



Impact of *Pendimethalin* on Mitotic Index and Root Growth Inhibition of *Allium cepa* (L.)

KEYWORDS

Allium cepa, Mitodepressive, Mitotic index, *Pendimethalin* effect.

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ABSTRACT *The cytotoxic, mitodepressive effect of herbicide Pendimethalin was observed on Allium cepa L., during 2013-2014 at Agra. The treatment included: control, higher concentration and lower concentration of Pendimethalin. After 2-3 days treatment, the recovery for higher concentration was done for 2 days, in which root growth was completely inhibited in higher concentration as compared to lower concentration and control. The overall effect was irreversible during recovery. However root growth inhibitory effect was reduced at lower concentration of Pendimethalin. The Mitotic Index showed that maximum cell division was obtained during 6:00 AM – 12:00 PM in control. Pendimethalin was found to arrest the maximum cell in Anaphase > Prophase > Metaphase > Telophase respectively.*

INTRODUCTION

Onion (*Allium cepa* L.) belongs to the bulb crops, and member of Alliaceae family. Total production of onion in India is about 163.09 Lakh Ton [3]. Onions are easy to store, to handle and to observe there microscopic and macroscopic parameters and widely used for the determination of cytotoxic, genotoxic and mutagenic effects of various substances. *Pendimethalin* (N-(1-ethyl propyl)-2,6-dinitro-3,4-xylidine) has the empirical formula $C_{13}H_{19}N_2O_4$, a selective pre-emergent herbicide a dinitroaniline group. *Pendimethalin* is known as mitotic inhibitor herbicide which can inhibit cell division in root tips. *Pendimethalin* has been classified by the US Environmental Protection Agency (EPA) as a Persistent Bioaccumulative Toxic (PBT) [8]. The US Environmental Protection Agency classifies *Pendimethalin* as a slightly toxic compound, class III, and a possible human carcinogen, group C [13]. The *Allium* test is an efficient cytological model for chromosomal aberration and mitotic activity assay of different environmental pollutants [12]. Thus, in present study we evaluated root growth inhibition and cytotoxic effects of *Pendimethalin*, by using *A. cepa* test.

MATERIAL AND METHODS

1. Bioassay

The present experiment was conducted to determine the cytological impact of herbicide *Pendimethalin* on *Allium*. Influence of different concentrations of *Pendimethalin* including Control (0 ml/L) Higher conc. (0.05 ml/L, 0.25 ml/L, 0.50 ml/L, 0.75 ml/L and 1 ml/L) and Lower conc. (0.01 ml/L, 0.02 ml/L, 0.03 ml/L, 0.04 ml/L and 0.05 ml/L) on growth and cell division during short term treatment using *Allium* test was done. Equal size of onion bulbs were used from a population of a commercial variety of onion and series of onion were grown in each tap water and distilled water (i.e. control) and test herbicide (i.e. different concentration of *Pendimethalin*). Before the test was started the outer scales of bulbs and the brownish bottom plate were removed and the ring of the primordial being left unbroken. The dried outer scales were carefully removed then the bulbs were used for the bioassay according to the standard procedures [10]. The peeled bulb then put into fresh water during the cleansing procedure to protect primordial from drying. Thereafter, the onion bulbs were placed in Gibson tube filled with tap water and distilled water control and *Pendimethalin*.

2. Growth Inhibition Analysis

The experiment was performed at constant room temperature and protected against direct sunlight. The onion bulbs were placed on all the treatments including control for 2-3 days until roots observed. After root development, 2-3 cm long roots were selected due to their high mitotic frequency. Similar experiment was conducted by reducing the concentration of *Pendimethalin*. After the treatment recovery was also done for 2 days and root growth was observed. After incubation and recovery, the root tips were cut off fixed for 12-18 hours in Carnoy's fixer (ethyl alcohol absolute: glacial acetic acid = 3:1). After fixing in Carnoy's fixer, the permanent microscopic slides were prepared and observations were recorded.

3. Mitotic Index

Mitotic index was considered as a dependable parameter for evaluating the cytotoxicity of various agents [6] and to estimate the frequency of cellular division [4, 7]. Mitotic index is a characteristic ratio for particular tissue, so any change in this ratio reflects the influence on the biochemical events inside the cell [1]. Mitotic index was calculated by using following formula-

$$\text{Mitotic index (MI)} = \frac{n}{N} \times 100$$

Where,

n = Total Number of dividing cells

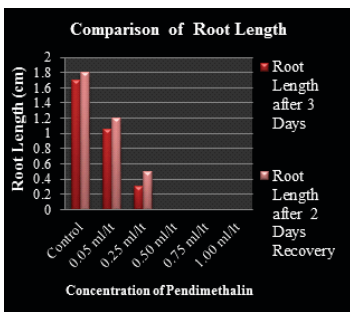
N = Total Number of cells

RESULTS AND DISCUSSION

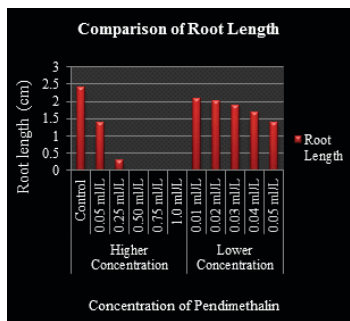
1. Effect on Root Growth

After 3 days of incubation at 18 - 25°C, the test tube filled with tap water and distilled water i.e. control (0 ml/L) showed elongation in root growth. However, at higher concentration of *Pendimethalin* (0.50 ml/L, 0.75 ml/L and 1.0 ml/L) root growth was completely inhibited, but root growth was observed in 0.05 ml/L and 0.25 ml/L concentration. When three days pre-treatment was followed by two days recovery, the result was observed without affecting root growth in concentration (0.05 ml/L and 0.25 ml/L) of *Pendimethalin* and complete inhibition in (0.50 ml/L, 0.75 ml/L and 1.0 ml/L) (Graph 1). Similar experiment was conducted by reducing the concentration of *Pendimethalin* (0.01 ml/L, 0.02 ml/L, 0.03 ml/L, 0.04 ml/L and 0.05 ml/L). After three days of incubation the root growth was observed in all concentrations. It can be interpreted that even small concentration of *Pendimethalin* affects the root growth of

onion confirming its growth promoting nature (Graph 2). Present finding is in conformity with earlier findings of Tylicky *et al.*, 2010 [12].



Graph 1. Comparison of Root Length after 3 days treatment and 2 days recovery.



Graph 2. Comparison of Root Length according to the different concentration of Pendimethalin.

2. Effect on Mitotic index

Effect of Pendimethalin on onion root tips was compared with control for Mitotic Index by perusing the Table 1. It has been observed that in control maximum cell division occurred

Table1. Estimation of Mitotic index in Different concentration of Pendimethalin during 6:00 AM to 12:00 PM.

S. No	Time	Control	Higher Concentration (ml/L)					Lower Concentration (ml/L)				
			0.05	0.25	0.50	0.75	1.0	0.01	0.02	0.03	0.04	0.05
1	6:00 AM	6.82	5.19	5.01	-	-	-	6.70	6.61	6.15	5.95	5.19
2	7:00 AM	7.03	6.39	6.23	-	-	-	6.99	6.80	6.69	6.41	6.39
3	8:00 AM	7.15	6.79	6.61	-	-	-	7.05	6.92	6.80	6.81	6.79
4	9:00 AM	7.32	6.99	6.83	-	-	-	7.21	7.13	7.09	7.03	6.99
5	10:00 AM	7.35	7.04	6.99	-	-	-	7.24	7.11	7.09	7.05	7.04
6	11:00 AM	7.38	7.06	7.01	-	-	-	7.28	7.19	7.10	7.09	7.06
7	12:00 PM	7.27	6.89	6.75	-	-	-	7.19	7.01	6.99	6.91	6.89

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between 6:00 AM - 12:00 PM during February to March with maximum temperature ranges from 15 – 25° C. Observations showed that lower concentration of Pendimethalin (0.05 ml/L and 0.25 ml/L) caused a strong mitodepressive effect on the meristematic cells of root tips of Allium cepa. The mitodepressive action resulted has significantly reduced mitotic index. However, higher concentration of Pendimethalin (0.50 ml/L, 0.75 ml/L and 1.0 ml/L) completely inhibited the cell division and mitotic aberrations could not be estimated because Mitotic Index was too low. The results show that Pendimethalin affects on toxicity and genotoxicity of onion. The root growth rate of treated onion is reduced as compared to its control. Concluding that Pendimethalin can alter the mitotic index after a certain period of time.

Growth is one of the best indicant for the evaluation of plant responses to environmental stress [9]. The decision of cell enter and/or exit cell division or differentiate depends on several cellular events and signals. These cellular events have long been of great interest to understand the bases of each one. Several agents stimulate the mitotic activity, while others cause mitodepressive and toxic activity. These mitodepressive drugs are effective against cells that are proliferating and some of them produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase [5, 3]. Significant reduction of MI may be due to the mitodepressive action of substances. Therefore, the chemicals used interfere in the normal cell cycle resulting in decrease in number of dividing cells [11].

CONCLUSION

It can be concluded that Pendimethalin has prominent role in reduction of root length of onion and causes mitodepressive effects and Mitotic Index significantly at its different mitotic phases.

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