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Cytotoxicity of two common pesticides using Allium cepa (onion) Assay

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Abstract

The use of pesticides for agricultural, domestic and industrial purposes is increasing alarmingly; and there is much concern in the society about their use. This is because pesticides are known to be generally toxic and can also affect non-target organisms. This study investigated the effect of two pesticides: glyphosate and paraquat dichloride using the Allium cepa assay. Onion roots were exposed to varying concentrations of both pesticides respectively, and squashes of the root tip cells were examined. Microscopic end points such as stickiness, bridge formation, micro nuclei, lagging chromosomes, binucleated cells, C- mitosis and damaged cells were observed. These chromosomal abberations are concentration-dependent. In general, paraquat dichloride is more toxic than glyphosate.

Keywords: *Alium cepa*, chromosome, pesticides, cytotoxic

Introduction

Pesticides are substances or mixture of substances intended for preventing, destroying or controlling any pest, and this includes both plants and animals (FAO, 2003). Pesticides can be classified by target organisms, chemical structure, and physical state (Gilden R.C. *et al.,* 2010). Many pesticides can be grouped into chemical families. The two largest classes of synthetic pesticides are insecticides, used to kill insects, and herbicides used to kills plants. Pesticide products very rarely consist of pure technical material. The active ingredient is usually formulated with other materials and this is the product sold, but it may be further diluted in use. The most frequently used products are formulations for mixing with water which are then applied as sprays (Mesnage *et al.,* 2014).

Glyphosate (*N*-phosphonomethyl) glycine) is an organic compound containing phosphorus, used as a broad-spectrum herbicide. It is used to kill undesirable plant, especially the angiosperm annuals and grasses that compete with cultivated plants. Glyphosate which is better known by the trade name **Roundup,** is absorbed through foliage, and minimally through roots, (Sprankle et al., 1975; National Pesticide Information, 2010) and transported to growing points. It inhibits a plant enzyme involved in the synthesis of three aromatic amino acids, and is therefore only effective on actively growing plants. The development of glyphosate resistance in weed species however is emerging as a costly problem. While glyphosate and formulations such as Roundup have been approved by regulatory bodies world-

wide, concerns about their effects on humans and the environment persist (Cressey, 2015).

Paraquat (*N*, *N*′-dimethyl-4, 4′-bipyridinium dichloride) on other hand is quick-acting and non-selective, killing green plant tissue on contact. It is also toxic to human beings and animals. It is linked to development of neurological disorders in man (Kamel, 2013). Paraquat was first manufactured and sold by Imperial Chemical Industries in early 1962, and is today among the most commonly used herbicides. As an herbicide, paraquat acts by inhibiting photosynthesis (Summers, 1980).

Problems with decreased susceptibility of weeds to herbicides has led to applying herbicides with different modes of action, along with cultural methods. One example is the "Double Knock regime with paraquat cleaning-up after glyphosate which was predicted to keep all fields free of glyphosate resistant ryegrass for at least 30 years (Walsh and Powels, 2007)

Despite all these benefits however, pesticides released into the environment have effects on human health and this has led to wide spread public concerns and controversial disputes. Residues of pesticides are known to remain in soil (Subbarao, 1999), water (Medina et al., 1999) and also in vegetables and fruits (Osman *et al.,* 2010) which constitute a risk for human health.

The most commonly used toxicological tests were carried out on mammals. Recently one of the fundamental concerns for both science and ethics has become use of animals in toxicity studies (Mukhopadhyay *et al*., 2004; Purchase, 1997). The principles of humane animal experimentation-replacement, reduction and refinement (known as the Three Rs) were defined by Russell and Burch (Russell and Burch, 1959). According to these principles, alternative test objects have been searched. Fiskesjo (1985) underlined that "a standard test for toxicity must be easy to perform and the results should be rapidly obtained and reproducible". Higher plants provide valuable genetic assay systems and fulfill these demands (Grant, 1994; Leme and Marin-Morales, 2009).

Among the plant species, *Allium cepa* is considered to be a suitable test system for evaluation of the genotoxic potential of pesticides (Asita and Matebesi, 2010; Bolle *et al*., 2004; Chauhan *et al*., 1998; Fernandes *et al*., 2007; Mustafa and Arikan, 2008; Yuzbasioglu *et al*., 2009). *Allium cepa* test has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa* (Yekeen *et al.,* 2013; Thais *et al*., 2007; Cabrera *et al*., 1994). According to Fiskesjo (1988) "results from the *Allium* test have shown good agreement with results from other test systems, eukaryotic as well as prokaryotic".

The aim of this study therefore is to study the cytotoxic effect of two common pesticides, namely –glyphosate and paraquat, using the Alium cepa assay.

Materials and methods

Healthy and cleaned onion bulbs of about the same size were selected and placed in plastic containers with distilled water to germinate for 48 hours. The onion bulbs were divided into three groups- the first was the control (in three replicates); the second, the five different treatment concentrations of glyphosate and the third group was the five different treatment concentrations for paraquate- all in three replicates

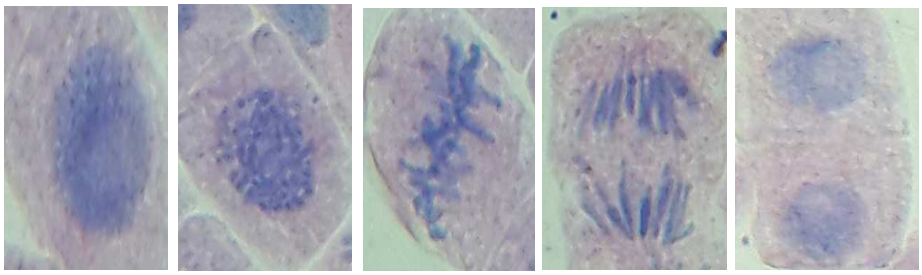
each. With the rootlets emerging, the bulbs were transferred to other clean containers containing the various treatments after the 2 day period. The 5 varying glyphosate concentrations were: 0.75g/L, 1.5g/L, 3.0g/L, 6.0g/L and 8.0g/L); and the concentrations for paraquate dichloride were: 0.43g/L, 0.86g/L, 1.7g/L, 3.4g/L and 5.0g/L. The bulbs of the control group however remained in distilled water. The exposure time of the onion bulb with emerged rootlets to the different treatment was 24 hours. This experiment was carried out in the dark. The rootlets of the onion bulbs were cut off and immediately fixed in ethanol: acetic acid (3:1) for 24 hours. The rootlets were then removed from the fixative and transferred to 70% ethanol in which they were kept under refrigeration (4°C) until used.

Slide preparation

The rootlets were taken out of the 70% ethanol. The root tips were cut and hydrolyzed in 1N HCL for 20minutes in a water bath at 60^{o}C. They were then rinsed in distilled water and stored in water with 5 drops of 1% Ferric Chloride for at least 24 hours before slide preparation. Squashes were made in a drop of acetocarmine stain, and viewed under a compound light microscope. Photomicrographs were taken with a Samsung digital camera using an X40 objective.

Result

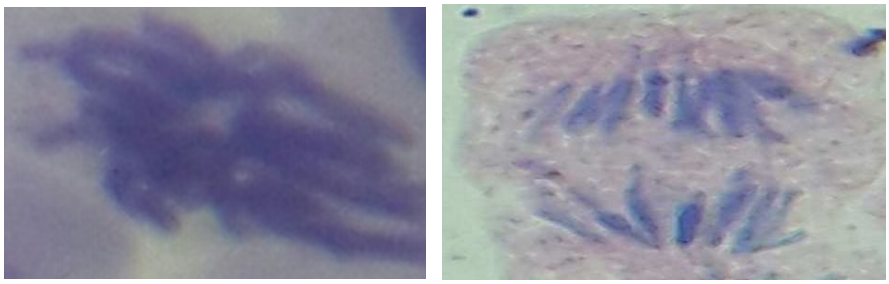
Slide preparation of onion root tips of the control showed normal mitotic division phases (Fig. 1) from interphase to telophase as expected.



A B C D E

Plate 1: Photomicrographs of normal mitotic phases in onion cells (Control), showing interphase (A), prophase (B), metaphase (C), anaphase (D), and telophase (E) stages

The various treatment concentrations of glyphosate induced various chromosomal aberrations and other forms of cell damage. For instance the treatment 0.75g/l of glyphosate caused irregular disjoining of the chromosomes at anaphase, resulting in lagging (Plate 2).



A B

Plate 2: Onion cell treated with 0.75g/l glyphosate showing lagging chromosomes at anaphase (A) compared to normal anaphase (B) in the control.

With the treatment 1.50g/l of glyphosate, disjoined telophases were observed (Plate 3). Other concentration treatments with glyphosate showed aberrations such as sticky chromosomes, C-mitosis and micro nuclei with 3.0g/l (Plate 4); damaged prophase and nuclear lesions with 6.0g/l (Plate 5) and also nuclear lesions were observed with 8.0g/l (Plate 6).

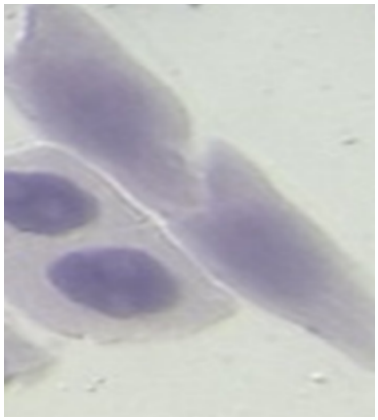
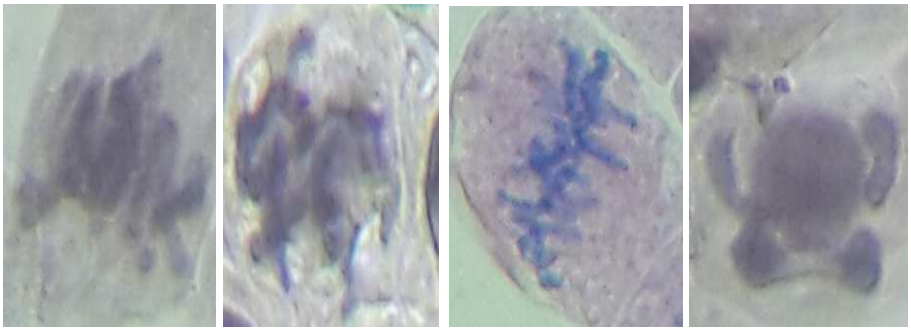
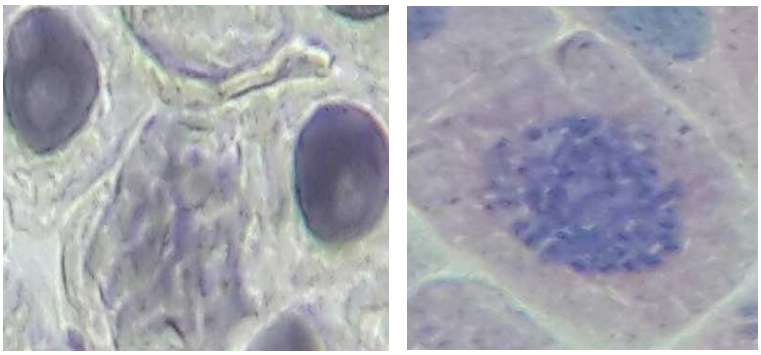


Plate 3: Abnormal telophase of onion cell treated with 1.5 g/L glyphosate



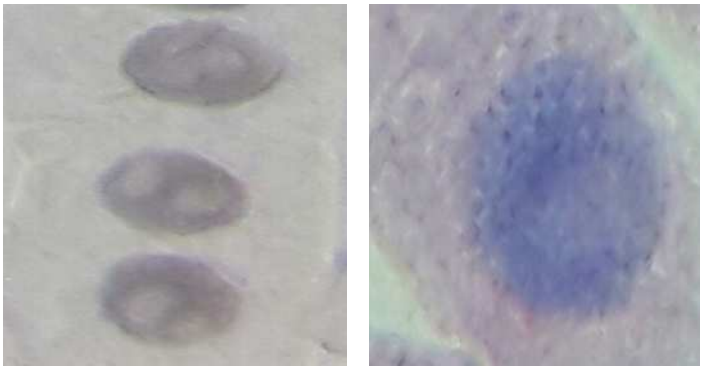
A **B** **C** **D**

Plate 4: Chromosomal abberations with 3.0g/l glyphosate showing, Sticky chromosomes (A), and C-mitosis (B) compared to normal metaphase (C); Micro nuclei (D)



A **B**

Plate 5: Damaged prophase and nuclear lesions of onion cells treated with 6.0 g/l of glyphosate (A) compared to normal interphase (B)

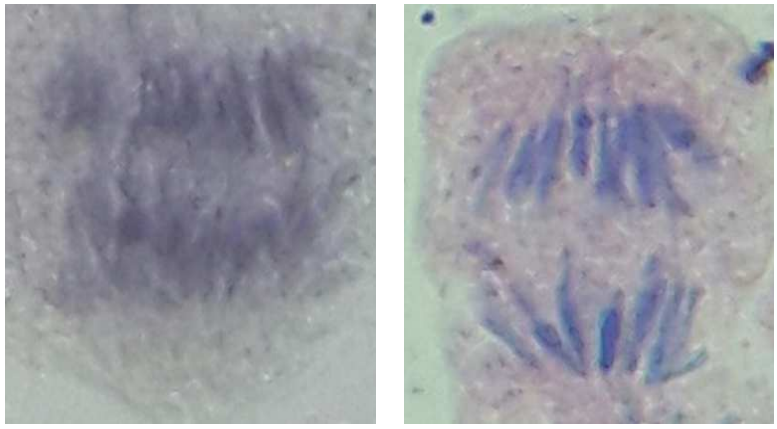


A **B**

Plate 6: Nuclear lesions in onion cells treated with 8.0 g/l of glyphosate, compared to normal interphase

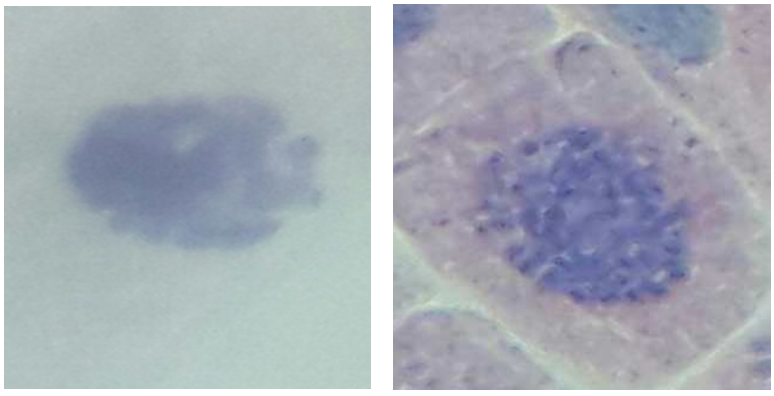
The varying concentration treatments of paraquat dichloride also induced various cytotoxic effects on the root meristem cells of *Allium cepa*. However, most of the cells in the slide preparations were damaged and unlike with the glyphosate treatments, very few clear mitotic stages were observed. Those observed include: Chromosome Bridge at anaphase (Plate 7), nuclear lesions (Plate 8), burnt cells (Plate 9), and binucleate cells (Plate10).

The degree of the effect of cytotoxicity was observed to be more with increase in the treatment concentrations of both pesticides, but more with paraquat dichloride. The higher concentrations caused burning of entire cells and their content.



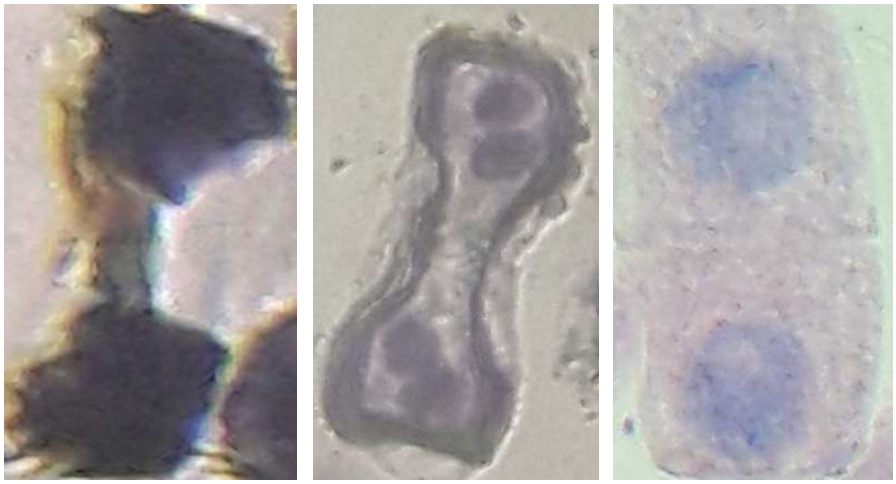
A **B**

Plate 7: Chromosome Bridge at anaphase (A) in onion cell treated with 0.43 g/l paraquat dichloride, compared to normal anaphase (B)



A **B**

Plate 8: Nuclear lesion in onion cell treated with 0.85 g/l paraquat dichloride (A) compared to the control (B)



A **B** **C**

Plate 9: Other cell damages induced by paraquate: (A) burnt cell at (1.7 g/l paraquat dichloride) and (B) binucleate onion cell (3.4g/l paraquate dichloride) compared to the control (C)

Discussion

The usefulness of the *Allium cepa* assay in assessing the cytotoxicity of glyphosate and paraquate dichloride has been well demonstrated in this study. Among the plant species, *Allium cepa* is considered to be a suitable test system for evaluation of the genotoxic potential of pesticides (Asita and Matebesi, 2010; Mustafa and Arikan, 2008 and Yuzbasioglu *et al*., 2009) among other things. This test uses a model that is adequately sensitive to detect innumerous substances that cause chromosomal alterations. Onion (*Allium cepa* L.) is an excellent *invivo*, where the root cells grow in direct contact with substance of interest. The mitotic phases are very clear in the onion and it has a stable chromosome number. There is diversity in the chromosome morphology and a stable karyotype; spontaneous chromosomal damages rarely occur. Furthermore, it gives clear and fast response to the toxic substances. (Firbas and Amon, 2013).

In this study, different chromosomal aberrations were seen at different mitotic stages as a result of the exposure of the onion cells to the two pesticides- glyphosate and paraquat dichloride, and they were seen in all the tested concentrations. The aberrations observed include: sticky metaphase chromosomes, Chromosome Bridge at anaphase, lagging chromosomes at anaphase, binucleate cells, nuclear lesions and damaged cells. Similar results were observed by Asita and Matebesi (2010), Faizah (2014) and Yekeen and Adeboye (2013) in Alium cepa root cells exposed to different pesticides.

According to Fiskesjo (1988) "results from the *Allium* test have shown good agreement with results from other test systems, eukaryotic as well as prokaryotic". The detected chromosomal aberrations may result from chromatin dysfunction (stickiness and bridge) or as a result of the effect of the pesticide on spindle appa-

ratus formation and thus resulted in cell division disturbances (Faizah, 2014). **Sticky chromosomes** are chromosomes that fail to condense completely at metaphase. Chromatin masses which are undistinguishable as chromosomes are seen as clumps in extreme cases. Cells having sticky chromosomes lack spindle fibres (Asita & Mokhobo, 2013). Stickiness may have resulted from increased chromosomal contraction and condensation or might result from the depolymerization of DNA and partial dissolution of nucleoproteins. Chromosome stickiness reflects toxic effects, usually of an irreversible type and probably leading to cell death (Khanna *et al.,* 2013; Turkoglu, 2007; Rencuzogullari *et al.,* 2001). **Chromosome bridges** result from chromosome and /or chromatid breakage and fusion or may be caused by stickiness of chromosomes which makes their separation and free movements incomplete; thus they remain connected by bridges (Ping et al*.,* 2012). **Micronuclei** can be spontaneously originated due to the development of the isolated chromosome that results from an unequal distribution of genetic material. However, their induction is commonly used to detect genetic damages derived from exposure to mutagenic chemicals. According to some authors, micronuclei can be formed as a result of acentric fragments or entire chromosomes not incorporated to the main nucleus during the cell cycle. Therefore, any substance that is able to promote micronuclei formation is said to be clastogenic or aneugenic (Meng and Zhang*.,* 1992). **Lagging chromosomes** are whole chromosomes that fail to move to either pole at anaphase. This effect arises from possible damage to the kinetochores. Turkoglu (2007) in his work on the genotoxicity of five food preservatives using *Allium cepa* assay reported that lagging chromosomes resulted due to failure of the chromosomes to get attached to the spindle fibre and to move to either of the two poles. **Binucleated cells** occur as a result of inhibition of the cytokinesis part of cell division. **C-mitosis is** exhibited by Mitotic cells that lack spindle fibres with unattached whole chromosomes lying scattered throughout the cell. The term c-mitosis was coined by Levan, and he said that colchicine prevents the assembly of the spindle fibres and results in scattering of the chromosomes over the cells (Levan, 1938).

In addition to the observed chromosomal aberrations, higher concentrations of both glyphosate and paraquat dichloride induced whole cell damage. This was more in paraquat dichloride as concentrations far below the field concentration induced whole cell damage. The concentrations of glyphosate and paraquat dichloride used in the field are 6.0g/L, and 3.4g/L respectively. These field concentrations which were part of the treatments used in this work, were harmful to the onions cells and could be harmful to animals and other plants that come in contact with them. Paraquat dichloride however was observed to be more toxic in its effect on cells than glyphosate.

In conclusion, the two pesticides; glyphosate and paraquat dichloride are both cytotoxic. The induction of stickiness, bridge formation, presence of micro nuclei, lagging chromosomes, binucleated cells, and C- mitosis indicates that glyphosate and paraquat dichloride can pose adverse effects on non-target organisms as well as the environment at large. Genotoxins induce genetic damage and the genetic activity of these chemicals result in abnormalities of the cell division process which is essential to growth and life. The following recommendations are therefore made:

- The use of these pesticides should be under control in agricultural fields since they could be harmful to non-target organisms that come in contact

with them**.** Individuals and organizations using these pesticides should adhere to safety measures by putting on their personal protective equipment (PPE) as this will prevent penetration of these pesticides into the body through oral, nasal and dermal routes.

- The use of alternatives to pesticides should be encouraged, some of which are: Methods of cultivation (cultivation practices), use of genetic engineering, methods of interfering with insect breeding and use of biological pest controls (such as pheromones and microbial pesticides). In addition, the environmental protection agency (EPA) register for reduced-risk conventional pesticides should be checked always.

Be that as it may, haven seen the cytotoxic effects of these pesticides (glyphosate and paraquat dichloride), recommendations should actually be that the use of these two chemicals be stopped completely. Nevertheless, because of the necessity of pesticides, an increased awareness and enlightenment of the public on the dangers associated with the use of these chemicals and the need for adherence to safety measures by putting on PPE while making use of them should be emphasized by the regulatory agencies saddled with this responsibility. Furthermore, if any of these two pesticides must be used glyphosate should be recommended as it is milder in toxicity. However, for resistant and stubborn weeds paraquat dichloride may be necessary; but great care must be taken in using it as it is more toxic.

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