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Cytotoxic effect of urea (Fertilizer) on onion root tip cells

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Abstract
These days demanding of Urea increases due to presence of inorganic element Nitrogen and also beneficial for plant growth, but long term uses of Urea becoming hazardous for soil, water and also health of aquatic animal, human being. Since it affect fertility of soil and cause to damage the organ of fish as well as cytotoxic effect. The present study was undertaken to evaluate its cytotoxic effect on onion root tip cells. A 2.5 g/l concentration of Urea were treated for two duration 72hrs. and 120 hrs.in onion root tip cells and control group was taken as no treatment. There is no significant difference in mitotic index at 72 hrs. and significant difference was observed in mitotic index at 120 hrs. Various type of chromosomal abnormalities were also observed including Multipolar spindle, Laggard, Anaphase bridge etc.

Keywords: Urea, cytotoxicity, mitotic index, chromosomal abnormalities

Introduction

In recent years, rapid growth of population and continuous decline in the amount of cultivated land and order to obtain higher crop production in agriculture to fulfill need of growing population farmer have use fertilizers. (Wang *et al*., 2010), [11]. Fertilizers is of two type – (i) natural (ii) synthetize. Synthetize fertilizer are generally in chemical nature. To increase yield of crop farmer were use chemical fertilizers. It has been found to be effectively only within the first years, often productivity is less on cultivated land, thus demanding consistent use on a long term basis (Ojeniyi *et al*,2009, Zhang *et al*., 2009) [6, 12]. To increase the growth of plants, we use additional nutrient, found in fertilizer. Nitrogen is one of the most limited elements that are essential for plant growth and development (Mostafa and Abo-Baker *et al*., 2010, Suriharn *et al*., 201, Undie *et al*.,2012) [5, 8, 10]. Urea fertilizers are most widely used in world because of its high solubility, high nitrogen content (46%), low cost and ease in handling and its account for over 50% of all nitrogen applied (Gilbert *et al*., 2006) [4].In order to yield higher production application chemicals fertilizers are done in excess amount (Stewart *et al*, 2005) Its application increase during peak season to yield high quality and quantity of crop, but regular and long term uses may cause deteriorate the quality of soil by acidifying (Clay *et al*., 1990) [2] There are harmful accumulation of nitrates and nitrites in plants such as lettuce, spinach and Telfaria whose leaves are eaten usually (Sonmez *et al*., 2014) Hydrolysis of urea by enzyme urease form ammonia ion,NH_4^+ and with help of microbe it further convert into NH_3, NO_2,NO_3 by of volatilization, nitrification, denitrification process (Svensen and Singh 1997; Silva *et al*. 2005; Di and Cameron 2008, Glibert *et al*., 2014) [3, 7, 9]. Specially paradoxical ions, Ammonium and nitrate two organic form use by soil may result toxicity symptom (Kavita *et al*., 2013). Symptom may inhibition seed germination and seedling germination by higher concentration of ammonium, may also characteristics manifestation in inhibition of primary root growth (Britto & Kronzucker *et al*., 2000). Similarly repress of lateral root development and activation of the lateral root meristem by high concentration of nitrate (Zhang and Forde *et al*., 2009) [12]. However, these type of chemical may cause possibilities mutation of somatic cells, which may lead to result of accumulation of abnormal genes in population by heriting in next generation or may occurrence of malignant cells in individuals.

Damaging of gene including alteration in nucleic acid and deoxyribonucleic (DNA by toxicants, result in activation of cell´s genome; thesetype of toxicity classified as "genotoxic". Genotoxic and cytotoxic effects of fertilizer on plants have been shown by (Koca *et al*., 2008) and (Khaldi *et al*., 2007) respectively.

In this study, the *Allium cepa* (onion) assay has been used to evaluate the cytotoxicity of urea using mitotic index parameters and root growth.

Material and method

Urea solution was prepared using 2.5 gm crystalled urea which was dissolve in 1 l of tap water. 5 bulb of onion (average wt. 40-45g) were taken. After growing 1 to 2 cm long roots, onion bulb were transferred into coupling jar with 2.5% treatment of urea. The set up was kept at room temperature for 72 hours and 120 hours. The control was also maintained with no treatment, contained only tap water. After completing given two hours, root were cut, washed, fixed in (1:3) Acetoalcohol for 24 hours and preserved in 70% alcohol for 24 hours. Acetacarmine stain squash preparations were made. About 2000 cells studied randomly. The data were analyzed and interpreted by calculating the Mitotic Index (TABLE – 1) of both control and treated group, statistically analyzed by following formula given below:-

Mitotic Index (MI) = Mitotic index =

$$\frac{\text{Number of dividing cells}}{\text{Total number of counted cells}}$$

Result

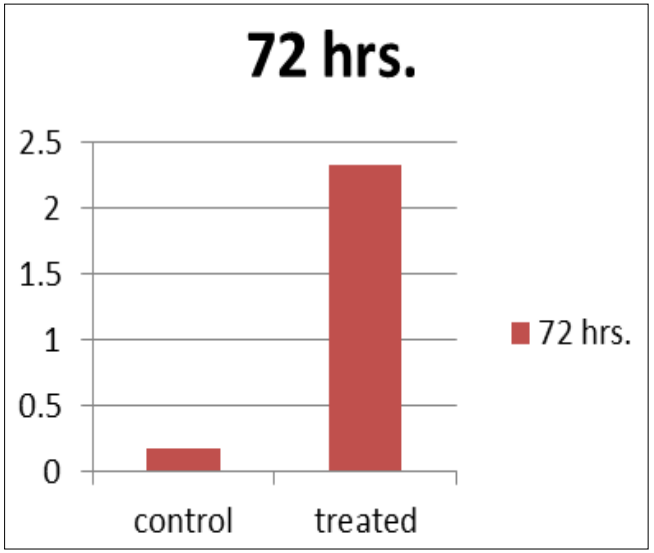
Statistical analysis

The data are expressed as Mean ± SE and statistical analysis was performed by using t- test.

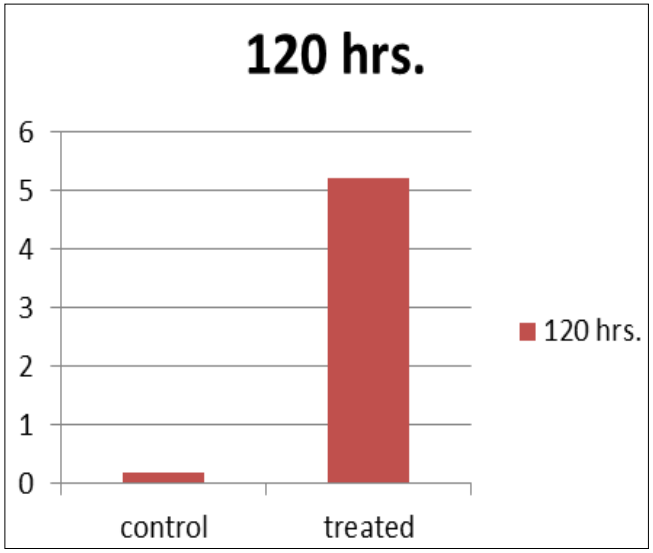
Table 1: Effect of urea (2.5%) on mitotic index in onion root- tip cells at 72 hrs. and 120 hrs**.**

Exp. variant	Total No. of screene-d cell	Total No. of dividin-g cell	Mitotic Index (Total)	Phase Distribution								Abnormalities									
				Prophase		Metaphase		Anaphase		Telophase		Multipolar		Laggard		Anaphase Bridge		Polyplo-idy		Total No. of Abnorm-alities	
			% ±S.E	No.	% ± S.E	No.	% ± S.E	No.	% ± S.E	No	% ± S.E	No.	% ± S.E	No.	% ± S.E	No.	% ± S.E	No	% ± S.E	No.	% ± S.E
control	2163	329	15.21 ± 0.77	168	7.7 ± 1.47	74	3.47 ± 0.9	50	2.3 ± 0.83	33	1.52 ± 0.67	4	0.18 ± 0.23	0	0	0	0	0	0	4	0.18 ± 0.23
Treated 72 hrs.	2823	385	13.63 ±1.38	223	7.89 ± 1.3	14	0.49 ± 0.33	49	1.73 ± 0.66	33	1.16 ± 0.53	48	1.70 ± 0.65	5	0.17 ± 0.62	8	0.28 ± 0.26	5	0.17± 0.2	66	2.33 ± 0.89
Treated 120 hrs.	3668	582	15.87 ±0.60	323	8.80 ± 1.1	23	0.62 ± 0.31	14	0.38 ± 0.24	31	0.84 ± 0.37	120	3.27 ± 0.73	25	68 ± 0.3	34	0.92 ± 0.40	12	0.32± 0.22	191	5.20 ± 0.36

For Mitotic Index	Total	For Abnormalities	Total
t- Difference between control and treated 72 hrs.	1.58	t- Difference between control and treated 72 hrs.	2.36
t- Difference between control and treated 120 hrs.	0.67	t- Difference between control and treated 120 hrs.	11.95



Graph (A)



Graph (B)

Fig 1: Histogram showing mitotic index in Onion Root Tips Cells at 96 Hrs. and 120Hrs. in control and Urea (2.5%) Treated group.

Discussion

There is no significant difference between control and treated group of urea at 72 and 120 hours treatment in mitotic index of onion root tip cells. (TABLE-1)

It indicates that there is no effect of urea at 2.5% concentration on onion root tip cells. But a significant difference were found in total abnormalities of chromosome in treated group of 120 hours (5.20± 0.36) than control (0.18± 0.23).The total abnormalities in 72 hours treated group (2.33±0.89) were not significant than control (0.18±0.23).

It showed that 2.5% concentration of urea caused abnormalities in onion root tip cells at higher duration (120 hours).The maximum chromosomal abnormalities were abserved at 120 hours treatment of urea than 72 hours.

This result found to be duration dependent because 72 hours duration causes no significant increase in chromosomal abnormalities than 120 hours duration.

Therefore, it can be concluded that long term treatment duration of urea cause cytoxic effect on chromosome of onion root tip cells.

Conclusion

Therefore it can be concluded that long term use of Urea is mitotic inhibitor since it causes several abnormalities in dividing cell. Lack of awareness, narrow access of farmers to soil testing facilities and inadequate motivation by extension people, this become general problem (Sultana *et al*., 2014).

Therefore people must be aware toward long term uses of fertilizers and should use alternative product, which may be eco-friendly.

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