Effect of [N-(Phosphonomethl)-glycine] (Glyphosate) Herbicide on Soil Microbial Population

Halima Isa[1*], Mohammed Bashir[1], Maryam Ibraheem[1] and Aisha Mahmud Marafa[2]

[1]*Department of Microbiology, Modibbo Adama University, Yola, Nigeria.*
[2]*School of Science Technology Adamawa State Polytechnique, Yola, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors MB, HI, MI and AMM designed the work, authors HI and MB managed literature searches, authors MI and AMM collected the samples and all authors conducted experimental analyses and laboratory procedures. Authors MI and MB wrote the first draft of the manuscript, author HI wrote the final draft of the manuscript.

Original Research Article

ABSTRACT

Aims: To determine the effect of [N-(phosphonomethl)-glycine] (glyphosate) herbicide on soil bacterial and fungal population.
Study Design: The effect glyphosate herbicide on soil microorganisms population on 2 different farm land was determined by Isolation of bacteria and fungi from untreated and Glyphosate herbicide treated soils using Nutrient agar and Potatoes dextrose agar (PDA) for the bacteria and fungi respectively. The number of bacteria and fungi present in both treated and untreated soil was then enumerated and the isolates determined.
Place and Duration of Study: The study is a cross sectional research and was conducted on two Farm lands located at Yola capital city of Adamawa state Nigeria wheresoil samples were collected and Microbiology laboratory of Modibbo Adama University Yola were the sample was processed and analyzed. The study was conducted from February to May of 2021.
Methodology: Bacteria and fungi were isolated from soil samples before and after treatment of the soils with N-(phosphonomethl)-glycine(Glyphosate) herbicide at different concentration, the bacteria and fungi populations isolated both before and after the treatment were compared.

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Corresponding author: E-mail: halimaisa@mauech.edu.ng, halimaisa@mautech.edu.ng;

Results: The study showed that Glyphosate herbicide caused reduction in the bacterial and fungal count from 3 days of treatment up to 15 days. The bacterial count reduced from 6.1×10^8 cfu/g in the untreated soil to 1.6×10^8 cfu/g on the treatment. Also the fungal count reduced from 1.0×10^8 cfu/g in the untreated soil to 5.0×10^7 cfu/g after 15days of soil treatment. Both the bacterial and fungal count continues to show a gradual decrease up to 15 days in the treated soil. However, several bacteria and fungi were isolated with *Bacillus* spp. and *Micrococcus* spp. having the bacteria with highest occurrence with 42(19.91%) and *Aspergillus*spp. as the fungi with the highest occurrence with 12(42.85%). Statistical analysis of the data obtained indicated that At 95% confidence level, there is a significant difference in the population of bacteria and fungi before and after the soil treatment *P-value*(T>t)=.001

Conclusion: The study revealed that Glyphosate herbicide has a negative effect on soil bacteria and fungi population.

1. INTRODUCTION

Soil microorganisms are very important as almost all chemical transformation in the soil take place with the help of soil microorganisms. These organisms play a key role in soil fertility they also improve the nutrients cycle in the soil and also help in plant growth and development. Several herbicides have effect on these microorganisms. Herbicides can be applied directly to the plants, applied to the soil, or sprayed on to the foliage. Herbicides are applied before, during, or after crop planting in row-crop farming to maximize crop production by diminishing the development of weed. Herbicides are also applied in ponds and lakes to control aquatic plants, in forests to prepare logged areas for replanting on to golf courses, lawn, parks, and other areas to clear out unwanted vegetation [1].

In Nigeria, herbicides have since effectively been used to control weeds in Agricultural systems [2]. As farmers continue to realize the usefulness of herbicides, larger quantity is applied to the soil. There are several types of herbicide which include: paraquat,butachlor,Glyphosate. Glyphosate [N-(phosphonomethI)- glycine] herbicide is a broad-spectrum, non-selective, post emergence herbicide that are widely used in agriculture [3]. It is the active ingredient in roundup and other weed killing formulation especially used in Agriculture and gardens maintenance [4]. However, lyphosate can impact negatively on the soil microbial community, the crops, and even the human population. Herbicides are known to cause changes on the microbial populations in the soil and the activities of species of microorganisms [5].

As a biologically active chemical is applied to ecological systems, it is inevitable that the systems will alter in response to the interference [6]. It is a common practice among local farmers to use high concentrations of herbicide with the hope to yield greater result. Unfortunately this herbicide either directly or indirectly may have effect on soil microorganisms. As such, investigation on the effects of high concentration of herbicide on soil bacterial population needs to be carried out in order to reduce the effects of the herbicide to the microbial population and the plants and enlighten the community on the danger they pose on soil fertility by using excess herbicide to the soil [1].

Glyphosate have been shown to alter the balance of soil microbial populations and metabolite. They also alter the balances of soil ecology [3]. Glyphosate adsorption is higher in low- pH system (pH of 6 and below) and that alkaline soil (PH > 7) tends to poorly adsorb Glyphosate, allowing for greater contamination of water. Glyphosate is less toxic than a number of other herbicide such as those from the organochlorine family [1].

The knowledge of the effects of Glyphosate is of paramount important and it will guide to the appropriate use of this herbicide to reduce its effect to the microbial population. This can be achieved by carrying out studies to assess the effects of this herbicide (Glyphosate) on soil bacteria and fungi population in farm soil.

2. MATERIALS AND METHODS

2.1 Sample Collection

Soil samples used in this study were collected from 2 different farm lands. The soil samples were collected from 0 – 10cm depth using auger and transferred to separate clean containers.

The samples were labeled and the containers sealed off after collecting the correct kilogram. The samples were transported to the laboratory for analysis. Part of sampling point was used as a control point.

2.2 Determination of Bacterial and Fungal Population in the Sample

One gram (1g) of soil sample was added to test tube containing 9ml of sterile distilled water and mixed properly. Serial dilution was conducted by transferring 1ml of the suspension into a separate test tubes containing 9ml of distilled water to obtain a tenth fold serial dilution. One (1) ml of aliquot from 10^{-8} diluted suspensions was poured into sterile clean Petri dishes followed by 20ml of sterile molten nutrient agar and potatoes dextrose ager (PDA) for bacteria and fungi isolation respectively. The PDA was supplemented with antibacterial inhibitor.The plates were shaken gently and allowed to gel and incubated in inverted positions at 37^{0}C and 25^{0}C – 28^{0}C for 24hrs for bacteria and fungi respectively (Araujo et al. 2003).After the incubation period, the colonies were counted using a colony counter and the number of colony forming unit per gram (cfu/g) of the soil sample was calculated and recorded.The colonies were sub cultured on to fresh media to obtain a pure culture [7].The same procedure was followed after treating the soil samples with different concentration of the herbicide to determine the microbial population after soil treatment. Exactly 3ml/100g of different concentration of the herbicide (10ml, 5ml, and 2.5m per litre) was applied, into F_1, F_2 and F_3 respectively. With F_0 serving as the control. Three (3) replicates were made for each treatment and the average result was used. The soil samples treated were allowed to stay for 3 -15 days before the bacterial and fungal population was determined [1].

2.3 Identification of the Isolates

The identification of the organisms was based on their morphological, and biochemical characteristics (for bacteria)as described by Cheesbrough 2006 [7]. The fungal isolated were also identified macroscopically and microscopically using lactophenol cotton blue stain. The differences in their structural appearance were observed and compared with fungal atlas.

2.4 Statistical Analysis

Student T-test was performed to determine if there is significant difference between the Bacterial and fungal population of the soil sample before and after the treatment on the two farms soils analyzed.

3. RESULTS AND DISCUSSION

3.1 Bacterial and Fungal Population in Farm 1

The result of the bacterial and fungal populations, in farm1 before and after treatments is represented in Table 1. The result revealed that the control has the highest microbial count of $4.7x10^{8}$cfu/g, while the lowest microbial count was obtained in F_3 with a microbial count of $6.0x10^{7}$cfu/g. However, the fungal count was also highest in the control (i.e. without herbicide) with $1.0x10^{8}$cfu/g, while sample F_3 has the lowest value with $5.0x10^{7}$ cfu/g.

3.2 Bacterial and Fungal Populations in Farm 2

The result of the bacterial and fungal populations, in the second farm before and after treatments is represented in Table 2. The result revealed that F_0 which is the control has the highest microbial count with $6.1x10^{8}$cfu/g, while the lowest microbial count was obtained in $F_3$with a microbial count of $1.6x10^{8}$cfu/g. However, the fungal count was also highest in the control with $1.2x10^{8}$fu/g, while sample F_3 has the lowest value with $4.0x10^{7}$cfu/g.

3.3 Percentage Distribution of Bacterial Species in Both Farms

Different types of bacteria were isolated in both farm 1 and farm 2. Among the bacterial isolate, B*acillus* spp. and *Micrococcus* spp. have the frequency of occurrence with 42(19.91%). While *Pseudomonas* spp. was found to be least bacteria isolated in both farm 1 and farm 2 as described in Table 3.

3.4 Percentage Distribution of Fungal Species in Both Farms

The percentage of distribution of fungal isolates from farm 1 and 2 are calculated in the Table 4. The result revealed that *Aspergillus* spp. was highly present in the farm 1 and farm 2 with 12(42.85%). Both *Penicillium* spp. and *Actinomycetes* were found to have 8(28.57%) in both the farms.

Table 1. Bacterial and fungal populations in farm 1

Glyphosate level	Bacterial population (cfu/g)		Fungal population (cfu/g)	
	No. of isolates	Bacterial population	No. isolates	Fungal population
(F_0)	47	4.7×10^8	10	1.0×10^8
(F_1)	32	3.2×10^8	8	8.0×10^7
(F_2)	18	1.8×10^8	7	7.0×10^7
(F_3)	6	6.0×10^7	5	5.0×10^7

Key: F_0 Control, F_1, soil sample treated with 2.5ml/L concentration, F_2 soil sample treated 5ml/L concentration, F_3 Soil sample treated with 10ml/L herbicide concentration, cfu/g colony forming units per gram

Table 2. Bacterial and fungal populations in farm 2

Glyphosate level	Bacteria population(cfu/g)		Fungal population(cfu/g)	
	No of isolates	Bacterial population	No isolates	Fungal population
(F_0)	61	6.1×10^8	12	1.2×10^8
(F_1)	40	4.0×10^8	9	9.0×10^7
(F_2)	28	2.8×10^8	7	7.0×10^7
(F_3)	16	1.6×10^8	4	4.0×10^7

Key: F_0 Control, F_1 soil sample treated with 2.5ml/L concentration, F_2 soil sample treated 5ml/L concentration, F_3 Soil sample treated with 10ml/L herbicide concentration, cfu/g colony forming units per gram

Table 3. Percentage distribution of bacteria species isolated from all the soil samples in both farm 1 and 2

Bacterial Species	No of Isolates	% Frequency
*Enterobacter*spp.	39	18.48
*Klebsiella*spp.	36	17.06
*Rhizobium*spp.	28	13.27
Micrococcus spp.	42	19.91
Bacillus spp.	42	19.91
Pseudomonas spp.	24	11.37
Total	211	100

Table 4. Percentage distributions of fungal isolate from farm 1 and 2

Fungal species	No of isolates	Percentage frequency (%)
*Penicillium*spp.	8	28.57
*Aspergillus*spp.	12	42.85
*Actinomycetes*spp.	8	28.57
Total	28	100

The attainment of soil management to maintain soil quality depends on the knowledge of how soils respond to agricultural practices. Therefore, recent interest in evaluating the quality of soil resources such as bacterial and fungal population has been stimulated by increasing awareness that soil is a critically important component of the earth's biosphere, functioning not only in the production of food and fiber but also in the maintenance of environmental quality [8]. On the other hand, feeding the ever-increasing human population is most challenging in developing countries because of soil degradation. For instance, in Sub-Saharan African countries, soil fertility depletion is the fundamental biophysical cause for declining per capita food production [9]. This challenge will continue as population pressure increases and degradation of soil resources is aggravated. Reversing this trend lies in the enhancement of sustainable development of the agricultural sector; however, the basis of sustainable agricultural development is good soil quality. Hence maintenance of soil quality is an integral part of sustainable agriculture. The rate of soil quality degradation depends on land use systems, soil types, topography, and climatic conditions. Among these factors, inappropriate land use aggravates the degradation of soil physicochemical and biological properties [10]. In

view of that, land use affects basic processes such as erosion, soil structure and aggregate stability, nutrient cycling, leaching, carbon sequestration, and other similar physical and biochemical processes [11].

Soil fertility index such as texture, pH, organic matter content, cation exchange capacity, exchangeable sodium percentage, available phosphorus, percentage nitrogen (%N) and percentage base saturation, are important soil chemical properties influencing nutrient availability and retention in soil [12].

In this work, a comparison of the effects of total bacteria and fungi count of the soil sample before and after application of the herbicide to the soil showed that Glyphosate herbicide caused reduction in the bacterial and fungal count from 3 days of treatment up to 15 days. The bacterial and fungal count reduced with increase in concentration of the herbicide.

The result obtained in this research agreed with the result of Araujo *et al.,* 2003 [3], who also observed a reduction in the microbial number after treatment of soil with Glyphosate herbicide. A number of reasons could be attached to this phenomenon, which may include the ability of the bacterial and fungal species present in the soil samples to grow in the presence of Glyphosate herbicide, the geographical location, the weather, concentration and quantity of Glyphosate applied onto the soil during the treatment.

This study is also in line with the research conducted by Bashir *et al.,* 2016 [1], were it was discovered that the Glyphosate herbicide completely eliminate bacterial and fungal growth in the soil. Although, in this study bacterial and fungal growths were not completely eliminated, but if concentration of the herbicide and the duration of the treatment increase, it is likely that the growth will be eliminated completely.

Various bacterial species were isolated but only *Aspergillus*spp. survived 15 days of treatment with Glyphosate herbicide. While *Actinomycetes*and *Penicillium*spp. disappear after 8 days due to the effects of the Glyphosate herbicide. This indicates that not all bacteria and fungi can withstand the application of this herbicide and this may lead to changes the type of in species of microbe organisms play a key role in soil fertility they also improve the nutrients cycle in the soil and also help in plant growth and development.

4. CONCLUSION

Glyphosate herbicide was found to have significant effects on the growth and survival of bacteria and fungi in the soil. The effects of Glyphosate herbicide on bacterial and fungal population revealed that these organisms are less resistance to Glyphosate herbicide and it affects their activities in the soil. However the exposure of microorganism to the Glyphosate herbicide can leads to a short and or long term changes on the growth and development of the microbial community in soil. Statistical analysis of the data obtained indicated that at 95% confidence level, there is a significant difference in the population of bacteria and fungi before and after the soil treatment *P-value* (T>t) =.001. In view of that, it is recommended that farmers should practice the various methods of incorporation of crop residues to the soils as they will play an important role in nutrient availability, preserve the soil microbes and soil aggregate formation which is required in order to enhance crop growth and minimize the use of inorganic fertilizers and their effects on soil quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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