Original Article

The effect of the application of different rates of herbicides on the residual level of the herbicides and their metabolites in harvested maize cobs

Joyce Chinyere Best-Ordinioha, E. A. Ataga[1], Best Ordinioha[2]

Green River Project, Nigerian Agip Oil Company, Port Harcourt, [1]Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, [2]Department of Preventive and Social Medicine, Faculty of Clinical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Abstract

Background: The use of herbicide in weed control has grown significantly in Nigeria in recent years. Most of the applications are indiscriminately carried out by illiterate farmers, and therefore pose a significant threat to the environment, crop yield and human health. This study assessed the effects of different application rates of the herbicides on the residual levels of the herbicides and their metabolites in harvested maize cobs.

Methods: The study was carried out in a plot of land at the University of Port Harcourt in 2013 and 2014, using a randomised complete block design with three replicates, and plot size of 2 m × 2 m. N-(phosphonomethyl) glycine was used to prepare the experimental plots, while different application doses of Primextra dual gold (2-chloro-4-(ethylamino)-6-isopropylamino-1,3-5-triazine and 2-chloro-N-(ethy-6-methy(phenyl)-N-(2-methoxyl-1-methylethyl acetamide) were applied to the plots, after planting the maize. The control plot was not treated with the herbicides. The residual levels of the herbicides and their metabolites in the cobs harvested from the plots were tested using gas chromatography, with Pulsed Flame Photometric Detector.

Results: The residue of the herbicides and their metabolites were barely detectable in the harvested maize cobs when the herbicides were applied at or below their recommended dose, but noted at up to 0.09 ppm, when applied at twice the recommended dose. The residues were also noted in some of the cobs harvested from the control plot, at a mean concentration of 0.0033 ppm, which is significantly <0.012 ppm recorded in the treated plot.

Conclusion: The residual concentrations of herbicides and their metabolites in harvested crops increased with increasing application dose of the herbicides. Proper education of farmers is, therefore, needed to safeguard the environment and public health.

Keywords: 2-chloro-4-(ethylamino)-6-isopropylamino-1,3-5-triazine, 2-chloro-N-(ethy-6-methy (phenyl)-N-(2-methoxyl-1-methylethyl acetamide, herbicides, N-(phosphonomethyl) glycine, Nigeria, residual level

Address for correspondence:
Dr. Joyce Chinyere Best-Ordinioha, P. O. Box 162 Omoku, Onelga, Rivers State, Nigeria. E-mail: ruralhealthforum@yahoo.com
Received: 12.09.2016, **Accepted:** 26.10.2016

Access this article online	
Quick Response Code:	Website: www.phmj.org
	DOI: 10.4103/0795-3038.237884

How to cite this article: Best-Ordinioha JC, Ataga EA, Ordinioha B. The effect of the application of different rates of herbicides on the residual level of the herbicides and their metabolites in harvested maize cobs. Port Harcourt Med J 2017;11:122-6.

Introduction

The use of herbicides in weed control has grown significantly in Nigeria in recent years.[1-3] This increase has been linked to the ease of application, and the effectiveness of the herbicides in controlling the common weeds in Nigerian farms.[2,3] These are in addition to the growing difficulty in hiring labour to carry out the traditional method of manually cutting off the weeds.[4]

Most of the applications of the herbicides are, however, carried out by illiterate farmers who apply the herbicides indiscriminately, mostly because they had not received any training on the judicious application of the herbicides.[2-4] This poses a significant threat to the environment, crop yield and on human health, as studies have indicated that such indiscriminate use of herbicides can leach into the environment, threatening non-target organisms,[5] harm soil microorganisms, thus affecting soil fertility[6] and can accumulate in harvested crops, possibly causing direct harm to humans, upon the consumption of the harvested crops.[7,8] There is, therefore, a need to carry out an elaborate study on the use of the herbicides in Nigeria, especially as the quoted studies and many other previous studies did not fully replicate the current field situation in Nigeria.

Several studies carried out in Nigeria, such as the one by Gushit *et al*.[7] had indicated that the use of the herbicides resulted in very small residual level of the herbicides and their metabolites in the harvested crops, but we want to postulate that this finding is most likely due to the use of small doses of the herbicides by the farmers, possibly due to financial constraints. We also want to postulate that the use of doses higher than the recommended dose of the herbicides would result in significant levels of the herbicides and their metabolites in the harvested crops. This study, therefore, assessed the effects of different application rates of the herbicides on the residual levels of the herbicides and their metabolites in harvested maize cobs. It is hoped that the study would not only answer the study hypotheses but also provide a useful insight into the public health implications of the use of the herbicides. The study was carried out in maize (*Zea mays* L) because it is a short duration crop, and one of the crops the herbicides are commonly used to cultivate in Nigeria.

Methods

Experimental site and location

The field studies were carried out in a plot of land at the University of Port Harcourt during 2013 and 2014 farming seasons, with the experiment laid out in a randomised complete block design with three replicates, and plot size of 2 m × 2 m. The experimental farm is a well-drained plot of land, with sandy loam soil, located at a latitude of 4.9027°N and a longitude of 6.9205°E. It is in the rainforest ecological zone of the Niger Delta region and like most other communities in southern Nigeria has two seasons, the rainy season and the dry season, with an average annual rainfall of about 3000 mm (118.1 in).

Land preparation and application of herbicides

The experimental site was fallowed for 2 years and was prepared for the experiment by the application of 'Round-Up', a proprietary N-(phosphonomethyl) glycine (glyphosate) herbicide, bought from the Port Harcourt office of the Rivers State Agricultural Development Programme. These non-till methods were adopted for the study because it has been shown to reduce leaching and erosion,[9] which is vital in containing the experimental herbicides within the experimental plots.

Glyphosate is the most widely used herbicide in the world, preferred for its low toxicity to mammals and its low environmental impact.[10] It was used in preparing the experimental plot for the study, not only for its low toxicity to mammals and the environment but also because of its broad spectrum, non-selective weed killing capacity and its proven effectiveness in non-till agriculture.[10] The glyphosate was applied to the experimental plot at the manufacturer's recommended rate and resulted in the complete burning off of the weeds. The experimental site was then left for 2 weeks, to allow the burnt grasses to completely dry before the maize seeds are planted in rows.

The maize variety used for the study, the swan-1-yellow was obtained from the Green River Project of the Nigerian Agip Oil Company and is widely cultivated in the farming communities of the Niger Delta region. The seeds were sown using the recommended seed rate of 25 kg/ha, at a planting distance of 75 cm between the rows, while an intra-row spacing distance of 25 cm was maintained on flat. Extra plants were thinned out at the early growth stage, to maintain one plant per hole after 2 weeks of planting.

Primextra dual gold, a proprietary herbicide containing 290 g/l of 2-chloro-N-(ethy-6-methy (phenyl)-N-(2-methoxyl-1-methylethyl acetamide (metolachlor) and 370 g/l of 2-chloro-4-(ethylamino)-6-isopropylamino-1,3-5-triazine (atrazine), manufactured by Syngenta Nigeria Limited, Lagos and purchased from a local dealer in Port Harcourt was then applied to the experimental plot, immediately after planting the maize. Atrazine is one of the most widely used 5-triazine herbicides, used either singly or in combination with other herbicides, as a pre-emergence herbicide in the control of broadleaf and grassy weeds;[11,12] while metolachlor, on the other hand, is commonly used in combination with other herbicides because it is a very selective herbicide, renowned for its activity against grassy weeds.[13]

The Primextra dual gold was applied to the experimental plots at five different rates corresponding to a quarter of the recommended dose, half of the recommended dose, the recommended dose, 1.5 times the recommended dose and twice the recommended dose. The information label of the Primextra dual gold used for the study showed that the product contains 290 g/l of metolachlor and 370 g/l of atrazine, with a recommended dose for maize farm of 3.2 l/ha, which is equivalent to 2.11 kg a.i./ha. Therefore, the herbicide was applied at 0.59 kg a.i./ha, 1.06 kg a.i./ha, 2.11 kg a.i./ha, 3.17 kg a.i./ha and 4.22 kg a.i./ha to correspond to a quarter of the recommended dose, half of the recommended dose, the recommended dose, 1.5 times the recommended dose and two times the recommended dose.

The control plot did not receive any herbicide application, before and after the maize seeds were planted. The plot was manually cleared with using the weeding hand hoe.

The evaluation of the herbicides and their metabolites in the harvested crop

The maize cobs from each of the experimental plots were harvested when they were deemed ripe enough and prepared for analysis. A cob harvested from 12 randomly selected maize plants from each of the experimental plots was used for the analysis, as specified by the Food and Agriculture manual for the evaluation of pesticide residues in food and feed.[14] The ears from the cobs harvested from each of the experimental plots were labelled and packaged in an iced cooler, to limit the enzymatic degradation of the herbicides and the metabolites[15] and then transported to the central laboratory of the University of Ibadan, where the maize ears were crushed and processed for the presence of the herbicides (glyphosate, atrazine and metolachlor) and their metabolites in the cobs, using gas chromatography, with Pulsed Flame Photometric Detector. This is consistent with the method employed by a similar Nigerian study.[16]

Data analysis

The data collected during the study were put in a database and then analysed using IBM's SPSS statistical package, Version 20 and Microsoft Excel, after checking for consistency and completeness. Summary measures were calculated for each outcome of interest, while the test of significance was conducted using the relevant statistical test, at 95% confidence interval, with $P \leq 0.05$ considered statistically significant.

Results

The mean concentrations of the herbicides and their metabolites in the harvested maize cobs in the first phase of the study are presented in Table 1, whereas the mean concentrations in the second phase of the study are presented in Table 2. The concentrations of the herbicides and their metabolites were barely detectable in the harvested maize cobs when the herbicides were applied at or below their recommended dose but were noted in increased concentration in the cobs harvested from the plots, in which the herbicides were applied at doses higher than the recommended.

The herbicides and their metabolites were also detected in some of the cobs harvested from the control plot that did not receive any herbicide application. The mean concentration of the herbicides and their metabolites was 0.0033 ppm in the control plot in both phases of the study compared to 0.012 ppm in both phases of the study in the treated plots.

The mean concentration of glyphosate and its metabolites is lower than those of the other herbicides and their metabolites. Glyphosate was not detected in any of the tasted maize cobs, while its metabolites were found in lower concentration compared to the metabolites of the other herbicides.

Discussion

The study showed that the concentrations of the herbicides and their metabolites were barely detectable in the harvested maize cobs when the herbicides were applied at or below

Table 1: Mean concentration of the herbicides and their metabolites in the maize cobs harvested in 2013

Treatment (kg a.i./ha)	A (ppm)	B (ppm)	C (ppm)	D (ppm)	E (ppm)	F (ppm)
0.53	ND	ND	ND	0.01	ND	0.01
1.06	ND	ND	ND	0.01	ND	ND
2.11	ND	ND	ND	0.03	ND	0.01
3.17	ND	0.01	0.01	0.06	0.01	0.02
4.22	ND	0.01	0.01	0.08	0.02	0.05
Mean of T	0.00	0.0033	0.004	0.038	0.006	0.018
Control	ND	ND	ND	0.01	ND	0.01

A: Glyphosate, B: Glyphosate metabolites, C: Atrazine, D: Atrazine metabolites, E: Metolachlor, F: Metolachlor metabolites, Mean of T: Mean concentration of the herbicide in the treated plots, ND: Not detected

Table 2: Mean concentration of the herbicides and their metabolites in the maize cobs harvested in 2014

Treatment (kg a.i./ha)	A (ppm)	B (ppm)	C (ppm)	D (ppm)	E (ppm)	F (ppm)
0.53	ND	ND	ND	0.01	ND	ND
1.06	ND	ND	ND	ND	ND	0.01
2.11	ND	ND	0.01	0.01	ND	0.01
3.17	ND	ND	0.01	0.05	0.02	0.03
4.22	ND	ND	0.01	0.09	0.03	0.06
Mean of T	0.00	0.00	0.006	0.032	0.01	0.022
Control	ND	ND	ND	0.01	ND	0.01

A: Glyphosate, B: Glyphosate metabolites, C: Atrazine, D: Atrazine metabolites, E: Metolachlor, F: Metolachlor metabolites, Mean of T: Mean concentration of the herbicide in the treated plots, ND: Not detected their recommended dose. The levels detected in this study are consistent with the levels recorded in a similar study conducted in maize[13,16] and leafy vegetables,[7] but lower than the levels recorded in root crops such as yam and cassava.[7] These differences could be attributed to many factors, especially those related to the persistence of the herbicides in the soil,[8,9] but the most probable reasons for the differences noted in this study relate to the timing of the application of the herbicides,[17] and the fact that root crops tend to bioaccumulate the herbicides and their metabolites.[7]

The study conducted by Cao *et al*.,[13] in which no herbicides and their metabolites were detected used a single herbicide that was applied once, whereas a total of three herbicides were used in this study for land preparation and for weed control. Araújo *et al*.[17] in a study conducted with glyphosate-resistant soybean showed that higher concentrations of glyphosate and its metabolites were detected in the harvested crop when the glyphosate was sprayed several times during the crop cycle, especially when the herbicide was sprayed during the flowering stage of the crop. This was not the situation in this study, as the herbicides were applied once and were not repeated irrespective of the presence of weeds in the plots.

Studies have shown the greater tendency of root crops to bioaccumulate the herbicides and their metabolites in their roots,[7] and for the other crops to accumulate them more in their stems and leaves and less in their grains.[17,18] A study carried out with glyphosate-resisted soybeans found that the concentration of glyphosate and its metabolites in the leaves and stems of the soybean ranged from 1.9 to 4.4 mg/kg, which is significantly higher than the concentration in the grains that ranged from 0.1 to 1.8 mg/kg.[19] The tendency of crops to accumulate the herbicides and their metabolites more in their roots, leaves and stem and less in their grains has been linked to the routes of absorption of the herbicides and the differences in the metabolic activities of the different parts of the plant.[18] After application, glyphosate and the other herbicides are absorbed by the foliage of the crop and translocated to the stems, leaves and roots of the entire plant, but further concentration of the herbicides in other parts of the plants is, however, dependent on the level of meristematic and metabolic activity of that part of the plant. The activity level in grains tend to be lower than those of the stem and leaves.[18]

The residual concentrations of the herbicides and their metabolites recorded in this study are significantly lower than the concentration found to have adverse effects on human beings following oral ingestion.[8] Different countries have established a range of 'acceptable' daily intake levels for human exposures to different herbicides, generally referred to in the US as the chronic Reference Dose (cRfD) or in the European Union as the acceptable daily intake (ADI). Levels of the herbicides in food products that are above these levels are considered dangerous to health, as they are able to cause several health effects in humans including cancer and renal and liver damages.[19,20] The current U.S. Environmental Protection Agency cRfD for glyphosate is 1.75 mg of glyphosate per kilogram body weight per day (mg/kg/day), which is five times lower than the current European Union. ADI of 0.3 mg/kg/day,[8] while the ADI for metolachlor is 0.092 mg/kg/day. The fact that the residue concentration recorded in this study is significantly lower than these ADI levels means that the use of the herbicides, in the way they have been used in the study, does not constitute a significant threat to public health.

This study, however, found that the residue concentration in the harvested cobs increased with increasing dose of the herbicides above the recommended dose. The mean residual concentration in the first phase of the study increased ten-fold from 0.003 ppm in the experimental plot, in which a quarter of the recommended dose was applied, to 0.03 ppm in the plot in which twice the recommended dose was applied. This indicates a potential public health threat, if the herbicides are used above the recommended dose, especially in Nigeria where the herbicides are used by increasing number of largely untrained subsistent farmers.[1,21] Both authors found that increasing number of farmers use the herbicides with little knowledge of the risk involved and are exposed to the chemicals, either when spraying or packaging the products. There is, therefore, an urgent need to carry out the necessary training on the safe use of the herbicides.

The mean concentration of glyphosate and its metabolites in the harvested maize cobs was less than the residual concentration of the other herbicides. Glyphosate was not detected in the harvested cobs in the first phase of the study, whereas atrazine was detected at a concentration of 0.004 ppm and metolachlor at a concentration of 0.006 ppm in the cobs. These differences can be attributed to the fact that glyphosate was used earlier than the other herbicides and therefore had more time to be degraded.[17,22,23] This is buttressed by the fact that whereas no glyphosate was detected in the harvested cobs, the concentration of the metabolites of glyphosate averaged 0.01 ppm in some of the treated experimental plots.

The study also found some of the metabolites of the herbicides in the cobs harvested in the control plot that did not receive any herbicide application albeit at levels that are significantly lower than the levels found in the treated plots. The mean residue concentration in the cobs harvested from the control plot in the first phase of the study was 0.0033 ppm compared to 0.012 ppm in the treated plots. This is consistent with the findings of other studies that also found the herbicides and their metabolites in other non-intended targets of the herbicides such as fishes and rivers.[5] Although the mean residue concentration in the cobs harvested from the control plot of this study was significantly lower than that of the treated plots and much lower than the level found to cause adverse health effects,[8] it, however, reveals the potential threat of the use of the herbicides to the environment. The contamination of the control plot of this study with the herbicides that were applied to the treated plots is likely to be through the leaching of the herbicides from the treated plot to the control plot. This is even as the experimental plots were deliberately not tilled, to reduce the leaching.[9] This further buttresses the potential threat of the use of the herbicides on the environment and public health, especially as important root crops such as cassava and yam have a greater tendency to bioaccumulate the herbicides.[7]

Conclusion

The residual concentrations of herbicides and their metabolites in harvested crops are low at the recommended doses of the herbicides but tend to increase with increasing application dose of the herbicides. Farmers therefore need to be trained on the proper dosing of the herbicides, to for stale the possible threat to the environment and public health.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Iyagba AG. Assessing the safety use of herbicides by horticultural farmers in Rivers state, Nigeria. Eur Sci J 2013;9:97-108.
2. Agahiu AE, Baiyeri KP, Ogbuji RO, Udensi UE. Assessment of status, perception of weed infestation and methods of weed control adopted by cassava farmers in Kogi state, Nigeria. J Anim Plant Sci 2012;13:1823-30.
3. Agahiu AE, Udensi UE, Tarawali G, Okoye BC, Ogbuji RO, Baiyeri KP. Assessment of weed management strategies and intercrop combinations on cassava yield in the middle belt of Nigeria. Afr J Agric Res 2011;6:5729-35.
4. Tijani AA. Pesticides use practices and safety issues: The case of cocoa farmers in Ondo state, Nigeria. J Hum Ecol 2006;19:183-90.
5. Ize-Iyamu OK, Asia IO, Egwakhide PA. Concentrations of residues from organochlorine pesticide in water and fish from some rivers in Edo state Nigeria. Int J Phys Sci 2007;2:237-41.
6. Ayansina AD, Oso BA. Effect of two commonly used herbicide on soil microflora at two different concentrations. Afr J Biotechnol 2006;5:129-32.
7. Gushit JS, Ekanem EO, Adamu HM, Chindo IY. Analysis of herbicide residues and organic priority pollutants in selected root and leafy vegetable crops in Plateau state, Nigeria. World J Anal Chem 2013;1:23-8.
8. Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, *et al.* Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. Environ Health 2016;15:19.
9. Ding G, Novak JM, Herbert S, Xing B. Long-term tillage effects on soil metolachlor sorption and desorption behavior. Chemosphere 2002;48:897-904.
10. Franz JE, Sikorski JA, Mao MK. Glyphosate: A Unique Global Herbicide. Wasington, DC: American Chemical Society, 1997.
11. Munier-Lamy C, Feuvrier MP, Choné T. Degradation of 14C-atrazine bound residues in brown soil and rendzina fractions. J Environ Qual 2002;31:241-7.
12. Hamid AA, Aiyelaagbe OO, Balogun GA. Herbicides and its applications. Adv Nat Appl Sci 2011;5:201-13.
13. Cao P, Wang X, Liu F, Zhao E, Han L. Dissipation and residue of S-metolachlor in maize and soil. Bull Environ Contam Toxicol 2008;80:391-4.
14. Itodo AU, Abdulrahman FW, Hassan LG, Maigandi SA, Itodo HU. Application of GC/MS quantification in the adsorption of herbicide by sorbents from two abundant nut shells. Researcher 2010;2:64-73.
15. Food and Agriculture (FAO) Manual, Plant Production and Protection Paper 197. Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed. Rome: FAO, 2009; 163-73.
16. Adegbite JB, Lajide L, Aladesanwa RD, Aiyesanmi FA, Abiodun OA, Adepeju AB, *et al*. Effect of herbicide application on residue content and nutritional composition of maize from a pilot maize farm. Am J Agric Sci 2016;3:35-9.
17. Araújo AS, Monteiro RT, Abarkeli RB. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 2003;52:799-804.
18. Komoba D, Gennity I, Sandermann H Jr. Plant metabolism of herbicides with C-P bonds: Glyphosate. Pestic Biochem Physiol 1992;43:85-94.
19. Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini GE. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol 2015;84:133-53.
20. Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol 2013;59:129-36.
21. Gushit JS, Ekanem EO, Adamu HM, Abayeh OJ, Malan D. Utilization pattern and risk assessment of herbicides usage by farmers, marketers and agricultural extension workers (AEWs) in Plateau state, Nigeria. Int J Agric Sci Res 2013;2:177-84.
22. Busse MD, Ratcliff AW, Shestak CJ, Powers RF. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol Biochem 2001;33:1777-89.
23. Mamy L, Barriuso E, Gabrielle B. Glyphosate fate in soils when arriving in plant residues. Chemosphere 2016;154:425-33.