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Phyto-toxic Influence of an Atrazine-based Selective Herbicide – Arda-force® on Onions (*Allium cepa L*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author DFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EO managed the analysis of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The constant impact on the environment occasioned by pollution, indiscriminate application of agricultural chemicals, security challenges and crisis in the Niger Delta ecological area of Nigeria has caused severe damage to plants, soil organisms and humans.
Aim and Methodology: In this research, onions (*Allium cepa L*) was exposed to varying concentrations of an atrazine-based selective herbicide Arda-force® to estimate the phyto-toxic effects on the plant species using the Organization for Economic Co-operation and Development, (OECD) protocol #208.
Results: The mean effective concentration (EC_{50}) using root growth inhibition produced indications of phyto-toxicity to the exposed species at a concentration of 0.55 ± 0.06 mg/L. Similarly, the maximum root growth inhibition efficiency relative to the control was 65% as recorded in the highest test concentration of 1.25 mg/L.
Discussion: The study indicated that constant application / indiscriminate use of the herbicide Arda-force® could cause deleterious influence on these plant and vegetable species, daily consumed by humans as a rich source of anti-oxidants.
Conclusion: This study concluded that atrazine-based herbicide Arda-force® used in this

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assessment resulted in phyto-toxic effects to *Allium cepa L*. At the exposed concentrations of the herbicide to non-target specie – *Allium cepa L.* that are integral parts of the ecosystems, the ‘‘harmless’’ status of atrazine acclaimed by the United State Environmental Protection Agency (USEPA) is still very much in doubt.

Keywords: Atrazine; onion (Allium cepa L); root growth inhibition; phyto-toxicity; selective herbicide.

1. INTRODUCTION

The last few decades have experienced a tremendous increase in the use and application of pesticides (herbicides). These chemicals provide a lots of benefits in the agricultural sector as well as many shortfalls. Some benefits of herbicides include: crop protection, food preservation, material preservation, disease control amongst others. Herbicides are used in the agricultural sector to manage unwanted vegetation (weeds), however, the uncontrollable use by farmers and non-farmers alike could cause significant harmful effects on non-target receptors in the environment [1]. With rising resistance of weeds to commonly used herbicides, different formulations are being developed periodically to meet weed challenges. One remarkable herbicide that has been formulated in recent times is atrazine - a member of the chlorophenoxy triazine family within the subclass of carbamates [2]. It is in the class of selective herbicide formulated for used in the agricultural sector to control annual grasses and broad-leaved weeds attacking crops such as corn, sugarcane, pineapple, sorghum amongst others [3].

Atrazine herbicide works by inhibiting the photosynthesis pathway and thus prevents the growth of weeds attacking specific viable crops grown for human consumption. The mechanism of action of the herbicide causes chlorosis - yellowing of leaves. Chlorosis is referred to as a condition indicating lack of nutrients (especially phosphorus) in the soil and drying of the leaves, which then collapses the stem and petioles [4,5,6]. Atrazine herbicide reduces the weed intensity substantially and record lower weed index and maximum weed control efficiency as compared to other herbicides. When applied to soil, it remains in the soil for a long period before it is been broken down in the soil over a period of one growing season. In addition to chlorosis and necrosis (tissues dead or degeneration) [7,8]. Minshall [9], found that herbicide formulation used in his study greatly reduced transpiration in the leaves of beans (*Phaseolus vulgaris*). However, in addition to its specific target weed, non-target environmental receptors/species are also affected. Key amongst them, are soil dwelling organisms – snails, earthworms, microorganisms etc. and plants – onions, phytoplankton, and invertebrates [10].

The effects of these herbicides are enormous as herbicide residues can still be found on some marketable crop produce. This is so because the herbicide molecules are usually very large and are not readily biodegradable [11]. The degradation rate (half-life of about 12 to 30 years for some formulations, during this period of breakdown, the chemicals can attack lipid tissues of species on a long-term period on the exposed organisms. Although some herbicide composition like the glyphosate are known to biodegrade within a few weeks, the question is if crops are harvested and consumed before the set degradation period of the herbicide, then we have bioaccumulation issues to contend with in organisms that consume the products including humans [12].

With the initial prohibition of atrazine herbicides and renewed approval for use subsequently, there has been a controversy that has lingered for years now. The United State Environmental Protection Agency (USEPA) review was criticized by Hayes et al. [13], who noted in his research that the decline in amphibian populations was majorly as a result of atrazine exposure in environmental media. The herbicide on exposure caused deleterious impact to aquatic species thirty times below the levels recommended and approved by USEPA and other International authorities. Hayes et al. [13,14], claims amongst others researchers still remain controversial [15,16]. This study considered the phyto-toxic effects of an atrazine-based herbicide –Arda-force® on onions (*Allium cepa linn*) with a view to buttress the claims presented by Solomon et al. [17] and Hayes et al. [13,14] on the harmful effects of atrazine-based herbicides.

2. MATERIALS AND METHODS

The study area was Ugbomro community which is made up of fresh water environment and is located in Ughelli North Local Government Area of Delta State Nigeria. The coordinates for sampling was latitude 5°33'59.6" N and longitude 5°49'59.8" E. The onions were purchased from Agbarho, a located in Ughelli North Local Government.

The Organization for Economic Co-operation and Development, (OECD) protocol #208 was used in this evaluation for the exposure of *Allium cepa L* to the herbicide [18]. Arda-force® is a selective atrazine-based herbicide containing 2-chloro-4-ethylamine-6-isopropylamino-S-triazine, empirical formula –($C_8H_{14}ClN_5$) as the major active ingredient (50% SC). As weed killer, the chemical is widely and currently used by farmers and non-farmers in unregulated concentration in the Niger Delta area of Nigeria.

The purple (stuttgarter) variety of onion (*Allium cepa L*) was the test specie used in this study with an average weight and length of 72.90 ± 0.38 g and 6.49 ± 0.05 respectively. The onions were air-dried for two weeks and in order to expose the meristematic tissues, dead roots at the base were carefully shaved off with the aid of a blade [18,19]. The bulbs were placed in distilled water to prevent the primordial cells from drying up. The bulbs were grown in distilled water for 48 hours, after which they were removed and placed on blotting paper to remove excess water before exposure to the prepared test chemical for the range-finding and subsequently the definitive test. A range-finding test with concentrations of 0.1 mg/L, 1.0 mg/L and 10.0 mg/L was first conducted so as to establish the concentrations to be used for the 96 hours root growth inhibition evaluation. From the results of the range-finding analysis, serial dilutions of the test herbicide was prepared into triplicates of five concentrations (1.25, 0.625, 0.3125, 0.15625 and 0.078125 mg/L) in addition to the controls. Each onions bulb was placed on the surface of each concentration of the herbicide contained in a 250 mL test vessel, which was kept in the dark for 96 h duration. At the end of the 96 hour experiment, the root of onion bulbs were prudently removed with a forcep and the lengths measured (in cm).

The phyto-toxic impact caused by the herbicide was appraised using percentage root growth inhibition in relation to the control, EC_{50} (the effective concentration where root growth amounts to 50% of the controls) and the morphology of the inhibited roots.

The root growth inhibition, and effective concentration EC_{50} was used to ascertain the vulnerability of the exposed onions to the test herbicide. Similarly, statistical analysis was used to test the average statistical variation between the controls and treated groups at significance level of $P = .05$.

3. RESULTS

The data generated from this study are depicted in Table 1 and Figs. 1-2. Table 1 showed the effects of root growth inhibition on the application of Arda-force®. The effective concentration (EC_{50}) for the test chemical determined after exposure was 0.55 ± 0.06 mg/L and was rated highly toxic using the OECD rating [20]. The safe concentration that would not cause damage to plants is estimated at 10% of the EC_{50} value which is 0.055 mg/L.The maximum root growth inhibition efficiency relative to the control of 65% was recorded in the highest test concentration of 1.25 mg/L while the minimum of 9% was obtained from the lowest test concentration of 0.078125 mg/L. The chemical had varying degree of effects on the *Allium cepa L*, namely, twisted roots, shirked and decoloured root most especially at the highest exposure concentrations. Some of the onions bulb was very soft and tender at test termination in concentrations of 0.625 and 1.25 mg/L.

Table 1. Average results of Ardaforce® exposed to *Allium cepa L*

Conc., mg/L	Average root length	SGR	% Growth rate	%RGIE
0	5.66 ± 0.50	0.0590	100	0
0.078125	5.16 ± 0.46	0.0538	91	9
0.15625	4.02 ± 0.15	0.0419	71	29
0.3125	3.62 ± 0.10	0.0377	64	36
0.625	2.54 ± 0.42	0.0265	45	55
1.25	1.96 ± 0.29	0.0204	35	65

Data were processed and expressed as mean ± SD of eight replicates; SGR = Specific growth rate; RGIE = Root growth inhibition efficiency

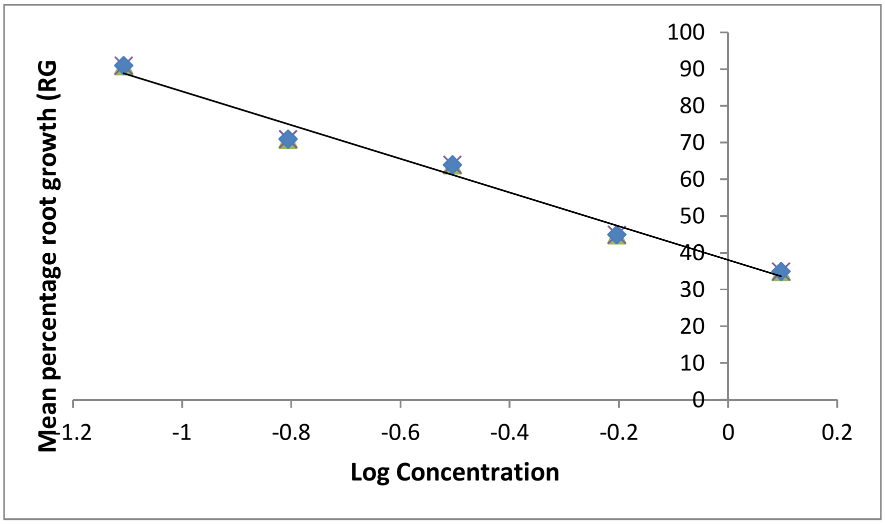

Fig. 1. Average percentage root growth of *Allium cepa L*exposed to Arda-force®

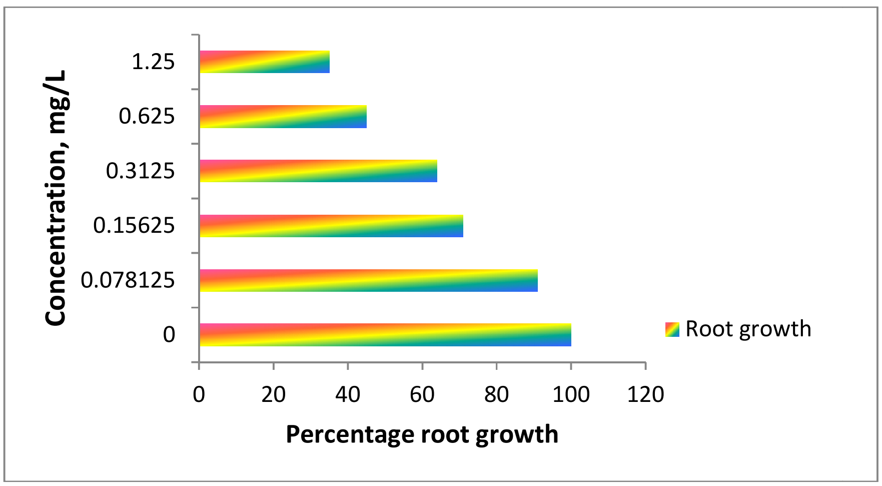

Fig. 2. Percentage root growth of *Allium cepa L* exposed to Arda-force®

4. DISCUSSION

From the last few decades, since the formulation of pesticides came into existence, the environment has suffered a myriad of effects / challenges from exposure to these chemicals [21]. The effects vary from exposure to organisms in the environment, accumulated effects on humans and environment media contamination amongst others. The benefits of herbicides are numerous and so does the negative consequences [2,22,23]. Over the last few decades, there has been a controversy on the application of atrazine-based herbicides. With the repeated banning and unbanning of the herbicide, it leave one to wonder if the regulatory authorities are sincere and right with their analysis to accept an otherwise banned herbicide [13,14,16]. The true be told, herbicides contain large molecules that would not biodegrade in a hurry, no matter the composition. Also repeated and frequent application could result in bioaccumulation of certain organic fractions in environmental media as well as organisms inhabiting the environment. Humans and lots of the higher species, who are usually the end users and consumers of a wide variety of crops and animals could easily get contaminated species in the local markets since individual obtain or buy their produce directly from farms or the local market. It is apparent that the use of herbicides such as Ardaforce™ in the environment could have 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 [2,24]. Base on their chemical properties, these herbicides used can bio-accumulate in food chains, enter organisms and consequently negatively influence human health.

Export products from some countries are sometimes rejected after analysis indicating herbicide residues. This had led to a tragic situation since most consumers are either not informed or simply do not take into consideration the resultant health implications of consuming crop and animals containing herbicides residues. The next decade would be saddled with a myriad of regulatory issues that needs to be resolved so that humans are not loaded with toxic substances without regards to their survival [16,25]. It have been reported that atrazine can cause liver, kidney, and heart damage in animals; it is possible that atrazine could cause these effects in humans, although this has not been ascertained. However, not enough information is available to definitely state whether atrazine causes cancer in humans. Similarly, as a potent endocrine disrupter, atrazine can interfere with hormonal activity of animals and humans at very minute concentrations [26].

The mechanism of action of the herbicide could be acute, inhibiting the natural process of photosynthesis and growth. Death in plant occurs as a result of starvation and oxidative stress caused by breakdown in the electron transport process. The higher the light intensity the more the oxidative damage. The health issues bewailing atrazine is a concern since atrazine is the most commonly detected herbicide in most groundwater in vulnerable regions of the world [27]. However, repeated application could lead to loss of biodiversity and could result in harm to humans who eat produce from contaminated farms [26].

The data from this study is in line with the views presented by Solomon et al. [17] and Hayes et al. [13,14] on the harmful effects of atrazine herbicides exposed in different matrixes and organisms. There are however, alternatives to chemical herbicides that would not cause damage to plants and soil animals. For long term weed management, reduced shallow tilling to a depth of 25 cm of soil in combination with the use of green manure will reduce weed population, increase crop yield and is good for the soil and its organisms (earthworms, snails, microbial communities). This is a good weed control method that can be used to overcome the need to use chemical herbicides. In addition, natural herbicides can be used as non-chemical alternative to chemical-based herbicide for weed control, although the chemical-based herbicides produce faster results when compared to natural herbicides. The herbicide atrazine was banned (removed from the market) or not allowed to be used in some crops, however, in Nigeria, different formulations of the herbicide can still be found and used in large quantity. Similarly, in Nigeria, the trend of uncontrollable application by specialists (farmers) and non-skilled individuals is increasing tremendously by the day with very little regulatory surveillance and monitoring to combat the likely consequences. The hazardous impact of atrazine application in combating weed menace in the future would still be in controversy if drastic measures are not taken in the nearest possible future.

5. CONCLUSION

We conclude from this study that the atrazine-based herbicide used in this appraisal Arda-force® had the potential to cause toxicological damage / impact on onions – a sensitive and viable anti-oxidant specie consumed daily by human. Extreme measures needs to be taken in the location these crops are harvested for sales and consumption. However, more assessments on atrazine-based herbicides still need to be evaluated to unravel or counter the controversy acclaimed by the United State Environmental Protection Agency (USEPA) that the herbicide - atrazine is ‘safe’ for use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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