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*Full Length Research Paper*

# Short-term phyto-toxicity consequences of a non-selective herbicide glyphosate (Roundup™) on the growth of onions (*Allium cepa Linn*.)

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**This study examined the phyto-toxic effects of a commonly named non-selective herbicide glyphosate (Roundup™) on onions (*Allium cepa Linn*.). The study was necessitated due to the indiscriminate use and release of Roundup™ for weed control in the Niger Delta soils of Nigeria. The Organisation for Economic Co-operation and Development (OECD) standard protocol # 208 was adopted. The short-term phyto-toxic consequence on onion (*A. cepa L.*) was determined after a 4-day exposure to varying concentrations of the test chemical at 0.625, 1.25, 2.5, 5 and 10 mg/L, respectively. The percentage growth rate decreased as percentage growth rate inhibition efficiency increased, which implies that the effect of the herbicide was concentration dependent. The mean percentage growth rate inhibition efficiency relative to the control was 28, 47, 61, 73 and 96%, respectively. The effective concentration ($EC_{50}$) for % Inhibition efficiency was 1.550 mg/L with a 95% confidence interval of 1.269 to 1.848 mg/L. The biological alterations on the onions in the test solutions varied in order of increasing concentrations. Some observations made include decolouration of the test solutions and stunted growth especially at the highest concentration of 10 mg/L. Other effects include: bulb deformation, tissue and root damage. There was significant difference between the exposed species and the control at $P < 0.05$. The use of the herbicide Roundup™ with such hazardous effects can harm plants especially onion which is a very viable food product of man. This could lead to likely distortion on the ecosystem balance of onions (*A. cepa*) and similar plants and subsequent effect on human, the major end user.**

**Key words:** Herbicide, glyphosate (roundup), onion (*Allium cepa Linn*.), phytotoxicity.

## INTRODUCTION

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling, mitigating or controlling any pest. They could interfere with the production, processing, storage, transport or marketing of

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food, agricultural commodities, wood products and animal feedstuffs (FAO, 2002; USEPA, 2007). Subclasses of pesticides and their target pest include: herbicides (weeds), insecticides (insects), pediculicides (lice), biocides (micro-organisms), algicides (algae), avicides (birds), molluscicides (snails), nematicides (nematodes) and rodenticides (rodents) (Gilden et al., 2010). Herbicides commonly known as weed-killers are pesticides, substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetation (Cork and Krueger, 1992). Herbicides may be selective and non-selective. Selective herbicides affect only certain types of plants (that is, they kill specific targets while leaving the desired crop relatively unharmed). Non-selective herbicides kill all plants with which they come into contact (that is, inhibits a very broad range of plant types). In Nigeria, herbicides have been used effectively to control weeds in agricultural systems (Adenikinju and Folarin, 1976). Herbicides could accumulate to toxic levels in soils and become harmful to microorganisms, plants, wildlife and man (Amakiri, 1982; Wilson and Tisdell, 2001). Herbicides may directly contact surface water *via* aerial drift, accidental spills, or surface runoff polluting water bodies thereby causing havoc to aquatic habitats amongst others. Over 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments and food. The amount of herbicide that migrates from the intended application area is influenced by the particular chemical's properties such as: propensity for binding to soil, vapour pressure, water solubility, and resistance to being broken down over time. Glyphosate (Roundup™), which was assessed in this study is one of the most widely used non-selective herbicide with applications in agriculture, forestry, industrial weed control, garden and aquatic environments (Baerson, 2002; Tomlin, 2006; Cavusoglu et al., 2011). In the soil environment, glyphosate is resistant to chemical degradation, stable to sunlight, relatively non-leachable, and has a low tendency to runoff. It is relatively immobile in most soil environments as a result of its strong adsorption to soil particles. It is also known to cause damage to soil organisms (Dalby et al., 1995).

Onion (*Allium cepa*) belongs to the bulb crops, a group belonging to the family of *Alliaceae*. It is one of the most important edible vegetable crops used for seasoning not only in Nigeria but all over the world. Weeds are one of the main plant protection problems in onion fields. They compete with onions for light, nutrients, water and space. Many researchers have reported that onion plants are poor competitors (Ghosheh, 2004; Carlson and Kirby, 2005; Qasem, 2006; Smith et al., 2011). This poor competitive ability with its initial slow growth and lack of adequate foliage (shallow roots and thin canopy) makes onions weak against weeds. In addition, their cylindrical upright leaves do not shade the soil to block weed growth (Wicks et al., 1973). Mainly, chemical control is applied against weeds in onion producing areas, but possible phyto-toxicity on onion is also a main problem. Root growth inhibition and adverse effects upon chromosomes provide indications of toxicity and genotoxicity (Rank and Nielsen, 1993; Bonciu, 2012; Olorunfemi and Ehwre, 2010).

The purpose of this study was to determine the deleterious effects of a commonly used non-selective herbicide; glyphosate (Roundup™) on the growth characteristics of onion (*A. cepa*). The use of herbicides in the environment has been of a great benefit in weed control however, their effects on onions and other plants as well as their fate in the environment is of important consideration due to the fact that humans are the major end users of plant produce. Onion (*A. cepa*) was chosen because they are relatively abundant in Nigeria and worldwide, available all the year round, sensitive to pollutants and are daily consumed by man and other organisms (Beeby, 2001; Rank and Nielsen, 1993).

## MATERIALS AND METHODS

### Test chemical

Roundup™ (Glyphosate, N-phosphonomethyl glycine), a non-selective herbicide was obtained locally from the vendors in Warri, Delta State, Nigeria. The test chemical is liquid, soluble in water and contains 360 g/L glyphosate. The chemical is currently used by farmers for weed control in the Niger Delta area of Nigeria.

### Test species

The test species was common onion (*A. cepa* L.) of the purple variety. The onions used had a mean size of 6.30 ± 0.08 cm in diameter with a mean weight of 78.33 ± 0.87 g.

### *A. cepa* assay

The *A. cepa* assay was assessed using the Organisation for Economic Co-operation and Development, (OECD) protocol 208 (OECD, 2003).

### Procurement and preparation of onions

Onion bulbs (*A. cepa*) of the purple variety with average size were purchased locally in Effurun, Warri, Delta State and the same batch of bulbs were used throughout the experiment. The onions were sun-dried for a week and the dried roots present at the base of the onion bulbs were carefully shaved off, with a sharp razor blade to expose the fresh meristematic tissues. Bulbs attacked by fungi were discarded at the beginning of the experiment. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up (Rank and Nielsen, 1993).

### Root growth inhibition evaluation

For root growth inhibition evaluation, the 4-days semi-static renewal assay started with a range finding test to determine the range of concentrations to be used for the definitive test. Freshly prepared

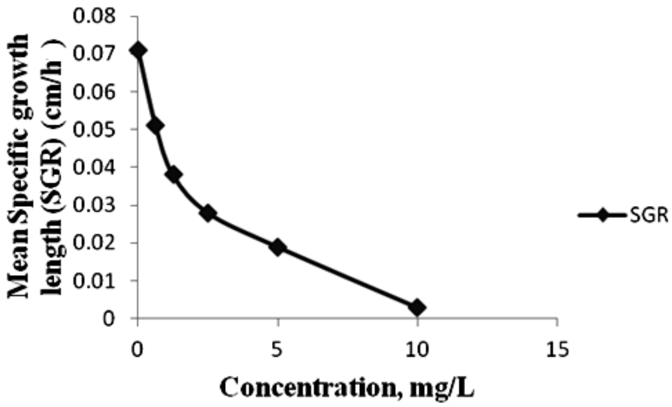


**Figure 1.** Means of specific growth length of *onions (Allium cepa)* exposed to glyphosate (Roundup™) in comparison with the control.

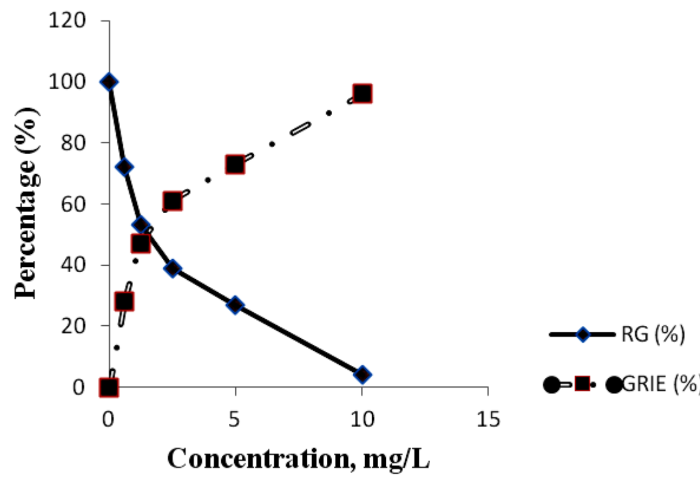


**Figure 2.** Means of percentage root growth relative of control (RG) in comparison with mean growth root inhibition efficiency (GRIE).

stock extracts of the test chemical was serially diluted into five concentrations of 10, 5, 2.5, 1.25 and 0.625 mg/L, respectively. Triplicate for each concentration and the control (tap water) were used for the 96 h bioassay. The negative control was set up with tap water of good quality. The tap water was ascertained to be with pH of 6.7±0.3 and had a relatively soft hardness concentration of 16 ± 0.01 mg $CaCO_3$/L and free from any chlorine compounds and toxic ions (Fiskesjo, 1985). The base of each of the bulbs was suspended on the extracts inside 200 mL beakers in the dark for 96 h. Test extracts were changed daily to maintain the same concentration. At the end of the exposure period, the roots of onion bulbs at each concentration were removed with forceps and their lengths measured (in cm) with a metre rule. From the weighted averages for each test concentration and the control, the percentage root growth inhibition in relation to the negative control and the $EC_{50}$ (the effective concentration where the percentage inhibition efficiency amounts to 50% of the controls) for each extract was determined (Fiskesjo, 1985). The effect of each sample on the morphology of growing roots was also examined.

### Statistical analysis

The effective concentration ($EC_{50}$) for root inhibition efficiency was used to determine the vulnerability of onions to the test chemical at 4 d (OECD, 2003). In addition, the analysis of variance (ANOVA) in Statistical Package for Social Science (SPSS) statistical software in Version 16.0 was also used to test the mean statistical difference between the controls and treated groups at significance levels of $P < 0.05$.

## RESULTS

The results of root growth inhibition evaluation of glyphosate (Roundup™) on onion (*A. cepa*) for the 4-days phytotoxicity assay are presented in Figures 1 to 3. There was significant growth in the control experiment for the test duration, which indicated that the test conditions were appropriate and thus growth inhibition recorded in the test solutions could be attributed to the effect of the test chemical. Although, the assay was carried out in the dark, at each test renewal, observations were made and recorded. At experimental renewal hour of 24, there was no growth in the highest concentration of 10 mg/L while the other concentrations recorded gradual growth. Figure 1 shows the mean root lengths of *A. cepa* cultivated in varying concentrations of the test chemicals and control. The mean root lengths of *A. cepa* obtained for the test chemical ranged from 0.29 ± 0.03 to 4.90 ± 0.12 cm while the control recorded 6.82 ± 0.07 cm. At test termination of the 4-days experiment, the mean percentage growth rate relative to the control recorded for the five concentrations 0.625, 1.25, 2.5, 5 and 10 mg/L were 72, 53, 39, 27 and 4%, respectively (Figure 1). There was a decrease in mean growth rate from the lowest concentration to highest concentration in the test chemical, an indication that growth rate was concentration dependent. The percentage growth rate between the control experiment and the test chemical was significantly different at levels of $P< 0.05$. However, the effect of the glyphosate was most exhibited at the highest concentration which recorded the highest percentage inhibition efficiency where growth was most inhibited. The percentage growth rate decreases as percentage growth rate inhibition efficiency increases, which implies that the effect of the herbicide was concentration dependent. The mean percentage growth rate inhibition efficiency relative to the control recorded 28, 47, 61, 73 and 96%, respectively (Figure 2).

In the various concentrations used in this assessment, the onion manifested varying degree of stress to the test chemical. Phytotoxicity of the test chemical was shown by decolouration of the test solutions and stunted growth in the highest concentrations. Daily observations showed that there was root damage in the test chemical vessels especially at higher concentrations. The roots were also characterized by malformations and these were mostly broken roots, twists, roots bent upwards and stunted growth. Other effects include: bulb deformation, tissue damage, etc. The effective concentration ($EC_{50}$) of the

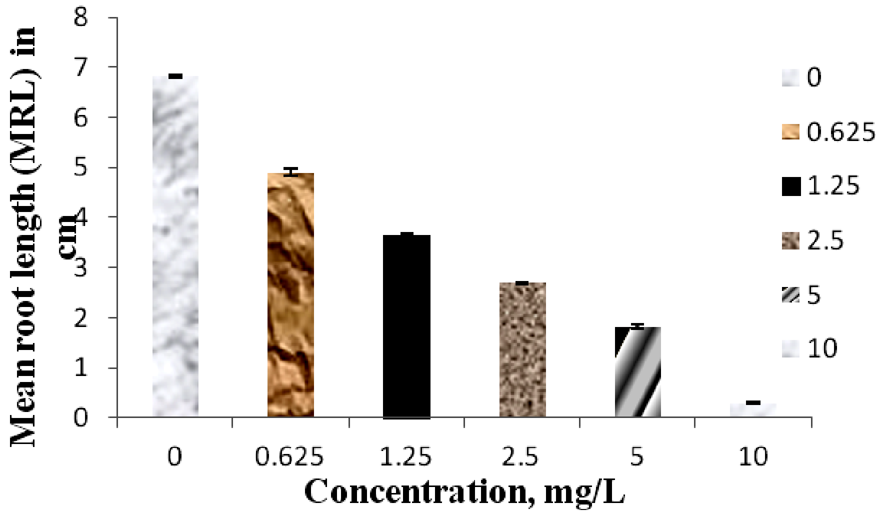


**Figure 3.** Means of root length of onions (*Allium cepa*) exposed to glyphosate (Roundup™) in comparison with the control.

chemical was evaluated using estimated 96 h $EC_{50}$ values in varying concentrations (Figure 3). From the result, 0.625 mg/L test solution has the minimum growth inhibition efficiency of about 28% while 10 mg/L has the maximum 96% in relation to the control. Safety factors are arbitrarily built in around the $EC_{50}$ values in order to arrive at environmentally tolerable concentrations. The concentration of a chemical in the environment should not exceed 10% of the $EC_{50}$. The estimated $EC_{50}$ value after 96 h for glyphosate (Roundup™) was 1.550 ± 0.04 mg/L with a safe limit of 0.155 mg/L and 95% confidence interval of 1.269 to 1.848 mg/L.

## DISCUSSION

As farmers continue to realize the usefulness of herbicides, larger quantities would be applied to the soil. However, the fate of these compounds in the environment is becoming increasingly important, since they could accumulate to toxic level and become harmful to plants as well as the environment (Wilson and Tisdell, 2001; Ayansina et al., 2003). However, most of the commonly used herbicides have not been characterized for their phytotoxicity consequence. The material safety data sheet (MSDS) of most chemicals / pesticides contain little or no details of their toxic levels to aquatic, terrestrial and plant species. Since a large number of herbicides are in use, there is significant concern regarding health effects to plants and man. From the data obtained for this assessment, there is the likelihood of this occurring. As the concentration of the pesticide is increasing, the percentage growth rate decreases while the percentage growth rate inhibition efficiency increases. This implies that uncontrollable use and release of the test chemical into the environment would lead to depletion of these viable species onion (*A. cepa*) serving as food for man. In addition to the health effects caused by herbicides, commercial herbicide mixtures often contain other chemicals, including non-active ingredients, which could have negative impacts on human health (Bonciu, 2012).

Herbicides present the only group of chemicals that are purposely applied to the environment with aim to suppress weed and animal pests and to protect agricultural and industrial products. However, the majority of herbicide is not specifically targeting the pest only and during their application they also affect non-target plants and animals (non-selective herbicides). Repeated application could lead to loss of biodiversity. Many herbicides are also not easily degradable and thus persist in soil, leach to groundwater, surface water and contaminate wide environment. Depending on their chemical properties they can bioaccumulate in food chains, enter organisms and consequently influence human health. The overall intensive non-selective herbicide application results in several negative effects in the environment that cannot be ignored (Carlson and Kirby, 2005; Olorunfemi and Ehwre, 2010). It has been proposed that glyphosate interferes with absorption and utilization of Mn, thus increasing a plants susceptibility to disease. However, the majority of research has not found reductions in Mn concentrations within plants following glyphosate applications (Bott et al., 2008; Nelson, 2009; Rosolem et al., 2009). From the discussion above, it is apparent that the use of herbicides in the environment has negative impacts on onion cells and likely soil nutrients which might affect the growth and yield of onions as well as other similar plant species (Kellogg et al., 2000; Cavusoglu et al., 2011).

However, due to the root growth and sensitive response to the tested chemical, *A. cepa* test could be useful for a water and soil quality assessment; that is, as a bio-indicator informing one of the environmental conditions in the event of application, spill and unintentional release.

## Conclusion

The indiscriminate use of herbicides for pest control would be difficult to eliminate in Nigeria since the regulators: National Agency for Food and Drug Administration and Control (NAFDAC) and the Federal Ministry of Agriculture and Rural Development (FMARD) have not enforced the characterization of all the organic and inorganic herbicides used in Nigeria prior to sale and use by farmers. However, only registration of the chemicals is ensured, this does not give details of the ecological damage the chemicals can cause to aquatic, terrestrial and plant species. Thus, the fate of these pesticides in the environment is an important consideration due to the fact that humans are the end users of plant produce. The assessment is with a view to safe-guiding the environment, its components and humans.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

This study acknowledges the thematic group of the University of Benin, Geo-Environmental and Climate Change Adaptation Research Centre, Benin City, Nigeria for their support and efforts.

**Abbreviations: NAFDAC,** National Agency for Food and Drug Administration and Control; **OECD,** Organisation for Economic Co-operation and Development; **$EC_{50}$,** effective concentration; **FMARD,** Federal Ministry of Agriculture and Rural Development.

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