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Phyto-toxic effects of atraforce®: A cotrazine based herbicide on onions (*Allium cepa* L)

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Abstract
The phyto-toxicological effects of a selective herbicide Atraforce® on onions (*Allium cepa* L) was assessed using the standard protocol of the Organization for Economic Co-operation and Development (OECD) # 208. The mean specific % growth rate relative to the control recorded was 77 ± 1.0, 70 ± 0.0, 60 ± 0.5, 47 ± 0.5 and 21 ± 0.44% with respect to the exposed concentrations (0.0625, 0.125, 0.25, 0.5 and 1.0 mg/L). The mean effective concentration (EC_{50}) for Atraforce® using root growth was 0.323 ± 0.02 mg/L, with a safe limit estimated at 0.0323 ± 0.04 mg/L. The Ecotoxicological Risk Assessment Matrix (ERAM) was used to classify Atraforce® as D 4 (P; E; C), that is 16 (P; E; C), which was considered high risk to plants, the environment and community based on the frequency of application/exposure. The results from this appraisal suggested that the use of the herbicide Atraforce® could cause deleterious effects on these viable plant and vegetable species.

Keywords: Atraforce®, growth impairment, onion (*Allium cepa* L), phytotoxicity, selective herbicide

1. Introduction

There is an escalating environmental contamination of the Niger Delta with chemicals that have not been ecologically tested for their safe use. These chemicals include pesticides and industrial chemicals. The ecological effects of pesticides and industrial chemicals to the ecosystem cannot be underestimated and evaluations need to be done to safe guide the organisms in the environment (water, soil, sediment). With little or no regular monitoring from regulators in Nigeria [Department of Petroleum Resources (DPR) and Federal Ministry of Environment (FME)], most end users dispose or use these chemicals indiscriminately [1].

Pesticides present the only group of chemicals that are purposely applied to the environment with the aim to suppress plant and animal pests and to protect agricultural and industrial products. Herbicides are a subclass of pesticides, however, majority of herbicides (weed killers) are not specifically targeting the pest only during their application, they also affect non-target plants and animals. Repeated application could lead to loss of biodiversity. The overall intensive herbicide application results in several negative effects in the environment that cannot be ignored [2-5]. Weeds are the most harmful pest that agriculture faces in terms of production. The need for tillage is reduced via chemical solutions to weed problems. Hence, it is argued that in addition to agricultural production, soil conservation is enhanced by herbicide use [6].

Herbicides may be selective and non-selective. Selective herbicides affect only certain types of plants (i.e. they kill specific targets while leaving the desired crop relatively unharmed). Non-selective herbicides kill all plants with which they come into contact (i.e. inhibits a very broad range of plant types) [7].

Atrazine is a member of the chlorophenoxy triazine family belonging to the subclass of carbamates. It is a systematic, selective, pre and post emergence broadleaf herbicide used for the control of weeds in crops like asparagus, corn, sorghum, sugarcane and pineapple [8]. It is used extensively in most parts of the world to control grass, sedge and broadleaf weeds during the cultivation of maize, wheat, sorghum, sugarcane and conifers [9, 10].

Triazine based herbicides (e.g. atrazine), functions are similar to plant growth hormones and grow cells without normal cell division, crushing the nutrient transport system of the plant, and in particular, triazines interfere with photosynthesis [11]. In addition to its intended effects on photosynthesis, atrazine has been shown in some scientific studies to negatively impact non-target organisms in the environment; including non-target plants, phytoplankton, and invertebrate.

Due to the widespread use of triazine herbicides in agriculture and its high exposure potentials for humans, the United States Environmental Protection Agency (USEPA) had conducted a special review on published and non-published data of several triazine herbicides [12, 13]. Atrazine is the second most widely used herbicide in the United State and most parts of the world. According to Nwani *et al*., [14], the herbicide atrazine is widely used in crops–weed control worldwide. In 2006, the United States Environmental protection agency (USEPA) stated that "the risks associated with pesticide residues pose a reasonable certainty of no harm''. The agency noted that atrazine does not adversely affect amphibian sexual development and that no additional testing was warranted [13].

The United States Environmental Protection Agency, however, opened a new review in 2009, which concluded that the agency's scientific bases for its regulation of atrazine are robust and ensure prevention of exposure levels that could lead to reproductive effects in humans. The study by the USEPA found that without atrazine the national corn yield would drop by six per cent, creating an annual loss of nearly two billion dollars. However, the herbicide degrades slowly in soil and often leaches into streams and rivers, where it does not readily dissolve. Atrazine is one of the most common contaminants in drinking water and an estimated thirty million people are exposed to trace amounts of the herbicide.

In 1962, Rachel Carson, a courageous American woman and scientist, wrote down her nature observation and pointed out sudden dying of birds caused by indiscriminate spraying of an organochlorine insecticide, dichlorodiphenyltrichloroethane (DDT). Her book, Silent Spring, became a landmark. It changed the existing view on pesticides and has stimulated public concern on pesticides and their impact on health and the environment. Silent Spring facilitated the ban of the DDT in 1972 in the United States [15].

Herbicides enter the soil via spray drift during foliage treatment, wash-off from treated foliage, release from granulates or from treated seeds in soil. Herbicides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man, plants and animal diseases. At the same time, the use of these chemicals have posed potential health hazard to aquatic and terrestrial lives. Herbicides are the major cause of concern for these environments because of their toxicity, persistency and tendency to bioaccumulate in organisms. They could leach into groundwater, surface water and contaminate soils and sediments. Due to their chemical properties and relatively low water solubility, they have high persistence in the environment that is they are not easily degradable (half-life of about 12 to 30 years for some formulations). They also have the potential to enter organisms and bioaccumulate in fatty tissues of living organisms in food chains and consequently influence human health [16].

Several researches have been done on various dangerous organic pesticides like Dieldrin, Endosulfan and Lindane, which are used in different domain (industrial and agricultural) to the detriment of the species in the environment and man. The problem of pesticide usage is not over in many countries even the old highly persistent, bioaccumulative pesticides that have been banned or restricted are still in use. Similarly, a lot of new products / formulations have been developed and used in large quantities. For many of these substances today we still do not have sufficient knowledge about their possible risks and adverse effects on the environment and human. Several of them appear to have a harmful environmental profile and significant levels have been detected in tissues of fish, periwinkles, snails and shrimp in areas where these persistent pesticides have been used [17- 20].

Onion (*Allium cepa* L) belongs to the bulb crops, a group belonging to the family of *Alliaceae*. It is one of the most important edible vegetable crops 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 [21-24]. 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 [25]. Mainly, chemical control is applied against weeds in onion producing areas, but possible phytotoxicity on onion is also a main problem. Root growth inhibition provide indications of toxicity to onions [26, 27]. This study assessed the phytotoxic effect of a selective herbicide Atraforce® on the growth of onion (*Allium cepa* L). This is with a view to ensuring that further damage is not done to these threaten viable and widely consumed vegetable species.

2. Materials and Methods

2.1 Test Chemical

Atraforce®, a selective herbicide was obtained locally from the vendors in Benin City, Edo State. The test chemical is liquid, soluble in water and contained atrazine (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 currently used by farmers and non-farmers alike in the Niger Delta area of Nigeria.

2.2 Test Specie

The test specie is the common onion (*Allium cepa* L) of the purple (Stuttgarter) variety. The onions used in this study were 6.25 ± 0.06 cm in diameters with a mean weight of 77.50 ± 0.47 g.

2.3 *Allium cepa* Assay

The *Allium cepa* assay was assessed using the Organisation for Economic Co-operation and Development, (OECD) protocol #208 [28a].

2.4 Preparation of Onions

The onions were air-dried for 2 weeks and the dried roots present at base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues. Any bulb attacked by fungi was not used for the experiment. The bulbs were then placed in distilled water to protect the primordial cells from drying up. The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water [29].

2.5 Root Growth Inhibition Evaluation

For the root growth inhibition evaluation, the 4 day semi-static renewal assay started with a range finding test to determine the range of concentrations to be used for the definitive test. Stock solutions of the test chemical were freshly prepared and serially diluted into five concentrations of 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/L. Each concentration and the control in triplicate were used for the 96 h bioassay. The control was set up with tap water and was ascertained to be of good quality with a pH value of 6.78 ± 0.3 and hardness concentration of 14 ± 0.01 mg $CaCO_3$/L. It was free from any chlorine compounds or toxic ions [30].

The base of each of the bulbs was suspended on the test solution in a 250 mL test tank in the dark for 96 h duration. At the end of the exposure period, the roots of onion bulbs in each concentration were removed with a forceps and their lengths measured (in cm) with a metre rule. From the means for each test concentration and the control, the percentage root growth inhibition in relation to the control and the EC_{50} (the effective concentration where root growth amounts to 50% of the controls) for each extract was determined [30]. The effect of each sample on the morphology of growing roots was also examined.

2.6 Ecotoxicological Risk Assessment (ERA)

To predict the effects of chemicals (herbicides) exposed organisms, the United State Environmental Protection Agency (USEPA) and Society of Environmental Toxicology and Chemistry (SETAC) standard guide/protocols for Ecotoxicological risk assessment matrix (ERAM) was used [31, 32, 33]. On the matrix, risk levels are classified as low, medium, or high categorized in a numbered format. Animals (A), plants (P), environment (E) and community (C) may be affected and using exposure concentration assessment, exposure duration assessment and the potency of the chemical assessment (toxicity effect assessment) classification could be done.

Each hazard is given a rating and this could be multiplied by the probability that these hazards would occur using the relationship:

Risk level = Hazard severity x likelihood of exposure

Hazard severity that is the consequence of the toxicological effects are indicated in Table 1.

For instance, if the release of the herbicide (hazard) could result in major damage or death, then it would be given a rating of 4. The next step is to consider how often each hazard is likely to occur due to exposure of the herbicide as indicated in Table 2.

Table 1: Hazard severity

	Effect of hazard	Toxic consequence
1	Slight effect on organisms	Practically non-toxic
2	Minor effect or damage to organisms	Slightly toxic
3	Localized effect or damage to organisms	Very toxic
4	Major effect (deaths)	Extremely toxic
5	Extensive effect (death of population)	Super toxic

Table 2: Likelihood of occurrence (exposure)

	Likelihood of exposure		Frequency of exposure
1	Seldom	A	Yearly
2	Frequent	B	Quarterly
3	Very likely	C	Monthly
4	Near certain	D	Weekly
5	Certain	E	Daily

For instance, if the release of the herbicide caused a major effect (deaths) (hazard) (4), was near certain to happen and since it had been used severally in the area and other locations on a weekly basis, then it must be given a rating of 4 or D. These two values (hazard severity and likelihood of exposure) are then multiplied together to get the risk level. The value should be entered into the risk assessment form (Table 3). That is:

4 (hazard) multiplied by 4 or D (occurrence - exposure) = 16 or D 4.

Since it is likely to affect plant (P), the environment (E), and community (C), it would be represented as D 4 (P; E; C) or 16 (P; E; C). Similarly, since risk levels can be classified as low, medium and high, it then means that any value within the green area 1 is considered low risk; the yellow area 2 is regarded as medium risk while the red area 3 is classified high risk. Since D 4 (P; E; C) or 16 (P; E; C) is under the red region, it can be regarded as high risk.

Table 3: Ecotoxicological Risk Assessment Matrix (ERAM)

	Consequence						Increasing Probability				
Severity			P	A	E	C	A	B	C	D	E
							Never experience the chemical in the area	Had been exposed / used in the area	Had been exposed/ used in the area and other locations	Had been exposed / used several times in the area	Had been exposed / used several times in the area and other locations
0	Practically non-toxic	>1000	No injury	No effect	No effect	No impact					
1	Practically non-toxic	>1000	Slight injury	Slight effect	Slight effect	Slight impact					
2	Slightly toxic	100-1000	Minor injury	Minor effect	Minor effect	Limited impact	Area 1				
3	Very toxic	10-100	Major injury	Localized effect	Localized effect	Considerable impact	Green				
4	Extremely toxic	1.0-10	Single fatality	Major effect (deaths)	Major effect	National impact		Area 2		Area 3	
5	Super toxic	<1.0	Multiple fatality	Extensive effect (kills)	Massive effect	International impact		Yellow		Red	

Abbreviations: LC50 median lethal concentration in mg/L. Data from OECD, [28b]

2.7 Statistical Analysis

The effective concentration EC_{50} for root inhibition was used to determine the susceptibility of onions to the test herbicide at 4-d [28b]. In addition, the analysis of variance (ANOVA) in Statistical Package for Social Science (SPSS) statistical software in Version 22.0 was also used to test the mean statistical difference between the controls and treated groups at significance level of $P = .05$.

3. Results

The results of the adverse effect of exposure of onion (*Allium cepa* L) to different concentrations of Atraforce® using root growth inhibition evaluation for the 4-day phytotoxic assay are presented in Table 4 and Figures 1-3. Throughout the duration of the experiment, the control onions were healthy with observable growth as the test assay progressed. An indication that the test conditions were appropriate; also implying that growth inhibition reported in the test solutions could be attributed to the effect of the test herbicide.

On the first day of exposure, decolouration of the onions was observed at the highest concentrations and with no observable growth as the days progressed. Slight growth was noted in the three lowest concentrations. Table 4 below showed the mean root lengths of *Allium cepa* cultivated in different concentrations of the test herbicide and control. The mean root lengths of *Allium cepa* obtained from test herbicide ranged from 0.98 ± 0.05 to 3.44 ± 0.04 cm while the control recorded 4.55 ± 0.03 cm at the end of the experimental period.

Table 4: Mean root length (RL) and mean root growth (%) of *Allium cepa* exposure to Atraforce®

Concentration (mg/L)	mean RL ± SD (cm)	Root growth (%)
Control	4.55 ± 0.03	100 ± 0.0
0.0625	3.44 ± 0.04	77 ± 1.0
0.125	3.13 ± 0.02	70 ± 0.0
0.25	2.71 ± 0.01	60 ± 0.5
0.5	2.14 ± 0.03	47 ± 0.5
1.0	0.98 ± 0.05	21 ± 0.44

RL= mean % growth rate relative to the control

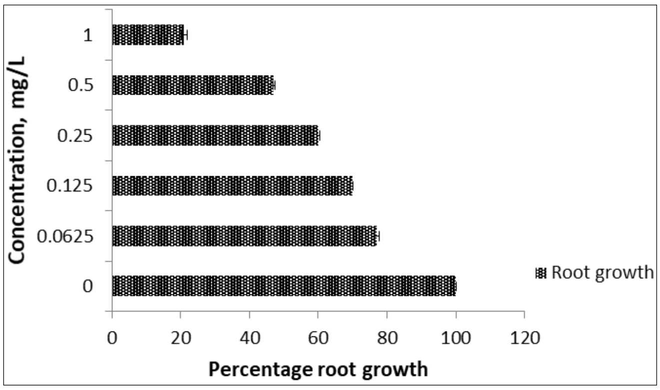

Fig 1: Mean (%) Root growth of Atraforce®

The mean % growth rate relative to the control recorded for the five concentrations 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/L were 77 ± 1.0, 70 ± 0.0, 60 ± 0.5, 47 ± 0.5 and 21 ± 0.44% respectively (Figure 1 and 2). The percentage growth rate between the control experiment and the test chemical was significantly different at levels of P <0.05. Phytotoxic effect of the herbicide on onions include: decolouration of the test solutions and stunted growth in a number of the higher concentrations, twisting of roots, bulb deformation and root damage amongst others. The effective concentration (EC_{50}) of the chemical was evaluated using estimated 96 h EC_{50} values of growth length in varying concentrations. The effective concentration (EC_{50}) for Atraforce® was determined at 0.323 ± 0.02 mg/L.

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 receiving environment should not exceed 10% of the EC_{50}. Thus, the estimated safe concentration for Atraforce® was 0.0323 ± 0.04 mg/L In this study, there was decrease in root growth as the concentration of the test solutions increased (Figure 3). This was indicative of the dependence of root growth on concentration. Thus, the higher the concentration of the test herbicide in the environment, the more the likely adverse effect and effect could be magnified either in acute or chronic exposures [34, 35].

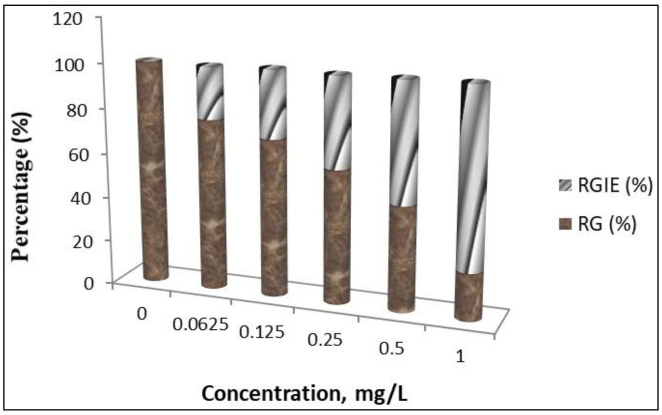

Fig 2: Mean (%) Root growth relative of control (RG) in comparison with mean root growth inhibition efficiency (RGIE)

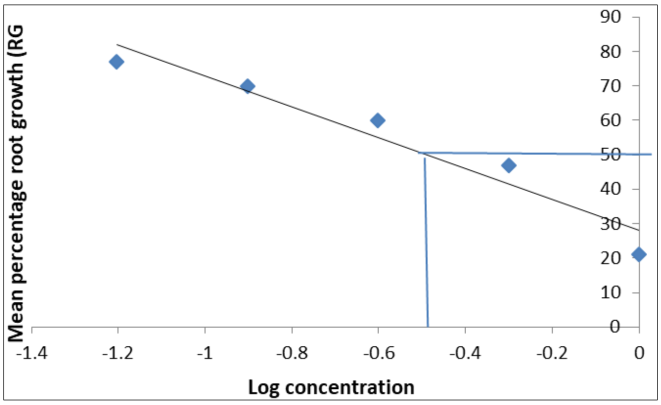

Fig 3: Mean (%) root growth (RG) of onions (*Allium cepa* L) against log concentration of Atraforce®

Ecotoxicological Risk Assessment (ERA) of the herbicide

The release of the herbicide (hazard) into the environment could result in major damage or death, which could be given a rating of 4 (Table 1). Considering the hazard a weekly exposure of the chemical could cause as indicated in Table 2. This exposure could result in likely to effects to animals (A), plant (P) and the environment (E), it would be represented as D 4 (A; P; E) or 16 (A; P; E) for Atraforce® using Table 3.

4. Discussion

Herbicides show adverse effects in all standard categories of toxicological testing, including short-term toxicity, long-term toxicity, genetic damage, effects on reproduction and carcinogenicity. Scientific studies have shown that herbicide formulations and metabolic products could cause the death of aquatic, terrestrial and plant species even at low concentrations [18, 19, 36]. Selective control means that the target weeds are controlled, with little or no injury to the crop. Atraforce® containing atrazine is well tolerated by actively growing corn and sorghum, which absorb and metabolize the herbicide and thereby detoxify it.

Despite the tremendous advantages of herbicide use, the disadvantages should not be ignored since the environment is at great risk due to daily unregulated application. It therefore calls for constant scientific evaluation and control of the use of these products. Because once applied after solving the problem at hand it generates a myriad of other problems. In this vein, it could contaminate air, water, soil, sediment and non-target vegetation in regions applied. The resultant effect could be harmful to plants and animals including beneficial soil microorganisms, insects and non-target species despite been selective [37]. Similarly, human beings are particularly sensitive targets for herbicides through crops from farms the herbicides have been applied. Chronic poisoning in human beings and mammals due to herbicides results from herbicide decay derivatives that are taken with the food chain [38].

Onions growth inhibition reported for this study was caused by the test herbicide since the control species recorded significant growth. Atraforce® is used for the post-emergence control of selected weeds in established lucerne, maize, sweetcorn and non-cropland situations and as such it is not expected to attack any plant but the specific weed of interest. However, studies have reported that atrazine is an endocrine disruptor, an agent that may alter the natural hormonal system in animals [39, 40, 41]. Despite the effects (hermaphroditism) noted in frog by Hayes *et al*., [41], which were exposed to thirty times below the levels recommended by the United State (0.1 µg/L to 30 µg/L), the United State still approved the continued use of atrazine, the same period that the European Commission chose to remove it from circulation. He concluded that atrazine could be a major factor in the decline in amphibian populations, a phenomenon observed all over the world. However, the herbicide is still in use in most parts of the world especially Nigeria. The USEPA review has been criticized by Hayes *et al*., [41] and this remains controversial [42, 43, 44]. However, more evaluations on the test herbicide still need to be understudied to counter this controversy.

5. Conclusion

This study provided data and information on the short-term toxicity of Atraforce® that may be a helpful user-guide on safe application levels for the herbicide. This report provide the impetus for further studies to evaluate any damage caused at environmentally relevant concentrations to non-target organisms that are integral parts of ecosystems since the safety of atrazine is still in question.

6. References

1. Ezemonye LI, Enuneku AA. Stage dependent acute toxicity of exposure of *Bufo maculatus* and *Ptychadena bibroni* tadpoles to cadmium. Journal of Applied Science and Technology 2006;11(1-2):78-82.
2. Trefry JH, Trocine RP, Naito KL, Metz S. Assessing the potential for enhanced bioaccumulation of heavy metals from produced water discharges to the Gulf of Mexico. Produced water 2. Environmental issues and mitigation technologies. Edited by Mark Reed and Stale Johnsen, Plenum Press, New York and London 1996, 339-354.
3. Van den Heuvel M, Power M, MacKinnon MD, Dixon DG. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*) I: Water quality characteristics and yellow perch physiological and population responses. Canadian Journal of Fish Aquatic Science 1999;56(7):1213-1225.
4. Baker BP, Benbrook CM, Groth E, Benbrook KL. Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets. Food Additives and Contaminants 2002;19(5):427-446.
5. Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (*Common carp*). International Journal of Environmental Science and Technology 2008;5(2):179-182.
6. Warnemuende EA, Patterson JP, Smith DR, Huang C. Effects of tilling no-till soil on losses of atrazine and glyphosate to runoff water under variable intensity simulated rainfall. Soil and Tillage Research 2006;19:26-30.
7. Cork DJ, Krueger JP. Pesticide biodegradation. In Encyclopedia of Microbiology. (Lederberg J. Ed) 1992, 357-361.
8. Rouchaud J, Gustin F, Callens D, Bulcke R. Atrazine soil metabolism in maize fields treated with organic fertilizers. Weed Research 1996;36(2):105-113.
9. Worthing CR, Walter JB. The pesticide manual. 7th Ed Uk. The Lavenham Press 1983, 589p.
10. Van den Brink, P. J., van Donk E., Gylstra R., Crum S. J. H. and Brock T. C. M. Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 1995;31(5):3181-3200.
11. Jones TW, Kemp WM, Estes PS, Stevenson JC. Atrazine uptake, phytotoxicity, release, and short-term recovery for the submersed aquatic plant, *Potamogeton perfoliatus*. Report to U.S. Environmental Protection Agency, Annapolis. NTIS, Springfield, VA 1982.
12. Wu TL. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. Journal of Environmental Quality 1980;9:459-465.
13. United States Environmental Protection Agency (USEPA). Atrazine Interim Reregistration Eligibility Decision (IRED) Report #20460. WASHINGTON, D.C 2006, 1-324.
14. Nwani CD, Magpure NS, Kumar R, Kudshwaha B, Kumar P, Lakra WS. Mutapenic and genotoxic assessment of atrazine-based herbicides to fresh water fish *Channa punctatus* (Bloch) using micronueclus and single cell gel electrophoresis. Environmental Toxicology and Pharmacology 2011;31:314-322.
15. Carson R. Silent Spring. Boston: Houghton Mifflin 1962.
16. Joseph B, Raj SJ. Effect curacron toxicity on the total serum content of *Cyprinus carpio*. Toxicology and Environmental Chemistry 2010;92:1889-1893.
17. Anasco N, Uno S, Koyama J, Matsuoka T, Kuwahara N. Assessment of pesticide residues in freshwater areas affected by rice paddy effluents in Southern Japan. Environmental Monitoring and Assessment 2010;160(1):371-383.
18. Ezemonye L, Tongo I. Sublethal effects of endosulfan and diazinon pesticides on glutathione-S-transferase (GST) in various tissues of adult amphibians (*Bufo regularis*). Chemosphere 2010;81(2):214-217.
19. Ogbomida ET, Ezemonye LIN. Elevation of testosterone level in periwinkle snail (*Tympanotonos fuscatus var radula*) exposed to Bis-Tributyltin Oxide (TBTO). The Bioscientist 2013;1(2):147-157.
20. Ogeleka DF, Onwuemene C, Okieimen FE. Toxicity potential of Grassate® a non-selective herbicide on snail (*Archachatina marginata*) and earthworm (*Aporrectodea longa*). Chemistry and Ecology 2017;33(5):446-462.
21. Ghosheh HZ. Single herbicide treatments for control of broadleaved weeds in onion (*Allium cepa*). Crop Protection 2004;23:539-542.

22. Carlson HL, Kirby D. Effect of herbicide rate and application timing on weed control in dehydrator onions. University of Florida, Intermountain Research and Extension Center 2005;115:4-8.
23. Qasem JR. Chemical weed control in seedbed sown onion (*Allium cepa* L.). Crop Prot 2006;25:618-622.
24. Cavusoglu K, Yalcin E, Turkmen Z, Yapar K, Cavusoglu K, and Cicek F. Investigation of toxic effects of the glyphosate on *Allium cepa*. Journal of Agricultural Sciences 2011;17:131- 142.
25. Wicks GA, Johnston DN, Nuland DS, Kinbacher EJ. Competition between annual weeds and sweet Spanish onions. Weed Sciences 1973;21:436-439.
26. Olorunfemi DI, Ehwre EO. Chromosomal aberrations induced in root tips of *Allium cepa* by squeezed garri extracts. Report and Opinion 2010;2(12):166-171.
27. Bonciu E, Cytological effects induced by Agil herbicide to onion. Journal of Horticulture, Forestry and Biotechnology 2012;16(1):68-72.
28. [28a] Organisation for Economic Co-operation and Development. Terrestrial plants, growth test" OECD Guideline for testing of chemicals 208, OECD, Paris 2003a, 1-19.
29. [28b] Organisation for Economic Co-operation and Development (OECD). Environment, Health and Safety Publications Series on Pesticides Persistent, Bioaccumulative, and Toxic Pesticides in OECD Member Countries Results of Survey on Data Requirements and Risk Assessment Approaches 2003;15:1-67.
30. Rank J, Nielsen MH. A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. Hereditas 1993;118:49-53.
31. Fiskesjo G. The Allium test as a standard in environmental monitoring. Hereditas 1985;102:99-112.
32. United State Environmental Protection Agency (USEPA). Framework for ecological risk assessment. ERA/630/R-92/001. Risk assessment forum. USEPA, Office of Research and Development (ORD) Publications, Washington D.C 1992, 20460.
33. United State Environmental Protection Agency (USEPA). Proposed guidelines for ecological risk assessment. Federal register 1996;61:47552-47631.
34. Society of Environmental Toxicology and Chemistry (SETAC). Ecological risk assessment. A technical issue paper. 1010 North 12th Avenue Pensacola, FL 32501-3367. USA 1997, 1-4.
35. Baerson SR, Rodirguez DJ, Biest NA, Tran M, You J, Kreuger RW *et al*. Investigating the mechanism of glyphosate resistance in rigid ryegrass (*Lolium rigidum*). Weed Science, 2002;50:721–30.
36. El-Nady M.F. and Belal E.B. Effect of phytotoxicity of pendimethalin residues and its bioremediation on growth and anatomical characteristics of *Cucumis sativus* and *Echinochloa crus-galli* plants. Asian Journal of Crop Science 2013;5:222-237.
37. Dimitrov BD, Gadeva PG, Benova DK, Bineva MV. Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. Mutagenesis 2006;21:375-382.
38. Sanders HO. Toxicity of pesticides to the crustacean *Gammarus lacustris*, Technical papers of the bureau of sport fisheries and wildlife, No. 25. US Dept. of Interior Fish and Wildlife Service, Washington D.C 2010.
39. Metin N, Kubas A, Hurma H and Erbay E R. Pesticide usage and its effects on the environment in Thrace region. The Journal of Environmental Protection and Ecology 2003;4:328-333.
40. Solomon KR, Carr JA, Du Preez LH, Giesy JP, Kendall RJ, Smith EE *et al*. Effect of atrazine on fish, amphibians, and aquatic reptiles: A Critical Review. Critical Reviews in Toxicology 2008;38:721-772.
41. Hayes T, Khoury V, Narayan A, Nazir M, Park A, Brown T *et al*. Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). Proceedings of the National Academy of Sciences 2010, 4612-4617.
42. Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T. Demasculinization and feminization of gonads by atrazine: Consistent effects across vertebrate classes. The Journal of Steroid Biochemistry and Molecular Biology 2011;127(1-2):64-73.
43. Vogel JR, Majewski MS, Capel PD. Pesticides in rain in four agricultural watersheds in the United States. Journal of Environmental Quality 2008;37(3):1101-1115.
44. Tillitt D, Papoulias DM, Whyte JJ, Richter CA. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas*). Aquatic Toxicology 2010;99(2):149-59.
45. Aviv R. A valuable reputation: After Tyrone Hayes said that a chemical was harmful, its maker pursued him. The New Yorker. Annals of Science, February 2014, 10.