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ALLELOTOXIC EFFECTS OF *PARTHENIUM HYSTEROPHORUS* L. LEAF AQUEOUS EXTRACT ON CYTOMORPHOLOGICAL BEHAVIOR OF *ALLIUM CEPA* L. AND *LENS ESCULENTA* MOENCH

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Abstract: *Parthenium hysterophorus* a species of Asteraceae, has gained a worldwide cognigence as an weed due to its invasiveness at many parts of the world. It is well known for its allelopathic effect to various neighboring plants. The allelopathic effect of Parthenium leaf extract has been noted to affect the ability of plant cell division, the chromosomal conformity as well as the growth of *Allium cepa* and *Lens esculenta*. The present study illustrated that with the increase of concentration of leaf extract the mitotic index decreases and the percentage of chromosomal abnormality increases correspondingly destabilizing the growth behavior of the studied plants.

Key words: Allelotoxicity; Leaf extract; *Parthenium hysterophorus*; *Allium cepa; Lens esculenta*.

INTRODUCTION

Parthenium hysterophorus L. a plant of Asteraceae form tropical America has attained a worldwide distribution due to its instinct invasiveness. Its success as a weed has led to scientific investigations at different corners of world to unravel the cause of it. Allelopathy has been considered to be one of the most potent factors for imparting invasiveness to the species [1-6].

Allelopathy is a phenomenon by which individuals of one plant species can negatively or positively influence the growth and other life processes of neighboring species. Some chemical exudates of the plant, known as allelochemicals, are responsible for this. Like chemicals have been detected for *Parthenium hysterophorus* L. also. Some of these are vancillic acid, anisic acid, cornopillin, parthenin etc. [7-11]. Deleterious influences of these allelochemicals on crop plants, forest plants are well documented by a most of workers [12-14]. In India during last two decades this species has increased its population fast. The species has shown least specification regarding the choice of site of growing, thus, it grows on fellow land, forests and equally well on nutrient rich arable land; quite competitively with cultivated crops [15,16]. Allelopathic effects of the species being a well-known fact. So the present study attempts to reveal the impact of the extract of leaf residues on the cell division, chromosome morphology and growth of two agricultural plants *Allium cepa* and *Lens esculenta*.

MATERIALS AND METHODS

Matured leaves of *Parthenium hysterophorus* were taken to collect leaf extract. Seed of *Lens esculenta* and bulb of *Allium cepa* were used as the plant materials to observe the effects of leaf extract.

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Collection of leaf

Matured leaves of *Parthenium hysterophorus* were collected mostly as the leaves that has just fallen on the earth or the most mature yellow senescent leaves about to be detached from plant.

Preparation of leaf extract

Forty (40) grams of such mature leaves were pounded and dippped in 500 ml. of double distilled water for 72 hours. The leaf extract that came out by that time was collected by straining out the debris of leaf tissues with a strainer. The solution, thus obtained, was taken as stock solution. Different concentrations of the extract were prepared by adding double distilled water to the stock solution according to need.

The concentrations used are:

- Concentrated (i.e. the stock solution 100%)
- 50%
- 25%
- 12.5%
- 6.25%

Treatment of seed of Lens esculenta and bulb of Allium cepa

The plant materials were taken in two different lots-one for cytological works and the other to study the germination and growth behavior. Same experimental sets were set for both of the above mentioned lots and also for both species.

Bulb of Allium cepa

Bulbs in different sets have treated with the respective concentrations of extract for 3 days, roots emerged were collected for cytological studies. In another lot bulbs treated similarly were transferred to

the sterile sand soil (4:1) mixture after treatment with the extract for 3 days.

Seeds of Lens esculenta

Seeds have been treated with the extract for consecutively 3 days and the roots emerged by that time were collected and fixed for cytological study. Also the seeds of the other lot of the same species and treated with same treatment were transferred to sterile sand and soil (4:1) mixture for growth study.

Cytological Study

Fixed root tips of the treated plant materials were stained with aceto-orcein and squashed with 45% acetic acid for cytological study. Mitotic index and abnormality percentage were calculated for all sets of experiments.

Mitotic index (MI) = (Dividing cells per field / Total no. of cells per field) x 100.

Abnormality percentage= (Abnormal cells per field / No. of dividing cells per field) x 100.

Germination percentage

Germination percentage of seeds of *Lens esculenta* was calculated for different sets of experiments as well as the Relative germination percentage was also calculated in the following way.

Germination percentage = (No. of seeds germinates / Total no. of seeds) x 100.

Relative germination percentage = (Germination Percentage of tested seed / germination percentage of control seeds) x 100.

Plant Growth

Growth behavior of *Lens esculenta* was studied in respect to the height of plant attained after certain intervals of time.

Relative height of plant

Number of leaves, length of leaves and relative length of leaves. In case of *Allium cepa*: The length of largest leaf and smallest leaf were noted at certain interval.

Number of leaves per plant was also counted after 7 days of planting.

Relative length of shoot = (Mean shoot length of tested plant/mean shoot length of control) x100.

Relative number of leaf= (Mean no. of leaf of tested plant / mean no. of leaf of control) x100.

RESULTS AND DISCUSSION

The effect of leaf extract on cell division was reflected by the reduction in the mitotic index, disturbances in cytokinesis, disturbances in spindle function etc. and the impact on chromosome was understood by the presence of morphological distortions of chromosomes. The account of impact of leaf extract on chromosome morphology was taken in respect to the dividing cells. The results in this regard are presented in the table–I-IV and Fig. 1-6.

The results shown that with the increase of the concentrations of leaf extract the mitotic index decreases and abnormality percentage increases. Similarly the anomalies of chromosomal morphology increases considerably and consistently for both of *Lens esculenta* and *Allium cepa* with the increase of concentration of leaf extract. It was also seen that the seeds of lentil and bulb of onion treated with stock solution (100%) could not able to produce roots and at the 12.5% concentration of leaf extract chromosome erosion is the highest and at 50% concentration chromosomal disturbance (Fig. 12-13) is the highest in both tested plants. In *Allium cepa* at 50% concentration all types of chromosomal abnormalities were occurred whereas in *Lens esculenta* all types of chromosomal abnormalities were occurred at 12.5% concentration of leaf extract. These abnormalities were occurred in all the divisional phases. The root cells of lentil were mostly affected in prophase and in anaphase at 50% concentration and 6.25% concentration of leaf extract respectively as well as at 12.5% concentration and 25% concentration metaphase stage was greatly affected. But the root cells of onion were mostly affected in metaphase at 6.25% and 50% concentration of leaf extract as well as at 12.5% and 25% concentration of leaf extract prophase stage was highly affected. The kinds of these anomalies met with are mostly-stickiness of chromosomes, erosion of chromatin contents of different extents, shortening of chromosomes etc.

Concurrently the germination percentage of seeds of *Lens esculenta* decreases in response of the increasing concentration of the leaf extract and so also the relative germination percentage like findings in earlier works were quite abound and the decrease of germination of wheat, black gram, soybean, maize, groundnut and green gram seed in response of parthenin [17-21]. The germination performance of seeds of *Lens esculenta* is shown in the Table V-VI, Fig. 7. All chosen indices of growth, height of plant, length of longest leaf, length of smallest leaf, highest number of leaf of *Lens esculenta* shown concomitant decrease with the increase of the concentration of leaf extract. Similar responses were noted in case of relative height of plant,

relative length of longest leaf, relative highest number of leaf as well.

In case of *Allium cepa* length of longest leaf, length of shortest leaf, highest number of leaf all became decrease with the increase of the concentration of leaf extract. Such findings of growth behavior in accordance with so many earlier experiment by different workers [22-26] witnessed reduction of growth of radical and plumule of *Ageratum conyzoides*, *Helianthus annuus*, *Zea mays* and other economically important crops by the influence of *Parthenium* leaf extract. The growth performance of these tested plants is reflected in the table –VII-X, Fig. 8-11.

Table I: Result of the cytogenetical effect of aqueous leaf extract of *Parthenium hysterophorus* at different concentrations on *Allium cepa* (mean±SD).

Concentration of leaf extract	Mitotic index (MI)	Abnormality Percentage	Types of abnormality			
			Spindle disturbance (%)	Chromosomal Disturbance (%)	Spindle & Chromosomal disturbance (%)	Erosion (%)
Control (0%)	24.32±1.44	3.75±1.18	69.31±1.66	28.33±1.89	2.38±1.02	Nil
6.25%	22.34±2.11	15.01±1.89	42.98±1.27	56.92±1.89	Nil	Nil
12.5%	21.72±0.98	22.25±2.03	33.31±1.11	12.45±2.21	Nil	53.98±1.14
25%	18.80±0.86	35.25±1.07	21.60±1.53	76.1±2.31	2.15±1.22	Nil
50%	17.33±0.69	53.29±2.12	6.88±1.03	81.18±3.01	10.21±1.23	2.45± 0.8

Table II: Result of the cytogenetical effect of aqueous leaf extract of *Parthenium hysterophorus* at different concentrations on *Lens esculenta* (mean±SD).

Concentration of leaf extract	Mitotic index (MI)	Abnormality Percentage	Types of abnormality			
			Spindle Disturbance (%)	Chromosomal Disturbance (%)	Spindle & Chromosomal Disturbance (%)	Erosion (%)
Control (0%)	22.59 ±2.36	3.87± 1.15	92.82 ±2.31	Nil	7.11± 1.51	Nil
6.25%	21.51 ±1.06	9.02 ±0.49	66.4 ±2.01	31.1 ±2.57	2.34 ±0.68	Nil
12.5%	19.18 ±1.10	25.85± 0.55	35.01± 1.78	14.83± 2.03	6.07 ±1.98	44.01± 1.11
25%	16.07 1.5	32.72± 3.07	14.40 ±2.01	84.72±2.43	Nil	Nil
50%	14.15 ±0.37	41.72 ±3.5	6.88 ±1.03	92.18 ±3.01	Nil	Nil

Table III: Result of the cytogenetical effect of aqueous leaf extract of *Parthenium hysterophorus* at different concentrations on *Allium cepa* (mean±SD).

Concentration of Leaf extract	Abnormality Percentage	Abnormality at M phase			
		Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
Control (0%)	3.25±1.18	20.08±0.69	29.11±1.15	38.17±1.66	23.55±0.78
6.25%	15.01±10.89	25.30±0.88	51.87±1.35	22.70±2.29	1.13±0.44
12.5%	22.25±2.03	35.13±1.27	33.31± 2.01	16.11±1.77	11.22±0.66
25%	35.25±1.07	39.78±2.89	37.4 ±2.72	19.57±1.66	6.25±2.56
50%	53.29±2.12	35.58±1.69	36.34± 2.99	23.08±1.22	4.99± 2.10

Table IV: Result of the cytogenetical effect of aqueous leaf extract of *Parthenium hysterophorus* at different concentrations on *Lens esculenta* (mean±SD)

Concentration of leaf extract	Abnormality Percentage	Abnormality at M phase			
		Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
Control (0%)	3.81±1.15	21.08±0.61	27.11±2.1	40.2±1.8	18.02±0.79
6.25%	9.02 ±0.49	21.94±1.88	25.07±2.28	39.20±2.33	14.28±0.88
12.5%	25.85±0.55	39.41±1.25	41.32±2.79	17.73±0.69	2.24±1.23
25%	32.72±3.04	42.31±2.5	44.8±1.18	12.19±0.814	1.82±0.85
50%	41.72±3.5	48.57±2.72	39.44±1.56	9.44±1.11	2.52±0.78

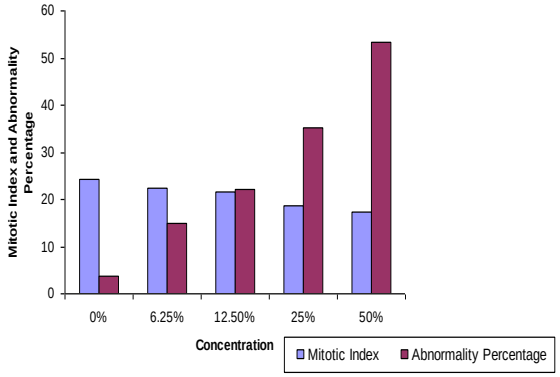

Figure 1: Mitotic Index and Abnormality percentage of Allium *cepa* at different concentration of leaf extract of *Parthenium hysterophorus*

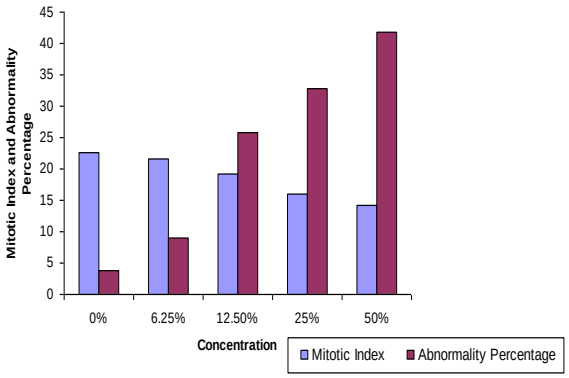

Figure 2: Mitotic Index and Abnormality percentage of *Lens esculenta* at different concentration of *Parthenium* leaf extract

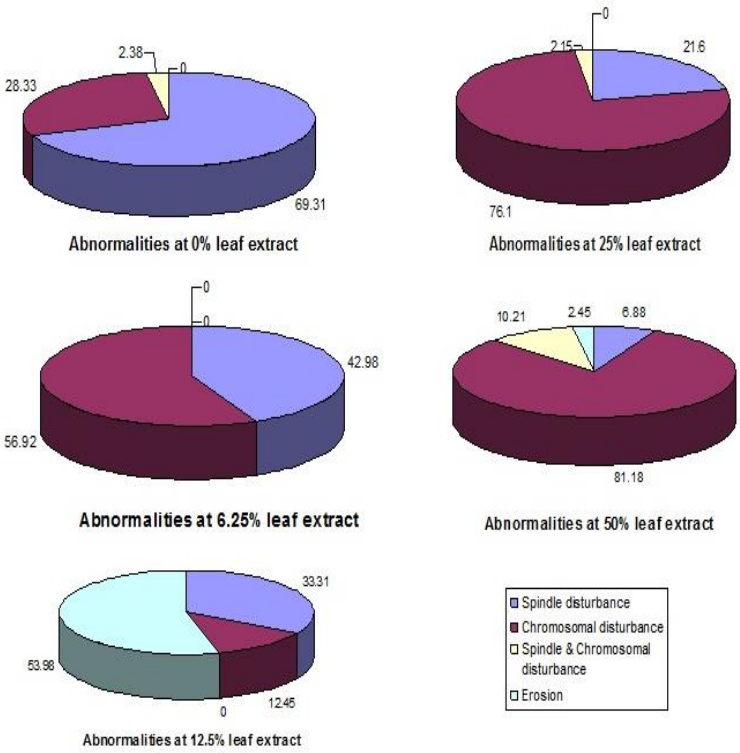

Figure 3: Different types of abnormalities in *Allium cepa* at different concentration of leaf extract of *Parthenium hysterophorus*

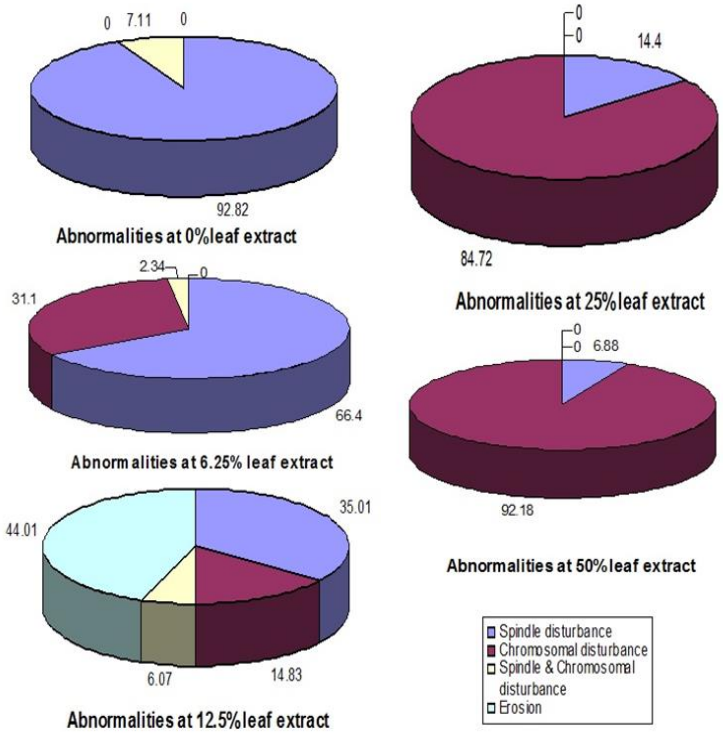

Figure 4: Different types of abnormalities in Lens *esculenta* at different concentration of leaf extract of *Parthenium hysterophorus*

Figure 5: Different types of divisional abnormalities in Allium *cepa* at different concentration of leaf extract of *Parthenium hysterophorus*

Figure 6: Different types of divisional abnormalities in *Lens esculenta* at different concentration of leaf extract of *Parthenium hysterophorus*

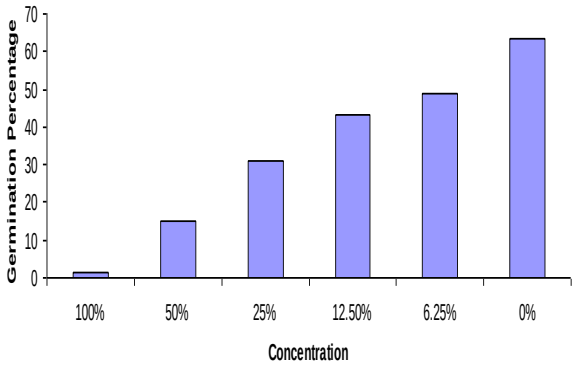

Figure 7: Germination Percentage of seeds of *Lens esculenta* in response to different concentration of Parthenium leaf extract

Table V: Result of Germination performance of *Lens esculenta* on 7th day in response of different concentration of leaf extract of *Parthenium hysterophorus* (mean±SD).

Concentration of leaf extract	Germination percentage
Control (0%)	63.4 ±3.63
6.25%	49 ±1.39
12.5%	43 ±2.09
25%	31.2± 2.58
50%	14.8± 2.7
Concentrate (100%)	1.6 ±0.44

Table VI: Result of Relative germination performance of *Lens esculenta* on 7th day in response of different concentration of leaf extract of *Parthenium hysterophorus*.

Concentration Of Leaf Extract	Relative Germination Percentage
Control (0%)	100
6.25%	77.21
12.5%	67.75
25%	49.16
50%	23.32
Concentrate (100%)	2.5

Table VII: Result of growth performance of *Lens esculenta* on 7th day in response different concentration of leaf extract of *Parthenium hysterophorus* (mean±SD).

Concentration of leaf extract	Length of shoot (cm)	Length of longest leaf (cm)	Length of shortest leaf(cm)	Highest number of leaf
Control (0%)	14.00±1.30	6.81±1.22	3.37±1.4	10.75 ± 0.59
6.25%	10.00±0.81	4.6 ±0.51	2.4±0.63	9 ±0.38
12.5%	8.25± 0.65	3.6 ±0.32	1.89± 0.41	7.5 ± 0.7
25%	7.25±0.95	2.9 ±0.42	1.59±0.21	5.5± 0.8
50%	6.5±0.57	2.1 ± 0.3	0.92 ±0.24	4 ± 0.51
Concentrate (100%)	2.5 ±0.4	1.2± 0.4	0.70±0.31	1.75 ± 0.41

Table VIII: Result of growth performance of *Allium cepa* on 7th day in response of different concentration of leaf extract of *Parthenium hysterophorus* (mean±SD).

Concentration of leaf extract	Length of longest leaf(cm)	Length of shortest leaf(cm)	Highest number of leaf	Lowest number of leaf
Control (0%)	13.87 ± 1.05	5 ± 0.91	5.75 ± 0.45	2.5 ±0.57
6.25%	11.87± 1.25	4.25 ± 0.64	5.5 ± 0.5	2.25± 0.45
12.5%	11 ± 1.84	3.5± 0.4	5.25± 0.81	1.75± 0.5
25%	8.5 ±1.08	3.05 ±0.48	4.75 ±0.62	1.5± 0.57
50%	7.8 ±0.62	2.5 ±0.4	4.25 ±0.55	1.25 ±0.45
Concentrate (100%)	0.75 ±0.21	0.25 ±0.07	1.25 ±0.27	0.5 ±0.23

Table IX: Result of relative growth performance of *Lens esculenta* on 7th day in response to different concentration of leaf extract of *Parthenium hysterophorus*.

Concentration of leaf extract	Relative Length of longest leaf(RLLF)	Relative length of longest leaf (RLLF)	Relative length of shortest leaf (RLSL)	Relative highest number of leaf (RHNL)
Control (0%)	100	100	100	100
6.25%	71.42	67.54	71.21	83.72
12.5%	58.92	52.86	56.08	69.76
25%	51.78	42.58	47.18	51.16
50%	46.42	38.83	27.29	37.20
Concentrate (100%)	17.85	17.62	20.77	13.95

Table X: Result of relative growth performance *Allium cepa* on 7th day in response of different concentration of leaf extract of *Parthenium hysterophorus.*

Concentration of leaf extract	Relative Length of longest leaf(RLLF)	Relative length of shortest leaf(RLSL)	Relative highest number of leaf (RHNL)	Relative lowest number of leaf (RLNL)
Control (0%)	100	100	100	100
6.25%	85.58	81	95.65	90
12.5%	79.3	70	91.30	70
25%	61.28	61	82.6	601
50%	56.23	50	73.91	50
Concentrate (100%)	5.4	5	21.73	20

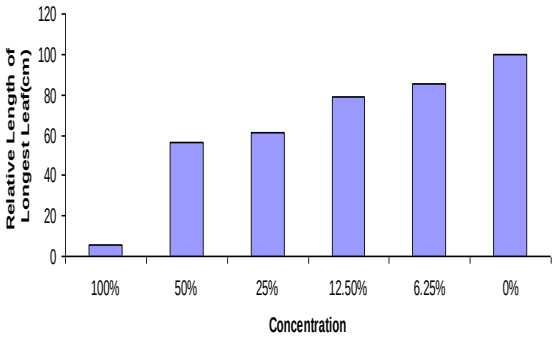

Figure 8: Relative growth of performance of *Allium cepa* at different concentration of Parthenium leaf extract after 7 days

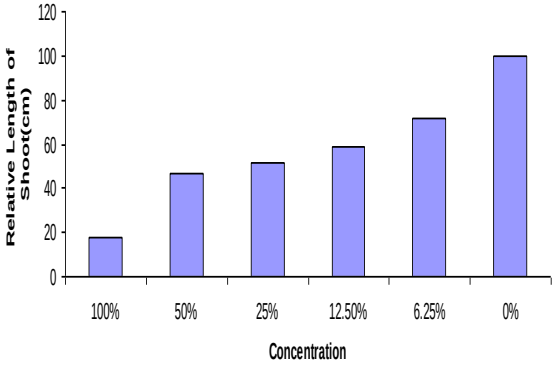

Figure 9: Relative growth of performance of *Lens esculenta* at different concentration of Parthenium leaf extract after 7 days

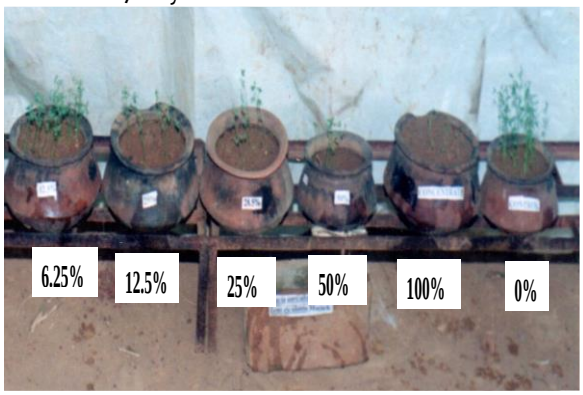

Figure 10: Growth performance of *Lens esculenta* in respect to concentration of leaf extract of Parthenium

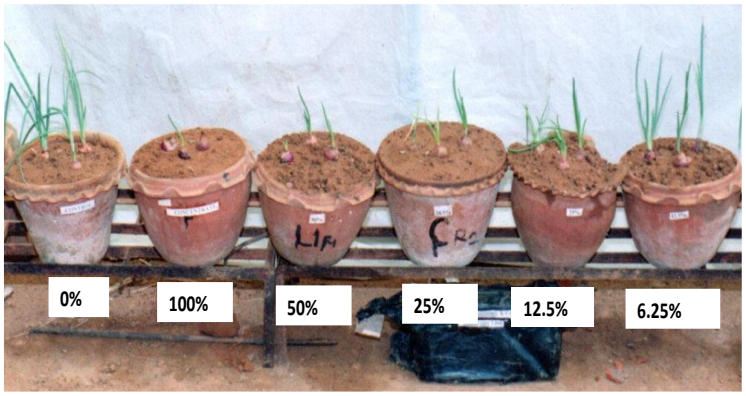

Figure 11: Growth performance of *Allium cepa* in respect to concentration of leaf extract of Parthenium

Figure 12: Chromosomal abnormalities (1) chromosomal disturbance at metaphase and (2) Spindle disturbance at anaphase

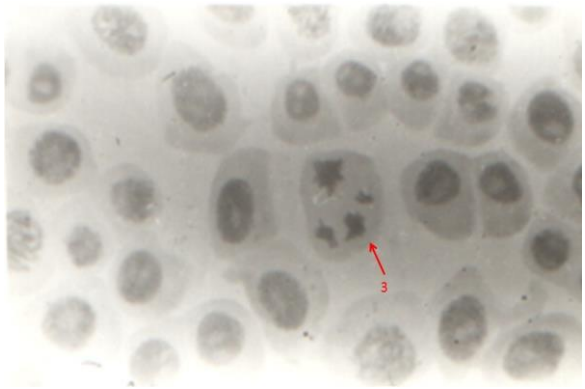

Figure 13: Chromosomal abnormalities (3) chromosomal erosion

CONCLUSION

The allelochemicals are present in the matured leaves of *Parthenium hysterophorus* indicate their potentiality in changing the pattern of cell division, chromosomal conformity of cell as well as growth behaviour of tested plants- *Allium cepa* and *Lens esculenta*. These allelochemicals may act synergistically or antagonistically on these tested crop plants. So it would be complicated and challenging to mark out the appropriate cause of the above result. However this might be due to some interaction between

allelochemicals produced by Parthenium and the tested crop plants. Future detailed studies may suggest to ascertain the mode of action, identification and isolation of such allelochemicals and may provide a basis for development of growth regulators and natural pesticides to boost up production in sustainable agriculture.

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