

Effects of Bayluscide on Mitosis in Root Tip Cells of *Allium cepa* L.

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ABSTRACT

A common practice among farmers is to spray pesticide on crops in order to protect them from herbivores to have an abundant production of crops. The purpose of this study was to find out the effects of Bayluscide on cell division in root tip cells of onions (*Allium cepa*). The study uses the Randomized Complete Block Design (RCBD), consisting of one control group and four experimental groups that were replicated thrice. Fifteen healthy onion bulb roots were used and subjected to 0%, 2%, 6%, 10% and 14% concentration of Bayluscide and distilled water. Excised root tips were prepared for slides, and cytological changes were examined under the microscope. The cells counted and observed were subjected to Analysis of Variance (ANOVA), Duncan Multiple Range Test and Mitotic Index. As reflected in the result, there was a significant decrease in cells undergoing cell division as the treatment concentration increases. Cells at the interphase stage increased in number as the concentration increases during the prophase, metaphase, anaphase, and telophase showed a marked decrease in a number. Cytological abnormalities noted were shrunken and a broken nucleus.

KEYWORDS

Biology, Bayluscide, Randomized Complete Block Design (RCBD), Tagbilaran City, Philippines

INTRODUCTION

Pesticides are used all over the world. In recent years, their applications have increased enormously because these have significantly enhanced agricultural yield through inhibition of disease-causing organisms and by making action against pests in the field and during storage of agricultural products (Asita & Makhalemele, 2009). A study conducted across Europe in its western and eastern part of the continent, findings showed that insecticide and fungicide usability had a consistent adverse effect on biodiversity in its potential for biological control. Thus, minimal use of pesticides is strongly recommended (Geiger, et al., 2010). Plants are most affected by pesticides whether they are subjected from a direct application such as uptake from soil and water or atmospheric drift. Yet, there is less explanation about the cell-cycle even if there are lots of studies at the molecular level on the dynamics that lead to the progression through the phases of cell cycle (De Veylder, Beeckman, & Inzé, 2007).

Allium cepa is considered a very good plant and a useful biomarker for environmental monitoring (Qin et al., 2010). It is an efficient test for chemical screening and in monitoring for genotoxicity of environmental contaminants (Feretti et al., 2007). Such study further added that the test has been used extensively to report genotoxicity of many pesticides showing compounds that can cause chromosomal aberrations in root tip cells of *Allium cepa*.

The Allium test, which was first introduced by Levan in 1938, is a short-term test with many advantages: low cost, ease of handling good chromosome conditions for the study of chromosome damage or disturbance of cell division (Fiskesjo, 1985). The standard procedure of treating the series of onions for each concentration of the verified chemical permits statistical considerations and sensitivity showing good correlation with other tests. Positive results indicates in the Allium test should be taken as a caution and a possible indication upon which the tested chemical may be possibly risking human health and our environment (Fiskesjo, 1985).

The determination of cytotoxicity is done by calculating the indices of mitosis in comparison to the indices of mitosis of the treated cells per dose of each pesticide with that of the group of negative control (Asita, et al., 2009). The mitotic index (MI) is characterized by the total number of dividing cell in a possible cell cycle has been utilized as a parameter to evaluate the cytotoxicity of chemical agents and at the same time will also be used as a sensitive test to estimate pollution levels (Leme, & Marin-Morales, 2009). Leme et al., (2009)

further stated that the cytotoxicity levels of an agent can be determined by the increase or decrease in the mi. MIs, which is lower than the negative control, can indicate alterations came out from the exposed organism's chemical action in the growth and development of exposed organisms. MI's increasing than the negative control are results of an increase in cell division which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues (Leme, et al., 2009). A possible dose of a test substance was adjudged to be cytotoxic if the mitotic index of treated cells at that concentration is half or less, compared to the mitotic index of the concurrent water treated cells (Asita et al. 2009).

The observed changes agrees with the study done by Abu et al. in 2006 which states that the inhibition of mitotic index and reduction in mitotic phase percentages indicated that the treatments done impeded with the natural sequence of cell division, thus, obstructing the amount of cells going through the prophase stage at high level of concentration. A lowered cell division index suggests an inhibitory effect at the interphase stage reported that the mitotic rate was closely related to the resultant level of ATP (Abu et al., 2006). In the study of Lamsal et al., 2010 it was found out that the chromosomal abnormality percentage in various stages of mitosis was significantly higher in all treatment periods and concentrations and the type of defects produced were spread in the prophase, the non-synchronization of condensation of chromosome may disturb prophase, shifting of equatorial plate, sticky chromosomes, c-metaphase, and sticky metaphase than that of the control.

In various endemic countries, Molluscicide has been extensively used for snail control as part of its strategic control programs. Bayluscide (Niclosamide), UPAC name of 5 chloro-N-(2-chloro-4- nitrophenyl)-2-hydroxybenzamide is one of the most effective molluscicides and there are many reports of its efficacy in decreasing the prevalence of snail invasion (Tesana et al., 2012).

This study aimed to investigate the effects of bayluscide, a molluscicide that is used in the farms whether such would have a harmful effect on plants such as *Allium cepa* l. (onions). The study determined the effect of bayluscide on the mitotic index, thus, by undergoing such investigation on whether the root tip cells of *Allium cepa* would cause the possible cellular abnormality. The unending harm of the endless use of pesticides and herbicides in agriculture, as well as the increase of pollution in ecosystems due to industrial development, justify the evaluation of the toxicity of these chemicals (Tartar et al., 2006).

METHODOLOGY

The research uses an experimental method comparing two groups under study. For the purpose of root initiation, the experiment utilized 20 selected medium sized clean, healthy bulbs of *Allium cepa* L. of the same size, weight and species (Tartar et al., 2006).

The onions were germinated in distilled water at room temperature in a 150 ml beaker filled with 25 ml of commercially available distilled water for 87 hours. Onions were suspended on top of the beaker with barbecue sticks allowing only the lower part of the onion bulb to be submerged in the distilled water. The set-up was placed in the Zoology laboratory with the same temperature and amount of sunlight.

Using the Randomized Complete Block Design (RCBD), 15 onion bulbs with the most number of roots were randomly selected to comprise the control and experimental groups respectively. When the roots reached 1.5-2 cm length they were treated with different concentrations of Bayluscide diluted with distilled water (2%, 6%,10% and 14%) for 12 hours as shown in Table 1. The controls were at the same time treated with distilled water for the same time periods. Each experimental set up was replicated thrice.

Table 1. Concentration of the Mixture of Bayluscide and Distilled Water

Set-up	Bayluscide	Distilled H ₂ O
Control	0 ml	100 ml
T1	2 ml	98 ml
T2	6 ml	94 ml
T3	10 ml	90 ml
T4	14 ml	86 ml

Twelve hours after, the root tips were washed thoroughly with running water and bulbs were placed in jars with distilled water for another 24 hours. This was used to determine how the treatment affected the mitosis of the root tip cells of *Allium cepa* L. Then the root tips were excised with a length of 0.2 cm each at 12:00 noon were maximal cell division occurs. The excised roots were placed in vials with Farmer's fluid (3 parts of ethanol: 1 part glacial acetic acid) in order to fix the chromosomes and left for another 24 hours. The Farmer's fluid was then replaced with hydrochloric acid that functions to clear and soften the root tips. Moreover, this set-up was left for 30 minutes.

Using the squash technique, excised root tips were placed on a glass slide and stained with methylene blue to make the cells very observable under the microscope. The slides were passed over a lighted alcohol lamp three times in order to fixate the cells. After which a cover slip is placed and sealed with a colorless nail polish. A total of 15 well-prepared slides with distinct mitotic figures were selected and scored in the study. Counting of cells and scoring of cytological abnormalities was done using an electric compound microscope with a total magnification of 500x beginning at the tip portion of the root and moving from left to the right side of the slide.

A total of 300 cells per slide adding up to 900 per treatment were examined and considered in calculating the mitotic index and frequency of cells with cytological abnormalities. These were subjected to ANOVA, Duncan's Multiple Range Test, and Mitotic Index.

A photomicroscope was then used to document the cells examined.

RESULTS AND DISCUSSION

An analysis of the mitotic index in root tip cells of *Allium cepa* showed that there was a marked decrease in cells undergoing cell division as the treatment concentration of Bayluscide increased. It was observed that cells at the interphase stage increased in number as the concentration increased; while cells at prophase, metaphase, anaphase and telophase stages decreased in number. Protein synthesis occurred at interphase stage and if this process is interrupted by the presence of chemical substance there is a possibility that a cell will not enter the prophase stage that is the first actual stage of cell division.

Effects also involved spindle apparatus disturbances since these structures are sensitive to chemical agents, and they appear only at the time the cell undergoes cell division. Due to disturbances of these apparatus, most of the cells were blocked at interphase and prophase stages.

Bayluscide exerts its effect starting at 2% concentration, but it is more effective in inhibiting cell division at the higher dosages. Although disturbances occurred at the mitotic division, the process of cell division still proceeded of but at a lesser rate. Therefore, the dosage of Bayluscide used cannot cause total inhibition of cell division.

Results showed agrees with the study conducted by Qin, R. et al. in 2010 which states that as the concentration of pesticides increases it causes a reduction in mitotic activity, induction of nucleolar alteration and alteration of several antioxidant enzyme activities in plant cells.

Table 2. Mitotic Indices in Root Tip Cells of *Allium cepa*

Set-up	Bayluscide Concentration	Replicates			Total	Mean
		1	2	3		
Control	None	186	194	202	582	194
Treatment 1	2%	19	65	32	116	38.67
Treatment 2	6%	44	30	25	99	33
Treatment 3	10%	32	21	25	78	26
Treatment 4	14%	14	24	19	57	19
Grand Total					932	
Grand Mean						62.134

The study also found out that Bayluscide caused cell abnormalities such as cells with shrunken and a broken nucleus starting at 2% concentration but was more pronounced in higher dosages. Bayluscide affected the cell membrane causing it to penetrate the cytoplasm of the cell and alteration of the cytoskeleton, responsibly maintain the shape of the cell. Due to changes occurring in the cytoplasm, the nucleus that is the most sensitive part of the cell was also altered. The final phase of damage resulted to a deranged metabolic performances of injured cell structures and is expressed in the inhibition of cell division, in activation and death or the distortion of the genetic processes. However from the concentration of Bayluscide used there were no chromosomal abnormalities observed. These results indicated that Bayluscide should be regarded as a mutagenic agent for plants. Hence, the use of this molluscicide should be under control in agricultural fields.

In a study conducted by Tartar et al. in 2012, showed that high number of abnormal cells were observed at high concentrations of the pesticides. Chromosome abnormalities increase proportionally with an increase in concentration and the time of exposure until they reach a point that, because of a decrease in the percentage of cell division chromosome abnormalities begins to diminish (Tartar, et al. 2006). Examination using a microscope of squashes of *Allium cepa* L. root tip cells showed that induced treatments of a number of mitotic abnormalities when compared with control and the increasing mitotic abnormalities were dependent on the increasing treatment periods and concentrations (Fidun, K., et al. 2009).

Table 3. Number of Cells with Abnormalities

Set-up	Replicates	# of Abnormal Cells	% of Abnormal Cells
Control	1	0	0
	2	0	0
	3	0	0
Total		0	0
Mean		0	
Treatment 1	1	133	44.33
	2	223	74.33
	3	97	32.33
Total		453	150.99
Mean		151	
Treatment 2	1	158	52.67
	2	194	64.67
	3	203	67.67
Total		555	185
Mean		185	
Treatment 3	1	111	37
	2	233	77.67
	3	259	86.33
Total		603	201
Mean		201	
Treatment 4	1	236	78.67
	2	207	69
	3	264	88
Total		707	235.67
Mean		236	

Table 4. Frequency of Cell Stages and Mitotic index in root Tip cells of *Allium cepa* L.

Set-up	Replicate	Interphase	Prophase	Metaphase	Anaphase	Telophase	# of mitotic cells	Mitotic Index
Control group	1	114	142	6	10	28	186	62.00%
	2	106	146	6	18	24	194	64.67%
	3	98	164	6	8	24	202	67.33%
Treatment 1 (2%)	1	281	16	1	0	2	19	6.33%
	2	235	64	0	1	0	65	21.67%
	3	268	32	0	0	0	32	10.67%
Treatment 2 (6%)	1	256	36	0	3	5	44	14.67%
	2	270	30	0	0	0	30	10.00%
	3	275	20	1	2	2	25	8.33%
Treatment 3 (10%)	1	268	28	2	1	1	32	10.67%
	2	279	21	0	0	0	21	7.00%
	3	275	22	1	0	2	25	8.33%
Treatment 4 (14%)	1	286	14	0	0	0	14	4.67%
	2	276	18	2	1	3	24	8.00%
	3	281	17	0	2	0	19	6.33%

Table 4 shows the different stages of the cell cycle where the first stage of cell cycle is the interphase that is known as resting stage of a cell, and the first actual cell division is the prophase stage. It was observed that as the concentration of Bayluscide was increased, more cells are arrested in the interphase stage and no longer entered the prophase stage resulting in its decreased numbers. However despite decreasing of mitotic index, it does not totally inhibit the cell division process considering that there were cells observed at the telophase stage that is the final phase of cell cycle.

The observed changes agrees with the study done by Abu et al. in 2006 which states that the inhibition of mitotic index and reduction in mitotic phases percentages indicated that the treatments that were introduces barred with the natural sequence of cell division, thus, interfering the amount of cells that came into the prophase stage at high concentrations. A lowered cell division index

suggests an inhibitory effect at the interphase stage came up that the mitotic rate was closely related to the resultant level of ATP (Abu et al., 2006). In the study of Lamsal, K., et al. in 2010 it was found out that the chromosomal abnormality percentage in various stages of mitosis was significantly higher in all treatment periods and concentrations and the type of defects produced were spread in the prophase, the non-synchronization of condensation of chromosomes, disrupted prophase, equatorial plate shifting, sticky chromosomes, C-metaphase, and sticky metaphase than that of the control.

Table 4 shows that in the Control group there were 186 mitotic cells with a 62% mitotic index. Replicate 1 shows that during the interphase there were 114 cells; prophase had 142, metaphase had 6, anaphase had 10 and telophase had 28. Replicate 2 interphase had 106 cells, prophase had 146, metaphase had 6, anaphase had 18 and telophase had 24 with 194 total mitotic cells and 64.67% mitotic index. Replicate 3 interphase had 98 cells, prophase had 164, metaphase had 6, anaphase had 8 and telophase had 24 with a total of 202 mitotic cells and 67.33% mitotic index.

Treatment 1 replicate 1 showed that in the interphase there were 281 cells, prophase had 16, metaphase had 1, anaphase 0, and telophase has 2 with a total of 19 mitotic cells with 6.33% mitotic index. Replicate 2 interphase had 281 cells, prophase had 64, metaphase had 0, anaphase had 1 and telophase had 0 with total mitotic cells of 65 and 21.67% mitotic index. Replicate 3 interphase had 286 cells, prophase had 32, metaphase, anaphase and telophase had 0 cells respectively with 32 total mitotic cells and 10.67% mitotic index.

Treatment 2 replicate 1 has a total of 44 mitotic cells with a mitotic index of 14.67%. In its interphase it had 256 cells, prophase had 36, metaphase had 0, anaphase had 3 and telophase had 5. Replicate 2 had 30 total cells with 10% mitotic index, interphase had 270 cells, prophase had 30, metaphase, anaphase and telophase had 0 respectively. Replicate 3 had 25 total of cells with a mitotic index of 8.33%, interphase had 275 cells, prophase had 20, metaphase had 1, anaphase had 2 and telophase had 2.

Treatment 3 replicate 1 had a total of 32 total cells with a mitotic index of 10.67%, its interphase had 268 cells, prophase had 28, metaphase had 2, anaphase had 1 and telophase had 1. Replicate 2 had 21 total cells with a mitotic index of 7%, its interphase had 279 cells, prophase had 21, metaphase, anaphase and telophase did not form any cell. Replicate 3 had 25 total mitotic cells, its interphase had 275 cells, prophase had 22, metaphase had 1, anaphase had 0 and telophase had 2.

Treatment 4 replicate 1 had 14 total cells with a mitotic index of 4.67%, interphase had 286 cells, prophase had 14, metaphase, anaphase and telophase did not form any cell. Replicate 2 had a total of 24 cells with a mitotic index of 8%, interphase had 276, prophase had 18, metaphase had 2, anaphase had 1 and telophase had 3. Replicate 3 had 19 total mitotic cells with a mitotic index of 6.33%, interphase had 281 cells, prophase had 17, metaphase had 0, anaphase had 2 and telophase had 0.

CONCLUSION

The result of the study showed that there was a marked decrease in cells undergoing cell division as the treatment concentration of Bayluscide increased. Bayluscide exerted its effect starting at 2% concentration, but it was more effective in inhibiting cell division at the higher dosages. Although disturbances occurred at the mitotic division, the process of cell division still proceeded but at a lesser rate. The dosage of Bayluscide used cannot cause total inhibition of cell division.

The study also found out that Bayluscide caused cell abnormalities such as cells with shrunken and broken nucleus starting at 2% concentration but was more pronounced in higher dosages. However, from the concentration of Bayluscide used there were no chromosomal abnormalities observed. These results indicated that Bayluscide should be regarded as a mutagenic agent for plants.

RECOMMENDATION

It is recommended that further studies will be conducted regarding the possible outcomes of the mutagenic effects of bayluscide.

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