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The Effect of Glyphosate Harbicide on Soil Fungi

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Abstract
Glyphosate herbicide is one of the herbicide used throughout the world and they are very important to agriculture. Despite the role of glyphosate herbicide to agriculture, they also posed direct or indirect threats to the health of humans and also to the nature and survival of soil microorganisms. This study was carried out to determine the effect of glyphosate herbicide on soil fungi. Enumeration of fungal population in the soil samples before and after treatment was carried out. The fungal count was found to be $2.8x10^3$cfu/g before the treatment with glyphosate herbicide and the number continue to decrease up to $0.4x10^3$cfu/g in the 15day of glyphosate herbicide treatment. The fungal population decreased upon treatment with glyphosate herbicide when compared to the control i.e. the untreated soil sample. Glyphosate herbicide causes greater reduction in fungal count because the fungal populations decreased gradually and complete disappearance of some species after 6 days of treatment and continue to decrease up to 15 days after treatment. Soil fungi were isolated from the soil before the application of glyphosate herbicide; they include *A. niger, A. flavus, Penicillium* spp., *Microsporium* spp. *Trychophyton* spp. upon application of glyphosate herbicide, not all the fungi isolated before treatment survive the effect of glyphosate herbicide. The most frequently isolated fungi that survive up to 15 days of treatment is *Aspergillus* species (*A. flavus* and *A. niger*) while *Microsporium* spp., *Trychophyton* spp. and *Penicillium* spp. disappeared completely after 9 days of treatment.
Keywords: Effect, Glyphosate, Herbicide, Soil, Fungi.

INTRODUCTION

The important component of the ecosystem is soil and it serves as a medium for plant growth through the activity of microbial communities. These soil microbial communities (like bacteria and fungi) play critical role in the decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (Zain *et al.,* 2013). Numerous studies have shown the effect of herbicides on soil microorganisms population that ultimately affect the rates of decomposing labile, celluloses and recalcitrant like lignin in a variety of ecosystems. However, microorganisms are a major portion of the biodiversity and biomass of soil; they are considered crucial to life and are present in very large numbers (UNEP, 2011). Studies about the impact of glyphosate on soil micro-organisms have provided contrasting results. Some soil-based studies have not found any threat to soil micro-organisms from glyphosate (Araujo *et al.,* 2003). Glyphosate is among the most popular herbicides registered for forest use for its broad effectiveness on competing vegetation, mild effect on conifers, rapid inactivation in soil, and low mammalian toxicity (Matt *et al.,* 2000). Glyphosate is an integral component of conifer release programs and has led to improvements in the growth of intensively managed forests (Powers and Reynolds, 1999).

Soil microorganisms are an ideal community to evaluate the target effects of glyphosate herbicide because they are affected both directly and indirectly by glyphosate (Matt *et al.,* 2000). Direct, toxic effects result from inhibition of amino acid synthesis via the shikimic acid pathway

Indirect effects of glyphosate may also be a driving force influencing the microbial community (Matt *et al.,* 2000). Long-term control of understory vegetation can reduce soil organic matter and nitrogen content (Busse *et al.*, 1996), both vital resources for microbial activity.

Glyphosate has an amino and phosphoric analogue of the natural amino acid glycine (Ella *et al.,* 2013). Glyphosate was first discovered to have herbicidal activity in 1970 by John Frank (Ella *et al.,* 2013). Glyphosate kills plants by inhibiting the enzymes 5-enolpyruvyl shikimate - 3- phosphate synthase (EPSPS) which catalyzes the reaction of Shikimate - 3 - phosphate (S3p) and phosphoenolpyruvate to form 5 - Enolpyruvyl - Shikimate - 3- phosphate (ESP). ESP is subsequently dephosphorylated to chorismate an essential precursor in plants for the aromatic amino acides:

phenylalanine, tyrosine and tryptophan biosynthesis is inhibited (Zablotowiez and Reddy, 2004; USEPA, 2006).

Glyphosate is less toxic than a number of other herbicides such as those from the organochlorine family (Williams *et al.*, 2000, USEPA, 2006). Ingestion of one or several mouthfuls of glyphosate has been found to cause fatalities in people despite immediate and intensive treatment (Ella *et al.,* 2013). Acute toxicity induced by glyphosate is enhanced when ingestion (100 ml or more) is accompanied by esophageal injuries (Chang *et al.*, 1999). Glyphosate has been shown to alter the balance of soil microbial populations and metabolites. They also alter the balances of soil ecology (Araujo *et al.*, 2003).

Glyphosate selectively binds with iron and aluminum oxides in the soil, but is released when phosphates are heavily introduced (Ella *et al.*, 2013). The glyphosate adsorption is highest in low – pH system (pH of 6 or below), and that alkaline soils (pH > 7) tend to poorly adsorb glyphosate, allowing for greater contamination of water. In the soil environment, glyphosate is resistant to chemical degradation such as hydrolysis and is stable to sunlight (Ella *et al.,* 2013). The primary metabolite of glyphosate is amino-methyl phosphonic acid (AMPA) which has a slower degradation rate than glyphosate. Glyphosate has been considered an environmentally safe herbicide because it is assumed to be inactivated quickly after spraying due to rapid sorption onto particles in the soil, and its fast degradation by microbes (Hagner *et al.,* 2015). In addition, the mechanism by which it kills plants (inhibiting the shikimic acid metabolic pathway) (Sviridov *et al.,* 2015) and it is thought to be unique to plants and some micro-organisms, including bacteria, algae and fungi, and thus theoretically not a threat to mammals (Gaupp-Berghausen *et al.*, 2015).

Furthermore, the half-life of glyphosate, which gives an indication of its persistence in the soil and water, is believed to be longer than previously thought (Myers *et al.*, 2016). Recent research suggests that the herbicide persists longer with the return of crop residues containing glyphosate to the soil (Mamy *et al.*, 2016). However, Stratton and Stewart (1992) discovered that higher concentrations of glyphosate herbicide treatment resulted in much lower microbial counts. Therefore, the low concentration of glyphosate herbicide in the soil when applied at the normal application rates is most unlikely to cause any detrimental effect on soil microbes and soil fertility.

MATERIALS AND METHODS

Source of Herbicide

Glyphosate herbicide was used in this study and the herbicide was obtained from a local herbicide dealer store in Girei market, Girei local government, Adamawa State.

Soil Treatment

The soil was treated with glyphosate herbicide using a knapsack sprayer at a dilution rate of 0.2 liter per $5cm^2$ of land in 20 liters of water which corresponds to company recommended rate of 5 liters per 1Hectre of land in 400 liters of water (Zain *et al.,* 2013).

Sample Collection

The soil samples were collected at 5cm depth from open demarcated field at Mautech, Yola area of Girei local government. Five grams (5g) of soil samples were collected using a knife and then covered with aluminum foil. Soil samples were collected immediately before treatment and at 3 days interval after treatment up to the 15 days. The collected soil samples were sieve using a 2-mm sieve, placed in polythene bags and transported to the laboratory for analysis (Haney *et al.*, 2002).

Isolation and Enumeration of Microbial Population

Isolation and enumeration of the microbial populations was conducted using specific media for the growth of fungi. The growth media was supplemented with antibacterial inhibitor, Potatoes Dextrose Agar (PDA) was used for the enumeration of fungi in the samples (Araujo *et al.,* 2003). The antibacterial inhibitor was added into sterilized media (121^0C for 15 min) accordingly, and mixed thoroughly on hotplate and stirrer (Jenway) before pouring into each Petri dish. Ten fold dilution of the soil suspension was made i.e. 1g of soil sample was dispensed into 9ml of distilled water. Serial dilation was carried out to reduce the concentration of microbes in the sample. Thereafter, 0.1ml of the suspension from 10^{-5} was dispensed into the Potatoes Dextrose Agar (PDA) by pour plate method. The PDA was supplemented with chloramphenicol to prevent bacterial growth and it was incubated at 28^0C for 72 hours.

After incubation, the plates were placed on a colony counter to determine the number of colony forming unit per gram of each sample. The number colonies obtained was multiplied by the inverse of the dilution factor to obtain the number of colony forming unit per gram of each soil sample (cfu/g) (Araujo *et al.,* 2003; Cheesbrough, 2006).

Fungal Identification

The fungal isolates were examined on the basis of their morphological characteristics. This involved the appearance, texture and pigmentation of the colonies.

The microscopic morphology of each isolates was studied in lactophenol cotton blue mounts as described by Cheesbrough (2006).

RESULTS

Total Fungal Count (TFC)

The effect of glyphosate on fungal population at the start and end of the work is shown in table 1 below, the highest fungal count was observed before treatment of the soil with glyphosate herbicide. The application of glyphosate herbicide resulted in low fungal count and the population decreased significantly during the first 3 days up to 6 days after treatment.

Table 1: Total Fungal Count (TFC) of Soil Before and After Treatment with Glyphosate Herbicide

Soil	Days	Total Fungal Count (CFU/g)
BT	-	$2.8x10^3$
AT	Day 3	$1.8x10^3$
AT	Day 6	$1.0x10^3$
AT	Day 9	$0.6 x10^3$
AT	Day 12	$0.6x10^3$
AT	Day 15	$0.4x10^3$

Key: BT = Before Treatment
AT = After Treatment

Fungi Isolated from the Soil

Effect of glyphosate herbicide on various fungal populations in the soil is presented in table 2. Before the soil treatment with glyphosate herbicide, various species of fungi were isolated. These include *Penicillium, Microsporium, Trychophyton and Aspergillus* species. However, after soil treatment with glyphosate herbicide it was observed that as the number of days increased the fungal population also decreased up to 6 days after treatment. Complete disappearance of some of the species such as *Penicillium, Microsporium and Trychophyton* was also observed after 9 days of soil treatment with glyphosate herbicide. The only surviving species up to 15 days of treatment ware *A. niger* and *A. flavus*.

Table 2: Fungal Isolate from Untreated and Treated soil

Soil	Days	*A. niger*	*A. flavus*	*Penicillium* spp.	*Microsporium* spp.	*Trychophyton* spp.
BT	-	+	+	+	+	+
AT	Day 3	+	+	+	+	+
AT	Day 6	+	+	+	+	+
AT	Day 9	+	+	-	-	-
AT	Day 12	+	+	-	-	-
AT	Day 15	+	+	-	-	-

Key: BT = Before Treatment
AT = After Treatment
+ = Present
- = absent

DISCUSSION

This work was designed to determine the effect of glyphosate herbicide on soil fungi. In this work, a comparison of the effect of total fungal count (TFC) of the soil sample before and after application of the herbicide to the soil showed that glyphosate herbicide caused reduction in the fungal population of the soil samples. There was a decrease in fungal count from 3 days of the treatment up to 15 days. The fungal count reduced from $2.8x10^3$cfu/g in the untreated soil to $1.8x10^3$cfu/g after three (3) days of soil treatment. The fungal count continues to show a gradual decrease up to day 15 in the treated soil. This could be attributed to the fact that fungi do not resist the effect of glyphosate herbicide. This study was in-line with the research conducted by matt *et al.,* (2000) were they discovered that the glyphosate herbicide completely eliminated fungal growth in the soil. The result obtained agrees with the result of Araujo *et al.,* 2003 who also observed a reduction in the microbial number after treatment of soil with glyphosate herbicide. A number of reasons have been advanced for this phenomenon. This depends on the ability of the fungal to grow in the presence of glyphosate

herbicide and the concentration of glyphosate used.

Prior to the application of glyphosate herbicide to the soil treatment, various fungal species were isolated include *Microsporium* spp., *Penicillium* spp., *Trychophyton* spp. and *Aspergillus* spp. *(A. niger and A. flavus)* but only *Aspergillus* spp. *(A. niger and A. flavus)* survived 15 days of treatment with glyphosate herbicide while *Microsporium* spp., *Penicillium* spp., and *Trychophyton* spp. disappeared after 6days of treatment due to the effect of glyphosate herbicide.

From the result obtained, it was also found out that glyphosate herbicide caused greater effect on fungal species because they disappear after 9days of treatment with only *Aspergillus* species *(A. niger and A. flavus)* grew very well in the present of glyphosate herbicide up to 15 days after treatment. However, *Microsporium, Penicillium and Trychophyton* species disappeared up to 15 days post treatment with glyphosate.

Statistical analysis to test the significant difference fungal populations before and after treatment of the soil with glyphosate herbicide indicated that there is a significant difference in the population of fungi before and after the glyphosate herbicide treatment with $2.8x10^3$cfu/g and $0.4x10^3$cfu/g respectively. Stata_11 version 419.11.701 at 95% confidence limit, p value is 0.0001 fungi.

CONCLUSION

The results of this study showed that glyphosate herbicide have a significant toxic effect on the growth fungi in the soil. The effect of the glyphosate herbicide on fungal population revealed that fungi are less resistant to the glyphosate herbicide and it can affect their activities in the soil. However, the exposures of microorganisms to the glyphosate herbicide can lead to a short and/or long term changes on the growth and development of the microbial community in soil.

RECOMMENDATION

With respect to the health and ecological disturbance that could be associated with the use of glyphosate herbicide, there is need for controlled importation and sell of glyphosate herbicide to farmers. Also the use of glyphosate herbicide should always be considered as the last resort not to be use when alternative of lesser hazard herbicides are available and also preference should be given to herbicide that have low toxicity and which persist for a short time.

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