ASSESSMENT OF THE EFFECTS OF HERBICIDE (GLYPHOSATE) APPLICATION ON POPULATION OF FARM SOIL BACTERIA

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ABSTRACT:

One of the most effective ways of increasing food production includes herbicides application to destroy weeds on farmlands. Annually, tones of herbicides are been applied to control harmful effects caused by weeds. Research has shown that sometimes, it is only about 5% of these chemicals reach the target weeds. Most of these herbicides affects the non-target parts of the agricultural ecosystem resulting in the destruction of bacterial population, which is contrary to the aim at which they are applied. Hence, careful attention is needed when dealing with herbicides on farmlands. In this work, research was conducted to determine the effects of glyphosate herbicide application on farm soil bacterial population at different application rates. Soil samples were collected at the depth of 0-15cm from Damaturu farm lands with the aid of Auger, the soils were placed in plastic pots measuring 18.3cm wide and 16 cm height. After three weeks of application, microbiological analyses were conducted using nutrient agar media for the growth and identification of bacteria. The results reveal that herbicide applications caused drastic reduction in bacterial population present in the soil when compared with the control. These findings showed that glyphosate application to kill weeds, have detrimental effects on bacteria in soil especially Rhizobium which is known to improve soil fertility by its nitrogen fixing abilities.

Keywords: Glyphosate, Herbicide, Bacteria, Rhizobium, population

INTRODUCTION

The use of herbicides in agriculture has tremendously assisted in improving the quantity of food production in our modern world of today, of which Nigeria is not an exception. Herbicides despite its importance has tremendous impacts on soil microorganisms whose activities influences overall soil fertility (Zain *et al.*, 2013). Glyphosate [N-(phosphonomethyl) glycine is one of the most widely used broad-spectrum organophosphorus herbicides (Gill *et al.*, 2017). It is a widely used herbicide in agriculture against perennial and annual weeds and in silviculture, domestic gardens, and urban areas (Zhang *et al.*, 2015). It is an essential component of non-selective and post-emergent herbicides used to protect the crop from grasses, annual broad-leaved weeds, woody plants (Conrad *et al.*, 2017). Herbicides are directly toxic to weeds having indirect effects to soil invertebrates (Singh *et al.*, 2020). During the past four decades, a large number of herbicides have been introduced as pre and post-emergent weed killers in many countries of the world. In Nigeria, herbicides have since effectively been used to control weeds in agricultural systems (Wang *et al.*, 2016). As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. But the fate of these compounds in the soils is becoming increasingly important since they could be leached; in which case groundwater is contaminated or immobilizes, and persists on the top soil (Ayansina *et al.*, 2019). These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and man (Simranjid *et al.*, 2020). There is an increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial communities present in soils, and these non-target effects may reduce the performance of important soil functions. These critical soil functions include organic matter degradation, the nitrogen cycle and methane oxidation (Sviridov *et al.*, 2015). Glyphosate has been reported to cause activation in soil urease and invertase both of which are important soil enzymes (Sannino and Gianfreda, 2001). Over the last decade, about 6.1 billion kilograms of the herbicide glyphosate have been applied worldwide (Benbrook, 2016). While pesticides serve useful purposes, concern has been expressed regarding their possible effects on the environment. Ubuoh *et al.*, (2012) gave the following four effects of herbicides on living organisms in the soil: a) they may be directly toxic to animals in soil; b) they may affect the soil organisms genetically to produce population resistant to the herbicides; c) they may have sub-lethal effects that result in alterations in behavior or changes in metabolic or reproductive activity; d) they may be taken into bodies of soil flora or fauna and pass on to the other organisms.

Research portrays that there is much more to be learned about the fate of glyphosate, including its sorption, degradation, and leaching. The fate depends upon the medium and varies a lot from soil to soil as well. This variability does not give a clear prediction, and results generate ambiguous conclusions. Apart from environmental risks, glyphosate is also associated with health risks. This makes for the requirement to develop an eco-friendly strategy for bioremediation (Simranjeet *et al.*, 2020). Glyphosate being a potent inhibitor of EPSPS in the shikimic pathway, exerts negative effects on non-target plants. Various ecosystems and their abiotic and biotic components including animals, plants, and microbes are adversely affected by the indiscriminate use of glyphosate (Amakiri, 2018).

The soil microorganisms like bacteria, fungi, algae and nematodes play important role in soil nutrition through their role in decay of plant and other organic matter in soil as nitrifiers. Anything that disrupts their activity could be expected to affect the nutritional quality of soils and would thus have serious consequences (Gaupp *et al.*, 2015). Also, microorganisms that live in soil can be killed not only by chemicals applied directly to the soil, but also by those that reach the soil in drift from aerial sprays or washed off foliage, which in turn affect the breakdown of some kinds of dead leaf material into its organic and inorganic constituents and in the incorporation of these material into the soil structure (Mishra *et al.*, 2001). Above all, in spite of inherent drawbacks of herbicides in terms of toxicity and environmental pollution, their use either on foliage or in soil cannot be totally dispensed in Damaturu community farms. However, continuous use of herbicide requires constant monitoring with respect to their persistence in soil and plants and effects on soil organisms in terms of ill effects and toxic residue. Soil microorganisms have a great contribution towards soil fertility. Any adverse impact of chemical on soil characteristics and microorganisms may lead to ultimate loss of soil fertility. It's on the basis of this that the aim of this paper is to assess the effects of Glyphosate (Herbicide) application on bacterial population in farm soil of Damaturu Metropolis in Yobe State.

METHODOLOGY

Sample Collection

The method described by Susmita and Behera, (2001) was utilized for collection of the soil samples. Soil samples used in this investigation were collected from Damaturu farm lands. Four sample points were randomly selected in uncultivated farm land designated (F0, F1, F2, and F3) and one sample (F4), was collected from another farm that has been cultivated with herbicide for the past five (5) years. All soil samples were collected from 0 – 15cm depth using auger and transferred to separate clean containers. All samples were labeled and the containers sealed off after collecting the correct volume.

Sample Preparation

Soil samples collected were diluted with Glyphosate after the recommended rate of application per hectare was obtained (5.56mg of active glyphosate ingredient per gram of soil). The land area was calculated to obtain accurate amount to be applied. The recommended rate obtained with respect to the land area was 0.8325ml of Glyphosate into 39.53ml of water. This was multiplied by 15(as multiplication factor) to obtain amount that will reach the bottom of the sample container. For the recommended rate therefore, 12.5ml of Glyphosate was diluted into 589ml of water. At ½ concentration (6.2mls) and (18.75mls) at 1½ herbicide concentration. The control, land with herbicides application for past five years where only treated with water at equal proportion.

Analytical Procedure

Part of soil samples collected were used as control without herbicide application after which the herbicide (Glyphosate) at different concentrations ½, at recommended dose 1, and 1½ the normal concentration was applied, into F1, F2 and F3 respectively. With F0 serving as the control and F4 as farm land cultivated with herbicides for the past five years. These gives a total of 5 sample points collected from two farm lands. Three (3) replicates were made for each treatment to give a total of fifteen (15) samples. The soil samples treated were allowed to stay for three (3) weeks before the analysis begin.

Preparation of Media

All media used for the isolation and counting were prepared according to manufacturer's instructions and specifications.

Isolation and Total Bacterial Count

After 24hrs of incubation, colonies were counted using a colony counter. Pure culture was isolated from this plate by streak plate method using inoculation loop. The isolation of the organisms was based on their morphological characteristics such as color, shape e.t.c. Some of the isolated organisms were designated as F0D1, F1B1, F3D3, and F4E2. The organisms then were subjected to different biochemical tests.

Gram Staining and Biochemical Test

Gram staining to differentiate between gram positive and negative bacteria was carried out using the procedure described by Ann *et al.*, (2005). Biochemical test such as Triple sugar Iron (TSI) and hydrogen Sulphite production test, oxidase, motility, Urea Hydrolysis (Urease) test, IMViC test were all carried out and the result recorded, using the procedures as described by Cheesbrough, (2000).

RESULTS

Bacterial Population

The bacterial population showed a decreasing trend in soils not treated with glyphosate to soils treated up to 5 years. Soils not treated with glyphosate at all which was the control had the highest number of bacteria while the least count of bacteria was encountered in soils that had glyphosate treatment in farm land for five 5 years.

Table 1: Mean of Bacterial population of farm soil to different treatments of glyphosate

Glyphosate levels	Mean of Bacterial Population x 10^{-8}
(F0)	15.25
(F1)	10.00
(F2)	7.00
(F3)	4.00
(F4)	1.75

Key: F0= Control sample, F1= Soil sample treated with ½ concentration, F2= Soil sample treated at recommended rate application, F3= Soil sample treated with 1½ herbicide concentration, F4= Soil sample treated with herbicide for the past five (5) years.

Table 2: Biochemical Test for Isolated Organisms

Isolate No.	GS	MR	VP	UR	IND	OX	CIT	MOT	H_2S	Gas	LAC	GLU	Suspected Organisms
F_0D_1	-	+	-	-	-	+	-	+	-	+	-	+	*Enterobacter spp.*
F_1B_1	-	+	+	Slw+	-	+	+	-	-	+	+	+	*Klebsiella spp.*
F_3D_3	-	-	-	-	-	+	-	+	-	-	+	+	*Rhizobium spp.*
F_4E_3	+	+	+	-	-	+	-	+	-	+	-	+	*Micrococcus spp.*

Key: GS= Gram stain MR= Methyl red, VP= Voges Proskeaur, UR= urease, LAC= Lactose, GLU= Glucose OX= Oxidase, CIT= Citrate, MOT= Motility, IND= Indole, GLU= Glucose, SLW= slow, Positive (+), Negative (-)

Table: 3 Group statistics indicating significance differences between mean populations of bacteria found in soil treated with glyphosate.

Treatments	Means differences	S.E. Difference	Sig. (2-tailed)
F0 &F1	5.25	1.702	0.022
F0 & F2	8.25	1.702	0.003
F0 & F3	11.25	1.797	0.001
F0& F4	13.25	1.458	0.000
F1& F2	3.00	1.543	0.097
F1& F3	6.00	1.633	0.010
F1& F4	8.25	1.250	0.001

F2 & F3	3.00	1.633	0.116
F2& F4	5.25	1.250	0.006
F3& F4	2.25	1.377	0.153

Key: **sig**: Significant difference, **S. E**= Standard Error.

Table 3: the group statistics indicated that there were significant differences between most of the treatments, except at F1& F2, F2 & F3 and F3& F4.

Bacteria Isolated from soils

Bacteria isolated from this study are Enterobacter, Klebseilla, Rhizobium and Micrococcus. The occurrences of these organisms are seen below. It was observed that the total number of organisms decreases as the herbicide concentration increases. The control sample (F0) has the highest population and soil sample treated with full strength glyphosate application for the past five years (F4) has the lowest number.

Table: 4 Bacterial Isolates (Occurrences) from soil samples treated with glyphosate herbicide.

Bacterial Isolate	F0	F1	F2	F3	F4
Enterobacter spp.	15	10	8	6	3
Klebsiella spp.	13	13	7	3	2
Rhizobium spp.	14	9	4	1	0
Micrococcus spp.	19	8	9	6	2
TOTAL	61	40	28	16	7

Table: 5 Total colony counts at different concentrations

Treatments	Colony Count	Bacterial Count (CFU/ml)
F0	61	6.1×10^7
F1	40	4.0×10^7
F2	28	2.8×10^7
F3	16	1.6×10^7
F4	7	7.0×10^6

Key: F0= Control sample, F1= Soil sample treated with ½ concentration, F2= Soil sample treated at recommended rate application, F3= Soil sample treated with 1½ herbicide concentration, F4= Soil sample treated with herbicide for the past five (5) years, CFU= Colony Forming Unit.

Discussion

The research found that there is a decline in the total number of bacterial population at ½, 1.0, 1½ and soil treated for the past five years when compared with the control soil sample. There were marked significant difference between the groups ($p < 0.05$). The results also evaluated the differences in the population of bacteria at recommended dose rate to farm soils treated at higher than the recommended dose application, it was found that the application of glyphosate at higher than the recommended rate alters the population by decreasing the number of some species of bacteria. Despite the reduction in the population of bacteria from samples treated at higher than the recommended dose application and samples treated for the past five years, the result revealed that there was no significant difference between the groups ($P > 0.05$). For control soil samples and soil samples treated with glyphosate for the past five years, it was shown that there was a wide significance difference. This means that there is a long-term effect of these herbicides on bacterial population.

Some species of bacteria such as Rhizobium despite their functions in nitrogen fixation, were totally affected due to the herbicide prolong exposure in the soil. However, other species such as *Micrococcus* and *Enterobacter* showed some resistance to herbicides even at higher concentrations. The result was in correlation with the research made by Ubuoh, *et al.,* (2012), on the effect of glyphosate on soil microbial spectrum at ½ liter, and 1.0 liter application. There result revealed a drastic reduction in bacterial population when compared to their control. Research by Baley *et al.,* (2005), also revealed that glyphosate is active against some soil borne pathogens. These results however, disagree with other research findings made by (Busse *et al.*, 2001; Hanley *et al.,* 2002; Ratcliff *et al.,* 2006.; Lupwayi *et al.*, 2009;) their studies indicated glyphosate at recommended field use rates has no detrimental effect on microorganisms and that the effect depends on the type of microbial response to the glyphosate being applied and how much actually reach the soil profile.

Conclusion

This research found that soil samples treated with glyphosate herbicides at different concentration including the recommended rate affected the number of bacterial population when compared with the control sample. It was concluded that soil samples treated with glyphosate herbicides has effects on bacterial population in farm soils of Damaturu metropolis.

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