

EFFECTS OF ELECTROMAGNETIC FIELDS (CREATED BY HIGH TENSION LINES) ON SOME INDIGENOUS PLANT SPECIES – VI. COMMELINACEAE, CONVULVULACEAE, CUCURBITACEAE, CYPERACEAE, EUPHORBIACEAE, GENTIANACEAE AND MALVACEAE

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ABSTRACT

The genotoxic effects of electromagnetic fields of high voltage power lines were studied in 33 specimens of thirteen species belonging to seven angiospermic families, i.e. Commelinaceae, Convolvulaceae, Cucurbitaceae, Cyperaceae, Euphorbiaceae, Gentianaceae and Malvaceae. The plant specimens were collected from under the high tension lines of different intensities (66 kV, 132 kV, 220 kV and 500 kV). Collection of the same species as control specimens was made from areas where there was no electromagnetic field. The aspects studied were PMC meiosis, meiotic products and pollen fertility. The study showed that with the increase in voltages and strength of magnetic field, the meiotic abnormalities and pollen sterility also increased. During the study of PMC meiosis quite higher percentages of meiotic abnormalities were observed, such as stickiness, pairing disturbances, precocity, laggards, bridges and multipolar divisions etc. These abnormalities were found to be significantly higher in test plants as compared to their respective controls. The meiotic products also showed abnormal products in few specimens. The study of pollen fertility showed significantly higher percentages of sterile pollen grains in test plants in comparison with their controls in most of the cases.

Key Words: Genotoxicity, meiosis, pollen mother cell, meiotic products, pollen sterility.

INTRODUCTION

A number of studies deal with the possible genotoxic effects of electromagnetic fields on humans, animals and plants. In case of humans an increase in chromosomal aberrations was observed (Garcia-Sagredo and Monteagudo, 1991; Othman *et al.* 2003; Vijayalaxmi and Obe, 2005) lymphocytic cells when they were exposed to or treated with EMFs. On the contrary, the studies of Cohen *et al.* (1986); Fiorani *et al.* (1992); McCann *et al.* (1998); Scarfi *et al.* (1999); Ansari and Hei (2000); and Skyberg *et al.* (2001) did not support this observation and according to them EMFs are non- cyto and geno toxic in nature.

In animals an increase in chromosomal translocations in the first meiotic prophase of male mice (Manikowska-Czerska *et al.* 1983), an enhancement in the aneuploidy of female mice (Mailhes *et al.* 1997), a delay in time of mitosis and induction of two developmental abnormalities; exogastrulation and collapsed embryos in sea urchin (Levin and Ernst, 1997), DNA damage in mice (Svedenstall *et al.* 1999); these are some of the genotoxic effects which were observed after exposure of these animals to EMFs of 2450 MHz, 5G 60 Hz and 10 mT-0.1 T.

It is evident from different studies that certain genotoxic effects may be observed in plants after their exposure to electromagnetic fields. During mitosis and meiosis both these fields have been indicated to cause a number of chromosomal aberrations such as stickiness, univalents and multivalent formation, precocious and lagging chromosomes, micronuclei formation, bridges, multipolar division etc. (Linskens and Smeets, 1978; Saxena and Gupta, 1987; Runthala and Bhattacharya, 1991; Haider *et al.*, 1994; Zaidi and Khatoon, 2003, 2012; Zaidi *et al.*, 2012; Hanafy *et al.*, 2006; Zhang *et al.*, 2007; Răcuciu, 2009; Aksoy *et al.*, 2010).

Most of the studies deal with the effects of the EMFs on only one species and generally performed in laboratory conditions. Our work deals with the genotoxic effects of high tension transmission lines on plants belonging to different species, growing in their natural ecosystem. Until now no such work has been published from Pakistan.

MATERIALS AND METHODS

The specimens were collected from different locations in and around Karachi and some adjoining districts (Thatta, Lasbella, Jamshoro) in the vicinity of high-tension lines of 66 kV, 132 kV, 220 kV and 500 kV (just below the lines and the area of approximately 10 Km around these lines).

The same species were also collected to serve as control from areas free from High-tension lines or where intensity of electromagnetic fields is less than 1 mG. The intensity of EMF was measured 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.

For meiotic behavior 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 were taken by Nikon Photomicroscope.

Slides for meiotic products were prepared from young anthers by the same technique. 100 or more meiotic products were observed in each case. Photomicrographs of meiotic product 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 were deposited in the Karachi University Herbarium (KUH).

Differences of meiotic abnormalities and pollen sterility between test plants and controls were statistically analyzed by Z-test (Zar 1996).

RESULTS AND DISCUSSION

The results of PMC meiosis at different stages with meiotic abnormalities are summarized in Table 1. The meiotic abnormalities occurring during different stages include stickiness, univalents and multivalent during diakinesis, stickiness and precocious chromosomes during metaphase I and II and stickiness and laggards anaphase I and II. of meiosis. These abnormalities were observed in test (exposed to EMFs) as well as in control plants (not expose to EMFs) both but their percentages were comparatively higher in test plants.

Stage wise different species showed highest abnormal cells in different stages. Such as highest number of abnormal cells of diakinesis stage i.e. 33.33% were observed in one specimen of *Seddera latifolia* (collected from 132 kV line) and almost 29% abnormal cells at the same stage were observed in *Croton bonplandianus* (collected from 66 kV line). At metaphase I and metaphase II stage the highest (almost 78% and 54%) abnormal numbers of cells were observed in *Citrullus colocynthis* (500 kV). The highest number of (almost 53%) abnormal cells at anaphase I stage was recorded in *Abutilon fruticosum*. Whereas in case of anaphase II stage highest abnormal cells were observed in *C. bonplandianus* i.e. 24%.

On overall basis highest abnormal percentage was observed in *A. fruticosum* (40.5%) followed by *C. bonplandianus* (38%) and *C. colocynthis* (38%) (Fig. 1).

The results of meiotic products and pollen sterility are summarized in Table 2. **Daids** were observed only in *S. latifolia*; whereas in rest of the specimens normal products i.e. tetrads were obtained. Whereas diploid pollens were found in *S. latifolia* (0.87%) and *C. colocynthis* (0.33%). The highest (almost 28%) percentage of sterile pollens was recorded in *Abutilon sepalum*, collected under the high tension line of 132 kV.

The statistical analysis of meiotic abnormality and pollen sterility are summarized in table 3 and 4 respectively. Figure 2 shows that in almost 63% cases the difference of meiotic abnormalities was significantly higher in test plants as compared to control plant, and in about 72% cases (Fig. 3) the difference of pollen sterility was significantly higher in test specimens.

The results of the present study are consistent with the results of some of the previous workers (Linskens and Smeets, 1978; Runthala and Bhattacharya, 1991; Hanafy *et al.*, 2006; Zhang *et al.*, 2007); according to which that EMFs of high tension transmission lines causes certain chromosomal aberrations in the PMC meiosis, besides this their effects also observed on the meiotic products which results in the formation of abnormal products such as diads. In addition to all these abnormalities an increase in the percentage of sterile pollen grains was also observed.

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 or non reduced gametes), which ultimately give rise to polyploids. These chromosomal aberrations can pass to the next generations and may result in the formation of abnormal generations which may be sterile.

As it is obvious that EMFs affect the plants, in the same way they can also affect other organisms including human beings who are living in the close vicinity of these lines. In our most of the urban areas and in the outskirts of the city human populations are living just under these lines or in very close vicinity. Therefore detailed genotoxic investigations should be carried out, in human populations living under high voltage power lines.

Table 1. Details of abnormalities in PMC meiosis.

S.#	Family and Plant name	Voltage & Voucher No.	F. I (mG)	D (%)	M.I (%)	M.II (%)	A.I (%)	A.II (%)	Overall Ab. (%)
I	Commelinaceae								
	<i>Commelina albescens</i>								
1	Hassk.	220 kV, SZ 780	16.2	15.38	23.33	5.88	7.14	14.94
		Control, SZ 858	<1	7.5	10	0	0	4.86
II	Convolvulaceae								
	<i>Convolvulus prostratus</i>								
2	Forssk.	132 kV, SZ 235	5.1	36.36	26.09	34.14
		132 kV, SZ 258	5.1	31.48	14.29	12.12	12.5	20.14
		Control, SZ 741	<1	20.24	8.7	8.57	3.03	12.63
3	<i>C. rhyniospermus</i> Hochst.ex Choisy	66 kV, SZ 38	31.5	8.33	3.95
		Control, SZ 877	<1	5.56	0	8.33	0	3.13
4	<i>Cressa cretica</i> L.	132 kV, SZ 83	14.3	31.75	29.41	30.92
		Control, SZ 111	<1	0	12.5	0	5.55
5	<i>Ipomoea carnea</i> Jacq.	132 kV, SZ 266	5.1	1.56	37.78	31.08	0	11.11	19.28
		Control, SZ 728	<1	0	8.45	12.5	3.7	10.71	7.14
6	<i>Seddera latifolia</i> Hochst. & Steud.	132 kV, SZ 60	12.5	33.33	9.1	16.66	0	16.55
		500 kV, SZ 837	22	25.64	22.92	23.81	5.88	0	21.23
		Control, SZ 141	<1	0	9.09	13.88	0	2.94	6.61
III	Cucurbitaceae								
7	<i>Citrullus colocynthis</i> (L.) Schrud.	132 kV, SZ 265	5.1	37.5	52.17	0	0	27.91
		500 kV, SZ 825	17.9	0	78.38	54.24	36.21	11.32	38.24
		Control, SZ 468	<1	0	6.67	8.33	2.86	0	3.14
IV	Cyperaceae								
8	<i>Cyperus rotundus</i> L.	220 kV, SZ 125	6.9	2.77	12.9	12	19.61	0	11.96
		Control, SZ 209	<1	0	1.74	3	2.1
V	Euphorbiaceae								
	<i>Croton bonplandianus</i>								
9	Baill.	66 kV, SZ 675	5.7	29.17	33.73	17.74	34.78	28.9
		66 kV, SZ 676	5.7	38.46	44.35	33.33	24.24	38.61
		Control, SZ 730	<1	0	22.86	25.58	5.88	0	13.91
10	<i>Phyllanthus niruri</i> L.	132 kV, SZ 188	4.6	26.83	13.51	20.51
		Control, SZ 208	<1	17.65	16.67	20	10.04
VI	Gentianaceae								
11	<i>Enicostemma hyssopifolium</i> (Willd.) Verdoon	132 kV, SZ 652	11.5	0	19.1	25	0	22.22	18.75
		Control, SZ 869	<1	4	13	4.35	0	7.23
VII	Malvaceae								
12	<i>Abutilon fruticosum</i> Guill.	220 kV, SZ 571	29.7	35	48.57	52.94	13.64	40.54
		Control, SZ 686	<1	0	13.33	0	5.83
13	<i>Abutilon sepalum</i> S.A.Hus. & S.R.Baq.	132 kV, SZ 95	4.8	25	22	25.64	22.22	23.57
		132 kV, SZ 224	5.1	0	17.24	14.29	31.58	0	14.21
		132 kV, SZ 489	15.8	0	36.61	32	12.24	0	29.1
		Control, SZ 916	<1	0	12.96	3.45	5.9	6.9	7.41

Note: D= Diakinesis, M. I = Metaphase I, M. II = Metaphase II, A. I & II = Anaphase I & II, Ab. = Abnormality, MFI = Magnetic field intensity, mG = Milli Gauss, SZ = Initials of name of collector of voucher specimens.

Table 2. Comparison of diads, diploid pollens and sterile pollens in test and control specimens.

S. #	Family and Plant name	Voltage & Voucher No.	F. I. (mG)	Diads (%)	Diploid Pollens (%)	Pollen Sterility (%)
I	Commelinaceae					
1	<i>Commelina albescens</i> Hassk.	220 kV, SZ 780	16.2	0	0	7.98
		Control, SZ 858	<1	0	0	2.44
II	Convolvulaceae					
2	<i>Convolvulus prostratus</i> Forssk.	132 kV, SZ 235	5.1	0	0	7.93
		132 kV, SZ 258	5.1	0	0	5.26
		Control, SZ 741	<1	0	0	1.66
3	<i>C. rhyniospermus</i> Hochst.	66 kV, SZ 38	31.5	0	0	5.53
	ex Choisy	Control, SZ 877	<1	0	0	1.64
4	<i>Cressa cretica</i> L.	132 kV, SZ 83	14.3	0	0	5.07
		Control, SZ 111	<1	0	0	1.32
5	<i>Ipomoea carnea</i> Jacq.	132 kV, SZ 266	5.1	0	0	16.67
		Control, SZ 728	<1	0	0	1.32
6	<i>Seddera latifolia</i> Hochst.	132 kV, SZ 60	12.5	1.57	0.87	10.43
	& Steud.	500 kV, SZ 837	22	0	0	15.2
		Control, SZ 141	<1	0	0	0
III	Cucurbitaceae					
7	<i>Citrullus colocynthis</i> (L.)	132 kV, SZ 265	5.1	0	0.33	4.15
	Schrad.	500 kV, SZ 825	17.9	0	0	10.65
		Control, SZ 468	<1	0	0	0.62
IV	Cyperaceae					
8	<i>Cyperus rotundus</i> L.	220 kV, SZ 125	6.9	0	0	3.19
		Control, SZ 209	<1	0	0	0.5
V	Euphorbiaceae					
9	<i>Croton bonplandianus</i> Baill	66 kV, SZ 675	5.7	0	0	4.26
		66 kV, SZ 676	5.7	0	0	4.21
		Control, SZ 730	<1	0	0	1.13
10	<i>Phyllanthus niruri</i> L.	132 kV, SZ 188	4.6	0	0	2.72
		Control, SZ 208	<1	0	0	0
VI	Gentianaceae					
11	<i>Enicostemma hyssopifolium</i>	132 kV, SZ 652	11.5	0	0	11.03
	(Willd.) Verdoon	Control, SZ 869	<1	0	0	1.1
VII	Malvaceae					
12	<i>Abutilon fruticosum</i> Guill.	220 kV, SZ 571	29.7	0	0	5.91
		Control, SZ 686	<1	0	0	1.32
13	<i>Abutilon sepalum</i> S.A.Hus.	132 kV, SZ 95	4.8	0	0	27.66
	& S.R.Baq.	132 kV, SZ 224	5.1	0	0
		132 kV, SZ 489	15.8	0	0	11.02
		Control, SZ 916	<1	0	0	0

FI = Field intensity, mG= Milli Gauss

Table 3. 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	Commelinaceae				
1	<i>Commelina albescens</i> Hassk.	220 kV, SZ 780	2.5	N.S	p>0.05
II	Convolvulaceae				
2	<i>Convolvulus prostratus</i> Forssk.	132 kV, SZ 235	2.86	N.S	p>0.05
		132 kV, SZ 258	1.6	N.S	p>0.05
3	<i>C. rhyniospermus</i> Hochst.ex Choisy	66 kV, SZ 38	0.33	N.S	p>0.05
4	<i>Cressa cretica</i> L.	132 kV, SZ 83	3.89	S	p<0.05*
5	<i>Ipomoea carnea</i> Jacq.	132 kV, SZ 266	4	S	p<0.01**
6	<i>Seddera latifolia</i> Hochst. & Steud.	132 kV, SZ 60	3.33	N.S	p>0.05
		500 kV, SZ 837	4.67	S	p<0.001***
III	Cucurbitaceae				
7	<i>Citrullus colocynthis</i> (L.) Schrad.	132 kV, SZ 265	5	S	p<0.001***
		500 kV, SZ 825	11.67	S	p<0.001***
IV	Cyperaceae				
8	<i>Cyperus rotundus</i> L.	220 kV, SZ 125	5.26	S	p<0.001***
V	Euphorbiaceae				
9	<i>Croton bonplandianus</i> Baill	66 kV, SZ 675	3.75	S	p<0.05*
		66 kV, SZ 676	6.25	S	p<0.001***
10	<i>Phyllanthus niruri</i> L.	132 kV, SZ 188	0.36	N.S	p>0.05
VI	Gentianaceae				
11	<i>Enicostemma hyssopifolium</i> (Willd.) Verdoon	132 kV, SZ 652	4	S	p<0.01**
VII	Malvaceae				
12	<i>Abutilon fruticosum</i> Guill.	220 kV, SZ 571	7	S	p<0.001***
13	<i>Abutilon sepalum</i> S.A.Hus. & S.R.Baq.	132 kV, SZ 95	4.25	S	p<0.01**
		132 kV, SZ 224	2.33	N.S	p>0.05
		132 kV, SZ 489	4.75	S	p<0.001***

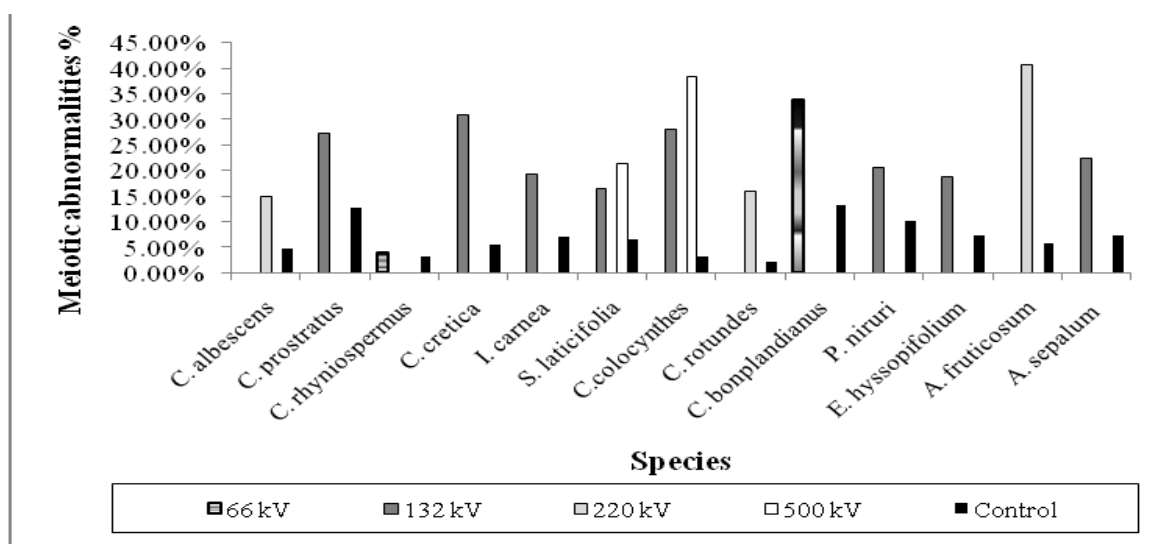


Fig.1. Species-wise comparison of meiotic abnormalities

Table 4. 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	Commelinaceae				
1	<i>Commelina albescens</i> Hassk.	220 kV, SZ 780	2.5	N.S	p>0.05
II	Convolvulaceae				
2	<i>Convolvulus prostratus</i> Forssk.	132 kV, SZ 235	1	N.S	p>0.05
		132 kV, SZ 258	2.5	N.S	p>0.05
3	<i>C.rhyniospermus</i> Hochst.ex Choisy	66 kV, SZ 38	4	S	p<0.01**
4	<i>Cressa cretica</i> L.	132 kV, SZ 83	5.7	S	p<0.001***
5	<i>Ipomoea carnea</i> Jacq.	132 kV, SZ 266	16	S	p<0.001***
6	<i>Seddera latifolia</i> Hochst. & Steud.	132 kV, SZ 60	5.5	S	p<0.001***
		500 kV, SZ 837	6.89	S	p<0.001***
III	Cucurbitaceae				
7	<i>Citrullus colocynthis</i> (L.) Schrad.	132 kV, SZ 265	2.8	N.S	p>0.05
		500 kV, SZ 825	9.17	S	p<0.001***
IV	Cyperaceae				
8	<i>Cyperus rotundus</i> L.	220 kV, SZ 125	6	S	p<0.001***
V	Euphorbiaceae				
9	<i>Croton bonplandianus</i> Baill	66 kV, SZ 675	4.22	S	p<0.01**
		66 kV, SZ 676	4.41	S	p<0.01**
10	<i>Phyllanthus niruri</i> L.	132 kV, SZ 188	2.5	N.S	p>0.05
VI	Gentianaceae				
11	<i>Enicostemma hyssopifolium</i> (Willd.) Verdoon	132 kV, SZ 652	10	S	p<0.001***
VII	Malvaceae				
12	<i>Abutilon fruticosum</i> Guill.	220 kV, SZ 571	5	S	p<0.001***
13	<i>Abutilon sepalum</i> S.A.Hus. & S.R.Baq.	132 kV, SZ 95	9.33	S	p<0.001***
		132 kV, SZ 489	5	S	p<0.001***

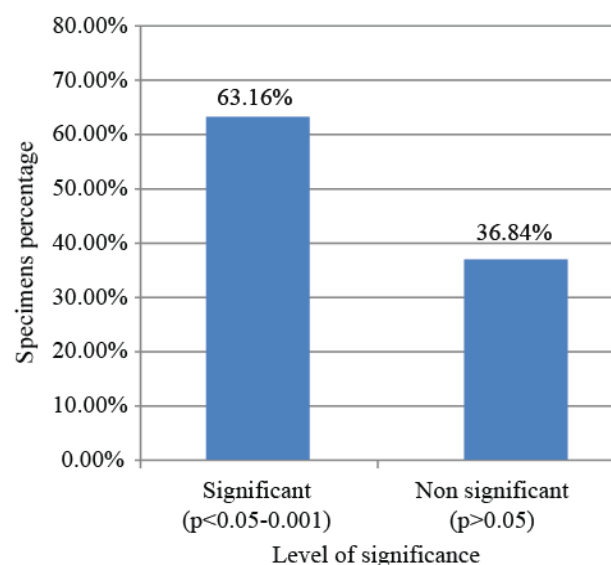


Fig.2. Comparison of significant and non-significant differences in the meiotic abnormalities of test and control specimens

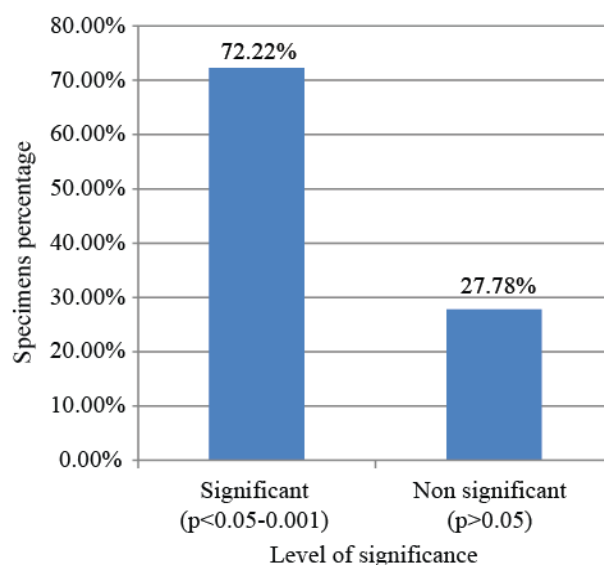


Fig.3. Comparison of significant and non-significant differences in the pollen sterility of test and control specimens.

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