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EFFECTS OF ELECTROMAGNETIC FIELDS ON SOME INDIGENOUS PLANT SPECIES–III. CAPPARACEAE AND CHENOPODIACEAE

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Abstract

To investigate the effects of electromagnetic fields of high tension wires 30 specimens of 11 species in two angiosperm families, Capparaceae and Chenopodiaceae were examined. The plants were collected under high tension wires of 132 kV, 220 kV and 500 kV within an area of about 10 meter. The same species as control specimens were also collected from areas free from electromagnetic fields. The plants were studied for PMC meiosis, meiotic products and pollen fertility. It is shown that with the increase in voltages of high tension wires, strength of magnetic field increases with increased meiotic abnormalities and pollen sterility. Significantly higher percentages of meiotic abnormalities were observed during PMC meiosis, such as stickiness, pairing disturbances, precocity, laggards, bridges and multipolar divisions etc. The differences of these abnormalities are found to be significantly different in test plants (exposed to EMFs) as compared to their respective controls (not exposed to EMFs). The study of meiotic products showed abnormal products in some cases; similarly pollen fertility showed comparatively higher percentages of sterile pollen grains in test plants in comparison with control plants. The pollen sterility of test plants is also significantly higher than control plants.

Introduction

The extensive use of electricity exposes living organisms (animals, plants, humans, microorganisms) to electromagnetic field of different intensities. The major causes of these EMFs are different household appliances, transmission lines, electrical trains etc. The first available important study was conducted by Werthiemer & Leeper (1979) who reported that EMFs cause cancer in humans who live close to or under electrical transmission lines. After their study a debate was started and until now thousands of studies available in literature showing the possible effects of EMFs on humans, animals, plants and microorganisms.

In plants most of the work has been done on the growth of plants, germination of seeds and genotoxicity caused by EMFs. According to some studies the rate of growth and germination of seed increased with the exposure to EMFs (Kato, 1988; Magone, 1996; Zhang & Hashinaga, 1997; Moon & Chung, 2000; Carbonell *et al.*, 2000; Reina *et al.,* 2001; Aladjadjiyan *et al*., 2002; Dardeniz *et al*., 2006; Dao-Liang *et al.*, 2009; Cakmak *et al.*, 2009). On the other hand some studies suggest an inhibition in growth and germination (Widacka & Jerzy, 1982; Selga & Selga, 1996; Peñuelas *et al*., 2004; Pazur *et al*., 2006) and some studies remain inconclusive (Celestino *et al.*, 1998; Mirta *et al*., 2007).

It is evident from different studies that EMFs of different intensities cause certain genotoxic effects in plants. During mitosis and meiosis both these fields are supposed to cause a number of chromosomal aberrations including stickiness, univalents and multivalent formation, precocious and lagging chromosomes, micronuclei formation, bridges, multipolar division etc. (Linskens & Smeets, 1978; Saxena & Gupta, 1987; Runthala & Bhattacharya, 1991; Haider *et al*., 1994; Zaidi & Khatoon, 2003, 2012; Hanafy *et al.*, 2006; Zhang *et al*., 2007; Răcucĭu, 2009; Aksoy *et al*., 2010).

With the exception of only two or three studies rest of the studies deal with the effects of these EMFs on only one species and the study was performed in most of the cases in lab conditions. Our work deal with the genotoxic effects of high tension transmission lines on plants belonging to different species, growing in their natural ecosystem under these high tension lines. Untill now no such work has been carried out and published in Pakistan (exclude this sentence).

Materials and Methods

The plants belonging to the family Capparaceae and Chenopodiaceae were collected from different plant populations in the vicinity of high-tension lines of 132 kV, 220 kV and 500 kV (just below the lines and the area of approximately 10 Km around these lines) (Table 1).

Table 1. List of localities.

S. #	District /Div.	Locality	Voltage
1.	Karachi	Bhitai colony, Korangi Creek	132 kV
2.	Thatta	Near Gharo, under the bridge, along National Highway	132 kV
3.	Thatta	Along National Highway, Bhambhore Museum turning	132 kV
4.	Thatta	Goth Gul Hasan Baloch, along National Highway	132 kV
5.	Thatta	10 Km from Ghaggar Phatak, along National Highway	132 kV
6.	Jamshoro	50 Km from Karachi, Kathore Bridge, Super Highway	220 kV
7.	Karacchi	On way to Port Bin Qasim, in front of Indus Motor Company	132 kV
8.	Lasbella	Shahanshah Balochistan Hotel, Opp. Hub Police Station	132 kV
9.	Thatta	Small mountain along National Highway, near Dhabeji	132 kV
10.	Karachi	2 Km before Gadap town and 2 Km after Gadap town	500 kV

Collection of the same species was also made as control specimens from areas free from High-tension lines or where intensity of electromagnetic fields is less than 1 mG, mostly from Karachi University Campus or other localities around Karachi. The intensity of EMF was measured in each case with the help of Lutron EMF-822A tester in milli Gauss (unit of magnetic field). Voucher specimens are deposited in Taxonomy and Cytology Unit, Department of Botany, University of Karachi.

For cytological studies young buds, mature buds and flowers were fixed on the spot in Carnoy's solution (absolute alcohol: glacial acetic acid, 3:1) for the study of meiotic behavior, meiotic products and pollen fertility respectively. To study the meiotic behavior of chromosomes, temporary slides were prepared from young anthers by usual squash technique with 1% propionic carmine as stain. Depending upon the availability at least 50 to a maximum of up to 200 pollen mother cells were studied for each observed meiotic stage. Photomicrographs of PMCs showing meiotic abnormalities with good contrast were taken by Nikon Photomicroscope.

Slides for meiotic products were prepared from young anthers by squash technique with 1% propionic carmine as stain. 100 or more meiotic products were observed in each case. Photomicrographs of normal meiotic product i.e. young microspore tetrad, and abnormal meiotic product i.e. diads were taken by Nikon Photomicroscope.

For the study of pollen fertility, slides of anthers from mature flowers were prepared by squash technique. Up to 1000 pollen grains were studied to score fertile, sterile, and diploid pollen grains. The voucher specimens have been deposited in the Karachi University Herbarium (KUH). Differences of meiotic abnormalities and pollen sterility between test plants and control plants were statistically analyzed by Z-test (Zar, 1996).

Results and Discussion

The results of meiotic abnormalities are summarized in table 2. A number of meiotic abnormalities were observed in different stages of meiosis, such as stickiness, univalents and multivalent during diakinesis, stickiness and precocious chromosomes during metaphase I and II and stickiness, laggards and multipolar division during anaphase I and II (Fig. 5a-k). These abnormalities are observed in test (expose to EMFs) and control plants (unexposed plants) both but their percentages are comparatively higher in test plants.

Table 2. Details of abnormalities in PMC meiosis (in terms of cells with abnormal meiotic stages).

S.#	Family and plant name	Voltage & Voucher #.	F. I (mG)	D %	Met.I %	Met.II %	Ana.I %	Ana.II %	Overall Ab. %
I	**Capparaceae**								
1	*Capparis decidua* (Forssk.) Edgew.	132 kV, SZ 300	10.5	…..	33.13	38.1	22.58	…..	31.47
		132 kV, SZ 349	15.8	…..	28.89	23.08	…..	16.67	24.07
		Control, SZ 142	<1	……	11.25	6.25	11.11	8.57	8.66
2	*Cleome brachycarpa* Vahl ex DC.	132 kV, SZ 401	12.5	0	40	31.82	30.56	…..	20.89
		Control, SZ 457	<1	0	18.6	11.11	4.08	…..	8.16
3	*C. scaposa* DC.	220 kV, SZ 432	39.8	27.42	29.59	12.28	8.7	13.33	22.35
		Control, SZ 465	<1	0	15.69	15.58	3.77	6.52	8.73
		Control, SZ 692	<1	0	15.07	13.33	3.03	5.88	9.09
4	*C. viscosa* L.	132 kV, SZ 419	12.5	…..	33.33	9.09	…..	…..	24.14
		132 kV, SZ 651	11.5	59.21	66.67	31.43	12	…..	54.44
		500 kV, SZ 828	26	65.31	73.44	66.67	46.5	…..	64.32
		Control, SZ 684	<1	…..	13.16	11.76	5.88	…..	10.57
5	*Gynandropsis gynandra* (L.) Briq.	132 kV, SZ 239	5.1	…..	37.5	0	37.78	…..	29.21
		500 kV, SZ 821	28	39.62	86.49	34.78	15	35.29	52.94
		Control, SZ 138	<1	…..	22.28	14.89	25.93	…..	20.16
6	*Maerua arenaria* (DC.) Hook.f. & Thoms.	132 kV, SZ 181	3.7	25	50.67	21.14	18.18	3.12	24.46
		Control, SZ 871	<1	0	24.49	15.91	11.54	5.66	10
II	**Chenopodiaceae**								
7	*Chenopodium album* L.	132 kV, SZ 267	5.1	8.16	39.62	72.22	30.36	24.14	31.72
		Control, SZ 900	<1	0	25	33.33	7.14	0	14.86
8	*C. murale* L.	132 kV, SZ 270	5.1	0	11.76	33.6	6.67	27.27	22.91
		Control, SZ 598	<1	…..	16.18	18.18	0	15.79	14.08
9	*Haloxylon stocksii* (Boiss.) Benth. & Hook.	132 kV, SZ 496	15.1	0	32.35	….	……	…..	14.38
		132 kV, SZ 761	5.6	0	58.97	18.75	18.92	16	25.81
		Control, SZ 884	<1	…..	4.08	3.13	0	0	2.23
10	*Salsola imbricata* Forssk.	132 kV, SZ 85	14.3	0	3.77	6.98	2.78	0	3.69
		132 kV, SZ 226	5.1	…..	16.67	13.33	…..	……	15.91
		132 kV, SZ 472	15.8	0	17.5	16.67	0	…..	7.83
		Control, SZ 881	<1	…..	2.53	6.56	0	0	3.08
11	*Suaeda fruticosa* Forssk.ex J.F.Gmelin	132 kV, SZ 264	5.1	…..	53.47	65.91	32.73	35.48	50.4
		Control, SZ 903	<1	…..	14.12	0	0	0	6.74

Note: F. I = Field intensity, mG = Milli Gauss, Diak. = Diakinesis, Met. I= Metaphase I, Met. II= Metaphase II, Ana I= Anaphase I, Ana II= Anaphase II, Ab = Abnormality

Stage wise highest abnormal cells of diakinesis stage were observed in two specimens of *Cleome viscosa* collected from 132 kV and 500 kV lines respectively; the percentages were 59% and 65% respectively. At metaphase I stage the highest (89%) number of cells was observed in *Gynandropsis gynandra*; it was then followed by 73% and 67% in *Cleome scaposa*. Highest number of (72%) abnormal cells at metaphase II stage was recorded in *Chenopodium album*, followed by 68% in *Cleome viscosa* and 66% in *Suaeda fruticosa*. In case of anaphase I again highest abnormal cells were observed in *Cleome viscosa* and their percentage was 47%. In case of anaphase II the highest (35%) abnormal cells were observed in two species i.e., *S. fruticosa* and *G. gynandra.*

Overall highest (64% and 54%) abnormal percentage was observed in two specimens of *C. viscosa* followed by 53% in *G. gynandra* and 50% in *S. fruticosa* (Fig. 1).

In Fig. 2 a comparison of mean meiotic abnormalities, mean pollen sterility and mean magnetic field strength at different voltages i.e 132 kV, 22 kV and 500 kV along with control is shown. From this figure it is obvious that with the increase in voltage from control to 500 kV a gradual increase in meiotic abnormalities, pollen sterility and magnetic field intensity takes place, with a slight discrepancy at 220 kV, since only one specimen was collected from this line.

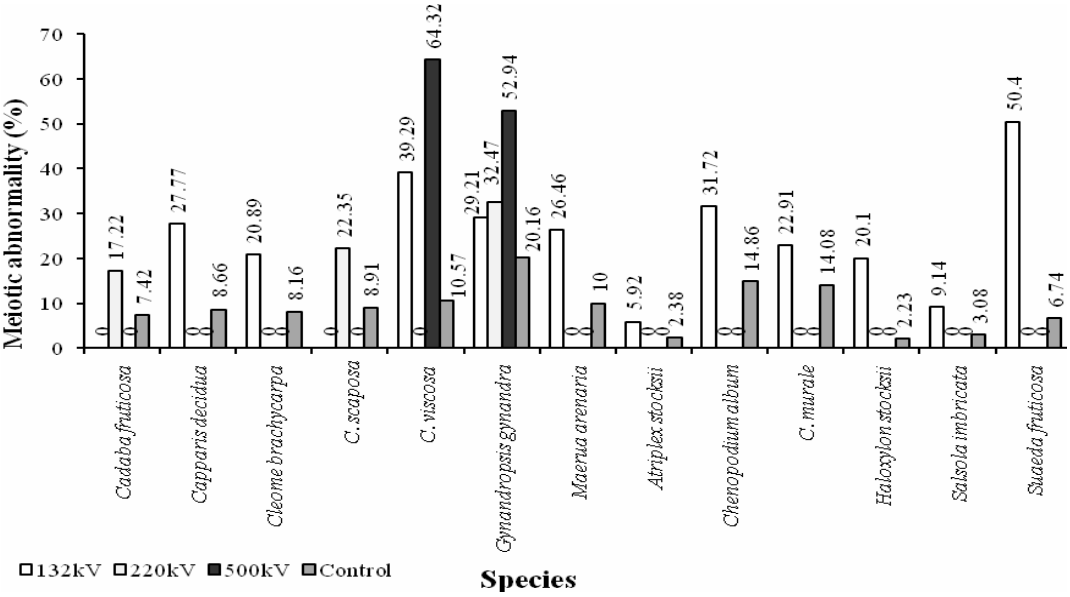

Fig. 1. Species-wise comparison of meiotic abnormalities in test specimens and control specimens.

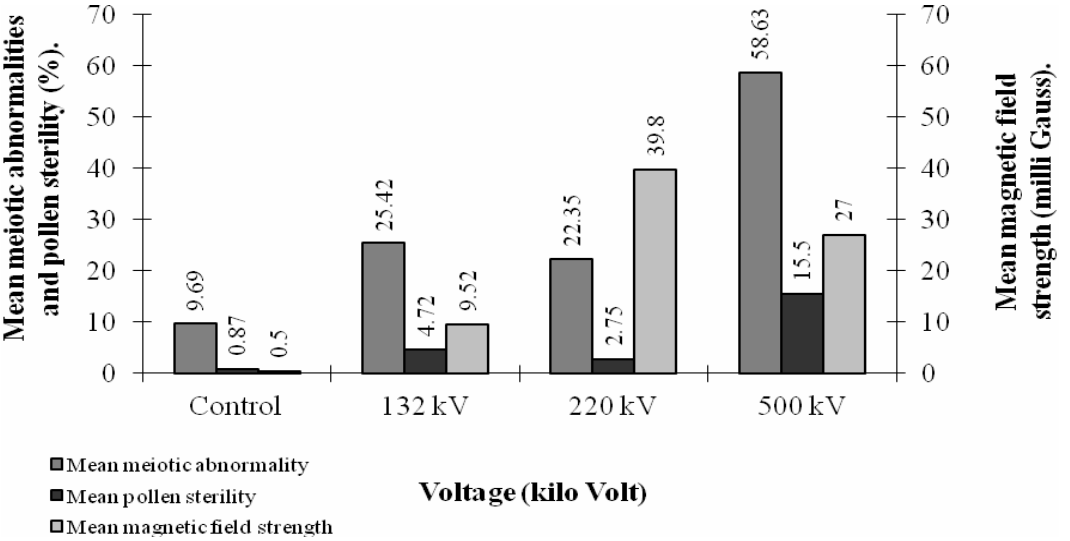

Fig. 2. Bar diagram showing comparison of mean meiotic abnormalities, mean pollen sterility and mean magnetic field strengths at control, 132 kV, 220 kV and 500 kV.

The results of meiotic products and pollen sterility are summarized in Table 3. Daids were observed in *Haloxylon stocksii* and *Suaeda fruticosa*, both were collected from 132 kV high tension transmission line (Fig. 5l); whereas in rest of the specimens normal products i.e. tetrads were obtained. The diploid pollens were found only in *H. stocksii.* The highest (20%) percentage of sterile pollens was recorded in *C. viscosa,* collected under the high tension line of 500 kV.

The statistical analysis of meiotic abnormality and pollen sterility are summarized in tables 4 and 5 respectively. Bar diagrams of these tables show that in almost 89% cases the difference of meiotic abnormalities was significantly higher in test plants as compared to control plants (Fig. 3) and in about 94% cases the difference of pollen sterility was significantly higher in test specimens (Fig. 4).

Our results agreed with the previous work (Linskens & Smeets, 1978; Runthala & Bhattacharya, 1991; Hanafy *et al.*, 2006; Zhang *et al*., 2006) that EMFs created by high tension lines causes certain chromosomal aberrations in the PMC meiosis, they also affect on the meiotic products and results in the formation of diads. In addition percentage of sterile pollen grains also increases.

On the basis of above results it may be concluded that in some cases, during the meiotic process above mentioned chromosomal aberrations ultimately result in the formation of abnormal meiotic products (diploid pollens and non reduced gametes), which give rise to polyploids. These chromosomal aberrations can pass to next generations and may result in the formation of abnormal generations which may be sterile.

Since EMFs affects the plants, they may also affect other living organisms found or living in the close vicinity of these high tension lines including human beings. Therefore a detailed investigation should be carried out, including human beings.

Table 3. Comparison of diads, hypertetrads, diploid pollens and sterile pollens in test and control specimens.

S.#	Family and plant name	Voltage & voucher #	F. I. (mG)	Diads %	Diploid pollens %	Pollen sterility %
I.	**Capparaceae**					
2.	*Capparis decidua* (Forssk.) Edgew.	132 kV, SZ 300	10.5	0	0	3.85
		132 kV, SZ 349	15.8	0	0	5.52
		Control, SZ 142	<1	0	0	1.75
3.	*Cleome brachycarpa* Vahl ex DC.	132 kV, SZ 401	12.5	0	0	10.89
		Control, SZ 457	<1	0	0	1.57
4.	*C. scaposa* DC.	220 kV, SZ 432	39.8	0	0	2.75
		Control, SZ 465	<1	0	0	1.23
		Control, SZ 692	<1	0	0	1.13
5.	*C. viscosa* L.	132 kV, SZ 419	12.5	…..	0	9.09
		132 kV, SZ 651	11.5	0	0	10
		500 kV, SZ 828	26	0	0	20.03
		Control, SZ 684	<1	0	0	0.5
6.	*Gynandropsis gynandra* (L.) Briq.	132 kV, SZ 239	5.1	0	0	2.46
		500 kV, SZ 821	28	0	0	10.98
		Control, SZ 138	<1	0	0	0.65
7.	*Maerua arenaria* (DC.) Hook.f. & Thoms.	132 kV, SZ 181	3.7	0	0	2.2
		Control, SZ 871	<1	0	0	0.4
II.	**Chenopodiaceae**					
9.	*Chenopodium album* L.	132 kV, SZ 267	5.1	0	0	3.09
		Control, SZ 900	<1	0	0	0.4
10.	*C. murale* L.	132 kV, SZ 270	5.1	0	0	3.49
		Control, SZ 598	<1	0	0	1.23
11.	*Haloxylon stocksii* (Boiss.)Benth. & Hook.	132 kV, SZ 496	15.1	0	0	4.76
		132 kV, SZ 761	5.6	2.43	1.32	3.43
		Control, SZ 884	<1	0	0	1.32
12.	*Salsola imbricata* Forssk.	132 kV, SZ 85	14.3	0	0	6.77
		132 kV, SZ 226	5.1	0	0	2.2
		132 kV, SZ 472	15.8	0	0	1.36
		Control, SZ 881	<1	0	0	0.22
13.	*Suaeda fruticosa* Forssk.ex J.F.Gmelin	132 kV, SZ 264	5.1	0.99	0	1.64
		Control, SZ 903	<1	0	0	0

Note: F.I.= Field Intensity, mG = Milli Gauss

Table 4. Statistical comparison of meiotic abnormalities (Z-test).

S.#	Family and plant name	Voltage & voucher no.	Z Test value	Z-Test status	Level of significance
I.	**Capparaceae**				
2.	*Capparis decidua* (Forssk.)	132 kV, SZ 300	7.3	S	p<0.001***
	Edgew.	132 kV, SZ 349	3.41	S	p<0.001***
3.	*Cleome brachycarpa* Vahl ex DC.	132 kV, SZ 401	4.33	S	p<0.001***
4.	*C. scaposa* DC.	220 kV, SZ 432	4.83	S	p<0.001***
5.	*C. viscosa* L.	132 kV, SZ 419	2.06	S	p<0.05*
		132 kV, SZ 651	10.75	S	p<0.001***
		500 kV, SZ 828	13.5	S	p<0.001***
6.	*Gynandropsis gynandra* (L.) Briq.	132 kV, SZ 239	1.5	N.S	p>0.05
		500 kV, SZ 821	6.8	S	p<0.001***
7.	*Maerua arenaria* (DC.) Hook.f.& Thoms.	132 kV, SZ 181	5.16	S	p<0.001***
II.	**Chenopodiaceae**				
9.	*Chenopodium album* L.	132 kV, SZ 267	4.25	S	p<0.001***
10.	*C. murale* L.	132 kV, SZ 270	2.36	S	p<0.05*
11.	*Haloxylon stocksii* (Boiss.) Benth. & Hook.	132 kV, SZ 496	4	S	p<0.001***
		132 kV, SZ 761	6.67	S	p<0.001***
12.	*Salsola imbricata* Forssk.	132 kV, SZ 85	0.58	N.S	p>0.05
		132 kV, SZ 226	4	S	p<0.001***
		132 kV, SZ 472	1.6	N.S	p>0.05
13.	*Suaeda fruticosa*Forssk.ex J.F.Gmelin	132 kV, SZ 264	13.75	S	p<0.001***

Note: S= Significant, N.S= Non-significant

Table 5. Statistical comparison of pollen sterility with the help of Z-test.

S.#	Family and plant name	Voltage & voucher no.	Z Test value	Z-Test status	Level of significance
I.	**Capparaceae**				
2.	*Capparis decidua* (Forssk.) Edgew.	132 kV, SZ 300	2.86	S	p<0.01**
		132 kV, SZ 349	4.2	S	p<0.001***
3.	*Cleome brachycarpa* Vahl exDC.	132 kV, SZ 401	12.86	S	p<0.001***
4.	*C. scaposa* DC.	220 kV, SZ 432	3.17	S	p<0.01**
5.	*C. viscosa* L.	132 kV, SZ 419	6.43	S	p<0.001***
		132 kV, SZ 651	6.43	S	p<0.001***
		500 kV, SZ 828	10	S	p<0.001***
6.	*Gynandropsis gynandra* (L.) Briq.	132 kV, SZ 239	3.33	S	p<0.001***
		500 kV, SZ 821	7.14	S	p<0.001***
7.	*Maerua arenaria* (DC.) Hook. f.&Thoms.	132 kV, SZ 181	3.03	S	p<0.01**
II.	**Chenopodiaceae**				
9.	*Chenopodium album* L.	132 kV, SZ 267	3.79	S	p<0.001***
10.	*C. murale* L.	132 kV, SZ 270	3.75	S	p<0.001***
11.	*Haloxylon stocksii* (Boiss.)	132 kV, SZ 496	4.44	S	p<0.001***
	Benth. & Hook.	132 kV, SZ 761	4	S	p<0.001***
12.	*Salsola imbricata* Forssk.	132 kV, SZ 85	6	S	p<0.001***
		132 kV, SZ 226	2.85	S	p<0.01**
		132 kV, SZ 472	1.67	N.S	p>0.05
13.	*Suaeda fruticosa* Forssk. ex J.F. Gmelin	132 kV, SZ 264	5	S	p<0.001***

Note: S= Significant, N.S= Non-significant

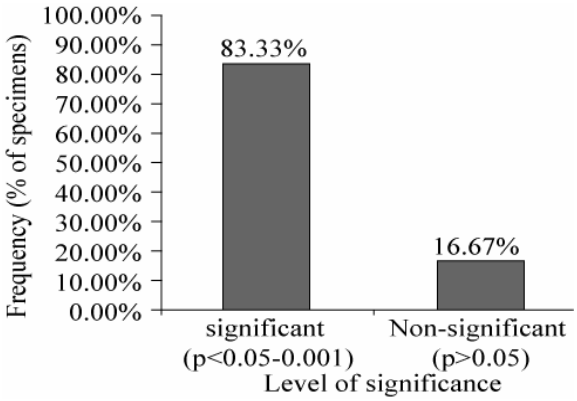

Fig. 3. Comparison of significant and non-significant differences in the meiotic abnormalities of test and control specimens.

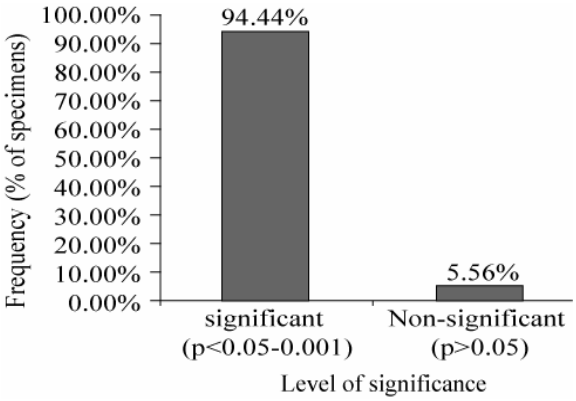

Fig. 4. Comparison of significant and non significant differences in the pollen sterility of test and control specimens.

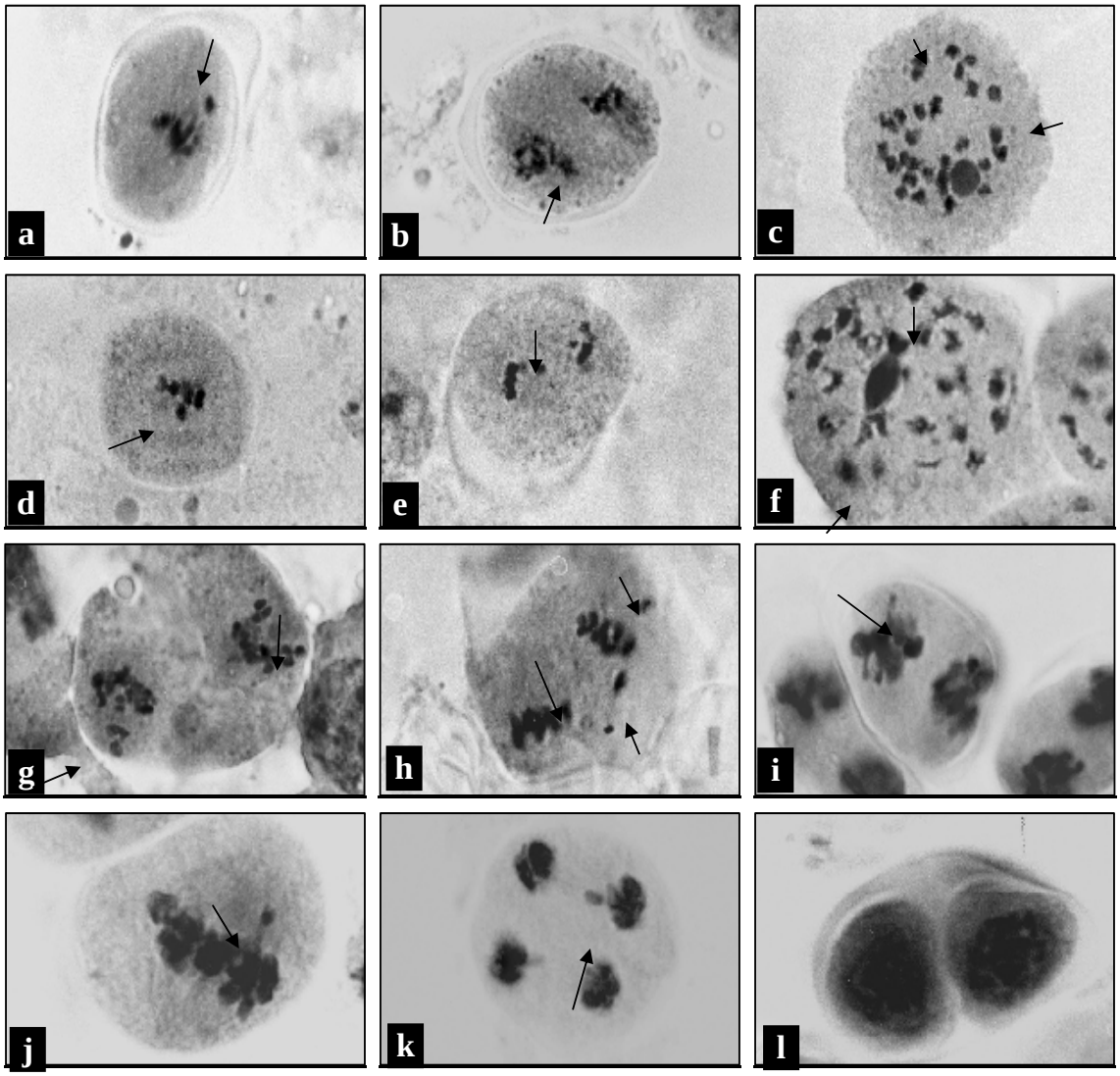

Fig. 5a, b: *Capparis decidua* X1000, **a.** Metaphase I showing stickiness and one precocious chromosome; b**.** Metaphase II with some precocious chromosomes. **c.** *Cleome brachycarpa* X100, Diakinesis with multivalents. **d, e**: *Cleome scaposa* X1000, **d.** Metaphase I with precocious bivalent, **e.** Metaphase II with precocious chromosomes. **f, g:** *Chenopodium album* X1000, **f**. Diakinesis showing disturbed pairing, **g.** Disturbed metaphase II. **h.** *Chenopodium murale* X1000, metaphase II with precocious chromosomes. **i-k**, *Suaeda fruiticosa* X1000: **i**. Metaphase I with stickiness and precocious chromosomes, **j**. Metaphase II with stickiness and precocious chromosomes, **k**. Anaphase II with stickiness and one lagging chromosome, **l**. Microspore diad.

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