THE EFFECT OF GLYPHOSATE (N-PHOSPHONOMETHYL GLYCINE) A NON-SELECTIVE HERBICIDE ON THE GROWTH OF SOME SELECTED SOIL FUNGI

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SUMMARY

Glyphosate (n-phosphonomethyl glycine) is one of the leading non selective herbicide used in weed management throughout the world. However, the excessive use of this herbicide portends serious danger not only to human health but affects the environment. Aside these, the nature and survival of various groups of microorganism including soil fungi may be affected. Thus, this study is aimed to investigate the effect of glyphosate (n-phosphonomethyl glycine) herbicide on the growth of soil fungi isolates from Ahmadu Bello University botanical garden. Isolation of soil fungi was carried out using serial dilution techniques. Fungal identification was achieved via microscopic and macroscopic features. Varying concentrations of glyphosphate (0, 0.01, 0.1, 0.5 and 1 % v/v) was incorporated on sterilized Potato Dextrose Agar (PDA) and the isolated fungi were grown on it. Radial mycelial growth of fungal isolates observed at 24 hours interval at 28 ± 2 °C then calculated. All treatments were laid in Complete Randomize Design and replicated three times. The results obtained were subjected to one way analysis of variance (ANOVA). The result obtained showed that three fungi; *Fusarium solani*, *Aspergillus niger* and *Trichoderma* spp were isolated from the soil samples. The radial mycelial growth of *Fusarium* sp., *Aspergillus niger* and *Trichoderma* sp. were observed to be significantly reduced with increase in the herbicide concentration at $P \leq 0.05$. Therefore, the results of this study suggest that excessive use of herbicides reduces the growth of soil fungi as such has a negative effect on ecosystem forms and function.

Keywords: Glyphosate, herbicide, mycelial growth, soil fungi

The most widely used herbicide in the world is glyphosate (Coupe *et al.*, 2012). Glyphosate N (phosphonomethyl) glycine, is the active ingredient in a non-selective systemic herbicide used worldwide (19). Soil is an important component of the ecosystem as it serves as a medium for plant growth, through the activity of microbial communities. Soil microbes (like bacteria and fungi) play critical role in the decomposition and nutrient cycling, breakdown of organic matter, release and availability of nutrients in soil and degradation of xenobiotic which in turn, affect Soil fertility and plant growth (Bending *et a*l., 2002). The non-target toxicity effects of herbicide in the soil may cause alteration of microbial population structure, function and soil health (Avidano *et al*., 2005). Soil fungus is the dominant organism among the soil microbial groups which is widely distributed in the upper layers of the soil (Zabaloy *et al*., 2008). Fungi are known to be extremely adaptable in different environment due to their ability to breakdown many complex substrates including herbicides. However, some microorganisms have varying sensitivity to a particular herbicides resulting in their reduced number and activity (Maciel *et al*., 2012). Ideal herbicides should have the quick ability to be degraded into non-toxic substances that ultimately exert less toxic effect on soil microbes (Araujo *et al*., 2003). Although, Glyphosate is one of the herbicide used throughout the world for weed management. It excessive usage poses serious danger not only to human health but also affects agricultural productivity and microorganisms including fungi (Durgadevi *et al*., 2017). Therefore, the study is aimed to investigate the effect of glyphosate (n-phosphonomethyl glycine) herbicide on the growth of soil fungi isolates from Ahmadu Bello University botanical garden.

MATERIALS AND METHODS

Sample collection

The soil sample was collected from Ahmadu Bello University Zaria botanical garden at the depth of 15cm using soil auger. Fifteen gram (15 g) of soil were taken and packed in a sterilized polythene bag and then taken to the laboratory for further analysis (Abdullahi *et al*., 2018)**.**

Source of the herbicide

Glyphosate herbicide was used in this study and the herbicide was obtained from a licensed herbicide distributor in Sabon Gari Local Government Area of Kaduna State.

Media preparation

Two hundred gram (200 g) of Irish potato was peeled and chopped, cooked using 2 L of distil water. The suspension was filtered through muslin cloth and agar and dextrose sugar (20 g each) was added into the filtrate and stored to obtain the Potato dextrose agar (PDA) which was sterilized using autoclave at 121 ±1^{0}C for 18 minutes in a portable autoclave, Griffin and George), cooled to 40 ±1^{0}C and amended with 0.25 g/l of chloramphenicol before 20 ml was dispensed into each sterile petri dish.

Isolation and identification of fungi isolates from the soil samples

The soil fungi were isolated by the serial dilution technique. One gram (1 g) of each soil sample was weighed and poured into a McCartney bottle containing 10 ml sterile distilled water and shaken vigorously for 5 minutes. Subsequently, 1 ml of the homogenized solution was taken using a sterile syringe and transferred to another McCartney bottle containing 9 ml sterile distilled water to make a 10^{-2} dilution. This process was repeated until a dilution of a thousand times (10^{-3}) and ten thousand times (10^{-4}) concentrations were achieved. For each dilution, 1 ml was pipetted into petri dish with potato dextrose agar (PDA) containing 1% streptomycin. The inoculated PDA petri dishes were then incubated at 28 ±1^{o}C and observed daily. Visible fungal colonies were transferred to freshly prepared Potato Dextrose Agar (PDA) amended with chloramphenicol (0.25 g/L) and also incubated in a Gallenkamp cooled incubator (England) at 28 ±1C for 5 days. The plates were observed daily for growth pattern of fungi (Zabaloy *et al*., 2012; Ubogu *et al*., 2015). The control was also incubated but without any soil suspension. Fungal morphology was observed by taking note of the colony features (growth pattern, shape and color) and microscopically by using sterile needle to collect a strand of mycelia, placed on a clean glass slide which was stained with lacto phenol cotton blue and then observed under microscope for conidia, conidiophores and arrangement of the spores (Lane *et al*., 2012; Zabaloy *et al*., 2012). These features were observed and compared with the fungal micrographs (Weaver *et al*., 2007).

Preparation of the various concentration of the herbicide

In addition to the control, various concentration of the herbicide was prepared by adding appropriate volume of the herbicide (aqueous solution (SL) Glyphosate 40% SL, Isopropylamine salt of glyphosate equivalent to 360 g/l glyphosate) undiluted as constituted and marketed by the manufacturer to the molten PDA at 45°C to obtain the following concentration 0, 0.01, 0.1, 0.5 and 1% v/v (16).

Determination of radial mycelial extension and percentage mycelial growth inhibitions due to herbicide

The radial growth of the fungal isolates on the PDA plates containing the herbicide at various concentration was determined obtaining 85 mm agar disc from the edge of actively growing pure culture of the individual fungal isolates and placing on the PDA plates containing the varying concentrations of herbicides after which measurements were then taken using a ruler (Ubogu *et al*., 2015).

The percentage mycelial growth inhibition of fungal isolates was determined by adopting the formula;

Percentage (%) mycelial growth inhibition = $\frac{Dc - Dr}{Dc} \times 100$

Where Dc = radial mycelial extension growth on the PDA without herbicide, Dr = radial mycelial growth on PDA with herbicide.

RESULTS

Macroscopic morphology of Fungal Isolates

Mycelia were observed to be wooly to cottony, spreading colonies on PDA. The color of the colony from the top view is white having a circular growth pattern (Plate I). Mycelia were observed to be black with a circular growth pattern (Plate II). Mycelia were observed to be uniformly dispersed in concentric rings forming a continuous lawn, cottony, dark green core with completely visible older mycelia zones of fertile conidiophores while younger mycelia zones were white coloured (Plate III).

Microscopic morphology of Fungal Isolates

Hyaline septate, conidiaphores, phialides, microconidia and macrocinidia were observed microscopically.

Microcinidia were observed to be cylindrical in shape while macroconidia produced were unbranched and have sickle shape (Plate IV).

Hyphae were observed to be septate and hyaline, conidial head were radiate initially and splitted into cloumns at maturity. Conidiaphores were long, smooth and hyaline which became darker at apex and terminated in circular and well pronounced globose vesicles. The conidia were brown-black and very rough globose (Plate V).

Fertile conidiophores with several levels of branching were observed. Branches near the tip bore a single phialide and that did not rebranched. Branches of conidiophore distal to the tips were longer than branches closer to the tips. Solitary phialides were cylindrical and swollen at the middle terminating in a single cell and subtended by an intercalary phialide (Plate VI).

Identification of Fungal Isolates

The result of the identification of fungal isolates when compared with the fungal micrographs and macrographs (18) indicated that they were *Fusarium* species, *Aspergillus niger* and *Trichoderma* species, respectively.

Plate I: Macroscopic view of *Fusarium* species

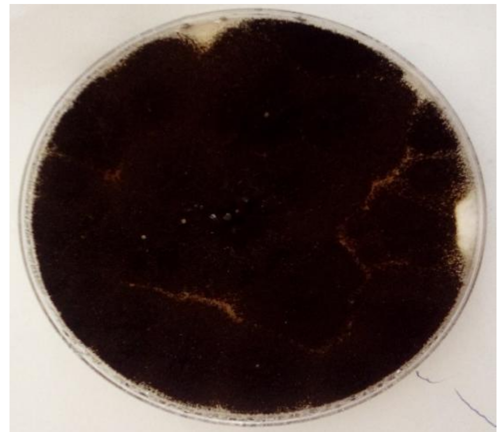


Plate II: Macroscopic view of *Aspergillus niger*

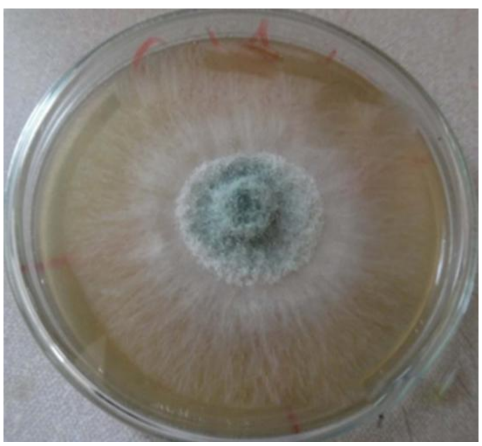
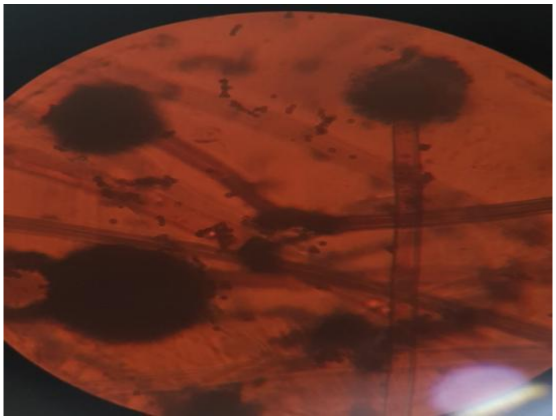


Plate III: Microscopic view of *Trichoderma* species

Plate IV: Microscopic view of *Fusarium* species



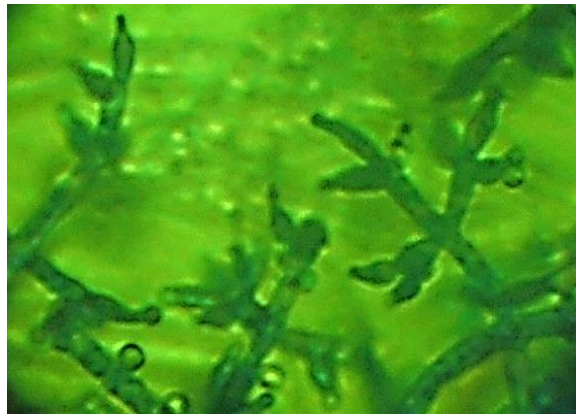


Plate V: Microscopic view of *Aspergillus niger* **Plate VI:** Microscopic view of *Trichoderma* species

Effect of Glyphosate on the Mycelia Growth of the Fungi Species

The effect of glyphosate on the mycelia growth of the *Fusarium solani* is shown on Table 1. On day 1 after inoculation, plates treated with 0.01 % glyphosate produced mycelia growth lengths which was statistically similar (P>0.05) with those of control (0 %). However, 0.1 %, 0.5 % and 1 % glyphosate treated plates were significantly lower (P<0.05) than all herbicide concentrations and the control. Records taken day 2 after inoculation showed that control and 0.01 % glyphosate treatment produced significantly higher (P<0.05) mycelia growth than the other treatments, with 1 % glyphosate treatments producing the least. The mycelia length produced at day 3 after inoculation indicated that control produced significantly higher mycelia growths in length and width than any other treatments, with 1 % glyphosate treatments producing the least.

The effect of the plant glyphosates on the mycelia growth of the *Aspergillus niger* is shown on Table 2. On day 1 after inoculation, plates treated with 0.01 % and 0.1 % glyphosate produced mycelia growth lengths, 2.2 and 2.0, respectively which were statistically similar with those of control (0 %) of 2.5 cm. However, 0.5 % and 1 % glyphosate treated plate had 1.5 cm and 1.2cm mycelia length which were significantly lower (P<0.05) than all other after all herbicide concentrations and the control. Records taken day 2 and day 3 after inoculation also showed that control, 0.01 % and 0.1 % produced significantly higher (P<0.05) mycelia growth in length and width than any other treatments. Meanwhile, 0.5 % and 1 % glyphosate treated treatments produced lesser growth though the least growth was observed with 1 % glyphosate amended treatments in day 2 and 3.

Table 1: Radial mycelia growth of *Fusarium* species on PDA as affected by varying concentration of glyphosate herbicide

Herbicide concentration	Day 1		Day 2		Day 3	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)
0%	$3.1^{a}\pm0.03$	$3.0^{a}\pm0.12$	5.1a$\pm$0.09	$4.8^{a}\pm0.19$	$8.3^{a}\pm0.13$	$8.^{a}\pm0.12$
0.01%	$2.8^{a}\pm0.11$	$2.9^{a}\pm0.13$	$4.8^{a}\pm0.18$	$4.7^{b}\pm0.29$	$8.0^{a}\pm0.28$	$7.8^{a}\pm0.25$
0.1%	$1.7^{b}\pm0.22$	$1.5^{b}\pm0.04$	$3.3^{b}\pm0.24$	$3.2^{b}\pm0.02$	$6.1^{b}\pm0.08$	$6.0^{b}\pm0.01$
0.5%	$1.4^{b}\pm0.19$	$1.3^{b}\pm0.21$	$2.8^{b}\pm0.10$	$2.6^{b}\pm0.06$	$4.8^{b}\pm0.13$	$4.7^{b}\pm0.09$
1%	$1.1^{c}\pm0.02$	$1.1^{c}\pm0.08$	$2.4^{c}\pm0.28$	$2.2^{c}\pm0.11$	$4.2^{c}\pm0.21$	$4.1^{c}\pm0.03$
LSD	0.90	0.80	1.30	1.20	1.80	1.70

Means followed by the same letter of superscript are not significantly different (P<0.05) along the column using Least significantly difference (LSD).

Table 2: Radial mycelia growth of *Aspergillus niger* on PDA as affected by varying concentration of glyphosate herbicide

Herbicide concentration	Day 1		Day 2		Day 3	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)
0%	$2.5^{a}\pm0.02$	$2.5^{a}\pm0.08$	$7.5^{a}\pm0.02$	$7.2^{a}\pm0.01$	$8.5^{a}\pm0.03$	$8.4^{a}\pm0.21$
0.01%	$2.2^{a}\pm0.07$	$2.2^{ab}\pm0.03$	$7.3^{a}\pm0.23$	$7.1^{a}\pm0.22$	$8.4^{a}\pm0.03$	$8.3^{a}\pm0.04$
0.1%	$2.0^{a}\pm0.01$	$2.0^{ab}\pm0.02$	$7.1^{a}\pm0.12$	$7.1^{a}\pm0.07$	$8.1^{a}\pm0.03$	$8.0^{a}\pm0.07$
0.5%	$1.5^{b}\pm0.03$	$1.8^{b}\pm0.14$	$5.1^{b}\pm0.19$	$5.1^{b}\pm0.03$	$6.3^{b}\pm0.08$	$6.2^{b}\pm0.25$
1%	$1.2^{c}\pm0.06$	$1.2^{c}\pm0.01$	$3.7^{c}\pm0.13$	$3.5^{c}\pm0.16$	$5.1^{c}\pm0.02$	$5.0^{c}\pm0.34$
LSD	0.40	0.42	1.25	1.10	1.12	1.11

Means followed by the same superscript are not significantly different ($P<0.05$) along the column using Least significantly difference (LSD).

The effect of the glyphosate herbicide on the mycelia growth of the *Trichoderma* species is shown on Table 3. On day 1 after inoculation, control (0 %) produced the highest mycelia growth length and width, 2.6 cm and 2.5 cm, respectively which were significantly higher ($P<0.05$) than all other glyphosate concentrations. However, in day 2 after inoculation, 0.01 % and 0.1 % glyphosate concentrations produced 8.5 cm and 8.4 cm mycelia length respectively while 8.5 cm and 8.3 cm width respectively, which were statistically similar to 8.5 cm mycelia growth with control (0 %) at ($P>0.05$). Meanwhile 1% glyphosate concentrations produced the least mycelia growth for day 1 and day2 after inoculation.

Table 3: Radial mycelia growth of *Trichoderma* species on PDA with varying concentration of glyphosate herbicide

Herbicide concentration	Day 1		Day 2	
	Length (cm)	Width(cm)	Length(cm)	Width(cm)
0%	$2.6^{a} \pm 0.07$	$2.5^{a} \pm 0.19$	$8.5^{a} \pm 0.12$	$8.5^{a} \pm 0.03$
0.01%	$2.5^{a} \pm 0.24$	$2.3^{a} \pm 0.11$	$8.5^{a} \pm 0.22$	$8.5^{a} \pm 0.12$
0.1%	$1.8^{b} \pm 0.18$	$1.6^{b} \pm 0.08$	$8.4^{a} \pm 0.04$	$8.3^{a} \pm 0.15$
0.5%	$1.2^{bc} \pm 0.31$	$1.0^{c} \pm 0.25$	$5.0^{b} \pm 0.01$	$4.8^{b} \pm 0.21$
1%	$0.9^{c} \pm 0.23$	$1.2^{bc} \pm 0.09$	$3.4^{c} \pm 0.12$	$3.3^{c} \pm 0.08$
LSD	0.60	0.50	1.43	1.26

Means followed by the same superscript are not significantly different ($P<0.05$) along the column using Least significantly difference (LSD).

DISCUSSION

Identification of fungal isolates

The macroscopic features of *Fusarium* species observed in this study were similar to a report by (Loekas *et al*., 2011). It was reported that the colony form of *Fusarium solani* isolated from soil sample was cotton-like with many aerial mycelia, white hyaline and circular growth pattern. In the same report, the micro-conidial wall was observed to be thick and oval while macro-conidia wall were thick and curve shape (Loekas *et al*., 2011).

The Macroscopic and microscopic features of *Aspergillus niger* observed in this study is similar in description to the report where it was observed that the colonies were black-dark brown colour and globular shape of the conidia was observed to be rough (Daini *et al*., 2011).

The Macroscopic and microscopic features of *Trichoderma* species such as the colony color and branching pattern observed in this study is similar with the observations by several researchers

(Sagarika *et al*., 2017; Abdullahi *et al*., 2018). Similar growth patterns were reported with different *Trichoderma* species isolated from soil sample. It was reported that the color of the colonies varied from light green to dark green (Sagarika *et al*., 2017). In another study, *Trichoderma longibrachiatum* isolated from soil samples collected from uncultivated land of Sabon Gari Local Government Area, Zaria Nigeria were observed to be having concentric rings with a dark green core radiating outward with a younger mycelia zone observed to be white in colour (Abdullahi *et al*., 2018).

Effect of glyphosate on mycelial growth of the fungal species

The results from this study clearly show the radial mycelial growth of the isolated fungi; *Fusarium* sp., *Aspergillus niger and Trichoderma* sp. decreased with increasing concentrations of the herbicide. It was reported that non-target toxicity effects of herbicides include the alteration of microbial population structure, function and soil health (Zabaloy *et al*., 2008). Araujo *et al*. (2003) observed that the administration of glyphosate on soil may exert at least temporary changes on the soil microbial activity. In yet another report, glyphosate herbicide was observed to have a significant toxic effect on the growth of some soil fungi (Bashir *et al*., 2018). Previous study demonstrated that repeated application of glyphosate in plots with conventional tillage reduced the number of micro-organisms especially the common saprophytic fungi in soil (Rose *et al.*, 2016). Zabaloy *et al.* (2012) reported that the pathogenic *Mucor* sp. and *Zygorhynchus* sp. were more susceptible to glyphosate exposure compared to *Aspergillus* sp. and *Penicillium* species. The increasing level of glyphosate in media inhibited the hyphal growth in *Mucor* sp. and *Zygorhynchus* sp. compared to *Aspergillus* sp. and *Penicillium* species. The dry matter of *Mucor* sp. and *Zygorhynchus* sp. was reduced by 50% even with 130 mg/L of glyphosate, whereas the same reduction level in *Aspergillus* sp. and *Penicillium* sp. needed a higher concentration of glyphosate. In yet another report by (Zain *et al*., 2013), it was observed that at higher concentration of paraquat and glufosinate ammonium high growth inhibition of 70%-100% in *Mucor* sp., *Aspergillus* sp. and *Penicillium* species was recorded. However, glyphosate was found to be less toxic to *Aspergillus* and *Penicillium* while causing inhibitory effect on *Mucor*. However, a positive effect of glyphosate in promoting the growth of soil microorganisms was also reported (Van der Heijden *et al.,* 2003). A higher fungal count was found in soil when supplemented with a low dosage of herbicide for the first time. In another report, Glyphosate application at a rate of 50 mg kg-1 was observed to increase the fungal population in soil (Ratcliff *et al*., 2006).

CONCLUSION

In conclusion, three soil fungi; *Fusarium* sp., *Aspergillus niger and Trichoderma* sp. were isolated from ABU botanical garden. The results from this study also show that glyphosate herbicide have a significant toxic effect on the mycelia growth of the fungi isolates. The highest inhibition of glyphosate herbicide on all the fungal isolates was recorded at 1% v/v of the herbicide which was the highest rate of concentration evaluated.

The indiscriminate and excessive use of glyphosate herbicides in the management and control of weeds should be discouraged. There is the need for controlled importation and sell of glyphosate herbicide to farmers; this is necessitated due to the health and ecological disturbance that is associated with the use of glyphosate herbicide. Also the use of glyphosate herbicide should always be considered as the last resort, it should not to be used when there are alternative to pest and disease management. It is recommended that only low concentrations of glyphosate herbicide should be used when necessary.

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