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ASSESSMENT OF THE EFFECTS OF GYPHOSATE HERBICIDE ON SOIL INVERTEBRATE POPULATIONS AND FUNGAL COLONIES IN BENUE STATE, NIGERIA

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Abstract

Herbicides containing glyphosate are widely used in agriculture, however, its negative consequences on soil invertebrate and fungal population is still unknown. The study was aimed to evaluate the effect of glyphosate herbicide on soil invertebrates and fungal population. The effects of glyphosate on some soil organisms were investigated in three locations (Ugbokolo, Ogene and Opialu) in Okpokwu LGA of Benue State. Soil invertebrates and fungi were sampled before spraying and after spraying by collecting fixed areas of soil sample in both the control and impact sites at the rate of $0kgha^{-}$,$^{1}0.84kgha^{-1}$ and $840kgha^{-1}$respectively. Samples were sorted for soil invertebrates and fungi. Results showed that fungi and invertebrates were isolated and identified. Fungal colonies were not affected significantly ($P < 0.05$) by the herbicide however, the number of isolates before application of glyphosate herbicides were higher in four out of the nine fungi sp while five out of nine fungi sp were higher after the application of glyphosate herbicides. Invertebrates soil fauna especially, taxa of Amphipoda (Landhopper), Haplotaxida (Earthworms), Julida (Millipedes), Lithobidda (Centipedes), Collembola (Springtails), Hymenoptera (Ants) and Isopoda (Woodlice) were affected significantly ($P > 0.05$) by the glyphosate herbicide. The effects of the herbicide on soil invertebrates were more severe in the month of November than in the month of July. In conclusion the study of the effect of glyphosate herbicide on soil invertebrate and fungal population provide better understanding of possible response of soil microbes to herbicides. Though, there was no significant adverse effect of glyphosate on soil fungi, however, the effect on soil invertebrates is enough reasons to discourage its continuous application.

Key Word: *Glyphosate, Herbicide, Soil, Invertebrate populations, Fungal colonies*

Introduction

Soil, an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. This soil microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (Singh *et al*.,1999;Chauhan *et al*., 2006;Tripathi *et al*.,2006; Pandey *et al*.,2007).However, soil micro-organisms are greatly influenced by factors including the application of herbicides (Pampulha *et al*., 2007), which are applied in modern agricultural practices to attain optimum crop yields (Zabaloy *et al*., 2008).

The effectiveness of glyphosate is based on the inhibition of 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS) in the shikimate acid pathway, which in turn interferes with the production of aromatic amino acids and secondary compounds required in the defense functions of plants and many microbes (Funke *et al* 2006; Kruger *et al*., 2013). The shikimate pathway is present in many taxa of soil fungi and other organisms, and in principle, glyphosate effects on soil organisms could either be intimate (when organisms growing in soil or on dead plant remains are subjected to glyphosate) or mediated through modified plant chemistry in withering plants. In greenhouse studies, glyphosate application has recently been found to affect microbial composition and enzymatic activity in plant rhizospheres (i.e. in the soil adhered to plant roots) as well as in bulk soil (Arango, 2014; Banks *et al*., 2014; Cherni, 2015; Schafer *et al*., 2014). Disrupting effects of glyphosate on earthworms and other invertebrates (Gaupp-Berghausen et al. –2015) and their interactions with symbiotic mycorrhizal fungi (Zaller *et al*., 2014; Helander 2018) have also been reported. However, there is much controversy about the effects of glyphosate on soil microbial communities and activities (Allegrini *et al* 2015; Hungria et al. 2014; Wolmarans *et al*. 2014) and notably, few studies have so far been carried out in field conditions (Druille *et al*. 2015). As the evidence of the effects of glyphosate-based herbicides on soils *In-situ* is scarce, and so far focuses entirely on microbes and earthworms, more attention is clearly required of the potential side-effects of these compounds on other key soil organisms and their associated ecosystem services (Gaupp-Berghausen *et al*. -2015; Zaller *et al*. 2014).

In general, glysophate and other herbicides were found to cause low mortality to Spiders and Beetles (Chiverton and Sotherton, 1991; Haughton *et al.*, 1999, 2001; Pekar, 2002; Pekar and Beneš, 2008). However glyphosate herbicides are toxic to amphibians particularly the Rana frogs (Cauble and Wagner, 2005). Glyphosate (Roundup), when applied to the soil were found not to exert any significant effect on the total count of soil fungi. However *Acremonium strictum*, *Aspergillus fumigatus* and *Penicillium glabrum* were significantly increased by high doses used and of *Penicillium glabrum* by the high dose only. However, *P. funiculosum* was completely eliminated (Abdel-Mallek *et a*l. 1994).It is thought that glyphosate herbicides can affect fungal population and soil invertebrate fauna negatively through direct toxicity effects and/or indirect effects through habitat modification. As the treated vegetation dies the decreased canopy cover can change the micro climate. The invertebrates often become more exposed to the desiccating sun and wind, accompanied by an increase in surface temperature and decrease in soil moisture (Toth *et. al*. 1996;Haughton *et. al*. 1999; Haughton *et. al*. 2001).

In Nigeria, most of the rural settlers are farmers and Okpokwu Local Government Area is a typical

example in Benue State where over 80% of the adult population engages in farming. From preliminary studies, it was observed that herbicides are extensively used for farming and the farmers in their ignorance use above the stipulated quantity of herbicides per application. These may persist relatively for long periods of time in the environment and may affect non-target organisms.

Materials and Methods

Procedure for Field Sampling

The soil samples were collected randomly from selected plots located in three towns in Okpokwu LGA of Benue state. At each experimental location, paired plots were established, each consisting of a 2×2-m quadrat. One plot of each pair in a location received herbicide spray and the other was set as a control which did not receive herbicide. The treatment and control plots of each pair were separated by 30 m. The control plots were located in a way to avoid possible cross-contamination. The plots had no previous history of any herbicidal application. The soil samples were collected from the upper 10 cm of the soil profile as proposed by Lal and Saxens(1982). Samples were transported in moistened cotton sacks, and extracted within 12 h of collection. No area was ever sampled twice, and samples were never taken adjacent to a previous one.

Treatment Procedure

Treatment included three levels of glyphosate application which were 0, 0.84 and 840 kg a.i./ha and four sampling days (1, 3, 7 and 30) after application. The glyphosate was applied to plots at the rates expressed above using Carbon dioxide pressurized knapsack sprayer. The sprayer had two nozzles (TT110.02), spaced 1.0m with pressure of 200 kpa and spray flow of 100L ha^{-1}. During the spraying the sky was clear and soil moist (Pereira *et al.*, 2008) instrument. The layout of the experiment was completely randomized design. Intermediate glyphosate concentration used was based on the recommended rate of glyphosate being 0.84 kg ha^{-1} (Mc Connell and Hossner, 1985; Sprinkle *et al* , 1995; Haney *et al* , 2000).The highest concentration used (100-fold) was selected to test effects since farmers sometimes use this or higher concentration other than the recommended field rate. A control treatment with no glyphosate was included for each soil.

Invertebrate Identification

All samples were extracted within 12 h of collection and the invertebrates extracted were stored in 70% ethanol. Using a simplified key for invertebrate identification (Cox, 1996), samples were sorted using a hand lense for the following taxa: Amphipoda (landhoppers), Hymenoptera (Ants), Collemobola (Springtails), Haplotaxida (earthworms), Isopoda (woodlice), Julida(millipede) and Lithobidda (Centipede).

Culturing of Fungi

The method adopted in this work is in line with that outlined by Rataliff *et al*. (2006). Fungi were extracted by agitating a 3g sub-sample of soil (oven dry equivalent) in 10 fold dilution of 0.15m sodium chloride (NaCl) with 3mm glass beads for 10 minutes. Serial dilutions of the soil samples

were plated in duplicate on malt extract agar for fungal propagules. Fungi were counted within 7 to 14 days incubation at 28^{o}C.

Microscopic Examination

A drop of 95% ethanol was placed on a microscope slide previously cleaned and using a sterile inoculating needle, small portion of the fungal growth was gently removed and placed on the ethanol. With the aid of another sterile inoculating needle, the fungus was gently spread out thinly. When most of the ethanol had evaporated, a drop of lactophenol cotton blue was added and covered with a cover slip. The preparation was observed with X40 objective and X100 oil Immersion objectives (Alice *et al*., 2006).

Identification of Fungi: The fungi were identified based on their cultural morphology and microscopic characteristics of their spores and hyphae using the simplified key to the classes of fungi (Division: Mycota) and fungus like organisms devoid of chlorophyll (Alexopoulus,1962).

Result

The result of fungi isolates of samples from all locations Ugbokolo, Ogene and Opialu revealed that no significant differences among the number of cells isolated however, *Aspergillus flavus, Geomyces pannorum, Penicillium sp. Rhizopus oryzae, Fusaium soloni* were higher in the number of isolates even after the application of glyphosate herbicides.

The result of invertebrates observed from samples from Ugbokolo, Ogene and Opialu revealed that significant difference exist in the number of invertebrates counted at varying concentrations in addition, there were reduction in the numbers counted for all the species of invertebrates after application of glyphosate herbicides as shown in tables 1 and 2 respectively.

Table: 1 Fungal Colonies Isolated from the Three Locations

	Number of colonies (cfu/ml)x10^2	
Fungi	Before glyphosate application	After glyphosate application
Aspergillus niger	37	34
Aspergillus flavus	22	26
Geomyces pannorum	11	14
Penicillium sp.	28	31
Cladosporium cladosporiodex	07	06
Rhizopus stolonifer	12	08
Rhizopus oryzae	17	18
Mucor hiemalis	21	17
Fusaium soloni	02	06

Table: 2 Fungal Colonies Isolated from the Three locations in the Month of July and November

	Number of colonies (cfu/ml) $x10^2$	
	July	**November**
Aspergillus niger	47	24
Aspergillus flavus	30	18
Geomyces pannorum	18	07
Penicillium sp.	39	20
Cladosporium cladosporiodex	09	04
Rhizopus stolonifer	15	05
Rhizopus oryzae	25	10
Mucor hiemalis	27	11
Fusaium soloni	08	00

Table:3 The mean number of invertebrates observed from the three locations

	Number of invertebrates per square metre	
Invertebrates	Before glyphosate application	After glyphosate application
Hymenoptera (Ants)	182	79
Amphipoda (landhopper)	36	10
Collembola (Springtails)	18	09
Lithobidda (Centipede)	28	12
Haplotaxida (Earth worm)	132	22
Julida (Millipede)	48	21
Isopoda (Woodlice)	26	14
Coleoptera (Beetle)	32	26

Table:4 The mean number of invertebrates observed from the three locations in the month of July and November

	Number of invertebrates per square metre	
	July	**November**
Hymenoptera (Ants)	201	60
Amphipoda (landhopper)	36	10
Collembola (Springtails)	20	07
Lithobidda (Centipede)	31	09
Haplotaxida (Earth worm)	131	23
Julida (Millipede)	55	14
Isopoda (Woodlice)	32	08
Coleoptera (Beetle)	39	19

Discussion

The application of the glyphosate herbicide had direct effect on invertebrates abundance since their number varied among the treated and control plots. The result of fungal cells isolated from Ugbokolo, Ogene and Opialu in the month of July showed no significant differences among the control, 0.84 kgha-1 and 840kgha-1 levels of the herbicide treatment. The result obtained in this investigation is in line with the work of Cliff *et al.* (2006) which showed that no significant main effect were found for fungal cells isolated in whitmore soil. A similar result was also obtained by Bahig *et al.*, (2008).The work of Wardle and Parkinson(1990), however, suggested an increase in the number of fungal cells directly or indirectly after glyphosate application, which is contrally to our result. The result is tested using One-way Analysis of Variance (ANOVA) ($P = 0.4589$) and Tukey-Kramer Multiple Comparisons Test ($P > 0.05$). These tests suggest that variations among column means is not significantly greater than expected. We, there by, accept the null hypotheses that the effect of glyphosate herbicide on soil fungi is merely due to chance

The result of the number of invertebrates observed fron soil smaples in Ugbokolo, Ogene and Opialu in the month of July showed a higher number of invertebrates at the control rate (0 $kgha^{-1}$), while the number of invertebrates where lower at the concentration of 0.840 $kgha^{-1}$ and 840 $kgha^{-1}$ of the glyphosate herbicide respectively. This results is in line with that of Kackson and

Pitue (2004) which show that an indirect effect on weeed species compositions and densities may likely affect invertebrate population than the direct effect of the herbicide.

Number of invertebrate population decresae in the month of July than November.The reason for this observation according to Smith and Aubin (1993) could be due to the relative dryness of the soil in the month of Novermber compared to that of July. Weaver *et al*. (2007), reported that glyphosate desipates relatively rapidly when applied to moist soil than dry soil and that the rate of glyphosate degradation does not appear to be largely dependent on soil pH or organic content, but likely to be temperature dependent. The result of the numbers of soil invertebrates from Ugbokolo, Ogene and Opialu in the month of November, showed that the number of invertebrates reduced with increase in the concentration of glyphosate herbicide. The effect of the glyphosate was also more servere in the month of November when compared to that of the month of July signifying that the glyphosate herbicide persist more in the month of November compared with that of July.

References

Abdel-Mallek A.Y., Abdel-Kader, M.I.A. and Shonkeir, A.M.A (1994). Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. *Microbiological Research* vol (149): 69-73

Allegrini, M., Zabaloy, M. C. and Gómez, E. V. (2015). Ecotoxicological assessment of soil microbial community tolerance to glyphosate. *Sci. Total. Environ.* **533**, 60–68

Arango, L.V. Katharina E.M B and Anton H. (2014). Effects of glyphosate on the bacterial community associated with roots of transgenic Roundup Ready® soybean. *Eur. J. Soil Biol.* **63**, 41–48

Bahig, A.E., Aly, E.A., Khaled, A.A.and Amel, K.A. (2008). Isolation, Characterization and Application of bacterial population from Agricultural Soil at Sohag Province, Egypt. Malaysian Journal of Microbiology Vol. 4 (2), pp 42-50

Banks, M. L., Kennedy, A. C., Kremer, R. J. and Eivazi, F. (2014). Soil microbial community responseto surfactants and herbicides in two soils. *Appl. Soil Ecol.* **74**, 12–20

Chauhan AK, Das A, Kharkwal H, Kharkw al AC, Varma A (2006). Impact of microorganisms on environment and health. In A.K. Chauhan & A. Varma (Eds.). Microbes: *Health and environment*, pp. 1-12, UK: Anshan

Cauble K. and Wagner, R.S. (2005). Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. *Bulletin of Environmental Contamination and Toxicology* 75, 429–435.

Cherni, A. E., Darine, T., Samir, C. and Fathi B. (2015).Effect of glyphosate on enzymatic activities, Rhizobiaceae and total bacterial communities in an agricultural Tunisian soil. *Water. Air Soil Pollut.* **226**, 2263

Chiverton PA, Sotherton NW. (1991). The effects on beneficial arthropods of the exclusion of herbicides from cereal crop edges. *Journal of Applied Ecology* 28, 1027–1039.

Druille, M., Cabello, M. N., García Parisi, P. A., Golluscio, R. A. and Omacini, M. (2015). Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. *Agric. Ecosyst. Environ.* **202**, 48–55

Funke, T., Han, H., Healy-Fried, M. L., Fischer, M. & Schönbrunn, E. (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. *Proc. Natl. Acad. Sci. USA* **103**, 13010–5

Gaupp-Berghausen, M., Rewald, M. H., Johann, G. and Zaller, J. Z. (2015). Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Sci. Rep. UK* **5**, 12886

Haughton AJ, Bell JR, Boatman ND, Wilcox A.(2001). The effect of the herbicide glyphosate on non-target spiders. Part II. Indirect effects on Lepthyphantes tenuis in field margins. *Pest Management Science* 57, 1037–1042.

Haughton, A.J., Wilcox, A., Chaney, K., (1999). The effects of different rates of glyphosate on non-target invertebrates in field margins. *Aspects of Applied Biology* 54, 185–190.

Helander, M. *et al*. (2018). Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback. *Sci. Tot. Environ.* **642**, 285–291

Hungria *et al*. (2014). Effects of the glyphosate-resistance gene and herbicides on soybean: field trials monitoring biological nitrogen fixation and yield. *Field Crops Res.* **158**, 43–54

Krüger, M., Shehata, A. A., Schrödl, W. & Rodloff, A. (2013). Glyphosate suppresses the antagonistic effect of *Enterococcus spp*. on *Clostridium botulinum*. *Anaerobe.* **20**, 74–8

Pampulha ME, Ferreira MA, Oliveira A (2007). Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. *J. Basic Microb.* 47:325-333

Pandey RR, Sharma G, Tripathi SK, Singh AK (2007). Litterfall, litter decomposition and nutrient dynamics in subtropical natural forest and managed plantations in northeastern *India. For. Ecol. Manag.* 240:96-106.

Pekar S, Beneš J. (2008). Aged pesticide residues are detrimental to agrobiont spiders (Araneae). *Journal of Applied Entomology* 132, 614–622.

Pekar S. (2002). Susceptibility of the spider Theridion impressum to 17 pesticides. *Journal of Pest Science* 75, 51–55.

Schafer, J. R., Hallett, S. G. and Johnson, W. G. (2014). Rhizosphere microbial community dynamics in glyphosate-treated susceptible and resistant biotypes of giant ragweed (*Ambrosia trifida*). *Weed Sci.* **62**, 370–381

Singh KP, Singh PK, Tripathi SK (1999). Litterfall, litter decomposition and nutrient release pattern in four native tree species raised on coal mine spoil at Singrauli, *India. Biol. Fertil. Soils* 29:371-378.

Smith, A.E., and Aubin, A.J., (1993). Degradation of ^{14}C glyphosate in sakatchewon soils. *Bull Environ. Contam toxicol*. 50: 499-505.

Tripathi SK, Sumida A, Ono K, Shibata H, Uemura S, Kodama Y, Hara T (2006). Leaf litterfall and decomposition of different above-and below ground parts of birch (Betula ermanii) tree and dwarf bamboo (Sasa kurilensis) shrub in a young secondary forest of Northern Japan. *Biol. Fertil. Soils* 43:237-246

Wardle, D.A. and Parkinson, D. (1990). Effects of three herbicides on soil microbial biomass and activity. *Plant Soil* 122: 21-28

Weaver, M.A., Krutz, L.J., Zabiotowicz, R.M. and Reddy, K.N. (2007). Effects of glyphosate on soil microbial communities and its Mississipi soil 63 (4): 88-93

Wolmarans, K. and Swart, W. J. (2014). Influence of glyphosate, other herbicides and genetically modified herbicide resistant crops on soil microbiota: a review. *S. Afr. J. Plant Soil* **31**, 177–186.

Zabaloy, M.C, Garland JL, Gomez, M.A (2008). An integrated approach to evaluate impacts of the herbicides glyphosate, 2-4-D and metsulfurom- methyt on soil microbial communities in the pampas region, Argentina. *J. Appl. Soil Ecol.* 40:1-12.

Zaller, J. G., Heigl, F., Ruess, L. & Grabmaier, A. (2014). Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model *Ecosystem. Sci. Rep.* **4**: 5634