PHYTOCHEMICAL STUDIES

(A) Phytochemical screening of the major plant constituents :-

1) *Cleome droserifolia* : The phytochemical screening of the alcoholic extract of the aerial parts of *C. droserifolia* plant was carried out. The results obtained in Table [1] indicate the presence of tannins, flavonoids, sterols, terpenes, glycosides and /or carbohydrates,, saponins, resins, alkaloids, and mucilage.

Table [1] : Phytochemical screening of the alcoholic extracts of the aerial parts of *C. droserifolia* and *P. oleracea*

TEST FOR	RESULT	
	C. droserifolia	*P. oleracea*
Tannins	+ve	+ve
Flavonoids	+ve	-ve
Sterols	+ve	+ve
Terpenes	+ve	+ve
Glycosides and /or carbohydrates	+ve	+ve
Saponins	+ve	-ve
Resins	+ve	+ve
Alkaloids	+ve	+ve
Mucilage	+ve	+ve

2) *Portulaca oleracea* : The results of the phytochemical screening of the alcoholic extract of *P. oleracea* aerial parts are shown in Table [1]. These results indicate the presence of tannins, resins, sterols, terpenes, alkaloids, glycosides and/or carbohydrates, and mucilage, while flavonoids and saponins are absent.

(B) Isolation and elucidation of structure of the pure chemical constituents :-

1. Compound (1) [mucilage isolated from *Cleome droserifolia*] :-

Compound (1) is an amorphous cream-coloured substance. It is suggested to be a polysaccharide in which lactose moeity is the main consisting sugar. This suggestion was confirmed by paper chromatography of the compound hydrolysate as shown in Fig. [3]. The solvent system used was n-butanol - acetic acid - water (4:1:5) and the reagent was aniline phthalate.

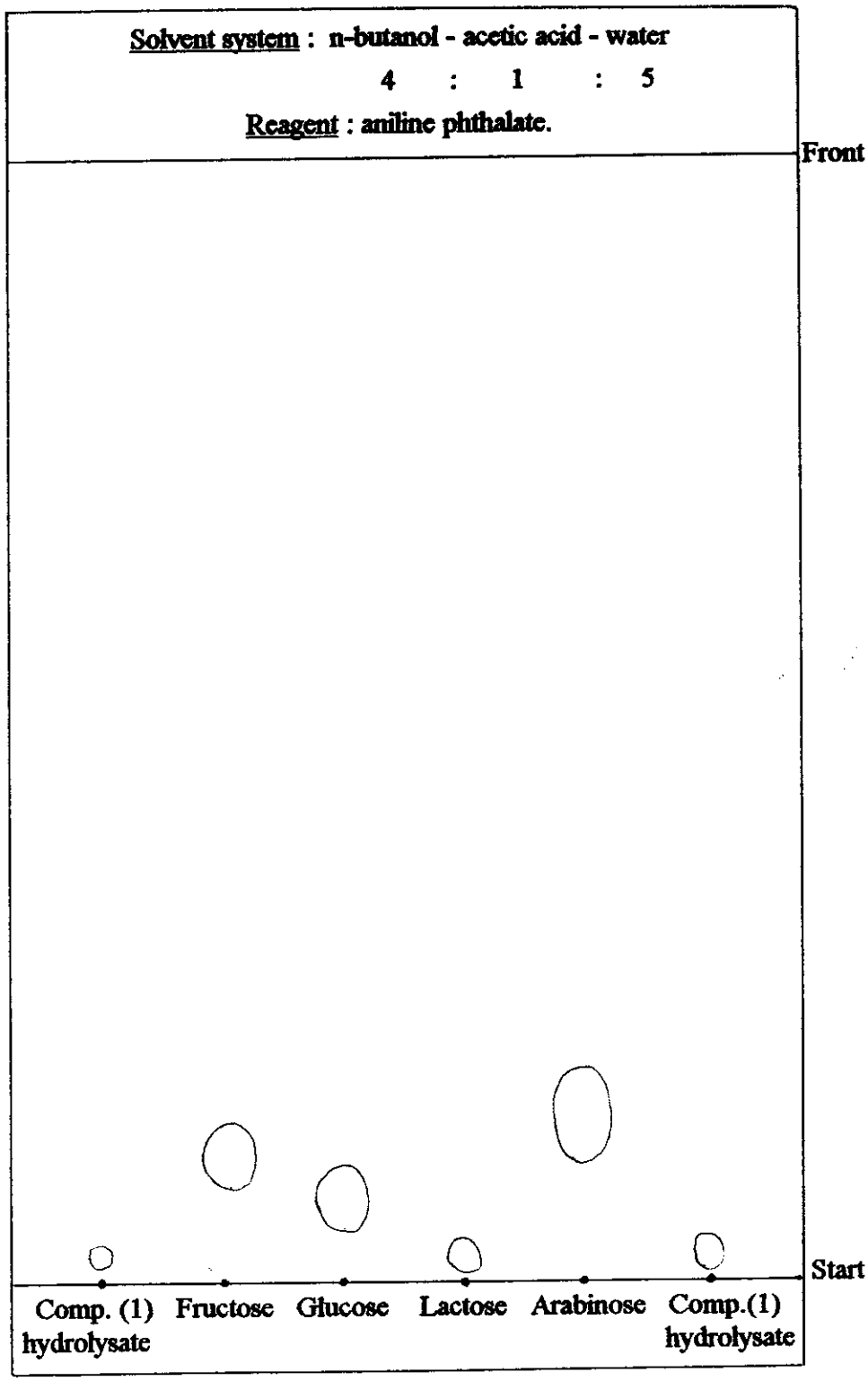


Fig. [3] : Ascending paper chromatogram of compound (1) hydrolysate

Mass spectrum data in Fig. [4] and Table [2] represent the possible fragmentations of compound (1).

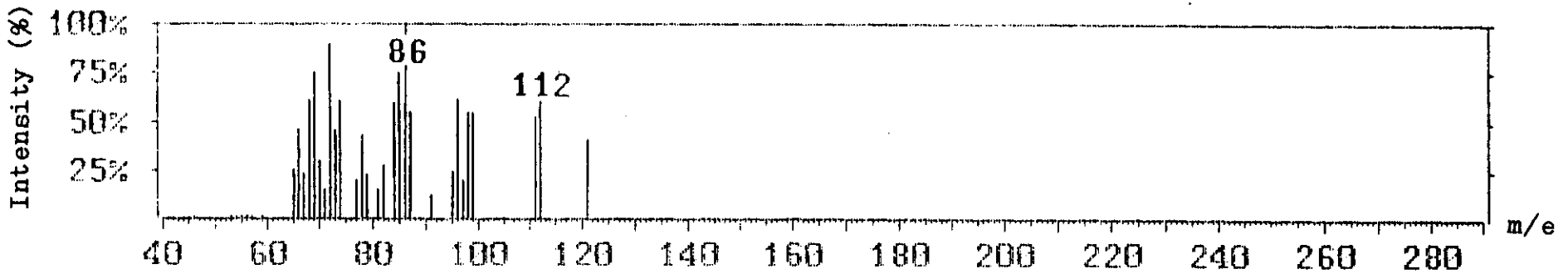


Fig. [4] : Mass spectrum analysis of compound (1)

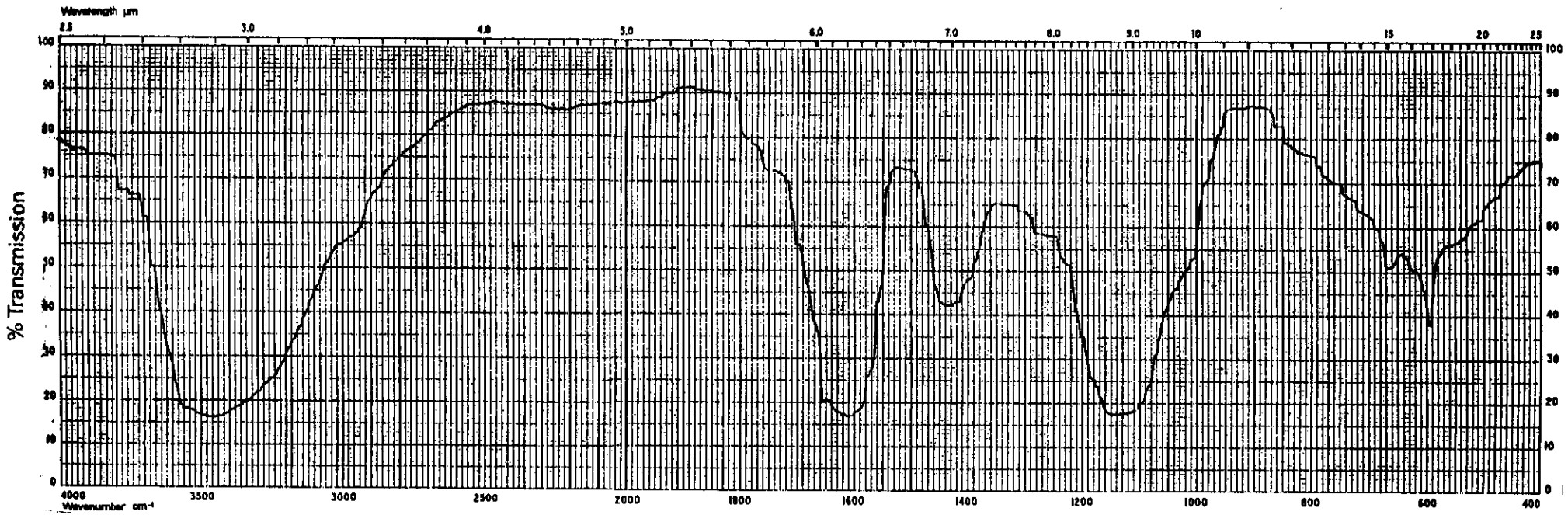
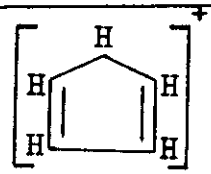
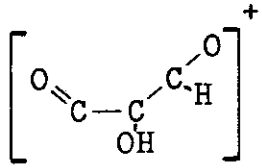
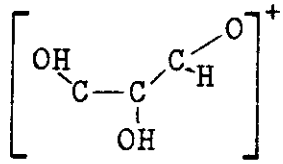
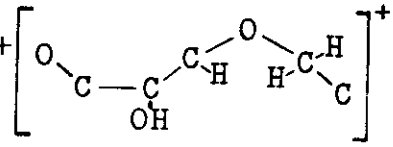
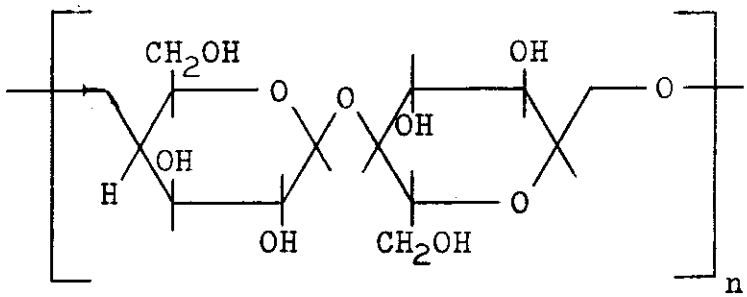


Fig. [5] : IR spectrum analysis of compound (1)

Table [2] : The possible fragmentations of compound (1)

m/e	Intensity	Fragment	
65	25	$(C_5H_5)^+$	
86	100	$(C_3H_2O_3)^+$	
87	55	$(C_3H_3O_3)^+$	
112	60.3	$(C_5H_4O_3)^+$	

IR spectral data confirmed the suggested structure [Fig. 5], where a strong peak at 3500 cm^{-1} represents the presence of the hydroxyl group. Also a strong peak at 1140 cm^{-1} represents the O-H linkage of the glucose-galactose moeity to form the lactose sugar.



α-Lactose 1-4 linkage

Compound (1)

2. Compound (2) [mucilage isolated from *Portulaca oleracea*] :-

Compound (2) is a cream-coloured amorphous substance. This compound is suggested to be a polysaccharide in which glucose moeity is the main consisting sugar.

Paper chromatography of compound (2) hydrolysate confirmed the presence of glucose [Fig. 6]. The used solvent system was n-butanol - acetic acid - water (4:1:5) and aniline phthalate as a reagent.

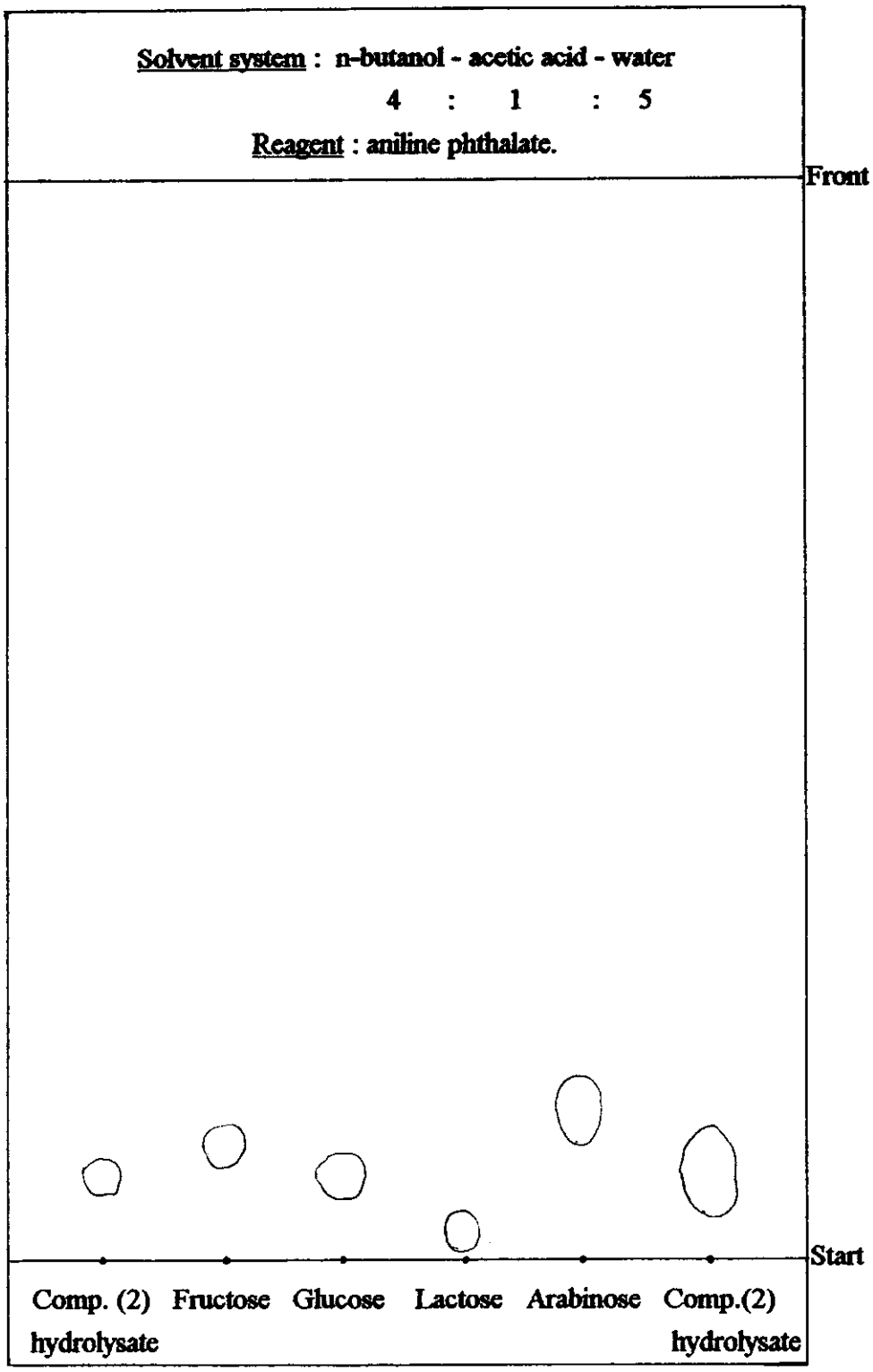


Fig. [6] : Ascending paper chromatogram of compound (2) hydrolysate

Mass spectrum data in Fig. [7] and Table [3] represent the possible fragmentations of the compound.

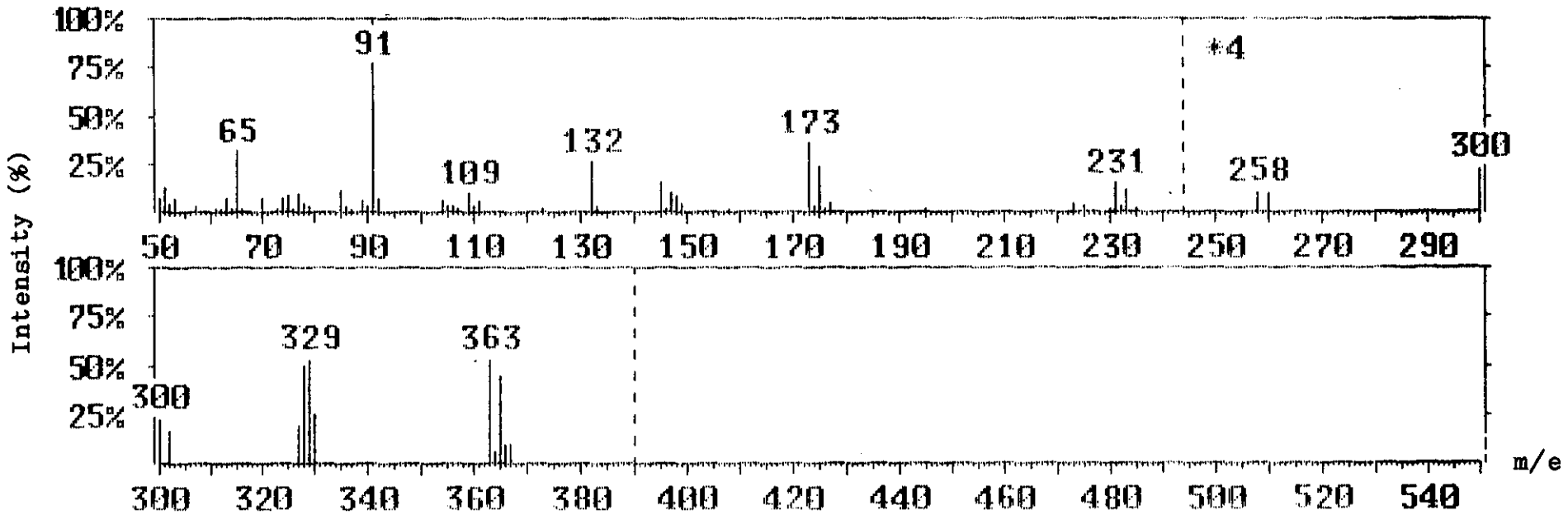
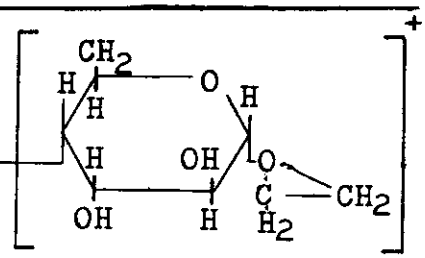
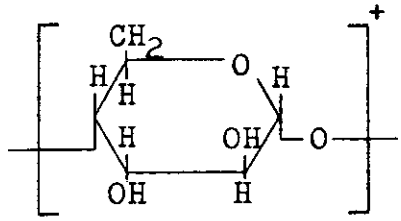
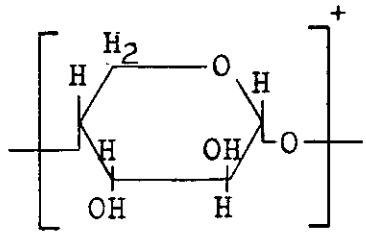
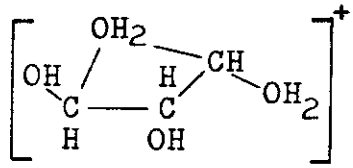
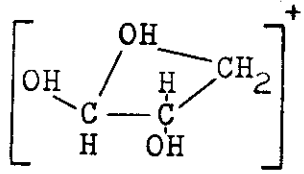
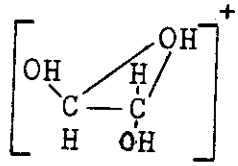
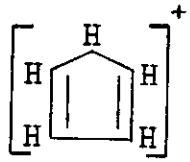
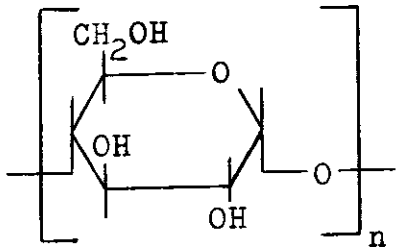


Fig. [7] : Mass spectrum analysis of compound (2)

Table [3] :The possible fragmentations of compound (2)

m/e	Intensity	Fragment
173	36	$(C_8H_{13}O_4)^+$ 
145	15.3	$(C_6H_9O_4)^+$ 
132	26.3	$(C_5H_8O_4)^+$ 
109	10.1	$(C_3H_9O_4)^+$ 
91	100.0	$(C_3H_7O_3)^+$ 
77	10.3	$(C_2H_5O_3)^+$ 
65	31.9	$(C_5H_5)^+$ 

IR spectrum data in Fig. [8] confirmed the suggested structure, where a medium peak at 3500 cm^{-1} represents the presence of the hydroxyl group. Also a very strong peak at 1000 cm^{-1} confirmed the structure of glucose moeity.



α- glucose 1-4 linkage

Compound (2)

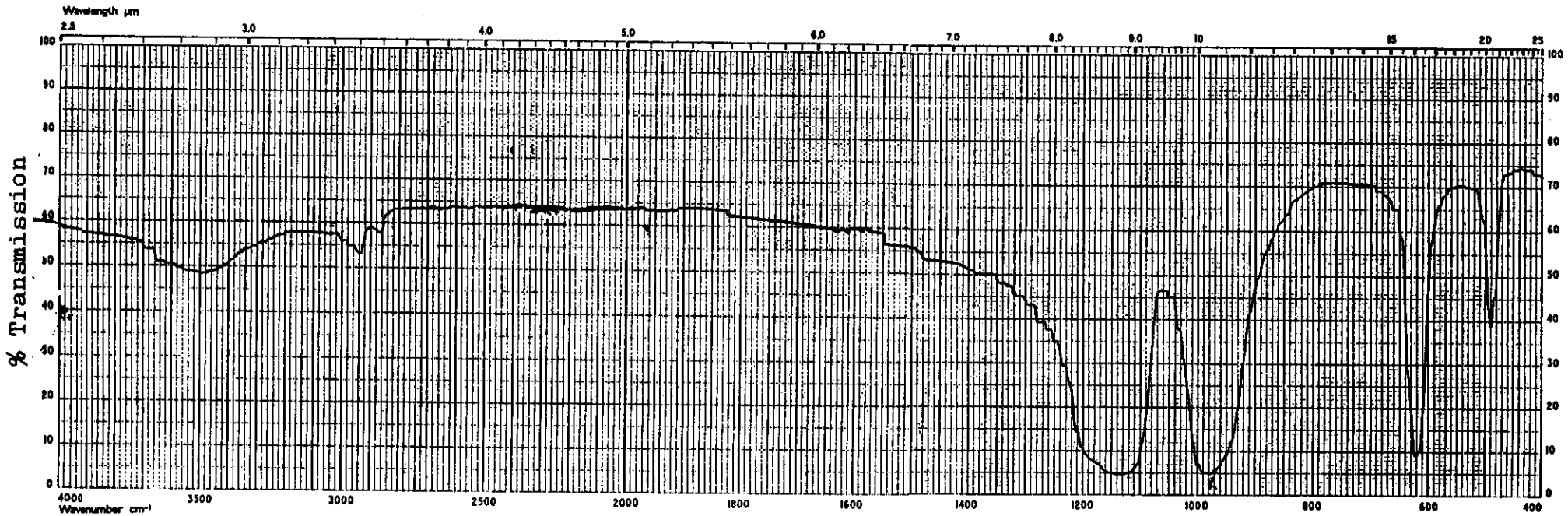


Fig. (8) IR spectrum analysis of compound (2)

CYTOLOGICAL STUDIES

I - LIGHT MICROSCOPY

i - Mitotic Investigations :-

A. Effects of plant extracts and chemicals on the rate of cell division (expressed as mitotic index MI) :-

1) Effect of *Cleome droserifolia* extract :-

The treatment of *A. cepa* root meristematic tissue with *C. droserifolia* extract resulted in a highly significant rreduction in the rate of cell division (MI) as compared to the control. This reduction was concentration rather than duration-dependent [Table 5]. Table [4] and Fig. [9] show that the direct treatment scored a maximum MI (6.8) after treatment with 0.25% *Cleome* extract for 4 hours [the lowest concentration for the shortest time duration tested] as compared to that of the control (8.6). The minimum scored MI was 2.2 after treatment with 3% extract [the highest applied concentration] for 4 hours. However, some treatments (1% extract for 8 hours, 1% for 24 hours and 3% for 8 hours) showed a-mitosis where cell division was stopped completely with no signs of toxicity. The latter - toxicity - occurred when roots blackened and lost their turgor. Toxicity occurred here when roots were treated with 3% extract for 24 hours [the highest concentration for the longest duration], and as appeared in Fig. [9] this toxic effect could not be recovered.

Within the same time duration of direct treatments, it is apparent that increasing the extract concentration caused a significant reduction in the MI values [Fig. 9]. However, the difference between the MI values of the 1% and 3% extract for 4 hours as well as 0.25% and 0.5% for 24 hours were not significant.

Table [4] : Mitotic index and phase index in Allium cepa root tip cells after direct and recovery treatments with Cleome extract

Treatment		Total number of counted cells		Mitotic Index		Phase Index of					
						Prophase		Metaphase		Ana-telophase	
Extract conc. (%)	Duration (hours)	D	R	D	R	D	R	D	R	D	R
Control		5919	5725	8.6	9.6	37.3	32.9	14.5	9.7	48.2	57.5
0.25	4	6265	5603	6.8	7.3	41.0	40.5	12.0	11.9	47.0	47.6
	8	5762	5788	4.6	6.6	37.1	30.7	15.7	21.7	47.2	47.6
	24	5266	5585	3.9	6.8	20.2	33.6	21.4	13.5	58.4	52.9
0.5	4	5377	5758	4.8	4.0	40.3	23.2	18.9	22.3	40.8	54.5
	8	5532	5799	2.5	8.2	27.7	33.6	10.2	16.6	62.1	47.8
	24	5461	5958	2.7	8.9	33.5	32.2	19.7	15.0	46.8	52.8
1.0	4	5368	5500	2.7	3.5	19.5	21.9	17.0	23.3	63.5	54.8
	8	A-M.	6026	A-M.	8.5	A-M.	35.5	A-M.	17.8	A-M.	61.7
	24	A-M.	5829	A-M.	8.8	A-M.	31.3	A-M.	16.0	A-M.	52.6
3.0	4	5598	5930	2.2	6.7	73.9	34.5	11.1	19.0	15.0	46.4
	8	A-M.	5791	A-M.	5.1	A-M.	41.1	A-M.	7.4	A-M.	51.5
	24	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC

conc. : concentration

D : Direct treatment ; R : Recovery treatment

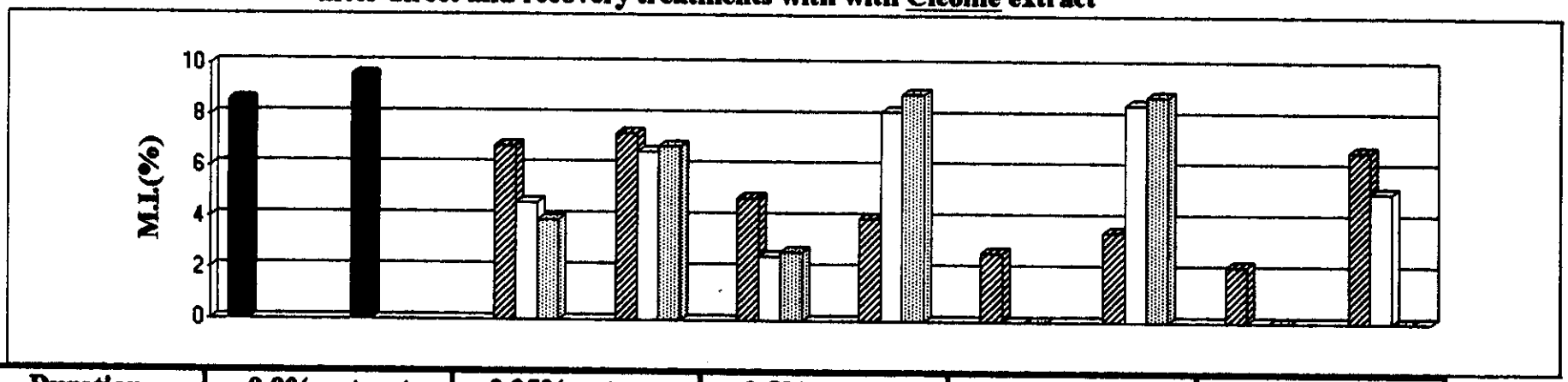
A-M. : a-mitosis

Table [5] : The effect of Cleome and Portulaca extracts on the mitotic index, phase index, percentage of total abnormalities and nucleoplasmic index in Allium cepa root tips

Treatment with	The effect of	Mitotic Index	Phase index of			Percentage of total abnormalities	Nucleoplasmic Index
			prophase	metaphase	ana-telophase		
Cleome extract	Concentration	H.S.	N.S.	N.S.	N.S.	H.S.	H.S.
	Duration	N.S.	N.S.	N.S.	N.S.	H.S.	H.S.
	Conc.-Duration interaction	H.S.	N.S.	N.S.	N.S.	H.S.	H.S.
Portulaca extract	Concentration	H.S.	N.S.	H.S.	H.S.	H.S.	H.S.
	Duration	H.S.	N.S.	S.	N.S.	H.S.	H.S.
	Conc.-Duration interaction	H.S.	N.S.	H.S.	N.S.	H.S.	H.S.

H.S : highly significant ; S. :significant ; N.S. : Non-significant (F-test).

Fig. [9] : Mitotic index (M.I) in Allium cepa root tip cells after direct and recovery treatments with with Cleome extract



Duration	0.0% extract		0.25% extract		0.5% extract		1.0% extract		3.0% extract	
	D	R	D	R	D	R	D	R	D	R
Control	8.6	9.6								
4 hours			6.8	7.3	4.8**	4.0**	2.7**	3.5**	2.2**	6.7*
8 hours			4.6**	6.6*	2.5**	8.2	A-M.	8.5	A-M.	5.1**
24 hours			3.9**	6.8	2.7**	8.9	A-M.	8.8	TOXIC	TOXIC

D : Direct treatment ; R : Recovery treatment.
A-M. : a-mitosis

L.S.D. (5%) = 1.803
L.S.D.(1%) = 2.384

* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

The test of recovery showed that all the treatments, except that of 0.5% for 4 hours, could be recovered and gave MI values higher than those of their direct ones. Moreover, some recovery treatments (0.5% for 8 hours, 0.5% for 24 hours, 1% for 8 hours and 1% for 24 hours) scored MI values very close to that of the control. However, the MI values of the recovery treatments seemed to be positively correlated with time duration in both of the 0.5% and 1% concentrations, whereas no distinct relation was observed with increasing concentration or time duration among the other treatments.

The recovered treatments gave significantly higher MI values than those of their direct origins in all the treatments except for those of 4 hours treatments with 0.25%, 0.5% and 1% [Fig. 9]. Moreover, direct treatments which exhibited a-mitosis (1% for 8 h and 24 h, and 3% for 8 h) could be highly recovered.

2) Effect of *Portulaca oleracea* extract :-

The treatment with *P. oleracea* extract resulted also in a highly significant reduction in the MI of *A. cepa* root tip cells. This reduction was concentration-dependent as well as duration-dependent [Table 5].

As shown in Table [6] and Fig. [10] the maximum scored MI of the direct treatments was 7.4 after treatment with 1% extract for 4 hours [the lowest concentration for the shortest duration] as compared to 8.7 for the control. On the other hand, the minimum MI value scored was 0.6 after treatment with 10% extract for 8 hours. However, the most effective treatments resulted in a-mitosis, as in case of 5% extract for 24 hours and 10% for 24 hours (the latter treatment could not be recovered).

Table [6] : Mitotic index and phase index in Allium cepa root tip cells after direct and recovery treatments with Portulaca extract

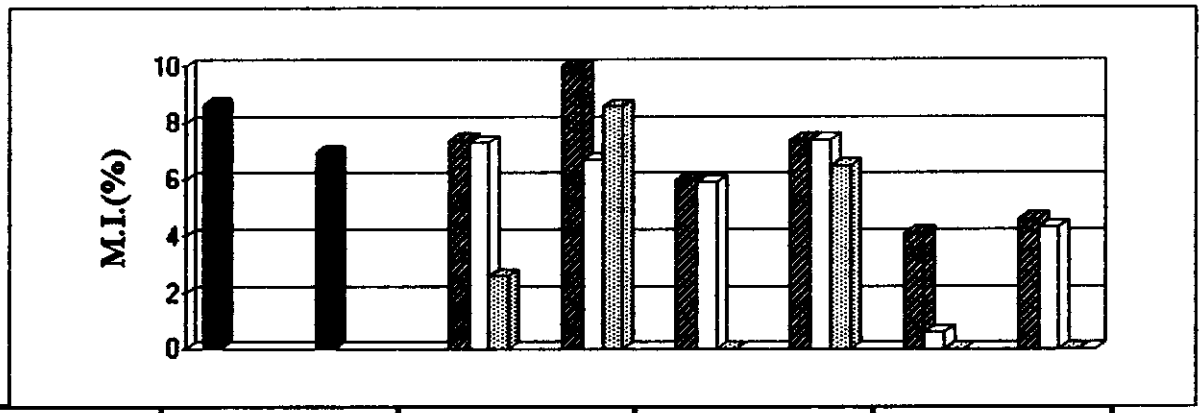
Treatment		Total number of counted cells		Mitotic Index		Phase Index of					
						Prophase		Metaphase		Ana-telophase	
Extract conc. (%)	Duration (hours)	D	R	D	R	D	R	D	R	D	R
Control		5623	5683	8.7	7	28.3	25.6	12.4	11.4	59.3	63
1.0	4	5602	5794	7.4	10	27.3	31.6	13.1	17.2	59.7	51.2
	8	5912	5729	7.3	6.7	27.2	28.8	16.6	12.1	56.2	59
	24	5679	5439	2.6	8.6	25	28.1	19.8	21.9	55.1	50
5.0	4	5578	5464	6	7.4	19.6	31.2	15.7	14.5	64.8	54.2
	8	5652	5831	5.9	7.4	23.9	28.6	19	17.2	57.1	54.2
	24	A-M.	5593	A-M.	6.5	A-M.	25.5	A-M.	19.9	A-M.	54.6
10.0	4	5341	5472	4.1	4.6	38.2	33.5	30	47.8	31.8	18.7
	8	5411	5731	0.6	4.3	45.3	34.8	10.3	16.5	44.4	48.7
	24	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.

conc. : concentration

D : Direct treatment ; R : Recovery treatment

A-M. : a-mitosis

Fig. [10] : Mitotic index (M.I.) in Allium cepa root tip cells after direct and recovery treatments with Portulaca extract



Duration	0.0% extract		1.0% extract		5.0% extract		10.0% extract	
	D	R	D	R	D	R	D	R
Control	8.7	7.0						
4 hours			7.4	10.0	6.0**	7.4	4.1**	4.6**
8 hours			7.3	6.7*	5.9**	7.4	0.6**	4.3**
24 hours			2.6**	8.6	A-M.	6.5*	A-M.	A-M.

D : Direct treatment ; R: Recovery treatment.
A-M. : a-mitosis

L.S.D.(5%) = 1.864
L.S.D.(1%) = 2.471

* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

The test of recovery showed that all the treatments, except that of the 1% extract for 8 hours and 10% for 24 hours (a-mitotic effect), were recoverable. Some of these recovered treatments (1% for 4 hours and 1% for 24 hours) gave MI values higher (10) or very close (8.6) to that of the control (8.7). Statistically, the difference between the MI value of any direct treatment and its recovered one was highly significant in all the treatments except for that of the 1% for 8 hours, 5% for 4 and 8 hours [Fig. 10].

3) Effect of plant chemical compounds :-

a- Effect of compound (1) [isolated from *C. droserifolia*] :-

Treating *A. cepa* root tips with compound (1) resulted in a highly significant reduction in the rate of cell division. Increasing concentration caused a more obvious decrease in the MI values. The difference between the MI values of the control and the 100 µg/ml treatment was significant, while that between the control and the other treatments (300 µg/ml and 500 µg/ml) were highly significant [Fig. 11 and Table 7].

b- Effect of compound (2) [isolated from *P. oleracea*] :-

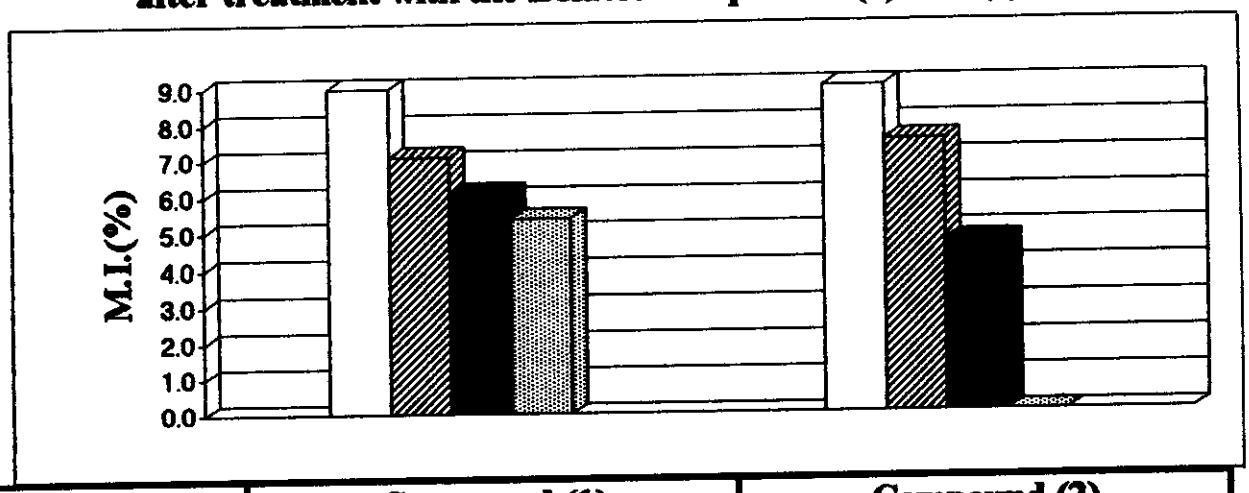
Treating *A. cepa* root tips with compound (2) showed also a mitodepressive effect more prominent than that of compound (1). The difference between the MI values of the control and the 100 µg/ml and 300 µg/ml treatments were significant and highly significant, respectively. The highest applied concentration (500 µg/ml) resulted in a-mitosis. At the same time, the difference between the MI values of any pair of treatments was highly significant [Fig. 11].

Table [7] : Mitotic index and phase index in Allium cepa root tip cells after treatment with the isolated compounds (1) and (2)

Treatment concentration	Compound (1) [isolated from C.droserifolia]					Compound (2) [isolated from P.oleracea]				
	Total number of counted cells	Mitotic index	Phase index of			Total number of counted cells	Mitotic index	Phase index of		
			prophase	metaphase	ana-telophase			prophase	metaphase	ana-telophase
Control	5731	9	26.7	16.8	56.5	5731	9	26.7	16.8	56.5
100 g/ml	5687	7.1	31.4	15.1	53.5	5477	7.5	26.8	21.4	51.8
300 g/ml	5670	6.1	28.3	15.7	56	5398	4.7	24.2	19.2	56.6
500 g/ml	5757	5.4	22.4	24.1	53.5	A-M.	A-M.	A-M.	A-M.	A-M.

A-M. : a-mitosis

Fig. [11] : Mitotic index (M.I.) in Allium cepa root tip cells after treatment with the isolated compounds (1) and (2)



Treatment	Compound (1) [isolated from C.droserifolia]	Compound (2) [isolated from P.oleracea]
Control □	9.0	9.0
100µg/ml ▨	7.1*	7.5*
300µg/ml ■	6.1**	4.7**
500µg/ml ▩	5.4**	A-M.
L.S.D.(5%) :	1.386	1.143
L.S.D.(1%) :	1.91	1.603

* : Significant to control at 0.05 level of probability (t-test).

** : Significant to control at 0.01 level of probability (t-test).

A-M. : a-mitosis

B - Effect on phase index (PI) :-

1. Effect of *C. droserifolia* extract :-

The treatment with *C. droserifolia* extract did not affect the percentages of phases significantly [Table 5]. Neither the change in concentration nor the expansion of time duration seems to affect the phase indices. The only significant change in PI throughout the treatments occurred when root tips were treated with the highest concentration (3%) for 4 hours [Table 4 and Fig. 12]. This treatment highly raised the prophase index on the expense of the ana-telophase index. The phase indices of the other treatments exhibited a fluctuated pattern which suggests that there was no distinct effect of *Cleome* extract on the phase indices.

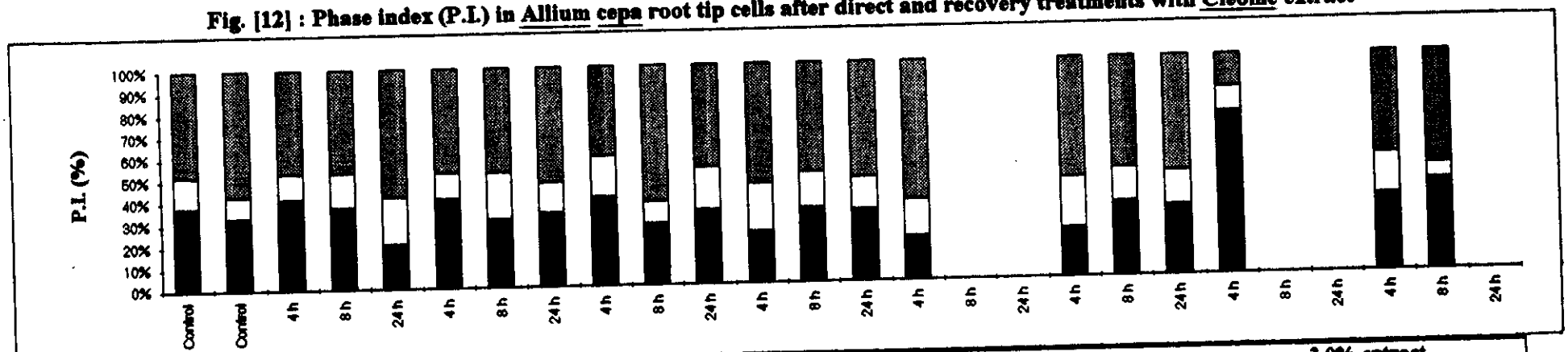
On the other hand, the phase indices of the recovery treatments showed a fluctuated pattern, where their values exceeded in some cases and receded in others, the values of their direct origins.

2. Effect of *P. oleracea* extract :-

The treatment of *A. cepa* root cells with *P. oleracea* extract did not affect the prophase index significantly. However, the treatment with 10% extract for 4 and 8 hours gave prophase index values (35.6 and 45.3, respectively) prominently higher - although non-significant - than those of the other lower concentration treatments [Table 6 and Fig. 13].

The frequencies of metaphase and ana-telophase were not affected by applying 1% and 5% *Portulaca* extract. Also 10% extract applied for 8 hours did not affect these phases significantly. Application of 10% extract for 4 hours resulted in a significantly higher metaphase index value (31.5) than that of the control on the expense of the ana-telophase

Fig. [12] : Phase index (P.I.) in Allium cepa root tip cells after direct and recovery treatments with Cleome extract



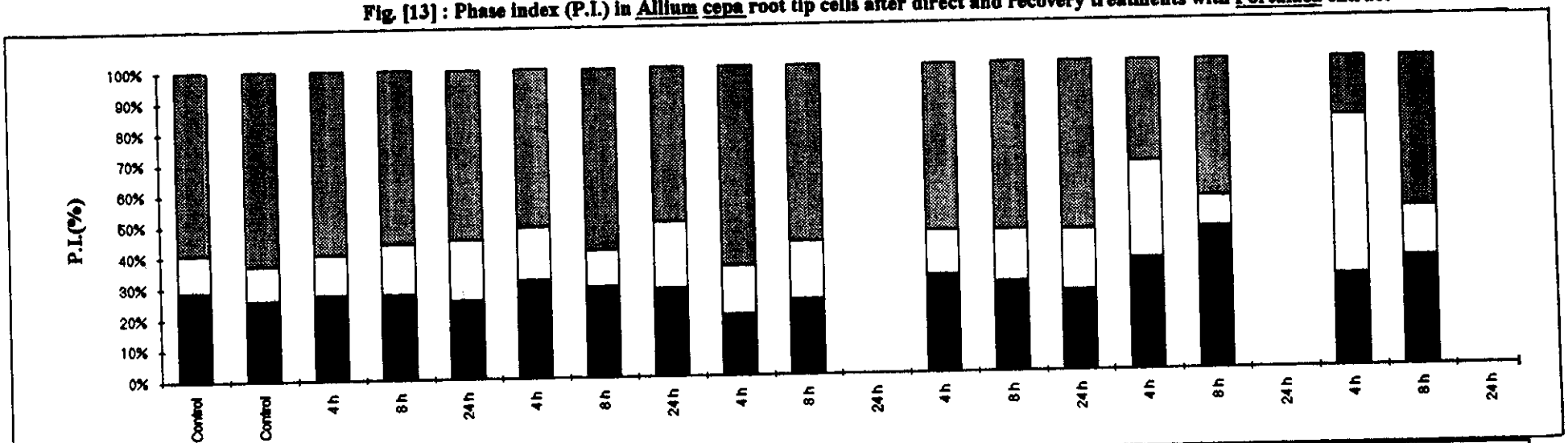
Phase Index of	0.0% extract		0.25% extract						0.5% extract						1.0% extract						3.0% extract					
	D	R	Direct treatment			Recovery treatment			Direct treatment			Recovery treatment			Direct treatment			Recovery treatment			Direct treatment			Recovery treatment		
	Control	Control	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h
Prophase	37.3	32.9	41.0	37.1	20.2	40.5	30.7	33.6	40.3	27.7	33.5	23.2	33.4	32.2	19.5	A-M.	A-M.	21.9	33.5	31.3	73.9**	A-M.	TOXIC	34.5	41.1	TOXIC
Metaphase	14.5	9.7	12.0	15.7	21.4	11.9	21.7	13.5	18.9	10.2	19.7	22.3	16.6	15.0	17.0	A-M.	A-M.	23.3	15.7	16.0	11.1	A-M.	TOXIC	19.0	7.4	TOXIC
Ana-telophase	48.2	57.5	47.0	47.2	58.4	47.6	47.6	52.9	40.8	62.1	46.8	54.5	50.0	52.8	63.5	A-M.	A-M.	54.8	50.8	52.6	15.0*	A-M.	TOXIC	46.4	51.5	TOXIC

D : Direct treatment ; R : Recovery treatment.
h : hours ; A-M. : a-mitosis

	Prophase Index	Metaphase Index	Ana-telophase Index
L.S.D. (5%) =	*24.988*	*17.771*	*26.536*
L.S.D. (1%) =	*33.038*	*23.496*	*35.085*

* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

Fig. [13] : Phase index (P.I.) in Allium cepa root tip cells after direct and recovery treatments with Portulaca extract



Phase Index of	0.0% extract		1.0% extract						5.0% extract						10.0% extract					
	D	R	Direct treatment			Recovery treatment			Direct treatment			Recovery treatment			Direct treatment			Recovery treatment		
	Control	Control	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h
Prophase	28.3	25.6	27.3	27.2	25.0	31.6	28.8	28.1	19.6	23.9	A-M.	31.2	28.6	25.5	35.6	45.3	A-M.	29.6	34.8	A-M.
Metaphase	12.4	11.4	13.1	16.6	19.8	17.2	12.1	21.9	15.7	19.0	A-M.	14.5	17.2	19.9	31.5*	10.3	A-M.	51.5**	16.5	A-M.
Ana-telophase	59.3	63.0	59.7	56.2	55.1	51.2	59.0	50.0	64.8	57.1	A-M.	54.2	54.2	54.6	32.9*	44.4	A-M.	19.0**	48.7	A-M.

D : Direct treatment ; R : Recovery treatment.
h : hours A-M. : a-mitosis

	Prophase Index	Metaphase Index	Ana-telophase Index
L.S.D. (5%) =	*23.788*	*17.908*	*22.725*
L.S.D. (1%) =	*31.538*	*23.743*	*30.129*

* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

index value (32.9) which was significantly low compared to its control [Fig. 13].

The recovery treatments gave PI values close to those of their direct origins. The significantly high metaphase index of the 10% extract for 4 hours direct treatment (31.5) was accompanied by a highly significant value (51.5) after recovery [Fig. 13]. The same picture was repeated in following ana-telophase percentage of the recovered roots, where all of which - except for that of the 10% extract for 4 hours - gave fluctuated proximate values which were close to that of the control. This same excepted recovery treatment gave ana-telophase index value (19.0) lower than that of its direct origin (32.9) which was significantly lower than that of the control [Fig. 13].

3. Effect of plants chemical compounds :-

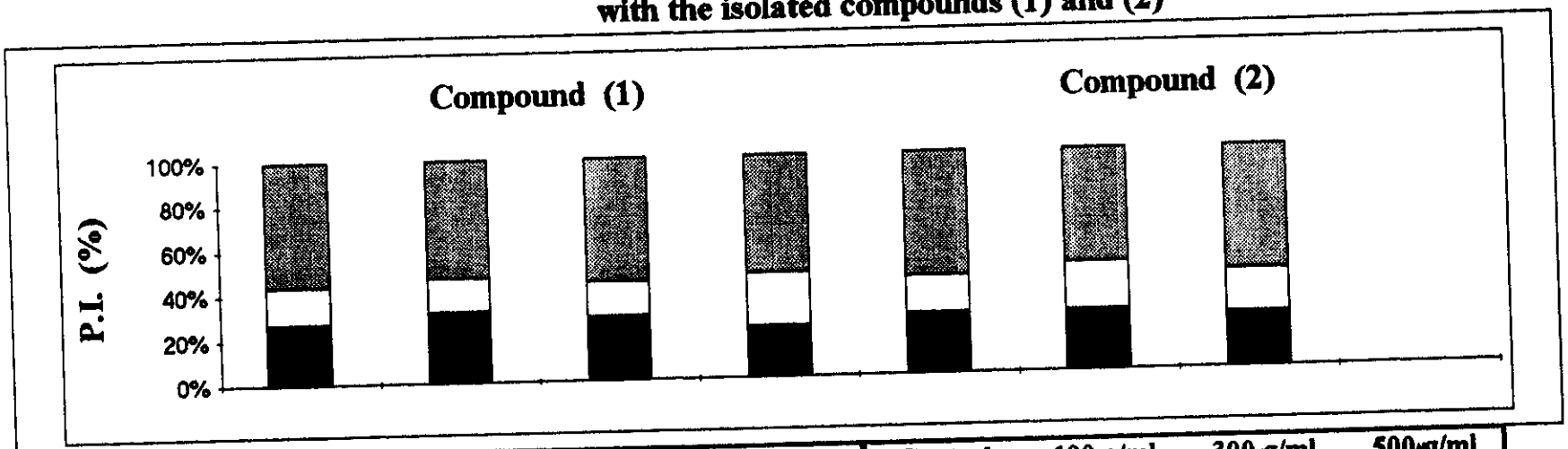
a) Effect of compound (1) [isolated from *C. droserifolia*] :-

The effect of compound (1) on the PI of *A. cepa* root cells was, in general, non-significant. Most of the treatments gave fluctuated PI values which were proximate and also close to those of their controls [Table 7 and Fig. 14]. The only significant deviation occurred after treatment with 500 µg/ml (the highest applied concentration) which gave metaphase index value (24.1) significantly higher than that of its control (16.8).

b) Effect of compound (2) [isolated from *P. oleracea*]:-

Treatment of *A. cepa* root cells with 100 µg/ml and 300 µg/ml did not affect the PI significantly in any phase [Table 7 and Fig. 14]. None of the treatments gave PI value significantly different from its control.

Fig.[14] : Phase indices (P.I.) in Allium cepa root tip cells after treatment with the isolated compounds (1) and (2)



Phase Index of	Control	100 µg/ml	300 µg/ml	500 µg/ml	Control	100 µg/ml	300 µg/ml	500 µg/ml
Prophase ■	26.7	31.4	28.3	22.4	26.7	26.8	24.2	A-M.
Metaphase □	16.8	15.1	15.7	24.1*	16.8	21.4	19.2	A-M.
Ana-telophase ▩	56.5	53.5	56.0	53.5	56.5	51.8	56.6	A-M.

A-M. : a-mitosis

	Prophase	Metaphase	Ana-telophase	Prophase	Metaphase	Ana-telophase
L.S.D. (5%) =	*6.966*	*5.782*	*9.774*	*8.611*	*9.521*	*13.325*
L.S.D. (1%) =	*9.598*	*7.967*	*13.467*	*12.073*	*13.348*	*18.682*

* : Significant to control at 0.05 level of probability (t-test).

C - Effect on the percentage of total abnormalities :-

1) Effect of *C. droserifolia* extract :-

Treating *A. cepa* root tips with *C. droserifolia* extract resulted in a statistically highly significant increase in the percentage of total abnormalities (PTA). This increase was concentration-dependent as well as duration-dependent [Table 5].

The highest scored PTA in direct treatments was 74.3% after treating root tips with 3% extract (the highest applied concentration) for 4 hours, and the minimum was 20.3% after treatment with 0.25% extract for 4 hours (the lowest concentration for the shortest duration applied) [Table 8]. Fig. [15] shows clearly that all direct treatments, except that of 0.25% extract for 4 hours, are significantly effective when compared to the control.

As for the effect of recovery, all the treatments except for those of the 0.5% extract were apparently recoverable. The values of the PTA in the recovered treatments were, in some cases, significantly lower than those of their direct origins.

2) Effect of *P. oleracea* extract :-

The directly treated *A. cepa* root cells with *P. oleracea* extract exhibited highly significant increases in the PTA. This effect was more pronounced on concentrating the extract solution as well as expanding the time duration of the treatments [Table 5].

The highest scored value of the PTA (68.9%) resulted after treatment with 10% extract (the highest applied concentration) for 8 hours, while the minimum one was 18.8% after treatment with 1% extract for 4 hours

Table [8] : Percentage of total abnormalities and its distribution in the different mitotic phases after direct and recovery treatments of Allium cepa root tips with Cleome extract

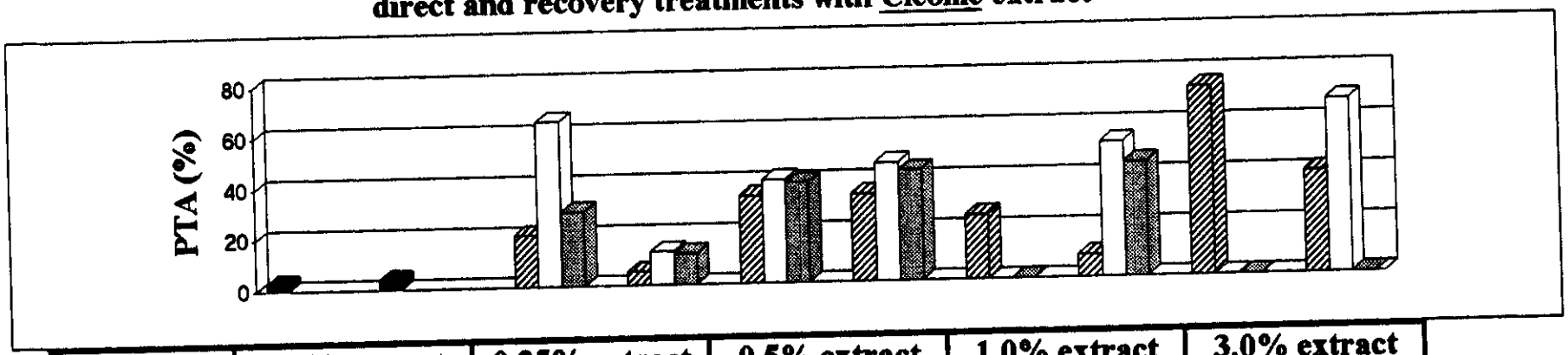
Treatment		Percentage of		Percentage of abnormal					
		total abnormalities		prophase		metaphase		ana-telophase	
Extract conc.(%)	Duration (hours)	D	R	D	R	D	R	D	R
Control		2.9	4.2	15.8	26.9	36.8	23.1	47.4	50
0.25	4	20.3	5.3	21.5	8.3	36.6	66.7	41.9	25
	8	64.9	13	50.5	29.8	20.5	50.9	29	19.3
	24	29.3	12.2	16.9	25.9	49.3	29.6	33.8	44.4
0.5	4	34.3	34.4	25	2.5	41.7	40.7	33.3	56.8
	8	40.6	46.5	46.9	41.8	21.9	23.8	31.2	34.4
	24	39.3	43.3	25.4	47.8	33.3	20.4	41.3	31.8
1.0	4	25.2	8.9	20.9	0	55.8	64.3	23.3	35.7
	8	A-M.	53.1	A-M.	52.6	A-M.	23.5	A-M.	23.9
	24	A-M.	45.1	A-M.	35.2	A-M.	27.3	A-M.	37.5
3.0	4	74.3	40.2	79.6	43.7	10.2	30.4	10.2	25.9
	8	A-M.	68.5	A-M.	53.1	A-M.	8.8	A-M.	38.1
	24	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC

D : Direct treatment ; R : Recovery treatment

conc. : concentration

A-M. : a-mitosis

Fig. [15] : Percentage of total abnormalities (PTA) in Allium cepa root tip cells after direct and recovery treatments with Cleome extract



Duration	0.0% extract		0.25% extract		0.5% extract		1.0% extract		3.0% extract	
	D	R	D	R	D	R	D	R	D	R
Control ■	2.9	4.2								
4 hours ▨			20.3	5.3	34.3**	34.4**	25.2*	8.9	74.3**	40.2**
8 hours □			64.9**	13.0	40.6**	46.5**	A-M.	53.1**	A-M.	68.5**
24 hours ▩			29.3*	12.2	39.3**	43.3**	A-M.	45.1**	TOXIC	TOXIC

D : Direct treatment ; R : Recovery treatment.
A-M. : a-mitosis.

L.S.D. 5% = 21.048
L.S.D. 1% = 27.828
* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

(the lowest concentration for shortest duration) [Table 9]. All treatments, except for the mildest one (1% extract for 4 hours), resulted in highly significant increases in PTA values over that of the control [Fig. 16]. Furthermore, concerning the same time duration, the values resulting from treatment with any pair of successive concentrations were significantly different, e.g. the values resulting from 1%, 5% and 10% extract for 4 hours were 18.8%, 36.4% and 55.3%, respectively, and it is obvious that the differences betwen these three values (17.6 and 18.9) are higher than the LSD at 5% (16.23). Generally, the PTA seems to be directly proportional with increasing concentration and time durations of the treatments.

The test of recovery showed that all the direct treatments were recoverable, i.e. the recovery treatments gave PTA lower than those of their direct origins. The only exception was that of the 10% extract for 4 hours which gave after recovery 69.4%, while the direct treatment scored 55.3%. The recovered treatments of the 1% and 5% induced a clear distinct trend directly proportional to the duration of the treatment, while the recovery treatments of the 10% extract exhibited a different behaviour, specially with the a-mitotic case after 24 hours duration.

3) Effect of plants chemical compounds :-

a- Effect of compound (1) [isolated from *C. droserifolia*] :-

The treatment with compound (1) caused a highly significant increase in the PTA [Table 10 and Fig. 17]. This increase was directly proportional to the concentration of the compound. The differences between the PTA of the control (9.0%) and that of any other treatment was highly significant. Comparing the 100 µg/ml and 300 µg/ml treatments, the

Table [9] : Percentage of total abnormalities and its distribution in the different mitotic phases after direct and recovery treatments of Allium cepa root tips with Portulaca extract

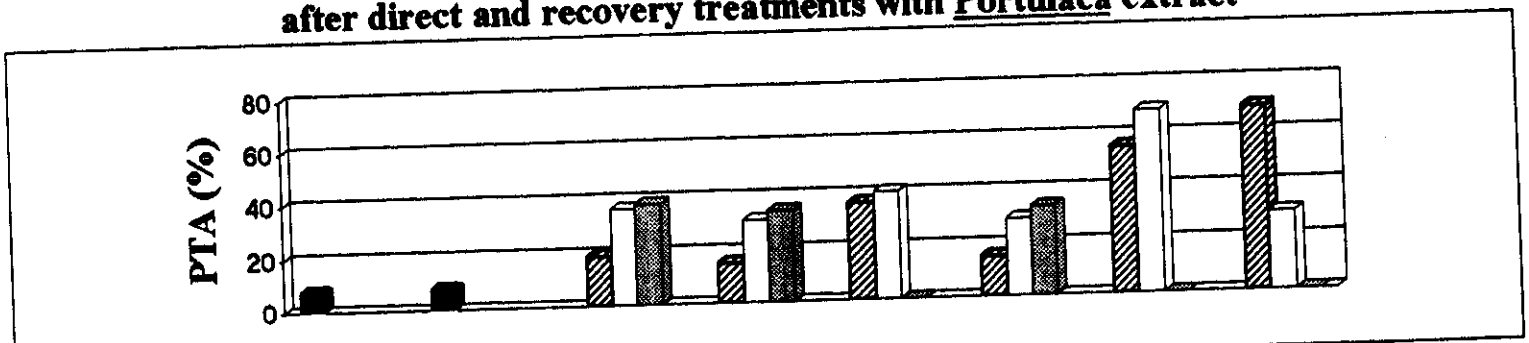
Treatment		Percentage of total abnormalities		Percentage of abnormal					
				prophase		metaphase		ana-telophase	
Extract conc.(%)	Duration (hours)	D	R	D	R	D	R	D	R
Control		7.9	8.5	19.5	37.1	29.3	28.6	51.2	34.3
1.0	4	18.8	15	36.9	30.6	28.3	32.7	34.8	36.7
	8	36.6	31.2	21.7	22.3	27.7	23.9	50.5	53.8
	24	38.3	34.3	25.4	18	35.6	36.5	38.9	45.5
5.0	4	36.4	14.8	23.1	26.1	30.8	23.2	46.1	50.7
	8	40.5	29	20.5	18.6	32.9	31	46.6	50.4
	24	A-M.	33.6	A-M.	26	A-M.	40.7	A-M.	33.3
10.0	4	55.3	69.4	49.3	36.9	34.1	53.9	16.7	9.2
	8	68.9	29.6	45.8	22	20.8	23.1	33.3	54.9
	24	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.

D : Direct treatment ; R : Recovery treatment

conc. : concentration

A-M. : a-mitosis

Fig. [16] : Percentage of total abnormalities (PTA) in Allium cepa root tip cells after direct and recovery treatments with Portulaca extract



Duration	0.0% extract		1.0% extract		5.0% extract		10.0% extract	
	D	R	D	R	D	R	D	R
Control	7.9	8.5						
4 hours			18.8	15.0	36.4**	14.8	55.3**	69.4**
8 hours			36.6**	31.2**	40.5**	29.0*	68.9**	29.6**
24 hours			38.3**	34.3**	A-M.	33.6**	A-M.	A-M.

D : Direct treatment ; R : Recovery treatment.

A-M. : a-mitosis

L.S.D. 5% = 16.23

L.S.D. 1% = 21.517

* : Significant to control at 0.05 level of probability (t-test).

** : Significant to control at 0.01 level of probability (t-test).

Table[10] : Percentage of total abnormalities and its distribution in the different mitotic phases after treatment of Allium cepa root tips with the isolated compounds (1) and (2)

Treatment concentration	Compound(1) [isolated from C.droserifolia]				Compound(2) [isolated from P.oleracea]			
	Percentage of total abnormalities	Percentage of abnormal			Percentage of total abnormalities	Percentage of abnormal		
		prophase	metaphase	ana-telophase		prophase	metaphase	ana-telophase
Control	9	28.4	28.4	43.3	9	28.4	28.4	43.3
100 μg/ml	24.6	26.7	21.9	51.4	33.7	28	30.5	41.5
300 μg/ml	39.8	37.7	23.5	38.8	39.1	25.7	28.6	45.7
500 μg/ml	44.9	25.8	29.8	44.4	A-M.	A-M.	A-M.	A-M.

A-M. : a-mitosis

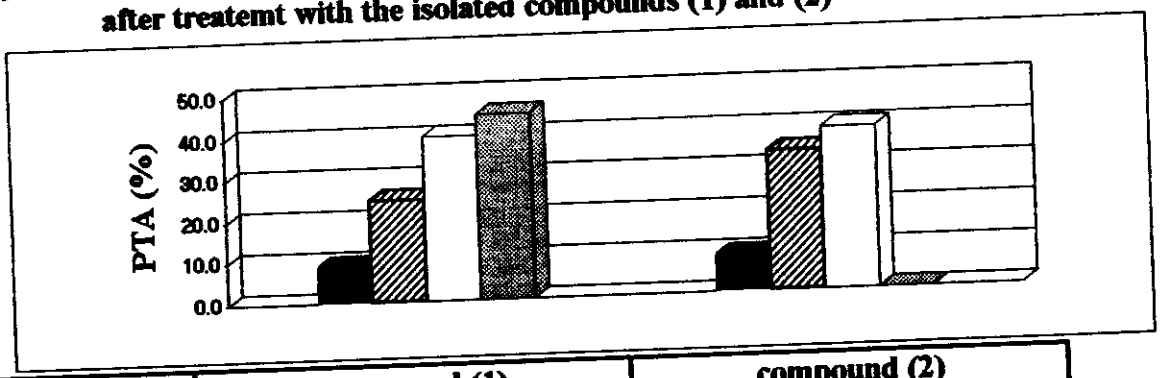
difference was also highly significant, while that between the 300 µg/ml and 500 µg/ml treatments was non-significant.

b- Effect of compound (2) [isolated from *P. oleracea*] :-

The PTA increased with the increase of concentration of the compound in the treating solution. The difference between the PTA of the control (9.0%) and that of the 100 µg/ml or 300 µg/ml treatment was highly significant, while that between the 100 µg/ml and 300 µg/ml treatments was non-significant [Table 10 and Fig. 17].

Treatment with 500 µg/ml of the compound resulted in a-mitosis. Statistically, treatment with compound (2) resulted in a highly significant increase in the PTA [Table 10 and Fig. 17].

Fig. [17] : Percentage of total abnormalities (PTA) in Allium cepa root tip cells after treatemt with the isolated compounds (1) and (2)



Treatment	compound (1)	compound (2)
Control	9.0	9.0
100 µg/ml	24.6**	33.7**
300 µg/ml	39.8**	39.1**
500 µg/ml	44.9**	A-M.
L.S.D. (5%) =	8.057	9.591
L.S.D. (1%) =	11.101	13.447

* : Significant to control at 0.05 level of probability (t-test).
** : Significant to control at 0.01 level of probability (t-test).

A-M. : a-mitosis

D - Effect on distribution of total abnormalities in the mitotic phases :-

1. Effect of *C. droserifolia* extract :-

In general, prophase seemed to be the most affected stage, since the percentage of abnormal prophase - in all the direct and most of the recovery treatments - were higher than those of the controls [Table 8]. The 4 hours direct treatment with 3% *Cleome* extract gave rise to the most pronounced effect on the distribution shown in Table [8]. This treatment resulted in the maximum and minimum readings (79.6% of prophase and 10.2% of both metaphase and ana-telophase, respectively) throughout the three phases. The maximum scored percentage of abnormal metaphase was 55.8% after 4 hours direct treatment with 1% extract and the maximum abnormal ana-telophase reading was 41.9% after 4 hours direct treatment with 0.25% extract.

After recovery, the maximum percentage of abnormality was recorded in metaphase (66.7%) followed by ana-telophase (56.8%), then prophase(53.1%) [Table 8].

2. Effect of *P. oleracea* extract :-

The amount of total abnormalities at prophase induced by any one of the direct treatments with *Portulaca* extract scored percentages higher than that of the control, thus, prophase seems to be the most affected stage [Table 9]. The maximum scored reading (50.5%), representing the highest percentage of abnormal ana-telophase, was induced by 8 hours direct treatment with 1% extract. The maxima of prophase and metaphase were 49.3% (after 4 hours direct treatment with 10% extract) and 35.6% (after 24 hours treatment with 1% extract), respectively.

The test of recovery revealed that ana-telophase was apparently the most affected stage followed by metaphase, if the increase in the percentages of abnormalities over that of the control was considered. Ana-telophase scored the highest percentage of abnormalities (54.9%) after 8 hours recovered treatment with 10% extract. The recovered treatment of 10% extract for 4 hours resulted in the highest percentages of abnormal prophase and metaphase (36.9% and 53.9%) on the expense of the abnormal ana-telophase percentages[Table 9].

3. Effect of plants chemical compounds :-

a) Effect of compound (1) [isolated from *C. droserifolia*] :-

It can be seen from Table [10] that the different applied concentrations of compound (1) caused slight increases in the percentages of abnormal ana-telophases. The maximum percentage of abnormality was 51.4% of ana-telophase after treatment with 100 µg/ml. The maxima of abnormal prophase and abnormal metaphase were 37.7% and 29.8% after treatment with 300 µg/ml and 500 µg/ml, respectively [Table 10].

b) Effect of compound (2) [isolated from *P. oleracea*] :-

Treating *A. cepa* root tip cells with compound (2) caused - as compound (1) - an increase in the percentage of abnormal ana-telophase on the expense of prophase and metaphase. The maximum scored percentage of abnormal ana-telophase was 45.7% after treatment with 300 µg/ml. The maximum scored percentage of abnormal prophase was 28.0% after 100 µg/ml treatment, and that of metaphase was 30.5% after treatment with the same concentration [Table 10].

Treatment with 500 µg/ml of compound (2) resulted in a-mitosis.

E - Induction of different types of abnormalities :-

The treatment of *A. cepa* root tip cells with *Cleome* extract, *Portulaca* extract and solutions of compound (1) and compound (2) induced the occurrence of some types of mitotic aberrations. Stickiness of the chromatin material, spindle disturbance, chromosome bridges, irregular prophase and despiralization of the chromosomes represent the main abnormalities recorded. The ratios of occurrence of these types throughout the mitotic cycle are shown in tables [11], [12] and [13]. These tables show clearly that stickiness was the most dominent type of the observed abnormalities, while despiralization represented the lowest ratio of occurrence.

1. Stickiness :-

Cleome extract induced high percentages of stickiness, specially at the recovery treatments of high concentrations (0.5%, 1% and 3%) for long time durations [Table 11]. Recovery of 8 hours treatments showed a regular increase of values with raising the extract concentration. The maximum percentage of stickiness (100%) was recorded after application of 3% extract for 8 hours recovered treatment. Direct treatments recorded a maximum percentage (96.9%) after 8 hours application of 0.25% extract.

Direct treatment with *Portulaca* extract showed a reduction of the stickiness percentage with increasing time duration when 1% and 5% extracts were applied. In contrast, all the recovery treatments and the 10% extract direct ones induced increases of stickiness percentages with lapse of treatment time [Table 12]. The maximum recorded percentage was 99.1% after 4 hours direct treatment with 5% extract.

Table[11] : Percentages of different types of abnormalities after direct and recovery treatments of Allium cepa root tips with Cleome extract

Treatment		Stickiness		Disturbance		Bridge		Irregular prophase		Despiralization	
Extract conc. (%)	Duration (hours)	D	R	D	R	D	R	D	R	D	R
0.25	4	77.2	90.9	12.7	4.5	5.1	4.5	2.5	0.0	2.5	0.0
	8	96.9	83.3	3.1	12.5	0.0	2.1	0.0	2.1	0.0	0.0
	24	82.0	63.0	3.3	23.9	13.1	10.9	1.6	2.2	0.0	0.0
0.5	4	62.0	76.8	13.9	8.7	13.9	14.5	6.3	0.0	3.8	0.0
	8	96.4	96.1	3.6	2.9	0.0	0.5	0.0	0.5	0.0	0.0
	24	77.4	96.3	11.3	3.7	11.3	0.0	0.0	0.0	0.0	0.0
1.0	4	79.4	61.5	14.7	7.7	2.9	30.8	2.9	0.0	0.0	0.0
	8	A-M.	98.4	A-M.	1.6	A-M.	0.0	A-M.	0.0	A-M.	0.0
	24	A-M.	99.6	A-M.	0.4	A-M.	0.0	A-M.	0.0	A-M.	0.0
3.0	4	86.4	95.2	10.2	2.4	2.3	2.4	1.1	0.0	0.0	0.0
	8	A-M.	100.0	A-M.	0.0	A-M.	0.0	A-M.	0.0	A-M.	0.0
	24	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC	TOXIC

conc. : concentration

D : Direct treatment ; R : Recovery treatment

A-M. : a-mitosis

Table[12] : Percentages of different types of abnormalities after direct and recovery treatments of Allium cepa root tips with Portulaca extract

Treatment		Stickiness		Disturbance		Bridge		Irregular prophase		Despiralization	
Extract conc. (%)	Duration (hours)	D	R	D	R	D	R	D	R	D	R
1.0	4	98.7	89.3	1.3	4.8	0.0	6.0	0.0	0.0	0.0	0.0
	8	91.7	90.0	7.6	7.3	0.0	1.8	0.6	0.0	0.0	0.9
	24	76.0	97.3	24.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0
5.0	4	99.1	83.3	0.0	11.7	0.9	5.0	0.0	0.0	0.0	0.0
	8	96.7	91.1	3.3	8.1	0.0	0.8	0.0	0.0	0.0	0.0
	24	A-M.	99.0	A-M.	0.0	A-M.	1.0	A-M.	0.0	A-M.	0.0
10.0	4	80.2	85.9	10.9	4.5	3.0	1.3	5.9	8.3	0.0	0.0
	8	95.0	93.5	5.0	3.9	0.0	1.3	0.0	1.3	0.0	0.0
	24	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.

conc. : concentration

D : Direct treatment ; R : Recovery treatment

A-M. : a-mitosis

Application of different concentrations of compound (1) and compound (2) induced absolutely high percentages of stickiness. Table [13] shows that 100 µg/ml of compound (1) caused 100% stickiness, while compound (2) caused the same percentage after application of 300 µg/ml. No relation between changing the concentration of either compound and the induction of stickiness could be expected, since the differences are very small.

Table [13] : Percentages of different types of abnormalities in Allium cepa root tips after treatment with the isolated compounds (1) and (2)

Treatment concentration	Stickiness		Disturbance		Bridge	
	Comp. (1)	Comp. (2)	Comp. (1)	Comp. (2)	Comp. (1)	Comp. (2)
100 ug/ml	100.0	98.8	0.0	1.2	0.0	0.0
300 ug/ml	99.4	100.0	0.0	0.0	0.6	0.0
500 ug/ml	99.4	A-M.	0.0	A-M.	0.6	A-M.

A-M. : a-mitosis

Plates [1, 2] show different grades of stickiness at different mitotic phases after treatment with the concerned extracts and compounds.

2. Spindle disturbance :-

Table [11] shows that direct treatment with *Cleome* extract induced variable degrees of spindle disturbance with a slight direct relation with concentration of the treatments. The test of recovery showed that all treatments, except that of 8 and 24 hours with 0.25% extract, induced

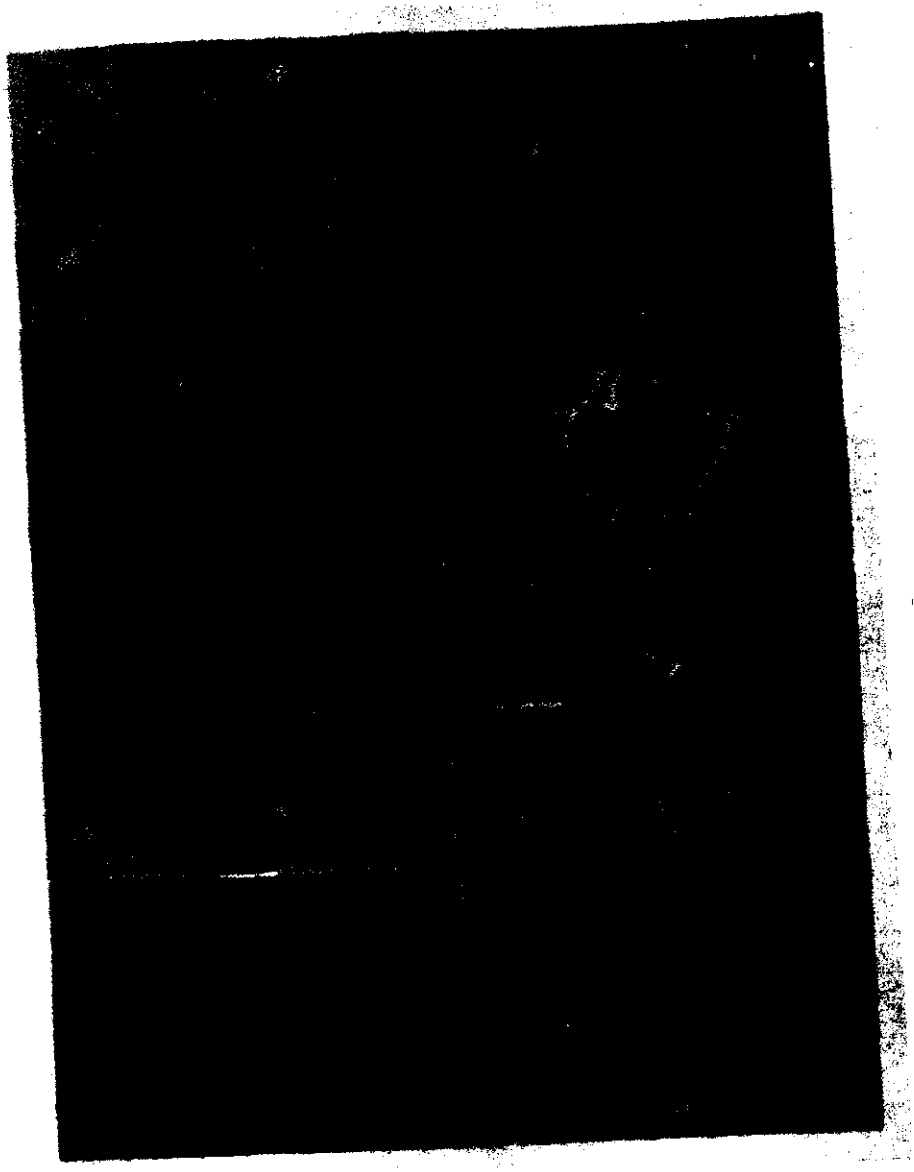


Plate [1] : Chromosome stickiness induced in *A. cepa* root tip cells :-

a. Sticky prophase : after treatment with 3% *Cleome* extract for 4 hours and recovery.

b. Sticky prophase : after treatment with 3% *Cleome* extract for 4 hours.

c. Severely sticky metaphase : after treatment with 0.25% *Cleome* extract for 4 hours and recovery.

d. Light stickiness at metaphase : after treatment with 0.5% *Cleome* extract for 4 hours.

e. Sticky metaphase : after treatment with 0.5% *Cleome* extract for 24 hours.

f. Severely sticky metaphase : after treatment with 0.5% *Cleome* extract for 8 hours.

g. Sticky metaphase : after treatment with 1% *Portulaca* extract for 24 hours.

h. Light stickiness at metaphase : after treatment with 0.25% *Cleome* extract for 4 hours.

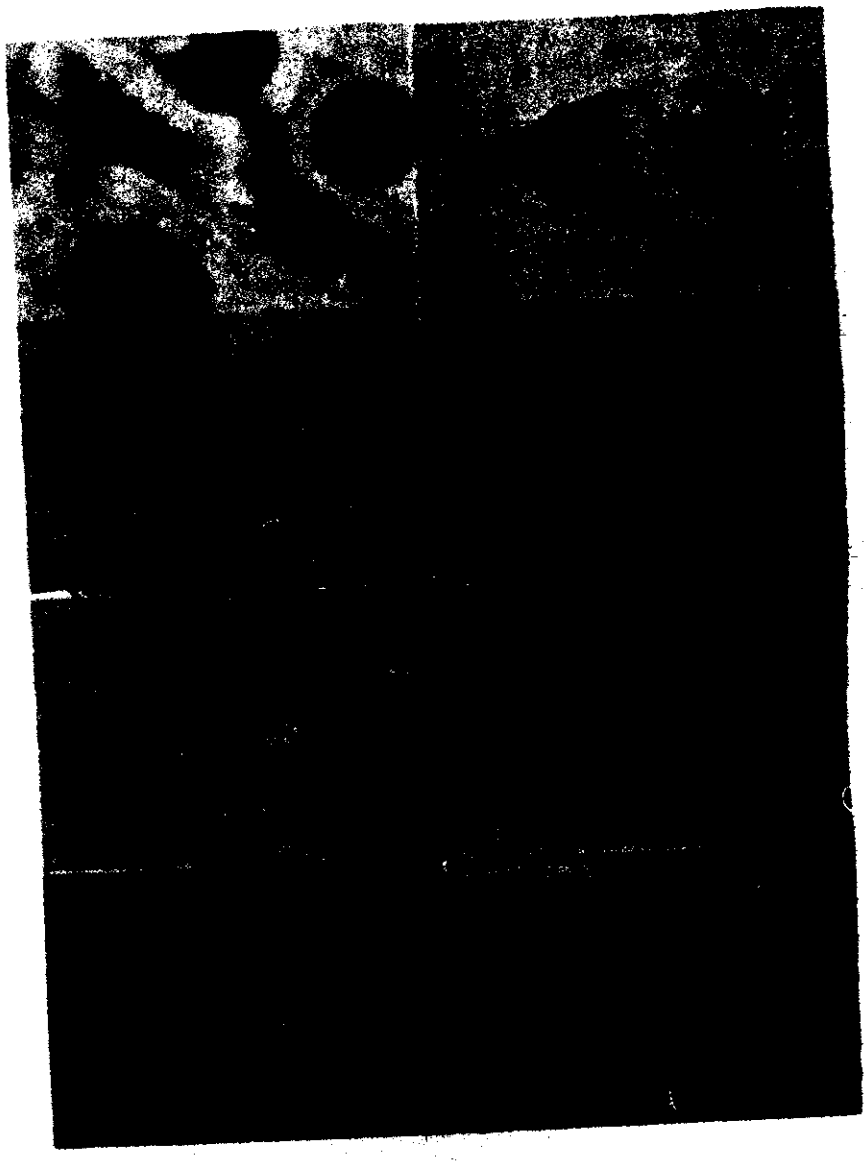


Plate [2] : Chromosome stickiness induced in *A. cepa* root tip cells :-

a. Sticky anaphase : after treatment with 0.25% *Cleome* extract for 8 hours.

b. Severely sticky anaphase : after treatment with 0.5% *Cleome* extract for 24 hours.

c. Severely sticky anaphase : after treatment with 0.5% *Cleome* extract for 24 hours and recovery.

d. Sticky anaphase : after treatment with 3% *Cleome* extract for 4 hours.

e. Sticky anaphase : after treatment with 100 µg/ml solution of Compound (1).

f. Light stickiness at telophase : after treatment with 1% *Cleome* extract for 8 hours and recovery.

g. Sticky telophase : after treatment with 1% *Portulaca* extract for 24 hours.

h. Sticky telophase : after treatment with 300 µg/ml solution of Compound (1).

lower percentages of disturbance after recovery than their direct ones. 14.7% was the maximum percentage of disturbance after 4 hours direct treatment with 1% *Cleome* extract, while 23.9% disturbance occurred in 24 hours recovery treatment with 0.25% extract.

1% and 5% *Portulaca* extracts induced a gradual increase of disturbance percentages with increase of time duration of direct treatments [Table 12], while 10% extract induced the reverse. Also, the occurrence of disturbance after recovery of 10% extract treatment recorded lower percentages than direct ones. 24 hours direct treatment with 1% extract induced the maximum percentage of disturbance (24.0%) [Table 12].

No disturbance was induced after treatment with compound (1) as seen in Table [13]. Compound (2) induced a low percentage of disturbance (1.2%) after treatment with 100 µg/ml only [Table 13].

Spindle disturbance prevents the normal movement of the mitotic chromosomes leading to : the disturbed alignment of chromosomes at metaphase or anaphase [Plate 3 : a-d], surpassing of some anaphase chromosomes [Plate 3 : e, f], or the diagonal withdrawal of chromosomes, i.e. diagonal anaphase [Plate 3 : g, h].

3. Bridges :-

After treatment with *Cleome* extract bridges were induced at most direct and recovery treatments with detectable percentages. However, the percentages were not correlated neither with concentration nor duration [Table 11]. 30.8% was recorded after recovery of 4 hours treatment with 1% extract as a maximum percentage of bridges occurrence.

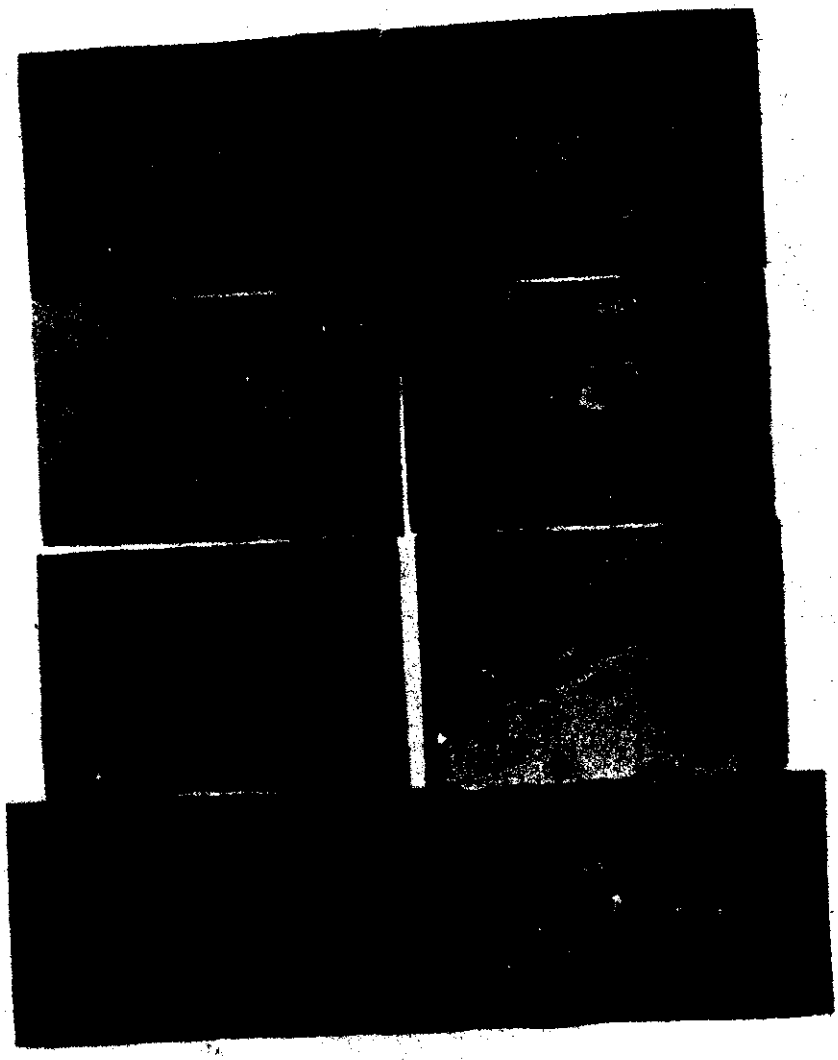


Plate [3] : Spindle disturbance induced in *A. cepa* root tip cells :-

a. Disturbed metaphase : after treatment with 1% *Portulaca* extract for 24 hours.

b. Disturbed metaphase : after treatment with 5% *Portulaca* extract for 8 hours and recovery.

c. Disturbed anaphase : after treatment with 3% *Cleome* extract for 4 hours.

d. Sticky disturbed anaphase : after treatment with 1% *Cleome* extract for 8 hours and recovery.

e. Sticky anaphase and surpassing chromosome : after treatment with 0.5% *Cleome* extract for 4 hours.

f. Surpassing chromosome : after treatment with 0.5% *Cleome* extract for 4 hours and recovery.

g. Diagonal anaphase : after treatment with 5% *Portulaca* extract for 8 hours.

h. Diagonal anaphase : after treatment with 100 µg/ml solution of Compound (2).

Bridge induction was more obvious after recovery following treatment with *Portulaca* extract than after direct treatment. Table [12] shows that all direct treatments, except that of 5% and 10% extracts for 4 hours, did not induce bridge formation. The maximum percentage of bridge occurrence was 6.0% after recovery of 4 hours treatment with 1% extract.

Compound (1) induced a very low percentage of bridges (0.6%) after treatment with either 300 µg/ml or 500 µg/ml solutions [Table 13]. On the other hand, compound (2) did not induce bridge occurrence at any of the used concentrations.

Plate [4] show bridges which occurred following treatment with the tested extracts and compounds.

4. Irregular prophase :-

Table [11] shows that treatment with *Cleome* extract have induced relatively low percentages of irregular prophase compared to the previous types of abnormalities. 6.3% was the maximum percentage induced by 4 hours treatment with 0.5% extract. The recorded values of percentages show no correlation with concentration or lapse of time of the treatments.

With *Portulaca* extract most of the irregular prophase cases were induced by the highest used concentration (10%) [Table 12]. The maximum percentage recorded was 8.3% after the recovery treatment of 10% extract for 4 hours.

Plate [5 : a-c] show some examples of the induced irregular prophase after treatment with the plants extracts only, since this abnormality was not induced by the two isolated compounds.

Plate [4] : Chromosome bridges induced in *A. cepa* root tip cells :-

a. Anaphase multi-bridge : after treatment with 0.5% *Cleome* extract for 4 hours.

b. Anaphase multi-bridge : after treatment with 1% *Cleome* extract for 4 hours and recovery.

c. Sticky anaphase with chromosome bridge : after treatment with 0.5% *Cleome* extract for 4 hours.

d. Sticky anaphase with chromosome bridge : after treatment with 0.25% *Cleome* extract for 24 hours and recovery.

e. Sticky anaphase with chromosome multi-bridge : after treatment with 3% *Cleome* extract for 4 hours.

f. Multi-bridged disturbed anaphase : after treatment with 0.5% *Cleome* extract for 4 hours and recovery.

g. Completely disturbed, multi-bridged anaphase : after treatment with 10% *Portulaca* extract for 4 hours.

h. Sticky, disturbed, multi-bridged anaphase : after treatment with 0.5% *Cleome* extract for 8 hours and recovery.

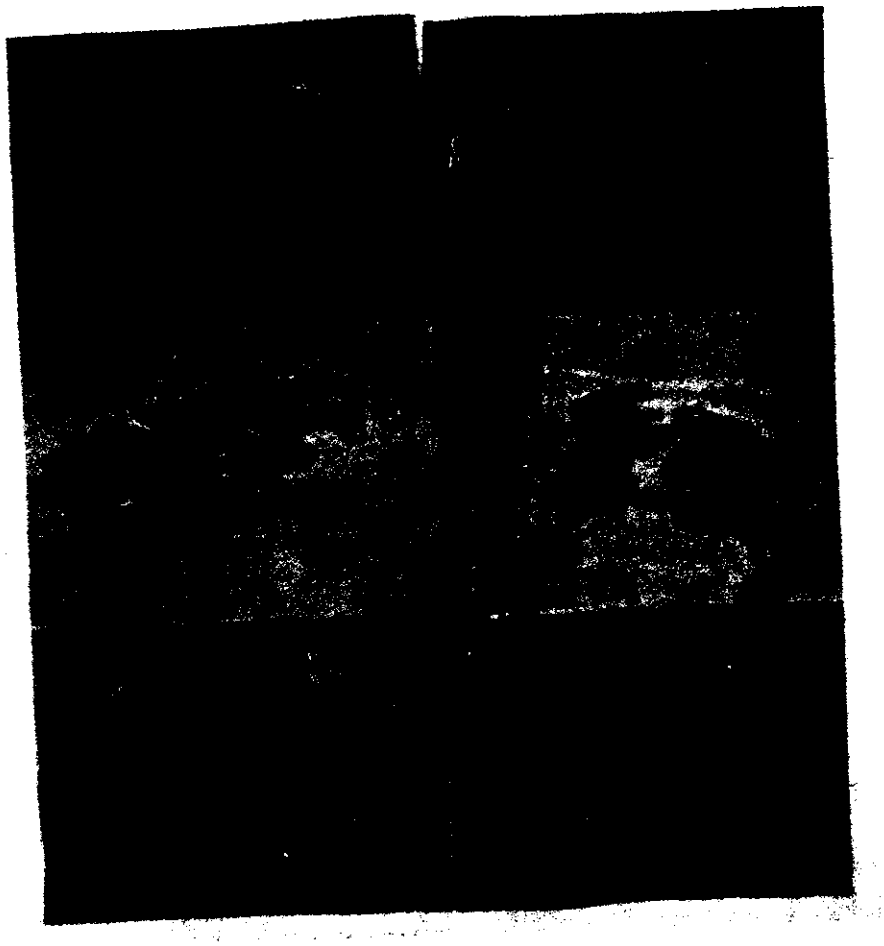


Plate [5] : Irregular prophase and chromosome despiralization induced in *A. cepa* root tip cells :-

a. Irregular prophase : after treatment with 1% *Portulaca* extract for 8 hours.

b. Irregular prophase : after treatment with 10% *Portulaca* extract for 4 hours and recovery.

c. Sticky, irregular prophase : after treatment with 0.5% *Cleome* extract for 4 hours.

d. Chromosome despiralization in severely sticky anaphase : after treatment with 0.25% *Cleome* extract for 4 hours.

e. Multi-bridged anaphase with chromosome despiralization : after treatment with 0.5% *Cleome* extract for 4 hours.

f. Chromosome despiralization : after treatment with 0.5% *Cleome* extract for 4 hours.

5. Despiralization :-

It was induced only by the shortest duration treatments of the lowest concentrations of *Cleome* extract (4 hours direct treatments with 0.25% and 0.5% extracts). The recorded percentages were 2.5% and 3.8%, respectively [Table 11].

Table [12] shows that only one treatment (recovered 1% *Portulaca* extract for 8 hours) induced despiralization, and its percentage did not exceed 0.9%.

Examples of the induced despiralization are shown in Plate [5 : d-f].

F - Distribution of the different types of abnormalities in each mitotic phase :-

1. After treatment with *Cleome* extract :-

Two types of abnormalities were induced at prophase, namely stickiness and irregular prophase. Stickiness constituted the most frequent one. Direct treatments for 8 hours with 0.25% and 0.5% extracts and 0.5% for 24 hours as well as recovery treatments for 4 hours with 0.25%, 0.5%

and 3%; for 8 hours with 1% and 3%; and for 24 hours with 0.5% and 1% extract had abnormal prophase cells composed entirely of the sticky type [Table 14]. Irregular prophase was less frequent and many treatments did not induce it at all. 45.5% was the maximum percentage of irregular prophase induced after 4 hours treatment with 0.5% extract [Table 14].

Metaphase abnormalities was confined in stickiness and spindle disturbance [Table 14]. Stickiness, again, recorded much higher percentages on the expense of disturbance. Four treatments, all are of the recovery ones, had metaphase abnormalities composed entirely of the sticky type, these are 0.25% extract for 4 hours, 1% for 8 and 24 hours, and 3% for 8 hours. The maximum percentage of disturbed metaphase recorded a frequency of 57.1% of the abnormal metaphase cells after 4 hours direct treatment with 3% extract [Table 14].

At ana-telophase stages stickiness, spindle disturbance, bridges, diagonal anaphase, surpassing chromosomes and despiralization were the induced types of abnormalities in a descending order. Table [14] shows clearly that stickiness was the most frequent type. Application of the lowest concentration of the extract (0.25%) induced percentages of stickiness after recovery lower than those of direct ones, while high concentrations (0.5%, 1% and 3%) induced higher percentages after recovery (with the exception of the 4 hours treatment with 1% extract). 100% of the ana-telophase cells were of the sticky type after recovery of the 8 hours treatment with 3% extract.

Bridge formation came to follow stickiness in the frequency of occurrence among the ana-telophase abnormalities, and these two abnormalities seemed to be negatively correlated to each other [Table 14].

Table[14] : Distribuation of the different types of abnormalities in each mitotic phase after direct and recovery treatments of Allium cepa root tips with Cleome extract

Treatment		Prophase				Metaphase				Ana-telophase											
Extract conc. (%)	Duration (hours)	Sticky		Irregular		Sticky		Disturbed		Sticky		Bridge		Disturbed		Diagonal		Surpassing		Despiral	
		D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
0.25	4	85.7	100.0	14.3	0.0	79.3	100.0	20.7	0.0	72.2	66.7	11.1	16.7	5.6	16.7	2.8	0.0	2.8	0.0	5.6	0.0
	8	100.0	92.3	0.0	7.7	93.9	92.0	6.1	8.0	93.8	50.0	0.0	10.0	2.1	0.0	4.2	40.0	0.0	0.0	0.0	0.0
	24	88.9	87.5	11.1	12.5	96.7	62.5	3.3	37.5	59.1	54.6	36.4	22.7	0.0	13.6	4.5	9.1	0.0	0.0	0.0	0.0
0.5	4	54.5	100.0	45.5	0.0	88.6	89.3	11.4	10.7	36.4	66.7	33.3	25.6	15.2	0.0	3.0	5.1	3.0	2.6	9.1	0.0
	8	100.0	98.8	0.0	1.2	91.7	92.0	8.3	8.0	94.1	95.8	0.0	1.4	5.9	1.4	0.0	1.4	0.0	0.0	0.0	0.0
	24	100.0	100.0	0.0	0.0	77.8	90.9	22.2	9.1	65.2	94.2	26.1	0.0	8.7	4.3	0.0	1.5	0.0	0.0	0.0	0.0
1.0	4	75.0	0.0	25.0	0.0	90.5	87.5	9.5	12.5	55.6	20.0	11.1	80.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0
	8	A-M.	100.0	A-M.	0.0	A-M.	100.0	A-M.	0.0	A-M.	93.2	A-M.	0.0	A-M.	3.4	A-M.	3.4	A-M.	0.0	A-M.	0.0
	24	A-M.	100.0	A-M.	0.0	A-M.	100.0	A-M.	0.0	A-M.	98.8	A-M.	0.0	A-M.	1.2	A-M.	0.0	A-M.	0.0	A-M.	0.0
3.0	4	98.4	100.0	1.6	0.0	42.9	91.2	57.1	8.8	70.0	91.4	20.0	8.6	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	A-M.	100.0	A-M.	0.0	A-M.	100.0	A-M.	0.0	A-M.	100.0	A-M.	0.0	A-M.	0.0	A-M.	0.0	A-M.	0.0	A-M.	0.0
	24	TOXIC																			

conc. : concentration

D : Direct treatment ; R : Recovery treatment

A-M. : a-mitosis

The maximum percentage of recorded bridges was 80.0% of the abnormal ana-telophase cells after the recovered treatment with 1% extract for 4 hours.

Table [14] shows that disturbed ana-telophase scored, in general, much higher percentages after treatment with the lower concentrations of the extract (0.25% and 0.5%) than higher ones (1% and 3%). 16.7% of the abnormal ana-telophase was the maximum percentage of disturbed ana-telophase after the recovery of 4 hours treatment with 0.25% extract.

The abundance of diagonal anaphase was slightly lower than that of disturbed ana-telophase. Diagonal anaphase recorded a maximum percentage (40.0% out of the abnormal ana-telophase cells) after recovery treatment of 0.25% extract for 8 hours.

Surpassing chromosomes and despiralization were induced at the lowest frequencies of occurrence. Table [14] shows that these abnormalities were induced only after 4 hours treatment with 0.25% and 0.5% extracts.

2. After treatment with *Portulaca* extract :-

Table [15] shows that prophase abnormalities were of three types; these are stickiness, irregularity and despiralization. Stickiness was so dominent on the expense of the other two types that 100% of the abnormal prophase cells at most treatments were of the sticky type. Irregular prophase was induced in few treatments, specially when the highest concentration (10% extract) was applied. Direct and recovery treatments with 10% extract for 4 hours induced the maximum percentages of irregular prophase. Despiralization was induced only after recovery of 8 hours treatment with 1% extract [Table 15].

Table [15] : Distribution of the different types of abnormalities in each mitotic phase after direct and recovery treatments of Allium cepa root tips with Portulaca extract

Treatment		Prophase						Metaphase				Ana-telophase							
Extract conc. (%)	Duration (hours)	Sticky		Irregular		Despiral		Sticky		Diturbed		Sticky		Bridge		Diagonal		Disturbed	
		D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
1.0	4	100.0	100.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	96.3	71.9	0.0	15.6	0.0	3.1	3.7	9.4
	8	97.0	95.7	3.0	0.0	0.0	4.3	100.0	92.6	0.0	7.4	85.2	86.7	0.0	3.3	14.8	6.7	0.0	3.3
	24	100.0	100.0	0.0	0.0	0.0	0.0	64.7	98.2	35.3	1.8	70.0	95.6	0.0	0.0	10.0	4.4	20.0	0.0
5.0	4	100.0	100.0	0.0	0.0	0.0	0.0	100.0	85.7	0.0	14.3	98.0	74.2	2.0	9.7	0.0	16.1	0.0	0.0
	8	100.0	100.0	0.0	0.0	0.0	0.0	100.0	89.7	0.0	10.3	93.0	88.9	0.0	1.6	7.0	9.5	0.0	0.0
	24	A-M.	100.0	A-M.	0.0	A-M.	0.0	A-M.	100.0	A-M.	0.0	A-M.	97.1	A-M.	2.9	A-M.	0.0	A-M.	0.0
10.0	4	85.4	75.0	14.6	25.0	0.0	0.0	72.5	92.0	27.5	8.0	85.0	87.5	15.0	12.5	0.0	0.0	0.0	0.0
	8	100.0	93.8	0.0	6.2	0.0	0.0	100.0	100.0	0.0	0.0	85.7	90.7	0.0	2.3	14.3	2.3	0.0	4.7
	24	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.	A-M.

conc. : concentration

D : Direct treatment ; R : Recovery treatment

A-M. : a-mitosis

Stickiness and spindle disturbance were the observed types of abnormalities at metaphase. Stickiness was highly frequent in most treatments (eight of which had metaphase abnormal cells composed entirely of the sticky type) [Table 15]. Some treatments induced disturbed metaphase cells of relatively low percentages. The maximum scored percentage of disturbed spindle constituted 35.3% of the abnormal metaphase cells which were induced by 24 hours direct treatment with 1% extract.

Types of abnormalities in the ana-telophase were stickiness, bridges, disturbed spindle and diagonal anaphase [Table 15]. Stickiness still the most frequent type although it did not reach 100% of the abnormal ana-telophase cells at any treatment as occurred in prophase or metaphase. Its percentage appearred to decrease with lapse of time within low concentrations (1% and 5%) direct treatments. 98.0% of the abnormal ana-telophase cells was the maximum percentage of stickiness; it was recorded after 4 hours direct treatment with 5% extract.

Bridge formation was induced after the recovery rather than direct treatments [Table 15] shows that only two direct treatments have induced bridge formation). The maximum percentage of bridges was 15.6% of the abnormal ana-telophase cells induced after the recovery of 1% extract treatment for 4 hours.

Diagonal anaphase was not induced after 4 hours direct treatment of any of the used concentrations; 16.1% was the maximum percentage of diagonal anaphase induced after the recovery treatment of 5% extract for 4 hours [Table 15].

Disturbed spindle was apparently induced after low concentration (1%) treatment, whereas higher concentrations (5% and 10%) caused no disturbance, with the exception of the recovery treatment of 10% extract for 8 hours [Table 15]. 20.0% was the maximum percentage of disturbance resulted after 24 hours direct treatment with 1% extract.

3. After treatment with plants chemical compounds :-

Prophase and metaphase abnormalities were only of the sticky type, as shown clearly in Table [16], after treatment with any concentration of either compound (1) or compound (2). Ana-telophase abnormalities were basically of the sticky type after treatment with either compound. However, bridge formation was induced at very low percentages (1.6% and 1.4%) after treatment with 300 µg/ml and 500 µg/ml of compound (1), respectively. Compound (2) did not induce bridge formation, meanwhile 100 µg/ml of it induced diagonal anaphase at a very low percentage (2.9%). However, the latter type of abnormality was not induced by any concentration tested of compound (1) [Table 16].

Table [16] : Distribution of the different types of abnormalities in each mitotic phase after treatment with the isolated compounds (1) and (2)

Treatment concentration	Prophase		Metaphase		Ana-telophase					
	Stickiness		Stickiness		Stickiness		Bridge		Diagonal	
	Comp.1	Comp.2	Comp.1	Comp.2	Comp.1	Comp.2	Comp.1	Comp.2	Comp.1	Comp.2
100 µg/ml	100.0	100.0	100.0	100.0	100.0	97.1	0.0	0.0	0.0	2.9
300 µg/ml	100.0	100.0	100.0	100.0	98.4	100.0	1.6	0.0	0.0	0.0
500 µg/ml	100.0	A-M.	100.0	A-M.	98.6	A-M.	1.4	A-M.	0.0	A-M.

A-M. : a-mitosis

G - Effect on the nucleoplasmic index (NP) :-

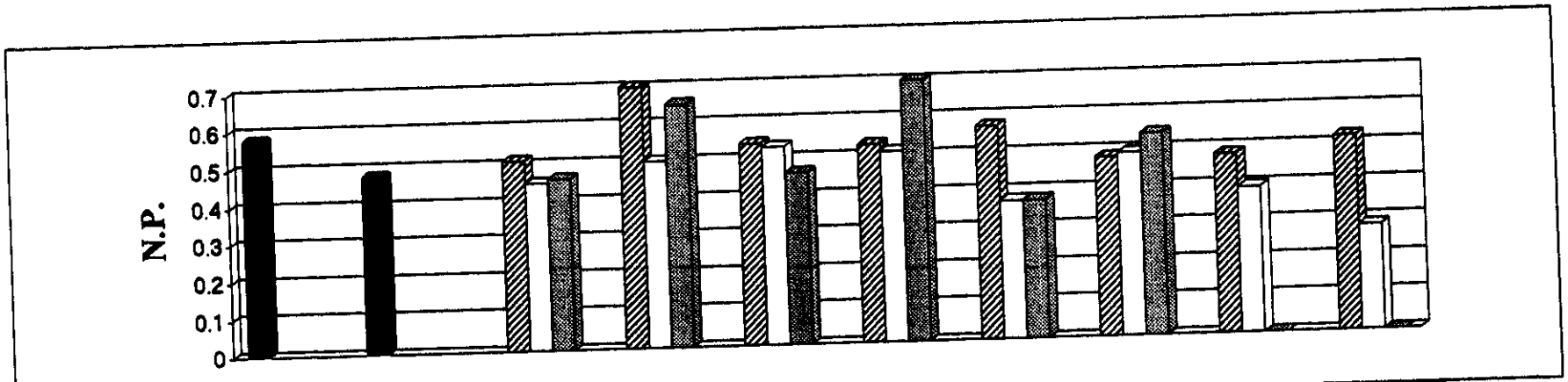
1. Effect of *C. droserifolia* extract :-

The treatment with *C. droserifolia* extract caused a highly significant decrease in the NP of *A. cepa* root tip cells [Table 5], specially when higher concentrations and longer time durations were applied.

The maximum scored NP in direct treatments was 0.57 resulted from 1% extract for 4 hours and the minimum was 0.37 from 1% extract for 8 and 24 hours [Fig. 18]. This Figure shows that the treatment with 0.25% extract for 8 and 24 hours and with 0.5% for 24 hours resulted in significantly reduced NP values, 0.45, 0..46 and 0.46, respectively, when compared to that of the control (0.58). Treatment with 1% extract for 8 and 24 hours and with 3% for 8 hours resulted in highly significant reduction in the NP values (0.37, 0.37 and 0.39). However, the NP values resulting by different direct treatments showed a slight effect of increasing duration of the treatment specially from 4 to 8 hours, but increasing concentration resulted in a fluctuated pattern.

The test of recovery showed that some of the NP values of the recovery treatments (1% extract treatments) appeared in an ascending trend with increase of time, while the others showed a fluctuating effect of both concentration and time. All the direct treatments which gave significantly reduced NP values, except that of the 3% extract for 8 hours, could be recovered. Moreover, some recovery treatments (0.25% for 4 hours and 0.5% for 24 hours) gave NP values (0.7 and 0.7) significantly higher than either that of the control or those of their direct ones. The treatment with 3% extract (the highest applied concentration) for 8 hours showed no sign of recovery (NP = 0.39 after direct treatment dropped to 0.28 after recovery).

Fig. [18] : Nucleoplasmic index (N.P.) in Allium cepa root tip cells after direct and recovery treatments with Cleome extract



Duration	0.0% extract		0.25% extract		0.5% extract		1.0% extract		3.0% extract	
	D	R	D	R	D	R	D	R	D	R
Control	0.58	0.48								
4 hours			0.51	0.70*	0.54	0.53	0.57	0.48	0.48	0.52
8 hours			0.45*	0.50	0.53	0.51	0.37**	0.49	0.39**	0.28**
24 hours			0.46*	0.65	0.46	0.70*	0.37**	0.54	TOXIC	TOXIC

D : Direct treatment ; R : Recovery treatment.

L.S.D. (5%) = 0.119

L.S.D. (1%) = 0.157

* : Significant to control at 0.05 level of probability (t-test).

** : Significant to control at 0.01 level of probability (t-test).

2. Effect of *P. oleracea* extract :-

A highly significant reduction in the NP values after treatment with *P. oleracea* extract was observed [Table 5]. This effect was concentration- and time duration-dependent, although there was no distinct gradation of NP values with the gradual increase of the extract concentration or time duration of the treatments.

The NP reached a maximum value (0.62) which was significantly higher than that of the control(0.54) after applying the lowest concentration (1%) for 8 hours direct treatment and a minimum value (0.26). at the highest concentration (10%) for 4 hours direct treatment [Fig.19]. All the direct treatments, except for that of the 1% extract for 8 hours, resulted in NP values significantly lower than that of the control (0.54).

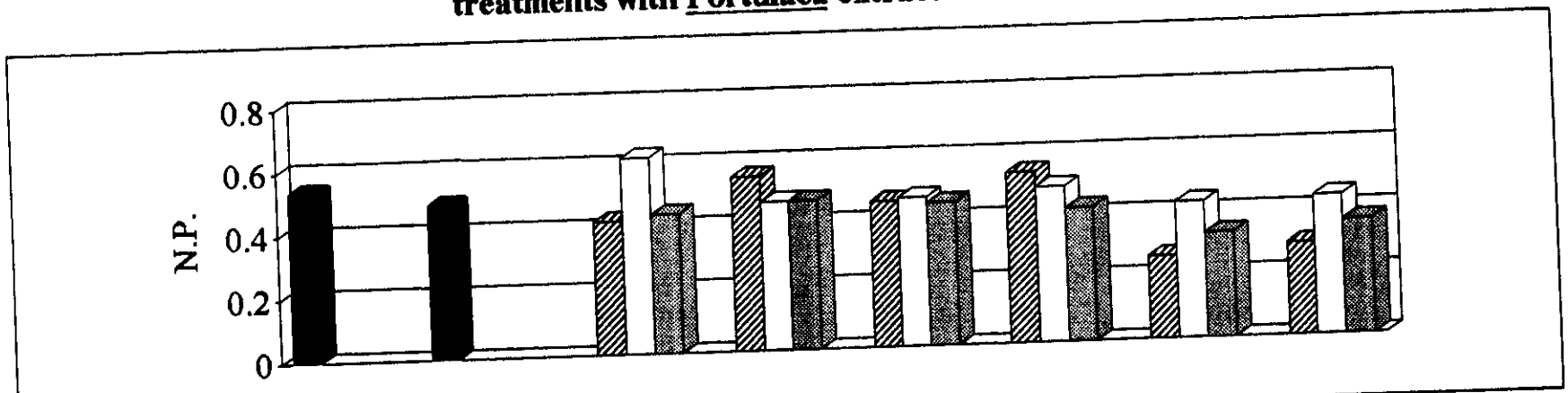
After recovery test, the NP values increased in all the treatments, except for that of the 1% for 8 hours and 5% for 24 hours, as compared to their direct origins. Nevertheless, only two of these treatments (1% and 5% extracts for 4 hours) showed to be really recovered, since the difference between the NP values of the direct treatments (0.42 and 0.46) and those of the recovery ones (0.55 and 0.54) were highly significant.

3. Effect of plants chemical compounds :-

a) Effect of compound (1) [isolated from *C. droserifolia*] :-

The treatment with different concentrations of compound (1) resulted in a highly significant, fluctuated increase of the NP of the treated specimens [Fig. 20]. The maximum scored NP (0.7) after treatment with 100 µg/ml was highly significant compared to that of the control (0.49), while the

Fig. [19] : Nucleoplasmic index (N.P.) in Allium cepa root tip cells after direct and recovery treatments with Portulaca extract



Duration	0.0% extract		1.0% extract		5.0% extract		10.0% extract	
	D	R	D	R	D	R	D	R
Control	0.54	0.49						
4 hours			0.42**	0.55	0.46**	0.54	0.26**	0.29**
8 hours			0.62**	0.47*	0.47*	0.49	0.43**	0.44**
24 hours			0.44**	0.47*	0.45**	0.42*	0.33**	0.36**

D : Direct treatment ; R : Recovery treatment

L.S.D. 5% = 0.056

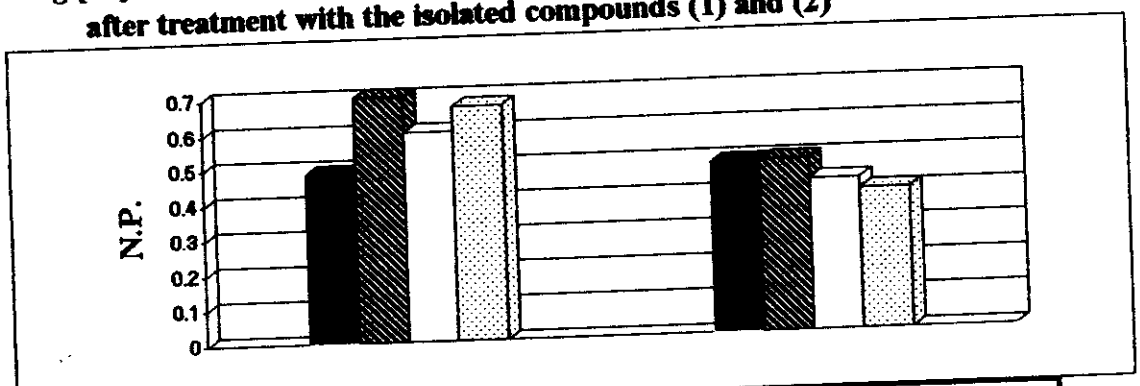
L.S.D. 1% = 0.075

* : Significant to control at 0.05 level of probability (t-test).

** : Significant to control at 0.01 level of probability (t-test).

minimum NP (0.6) after treatment with 300 µg/ml was also significantly higher than that of the control. The treatment with 500 µg/ml resulted in a medium NP value (0.67).

Fig.[20] : Nucloeplamic index (N.P.) in Allium cepa root tip cells after treatment with the isolated compounds (1) and (2)



Treatment	Compound (1)	Compound (2)
Control	0.49	0.49
100 µg/ml	0.70**	0.49
300 µg/ml	0.60**	0.43
500 µg/ml	0.67**	0.40*
L.S.D. (5%) =	0.085	0.076
L.S.D. (1%) =	0.117	0.106

*** : Significant to control at 0.05 level of probability (t-test).**
**** : Significant to control at 0.01 level of probability (t-test).**

b) Effect of compound (2) [isolated from *P. oleracea*] :-

A non-significant decrease of the NP values was scored on treating *A. cepa* root tips with differrent concentrations of compound (2) [Fig. 20]. Treatment with 100 µg/ml (the lowest concentration) resulted in NP value equal to that of the control (0.49). Increasing the compound concentration to 300 µg/ml resulted in a medium value (0.43) and the minimum value (0.4) arose after treatment with the highest concentration (500 µg/ml). This minimum NP value was significantly different from that of the control.

ii - Meiotic Investigations : -

Treating the flower buds of *V. faba* with solutions of either *Cleome* or *Portulaca* extracts resulted in meiotic aberrations of different types and ratios. The time duration of the different treatments was constant (3 hours), but fixation of the flower buds was carried out after 24 and 48 hours to test the degree of effects persistance.

A- Percentage of total abnormalities (PTA) :-

Table [17] and Fig. [21] show that application of 1% and 3% *Cleome* extracts induced highly significant PTA values when compared to that of the control. These PTA values were inversely proportional to the extract concentration and also to the elapsed time (24 or 48 hours) before fixation of the flower buds. With *Portulaca* extract the PTA values were similarly highly significant when compared to the control and inversely proportional to the extract concentration. Meanwhile, the lapse of time between treatment and fixation was inversely proportional to the PTA induced when 2.5% extract was applied and directly proportional with the 10% one.

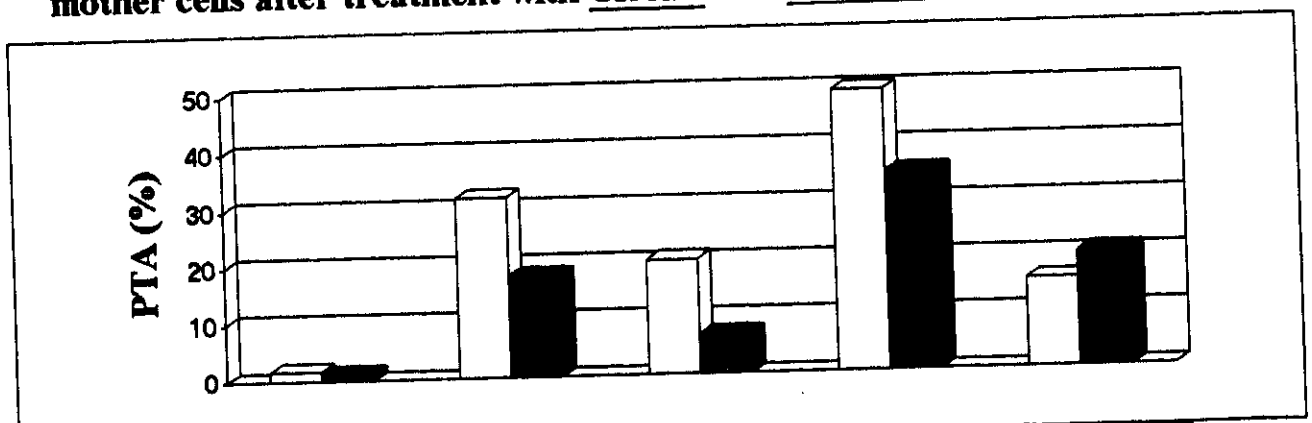
Treatment with 1% *Cleome* extract followed by 24 hours prior to fixation induced the highest PTA (31.8%), while the 3% extract treatment-48 hours induced the lowest PTA (6.8%). The maximum and minimum PTA induced by *Portulaca* extract were 49.5% after 2.5% extract-24 hours and 15.6% after 10% extract-24 hours, respectively [Table 17 and Fig. 21].

Table [17] : Percentages of meiotic abnormalities in Vicia faba plant after treatment with Cleome and Portulaca extracts

Treatment		Total counted PMCs	PTA	First division				PTA in 1st division	Second division				PTA in 2nd division
				Diak. & metap.I		Ana-telophase I			Metaphase II		Ana-telophase II		
Extract Conc.	Time lapse between treatment and fixation			PMCs (No.)	Abn.(%)	PMCs (No.)	Abn.(%)		PMCs (No.)	Abn.(%)	PMCs (No.)	Abn.(%)	
Control	24 hours	2095	1.7	584	1.7	512	4.9	3.2	56	0	943	0	0
	48 hours	2074	1	666	0.6	44	0	0.6	566	2.7	798	0.3	1.3
Cleome extract 1.0%	24 hours	1861	32	117	54.7	244	59	57.6	94	20.2	1406	25.9	25.5
	48 hours	1156	18	407	31.5	286	3.5	19.9	191	20.9	272	10.3	14.7
Cleome extract 3.0%	24 hours	1187	20	463	22.9	255	39.6	28.8	94	13.8	375	5.3	7
	48 hours	2002	6.8	63	7.9	605	16.7	15.9	135	13.3	1199	1	2.3
Portulaca extract 2.5%	24 hours	960	50	256	21.5	333	31.2	27	96	88.5	275	84	85.2
	48 hours	1318	35	270	67.4	307	25.7	45.2	208	39.9	533	21.8	26.9
Portulaca extract 10.0%	24 hours	1569	16	383	23.8	735	13.9	17.3	57	54.4	394	5.1	11.3
	48 hours	803	20	476	31.5	53	20.8	30.4	8	0	266	0.4	0.4

Conc. : Concentration
PMCs : Pollen mother cells
PTA : Percentage of total abnormalities
Diak. & Metap.I : Diakinesis and metaphase I
Abn.(%) : Percentage of abnormalities

Fig. [21] : Percentage of total abnormalities (PTA) in Vicia faba pollen mother cells after treatment with Cleome and Portulaca extracts



Time lapse between treatment and fixation (hours)	Control	Cleome extract		Portulaca extract	
		1.0%	3.0%	2.50%	10.0%
24 □	1.7	31.8**	20.2**	49.5**	15.6**
48 ■	1.0	17.8**	6.8 **	34.9**	20.2**

L.S.D. (5%)	=	0.312	0.378
L.S.D. (1%)	=	0.422	0.513

** : Significant to control at 0.01 level of probability (t-test).

B- The percentage of abnormalities of the 1st and 2nd divisions :-

Comparing the percentages of abnormalities of the 1st division after treatment with *Cleome* extract, it can be seen that increasing the extract concentration was accompanied by a decrease in the PTA [Table 17]. Also, increasing the time lapse after treatment (from 24 to 48 hours) causes a decrease in the PTA. Exactly, the same trend was observed in the 2nd division. Comparing the PTA of the 1st division with those of the 2nd division, it can be seen that the PTA of the 1st division were higher than those of the 2nd one.

Table [17] shows that the PTA of the 1st division after treatment with *Portulaca* extract were negatively correlated with concentration of the extract and positively correlated with the time lapse between treatment and fixation. In the 2nd division the PTA were negatively correlated with either concentration or time lapse. When the PTA of the 1st and 2nd divisions are compared, it can be noted that the percentages of the 1st division are higher than those of the 2nd one in all the treatments except for that of 2.5% extract-24 hours, where the reverse was recorded.

C - Types of abnormalities :-

1) Stickiness :-

After treatment with either extract, stickiness was the most frequent type of abnormalities. Its percentages of occurrence fluctuated with the increase of concentration of *Cleome* extract and also with the increase of time lapse between treatment and fixation [Table 18]. After application of the *Portulaca* extract stickiness percentage increased with increasing the extract concentration and fluctuated with increasing the time lapse after treatment from 24 to 48 hours. 94.5% was the maximum

Table [18] : Percentages of different types abnormalities after treatment of Vicia faba flower buds with Cleome and Portulaca extracts

Treatment		Stickiness	Bridge	Un-oriented chromatin material and chromosome lagging	Disturbed spindle	Multi-nucleate cells
Extract Conc.	Time lapse between treatment and fixation					
Cleome extract 1.0%	24 hours	94.5	0.0	0.0	5.6	0.0
	48 hours	78.6	17.0	4.4	0.0	0.0
Cleome extract 3.0%	24 hours	65.9	12.5	3.3	18.3	0.0
	48 hours	91.2	5.9	0.0	0.0	2.9
Portulaca extract 2.5%	24 hours	38.1	10.3	5.1	33.1	13.5
	48 hours	90.7	1.1	8.3	0.0	0.0
Portulaca extract 10.0%	24 hours	97.5	0.4	2.1	0.0	0.0
	48 hours	95.1	1.2	3.1	0.0	0.6

conc. : concentration

percentage of stickiness scored after 1% *Cleome* extract-24 hours and the minimum was 65.9% after 3% extract-24 hours. The maximum percentage after *Portulaca* extract treatments was 97.5% after 10%-24 hours and the minimum was 38.1% after 2.5%-24 hours.

Although stickiness was recorded in all the meiotic phases, as seen in Table [19], it was not induced at ana-telophase II after 1% and 3% *Cleome* extract-48 hours, 10% *Portulaca*-48 hours. It disappeared also at metaphase II after 10% *Portulaca*-48 hours. Nevertheless, 100% of the meiotic abnormalities brought about in many different phases were of the sticky type, whether under treatment with *Cleome* or *Portulaca* extracts. Also, it can be noted that stickiness percentages in diakinesis and metaphase I and metaphase II were majorly higher or equal to that of ana-telophase I and ana-telophase II, respectively, with the exception of the 10% *Portulaca* extract treatment-24 hours, where the percentage of stickiness in ana-telophase I was higher than that in diakinesis and metaphase I.

Stickiness at different meiotic phases after treatment with *Cleome* or *Portulaca* extracts are shown in Plates [6,7].

2) Bridges :-

From Table [18] it is apparent that bridge formation was induced at a relatively low percentages if compared to stickiness, but at higher percentages than the other types of abnormalities. No distinct correlation between time lapse, or concentration of either extracts and the percentage of bridge induction was observed.

Bridge formation was induced at ana-telophase I and ana-telophase II after treatment with either extracts [Table 19]. When *Cleome* extract was

Table [19] : Distribuation of different types of abnormalities in the meiotic phases after treatment of Vicia faba flower buds with Cleome and Portulaca extracts

Treatment		First division								Second division							
Extract Conc.	Time lapse between treatment and fixation	Diakinesis and Metaphase I			Ana-telophase I					Metaphase II			Ana-telophase II				
		Sticky	Un-oriented bivalents	Disturbed	Sticky	Bridge	Lag	Disturbed	Multi-nucleate	Sticky	Un-oriented chromosome	Disturbed	Sticky	Bridge	Lag	Disturbed	Multi-nucleate
1.0% *Cleome* extract 3.0%	24 hours	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	90.9	0.0	0.0	9.1	0.0
	48 hours	93.0	7.0	0.0	30.0	70.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
	24 hours	92.5	7.5	0.0	43.6	21.8	0.0	34.6	0.0	61.5	0.0	38.5	40.0	40.0	0.0	20.0	0.0
	48 hours	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	66.7	0.0	0.0	33.3
2.5% *Portulaca* extract 10%	24 hours	100.0	0.0	0.0	58.7	41.3	0.0	0.0	0.0	60.0	28.2	11.8	6.1	2.6	0.0	63.5	27.8
	48 hours	98.4	1.6	0.0	75.9	3.8	20.3	0.0	0.0	91.6	8.4	0.0	87.9	1.7	10.3	0.0	0.0
	24 hours	94.5	5.5	0.0	99.0	1.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
	48 hours	96.7	3.3	0.0	81.8	18.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

Conc. : concentration

Plate |6| : Chromosome stickiness induced in *V. faba* PMCs :-

a. Sticky metaphase I : after treatment with 2.5% *Portulaca* extract and 48 hours recovery.

b. Severely sticky metaphase I : after treatment with 10% *Portulaca* extract-48 hours.

c. Sticky star metaphase I : after treatment with 10% *Portulaca* extract-24 hours.

d. Sticky diakinesis : after treatment with 10% *Portulaca* extract-48 hours.

e. Sticky anaphase I : after treatment with 2.5% *Portulaca* extract-48 hours.

f. Sticky anaphase I : after treatment with 2.5% *Portulaca* extract-48 hours.

g. Sticky telophase I : after treatment with 1% *Cleome* extract-24 hours.

h. Sticky telophase I : after treatment with 2.5% *Portulaca* extract-24 hours.

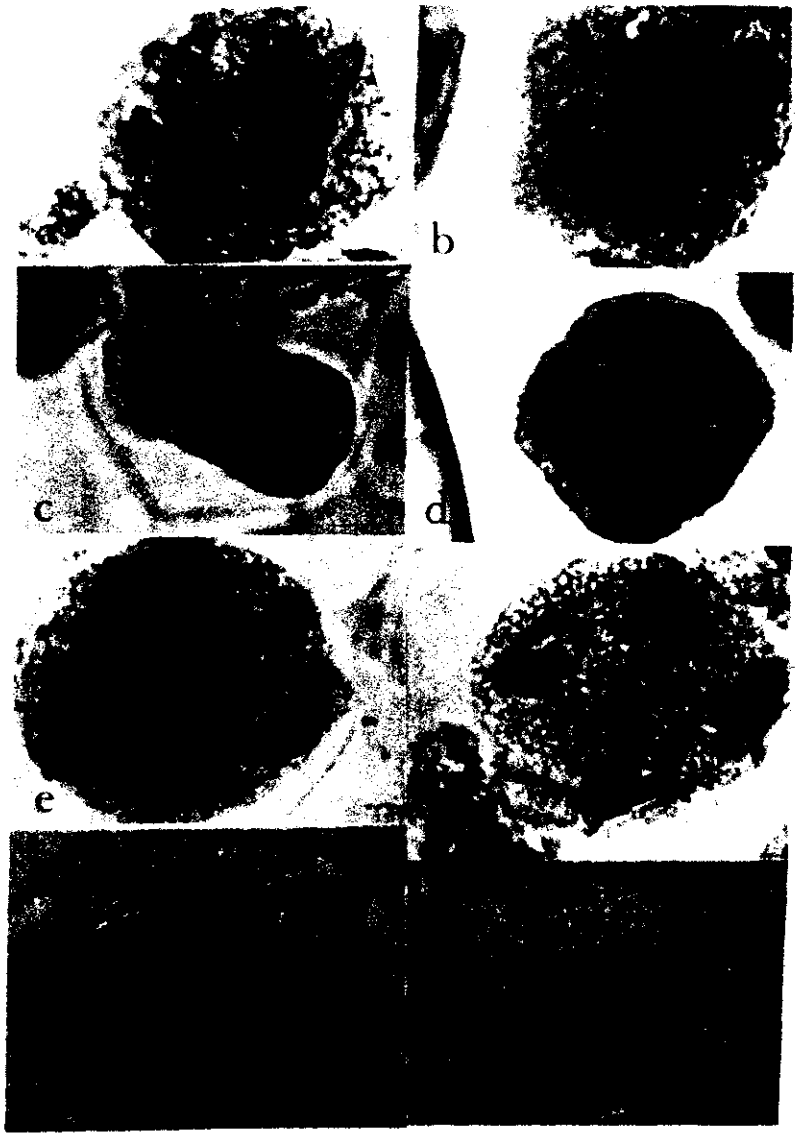


Plate [7] : Chromosome stickiness induced in *V. faba* PMCs :-

a. Sticky metaphase II : after treatment with 2.5% *Portulaca* extract-48 hours.

b. Sticky bridged anaphase I : after treatment with 10% *Portulaca* extract-24 hours.

c. Sticky anaphase II : after treatment with 3% *Cleome* extract-24 hours.

d. Sticky anaphase II : after treatment with 10% *Portulaca* extract-24 hours.

e. Sticky anaphase II : after treatment with 2.5% *Portulaca* extract-48 hours.

f. Sticky anaphase II : after treatment with 2.5% *Portulaca* extract-48 hours.

g. Very light sticky anaphase II : after treatment with 3% *Cleome* extract-24 hours.

h. Very light sticky anaphase II : after treatment with 10% *Portulaca* extract-24 hours.

applied, the percentages of bridge induction were higher at ana-telophase II (0.0%, 100%, 40% and 66.7%) than at ana-telophase I (0.0%, 70%, 21.8% and 0.0%). Application of *Portulaca* extract gave rise to the reverse result, i.e. the percentages of bridge induction at ana-telophase I (41.3%, 3.8%, 1.0% and 18.2%) were higher than those at ana-telophase II (2.6%, 1.7%, 0.0% and 0.0%) [Table 19].

Plate [8] illustrates different forms of bridges that resulted after treatment with the tested extracts.

3) Un-oriented chromatin material and chromosome lagging :-

Some *Cleome* extract treatments (1% extract-48 hours and 3%-24 hours) were inductive to detectable percentages of un-oriented bivalents only [Tables 18 and 19]. On the other hand, all *Portulaca* extract treatments were inductive to un-oriented chromatin and chromossome lagging, with a decrease in their percentages with increasing the extract concentration and, in the same time, increasing the time lapse caused an increase in these percentages [Table 18].

Table [19] shows that un-oriented bivalents were induced after all the *Portulaca* extract treatments, except that of 2.5% extract-24 hours, while at metaphase II un-oriented chromosomes were induced only after the 2.5% extract treatments. Lagging chromosomes, on the other hand, were induced only after the treatment of 2.5% *Portulaca* extract-48 hours, scoring at ana-telophase I 20.3% which dropped to 10.3% at ana-telophase II [Table 19].

Some examples of un-oriented bivalents induced by the extracts treatments are shown in Plate [9] .

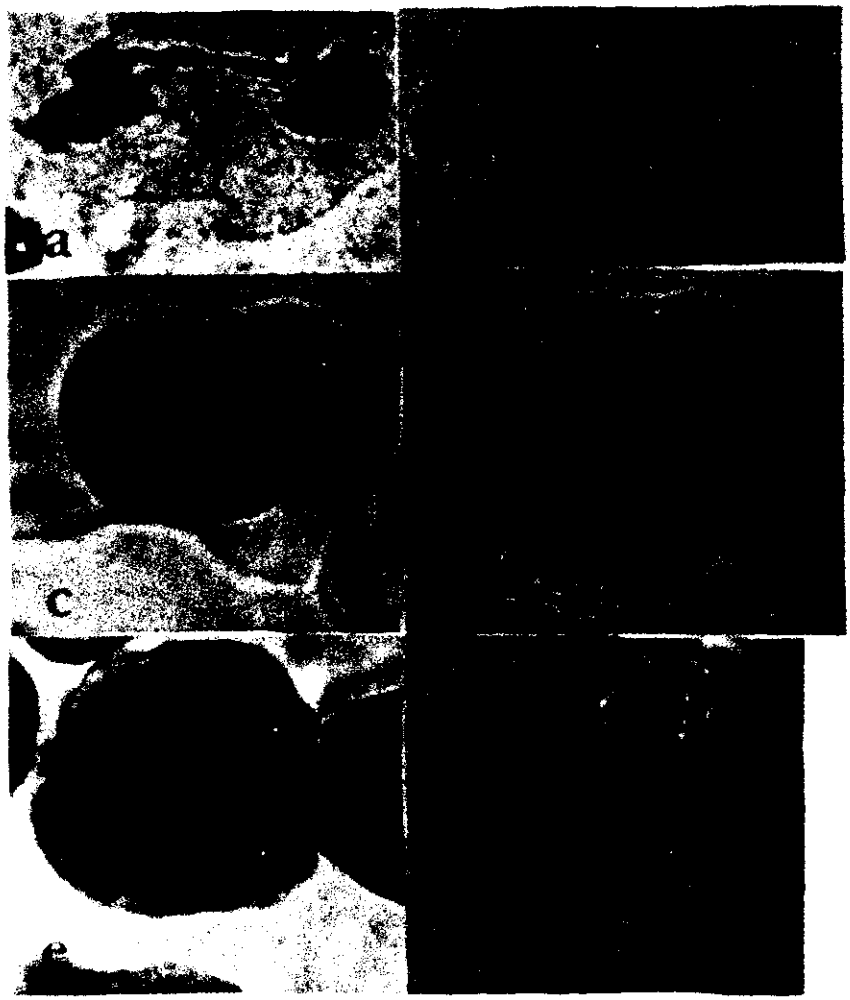


Plate [8] : Chromatin bridges induced in *V. faba* PMCs : -

a. Chromosome bridge at anaphase I : after treatment with 1% *Cleome* extract-48 hours.

b. Chromosome bridge at anaphase I : after treatment with 1% *Cleome* extract-48 hours.

c. Chromatid bridge at telophase I : after treatment with 3% *Cleome* extract-24 hours.

d. Chromatid bridge at telophase I : after treatment with 3% *Cleome* extract-24 hours.

e. Sticky bridged telophase I : after treatment with 10% *Portulaca* extract-24 hours.

f. Double bridge at anaphase II : after treatment with 2.5% *Portulaca* extract-48 hours.

Plate [9] : Un-oriented bivalents induced at metaphase I of *V. faba* PMCs : -

a & b After treatment with 1% *Cleome* extract-48 hours.

c & d After treatment with 2.5% *Portulaca* extract-48 hours.

e. Double un-oriented bivalent at metaphase I : after treatment with 10% *Portulaca* extract-48 hours.

f, g & h After treatment with 10% *Portulaca* extract-48 hours.

4) Disturbed spindle :-

This type of abnormality was induced only after 24 hours time lapse following treatments with 1% and 3% *Cleome* extract [Table 18]. Increasing the concentration of *Cleome* extract increased the percentage of spindle disturbance. *Portulaca* extract induced this abnormality only after 2.5% extract-24 hours.

In the 1st division, as seen in Table [19], spindle disturbance was absent from diakinesis and metaphase I of all the treatments with *Cleome* or *Portulaca* extracts. At ana-telophase I it was induced only after 3% *Cleome* extract-24 hours with a percentage of 34.6%. In the 2nd division, 38.5% of the abnormal metaphase II after 3% *Cleome* extract-24 hours, and 11.8% after 2.5% *Portulaca* extract-24 hours, were of the disturbed spindle type. At ana-telophase II, 1% and 3%-24 hours *Cleome* extract treatments induced 9.1% and 20.0% disturbed spindle of the total abnormalities, respectively. *Portulaca* extract (2.5%-24 hours) induced 63.5% disturbed spindle [Table 19].

Examples of spindle disturbance induced are illustrated in Plate [10].

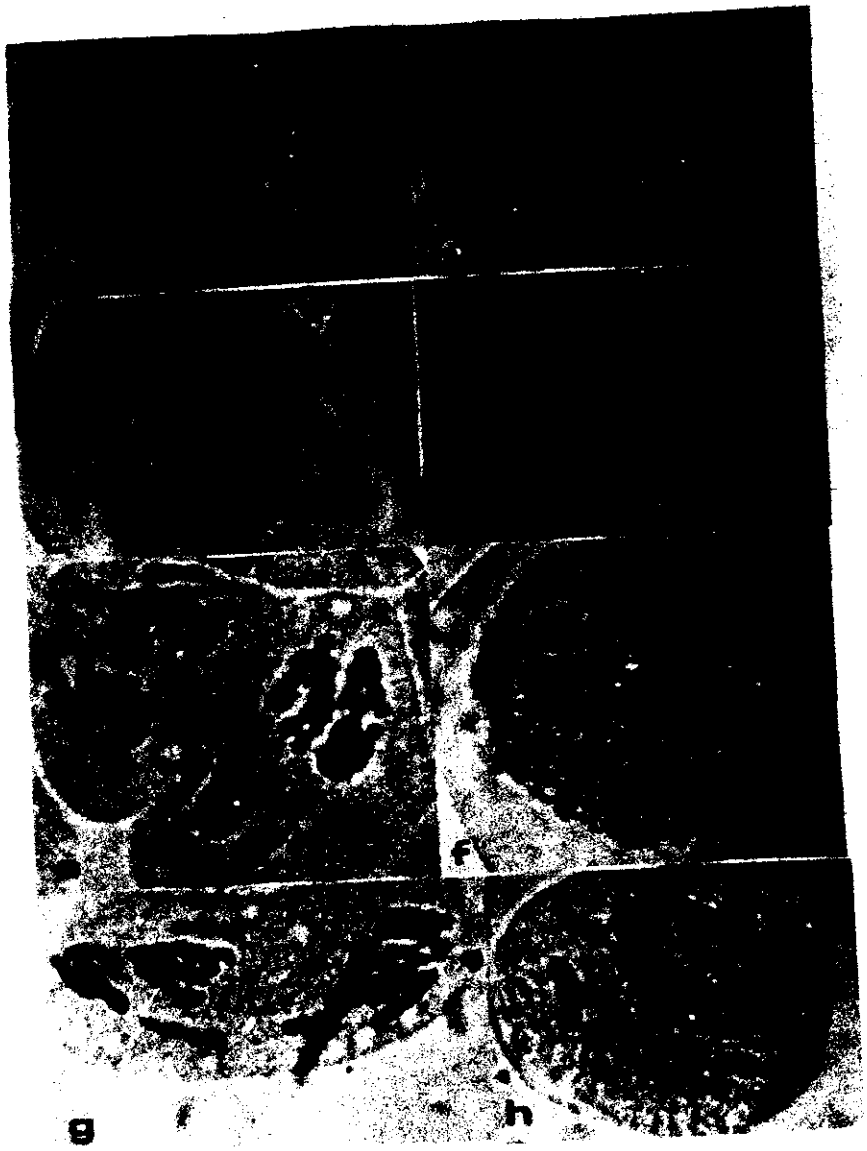


Plate [10] : Spindle disturbance induced in *V. faba* PMCs : -

a. Sticky disturbed metaphase I : after treatment with 10% *Portulaca* extract-24 hours.

b. Sticky disturbed metaphase I : after treatment with 10% *Portulaca* extract-24 hours.

c. Sticky disturbed metaphase I : after treatment with 2.5% *Portulaca* extract-48 hours.

d. Sticky disturbed metaphase I : after treatment with 2.5% *Portulaca* extract-48 hours.

e. Disturbed anaphase I : after treatment with 3% *Cleome* extract-24 hours.

f. Disturbed anaphase I with chromosome bridge : after treatment with 3% *Cleome* extract-24 hours.

g. Disturbed anaphase I : after treatment with 3% *Cleome* extract-24 hours.

h. Disturbed, multi-bridged anaphase I : after treatment with 3% *Cleome* extract-24 hours.

5) Multinucleate cells :-

Cells with 5, 6, 7 or 8 nuclei were observed after some treatments of *Cleome* or *Portulaca* extracts [Plate 11]. Only one treatment with *Cleome* extract (3%-48 hours) and two treatments with *Portulaca* extract (2.5%-24 hours and 10%-48 hours) were inductive to multinucleate cells [Table 18].

Multinucleate cells appearance was restricted at ana-telophase II [Table 19]. It was induced after the application of 3% *Cleome*-48 hours (33.3% of the abnormalities), 2.5% *Portulaca*-24 hours (27.8%) and 10%-48 hours (100%).

D- Percentages of abnormalities in spore tetrads and pollen grains :-

Table [20] and Fig. [22] show that treatment with *Cleome* extract induced abnormalities in both spore tetrads and pollen grains. Abnormalities of spore tetrads were only induced after treatment with 1% extract-48 hours (0.9%) and 3%-24 hours (6.5%), and they were all of the abnormally arranged type. It can be noted from Fig. [22] that the scored PTA values are highly significant when compared to the control. Pollen grains abnormalities were observed in all the *Cleome* extract treatments with rather low percentages, although three of them (5.3%, 2.5% and 1.8%) were highly significant when compared to that of the control. Increase of the extract concentration was accompanied by a decrease in the percentage of abnormalities. Effect of time lapse on the percentage of abnormalities was not regular. Deformation of pollen grains was the only type of abnormalities observed.

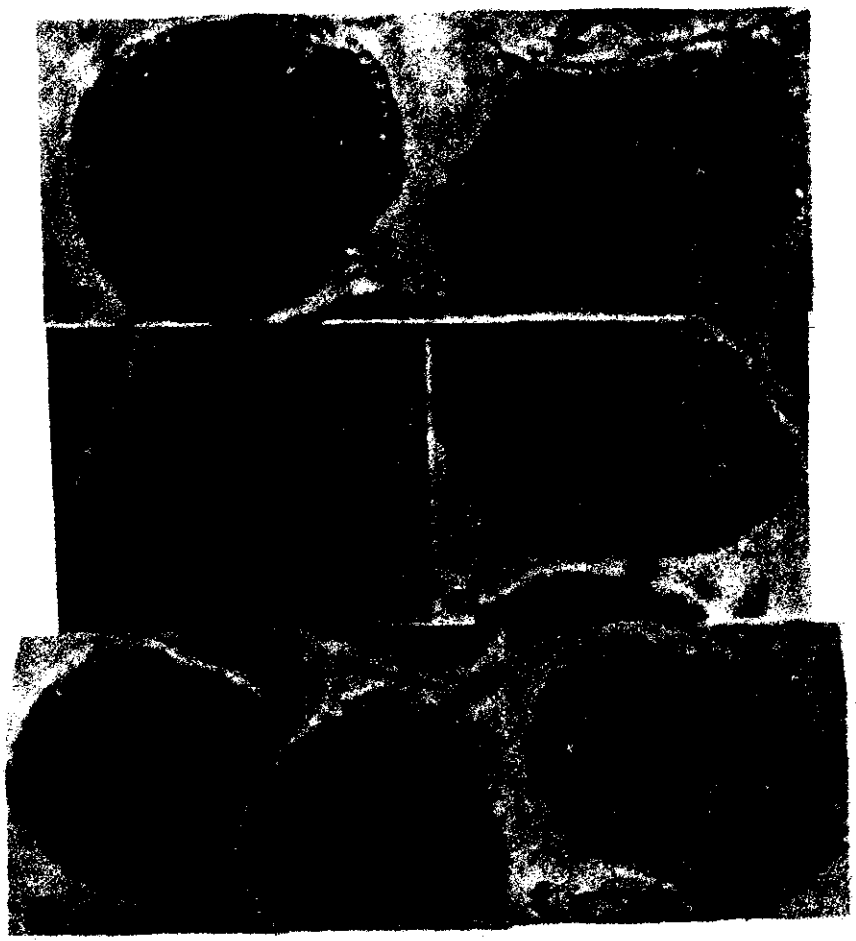


Plate [11] : Multi-nucleate cells induced in *V. faba* PMCs : -

a & b : After treatment with 3% *Cleome* extract-24 hours.

c & d : After treatment with 2.5% *Portulaca* extract-24 hours.

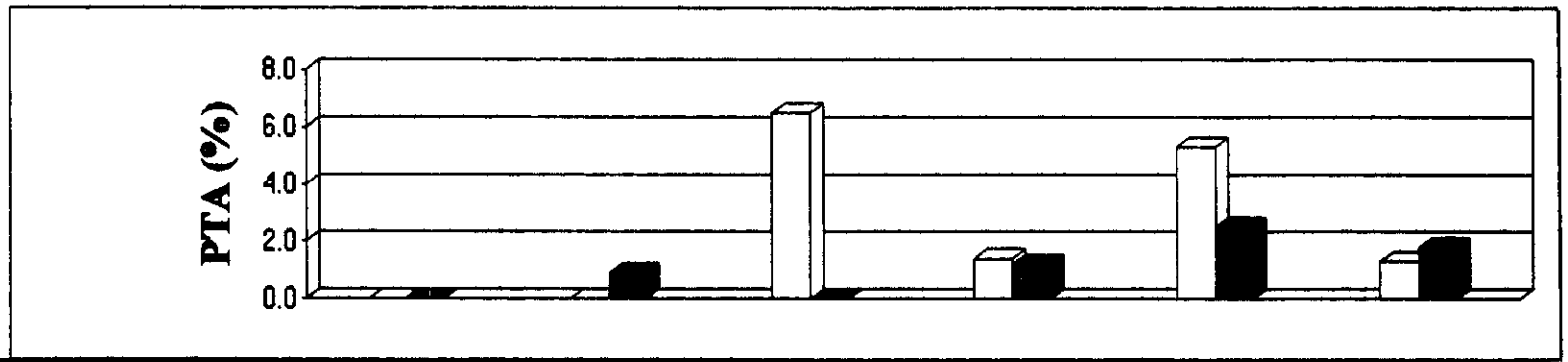
e & f : After treatment with 10% *Portulaca* extract-48 hours.

Table [20]: Percentages of abnormalities in spore tetrads and pollen grains of Vicia faba after treatment with Cleome and Portulaca extracts

Treatment		Spore tetrads					Pollen grains				
Extract Conc.	Time lapse between treatment and fixation	Number of spore tetrads	PTA	Types of abnormalities (%)			Number of pollen grains	PTA	Types of abnormalities (%)		
				Abn. arranged	Deformed	Sticky			Deformed	Un-stained	Sticky
Control	24 hours	225	0.0	-	-	-	1037	1.4	100.0	0.0	0.0
	48 hours	1074	0.0	-	-	-	2429	1.2	100.0	0.0	0.0
1.0% *Cleome* extract	24 hours	417	0.0	-	-	-	605	5.3	100.0	0.0	0.0
	48 hours	544	0.9	100.0	0.0	0.0	646	2.5	100.0	0.0	0.0
3.0% *Cleome* extract	24 hours	216	6.5	100.0	0.0	0.0	752	1.3	100.0	0.0	0.0
	48 hours	230	0.0	-	-	-	566	1.8	100.0	0.0	0.0
2.5% *Portulaca* extract	24 hours	288	1.7	0.0	0.0	100.0	521	89.1	1.9	26.9	71.1
	48 hours	221	17.6	0.0	12.8	87.2	463	1.7	100.0	0.0	0.0
10.0% *Portulaca* extract	24 hours	563	0.0	-	-	-	549	1.1	100.0	0.0	0.0
	48 hours	1004	0.7	0.0	100.0	0.0	1533	18.3	43.1	56.9	0.0

Conc. : Concentration
PTA : Percentage of total abnormalities
Abn. arranged : abnormally arranged

Fig.[22] : Percentage of total abnormalities (PTA) in spore tetrads and pollen grains of Vicia faba after treatment with Cleome extract



Time lapse between treatment and fixation (hours)	Spore Tetrads			Pollen Grains		
	Control	1.0% extract	3.0% extract	Control	1.0% extract	3.0% extract
24 ■	0.0	0.0	6.5**	1.4	5.3**	1.3
48 □	0.0	0.9**	0.0	1.2	2.5**	1.8**

L.S.D. (5%)	=	0.159	0.218
L.S.D. (1%)	=	0.223	0.306

** : Significant to control at 0.01 level of probability (t-test).

Abnormalities of spore tetrads and pollen grains were recorded after all the treatments with *Portulaca* extract, except for that of the 10% extract-24 hours where no abnormal spore tetrads were observed [Table 20 and Fig. 23]. The percentages of abnormalities of spore tetrads were negatively correlated with the extract concentration and positively correlated with the time lapse. Statistically, the induced percentages were highly significant when compared to the control. The maximum percentage of spore tetrads abnormalities was 17.6% after 2.5% extract-48 hours; 87.2% of this percentage was of the sticky type and the rest was of the deformed one. Treatment with 2.5%-24 hours induced 1.7% abnormality (stickiness only). The treatment with 10%-48 hours induced 0.7% abnormality (deformation only).

The percentages of abnormalities in pollen grains took a descending then an ascending patterns with increasing either the concentration or time lapse after treatment [Table 20]. The maximum, and also highly significant, percentage of abnormal pollen grains was 89.1% after 2.5% extract-24 hours [Fig. 23]; 71.1% of this percentage was stickiness, 26.9% unstained pollen grains and 1.9% deformation [Table 20]. The treatment with 2.5% extract-48 hours and 10%-24 hours induced low percentages of abnormalities (1.7% and 1.1%, respectively) and were composed of deformed pollen grains only. The last treatment, 10%-48 hours, induced the highly significant percentage (18.3%) of abnormality [Fig. 23]; 56.9% of which were unstained pollen grains and 43.1% deformed [Table 20].

Some types of abnormalities in spore tetrads and pollen grains are shown in Plates [12,13].

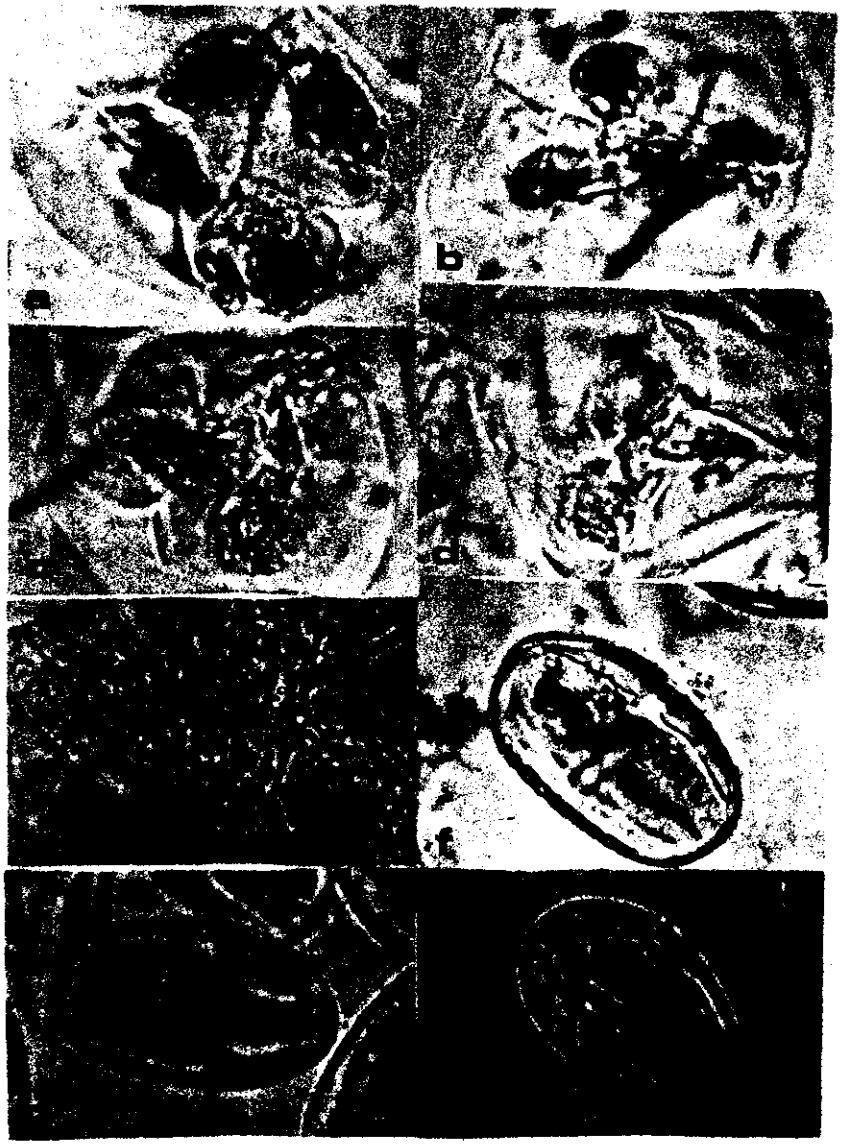


Plate [12] : Induced aberrations in spore tetrads and pollen grains of *V. faba* flower buds :-

a. Abnormally arranged spore tetrads : after treatment with 1% *Cleome* extract-48 hours.

b. Abnormally arranged spore tetrads : after treatment with 3% *Cleome* extract-24 hours.

c. Deformed spore tetrads : after treatment with 2.5% *Portulaca* extract-48 hours.

d. Deformed spore tetrads : after treatment with 10% *Portulaca* extract-48 hours.

e. Unstained pollen grains : after treatment with 10% *Portulaca* extract-48 hours.

f, g & h : Pollen grains with sticky nuclei : after treatment with 2.5% *Portulaca* extract-24 hours.

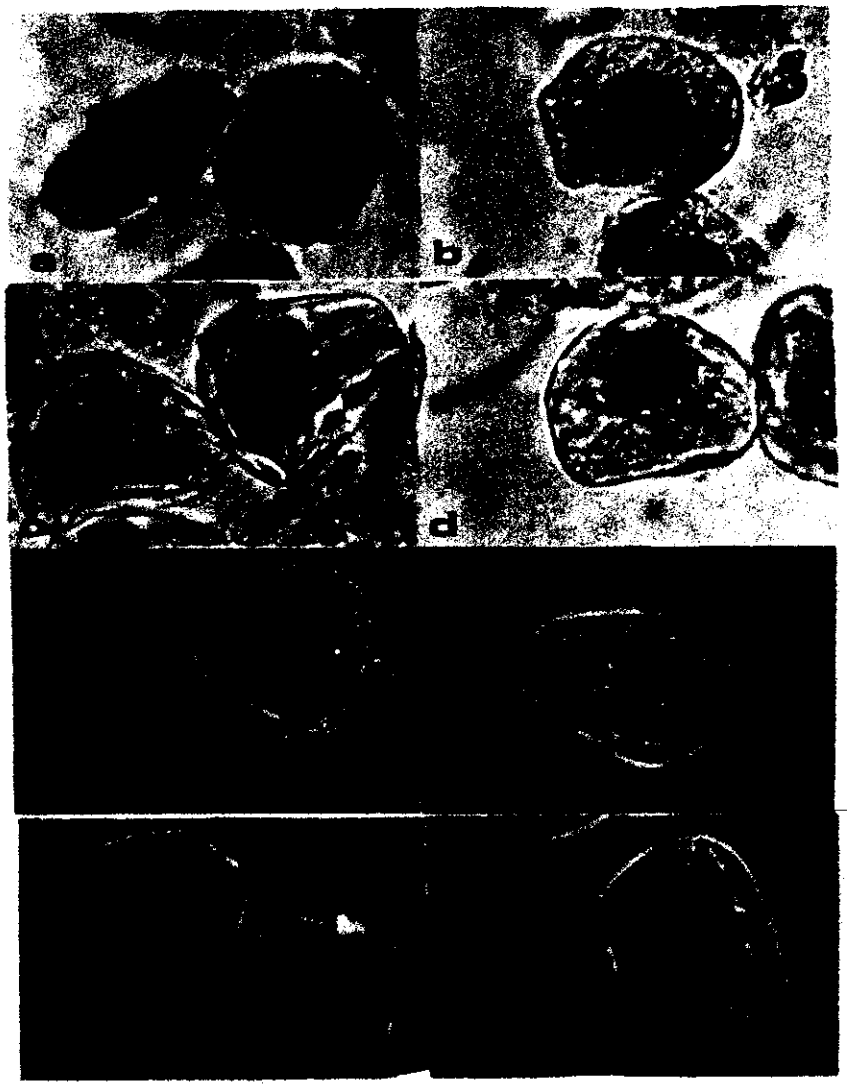


Plate [13] : Induced deformation in pollen grains of *V. faba* flower buds :-

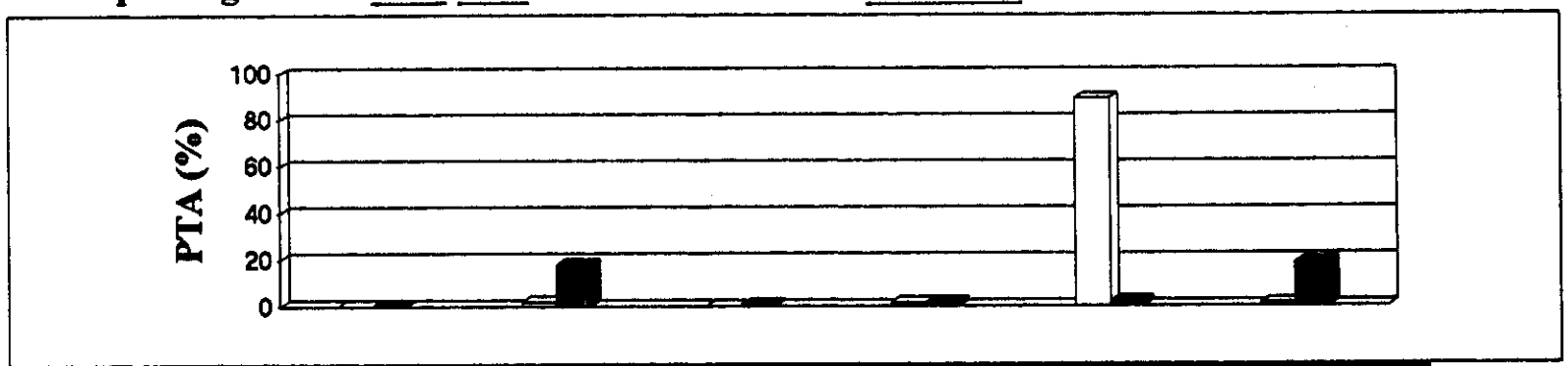
a &b : After treatment with 10% *Portulaca* extract-24 hours.

c & d : After treatment with 1% *Cleome* extract-48 hours.

e & f : After treatment with 3% *Cleome* extract-24 hours.

g & h : After treatment with 10% *Portulaca* extract-48 hours.

Fig.[23] : Percentage of total abnormalities (PTA) in spore tetrads and pollen grains of Vicia faba after treatment with Portulaca extract



Time lapse between treatment and fixation (hours)	Spore Tetrads			Pollen Grains		
	Control	2.5% extract	10.0% extract	Control	2.5% extract	10.0% extract
24 ■	0	1.7 **	0	1.4	89.1**	1.1
48 □	0	17.6**	0.7**	1.2	1.7	18.3**

L.S.D. (5%) = 0.218 0.509

L.S.D. (1%) = 0.306 0.714

** : Significant to control at 0.01 level of probability (t-test).

II - ELECTRON MICROSCOPY

Ultrathin sections of *Vicia faba* root tips treated with a number of concentrations of the aqueous extracts of *Cleome droserifolia* and *Portulaca oleracea* were examined under the electron microscope. Distinct cytoplasmic organelles, such as mitochondria, dictyosomes and the endoplasmic reticulum (ER) were looked at carefully to compare their structures to those of the untreated (control) root tip cells.

(A) Effect of treatment with *Cleome* extract :

Treatment with 0.5% *Cleome* extract induced an irregular topography of the mitochondrial surface accompanied by the appearance of an electron-lucid centre [Plate 16]. This may be the cause of rupture of some mitochondrial membranes [Plates 16 & 17]. Golgi vesicles were slightly swollen, and the ER saccules appearred exhausted or weakly organized [Plate 16]. This treatment induced also the formation of autophagic vacuoles [Plate 17].

Almost the same types of ultrastructural changes were induced by treatment with 1% *Cleome* extract. Some mitochondria showed the electron-lucid hollow centre [Plate 18], others showed rupture of their membranes [Plates 19 & 20]. Dictyosomes appearred with much more enlarged vesicles [Plates 18 &20] than those observed after the 0.5% extract treatment. The ER after this treatment also showed indistinct, apparently weakly-developed cisternae [Plates 18, 19 & 20]. Many autophagic vacuoles could be seen [Plates 18 & 19], perhaps containing rudiments of mitochondria which could be evidenced by the apparent swollowed body [Plate 19].

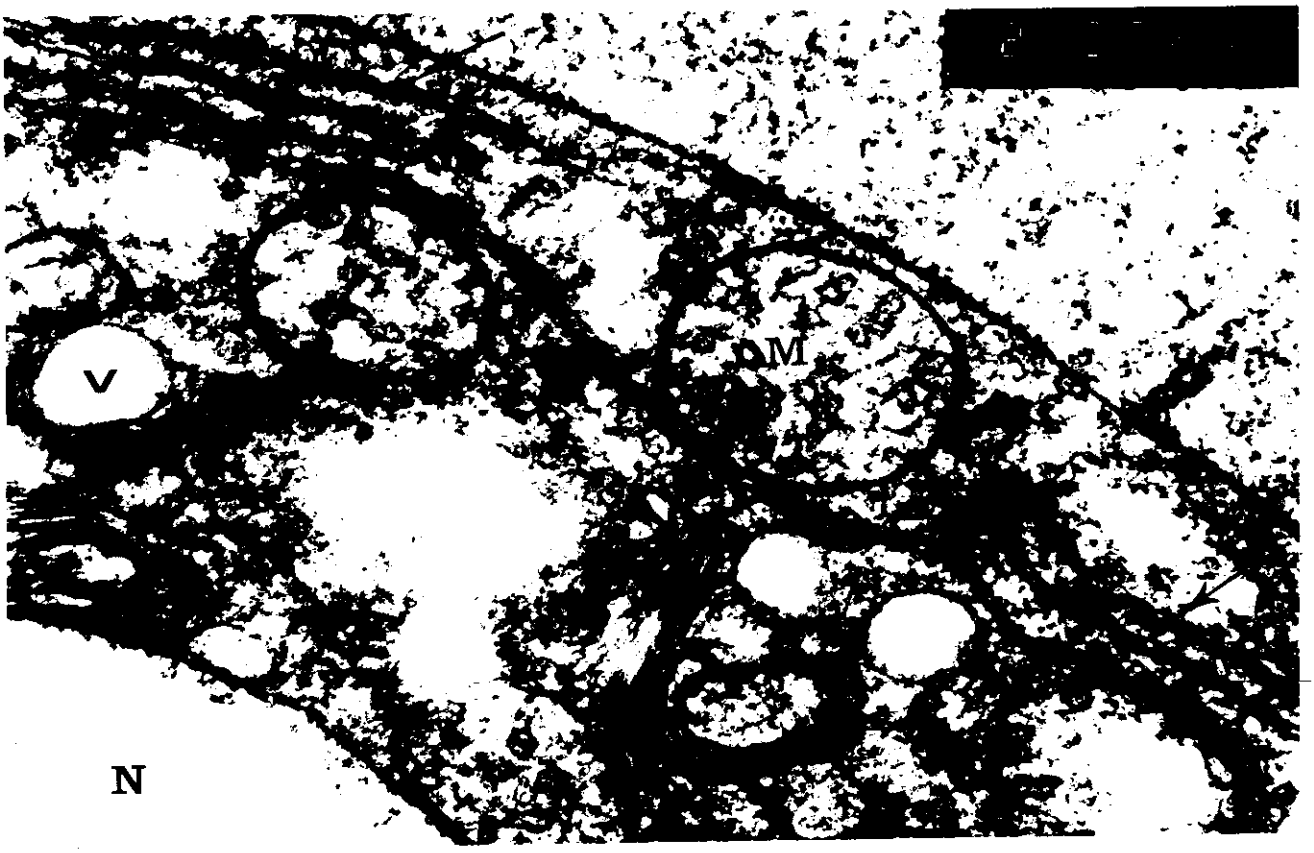


Plate [14] : Some cytoplasmic organelles in a control cell of *V. faba* root tip. Mitochondria {M}; endoplasmic reticulum {arrows}; vacuoles {V}; nucleus {N}. [X 108,000].

Plate [15] : Mitochondria {M}, and dictyosomes {D} in the cell cytoplasm of untreated (control) root meristem of *V. faba*. [X 108,000].

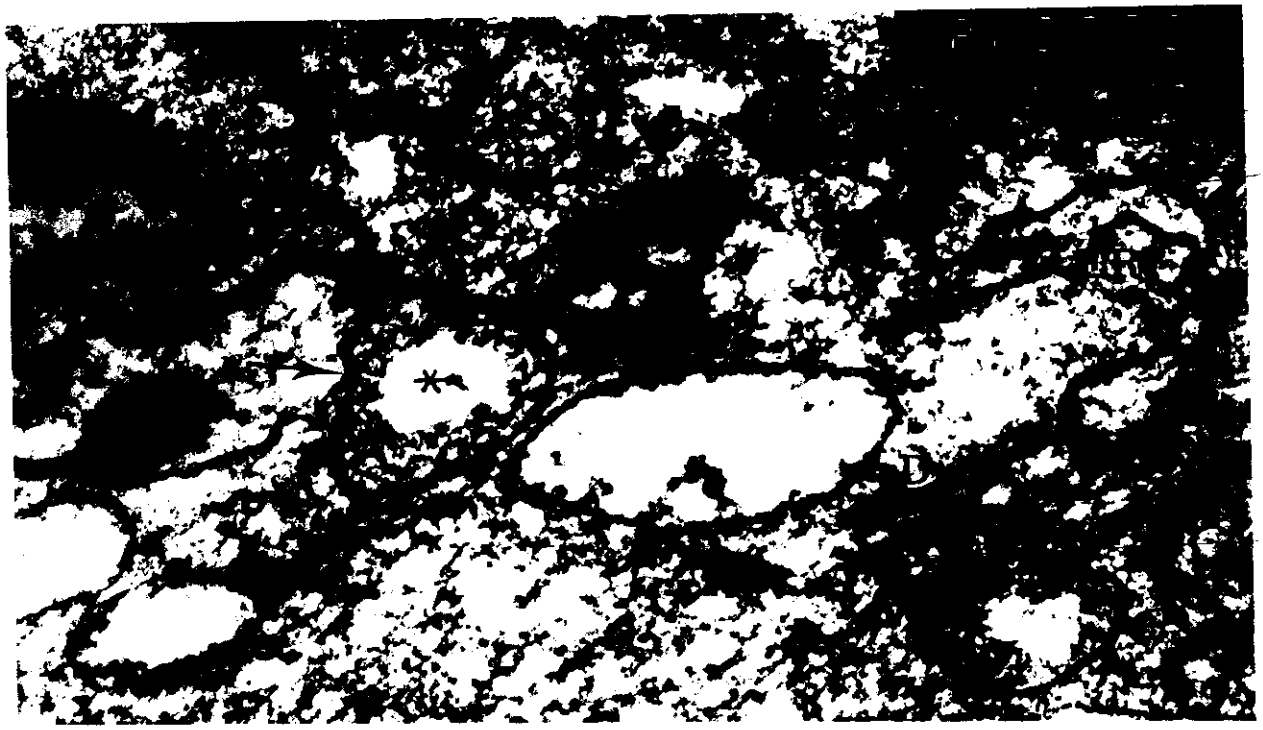


Plate [16] : Effect of 0.5% *Cleome* extract on the cytoplasmic organelles of *V. faba* root meristem. Irregular outline of the mitochondrial membrane {arrows} and another one ruptured {arrow heads}. Mitochondria with an electron-lucid hollow centre {*}. Swollen vesicles of the dictyosome {D}. Weakly organized endoplasmic reticulum {ER}. Vacuole {V}. [X 80,000].

Plate [17] : 0.5% *Cleome* extract-treated cells of *V. faba* root meristem. Note the ruptured membranes of mitochondria {M}, and the formation of an autophagic vacuole {AV}. [X 80,000].

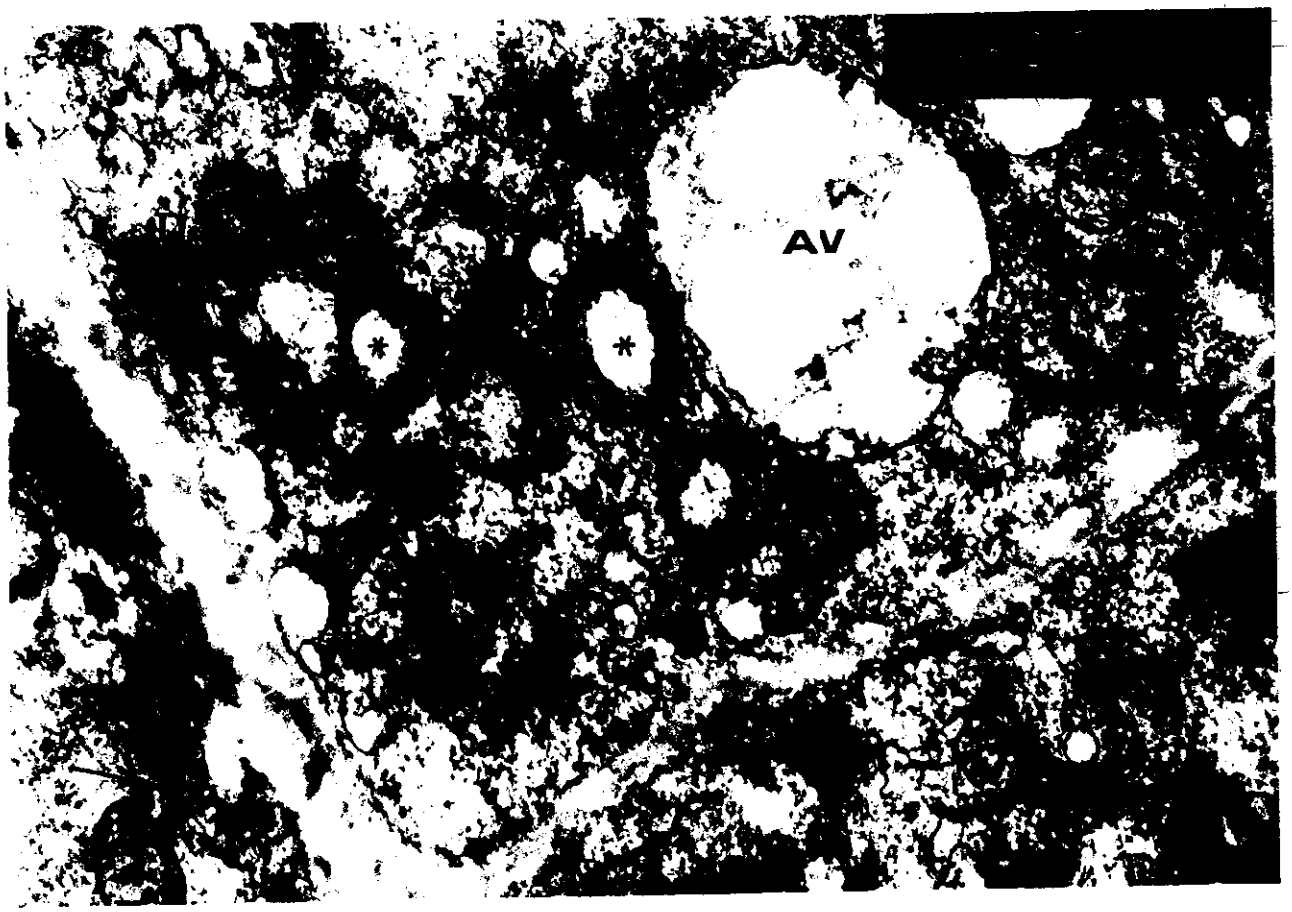


Plate [18] : Action of 1% *Cleome* extract treatment. Some mitochondria with electron-lucid hollow centre {*}. Dictyosome {D} with swollen vesicles. An autophagic vacuole {AV} containing some bodies (may be mitochondria) is notable. Weakly organized ER {arrows}. [X 56,000].

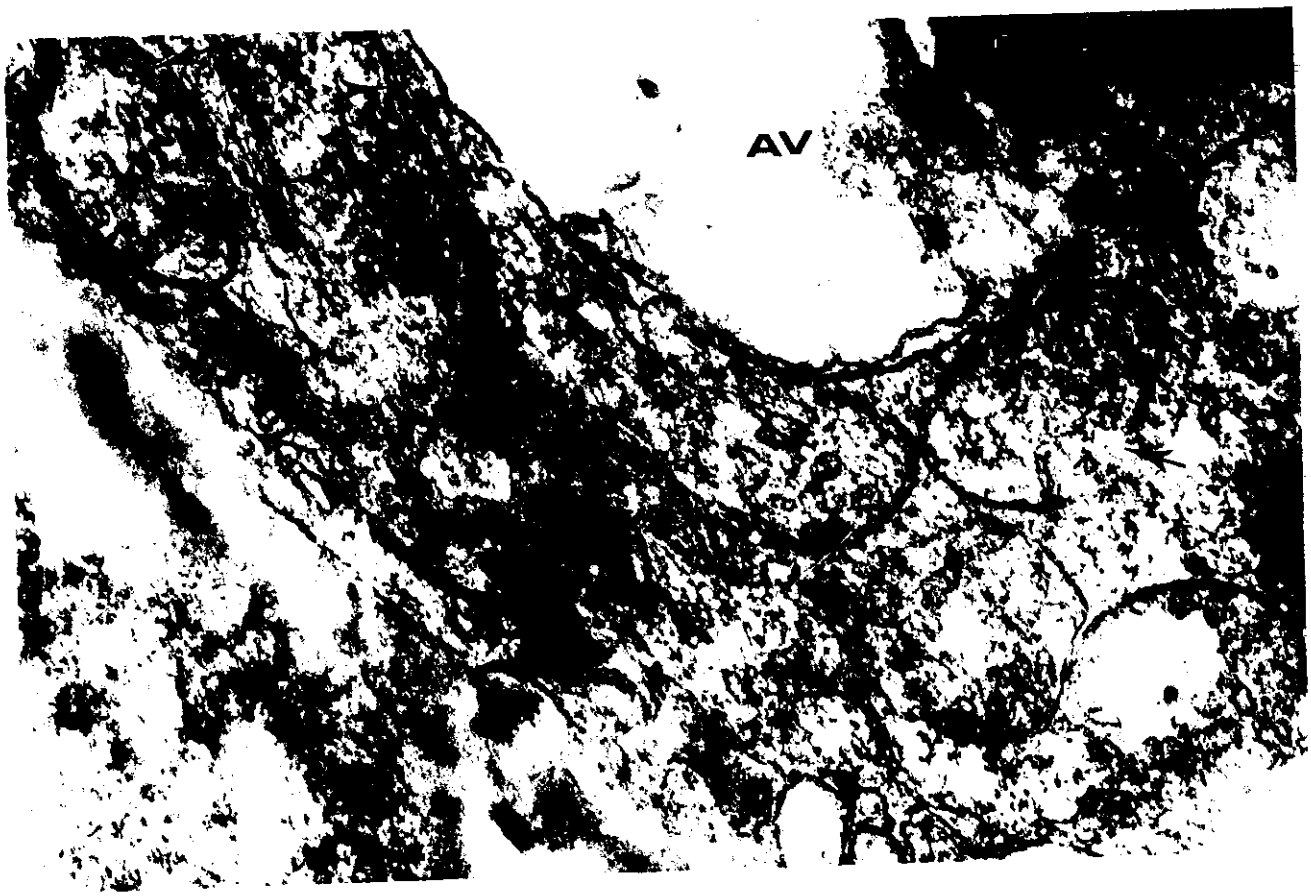


Plate [19] : Effect of 1% *Cleome* extract treatment. Mitochondria appear with incomplete or ruptured membranes {arrows}. An autophagic vacuole {AV} in the upper right side of the plate, with rudiments of a mitochondrion {*}. The ER {arrow heads} is weakly organized. [X108,000].

Plate [20] : Effect of treatment with 1% *Cleome* extract. Highly enlarged vesicles {*} of dictyosomes. Weakly organized endoplasmic reticulum {ER}, and mitochondrion with ruptured membrane {arrows}. [X 160,000].

Treatment with 3% *Cleome* extract proved to be extractive for the cytoplasmic components, since no ultrastructural details could be detected in spite of repeating the trials.

(B) Effect of treatment with *Portulaca* extract :

After 1% *Portulaca* extract treatment, dictyosomes showed remarkable changes. Their cisternae became electron-dense [Plates 21 & 23], and their vesicles appearred notably swollen [Plates 21, 22 & 23]. Some dictyosomes [Plate 23] appearred abnormally enlarged. Many mitochondria showed unusual irregular outline [Plate 21]. Autophagic vacuoles could be observed [Plate 22] enclosing bodies, which appearred to be mitochondria and ER segments. Many small, nearly spherical, membrane-bounded structures that may be Golgi vesicles were scattered in the cytoplasm [Plate 22]. Hanchey *et al.* (1968) noted these structures and suggested to identify them as "spherosomes". No clearly distinct alterations of the ER could be noted.

The main effect of the 5% *Portulaca* extract on the ER was contrary to that induced by the extract of 1% concentration. Long saccules of the ER tended to form loops [Plate 24] which may completely sequestrate parts of the cytoplasm [Plate 25] that may lead to the development of autophagic territories. Formation of concentric layers of the ER saccules, that may or may not enclose other cytoplasmic organelles, could be observed [Plate 26]. Many autophagic vacuoles were also noted [Plates 24, 25 & 26].

Treatment with the highest concentration of *Portulaca* extract (10%) induced effects, some of which were similar and others different from those induced by the lower concentrations (1% and 5%). Loops of ER saccules which enclose parts of the cytoplasm were still observed. Some

Plate [21] : Ultrastructural changes induced in *V. faba* root tip cells after treatment with 1% *Portulaca* extract. Dictyosomes with swollen vesicles {arrows}, and electron-dense cisternae are remarkable. Note also the irregular outline of mitochondria {M}. [X 56,000].

Plate [22] : Treatment with 1% *Portulaca* extract of *V. faba* root tip cells. An autophagic vacuole {AV} containing some organelles (apparently mitochondria and ER segments) can be observed. Dictyosomes with swollen vesicles {arrows} are also noted. Note the so-called "spherosomes" {arrow heads}. [X 80,000].

Plate [23] : Effect of 1% *Portulaca* extract treatment. Enlarged dictyosomes with remarkable swollen vesicles {arrows} and electron dense cisternae. [X 80,000].

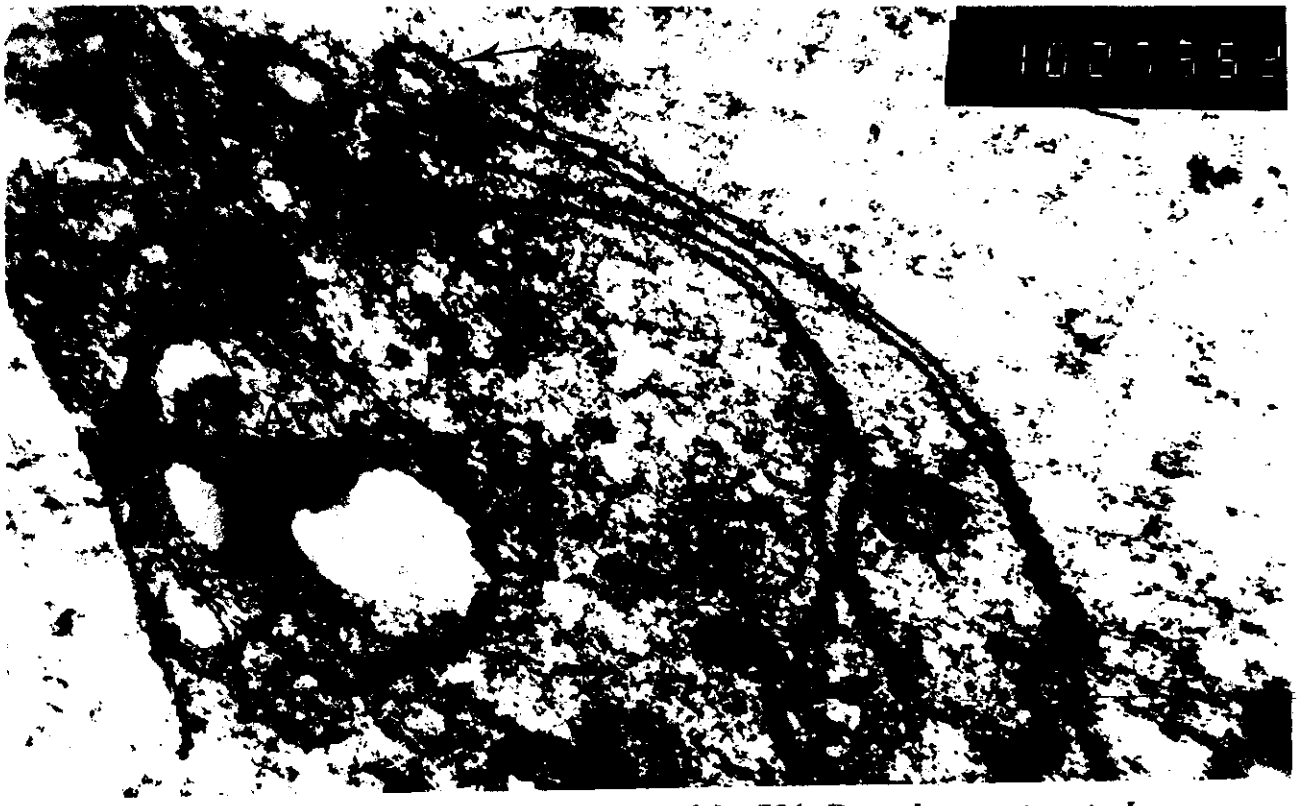


Plate [24] : Effect of treatment with 5% *Portulaca* extract. Long saccules of the ER which appear to form loops {arrows}. Autophagic vacuoles {AV}. Mitochondria {M}. [X 40,000].

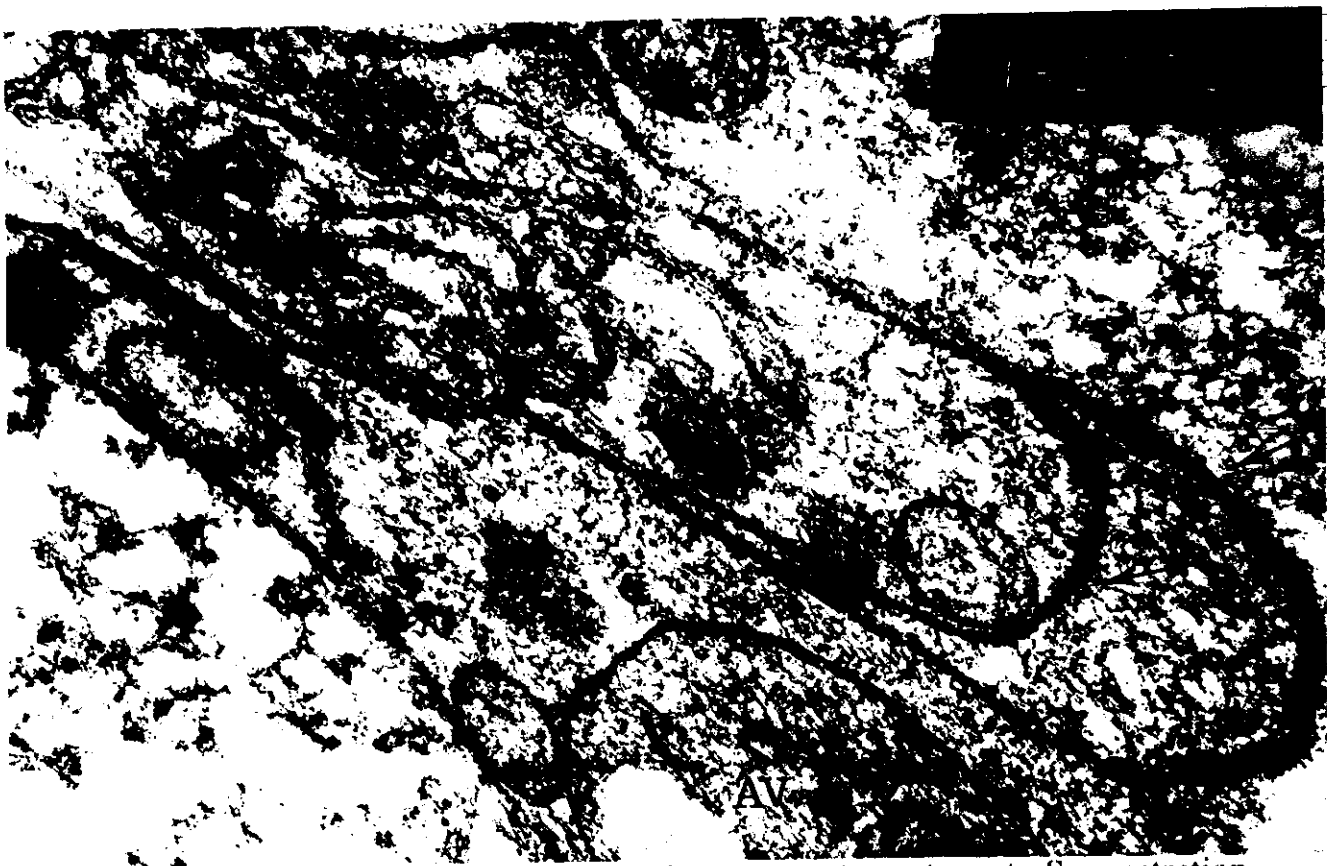


Plate [25] : Effect of 5% *Portulaca* extract treatment. Sequestration of autophagic territories made by loops of endoplasmic saccules {arrows}. An autophagic vacuole {AV}. [X 56,000].

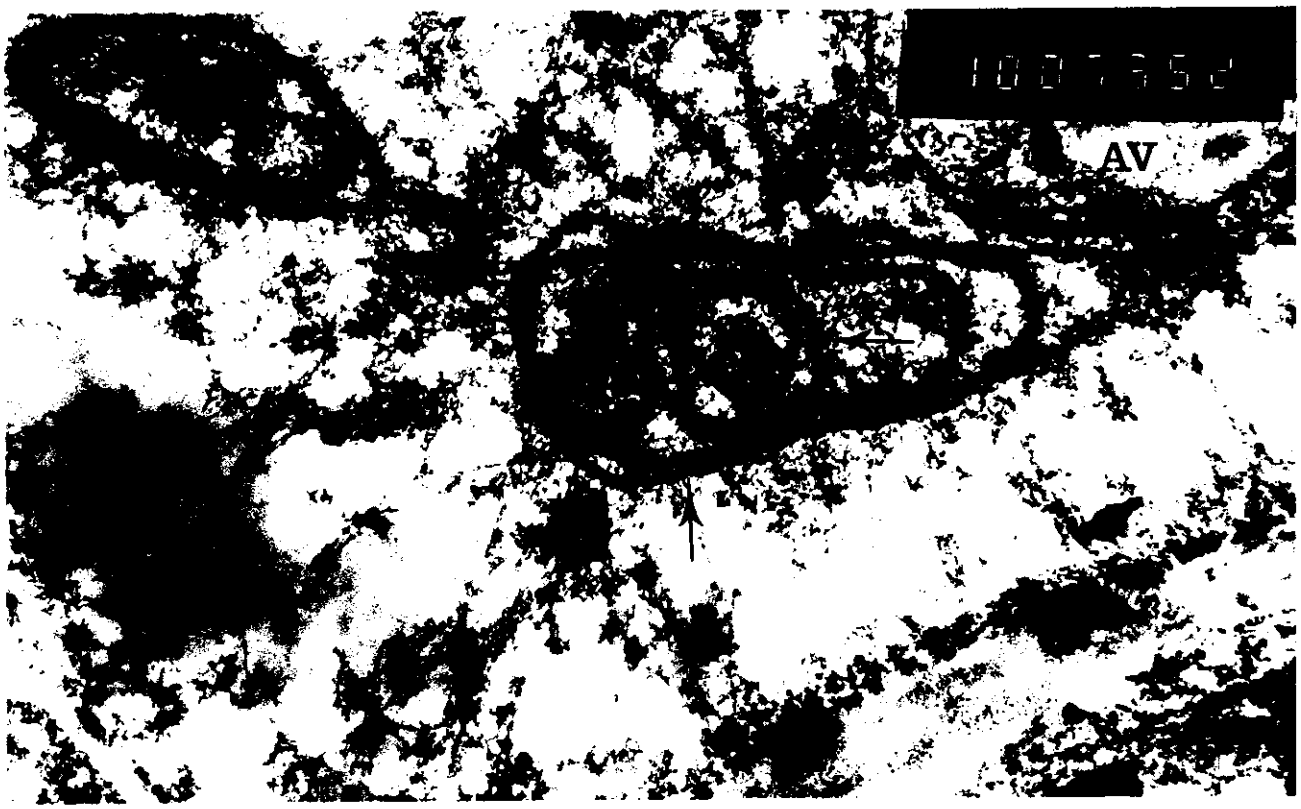


Plate [26] : Effect of 5% *Portulaca* extract treatment. Concentric layers of ER cisternae {arrows} enclosing some cytoplasmic organelles. An autophagic vacuole {AV}. [X 40,000].

mitochondria became abnormally enlarged [Plate 27] and many appearred with ruptured membranes [Plates 27 & 28]. Dictyosomes showed significantly increased number of cisternae, which also seemed elongated and electron-dense [Plates 27 & 28]. Notable reduction in the vesicles' volume of some dictyosomes was oberved [Plate 27]. The ER saccules exhibited irregular thickness [Plate 27] and many were arranged in roughly parallel profiles [Plate 28]. However, some ER saccules formed loops containing parts of the cytoplasm.

Concluding remarks :

Treatment of *V. faba* root tip cells with *Cleome* and *Portulaca* extracts induced many similar effects on the structure of the cytoplasmic organelles. However, some effects may increase with increasing the extract concentration of one plant and decreased with the other, e.g. the volume of Golgi vesicles which increased with increasing the extract concentration of *Cleome* decreased with increasing that of *Portulaca*.

The effect of *Cleome* extract on mitochondria was drastic, causing rupturing of their membranes which increased with the extract concentration.

Both of the extracts induced the formation of autophagic vacuoles with varying degrees of occurrence.

The main distinctive effect induced by the two extracts was on the ER. While *Cleome* extract caused exhaustion of the ER, *Portulaca* extract induced the formation of loops by ER saccules which may develop to autophagic territories and, in high concentration treatment, they became arranged in roughly parallel profiles.

Plate [27] : Effect of 10% *Portulaca* extract treatment. An abnormal enlargement of some mitochondria {M} with ruptured membrane {double arrow}. Dictyosomes showing a significant increase in the number of cisternae, with some elongated, and very small vesicles {arrow heads}. Irregular thickness of the ER saccules {arrows}. [X 80,000].

Plate [28] : Effect of 10% *Portulaca* extract treatment. The roughly parallel profiles arrangement of ER, with some saccules enclosing other organelles. Ruptured mitochondria {M}. Numerous dictyosomes with long, electron-dense and also numerous cisternae {arrows}. [X 56,000].