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RESEARCH ARTICLE

CYTOLOGICAL CHANGES IN LENTIL IN RESPONSE TO ALLELOPATHIC EFFECT OF *XANTHIUM STRUMARIUM* L.

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Abstract

In the present study allelopathic potential of *Xanthium strumarium L.* was accessed to induce cytological changes in Lentil (*Lens esculenta* Moench.). For seeds of Lentil were treated with different concentrations (100%, 50% and 25%) of leachates of different plant parts, viz., leaves of young, mature, and senesced stages, flower, stem and seeds of *Xanthium strumarium L* for 24 hours. Reduction in mitotic index along with increment in abnormality index in a dose dependent manner was recorded in root tip meristem of Lentil over the control. 100% concentration of seed lachates caused maximum reduction of mitotic index with higher abnormality index. Types of abnormalities induced fragmentation of chromosome in prophase, stickiness of chromosome in metaphase and anaphase, C-metaphase, disturbed polarity at anaphase, Chromosome Bridge at both anaphase and telophase and laggard chromosome at telophase. Pronounced inhibitory effects were also recorded in cell size.

INTRODUCTION

The interaction between plants, plant and insect, plant and microorganisms and plant and animals are always going on in environment through bioactive natural chemicals. The phenomenon is known as allelopathy and the chemicals released by one organism influencing the growth; reproduction and survival of others are known as allelochemicals. When these allelochemicals cause benefit to the target organism, it is called positive allelopathy. The inhibitory or harmful effect produced by the allelochemicals on organism is known as negative allelopathy.

Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory **(**Stamp, Nancy and March 2003). The term allelopathy signifies that interaction or inhibition of growth **(Molish, 1937)** of organisms, by the release of chemicals from different parts of plants or other organisms by leaching, root exudation, volatilization residue decomposition and other processes. These interactions are widely known in different groups of plants such as algae, lichens, crops, as well as annual and perennial weeds **(Rice, 1984; Putnam, 1985; Horseley, 1991; Lawrey, 1993; Inderjit and Dakshini, 1994 a and b; Bhakat*et al*., 2005; Inderjit, 2005).** The chemicals of the different plant parts in the environment play significant role in adaptation of specis and organization of community **(Chow, 1989)**.

Xanthium strumarium L. common cocklebur is a weed species belonging to the family Asteraceae family. The species is monoecious, with the flowers borne in separate unisexual heads **(Everitt*et al.*, 2007)**. The plant has some medicinal properties **(Kamboj and Saloha, 2010)** and has been used in traditional medicine in South Asia and China. Small quantities of parts of the mature plant may be consumed; the seeds and seedlings should not be eaten in large quantities because they contain significant concentrations of the extremely toxic chemical

carboxyatratyloside. The mature plant also contains at least four other toxins **(Islam *et al*., 2009)**. Animals have also been known to die after eating this plant.

Lentil (*Lens esculenta* Moench.) is an important pulse crop belonging to family Fabaceae, locally called mosoor dal, is excellent source of high quality protein, essential amino and fatty acids, fibers, minerals and vitamins. Being leguminous this pulse crops improve soil health by enriching nitrogen status through nitrogen fixation. Although there are few reports on cytological studies in different plants under allelopathic stress **(Sudharsan and reddy 1971; Kalearity and Malallah 1980; Shehab 1979, 1980, etc),** no work has been done regarding cytological changes of Lentil crop plants over *Xanthium strumarium*. Therefore, present investigation was done to determine the cytological effects of leachates of *Xanthium strumarium* in root tip meristem of Lentil.

2. Material and Methods

Dried and healthy seeds of *Lens esculenta* Moinch, collected from local farmers of Burdwan were sutface sterilized with 0.1% mercuric chloride for 90 seconds followed by repeated washing, in sterile double distilled water and soaked in different concentration of *Xanthium* leaf leachates.

2.1 Preparation of leachates

Leaves, stem, flower and seeds of *Xanthium* were collected from its natural habitat in the Golapbag campus of the University of Burdwan separately and were surface sterilized with 0.1% mercuric chloride followed by repeated washing in double distilled water. 10 grams of each of the plant part were soaked in 100 ml sterile double distilled water for 24 hours. The leachates were filtered and were considered as 100% stock solution from which 50% and 25% leachate solutions were made by using sterile double distilled water.

After 24 hour soaking the seeds were kept in separate petri dises for another 24 hrs, then newly immersed root tips were fixed in acid alcohol fixative at 30° tempareture for 24 hours. Then placed in 9:1 aceto-orcin (2%) : 1 (N) hydrochloric acid, warmed gently for 5 seconds and kept for 24 hours. Stained root tips were gently squashed in 45% acetic acid on microscopic slides

Pretreated seeds were sown in petri dishes lined by double layer of filter papers (Whatman no: 1) at 30° C. After germination root tips (1cm length) were fixed in Carnoy's fixative I for 24 hours at 4° C. The fixed root tips were kept in 9:1 acetic orcein : 1 (N) hydrochloric acid followed by gentle warming. After 24 hours. Stained root tips were squashed in 45% acetic acid on slide.

.2.2 Microscopic Study

With the help of Light Microscope (Leica DM 3000 Germany) parameters like mitotic index, abnormality index, chromosomal structural aberrations and cell size in root tip meristem were studied following **Nandi and Dalal (2014).** Data were analysed statistically including mean value and standard error following **Panse and Sukhatme (1978).**

3. Result

The results (Table-1) revealed that the mitotic index of the root tip meristem of lentil in the treatment lots of 25% leachates from the plant parts, viz., leaves of three different maturity stages, stem, flower and seeds of *Xanthium strumarium L.* increased sharply over the control. But beyond this concentration of leachates of plant parts mitotic index decreased gradually and significantly an almost all the cases. The highest mitotic index was noted in 25% leachate of *Xanthium* mature leaf. While the lowest one was recorded in root tip cells at 100% concentration of leachate of *Xanthium* seeds. The highest and significant abnormality indices were seen in 100% leachate solution from mature leaves and senesced leaves, abnormality index was found to increase progressively with increasing doses of leachate solutions.

Although a few cells in the control group showed abnormalities like c-metaphase, sticky chromosome at metaphase and disturbed polarity at anaphase, percentage of abnormal cells increased with increasing concentrations of leachate solutions. The maximum abnormal cells were found in 100% concentration. Types of abnormalities observed in the root tip cells of all the treated groups included fragmented chromosomes at prophase , c- metaphase, sticky chromosome and vagrant chromosome at metaphase, sticky chromosome at anaphase, disturbed polarity and bridge at anaphase, laggard and bridge at telophase. Seed leachate of 100% concentration induced maximum percentage of abnormal cells containing fragmented chromosome, c-metaphase, sticky chromosome at metaphase, chromatin bridge and higher percentage of cells with sticky chromosomes at anaphase and laggard chromosomes at telophase.

Cell sizes (both length and width) reduced in a dose dependent manner of plant part leachate solutions. Although all types of leachates caused higher reduction in cell size at 100% concentration, seed leachate affected mostly the length and width of the cell at the same concentration. But 25% leachate solution showed slight increment in size over the control.

Table 1: Effect of different concentration of plant part leachates of *Xanthium strumarium* on cell division and cell size in root tip cells of Lentil .Each value represent mean ±S.E. *Significant at P 0.05 **Significant at P 0.01

Plant part leachate	Concentration	Mitotic index (%)	Abnormality index (%)	Cell length(µm)	Cell width(µm)
Young Leaf	**100%**	**18.2813±2.127**	**1.3048±0.590**	**31.67±0.43**	**23.90±0.09**
	50%	**19.778±2.811**	**1.223±0.498**	**33.98±0.13**	**24.77±0.12**
	25%	**26.545±2.843**	**1.051±0.385**	**34.65±0.24**	**25.78±0.13**
Mature Leaf	**100%**	**18.098**±2.567**	**1.998*±0.453**	**26.97±0.45**	**21.97±0.32**
	50%	**19.678±2.096**	**1.786±0.098**	**31.97±0.53**	**23.76±0.12**
	25%	**27.089±2.509**	**1.655±0.432**	**37.09±0.23**	**31.09±0.32**
Senesced Leaf	**100%**	**18.999±2.009**	**1.998*±0.453**	**29.32±0.34**	**21.99±0.33**
	50%	**19.675±2.901**	**1.654±0.099**	**33.89±0.43**	**24.78±0.77**
	25%	**25.234±2.340**	**1.071±0.560**	**38.08±0.55**	**28.98±0.54**
Flower	**100%**	**16.342*±2.789**	**1.874*±0.453**	**23.78±0.67**	**19.87±0.61**
	50%	**18.675±2.765**	**1.865±0.456**	**32.65±0.44**	**27.98±0.76**
	25%	**24.543±2.098**	**1.587±0.876**	**39.09±0.65**	**29.56±0.16**
Stem	**100%**	**19.563*±1.304**	**1.985±0.563**	**24.67±0.45**	**17.07±0.23**
	50%	**22.079±1.116**	**1.556±0.543**	**26.89±0.65**	**20.98±0.45**
	25%	**24.678±1.111**	**1.223±0.765**	**30.88±0.23**	**23.76±0.34**
Seed	**100%**	**15.006±1.223**	**1.995±0.546**	**14.96±0.56**	**17.65±0.45**
	50%	**19.678±1.227**	**1.975±0.541**	**18.76±0.23**	**21.56±0.34**

	25%	**24.089±1.663**	**1.456±0.811**	**34.14±0.67**	**25.09±0.61**
Control Double Distilled water	**Control**	**22.987±0.871**	**1.001±0.841**	**34.56±0.67**	**27.78±0.54**

Among the stage wise abnormalities recorded in different treatment lots, seed leachates of *Xanthium* of 100% concentration induced maximum fragmented chromosomes at prophase (Table 2). Leachate solutions of mature and old leaves also produced higher percentage of chromosomal breakage than that of young leaf, flower and stem, although gradual escalation of fragmentation of chromosomes was observed with increasing concentrations of leachates in all the tested lots. The dose dependent increment of c-metaphase and sticky chromosomes at both metaphase and anaphase, chromosomal bridge at anaphase and telophase were observed in all the treated groups. Seed leachate with 100% concentration produced maximum c-metaphase and stickiness of chromosomes at metaphase, while highest frequency of anaphasic chromosomal stickiness at 100% concentration of flower leachate was noted. Seed leachates at 100% concentration also caused maximum chromosomal bridge in anaphase and laggard chromosome at telophase. Vagrant chromosomes were occasionally observed along with stickiness of chromosomes at metaphase in the treated groups. Disturbed polarity at anaphase was frequently observed.

Table 2: Stage wise chromosomal abnormalities (%) in root tip meristem of lentil induced by different concentrations of *Xanthium strumarium* plant part leachates. Each value represent mean ±S.E. ; Ddwt= Double distilled water

Plant part Leachate	Total no of cells observed	Concentrations (%)	Prophase	Metaphase			Anaphase			Telophase		
			Breakage	C-Metaphase	Sticky chromosome	Vagrant Chromosom	Sticky Chromosom	Disturbed polarity	Chromosomal Bridge	Chromosomal Bridge	Laggard chromosome	Unequal separation
Xanthium Young Leaf	1025	100	5.67±0.2	8.23±0.3	5.66±0.4	1.90±.06	4.67±0.3	1.90±.03	2.03±.06	2.22±.02	1.23±.02	1.67±.09
	1109	50	5.23±0.4	7.88±0.2	4.77±0.3	1.08±.07	3.76±0.3	1.22±.02	1.10±.03	1.24±.03		
	1198	25	4.33±0.6	7.45±0.3	3.78±0.4		2.87±0.4	1.34±.05		1.34±.04	1.08±.03	
Xanthium Mature Leaf	1098	100	6.90±0.5	9.54±0.3	5.21±0.5	2.33±.05	4.78±0.2	1.87±.04	2.56±.05	2.34±.04		
	1200	50	5.66±0.6	9.32±0.4	4.32±0.34	1.89±.02	5.76±0.2	2.89±.06	1.45±.04	1.10±.02		
	1100	25	4.98±0.2	7.01±0.2	3.90±0.3	1.09±.03	3.78±0.2	3.78±.03		2.09±.03	1.67±.03	1.34±.01
Xanthium Old Leaf	1176	100	6.76±0.2	9.56±0.2	5.78±0.3	2.76±.08	5.89±0.3	1.23±.04	3.78±.03	3.23±.04		
	1107	50	5.90±0.3	9.54±0.3	6.98±0.4		6.89±0.3	1.09±.05				
		25	4.70±0.2	8.34±0.2	3.98±0.3		4.87±0.4	2.89±.04				
Xanthium Flower	1099	100	5.89±0.2	7.90±0.23	6.78±0.4	1.76±.01	8.09±0.4	1.67±.06	2.67±.06	3.98±.05	1.23±.05	1.05±.02
	1019	50	4.87±0.3	7.52±0.12	5.09±0.4		3.87±0.2	1.02±.03	1.12±.04	1.23±.06		
	1043	25	3.87±0.3	6.56±0.4	3.87±0.2		2.78±0.2	2.09±.04				
Xanthium Stem	1097	100	5.23±0.2	9.89±0.3	7.89±0.3	1.79±.05	5.65±0.2	1.24±.03	3.45±.05	2.76±.07	2.34±.03	
	1107	50	4.23±0.2	8.98±0.2	6.55±0.3		4.78±0.3	3.45±.04	2.13±.03	1.23±.02		
	1029	25	3.55±0.3	7.90±0.3	2.76±0.4		4.10±0.4	3.70±.02				
Xanthium Seed	1056	100	7.98±0.3	9.78±0.5	8.45±0.4	1.34±.03	7.54±0.6	2.09±.05	4.23±.04	2.34±.02	2.78±.02	1.09±.03
	1075	50	5.99±0.2	8.56±0.3	2.97±0.3		5.67±0.2	1.45±.03	2.76±.04	3.45±.02	1.23±.04	
	1034	25	3.34±0.4	7.90±0.3	4.76±0.3		4.65±0.2	1.09±.04	1.08±.03	1.56±.03	1.01±.03	
Ddwt	1042	Control	1.77±0.2	2.09±0.3	2.10±0.4			1.15±.03				

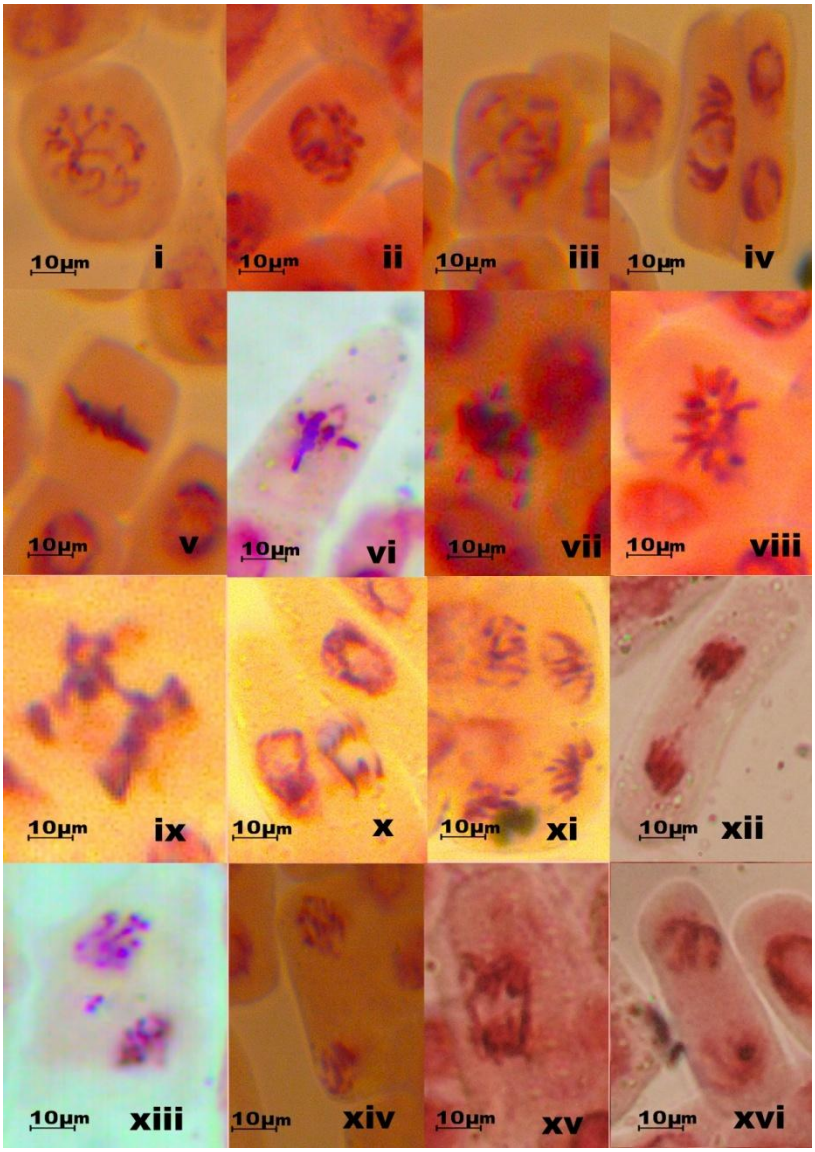

Figure 1: Photograph showing cytological changes induced by allelopathic effect of *Xanthium strumarium*on root tip meristem of *Lens esculenta*

i)Prophase (24 hours double distilled water treated) ii) condensed prophase (100% Xanthium leaf leachate) iii) prophase with breakage (100% Xanthium flower leachate) iv) abnormal prophase (100% Xanthium stem leachate) v) Sticky metaphase (50% Xanthium flower leachate) vi) Sticky metaphase with vagrant chromosome (100% Xanthium mature leaf leachate) vii) Metaphase with breakage (25% Xanthium stem leachate) viii) C-metaphage (100% Xanthium flower leachate)ix) Anaphage bridge (100% old leaf leachate)x) Anaphage bridge (25% Xanthium flower leachate)xi) Anaphage with breakage (100% Xanthium mature leaf leachate)xii) Anaphage with eroded chromatid and stickiness (100% Xanthium flower leachate)xiii) Lagard telophase(100% Xanthium mature leaf leachate)xiv) Telophase (50% Xanthium Flower leachate)xv) Anaphase with bridge (50% Xanthium mature leaf leachate)xvi) Telophage (50% Xanthium stem leachate)

4. Discussion

The leachates of different plant parts of *Xanthium strumarium* showed a wide variation in the inhibition of cell division of Lentil along with progressive abnormalities over the control.

But due to positive allelopathic effect of the chemicals present in the lechates of 25% concentration slight enhancement of cell division and cell size with lower abnormality index in root tip meistem of Lentil was observed. The result of mitotic inhibition along with abnormalities induced by allelochemicals of different plants are in conformity with the works of **Mohamed and El-Ashry, 2012** on *Pisum sativum* by *Brassica nigra* extract; **Shehab 1979**, on *Allium cepa* by extracts of *Pulicarya crispa*; **Adam and Farah 1989** on *Vicia faba* by extracts of *Cymbopogon proximus*; **George and Geethamma 1990** on *Allium cepa* by extracts of *Ricinus communis*; and **Madeiros and Takahashi 1987** on *Allium cepa* by extract of *Luffa operculata.* It indicates that the lechates lead to disturbance in cell cycle resulting reduced number of dividing cells. The leachates also might have effects in blocking the DNA or protein synthesis, which may be the cause of reduced mitotic index **(Mohamed and El Ashry, 2012).**

Cytological abnormalities are indicative of stress effects on chromosome. Here allelopathic stress induced by leachates of different plant parts of *Xanthium strumarium* caused different types of chromosomal abnormalities among which stickiness of chromosome was most common and frequency of cells with chromosome stickiness were seems to increase progressively with increasing concentration of leachates at both metaphase and anaphase. Seed and flower leachate produced maximum stickiness at metaphase and anaphase respectively. Stickiness may occur due to change in cytochemically balanced reactions **(Jaya Balan and Rao , 1987)** and may also be due to dissociation of nucleoproteins and alteration of the patterns of organization **(Evans, 1962),** which might have occur due to reduced cell moisture **(Singh, 2014). Mohamed and El Ashry, 2012** pointed out that stickiness might be due to ability of the extracts to cause DNA depolymerization and partial dissociation of nucleoproteins, breakage and exchanges of the basic folded unit of the chromatids and stippling of the protein covering of DNA in chromosomes as shown by **Onyenwe, 1983**. Fragmented chromosomes observed in prophase indicate clustogenic caused by the leachates. The presence of chromosome fragments indicates chromosomal breaks and may be consequence of chromosomal bridge at anaphase and telophase **(Singh, 2003).** Leachates affected spindle activity causing c-metaphase, laggard and disturbed polarity at anaphase because different workers **(Liu *et al.*, 2003/2004 and Zang *et al. 2009)** pointed out that disturbance in spindle activity caused C-metaphase, laggard, disturbed polarity, etc like features. All the irregularities in mitotic cell division indicated cytotoxic effect under allelopathic stress induced by leachates of different plant parts of *Xanthium strumarium* on Lentil. The chromosomal irregularity may lead to cell death. Among all the experimental leachate types seed leachate solution of 100% concentration showed maximum toxicity which might be due to maximum release of accumulated harmful allelochemicals from the seeds through leachate.

5. Conclusion

The mutagenic potential of *Xanthium strumarium* plant cannot be underestimated. The experimental results clearly indicate that the leachate of *Xanthium strumarium* may inhibit the growth of the lentil plant because growth is intimately related with the cell division. On the other hand the observed chromosomal abnormalities represent the direct effect of allelochemicals present in the leachate on the genetic material. The chromosome pattern reflects irreversible toxic effect leading to cell death. Consequently the genetic purity of genotype may be disturbed due to this allele-chemicals.The careful analysis of allelochemicals in the leachate is necessary which will be helpful in planning cultivation of the crop.

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