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Research paper

Genotoxic Effects of Cobalt Chloride on Mitotic Index of *Allium cepa* L.

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ABSTRACT

Root meristem cells are highly sensitive to genetic damage by chemicals; hence, present work is undertaken to assess the effect of different concentrations of Cobalt chloride on mitotic index of Allium cepa L. Experiments were conducted by using onion bulbs. These bulbs are planted in paper cups containing sand and soil mixture (2:1 ratio) and plants were treated with different molarity (M) of Cobalt chloride. The roots were macerated to analyze the mitotic index and chromosomal abnormalities. The mitotic index was 11.55%, 11.33% and 10.988% for the treatment with 0.5, 1.0, and 1.5M $CoCl_2$ respectively and it was least (9.1%) with 2.5M of $CoCl_2$. Maximum interphase cells (409) were observed with 2.5M, compared with the control and other treatments (0.5M: 398 cells, 1.0M: 399 cells and 1.5M 401 cells). The plants treated with 1.5M and 2.0M $CoCl_2$ have the highest number of metaphase cells (16) and less number of cells with anaphase observed in plant roots treated 2.5M CoCl2 (2 cells) followed by 0.5M and 2.0M $CoCl_2$. The total chromosomal abnormalities in the root tips treated with 2.5M $CoCl_2$ are 21(51.2%) was observed. Micronucleus of budding nucleus have an approximate half percentage 9 (21.9) whereas C-mitosis and chromosome stainless have the same proportion 3(7.3). It was noted that, the unequal sized 5 nuclei (12.1%), the anaphase bridge has the lowest percentage 1(2.04%) on the other hand 2.5M CoCl2 conc., has only 1(1.91) of C-mitosis and chromosome stainless. The study highlights that, the higher concentration of heavy metals have negative effect on the mitotic index of root meristematic cells.

1. Introduction

Heavy metals (HM) toxicity becomes global problem and has toxic impact on plants, animals and microorganisms. Heavy metals will remain in the soil for a longer period and they categorized as genotoxic agents. The higher levels of heavy metals in plants suppress the metabolism and translocation of reserve food materials to the growing regions and their subsequent utilization (Abubacker and Sathya, 2017). Heavy metals are the cofactors of several enzymes and they involved in several plant metabolism. Their toxicity for plants is expressed in many ways for example they are responsible to cause chlorosis, necrosis, and changes in plant morphological as well as physiological characteristics. The accumulation of heavy metals cause damages, alterations to the genetic material occurring over a cell cycle (Shahaby *et al*., 2003). The Allium test that involves the length of the root and chromosome aberration measurements proves to be an effective model system for measuring the environmental cytogenetic potential of pollutants (Leme, *et al*., 2009). The *Allium* test is an excellent indicator for analyzing anti proliferative effects, on plant medicinal extracts (Firbas and Amon, 2014). *Allium* chromosomes have been studied for

their diversity in size, structure and number (Awe and Akpan, 2017). Hence, present research was undertaken to assess the effect of Cobalt toxicity on mitotic index of the *Allium cepa* L.

Allium cepa L. belongs to the family Alliaceace which is one of the most important monocotyledonous vegetable crops. Allium plants are known as source of many different cobalt chloride compounds, which have similar metabolic pathway with phytochlorines and cystene as a basic precursor of phytochelatis. *Allium* species serves as food and spice crops, the knowledge on their heavy metal uptake and distribution as well as evaluation of the potential risks in the food chains should be a major concentration.

2. Materials and Methods

2.1 Onion Preparation

Small onion (*Allium cepa*) bulbs of the uniform size, weighing about 10-13g, were denuded and scarped by removing the loose outer scales so that root primordia are immersed in the specific distilled water. Onion soaked for before starting the experiments. They are allowed to sprout and produce roots, and then they are treated with different concentrations of $CoCl_2$ solutions 0.5M, 1.0M, 1.5M, 2.0M and 2.5 molarity (M) and distilled water.

2.2 Cytological study

Fifteen roots in each group were cut and fixed in 95% ethanol and 98% acetic acid (3.2) for 1hour and hydrolyzed in 1M hydrochloric acid, 95% ethanol and 99.8% acetic acid (5.3) for 5 min at 60^{o} C at the end of each time internal (24 hour). For the observation of changes in cell division, chromosome dissolves micronucleus formation, 10 root tips were squashed in cobalt fusion solution. Mitotic index and frequency of cells with chromosome disorder were used for the investigation. With this purpose the number of dividing cells and the nucleus of the different disorder per 1000 observed cells were determined.
Total chromosomal abnormality calculated by the following formula;

$$Total\ abnormality\ (\%) = \frac{Total\ number\ of\ aberrant\ cells}{Total\ number\ of\ cells\ of\ division}$$

2.3 Mitotic index

Mitotic index is a major of cellular proliferation. The percentage of cells undergoing mitosis in a given population of cells. Durations of the cell cycle and mitosis vary in different cell types an elevated mitotic index indicates more cells or dividing.

$$Mitotic\ Index = \frac{(P + M + A + T)}{N} \times 100$$

3. Results and Discussion

The effect of cobalt chloride on the root growth of *Allium cepa* L., varies with different concentration of $CoCl_2$ solution used (0.5M, 1.0M, 1.5M, 2.0M and 2.5M). The higher concentration of cobalt chloride obliviously decreased the root growth with increasing concentration and duration of the treatment.

The root growth rate is different for each treated concentration and control. Five replications were performed for each concentration and control to calculate mean root lengths with standard errors of the mean at selected time periods. The growth rate of root tips of onion treated with distilled water (control) is 40.63% compared to other plants treated with different cobalt chloride concentration (0.5, 1.5, 2.0, and 2.5M). After 72hrs the growth rate of root tips of onion treated with $CoCl_2$ conc. of 0.5, 1.0, 1.5, 2.0 and 2.5M were 40.63%, 35.86%, 21.05%, 15.15% and 12.9% respectively. The effect of dilute $CoCl_2$ conc., on the mitotic index of the root tips and the total cells examined classified into interphase and dividing cells (prophase, metaphase, anaphase and telophase).

The mitotic index of plants treated with different molarity of $CoCl_2$ showed significant differences with control plant root tips. The percentage of mitotic index in the control group (12%) was higher than that of the plants treated with different concentrations of cobalt chloride i.e., 0.1, 1.0, 2.0 and 2.5M. The lesser mitotic index (9.1) was observed in the plants treated with 2.5M of $CoCl_2$. The percentage of mitotic index in 0.5, 1.0 and 1.5M $CoCl_2$ conc., is 11.55%, 11.33% and 10.988% respectively. There was a significant difference between Interphase and dividing cell (prophase, anaphase, telophase and metaphase). Interphase has the highest number (409) cells in the plants treated with 2.5M while the control has the lesser number of interphase cells. It was almost similar with the other treatments (0.5M has 398 cells; 1.0M has 399 cells and 1.5M 401 cells).

3.1 Observation of cells respective phase

Among dividing cells, prophase was observed in more number of tips compared to metaphase, anaphase and telophase. The root tips grown with 0.5M $CoCl_2$ concentration has the maximum number of prophase (33 cells) and with 1.5M $CoCl_2$ concentration has the less number of cells with prophase (24 cells). The plants treated with 1.5 and 2.0M $CoCl_2$ has the highest number of metaphase cells (16) followed by control group (14 cells). The less number of cells with anaphase was observed with 2.5M $CoCl_2$ conc. (2 cells) followed by 0.5M (5 cells), 2.0M $CoCl_2$ concentration and control group has the same number (8 cells). The more number of anaphase was observed in plant roots treated with 1.0M $CoCl_2$ concentration (9 cells). At 2.5M $CoCl_2$ concentration

least number of cells with telophase (3 cells) was observed followed by 2.0M $CoCl_2$ concentration (4 cells), control group (5 cells) and 0.5M $CoCl_2$ (6 cells) but the comparatively more number of cells with telophase was observed with 1.5M $CoCl_2$ (7 cells).

Various chromosomal abnormalities induced by different concentration of $CoCl_2$ in meristematic cells of *Allium cepa* L., root tips were studied. It was observed that no chromosomal abnormalities with control plants treated with distilled water (0%). The total chromosomal abnormalities in the root tips treated with 2.5M $CoCl_2$ are 21(51.2%). Micronucleus of budding nucleus have an approximate half percentage 9 (21.9) whereas C-mitosis and chromosome stainless have the same proportion 3(7.3). It was observed that, unequal sized 5 nuclei (12.1%), the anaphase bridge has the lowest percentage 1(2.04%) on the other hand 2.5M $CoCl_2$ conc. has only 1(1.91) of C-mitosis and chromosome stainless and these abnormalities for 0.5M $CoCl_2$ were 2(3.8%) observed.

Micronucleus, budding nucleus, unequal sized nucleus and C-mitotic have the lowest percentage (1.9%) in the plants treated with 1.0M $CoCl_2$ concentration followed by anaphase bridge 2 (3.9%), but it was observed that, the chromosome stickiness has the highest percentage 3(5.8%) at the same concentrations. The total chromosomal abnormalities were 8 (15.6%) in the dividing cells of root meristematic cells with 2.0M $CoCl_2$ concentration. The unequal sized nucleus and anaphase bridge has the similar percentage 1(2.04%) likewise C-mitosis and chromosome stains have the same number 2(4.08%) in the root tips of the plants treated with 2.0M $CoCl_2$ concentration. The micronucleus and budding nucleus have the highest percentage 4(8.1%); total abnormalities for 2.5M $CoCl_2$ concentration were reported.

3.2 Micronucleus Formation

It was seen that 24 hrs exposure to 2.5M $CoCl_2$ significantly induced micronucleus (MN) formation composed with controls significant increase in quality of MN was observed in root tip cells of *Allium cepa* L., after exposure to cobalt. Micronucleus formation was considered to be the consequences of genotoxic events.

Normally the cells of *Allium cepa* L., contains one nucleus in toxic effect of $CoCl_2$ on micronucleus (MN) varied with treatment time in the presence of $CoCl_2$ for 24hr, nucleus consisting of nuclear material ,with bud shaped excrescences on the main exclass, protected from the nucleus, bud without an obvious constriction or bridge between the protecting nuclear material and nucleus. Gradually nuclear buds (NBUD) which appeared like a MN (micronucleus)-contained a narrow nuclear classic construction to the main nucleus or a reliably is the main nucleus. Then the nucleuses were well repeated from the main nucleus .the quantity of micronucleus induced increase with a prolonging the treatment.

Table 1 Effect of different concentrations of Cobalt solution on root length of *Allium cepa* at different time intervals

Concentration	Mitotic root length			Change rate at 72 hrs
	24 hrs	48 hrs	72 hrs	
0M	1.28±0.08	1.42±0.1	1.8±0.8	40.36%
0.5M	0.92±0.03	1.05±0.5	1.25±0.5	35.86%
1.0M	0.95±0.05	0.96±0.01	1.15±0.05	21.05%
1.5M	0.82±0.04	0.85±0.07	0.95±0.05	15.15%
2.0M	0.62±0.03	0.5±0.06	0.7±0.04	12.9%
2.5M	0.40±0.02	0.3±0.05	0.5±0.03	09.8%

Table 2 Effect of different concentration of $CoCl_2$ on the mitotic index of examined root tip cells of *Allium cepa*

$CoCl_2$ conc.	Total cell examined	Number of cells at Interphase	Number of cells at Prophase	Number of cells at Metaphase	Number of cells at Anaphase	Number of cells at Telophase	Mitotic index
0M	200	150	27	14	8	5	12%
0.5M	200	162	24	16	7	7	11.5%
1.0M	200	178	31	4	9	8	11.3%
1.5M	200	180	33	8	8	4	10.8%
2.0M	200	187	29	7	6	6	9.11%
2.5M	200	190	30	8	2	3	8.10%

Table 3 Chromosome abnormalities induced by $CoCl_2$ in *Allium cepa*

$CoCl_2$ conc.	Mitosis & budding nucleus	Unequal Sized nucleus	C-mitosis	Anaphase Bridge	Chromosome stickiness	Total abnormalities
0M	0(0)%	0(0)%	0(0)%	0(0)%	0(0)%	0(0)%
0.5M	0(0)%	0(0)%	1(1.9)%	0(0)%	1(1.9)%	2(3.8)%
1.0M	1(1.9)%	1(1.9)%	1(1.9)%	2(3.9)%	3(.8)%	8(15.6)%

1.5M	4(8.0)%	1(2.04)%	2(4.08)%	1(2.04)%	2(4.08)%	11(22.4)%
2.0M	9(21.9)%	5(12.1)%	3(7.3)%	1(2.4)%	3(4.09)%	12(2.4)%
2.5M	11(2.8)%	6(11.8)%	4(8.0)%	1(2.8)%	4(6.2)%	21(51.2)%

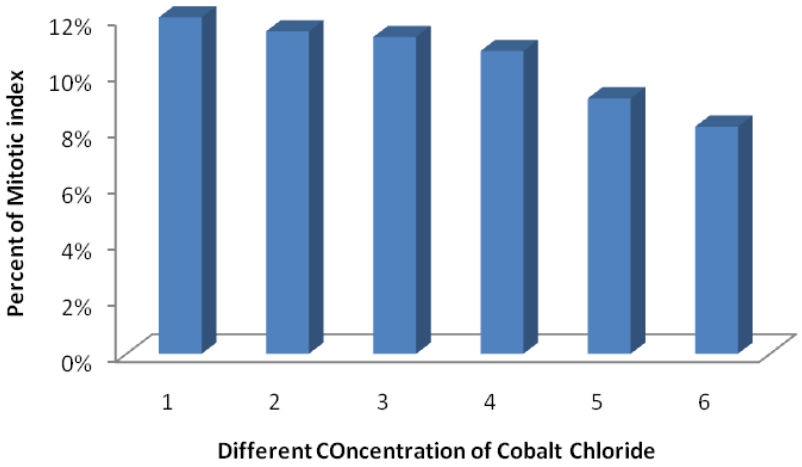

Fig. 1 Effect of different concentrations of cobalt chloride on mitotic index

In the present experiment roots of *Allium cepa* are treated with different concentrations of $CoCl_2$ and distilled water as control for selected time periods (24, 48, 72hrs). The degree to which one mechanism affects the plants over the other depends on a number of factors, including the species, genotype, plant age, ionic strength and composition of the salinitizing solution and the organ concerned (Munns, 2002).

Significant increase in the micronuclei formation was noticed in root tips of *Allium cepa* L., exposed to different cobalt chloride solution (Table1). Cobalt chloride also induced irregularly-shaped micronuclei as a genotoxicity. The abnormal chromosomal structures such as nuclear buds, found in *Allium cepa* as per the earlier researchers Fernandes *et al*., (2007); Leme and Marin Morales, (2009) and Hajmoradi and Kakei (2021). It was observed by Pohren *et al*., (2013) in their study the reduction in root length of *Allium cepa* L., with increasing heavy metal concentration. This effect can be related to its ability to promote alternations in the root cells of *Allium cepa* such as cells bearing C-mitosis, anaphase bridges, chromosome stickiness and micronuclei. This is in agreement with the early findings of Liu *et al*., (2015) and Zhang *et al*., (2011) Shahaby et *al*., (2003). The present experimental results were similar with the studies conducted by the Zhang *et al*., (2011). The percentage of mitotic index in the control group (12%) is higher than other treated groups (0.5, 1.0, 1.5, 2.0, and 2.5M), lower mitotic index (9.11%) was observed with 2.5M of $CoCl_2$. Among dividing cells prophase has a higher number compared to metaphase, anaphase and telophase. It is determined as the doping testing has biochemical processes (Lopez- Millian, 2009). In this investigation reduction in root length of *Allium cepa* was observed with increasing cobalt concentration.

The mechanisms responsible for micronuclei formation have not been yet fully understood as Fenech (2011), proposed that micronuclei can be result of DNA double strand breaks. This led to symmetrical and asymmetrical chromatid and chromosome exchanges, or fragments that failed to be included in the daughter nuclei at the completion of telophase during mitosis. This was because the lack of spindle fibers attachment during the segregation process in anaphase. Other authors that support such suggestion include the elimination of genetic material from micronuclei as mini cells and the presence of highly compacted genetic material within micronuclei (Fernandess *et al*., 2007). It was assumed that such micronuclei would be the result of an event of elimination of the exceeding genetic material. It is also assumed that there was relevance between early apoptosis events and formation of micronuclei after treatment with cobalt chloride from the present research, if a cell under goes a process of genetic material amplification and such exceeding material is expulsed through micronuclei is possible that the cells reestablish their viability, since their nuclear content would be normalized. Nevertheless if the micronucleus is composed of chromosomal losses or chromosomal fragments, depending on the nature of the material lost, it can lead to a cell's death process (Fernandez *et al*., 2007). The nuclear buds and the process of micronuclei formation is an initial process of elimination or they could dead to cell's death. Nevertheless, we cannot do any affirmation however it is possible to establish that the high number of cells bearing micronuclei can be an indicator of maintenance of cells physiology after exposure to $CoCl_2$. The results obtained from root growth and cytological analysis of *Allium cepa* suggested that different concentration of $CoCl_2$ showed inhibitory

effect on root growth, decreases cell division. The presented data can be a good base for the future phytoremediation purposes or for bio-fortification of crop plants by essential micronutrients which are required for human health.

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