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Evaluation of the Toxicity of Urea Using Allium cepa L. Assay

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Abstract
Considering the present abuse of fertilizer application today, an experiment was conducted to evaluate the toxic effects of urea on plants using the *Allium cepa* L. assay. Onion bulbs were first placed in water for 48 hours to initiate root growth, then they were placed in different concentrations of urea (0%, 0.005%, 0.01%, 0.025%, 0.05%) for 24 hours. Both macroscopic and microscopic parameters show the toxic effect of urea. Root length and root number dropped in value with increase in treatment concentration. Likewise, the actively dividing mitotic cells also reduced in number with treatment concentration, thus giving rise to a drop in the mitotic index and a corresponding increase in mitotic inhibition with increase in treatment concentration of urea. These results all gave rise to a visible drop in root mass of the onion bulbs.
Keywords: Urea, *Allium cepa* assay, toxicity, mitotic index
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Introduction

In recent years, due to rapid population growth and a continuous decline in the amount of cultivated land area, the rate of fertilizer application keeps on rising in order to obtain higher crop production in agriculture (Wang *et al.,* 2010). This is because the use of chemical fertilizers to increase yield has been found to be effective only within the first few years, after which the land becomes less productive, thus demanding consistent use on a long-term basis (Ojeniyi *et al*, 2009; Zhang *et al.,* 2009). Moreover, though the addition of nutrients found in fertilizer increases plant growth, too much of it can negatively affect their growth and overall health.

Large amounts of chemical fertilizers used during the peak season make both crop production quantity and quality to deteriorate. This is particularly the case with too much nitrogen.

Meanwhile, nitrogen is one of the major limiting elements that are essential for plant growth and development (Mostafa and Abo-Baker, 2010; Suriharn *et al*., 2011; Undie *et al*., 2012).

Of all the nitrogen fertilizers, Urea is the most widely used in the world because of its high solubility, high nitrogen content (46%), low cost and ease in handling; and it accounts for over 50% of all nitrogen applied (Gilbert, 2006).

Urea (N-source) added in soil undergoes N-transformations due to the presence of enzymes, microbes and soil microflora, resulting in the formation of ammonium/ammonia and finally nitrate through hydrolysis and nitrification, respectively (Kavita *et al.* 2013). The soil pH increases temporarily in areas were urea has been applied due to hydrolysis of the urea by urease enzymes (Clay *et al.* 1990), and thus, accumulation of NH3 produced induces NH3 toxicity. Ammonium and nitrate are known to be paradoxical ions; plants take up nitrogen in these two inorganic forms, but both can result in toxicity symptoms when used as an exclusive nitrogen source (Kavita *et al.* 2013).

Higher concentrations of ammonium inhibit seed germination and seedling germination. Another characteristic manifestation is the inhibition of primary root growth (Britto and Kronzucker 2002). Similarly, nitrate at higher concentrations represses lateral root development prior to activation of the lateral root meristem (Zhang and Forde 2000). Plant growth characters are controlled by cell division, which are affected by treatment with agrochemicals. However, the subtle danger of widespread use of such chemicals lies in the possibility of their causing mutation of somatic cells, resulting in the accumulation of heritable abnormal genes in the population or the formation of malignant cells in the individuals. The toxicants that act on deoxyribonucleic acid (DNA) cause damage to the genome, including alterations in the nucleic acids, and result in the modification or activation of a cell's genome; these toxicants are classified as "genotoxic". Genotoxic and cytotoxic effects of fertilizers on plants have been shown by Koca (2008) and Khaldi *et al*. (2012), respectively.

Generally, excessive amounts of chemical fertilizers are applied to vegetables for instance, in order to achieve a higher yield (Stewart *et al*., 2005). This is especially so with plants such as lettuce, spinach and *Telfaria* whose leaves are eaten and usually, there are harmful accumulation of nitrates and nitrites in these crops (Sönmez *et al*., 2007).

Farmers hardly use fertilizer based on the recommendation from authorities such as Soil Resource Development Institute (SRDI) and Department of Agriculture Extension (DAE). This is a general problem, and it might be due to lack of awareness, narrow access of farmers to soil testing facilities and/or inadequate motivation by extension people (Sultana *et al*., 2014).

In this study, the *Allium cepa* assay has been used to evaluate the cytotoxicity of Urea using mitotic index parameters and root growth. The *A. cepa* assay is an efficient test for in situ monitoring for toxicity of

environmental contaminants, and has been widely used to study genotoxicity and cytotoxicity of pesticides and fertilizers (Ma *et al*., 1994; Fernandes *et al.,* 2007; Kavita *et al.,* 2013). It is considered an excellent material for the assay of chromosomal aberration following chemical treatment. Its root meristem represents a normal proliferating plant cell population that is sensitive to changes in environmental conditions. The *A. cepa* assay provides a rapid procedure for screening chemicals which pose as environmental hazards.

Materials and methods

Dry and medium onion (*Allium cepa)* bulbs of about the same size, were locally obtained. The loose scaly part of the onion bulbs were carefully peeled off and the dead roots at their base carefully scraped away without destroying the root primordia.

Fifteen onion bulbs (between 110-120g) were then placed on plastic containers filled with distilled water so that only the base of the bulbs touch the distilled water for 48 hours to induce root growth, and also to determine the viability of the onions. The level of the water was maintained in each container so that the roots were always in contact with water. After 48 hours in water, the number of roots per onion bulb was recorded, and three roots from each onion bulb were colour-labelled to identify them, and their lengths recorded.

The cytotoxicity of urea was evaluated using *Allium cepa* assay. The different concentrations of urea in distilled water used were: 0%, 0.005%, 0.01%, 0.025% and 0.05%. The Control contained only distilled water.

After 48 hours in water, the onion bulbs were transferred into containers with the different treatments, each replicated three times. The set up was kept in the dark at room temperature. After 24 hours in the different treatments, the number of roots per onion bulb were recorded again, and the lengths of the labelled roots noted. A few roots were immediately harvested from each bulb with forceps from the base of the onion between 11am and 1pm (Ambrocio and Ian, 2011). The roots were fixed immediately in Carnoy's fluid (1 part of glacial acetic acid : 3 parts of ethanol) and placed in a refrigerator for 24 hours at 4^0C. The roots were then preserved in 70% ethanol in the refrigerator until use.

Slide Preparation

The onion roots were taken out of ethanol, rinsed and dried on filter paper.

The root tips were hydrolyzed in 1N hydrochloric acid in a water bath at 60^0C for 5 minutes to soften cell walls and make the root tip malleable. Then they were rinsed, dried and placed in a solution of 1% Ferric Chloride for one hour before slide preparation. Squashes were made in a drop of acetocarmine stain, and viewed under a compound light microscope. The mitotic study was carried out on temporary slides by observing the dividing cells and different types of division anomalies under low and high magnification. Photomicrographs were taken with a Samsung digital camera using an x40 objective.

The mitotic index for each treatment was calculated as follows:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells} \times 100}{\text{Total number of cells observed}}$$

Results

Macroscopic Parameters

The evaluation test on the effect of urea on *Allium* cells show that after 24 hours of the urea treatment, the Control had the highest values for root number (Table 1) and net root length (Table 2); and these values dropped with increase in Urea concentration.

Table 1: Onion root number before and after Urea treatment

Urea concentrations (%)	After 48hrs in water, Average Root number	After 24hrs in treatment, Average Root number	Net root number
Control	78.33±13.80	96.00±15.13	17.67
0.005	78.00 ± 4.00	88.33 ± 5.51	10.33
0.01	64.67 ± 8.08	73.00 ± 10.54	8.33
0.025	62.00 ± 14.18	69.00 ± 14.00	7.00
0.05	69.33 ± 10.26	73.00 ± 10.58	3.67

Table 2: Onion root length before and after Urea treatment

Urea concentrations (%)	After 48hrs in water, Average Root length (cm)	After 24hrs in treatment, Average Root length (cm)	Net increase in root length (cm)
Control	2.11± 0.05	3.05±0.68	0.94
0.005	2.47±0.07	3.04±0.02	0.57
0.01	2.59±0.03	2.99±0.02	0.40
0.025	2.87±0.12	3.21±0.07	0.34
0.05	2.77±0.06	2.98±0.09	0.21

Fig. 1 below shows the difference in root mass dropping drastically with treatment, even with the lowest treatment concentration. There appears however not to be as much difference in effect between treatment concentrations, except for the onion bulb in the highest concentration that is most affected and has very small root mass.

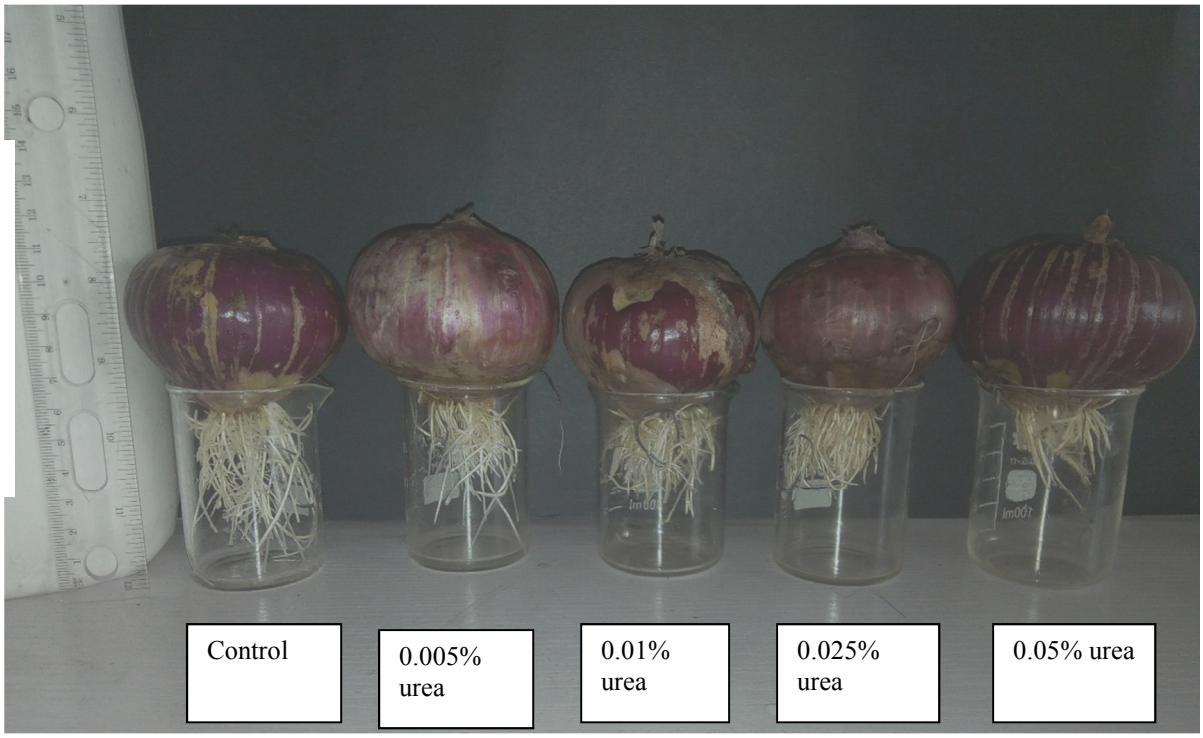

Fig. 1: Onion root growth response to treatment concentrations of urea

Microscopic Parameters

It was observed that the number of dividing cells per microscopic field decreased with increasing concentration of urea (Table 3). In addition, some abnormal mitotic stages were also observed in the treatment concentrations, especially in the highest (0.05%); they include: anaphase bridges, laggards, and aberrant metaphase and anaphase cells (Fig. 2).

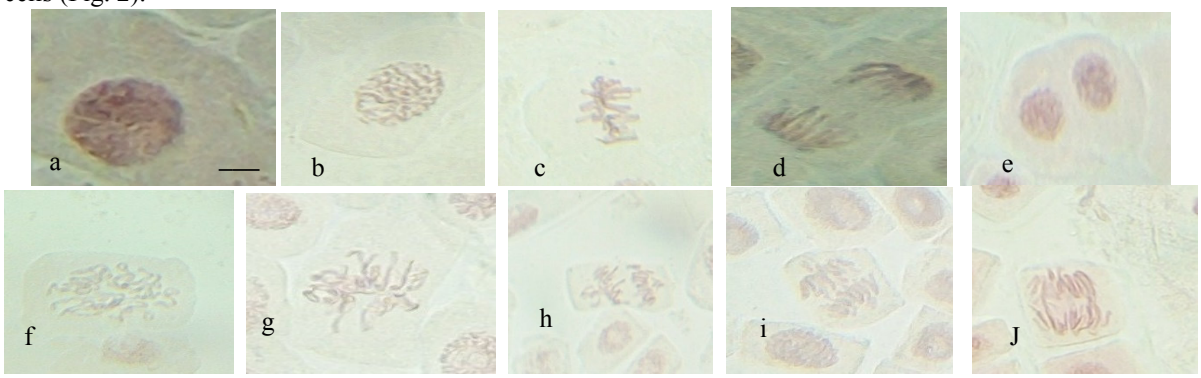

Figure 2: (a-e) normal stages of mitosis (a) interphase (b) prophase (c) metaphase (d) anaphase (e) telophase (f-J) aberrant mitotic stages (f) chromosome fragmentation at metaphase (g) scattered metaphase (h and i) precocious movement of chromosomes at anaphase (j) anaphase bridge (a-j, bar = 25µm)

Table 3: Mitotic indices and mitotic inhibition of onion root tips showing treatment effect

Treatment (% of urea)	Mitotic index (%)	Mitotic Inhibition (%)
Control	51.18 ± 0.56	-
0.005	36.31 ± 0.98	29.05
0.01	22.22 ± 0.72	56.58
0.025	15.17 ± 0.84	70.36
0.05	8.55 ± 0.63	83.29

The mitotic index shows a reduction in value with increase in treatment concentration (Table 3). Likewise, mitotic inhibition increased with treatment concentration.

Discussion

The toxicity of urea on plants, even at the relatively low concentrations used in this study (0.005% – 0.05%), was demonstrated using the *Allium cepa* assay. Urea affected negatively the root length and number with increasing concentration (Table 1and 2). Root lengths and root numbers decreased with increasing concentrations of urea that the onion bulbs were exposed to. The mitotic indices likewise dropped and mitotic inhibition increased with increase in treatment concentration (Table 3). The inhibition of primary root growth by inhibiting cell elongation and division are ammonium/ammonia toxicity symptoms as reported by Qin *et al*. (2011) in Arabidopsis. Similarly, work done by Kavita et al. (2013) indicate that the average root length and number of onion bulbs reduce with increasing concentrations of urea. The treatment concentrations of urea affected the number of roots of the onion bulbs they were exposed to significantly.

In addition to inhibiting seed germination and seedling germination, higher concentrations of ammonium inhibit primary root growth (Britto and Kronzucker 2002). Similarly, nitrate at higher concentrations represses lateral root development prior to activation of the lateral root meristem (Zhang and Forde 2000).

The meristematic regions of onion roots are very sensitive as it is the region of water absorption and mineral uptake. So, even at the relatively low concentrations of urea used in this study, the negative effect can be seen in the root mass development of the onion bulbs (Fig. 1). Therefore, it can be deduced that toxic substances, such as urea, coming in direct contact with dividing cells, can negatively affect the mitotic index of onion roots, and can have deleterious effects on plants, and may also affect the consumers of the affected plants, especially leaves. The most common chromosomal aberration observed with the treatments were irregular metaphase chromosomes, precocious movement of anaphase chromosomes and bridges (Fig. 2). Plant growth characters are controlled by cell division, which are affected by treatment with agrochemicals. However, the subtle danger of widespread use of such chemicals lies in the possibility of their causing mutation of somatic cells, resulting in the accumulation of heritable abnormal genes in the population or the formation of malignant cells in the individuals.

The fertilizer urea is a low cost, high N (46%) containing fertilizer. Urea hydrolyses to yield NH_4^+ and carbon dioxide. Uptake of NH_4^+ by plants releases H^+ which lowers the pH of the rhizosphere, leaving it acidic (Bindraban et al. 2015). Higher levels of NH_4^+ generate salinity stress as it reduces the uptake of other cations due to cation competition (Speer and Kaiser 1994; Britto and Kronzucker 2002; Britto and Kronzucker 2013). When NH_4^+ is supplied as prime N source, many plant species being sensitive, developed toxicity symptoms (Kronzucker et al. 2001; Lanquar et al. 2009; Rogato et al. 2010).

Plant injury has been traced to ammonia derived from organic sources such as urine, chicken manure and animal manure (Zhou *et al,* 2000). In addition, inorganic sources such as ammonium salts have been reported to be injurious to plants when placed too close to the root zone (Britto and Kronzucker 2002).

Conclusion and Recommendation

The toxicity of urea, even at relatively low concentrations, when in direct contact with the roots of onion, has been established in this study. The damage would definitely be much more at higher concentrations. With the indiscriminate application of fertilizers that is common today, more so, at peak seasons for different crops, especially leafy vegetables such as Telfaria, abuse is imminent. This is because nitrogen is a major nutrient requirement for the production of leafy vegetables. When there is a high nitrogen supply in leafy vegetable crops, nitrogen mobile form concentrations (i.e., nitrate, ammonium) increase in leaves, thus becoming hazardous to human health. A high-nitrate diet is an important factor in the development of several human diseases such as methahemoglobinaemia, and gastric and bladder cancer (Parks *et al.,* 2012).

Plants grown with high levels of Nitrogen fertilizer consist of carcinogenic substances such as nitrosamines, especially plants such as lettuce, spinach and *Telfaria occidentalis* whose leaves are eaten, and usually there are harmful accumulation of nitrates and nitrites in these crops (Sönmez *et al*., 2007).

It is recommended that farmers seek alternative long term methods of increasing soil nutrient content for plant growth as chemical fertilizers are detrimental to soils, plants, the environment and humans. Moreover, application of organic manure significantly impacts the physical, biological and chemical properties of the soils better, and

this is mostly due to increased soil organic matter content from manure application. Organic manure provide the major plant nutrients and many of the required secondary nutrients as well.

Soil testing, which is a valuable tool for determining the fertilizer inputs required for efficient and economic production, should be carried out.

Where the use of inorganic fertilizers (for example urea) to improve crop yield must be used, direct contact with the plant roots should be avoided; and it should be controlled or supervised by agricultural extension workers.

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