the weighed Whatman filter paper were determined before dipping into water until soil becomes saturated. The soil sample was then placed in a humid enclosure to drain off the excess water before weighing again, and the WHC was expressed in percentage as follows:

$$\text{Water Holding Capacity} = \frac{\text{Mass of water contained in saturated soil}}{\text{Mass of saturated soil}} \text{ x } 100$$

Soil preparation for herbicide treatments: The study was carried out using microcosm designed in 500cm plastic. An organophosphate (glyphosate) and quaternary ammonium (paraquat) herbicides were respectively used for the treatment of soil samples.

The method described by Zain *et al*. was adopted for the treatment procedure with little modification[24]. Five herbicide

concentrations were used for the treatment of soil samples. The applied herbicide treatment rates were half the normal field application rate (0.5FR), recommended field rate (FR), two times the field application rate (2FR), four times the field application rate (4FR) and eight times the field application rate (8FR). The corresponding concentration for each of the herbicide treatment rate of 0.5FR, FR, 2FR, 4FR and 8FR, respectively were: i. Glyphosate (µg/kg): 2.75, 5.50, 11.00, 22.00, 44.00, ii. Paraquat (µg/kg): 3.13, 6.25, 12.50, 25.00, 50.00.

The treatment rates were calculated as follows:

$$X\ (\mu g/kg)\ soil = \frac{\text{Recommended application rate (g a.i./ha)}}{\text{Amount of a.i.in formulation (g a.i./L)x } 450\frac{L}{ha}} \text{ X } \frac{1000000\ \mu g}{1kg}$$

Where; X = treatment rate, "g a.i" represent the gram of active ingredients present in pesticide formulation.

Fifty milliliters (50 ml) volumes of herbicide formulations were applied to 500g of soil samples by hand-spraying. Each treatment was replicated thrice and the deionised water treated soil served as control. The treated soils were mixed thoroughly by constant shaking for 5min and incubated at room temperature in the dark. The moisture content of soil samples were maintained at 50% of the maximum water holding capacity by spraying with deionized water as needed.

Effect of herbicide on soils' culturable bacterial and fungal population: The culturing and enumeration of bacterial and fungal population was carried out using pour plate technique on nutrient agar (NA) and potatoe dextrose agar (PDA) respectively. The NA was supplemented with 0.1g/L cyclohexamide; while potato dextrose agar plate (PDA) was supplemented with 30mg/L streptomycin sulphate. The media used were prepared according to manufacturer's instruction.

The impact of herbicides on bacterial and fungal population was observed at the 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 30^{th} and 35^{th} days period of exposure. A sterile 6mm cork-borer was aseptically used to take 3 sub-soil samples from each microcosm. They were mixed together to form a composite soil sample. One gram of the representative soil treatment was then used to make a serial dilution under aseptic condition up to 10^{-7} fold dilution factor and 0.1ml of 10^{-4} and 10^{-7} were inoculated on agar media plates in triplicates. The seeded plates were inverted and incubated at room temperature ($28\pm2^{o}C$). The distinct colonies that formed on the plates were counted with colony counter after 48h and 7 days of incubation for bacteria and fungi respectively. The counted colonies values were expressed in colony forming unit (cfu/g) of dry weight of soil sample.

Statistical analysis: The IBM Statistical Package for Social Sciences (SPSS) version 23 was used to carry out the statistical analysis of obtained data. The data on bacterial and fungal counts of various herbicides treated soils were analyzed using analysis of variance (ANOVA) and further subjected to Duncan multiple range test (DMRT) to compare the mean values for significant difference. Differences were considered statistically significant at $P \leq 0.05$.

Results and discussion

Impact of glyphosate pesticide on bacterial populations: The mean viable bacterial counts in glyphosate-treated soils and untreated control soils over the period of exposure were represented in Figure-1. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean viable bacterial counts ranged from $95.7x10^6$ cfu/g, $112.7x10^6$ cfu/g, $123.0x10^6$ cfu/g, $126.7x10^6$, $129.7x10^6$ cfu/g to $138.0x10^6$ cfu/g of soil samples for 8FR, 4FR, 0.5FR, FR, C and 2FR respectively (Figure-1). Although, there were differences in mean viable bacterial counts in all the glyphosate treated soil with values; the result of statistical analysis showed that these differences were not statistically significant ($p > 0.05$).

At the ten (10) days of exposure, the mean viable bacterial counts ranged from $90.3x10^6$ cfu/g, $106.0x10^6$ cfu/g, $120.7x10^6$ cfu/g, $121.7x10^6$ cfu/g, $134x10^6$ cfu/g to $143.3x10^6$ cfu/g of soil samples for 8FR, C, 0.5FR, 4FR, FR, and 2FR respectively (Figure-1). The observation showed that there were relative increases in bacterial counts in all the glyphosate treated soils (except 8FR). Statistically, all the treatments did not show any significant impact on bacterial counts ($p > 0.05$) except the 2FR glyphosate treated soil that showed significance increases in bacterial counts of the glyphosate treated soil ($p < 0.05$).

The mean viable bacterial counts observed in fifteen (15) days of exposure ranged from $106.7x10^6$ cfu/g, $109.3x10^6$ cfu/g, $123.0x10^6$ cfu/g, $135.0x10^6$ cfu/g, $138.0x10^6$ cfu/g to $146.0x10^6$ cfu/g. The results showed that the relative increases in bacterial counts observed in 4FR, FR, and 2FR respectively were highly significant ($p<0.01$); while the 0.5FR and 8FR showed no significance differences in bacterial counts of glyphosate treated soil ($p > 0.05$).

At the twenty (20) days of exposure, the mean viable bacterial counts ranged from $108.7x10^6$ cfu/g, $109.7x10^6$ cfu/g, $126.7x10^6$ cfu/g, $141.7x10^6$ cfu/g, $144.0x10^6$ cfu/g and $144.3x10^6$ cfu/g for 8FR, C, 0.5FR, FR, 2FR, 4FR respectively. The statistical analysis indicated that the relative increases in bacterial counts as observed in FR, 2FR and 4FR were significant when compared to untreated control soil ($p < 0.05$).

The mean viable bacterial counts as observed in twenty-five (25) days of exposure ranged from $98.7x10^6$ cfu/g, $110.0x10^6$ cfu/g, $112.7x10^6$ cfu/g, $129.0x10^6$ cfu/g, $135.3x10^6$ cfu/g to $136.7x10^6$ cfu/g of soil samples for C, 8FR, 0.5FR, FR, 2FR and 4FR respectively. The relative increases in bacterial counts

observed in FR, 2FR and 4FR were statistically significance ($p < 0.05$); while no significance difference was observed in bacterial counts of the soil treated with 0.5FR, 8FR of glyphosate ($p > 0.05$).

The mean viable bacterial counts in thirty (30) days of exposure ranged from $110.0x10^6$ cfu/g, $121.0x10^6$ cfu/g, $124.3x10^6$ cfu/g, $140.3x10^6$ cfu/g, $147x10^6$ cfu/g to $152.3x10^6$ cfu/g for C, 0.5FR, 8FR, FR, 2FR and 4FR respectively. The 2FR and 4FR were the only observed treatments rate that showed significant increase in bacterial counts of glyphosate treated soils ($p < 0.05$).

At the thirty-five (35) days of exposure, the mean viable bacterial counts ranged from $107.3x10^6$ cfu/g, $115.3x10^6$ cfu/g, $119.3x10^6$ cfu/g, $127.3x10^6$ cfu/g, $137.0x10^6$ cfu/g to $140.0x10^6$ cfu/g for C, 0.5FR, 8FR, FR, 2FR and 4FR respectively. The differences in the mean viable bacterial counts among all the treatments were not statistically significant ($p > 0.05$).

Degree of impact of glyphosate treatments on bacterial population relative to control over the period of exposure: The degree of impact of glyphosate pesticide treatments on bacterial populations was presented in percentage response (inhibition or stimulation) as shown in Figure-2. From the result, observation showed that glyphosate did not have any significant inhibitory effect on bacterial population for every treatment level.

On the soil samples treated with half of the field rate (0.5FR), the only mild inhibitory effect (5.2%) on bacterial population was observed at 5 day of exposure period (DEP); while there were progressive stimulatory effects throughout the remaining period of exposure. However, the highest relative percentage increase (17.5%) in bacterial population was observed at 25 DEP.

The degree of impact of glyphosate treatments on soil bacterial population for FR, 2FR and 4FR followed the same trend as observed in 0.5FR treatment level with highest stimulatory effect for FR (37.5%), 2FR (53.7%) and 4FR (55.0%) observed at 25 DEP.

The highest treatment level of glyphosate pesticide (8FR) showed a progressive decrease in inhibitory effect from 5(26.2%) DEP to 20 (0.9%) DEP (Figure-2).

Generally, in glyphosate treated soil the slight inhibitory effects (5.2, 2.3 and 13.1) for 0.5FR, FR, and 4FR respectively were observed only in the first five days of exposure; while the inhibitory effects was noticed till twenty days of exposure period for highest glyphosate treatment rate. Progressive increases in bacterial population were observed for all the glyphosate treatment levels over the period of exposure.

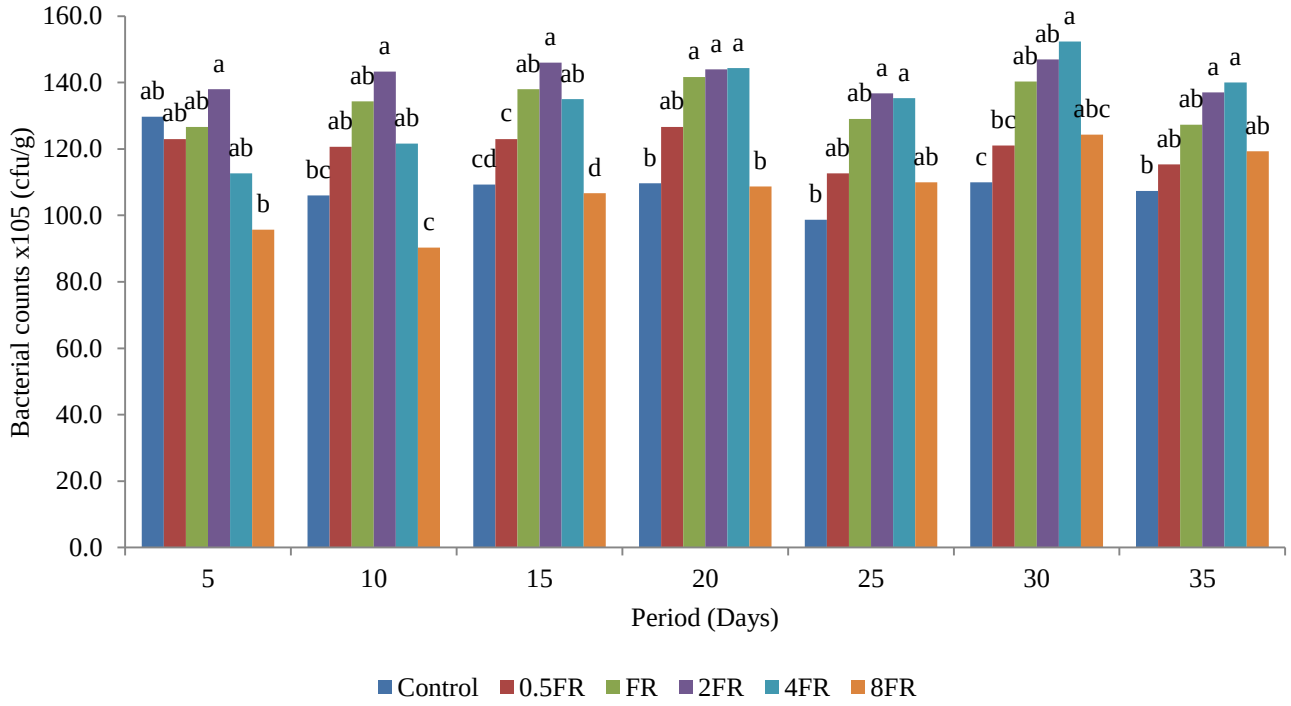

Figure-1: Mean viable bacterial counts in soil treated with glyphosate pesticide.

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

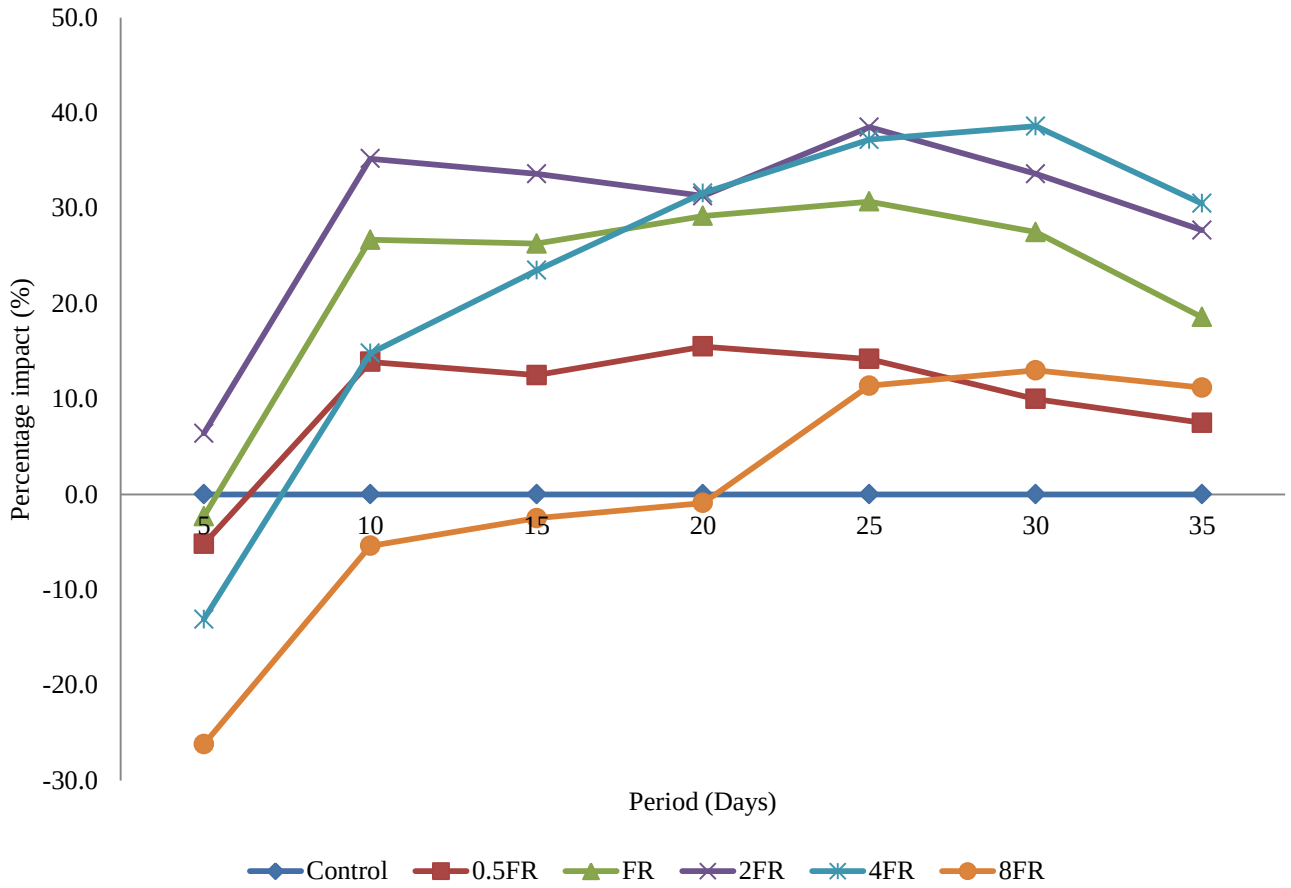

Figure-2: Degree of impact of glyphosate treatments on bacterial population relative to control over the period of exposure.

Impact of paraquat pesticide on the bacterial populations: The mean viable bacterial counts in paraquat-treated soils and untreated control soils over the period of exposure were represented in Figure-3. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean viable bacterial counts ranged from $36.3x10^6$ cfu/g, $63.0x10^6$ cfu/g, $120.3x10^6$ cfu/g, $137.7x10^6$, $139.7x10^6$ cfu/g to $172.0x10^6$ cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively (Figure-3). Observation from the result showed that the 0.5FR, FR and 2FR had no significant impact on bacterial counts ($p > 0.05$); while the 4FR and 8FR significantly reduces the bacterial counts of the paraquat treated soil ($p < 0.05$).

At the ten (10) days of exposure, the mean viable bacterial counts ranged from 41.7 x 10^6 cfu/g, 63.7 x 10^6 cfu/g, 104.7 x 10^6 cfu/g, 113.0 x 10^6 cfu/g, 133.7 x 10^6 cfu/g to 124.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively (Figure-3). The impact of paraquat on the mean viable bacteria counts showed similar effects as observed in 5 days of exposure with significant decreased in bacterial counts of soil treated with 4 times and 8 times the normal field rate of paraquat ($p < 0.05$).

The mean viable bacterial counts as observed in fifteen (15) days of exposure ranged from 56.3 x 10^6 cfu/g, 67.7 x 10^6 cfu/g, 107.0 x 10^6 cfu/g, 110.7 x 10^6 cfu/g, 115.7 x 10^6 cfu/g to 120.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Figure-3). The impact of paraquat on the mean viable bacterial counts followed the same trend as observed in the 5 and 10 days of exposure. The 4FR and 8FR paraquat treatment significantly reduces the mean bacterial counts of soil ($p < 0.05$).

At the twenty (20) days of exposure, the mean viable bacterial counts ranged from 48.0 x 10^6 cfu/g, 77.7 x 10^6 cfu/g, 89.0 x 10^6 cfu/g, 106.0 x 10^6 cfu/g, 108.3 x 10^6 cfu/g and 108.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Figure-3). Statistical analysis showed that the paraquat treatment levels of 4FR and 8FR significantly reduced the bacterial counts of the soil samples ($p < 0.05$).

The mean viable bacterial counts in twenty-five (25) days of exposure ranged from 60.7 x 10^6 cfu/g, 95.0 x 10^6 cfu/g, 100.7 x 10^6 cfu/g, 118.3 x 10^6 cfu/g, 124.7 x 10^6 cfu/g to 128.3 x 10^6

cfu/g for 8FR, 4FR, 2FR, C, 0.5FR, FR respectively. There were no significant differences in bacteria counts of the paraquat treated soils ($p > 0.05$). However, the highest treatment rate (8FR) significantly reduces the bacteria counts of the soil ($p < 0.05$).

The mean viable bacterial counts as observed in thirty (30) days of exposure ranged from 48.3 x 10^6 cfu/g, 69.3 x 10^6 cfu/g, 95.0 x 10^6 cfu/g, 104.0 x 10^6 cfu/g, 106.0 x 10^6 cfu/g to 112.7 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, C, FR and 0.5FR respectively. Similar effects that were noticed at 25 days of exposure were also observed for 30 days. The highest treatment rate (8FR) was the only paraquat treatment that significantly reduces the bacteria counts of the soil ($p < 0.05$).

At the thirty-five (35) days of exposure, the mean viable bacterial counts ranged from 54.0 x 10^6 cfu/g, 73.7 x 10^6 cfu/g, 100.7 x 10^6 cfu/g, 102.3 x 10^6 cfu/g, 108.7 x 10^6 cfu/g to 111.7 x 10^6 cfu/g for 8FR, 4FR, 2FR, C and 0.5FR respectively. The differences in the mean viable bacterial counts among all the paraquat treatments were not significant ($p > 0.05$) except for the highest treatment rate (8FR) that significantly reduce ($p < 0.05$) the mean viable bacterial counts of the treated soil.

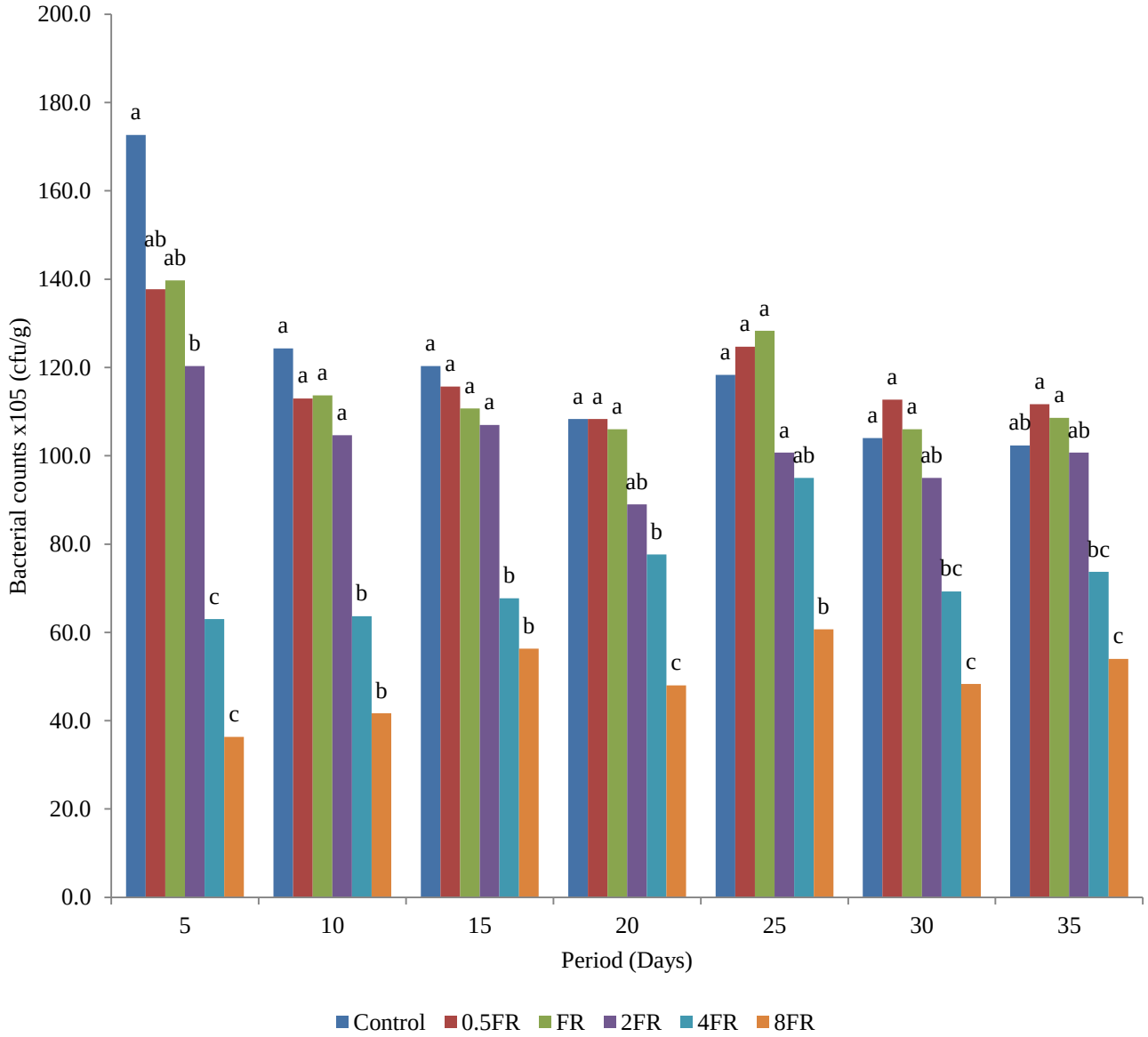

Figure-3: Mean viable bacterial counts in soil treated with paraquat pesticide.

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

Degree of impact of paraquat treatments on bacterial population relative to control over the period of exposure: The degree of impact of paraquat pesticide treatments on bacterial populations was presented as percentage response (inhibition or stimulation) as shown in Figure-4.

On the soil samples treated with half of the field rate (0.5FR), the inhibitory effects on bacterial population were observed at 5, 10 and 15 days of exposure period with the highest inhibitory effect (19.1%) observed at 5 day of exposure period (DEP); while stimulatory effects (5.4%, 8.4% and 9.3%) were observed at 25, 30 and 35 DEP respectively (Figure-4).

The field rate (FR) treatments showed the inhibitory effect on bacterial population at 5, 10, 15 and 20 DEP, while stimulatory effects (8.5%, 1.9% and 6.2%) were observed at 25, 30 and 35 DEP respectively.

The higher paraquat treatments rate (2FR, 4FR and 8FR) had the inhibitory effects on bacterial populations of treated soil throughout the experimental period. The highest inhibitory effect (30.3) recorded for paraquat treatments of concentration 2 times the field rate was observed in the first five days of exposure, while the least inhibition was observed at 35 days of exposure.

The highest inhibitory effects for 4FR and 8FR (63.5% and 78.9% respectively) was observed at the 5 days of DEP.

Generally, the inhibitory effects of paraquat pesticides on bacterial population increases with increase in soil treatment rate. The progressive reductions in the inhibitory effects of paraquat on soil viable bacterial population were observed over the period of exposure. Half the normal field rate (0.5) and the field rate of paraquat treatments showed marked recovery on 20^{th} and 25^{th} days of exposure respectively.

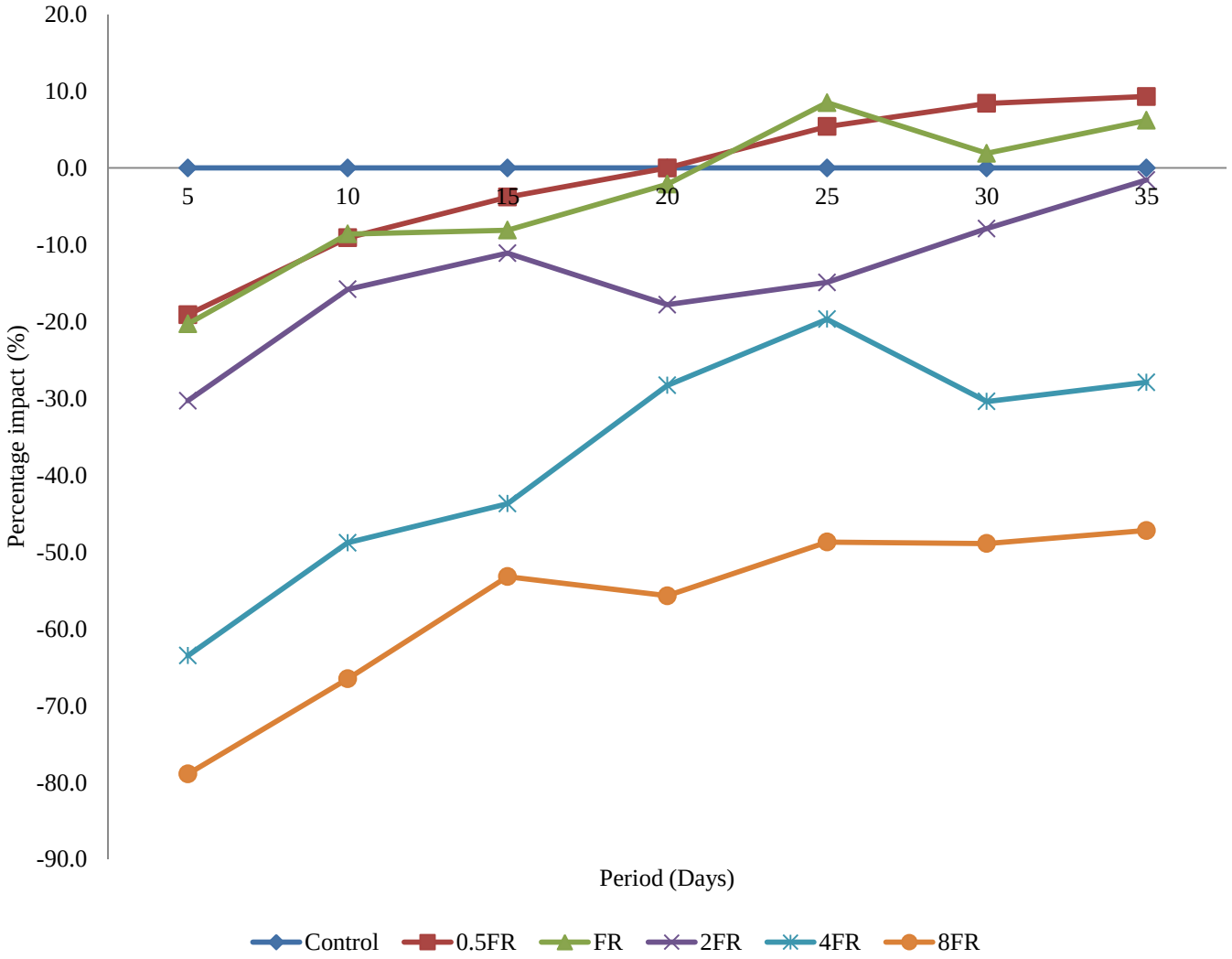

Figure-4: Degree of impact of paraquat treatments on bacterial population relative to control over the period of exposure.

Impact of glyphosate pesticide on the mean fungal populations: The mean fungal counts in treated soils and untreated control soils over the period of exposure were represented in Figure-5. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean fungal counts ranged from $13.x10^4$ cfu/g, $14.7x10^4$ cfu/g, $27.3x10^4$ cfu/g, $30.7x10^4$, $36.0x10^4$ cfu/g to $45.7x10^4$ cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Figure-5). Result showed that the mean differences in fungal counts of 0.5FR, FR and 2FR treated soils did not show any significant differences ($p>0.05$). However, 4FR and 8FR significantly reduced the fungal population of the treated soils ($p < 0.05$).

At the ten (10) days of exposure period, the mean fungal counts ranged from $22.3x10^4$ cfu/g, $26.3x10^4$ cfu/g, $27.3x10^4$ cfu/g, $30.3x10^4$ cfu/g, $30.7x10^4$ cfu/g to $31.7x10^4$ cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR (Figure-5). Significant reduction in fungal population were observed in 2FR, 4FR and 8FR ($p<0.05$) respectively. The 0.5FR and FR did not show any significant on fungal population of the treated soil.

The mean fungal counts observed in fifteen (15) DEP ranged from $21.0x10^4$ cfu/g, $25.0x10^4$ cfu/g, $29.3x10^4$ cfu/g, $31.0x\ 10^4$ cfu/g, $31.3x10^4$ cfu/g to $32.3x10^4$ cfu/g of soil samples for 8FR, 4FR, 2FR, C, FR and 0.5FR respectively (Figure-5). The effects of glyphosate treatments level on soil fungal count were only significant for 4FR and 8FR glyphosate treated soils ($p<0.05$).
At the twenty (20) days of exposure, the mean fungal counts ranged from $18.0x10^4$ cfu/g, $26.0x10^4$ cfu/g, $28.3x10^4$ cfu/g, 30.0×10^4 cfu/g, 30.3×10^4 cfu/g to 31.7×10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Figure-5). No significant differences observed in fungal counts of all the treatment levels ($p > 0.05$) except in the highest treatment rate (8FR) that showed significant reduction in fungal population ($p < 0.05$).

The mean fungal counts in twenty-five (25) days of exposure ranged from 19.3×10^4 cfu/g, 23.7×10^4 cfu/g, 28.7×10^4 cfu/g, 29.0×10^4 cfu/g, 29.3×10^4 cfu/g to 30.0×10^4 cfu/g for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively. The significant decrease in fungal counts was only observed in the highest glyphosate (8FR) treated soils ($p < 0.05$).

The mean fungal counts as observed in thirty (30) days of exposure ranged from 19.7×10^4 cfu/g, 25.0×10^4 cfu/g, 29.0×10^4 cfu/g, 29.7×10^4 cfu/g, 29.7×10^4 cfu/g to 30.7×10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, C, 0.5FR and FR respectively. Significant impact was observed in the soil treated with 8FR glyphosate while all other treatment levels did not showed any significant differences in the mean fungal counts of all the glyphosate treated soil samples.

At the thirty-five (35) days of exposure, the mean fungal counts ranged from 22.0×10^4 cfu/g, 26.3×10^4 cfu/g, 29.0×10^4 cfu/g, 29.7×10^4 cfu/g, 30.0×10^4 cfu/g to 30.7×10^4 cfu/g for 8FR, 4FR, 2FR, FR C and 0.5FR respectively. Result showed that there were no significant impacts of glyphosate treatments on the mean fungal counts of soils samples ($p > 0.05$), except in the highest treatment rate (8FR) that showed significant reduction in fungal population ($p < 0.05$).

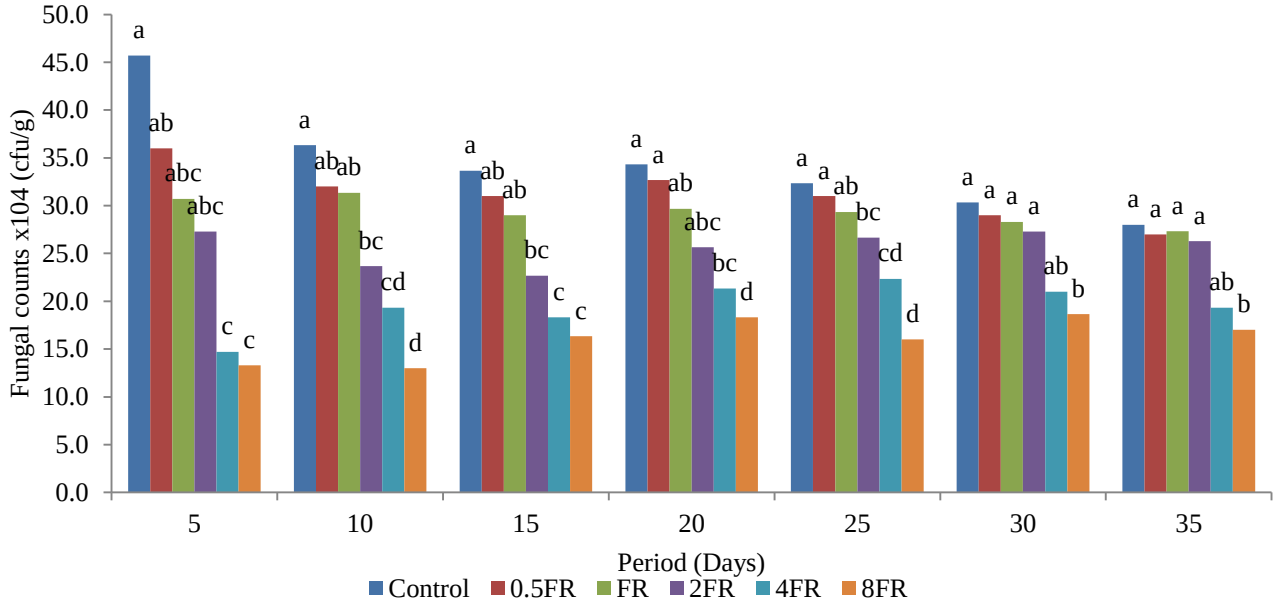

Figure-5: Mean fungal counts in soil treated with glyphosate pesticide.

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c, d).

Degrees of impacts of glyphosate treatments on fungal population relative to control over the period of exposure: Glyphosate pesticide treatments showed the various degree of inhibition on fungal counts in all the treated soil samples (Figure-6).

On the soil samples treated with half of the field rate (0.5FR), the highest inhibitory effects (21.2%) on fungal population was observed at 5 days of exposure period while the least inhibitory effect (3.6%) was observed at 35 day of exposure period (DEP).

The field rate (FR) treatments had the highest inhibition (32.8%) on fungal population at 5 DEP, while the least inhibitory effect (2.5%) was observed at 35 DEP (Figure-6).

The similar trend of inhibitory effects on soil fungal colonies as observed in 0.5FR and FR were also observed in higher glyphosate treatments rate (2FR, 4FR and 8FR) with the highest inhibitory effects (40.3%, 67.6% and 70.9% respectively) noticed in the first five days of exposure, while the least inhibition (6.1%, 31.1% and 39.3%) observed at 35 days of exposure.

Generally, the inhibitory effects of glyphosate pesticides on fungal population increases with increase in pesticide treatment rate. The progressive reductions in the inhibitory effects of glyphosate on soil fungal population were observed over the period of exposure.

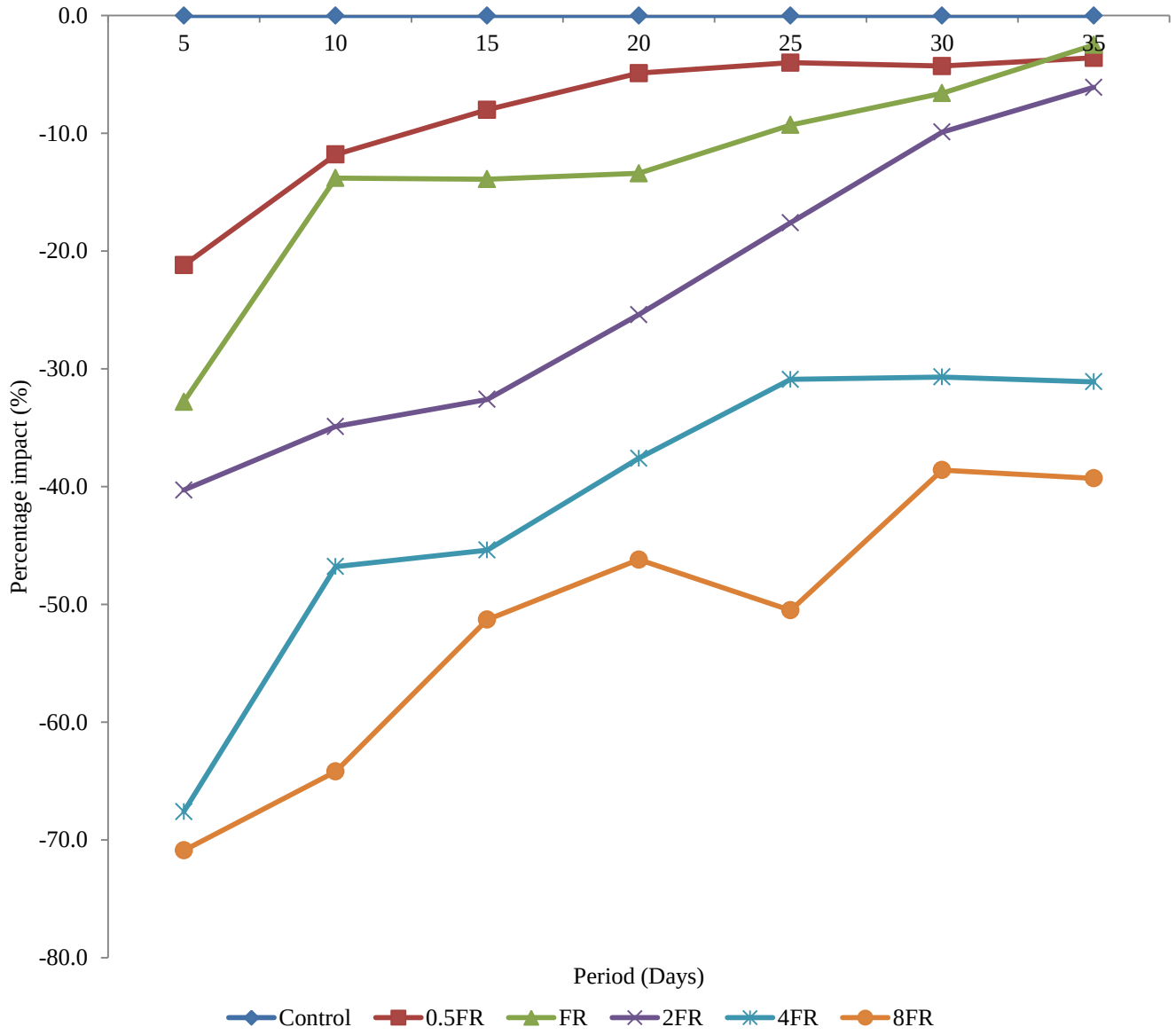

Figure-6: Degree of impact of glyphosate treatment levels on fungal population relative to control over the period of exposure.

Impact of paraquat pesticide on the mean fungal populations: The mean fungal counts in treated soils and untreated control soils over the period of exposure were represented in Figure-7. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean fungal counts ranged from 11.0 x 10^4 cfu/g, 21.7 x 10^4 cfu/g, 28.3 x 10^4 cfu/g, 30.7 x 10^4, 37.7 x 10^4 cfu/g to 38.0 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Figure-7). Result showed that the mean differences in fungal counts of 0.5FR to 4FR treated soils did not show any significant differences ($p > 0.05$). However the highest paraquat treatment level (8FR) significantly reduced the fungal population of the treated soils ($p < 0.05$).

At the ten (10) days of exposure period, the mean fungal counts ranged from 15.0 x 10^4 cfu/g, 24.3 x 10^4 cfu/g, 27.0 x 10^4 cfu/g, 31.7 x 10^4 cfu/g, 32.7 x 10^4 cfu/g to 34.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and C respectively (Figure-7). Significant reduction in fungal population was only observed in the soil samples treated with the highest paraquat concentration (8FR) ($p < 0.05$) respectively. There was no significant difference in the mean fungal counts of the soil samples of all other treatment levels ($p > 0.05$).

The mean fungal counts observed in fifteen (15) DEP ranged from 17.0 x 10^4 cfu/g, 19.3 x 10^4 cfu/g, 26.7 x 10^4 cfu/g, 33.3 x 10^4 cfu/g, 33.7 x 10^4 cfu/g to 34.0 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively (Figure-7). The effects of paraquat treatment levels on soil fungal count were only significant for 8FR paraquat treated soils ($p < 0.05$). At the twenty (20) days of exposure, the mean fungal counts ranged from 21.3 x 10^4 cfu/g, 23.7 x 10^4 cfu/g, 29.7 x 10^4 cfu/g, 32.3 x 10^4 cfu/g, 34.3 x 10^4 cfu/g to 35.3 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Figure-7). No significant differences observed in fungal counts of all the treatment levels ($p > 0.05$) except in the highest treatment rate (8FR) that showed significant reduction in fungal population ($p < 0.05$).

The mean fungal counts in twenty-five (25) days of exposure ranged from 20.7 x 10^4 cfu/g, 23.3 x 10^4 cfu/g, 23.3 x 10^4 cfu/g, 23.7 x 10^4 cfu/g, 25.3 x 10^4 cfu/g to 27.0 x 10^4 cfu/g for FR, 4FR, 2FR, FR, C and 0.5FR respectively. The significant decrease in fungal counts was only observed in the highest paraquat (8FR) treated soils ($p < 0.05$).

The mean fungal counts as observed in thirty (30) days of exposure ranged from 19.7 x 10^4 cfu/g, 20.7 x 10^4 cfu/g, 27.3 x 10^4 cfu/g, 29.0 x 10^4 cfu/g, 29.3 x 10^4 cfu/g to 29.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively. No significant impact ($p > 0.05$) of paraquat pesticides treatments on soil fungal population at 30 DEP.

At the thirty-five (35) days of exposure, the mean fungal counts ranged from 19.0 x 10^4 cfu/g, 23.7 x 10^4 cfu/g, 27.7 x 10^4 cfu/g, 29.3 x 10^4 cfu/g, 29.3 x 10^4 cfu/g to 30.0 x 10^4 cfu/g for 8FR, 4FR, 2FR, 0.5FR FR and control respectively. Result showed that there were no significant impacts of paraquat treatments on the mean fungal counts of soils samples ($p > 0.05$) among all the treatment levels.

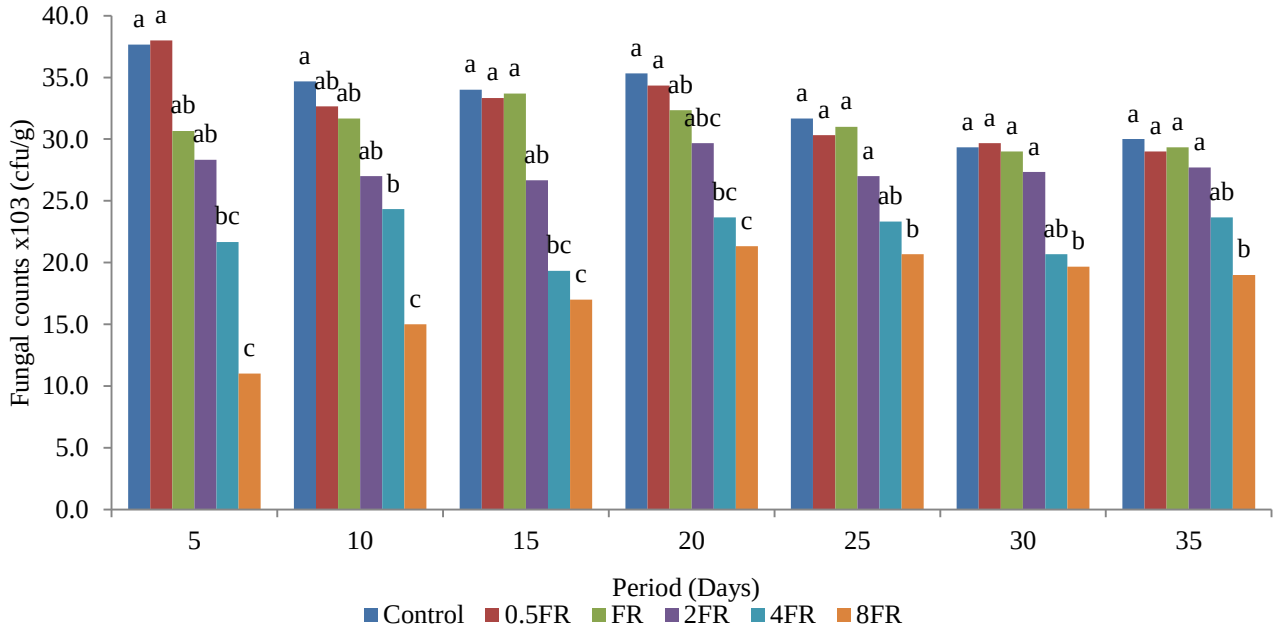

Figure-7: Mean fungal counts in soil treated with paraquat pesticide.

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

Degree of impacts of paraquat treatments on fungal population relative to control over the period of exposure: Paraquat pesticide treatments showed various degree of inhibition on fungal counts in all the treated soil samples (Figure-8).

On the soil samples treated with half of the field rate (0.5FR), the highest inhibitory effects (5.0%) on fungal population was observed at 10 days of exposure period while slight stimulatory effects (0.8% and 1.0%) were observed at 5 and 35 day of exposure periods (DEP) respectively (Figure-8).

The field rate (FR) treatments had the highest inhibition (18.6%) on fungal population at 5 DEP, while the least inhibitory effect (0.9%) was observed at 15 DEP.

The highest inhibitory effects (24.7%, 42.4% and 70.8%) of the higher paraquat treatment levels (2FR, 4FR and 8FR respectively) on fungal colonies were observed in the first five days of exposure.

Generally, the inhibitory effects of paraquat pesticides on fungal population increases with increase in pesticide treatment rate. The progressive reductions in the inhibitory effects of paraquat on soil fungal population were observed over the period of exposure.

Discussion: The results of the present study revealed that these herbicide treatments had differential effects on soil culturable bacterial and fungal populations and the effect is affected by the pesticide type, rate of application and length of exposure.

In glyphosate pesticide treated soils, there were indications that the glyphosate strongly stimulated the bacterial populations of the soil samples during the period of experiment. The glyphosate treatment concentration equivalent to double the recommended field rate (FR) value was observed to cause a marked increase of bacterial population at 10 day of exposure period. Likewise, significant increases in bacterial population were observed in the soil samples treated with FR, 2FR and 4FR glyphosate pesticide from 20 day of exposure to the last day of experiment. Contrarily, significant reduction in bacterial populations was initially observed in the soil treated with highest glyphosate treatment rate (8FR) within the first 10 day of exposure period with marked recovery after 10 days of exposure period. On the other hand no significant effect ($p > 0.05$) was observed on fungal population in the soil treated with glyphosate concentration equivalent to double the field application rate and below (0.5FR, FR and 2FR). While, significant reduction ($p < 0.05$) that lingered throughout the exposure period in the population of culturable fungal was observed in the soils treated with highest treatment rate (8FR).

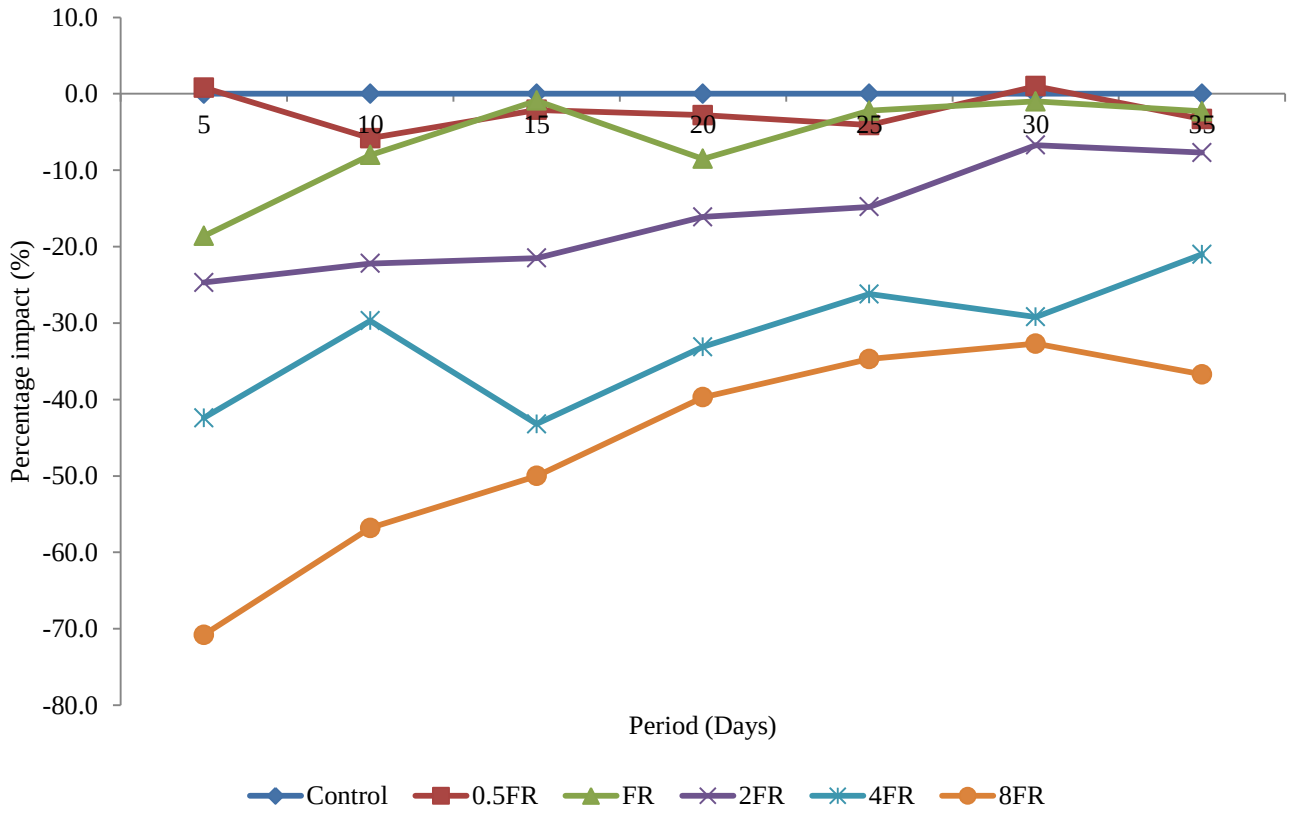

Figure-8: Degree of impact of paraquat treatment levels on fungal population relative to control over the period of exposure.

The observation of the effect of glyphosate on bacterial population was in line with the findings of Busse *et al*. who observed that the application of glyphosate formulation significantly stimulated the growth of soil bacteria and noticed corresponding increases in bacterial population with increases in glyphosate treatment rate[25]. However, they observed that fungal population remained unchanged regardless of the glyphosate application rate[25]. In the forest soil, the commercial formulation of glyphosate pesticide at recommended field rate was reported to have a minor effect on microbial community structure and produces a non-specific, transient stimulation of bacteria at higher concentration[26]. The stimulatory effect of glyphosate pesticide on soil bacteria was reported by Partoazar *et al*. who observed significant increase in bacterial counts in the soil treated with glyphosate at recommended field rate[27]. In 2016, Adomako and Akyeampong observed a gradual increase in bacterial population after treatment with glyphosate pesticide at the concentration rate that double the field application rate[28]. A significance increases in the population of bacterial obtained from farmer's field in Akure, Ondo State, Nigeria was also reported in the soil treated with glyphosate formulation[29]. Seasonal variation in bacterial numbers in the soil treated with glyphosate formulation has also been observed[30].

On the other hand, Ubuoh *et al*. observed a strong inhibitory effect on soil microbial population in the farm land treated with ½ litre and 1 litre of glyphosate formulations respectively[31]. Sebiomo *et al*. reported the significant negative effect on soil bacterial, fungal and actinomycetes populations from the soil samples treated with atrazine, primeextra, paraquat and glyphosate pesticide formulations[32]. In a microcosm study of effects of selected herbicides on soil microbial population, Zain *et al*. observed a significant adverse effect of glyphosate formulation on population of soil bacteria and fungi at concentration as low as half of the recommended field rate, but the effect was observed to disappeared at 20 days of treatment period[24]. Resent study have shown significant reduction in both bacterial and fungal population in farm land soil treated with glyphosate formulation[33].

The stimulatory effect of glyphosate on soil bacteria could be attributed to the ability of bacteria to be able to have some level of tolerance to glyphosate formulation and its potential use as a possible nutrient source. Glyphosate pesticide is an organophosphate ($C_3H_8NO_5P$) compound comprising of simple amino acid and can be used as a source of carbon (C), nitrogen (N) or phosphorus (P) by soil microorganisms[34]. Studies have demonstrated a strong correlation between the rate of glyphosate degradation and the population size of bacteria in the soil[35,36,37].

In Paraquat treated soil samples, significant reduction in bacterial and fungal populations was only observed in the soil treated with higher concentration. Likewise, progressive decline in the percentage inhibition rate of paraquat pesticide treatment on soil bacterial and fungal populations was observed throughout the period of exposure.

Report had shown that the soil samples that had been treated with paraquat pesticides contain lower populations of microorganisms including fungi[38]. Similarly, significant reduction in bacterial and fungal populations in the soil samples treated with high concentration of paraquat pesticide had earlier been reported with about 59.3 % reduction in fungal populations after the 6th day of treatment[24]. Adomako and Akyeampong, observed significant reduction in fungal population of the soil sample treated with concentration of paraquat above field application rate throughout the fifteen days of exposure period[28]. A research has shown that the application of paraquat to a farm land courses about 35 % decreases in the population of fungal population in the soil samples[29].

Paraquat compound is known to be strongly bounded to soil components which could reduce its bioavailability and account for its relatively mild effect at lower concentration[39,40]. However, its toxicity at higher concentration could be explained by its ability to readily convert to highly reactive paraquat radicals in the presence of molecular oxygen (O_2) and consequently the production of superoxide radicals (O_2^-) which is highly toxic to cell[10]. The superoxide radicals are very reactive and possess greater affinity to react and disrupt many processes in the cells and cell membranes. Since molecular oxygen is readily available in the upper layer of the soil and loosely parked soil as used in this research, the microorganisms could be vulnerable to paraquat reactive toxicity.

Generally, application of pesticide at normal field rate poses low or no risk to bacterial and fungal populations of the soil system as observed in this study. However, there were indications that the response of soil bacteria and fungi varied with the type of pesticide used and also strongly affected by the application rate as well as duration of exposure period. Kalia and Gosal, has identified the chemical dosage, the soil properties and various environmental features as part of the main factors that could determine the response of soil microorganisms to the pesticide pollution[41].

Conclusion

This research has shown the effect of herbicide application on the population of non-target soil culturable bacteria and fungi. The outcome as shown that the application of glyphosate formulation at recommended field rate increases the population of bacteria and relatively no effect on fungal population. On the other hand, paraquat formulation was observed to have no effect on the population of both microorganisms. Generally, above field recommended rate paraquat had significant inhibitory effect on the on population of non-target soil culturable bacteria and fungi. Therefore, it is encouraged that the recommended concentration of herbicide formulations should be applied for weed control.

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