

## Cyto-toxic effects of *Chromolaena Odorata* on mitosis in onion (*Allium cepa* L.) Root tip

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### ABSTRACT

*Chromolaena odorata* (Synonym- *Eupatorium rugosum*) is a member of Family Asteraceae and known as white snakeroot. It is locally called as jungle-modi as it grows rapidly in disturbed areas and inhibits germination of other plants in the vicinity. To understand its effects on cell division, 10, 20 and 50% aqueous extract of leaves of *E. rugosum* were used to treat the roots. Onion bulbs with 48 hr old roots were exposed to the extracts for 1 hrs and then fixed in Cornoy's alcoholic acetic acid. The squash prepared in 1% aceto-carmine were analysed under 40X objective lens for mitotic index and abnormalities. Experiments showed that mitotic index was maximum in control roots, and decreased in treated roots. *E. rugosum* induced mitotic abnormalities namely scattering of chromosomes at anaphase, formation of bridge, laggard and rings etc. in the root tips.

**Key Words:** *Chromolaena odorata*, cell division, mitotic index, cytotoxic effect

### INTRODUCTION

In recent decades studies on cytotoxic effects has gained much attention. Selectively cytotoxic compounds are useful in treatment of cancer. Number of plants also exhibits cytotoxic effects. Mitotic index is used as indicator of adequate cell proliferation biomarker. Inhibition of mitosis can be used to understand cytotoxic effects. Inhibition of mitotic index could be considered as cellular death or a delay in the cell proliferation kinetics (Rojas et al., 1993). *Chromolaena odorata* is a member of family Asteraceae locally known as *Jangal-modi*. It is a common weed that grows as opportunist on freshly disturbed areas like road sides, cleared zones in forest etc. It is known to inhibits seed germination and growth of other plants in its vicinity. Onion (*Allium cepa*) is one of the well known experimental material for study of cytological effects. The present study was undertaken with the null hypothesis that 'There are no effects of *Eupatorium rugosum* on mitotic index in *Allium cepa*'. The experiment was aimed to study effects of *Eupatorium rugosum* leaf extracts on mitotic index in *Allium cepa* and also to understand relation between concentration of leaf extract and cytotoxic effects.

## MATERIAL METHODS

### Preparation of plant extract:

Fresh leaves of *Chromolaena odorata* collected from the wild plants growing in the campus area were washed thoroughly with distilled water and blotted dry. Ten gm of fresh leaves were crushed in mortar and pestle in distilled water, filtered through muslin cloth. The filtrate was made 100 ml in volumetric flask with distilled water and used as stock solution (100 %).

### Experimental design:

All the experiments were carried out in October 2017 under laboratory conditions at room temperature i.e. 22 °C (minimum) to 29 °C (maximum). A total of 12 bulbs of onion (*Allium cepa*) were initially grown for induction of roots on a coupling jar filled with distilled water. After 48 hrs, 3 bulbs each were transferred to 10%, 20% and 50% dilutions of the aqueous plant extract for treatment. The roots were treated for 1 hr by transferring them to the plant extracts at 8.30 AM. Roots from control and treated bulbs were removed at 9.30 AM, fixed in Cornoy's fixative (1:3 Acetic alcohol) for 24 hours and preserved in 70 % alcohol in refrigerator.

### Mitotic studies and analysis:

Preserved root tips were thoroughly washed in distilled water and stained with 1 % aceto-carmin solution with few drops of 1N HCl. Slides were observed under binocular light microscope at 400 X magnification to

record number of cells under cell division. Mitotic index was calculated by the formulae

$$\% \text{ Mitotic Index (MI)} = \frac{\text{Number of cells under cell division}}{\text{Total number of cells examined}} \times 100$$

Phase Index was calculated as

$$\% \text{ Phase Index (PI)} = \frac{\text{Number of cells in the phase}}{\text{Total number of cells examined}} \times 100$$

Photomicrographs were taken at 1000 X magnification under oil immersion with digital eye piece (2 Mega Pixel) camera. The data obtained was transferred to statistical analysis by MS-Excel 2007.

## RESULTS & DISCUSSION

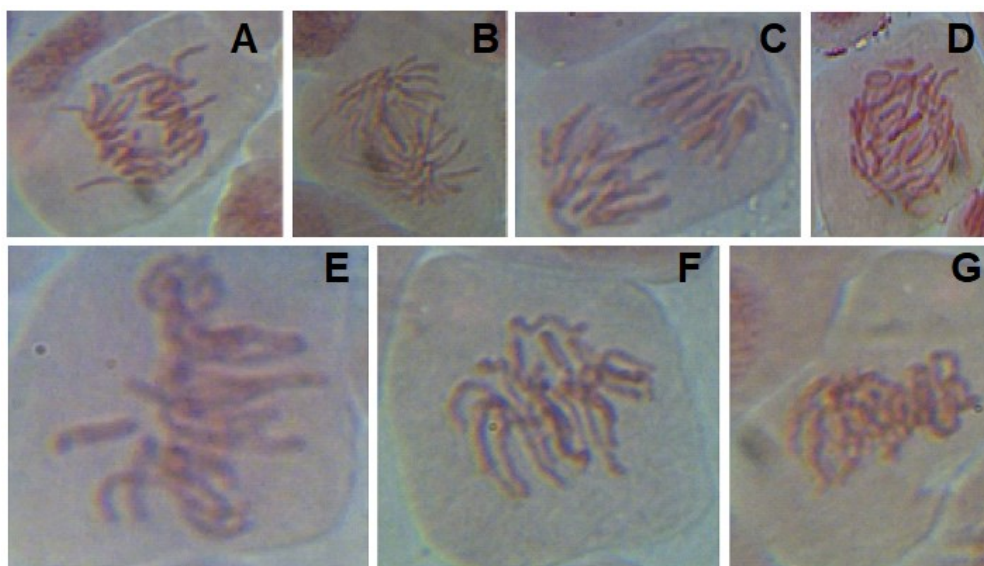
The *C. odorata* has 23.75 % dry weight. The studies showed that maximum cells were under normal cell division in control treatment and the number reduced with increase in the concentration of leaf extract. In higher concentrations cells enter in the cell division but were unable to enter in to metaphase and anaphase. This may be caused due to damage to the spindle apparatus. The results of 3270 cells observed during this experiment were shown in following tables. The studies revealed adverse effects of *C. odorata* leaf extract on the mitotic index. Furthermore, the number of cells under various cell division phases also declines with increase in the concentration of the leaf extract.

**Table 1. Number of onion cells in various phases of cell division after treatment of *C. odorata* leaf extract.**

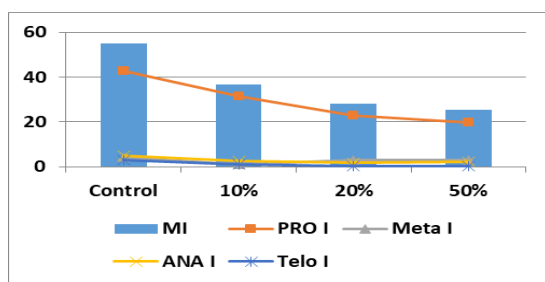
	Total Cell examined	Cell under division	Cell in prophase	Cells in metaphase	Cells in anaphase	Cells in telophase
Control	521	286	223	22	26	15
10%	589	216	186	7	16	7
20%	1117	314	257	35	20	2
50%	1043	266	205	32	25	4

**Table 2. Effect of *C. odorata* extract on % M.I. and % Phase Index in onion root tip**

	% MI	% PRO I	% Meta I	% ANA I	% Telo I	Total Cell examined
Control	54.89	42.80	4.22	4.99	2.88	521
10%	36.67	31.58	1.19	2.72	1.19	589
20%	28.11	23.01	3.13	1.79	0.18	1117
50%	25.50	19.65	3.07	2.40	0.38	1043



**Fig. 2.** Formation of abnormalities during metaphase and anaphase in onion root tip as an effect of *C. odorata* leaf extract. **A.:** Laggards, **B.** Scattered Anaphase, **C& D:** Bridge formation at anaphase, **E.:** Laggards, **F.** Scattered metaphase, **G:** Metaphasic rings.



**Fig. 1.** Effect of *C. odorata* extract on % M.I. and % Phase Index in onion root tip

The *C. odorata* also induced abnormalities in the cell division. The abnormalities were noted at metaphase and anaphase. Earlier studies by various scientists showed that plant extracts induced mitotic abnormalities like anaphase bridge, fault polarization of anaphase, chromosomal fragmentation etc in root mitosis of *Allium cepa* (Nwangburuka and Oyelana, 2011; Alege and Ojomah, 2014; and Yasin and Ozatab, 2014).

## CONCLUSION

It is clear from these studies that *C. odorata* inhibits mitosis in *Allium cepa* root tips. Experiments showed that *C. odorata* leaf extracts are cytotoxic and it has adverse effects mitosis.

As *C. odorata* inhibits cell division, it may be used for checking uncontrolled cell division like as in tumour formations and cancer treatments. The leaf extract may also be applied as herbicide to control the weeds.

However, further studies are necessary to understand the recovery response of the root tips, effects on mitotic index in other crop plants, induction of selective cytotoxicity and most effective cytotoxic concentrations.

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