Chapter 3

Determination of Relative Allelopathic Vigour of Three Weeds of West Bengal by Cytological Approach.

INTRODUCTION:

An investigation was carried out for the determination and evaluation of the allelopathic potential of the three weeds already mentioned in chapters-1 and 2, designing an experiment with cytological approach . Leaf extracts and leachates in the same concentrations as taken and already discussed in chapter-1and 2 from the three weeds are tested on *Allium cepa* and *Vigna radiata* root tips. Cytological evaluation of allelopathic potential is done by microscopic study of allelochemical induced alterations of cytological characters with particular reference to various anomalies during cell-division and chromosomal structural abnormalities like condensation, pole deviation, anaphase bridge, clumping, bi-nucleation ,depurination, fragmentation, stickiness, multinucleate cells, late separation with micronuclei formation etc. Comparison and statistical analysis of Chromosomal Abnormality Index help reveal the hierarchial order of allelopathic vigour. The weeds induce chromosomal abnormalities i.e. high- Chromosomal Abnormality Index (C.A.I) in both *Allium cepa* and *Vigna radiata root tips*.

Materials and Methods:

PHOTOPLATE-A	PHOTOPLATE-B	PHOTOPLATE-C
		
Desmostachya bipinnata **(Poaceae)**	***Parthenium hysterophorus*** **(Asteraceae)**	***Alternanthera sessilis*** **(Amaranthaceae)**
PHOTOPLATE-D		**PHOTOPLATE-E**
	**Bioassay Materials**	
Root tips (*Allium cepa*)		**Root tips (*Vigna radiata*)**

A)Preparation of Root tip (*Allium cepa*):

1) About 40 fresh and viable bulbs of *Allium cepa* (L) were taken and the dried roots were cut from their base of stem disc with a sharp blade and washed thoroughly and placed at 0.5 inch deep wet sand in a clay pot and kept in a well ventilated place at room temperature (30 ± 2°C).

2) After 3 days, at about 1 cm long rooted condition *Allium* blubs were pulled out carefully from the sand and thoroughly washed.

3) Test tubes (10 ml) were filled with leaf extracts of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* of different concentrations as shown in Table-32 , and the bulbs were placed on the test tube in such a way that the roots only immersed within the solution and were kept in room temperature for 18 hrs.

4) The bulbs were then pulled out from the test tubes and rinsed thoroughly with distilled water. The root tips were cut at 2-3 mm of length and fixed overnight in acetic acid and ethanol mixture (1:3).

5) Then root tips were kept for 15 min in 45% acetic acid (For storing root tips were transferred to 70% ethanol and kept in a refrigerator).

6) The material was then transferred to a mixture of 2% aceto-orcein and 1(N) HCl and warmed gently for 5 seconds and kept for 45 minutes in staining mixture.

7) Clean, dry , grease free slides were taken and the tip portion (deep coloured portion of the roots were placed in a drop of 45% acetic acid, covered with a cover glass and was tapped with a match-stick to scatter the cells uniformly.

8) The squash was taken in the fold of a blotting paper to give uniform pressure to spread the material. Excess fluid was blotted out, sealed with paraffin and the slide was finally observed under low and high power (40X and 100X).objective of binocular compound microscope.

B) Preparation of Root tip (*Vigna radiata*):

1) Fully viable green gram seeds were taken and immersed in a beaker half filled with double distilled water and kept in a well ventilated place at room temperature.

2) After sprouting of the plumule, seeds were transferred to moist sand and saw dust mixture (1:1) and kept at room temperature (30 ± 2°C).

3) After 2 days primary roots 1.0 mm to 1.5 mm long were pulled out from the sand saw dust mixture carefully and washed thoroughly with distilled water . Test tubes (10 ml) were then filled with leaf extract and leachates of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* of different concentrations as shown in Table 33 , and the bulbs were placed on the test tube in such a way that the roots only got immersed within the solution and kept in room temperature for 18 hrs.

4) The set ups were kept in a well ventilated and illuminated place at room temperature (30 ± 2°C) for 18 h. The roots were then pulled out from the sand solution mixture and thoroughly rinsed with distilled water.

5) Root tips were cut at 2-3 mm of length and fixed overnight in acetic acid and ethanol mixture

(1:3) and stored in 70% alcohol for subsequent use.

6) Then root tip squashes were made by the haematoxylin technique of Marimuthu and Subramanium (1960) and slightly modified in few steps to make it suitable for cytological investigation.

7) Root tips were hydrolyzed in 0.1 N HCl for 25 min at 70°C and then thoroughly washed with distilled water. The roots were then treated with 4% iron alum (Ferric ammonium sulphate) for 10 min and rinsed thoroughly with double distilled water according to the technique of Wigglesworth (1952).

8) The roots were then transferred to 0.5% haematoxylin stain and kept for 3 h and subsequently washed with water. To soften the tissue , roots were treated with 45% acetic acid for 5 seconds according to the technique of Wittmann (1962).

9) Clean dry and grease free slides were taken and root tips were squashed with the help of cover slip and tapped with match-stick to spread the cells, sealed with paraffin and observed under low and high power objective of binocular compound microscope.

Results:

The Abnormality Index (%) is calculated by the following formula:

$$\text{Abnormality Index} = \frac{\text{Total number of abnormal cells}}{\text{Total number of dividing cells}} \times 100\%$$

(Conc. In weight/volume = w/v, LE = Leaf Extract, LL= Leaf Leachate.)

(D= *Desmostachya bipinnata*; P= *Parthenium hysterophorus*; A= *Alternanthera sessilis*.)

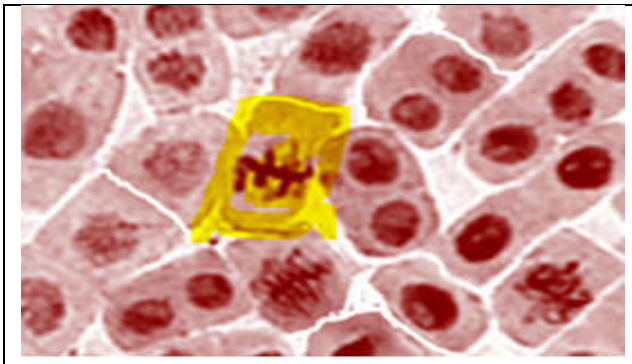
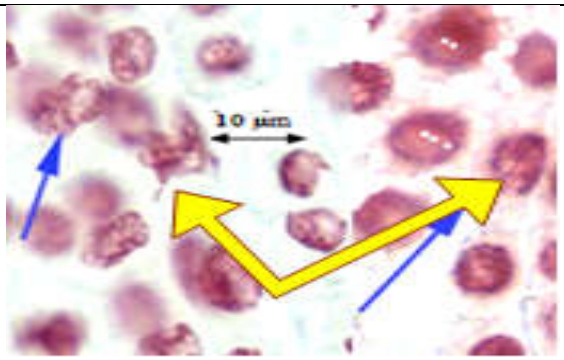
Table-32: Effect of seed pretreatment with leaf extracts and leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* on chromosomal aberrations of *Allium cepa* root tips.

Treatments in concentrations	Total dividing cells	Total cells counted	Condensation	Asynchronous division	Pole deviation	Anaphase bridge	Clumping	Binucleate cells	Depurination	Stickiness	Fragmentation	Micronuclei	Total abnormal cells counted	Chromosomal Abnormality Index (%)
Control	355	2976	0	0	0	0	0	0	0	0	0	0	0	**0**
DLE(1:05)	195	2850	3	2	1	3	2	2	2	2	2	2	21	**10.76**
DLE(1:10)	193	2917	2	3	1	4	3	1	1	1	1	1	18	**9.32**
DLE(1:20)	203	3096	2	1	0	1	2	3	2	0	2	1	14	**6.89**
PLE(1:05)	202	2644	2	1	0	2	1	2	1	2	1	2	14	**6.93**
PLE(1:10)	205	2838	2	1	1	2	2	2	2	0	1	1	14	**6.83**
PLE(1:20)	220	2844	1	0	1	2	1	2	2	2	1	1	13	**5.90**
ALE(1:05)	163	2152	1	1	0	1	1	2	1	1	1	1	10	**6.13**
ALE(1:10)	149	1964	1	1	1	1	1	1	0	0	1	1	8	**5.36**
ALE(1:20)	112	1640	1	0	1	0	1	1	0	0	1	0	5	**4.46**
LSD (p≤0.05)														**3.39**
DLL(1:05)	205	2868	2	1	0	3	2	2	2	2	2	2	18	**8.78**
DLL(1:10)	192	2851	2	2	0	3	2	1	2	0	2	2	16	**8.33**
DLL(1:20)	195	2960	1	2	0	1	2	2	3	0	1	1	13	**6.67**
PLL(1:05)	216	2853	1	1	0	3	1	2	1	2	1	2	14	**6.48**
PLL(1:10)	207	2630	1	1	0	2	1	2	1	1	1	2	12	**5.79**
PLL(1:20)	210	2780	1	0	0	3	2	1	2	0	1	2	12	**5.71**
ALL(1:05)	150	2238	1	1	0	1	1	2	1	1	0	1	9	**6.00**
ALL(1:10)	153	1973	1	1	0	1	1	1	1	1	1	0	8	**5.22**
ALL(1:20)	120	1794	0	0	0	0	1	1	0	1	1	0	4	**4.00**
LSD (p≤0.05)														**0.48**

Table-33: Effect of seed pretreatment with leaf extracts and leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* on chromosomal aberrations of *Vigna radiata* root tips.

Treatments in concentrations	Total dividing cells	Total cells counted	Late separation	Anaphase bridge	Clumping	Binucleate cells	Depurination	Stickiness	Multinucleate cells	Micronuclei	Total abnormal cells counted	Chromosomal Abnormality index (%)
Control	355	2976	0	0	0	0	0	0	0	0	0	**0**
DLE(1:05)	196	2863	3	6	4	3	3	2	2	2	25	**12.75**
DLE(1:10)	191	2917	3	4	5	3	3	1	1	2	22	**11.51**
DLE(1:20)	192	3338	2	3	4	2	2	2	1	1	17	**8.85**
PLE(1:05)	207	2798	2	4	3	3	3	1	3	2	21	**10.14**
PLE(1:10)	195	2815	3	4	3	2	2	1	2	2	19	**9.74**
PLE(1:20)	178	2963	2	3	2	3	1	1	2	1	15	**8.42**
ALE(1:05)	207	2798	2	5	4	3	3	1	3	2	23	**9.87**
ALE(1:10)	195	2815	3	4	3	2	2	1	2	2	19	**9.04**
ALE(1:20)	178	2936	2	3	2	3	1	1	2	1	15	**7.65**
LSD ($p \leq 0.05$)												**0.77**
DLL(1:05)	205	2756	3	5	4	3	3	2	3	3	26	**12.68**
DLL(1:10)	190	2852	3	4	4	1	4	1	1	2	20	**10.52**
DLL(1:20)	197	2860	2	3	3	2	1	2	2	2	17	**8.62**
PLL(1:05)	202	2449	3	4	2	2	1	2	2	2	18	**8.91**
PLL(1:10)	205	2984	2	5	3	1	3	1	0	1	16	**7.80**
PLL(1:20)	207	2850	1	4	2	1	1	1	2	1	13	**6.28**
ALL(1:05)	309	2794	4	6	3	3	3	2	3	2	26	**8.41**
ALL(1:10)	287	2811	3	5	4	1	4	1	1	2	21	**7.31**
ALL(1:20)	309	2773	2	3	3	2	1	2	2	2	17	**5.50**
LSD ($p \leq 0.05$)												**0.49**

Photographs (X 100) showing the Chromosomal Aberrations as shown (PHOTOPLATES 25-32 *) in treated samples & normal chromosomal behavior in CONTROL -1 and CONTROL-2 sample (PHOTOPLATE 29 & 30)-of Allium cepa root tip cells.*

<table>
<tr><td>  </td><td> 
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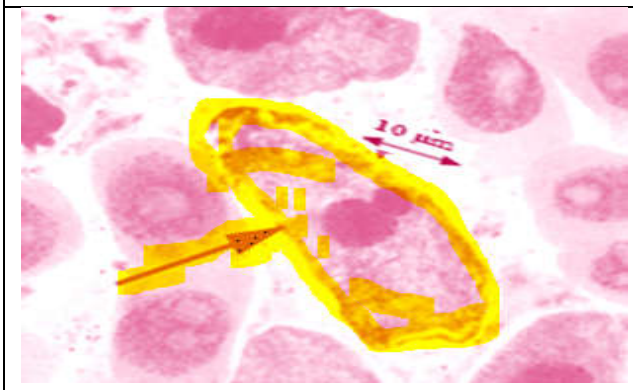
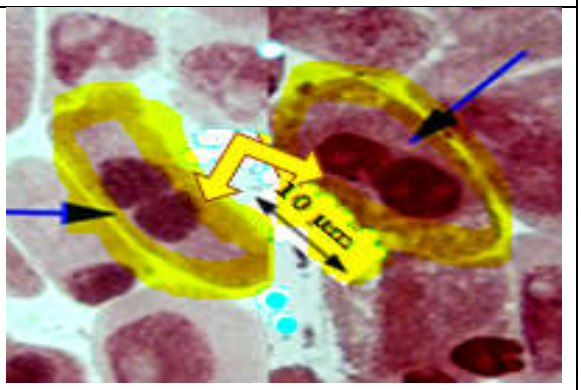
<tr><td>PHOTOPLATE-25 (X 100) (Chromosome Stickiness)</td><td>PHOTOPLATE- 26 (Depurination) (X 100)</td></tr>
<tr><td> 
</td><td> 
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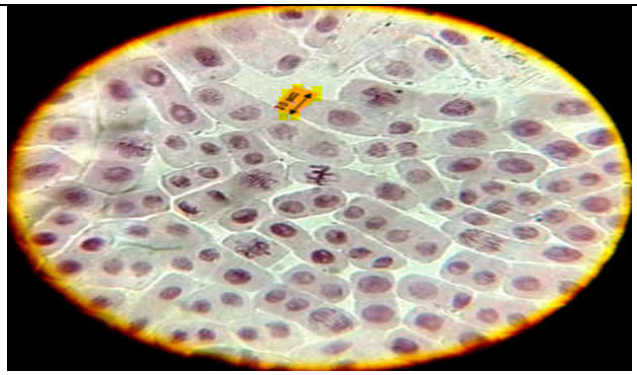
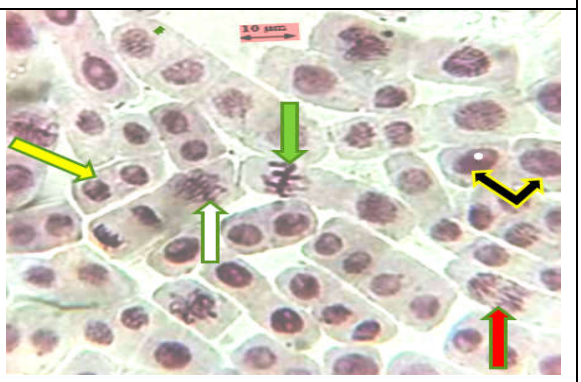
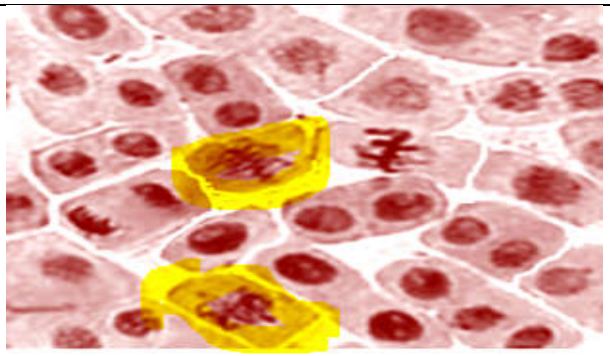
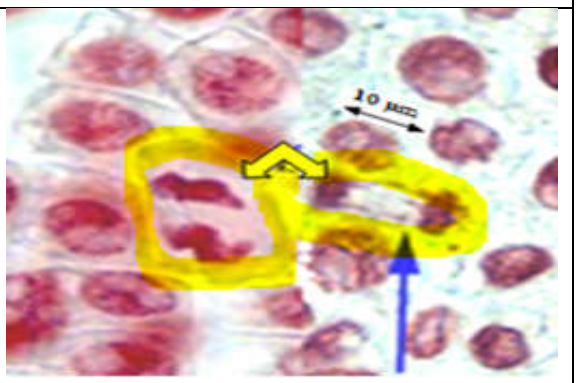
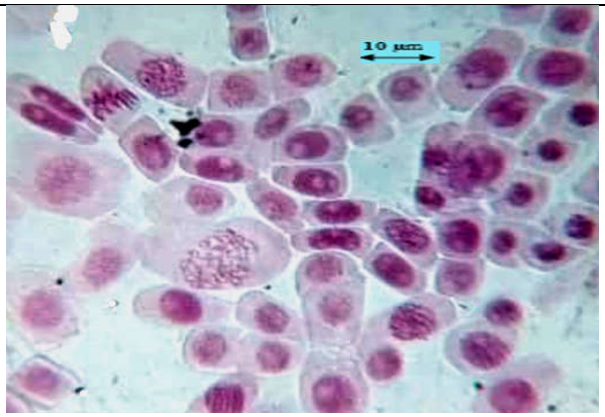
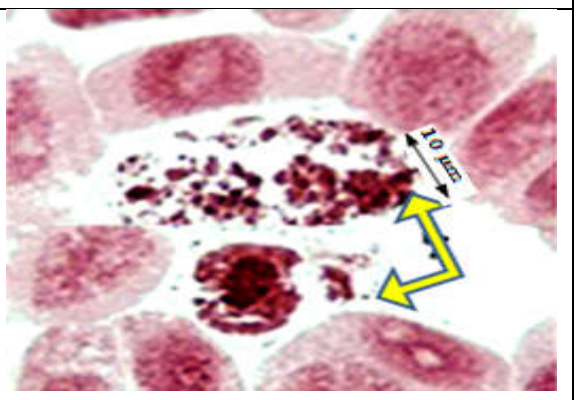
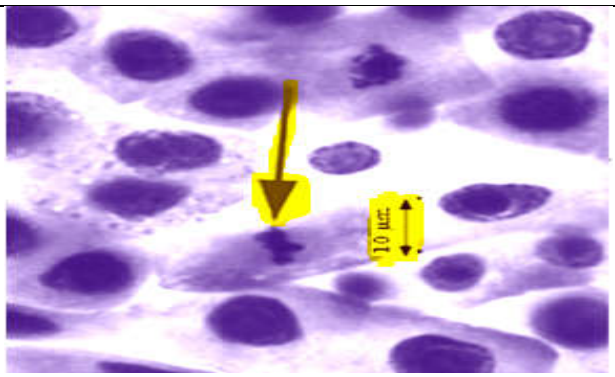
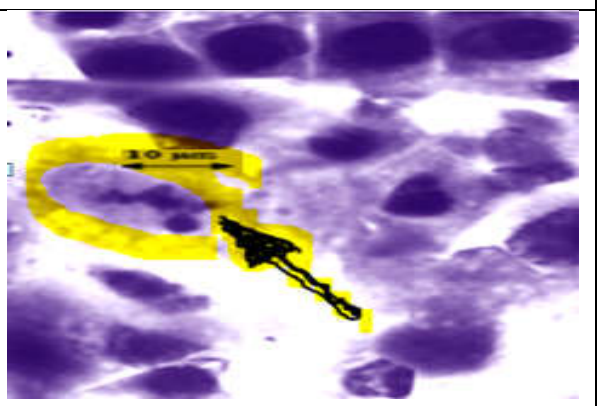
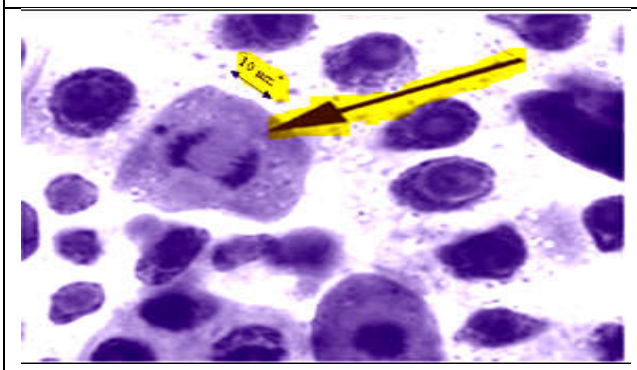
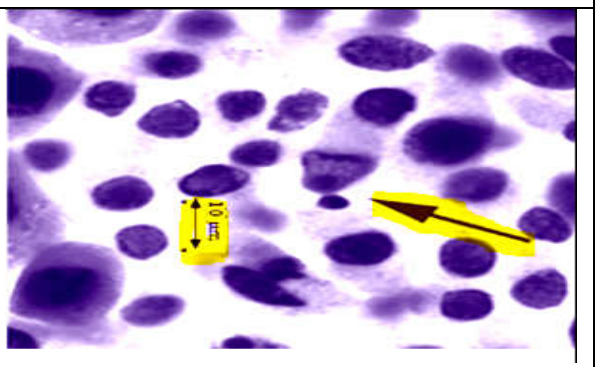
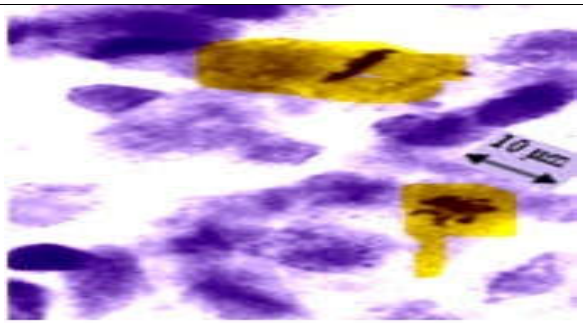
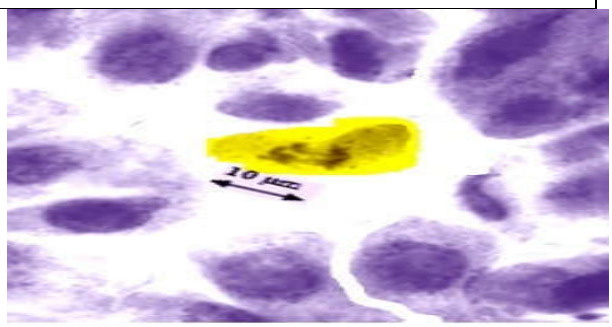
<tr><td>PHOTOPLATE-27 (Micronucleus). (X 100)</td><td>PHOTOPLATE-28 (Binucleate cells) (X 100)</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td><u>CONTROL</u>-1 (X 40) Normal Mitosis with Metaphase) PHOTOPLATE-29</td><td><u>CONTROL</u>-2 (X 40) (Normal Mitosis with Anaphase) PHOTOPLATE-30</td></tr>
</table>

PHOTOPLATE-31	PHOTOPLATE-32
	
Highlighted cells in yellow: **(Chromosomal Clumping). (X 100)**	**Highlighted cells in yellow:** **(Anaphase Bridge). (X 100)**
PHOTOPLATE-33	**PHOTOPLATE-34**
	
(X 40) (Normal Mitosis with Prophase)	**(Chromosome fragmentation). (X 100)**

Photographs (X 100) showing the Chromosomal Aberrations as shown (PHOTOPLATES 33 -44) in treated samples & normal chromosomal behavior in CONTROL (A & B) *(PHOTOPLATE 39 & 40)*- of *Vigna radiata* root tip cells.

PHOTOPLATE-35 (Chromosome Stickiness)	PHOTOPLATE-36 (Multinucleation)
	
PHOTOPLATE- 37 (Late separation with micronucleus).	PHOTOPLATE-38 (Binucleate cells)
	
CONTROL-A PHOTOPLATE 39 Highlighted cells (Normal Mitosis with Metaphase)	**CONTROL-B PHOTOPLATE-40** Highlighted cells (Normal Mitosis with Anaphase)
	

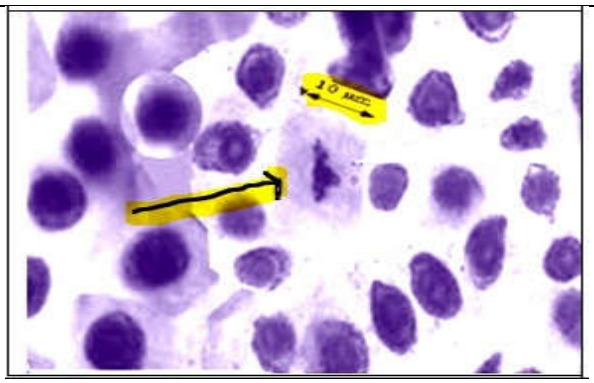
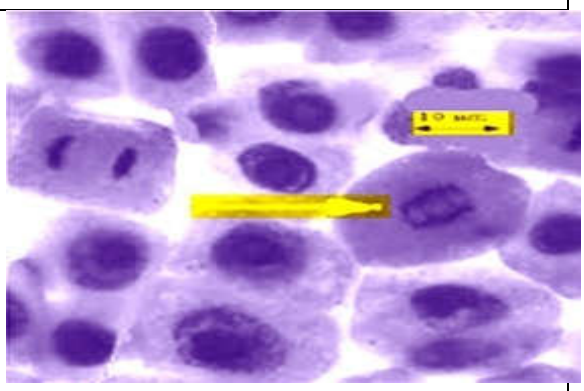
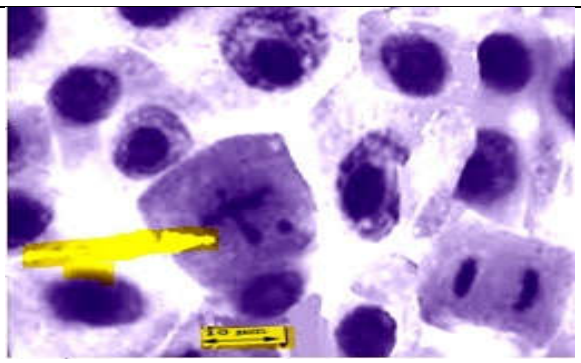
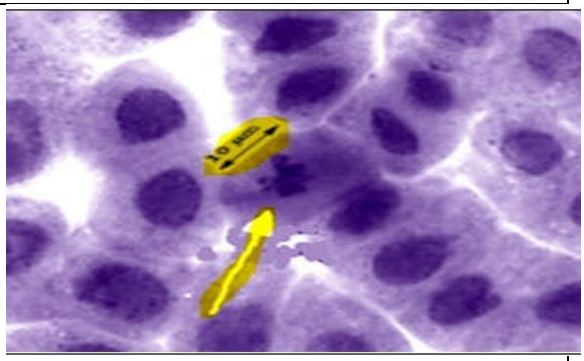
PHOTOPLATE-41 (Clumping)	PHOTOPLATE-42 (Anaphase Bridge)
	
(Late separation with micronucleus)	(Chromosome fragmentation)
	
PHOTOPLATE-43	**PHOTOPLATE- 44**

Figure-10 **Comparative Histogram of effect of seed pretreatment with leaf extracts and leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* on chromosomal aberrations of *Allium cepa* root tips.**

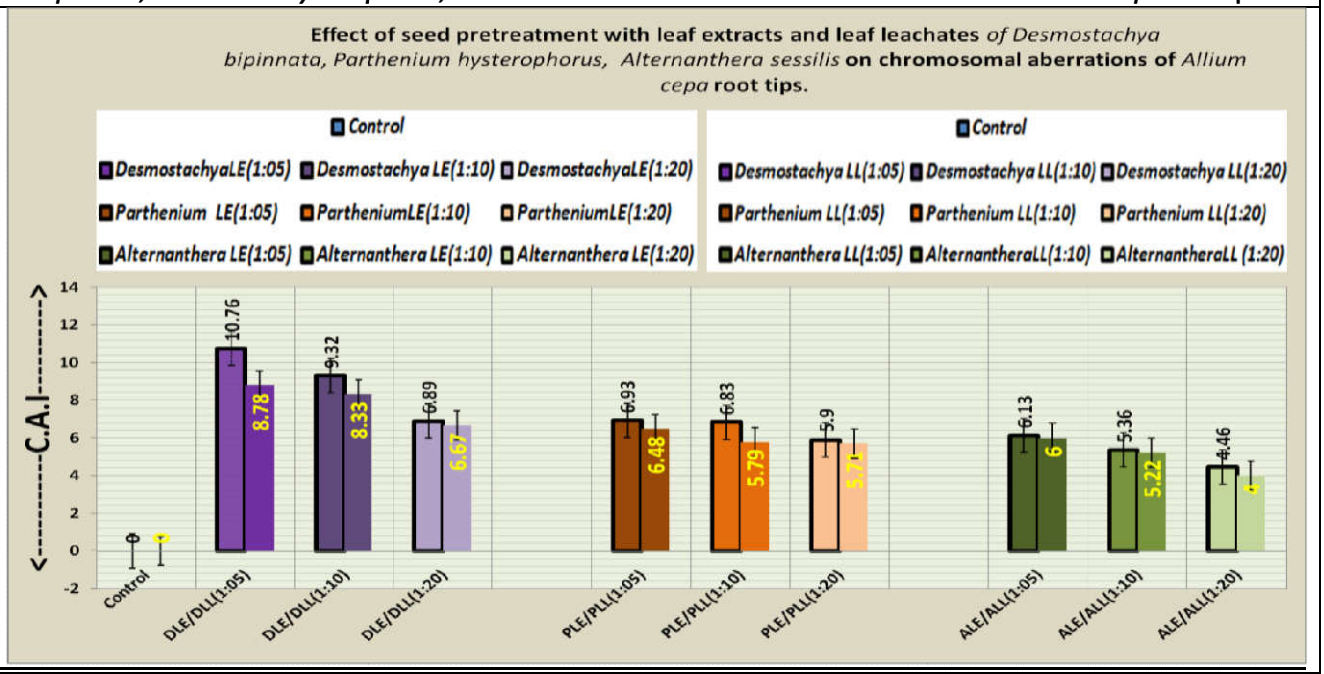
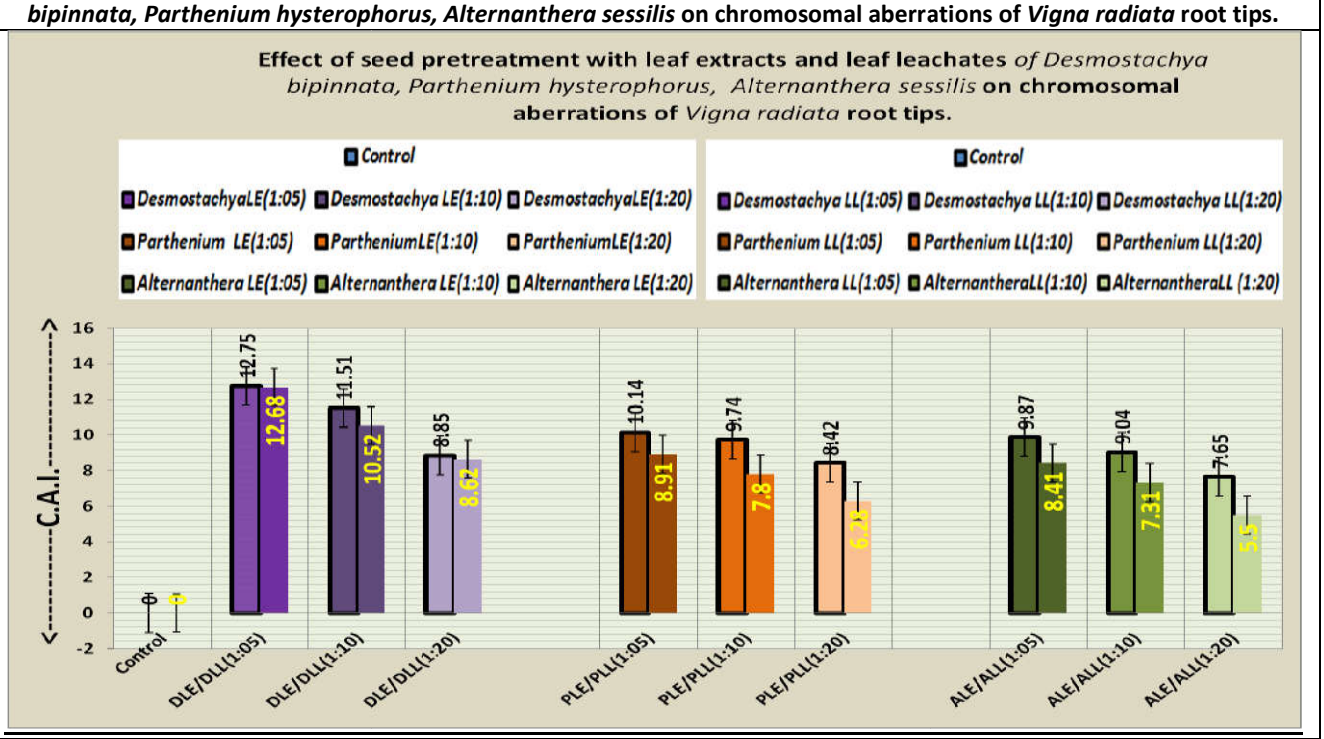


Figure-11 **Comparative Histogram of effect of seed pretreatment with leaf extracts and leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus, Alternanthera sessilis* on chromosomal aberrations of *Vigna radiata* root tips.**



Statistical analysis: (Table 32)

Analysis of Variance
Groups: Control, *Desmostachya* extract,, *Parthenium extract, Alternanthera extract, Desmostachya* leachates, *Parthenium* leachates, *Alternanthera* leachates

ANOVA					
Chromosomal Abnormality Index (%)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.067	6	12.511	11.482	.000
Within Groups	13.075	12	1.090		
Total	88.142	18			

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Total dividing cells	Between Groups	44183.193	6	7363.865	36.345	.000
	Within Groups	2431.333	12	202.611		
	Total	46614.526	18			
Total cells counted	Between Groups	3391018.667	6	565169.778	20.873	.000
	Within Groups	324925.333	12	27077.111		
	Total	3715944.000	18			
Condensation	Between Groups	7.439	6	1.240	5.579	.006
	Within Groups	2.667	12	.222		
	Total	10.105	18			
Asynchronous division	Between Groups	6.667	6	1.111	2.500	.083
	Within Groups	5.333	12	.444		
	Total	12.000	18			

Pole deviation	Between Groups	2.105	6	.351	2.105	.128
	Within Groups	2.000	12	.167		
	Total	4.105	18			
Anaphase bridge	Between Groups	16.351	6	2.725	3.504	.031
	Within Groups	9.333	12	.778		
	Total	25.684	18			
Clumping	Between Groups	6.632	6	1.105	6.632	.003
	Within Groups	2.000	12	.167		
	Total	8.632	18			
Binucleate cells	Between Groups	3.965	6	.661	1.699	.204
	Within Groups	4.667	12	.389		
	Total	8.632	18			
Depurination	Between Groups	8.772	6	1.462	5.263	.007
	Within Groups	3.333	12	.278		
	Total	12.105	18			
Stickiness	Between Groups	2.526	6	.421	.505	.793
	Within Groups	10.000	12	.833		
	Total	12.526	18			
Fragmentation	Between Groups	3.789	6	.632	3.789	.024
	Within Groups	2.000	12	.167		
	Total	5.789	18			
Micronuclei	Between Groups	7.193	6	1.199	4.316	.015
	Within Groups	3.333	12	.278		
	Total	10.526	18			
Total abnormal cells counted	Between Groups	402.526	6	67.088	13.880	.000
	Within Groups	58.000	12	4.833		
	Total	460.526	18			

Linear Regression Analysis:

ANOVA[a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	91.570	14	6.541	48.867	.001[b]
	Residual	.535	4	.134		
	Total	92.105	18			
a. Dependent Variable: Chromosomal Abnormality Index (%)						
b. Predictors: (Constant), Groups, Binucleate cells, Anaphase bridge, Stickiness, Pole deviation, Fragmentation, Asynchronous division, Depurination, Micronuclei, Total dividing cells, Condensation, Clumping, Total cells counted, Total abnormal cells counted						

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.022	2.048		1.476	.214
	Total dividing cells	.006	.011	.144	.601	.580
	Total cells counted	-.002	.001	-.366	-1.298	.264
	Condensation	-.166	.599	-.055	-.277	.796
	Asynchronous division	-.371	.610	-.134	-.609	.576
	Pole deviation	-.512	.719	-.108	-.713	.515
	Anaphase bridge	-1.218	.576	-.643	-2.113	.102
	Clumping	.400	.981	.122	.408	.704
	Binucleate cells	-.711	.572	-.218	-1.244	.281
	Depurination	-.384	.638	-.139	-.602	.579
	Stickiness	-.294	.800	-.108	-.367	.732
	Fragmentation	-.849	.735	-.213	-1.154	.313
	Micronuclei	-.134	.762	-.045	-.175	.869
	Total abnormal cells counted	.998	.627	2.231	1.590	.187
	Groups	.047	.135	.039	.350	.744
a. Dependent Variable: Chromosomal Abnormality Index (%)						

Statistical analysis: (Table 33)

Analysis of Variance
Groups: Control, *Desmostachya* extract,, *Parthenium extract, Alternanthera extract, Desmostachya* leachates, *Parthenium* leachates, *Alternanthera* leachates

ANOVA					
Chromosomal Abnormality index (%)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115.733	6	19.289	8.227	.001
Within Groups	28.135	12	2.345		
Total	143.869	18			

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Total dividing cells	Between Groups	46900.772	6	7816.795	71.531	.000
	Within Groups	1311.333	12	109.278		
	Total	48212.105	18			
Total cells counted	Between Groups	157445.298	6	26240.883	.967	.486
	Within Groups	325475.333	12	27122.944		
	Total	482920.632	18			
Late separation	Between Groups	7.754	6	1.292	2.326	.101
	Within Groups	6.667	12	.556		
	Total	14.421	18			
Anaphase bridge	Between Groups	18.281	6	3.047	2.493	.084
	Within Groups	14.667	12	1.222		
	Total	32.947	18			

Clumping	Between Groups	17.614	6	2.936	6.605	.003
	Within Groups	5.333	12	.444		
	Total	22.947	18			
Binucleate cells	Between Groups	9.123	6	1.520	2.737	.065
	Within Groups	6.667	12	.556		
	Total	15.789	18			
Depurination	Between Groups	7.860	6	1.310	.943	.500
	Within Groups	16.667	12	1.389		
	Total	24.526	18			
Stickiness	Between Groups	3.439	6	.573	2.579	.077
	Within Groups	2.667	12	.222		
	Total	6.105	18			
Multinucleate cells	Between Groups	6.491	6	1.082	1.498	.259
	Within Groups	8.667	12	.722		
	Total	15.158	18			
Micronuclei	Between Groups	4.772	6	.795	2.863	.057
	Within Groups	3.333	12	.278		
	Total	8.105	18			
Total abnormal cells counted	Between Groups	433.965	6	72.327	4.858	.010
	Within Groups	178.667	12	14.889		
	Total	612.632	18			

Linear Regression Analysis:

ANOVA[a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	151.848	11	13.804	52.611	.000[b]
	Residual	1.837	7	.262		
	Total	153.684	18			
a. Dependent Variable: Chromosomal Abnormality index (%)						
b. Predictors: (Constant), Groups, Clumping, Multinucleate cells, Total dividing cells, Stickiness, Total cells counted, Late separation, Anaphase bridge, Binucleate cells, Depurination, Micronuclei						

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	10.495	5.514		1.903	.099
	Total dividing cells	-.024	.003	-.431	-8.323	.000
	Total cells counted	-.001	.002	-.030	-.296	.776
	Late separation	-.120	.292	-.037	-.410	.694
	Anaphase bridge	.303	.246	.140	1.233	.258
	Clumping	-.134	.380	-.052	-.352	.735
	Binucleate cells	.581	.312	.186	1.862	.105
	Depurination	.645	.316	.258	2.046	.080
	Stickiness	.991	.394	.197	2.513	.040
	Multinucleate cells	-.560	.359	-.176	-1.559	.163
	Micronuclei	1.956	.775	.449	2.523	.040
	Groups	-.362	.101	-.234	-3.568	.009
a. Dependent Variable: Chromosomal Abnormality index (%)						

Excluded Variables[a]						
Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	Total abnormal cells counted	b .	----.	. ----.	----.	.000
a. Dependent Variable: Chromosomal Abnormality index (%)						
b. Predictors in the Model: (Constant), Groups, Clumping, Multinucleate cells, Total dividing cells, Stickiness, Total cells counted, Late separation, Anaphase bridge, Binucleate cells, Depurination, Micronuclei						

DISCUSSION:

Effect on chromosomal aberrations of *Allium cepa and Vigna radiata* root tips:

(Table 32 & 33):

Leaf extracts and leachates of *Desmostachya bipinnata*, *Parthenium hysterophorus* and *Alternanthera sessilis* induced chromosomal abnormalities in *Allium cepa* root tips.They exhibited many chromosomal abnormalities such as formation of condensation, asynchronous division, pole deviation, anaphase bridge, clumping, bi-nucleated cells ,depurination, fragmentation, micronuclei (Table 32) and late separation, anaphase bridge, clumping, binucleate cells, depurination, stickiness, multinucleate cells, late separation with micronucleus (Table 33).

Control samples in both the cases showed the normal mitotic division whereas the treated samples exhibited various abnormalities. It was shown that the value of aberration percent C.A.I.% were higher in the treated samples of *Desmostachya sp* than *Parthenium sp.* followed by *Alternanthera sp* with increasing order of concentration of extracts i.e.(1:5>1:10>1:20) . C.A.I. % induced by the leaf extracts and leachates of each of the three plants may be attributed to the effect of allelochemicals like quercetin, quercitrin, kaempferol common to the three plants which can inhibit topoisomerase I and II enzyme activity and interfere with the replication and transcription process and inhibit the process of the religation of DNA, thereby inhibiting receptor plant's mitosis, interfere with normal plant cell's DNA repair and destroy the structure and function of plant cell spindle fibers, leading to- chromosomal aberrations, micronuclei, chromosomal fragmentation and chromosome anaphasic bridges.

The results show that the allelochemicals of *Desmostachya are* maximum damaging as compared to *Parthenium* and *Alternanthera* .

. The results of this study show that, *Desmostachya bipinnata* has strong allelopathic effects by changing the plant cell membrane permeability and function, affecting the process of plant photosynthesis, inhibition of cell mitosis, interference with the normal process of repair of DNA

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