ECOTOXICOLOGICAL AND RISK ASSESSMENT OF GLYPHOSATE ON THE ACTIVITY OF DUCKWEED

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Abstract
Ecotoxicological effect and risk assessment of a pesticide residue (glyphosate) was studied. The effect of different concentrations (0.2-0.8ppm) of glyphosate on the plant (Duckweed) was used for the study by collecting and culturing duckweed for a period of one week and thereafter exposing the duckweed to different concentrations of glyphosate for 8 days. The pigment content and biomass were monitored to assess the effects of the glyphosate on the plant. The results revealed that all the concentrations show adverse effect on the plant pigment content as well as on the biomass productivity as the days progressed and the concentration increases. This study has shown that the residue of glyphosate could be dangerous to the ecosystem at concentration as low as 0.2ppm which is actually the allowable level of glyphosate in plant leaf.

Keywords: Ecotoxicology, duckweed, glyphosate, pigment, biomass

Introduction

Ecotoxicology is the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context [1]. The ultimate goal of this approach is to be able to predict the effects of pollution so that the most efficient and effective action to prevent or remediate any detrimental effect can be identified. In those ecosystems that are already impacted by pollution, ecotoxicological studies can inform as to the best course of action to restore ecosystem services and functions efficiently and effectively [2].

Glyphosate [N-(phosphonomethyl)glycine] is a non-selective amino-phosphonate acid-type herbicide, systemic and post-emergence which was introduced into commerce in 1973 [3]. Sale of this herbicide represents about 60% of the world market of herbicides, totaling 1.2 billion dollars per year, making this product the non-selective herbicide most extensively applied, mainly due to its broad spectrum for the elimination of weeds. Nowadays, especially with biotechnology support, some plants have become resistant to glyphosate, increasing its consumption by approximately 20% per year [4]. Formulations of glyphosate include an acid, monoammonium salt, diammonium salt, isopropylamine salt, potassium salt, sodium salt, and trimethylsulfonium or trimesium salt [5]. Technical grade glyphosate is used in formulated products, as well as the isopropylamine, sodium, and monoammonium salts. The isopropylamine salt has been reported to be the most commonly used in formulated products [6]. Evidence has shown that a significant proportion of the population could have glyphosate in their bodies. It has been observed that despite the fact that glyphosate is the world´s best-selling chemical herbicide and glyphosate-containing herbicides are the most widely-used herbicides in Europe, very little testing is done for glyphosate residues in food, feed, or water, studies has not been conducted on glyphosate residue effect on the body [7].

Though previous researches seemed to establish that the risk of plant injury from glyphosate residues was low, new studies have revealed that soil residues are potentially phytoactive and there is need for more information regarding the response of plants to glyphosate residues [8]. The overall aim of this research work is to study the ecotoxicological effect and the risk assessment of pesticide (glyphosate) residue using the effect on the activity of duckweed (*Lemna* minor).

Materials and Methods

Collection and culture of plant samples

Five grown duckweed (*Lemna* minor) species were selected for experimental purpose. The duckweed plant was identified by the Department of Plant Biology, University of Ilorin. Healthy and uninfected duckweed species were collected at their stage of maturity and cultured in the laboratory in the growth media of zero concentration of pesticide residue with a 12:12hrs light-dark cycle. Fresh leaf samples were washed thoroughly first with tap water followed by distilled water in the laboratory. They were allowed to dry and analyzed for the determination of chlorophylls (Ch-a and Ch-b) and carotenoids content.

Preparation Glyphosate and Treatment of plant samples

Five different concentrations were prepared (0ppm, 0.2ppm, 0.4ppm, 0.6ppm, and 0.8ppm) from the stock solution of glyphosate salt using the serial dilution. The prepared concentrations are then applied on the selected plants at room temperature under 12:12hrs light-dark cycle and analyzed for the effect on chlorophyll content and carotenoids in the plant.

Biomass Productivity

Plant samples were dried to a constant weight in an oven at 100 ºC for 24 hr. The dry weight of plants for each concentration and exposure time was expressed as percentage decrease of biomass relative to controls.

Total Chlorophyll and Carotenoid Contents of the Plant

The chlorophyll and carotenoid content of treated and control plants were measured by the absorption spectra of frond extracts using UV spectrophotometer (JENWAL 7300). The absorbance of pigment extract was measured at 663nm (A663), 645nm (A645) and 470nm (A470) [9].

Analytical procedure

Fresh plant leaf samples [0.5g] were taken, and homogenized in a mortar with a pestle with acetone[10 ml]. The homogenized sample mixture was centrifuged at 10,000 rpm for 15min at 40ºC and the supernatant was separated and 0.5ml of it mixed with 4.5ml of acetone. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b and carotenoids content with a spectrophotometer (Parkin). Chlorophyll-a, chlorophyll-b and carotenoid contents in mg/L were then calculated from the absorbance using the formula below [9];

$$Ch_a = 12.25A_{663} - 2.79A_{645}$$

$$Ch_b = 21.5A_{645} - 5.1A_{663}$$

$$C_{x+c} = \frac{(1000A_{470} - 1.82ch_a - 85.02ch_b)}{198}$$

Where; Ch_a = Chlorophyll a

Ch_b = Chlorophyll b

C_{x+c} = Carotenoid

Results and Discussion

The result of total chlorophyll-a, chlorophyll-b and carotenoid contents of the duckweed after exposure to different concentrations of glyphosate for several days are presented in Figures 1, 2 and 3 respectively. The variations in the weight of the plant were also observed and the results are presented in Figure 4.

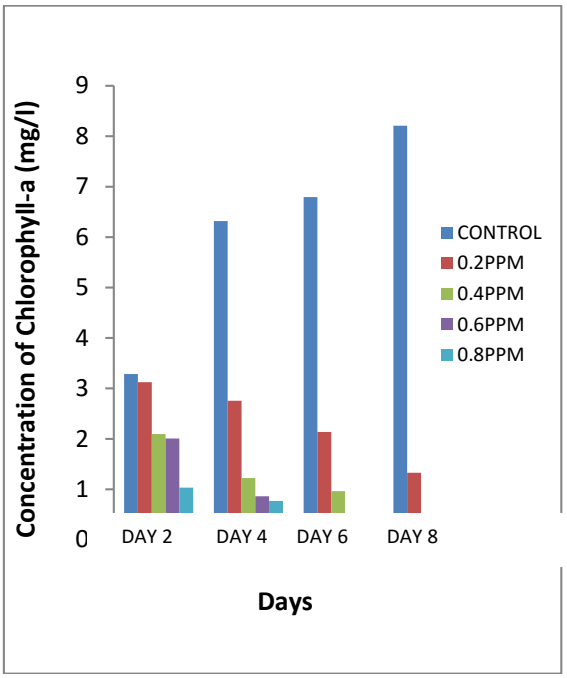

Figure 1:Total Chlorophyll-A Content with Respect to the Days

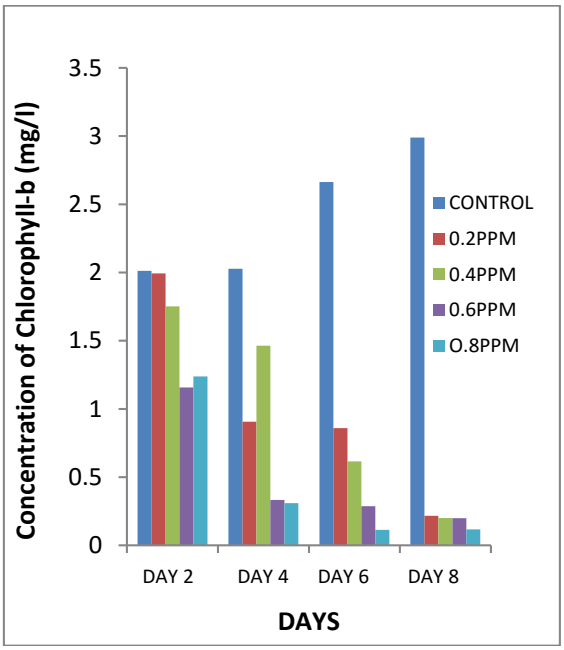

Figure 2: Total Chlorophyll-B Content with Respect to Days

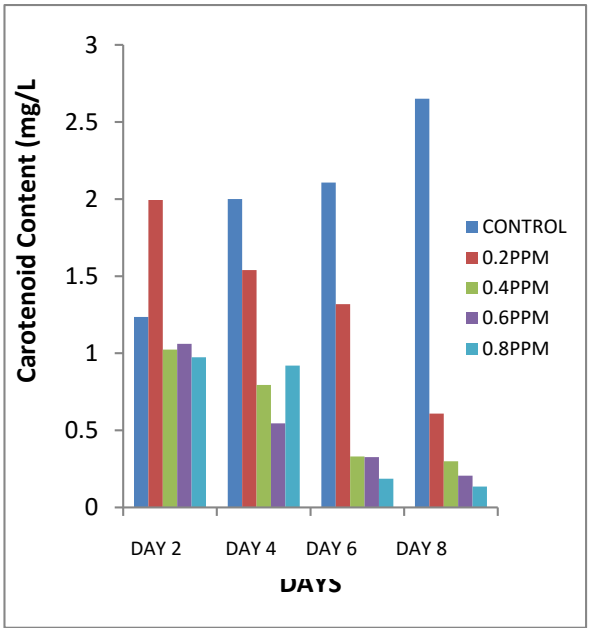

Figure 3: Total Carotenoid Content with Respect to Days

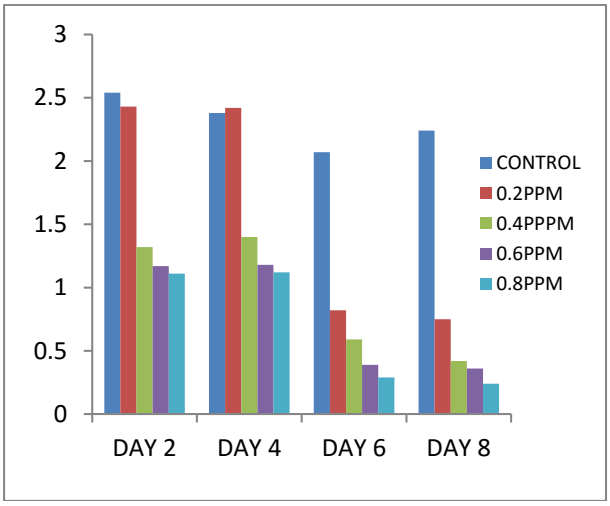

Figure 4: Variation of Weight (g) of Plants

From the results, it was observed that the chlorophyll-a and carotenoid contents of the control increased from day 2 to day 8. This revealed that the plant kept growing with no indication of injury. On the other hand, the plant samples containing different concentrations of the glyphosate showed a considerable visual signs of injury as the days progressed. These injuries became noticeable from the third day of application of glyphosate residue at different concentrations. In fact, it was observed that the plant fronts were drying as from the third day and the severity increased with increase in concentration.

At lower concentrations of glyphosate (0.2 ppm and 0.4 ppm), no severe injuries was done on the plant which may eventually lead to plant death, but at higher concentrations (0.6 ppm and 0.8 ppm) the injury become fatal as some fronds started turning yellow. These injuries were ascertained by the analysis of the chlorophyll and carotenoid contents in the plant which were found to be deceasing with increasing concentration of the glyphosate as days progressed. For example the carotenoid content of plant with 0.8 ppm glyphosate decreased from 0.974 to 0.136 (Figure 3) and can be attributed to the hazardous effect of the glyphosate [10, 11]. These findings were similar to those reported elsewhere that high concentrations of glyphosate has a negative effect on chlorophyll content of Congo grass [12].

Similarly, the weight of the control decreased slightly from 2.54g to 2.24g after the eighth day whereas the weight of plants with glyphosate decreased greatly, for example, with just 0.2 ppm of glyphosate the weight decreased from 2.43g to 0.75g. It was also observed that the higher the glyphosate concentration exposed to plant the more the loss in the weight of the plant. This further revealed that glyphosate has a negative effect on the growth of plant just as reported earlier [13].

Conclusion

This research studied the effect of various concentrations of glyphosate residue on the duckweed and it has revealed that at concentration higher than the allowable concentration of glyphosate residue (0.2ppm), there is significant hazardous effect on the plant pigment and the biomass productivity of the plant. The decrease in the chlorophyll content and the biomass productivity of the plant lead to lower nutrient and most probably death of the plant, and this will negatively affect organisms which depend on the plants for food. This will also result in decrease of the production of oxygen to the ecosystem. It is therefore recommended that the minimum level of the glyphosate should be maintained as recommended by either Environmental Protection Agency or World Health Organization.

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