Pak. J. Bot., 35(5): 743-755. 2003.

EFFECTS OF ELECTROMAGNETIC FIELDS (CREATED BY HIGH TENSION LINES) ON THE INDIGENOUS FLORAL BIODIVERSITY IN THE VICINITY OF KARACHI- I: STUDIES ON PMC MEIOSIS, MEIOTIC PRODUCTS AND POLLEN FERTILITY

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Abstract

To study the effect of electromagnetic fields of high-tension lines on plants, 45 species belonging to 18 genera and 11 families from different localities in and around Karachi under high-tension lines ranging from 66,000 to 2,20, 000 volts have been collected and studied. Besides this, collection of same species was also done from areas free from electromagnetic waves as control. Considerable abnormalities in the meiotic behaviour of these plants with an increase in voltage have been observed in comparison to the control plants. The difference in the frequency of meiotic abnormalities has been found to be statistically significant between test and control plants. Besides this a decrease in pollen fertility is also observed with the increase in voltages. The specimens collected from the vicinity of more than 100,000 volts lines (*i.e.* 1,32,000 & 2,20,000 volts) showed a tendency to produce certain percentage of diads and diploid pollen grains.

Introduction

With the modernization consumption of electricity has greatly increased. Every electrical appliance and electricity line produces electromagnetic fields (EMF). Not only humans but the natural ecosystem as well are exposed to electromagnetic fields of high-tension lines and cables. A debate is going on since last 15 to 20 years on the relationship between EMFs and different types of cancer in humans. An association between leukemia and cancer with electromagnetic fields was found among people living near high-tension lines (Theriault & Li, 1997; Tynes *et al.*, 1997). Some cases of breast cancer were also reported in female radiotelephone operators (Tynes *et al.*, 1996).

Some work has also been done on the effect of EMF on plants, such as Zhang *et al.* (1997) studied the effect of EMF on the germination and early growth of some vegetable seeds and found that EMF caused an increase in germination. Similarly Magon (1996) studied the effect of EMF on *Spirodela polyrhiza* (L.) Schleiden and observed shorter life span and fewer daughter plants in them.

However any significant work has not been done on the effects of EMFs on the biodiversity of natural ecosystems. The present work includes studies on meiotic behavior, meiotic product, and pollen fertility in different plant species from the natural populations growing in the close vicinity of high-tension lines.

Materials and Methods

The material was collected from different plant populations in the vicinity of high-tension lines in the following localities:

i) Karachi University campus, near boundary wall (66,000 V, collections made on 1-12-1999, 09-12-1999, 21-03-2001.)
ii) Pipri, National highway (1,32,000 V, collections made on 29-02-2000).
iii) Bhitai colony, Korangi creek (1,32,000 V, collection made on 11-07-2000).
iv) Road island near Rabia Duplex off University Road, Karachi (1,32,000 V, collection made on 14-01-2001).
v) Port Qasim turning, National Highway (2,20,000 V, collections made on 18-07-2001, 27-12-2001).
vi) Korangi Industrial area, Karachi (2,20,000 V, collections made on 27-12-2001).
vii) Super highway, 30km from Karachi (2,20,000 V, collection made on 15-03-2002).
viii) Super highway, 45km from Karachi (2,20,000 V, collection made on 15-03-2002).
ix) Gharo, National highway, Thatta (1,32,000 V, collection made on 02-07-2002).
x) Gharo, farmhouse, Thatta (1,32,000 V, collection made on 02-07-2002).
xi) National highway, near Bhambore museum turning (1,32,000 V, collection made on 06-09-2002).

Collection of the same species as control was also made from the areas not exposed to any high-tension line (*i.e.* places where was no high tension line of at least 3 km), mostly from Karachi University campus and some other areas in and around Karachi. For cytological studies, young buds were fixed in Carnoy's solution (absolute alcohol: glacial acetic acid, 3:1). Some full-grown buds and flowers were also fixed in the same solution to study the pollen fertility. Voucher specimen is kept in each case.

For the study of meiotic behaviour of chromosomes, slides were prepared by squash technique with 1 % propionic carmine. Twenty or more cells were counted for the study of each meiotic stage and photographs of meiotic abnormalities in these stages were taken by a Nikon photomicroscope (Figs. 2-27). For the study of pollen fertility, anthers from fully mature buds were squashed in 1 % propionic carmine, left for 10-20 minutes for staining and then observed under microscope. The dark stained pollens were counted as fertile whereas light stained or unstained as sterile. The fertile pollen grains were also photographed by Nikon photomicroscope. The voucher specimens have been deposited in the Karachi University Herbarium (KUH).

Comparison of test plants and control plants was performed statistically to find out if difference is significant, Z–test was performed with the help of following formula:

$$Z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\dfrac{\hat{p}_1 \times \hat{q}_1}{n_1} + \dfrac{\hat{p}_2 \times \hat{q}_2}{n_2}}}$$

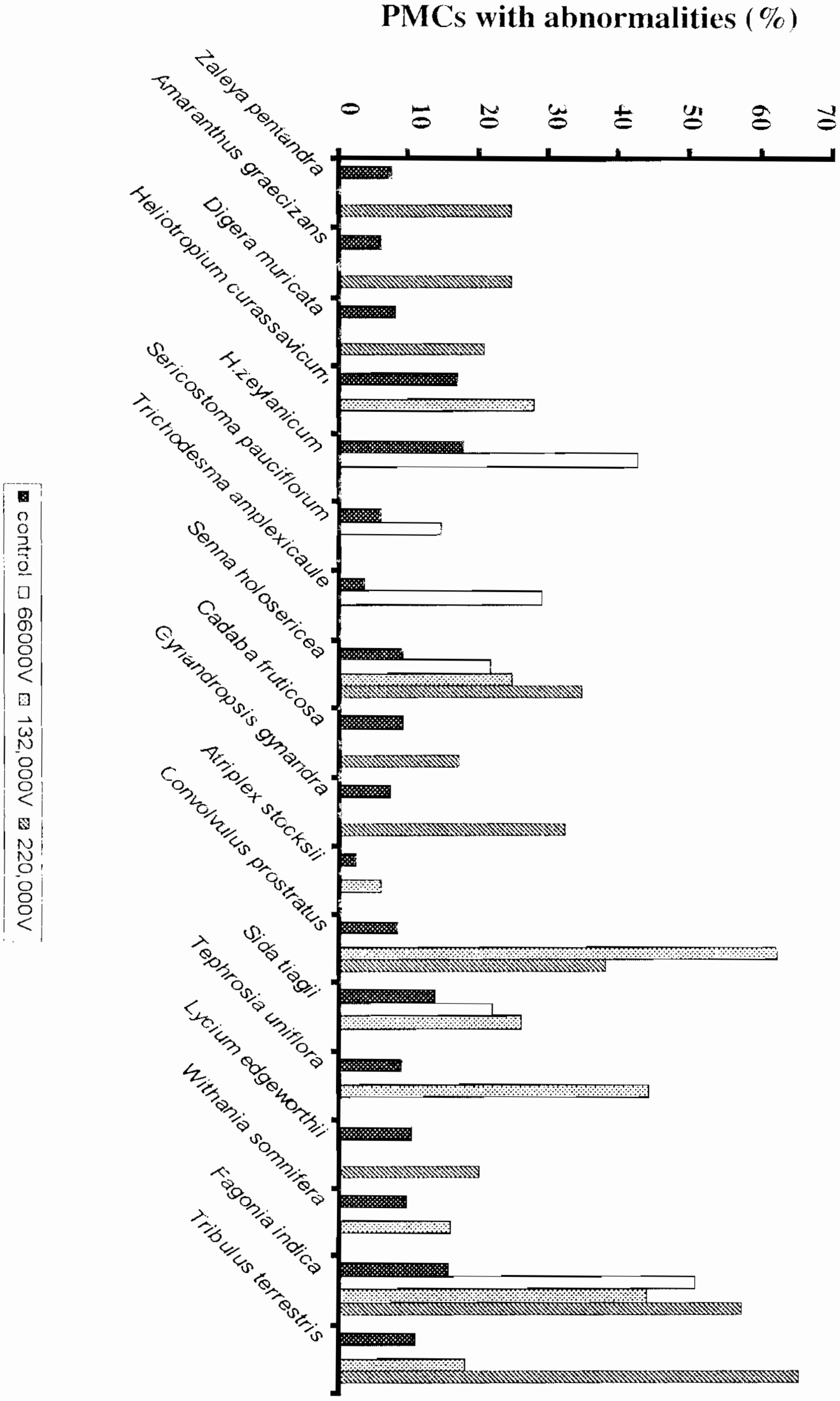
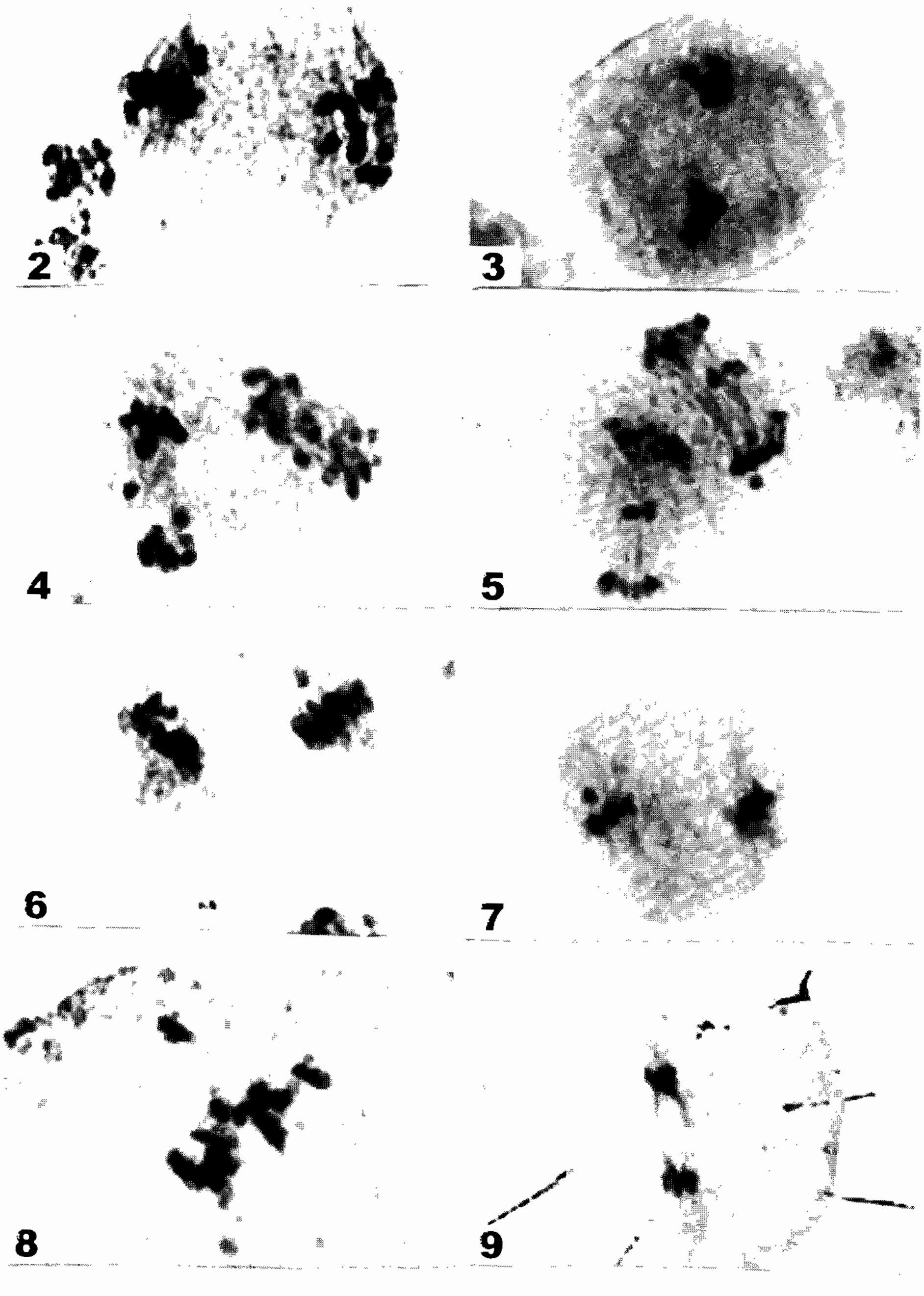
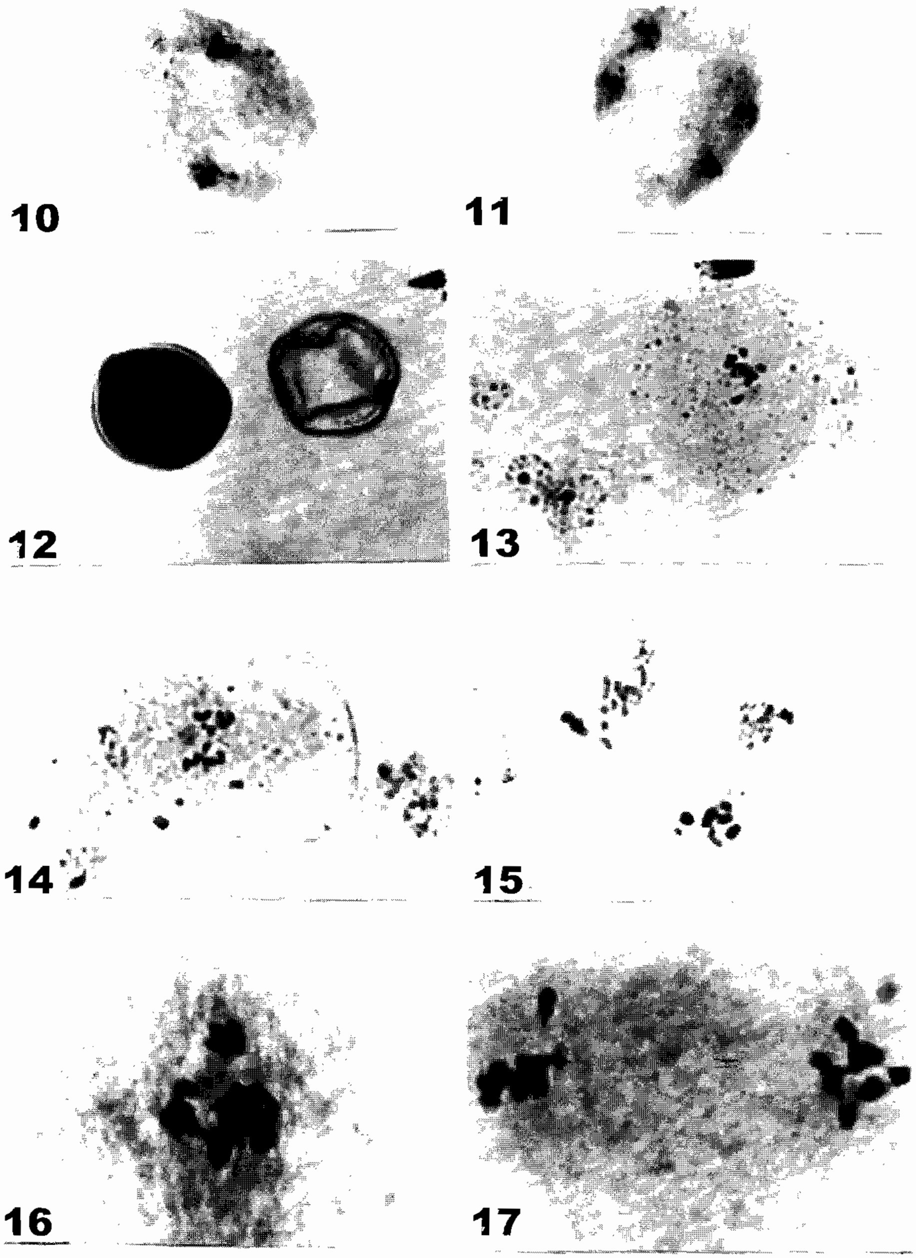


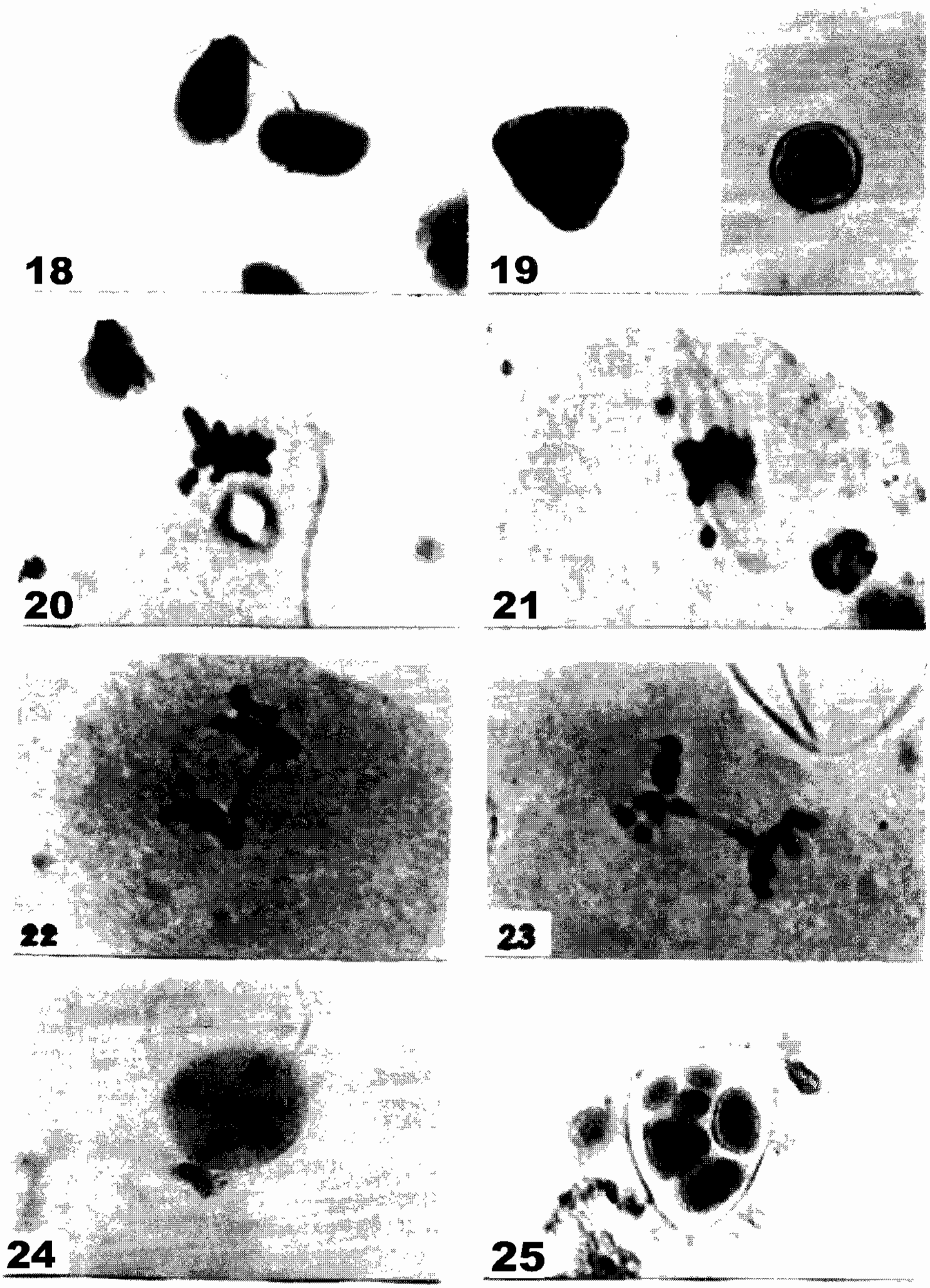
Fig. 1. Comparison of abnormalities of EMF-affected plants with controls.



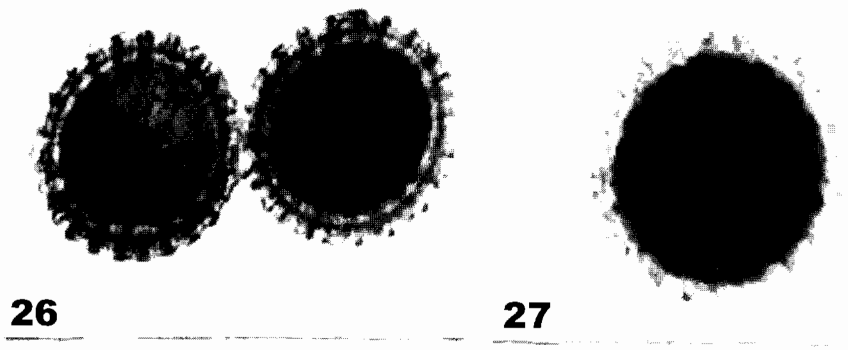
Figs. 2-9. Abnormalities in meiotic behaviour: 2, 3. *Heliotropium zelaynicum* (SZ 33), Precocious and disturbed metaphase II, 4-5. *Heliotropium zelaynicum* (SZ 33), laggards during anaphase II, 6-7. *Trichodesma amplexicaule* (SZ 115), Precocious metaphase–II, 8-9. *Senna holosericea* (SZ 91) precocious metaphase-I, bridge formation during anaphase-I.



Figs. 10-17. Abnormalities in meiotic behaviour: 10-12. *Senna holosericea* (SZ 91), precocious metaphase-II, laggards at anaphase-II, fertile & sterile pollen grains, 13-15. *S. holosericea* (SZ180), precocious metaphase-I, laggards during anaphase-I, & disturbed metaphase-II, 16,17. *Tephrosia uniflora* (SZ 64), precocious metaphase-I & metaphase-II.



Figs. 18-25. Abnormalities in pollen fertility and meiotic behaviour: 18, 19. *Tephrosia uniflora* (SZ 64) diads, sterile and fertile pollen grain, 20-23. *Fagonia indica* (SZ 53), precocious metaphase-I, Bridge formation during anaphase-I, 24, 25. *F. indica* (SZ 106), bridge formation during anaphase-II.



Figs. 26-27. *Tribulus terrestris* (SZ 145), abnormal meiotic product, haploid sterile and fertile pollen grain, diploid pollen grain.

Results and Discussion

Various abnormalities observed in pollen mother cells' (PMC) meiosis are shown in Figs. 1 to 27. The Table 1 reveals that the plants exposed to high-tension lines showed considerable meiotic abnormalities including stickiness in bivalents during diakinesis, precocity and disturbance during metaphases I & II, lagging chromosomes and bridge formation in anaphases I & II. Fig. 1 shows steady increase in abnormalities with an increase in the voltage of high-tension lines. However, different species showed different percentages of disturbed cells. The highest percentage of disturbed diakinesis was observed in *Senna holosericea* (SZ 121) *i.e.* 54 % which was collected under the high tension lines of 2,20,000 V, the highest percentages of disturbed metaphase I were detected in *Heliotropium zelaynicum* (SZ 33) which is 84 % and *Fagonia indica* (SZ 53) 77 %. Highest abnormality in metaphase II *i.e.* 58 % was observed in *Fagonia indica* (SZ 152), growing under the high-tension line of 2,20,000 V. Highest percentage of abnormal anaphase I was observed in *Cadaba fruticosa* (SZ 171) *i.e.* 100 % and *Tribulus terrestris* (SZ 145) 73 %, both were collected under the high-tension line of 2,20,000 V. The latter also showed the highest incidence of abnormal anaphase II (*i.e.* 69 %). The comparison between overall meiotic abnormalities in the test specimens and the respective control specimens is found to be statistically significant in most cases (Table 3).

Besides meiotic abnormalities, the plants also showed decrease in pollen fertility. The percentage of sterile pollen grains increased with the increase in voltage. Highest percentage of abnormal pollen grains (42 %) was observed in *Tribulus terrestris* (SZ 145). Usually the sterile pollen grains were smaller in size than fertile pollen grains (Figs. 11 & 18). Some species showed production of microspore diads due to the failure of meiosis-II (Fig. 17) along with normal tetrads, and certain percentage of diploid pollen grains at voltages higher than 100,000 V. The highest percentages of diads (36 %), diploid pollen grains and abnormal meiotic products were observed in *T. terrestris* (SZ 145), which was collected under the high-tension lines of 2,20,000 V (Figs. 26-27).

Table 1. Percentage of PMCs with meiotic abnormalities.

S. #	Name of species	Voltage V	Voucher #	Diaki-nesis	Met.I	Met.II	Ana.I	Ana.II	Overall abnormality
1.	Aizoaceae								
	Zaleya pentandra (L.) Jeffray	2.20.000	SZ 132	-----	-----	20.68	22.22	28.94	24.71
	(n = 9)	control	SZ 108	-----	15.49	-----	8.69	-----	7 69
2.	Amaranthaceae								
	Amaranthus graecizans L.	2.20.000	SZ 124	-----	30.55	8.33	31.04	-----	24.72
	(n=16)	control	SZ 143	-----	15.91	-----	-----	-----	5.88
	Digera muricata (L.) Mart.	2.20,000	SZ 123	15.79	14.42	28.68	9.09	-----	20.99
	(n = 9)	control	SZ 134	2.44	1.23	6.45	30	-----	8.19
3.	Boraginaceae								
	Heliotropium curassavicum L.	1,32.000	SZ 78	23.76	42.99	34.15	-----	-----	27.97
	(n =13)	control	SZ 137	-----	12.50	30.63	-----	-----	16.88
	H. zeylanicum(Burm.f.)Lam.	66.000	SZ 33	-----	83.56	22.23	21.43	48.72	46.25
	(n = 16)	66,000	SZ 35	-----	59.46	42.30	8.33	19.15	39.09
		control	SZ 69	-----	27.95	23.07	27.50	-----	25.29
	Sericostoma pauciflorum Stocks	66,000	SZ 114	-----	25.93	-----	23.08	-----	14.74
		control	SZ 74	-----	10.71	6.12	-----	-----	6.09
	Trichodesma amplexicaule Roth	66000	SZ 115	33.33	47.37	22.09	36	29.69	28.88
	(n =10)	control	SZ 67	-----	7.08	-----	2.41	-----	3.69
4.	Caesalpiniaceae								
	Senna holosericea Fres.	66.000	SZ 112	13 05	27.17	21.67	-----	22.41	21.78
		1,32.000	SZ 91	-----	34.13	10.78	-----	12	21.27
		1,32.000	SZ 92	33	24.37	32.25	40.74	20	27.02
		1,32,000	SZ 180	17.39	32.53	28.57	10	-----	26.09
		2.20.000	SZ 121	53.73	31.03	42.42	25.53	18.37	34.63
		control	SZ 103	2.63	13.72	-----	-----	-----	9.25
5.	Capparidaceae								
	Cadaba fruticosa (L.) Druce.	2,20.000	SZ 171	-----	20.69	20.86	20.73	-----	17.22
		control	SZ 211	-----	8.70	9.78	62	5.97	7.42
	Gynandropsis gynandra (L.) Briq.	2.20,000	SZ 122	35.24	48.98	23.84	22.92	9.09	32.47
	(n = 17)	control	SZ 138	-----	22.28	14.89	25.93	-----	20.16

Table 1. (Cont'd.)

S. #	Name of species	Voltage V	Voucher #	Diaki-nesis	Met.I	Met.II	Ana.I	Ana.II	Overall abnormality
	Atriplex stocksii Boiss.	1,32,000	SZ 98	4.46	8.70	-----	6.67	-----	5.92
		control	SZ 140	-----	-----	-----	7.41	-----	2.38
7.	Convolvulaceae								
	Convolvulus prostratus Forsk.	1,32,000	SZ 65	100	32	-----	-----	-----	62.22
		2,20,000	SZ 160	-----	41.66	6.12	48.57	33.33	38.22
		control	SZ 49	-----	-----	10	13.51	-----	8.41
8.	Malvaceae								
	Sida tiagii Bhandari	66000	SZ 40	21.05	25.65	30.31	7.41	-----	21.89
	(n = 14)	1,32,000	SZ 59	-----	30	-----	20	-----	26.15
		control	SZ 76	-----	23.64	-----	8.57	-----	13.91
9.	Papilionaceae								
	Tephrosia uniflora Pers.	1,32,000	SZ 64	-----	60.53	32.73	-----	-----	44.09
		control	SZ 107	-----	-----	15.79	-----	-----	8.82
10.	Solanaceae								
	Lycium edgeworthii Dunal	2,20,000	SZ 131	-----	23.08	23.53	18.92	11.11	20.09
	(n = 12)	control	SZ 135	-----	10.40	15.22	5.91	7.31	10.45
	Withania somnifera (L.) Dunal	1,32,000	SZ 93	-----	25	-----	-----	-----	14.28
		1,32,000	SZ 94	-----	20.71	16.91	25	25	17.82
		control	SZ 116	-----	7.96	17.27	-----	-----	9.62
11.	Zygophyllaceae								
	Fagonia indica L.	66,000	SZ 41	-----	64.05	34.50	32.67	-----	50.68
	(n = 9)	1,32,000	SZ 53	-----	77.33	18.18	57.14	26.08	43.82
		2,20,000	SZ 152	40.74	75.81	57.89	56.15	-----	57.17
		control	SZ 106		21.42	14.63	13.47	14.29	15.74
	Tribulus terrestris L.	1,32,000	SZ 189	31.25	28.57	-----	-----	-----	30
		2,20,000	SZ 145	-----	52	62.96	72.97	69.23	65.22
		control	SZ 184	-----	16.42	10.20	-----	-----	10.88

Table 2. Pollen fertility & meiotic products in affected specimens compared with control specimens (%).

S. #	Name of species	Voltage V	Voucher #	Diads	Sterile *P. grains*
1.	Aizoaceae				
	Zaleya pentandra (L.) Jeffray	2,20,000	SZ 132	-----	-----
	(n = 9)	control	SZ 108	0.23	0.65
2.	Amaranthaceae				
	Amaranthus graecizans L.	2,20,000	SZ 124	-----	3.11
	(n=16)	control	SZ 143	-----	1.14
	Digera muricata (L.) Mart.	2,20,000	SZ 123	-----	2.30
	(n = 9)	control	SZ 134	-----	0.92
3.	Boraginaceae				
	Heliotropium curassavicum L.	1,32,000	SZ 78	-----	7.23
	(n =13)	control	SZ 137	-----	2.33
	H. zeylanicum(Burm.f.)Lam.	66,000	SZ 33	-----	7.84
	(n = 16)	66,000	SZ 35	-----	1.96
		control	SZ 69	-----	3.59
	Sericostoma pauciflorum Stocks.	66,000	SZ 114	-----	0.91
		control	SZ 74	-----	
	Trichodesma amplexicaule Roth.	66,000	SZ 115		4.86
	(n =10)	control	SZ 67	-----	2.41
4.	Caesalpiniaceae				
	Senna holosericea Fres.	66,000	SZ 112	-----	2.04
		1,32,000	SZ 91	0.57	4.05
		1,32,000	SZ 92	-----	-----
		1,32,000	SZ 180	-----	1.73
		2,20,000	SZ 121	-----	2.91
		control	SZ 103	-----	0.48
5.	Capparidaceae				
	Cadaba fruticosa (L.) Druce.	2,20,000	SZ 171	0.22	1.42
		control	SZ 211	-----	0.35
	Gynandropsis gynandra (L.) Briq.	2,20,000	SZ 122	-----	0.91
	(n = 17)	control	SZ 138	-----	0.65

Table 2. (Cont'd.)

S. #	Name of species	Voltage V	Voucher #	Diads	Sterile *P. grains*
6.	Chenopodiaceae				
	Atriplex stocksii Boiss.	1,32,000	SZ 98	-----	4.31
		control	SZ 140	-----	-----
7.	Convolvulaceae				
	Convolvulus prostratus Forsk.	1,32,000	SZ 65	-----	1.85
		2,20,000	SZ 160	-----	4.59
		control	SZ 49	-----	1.85
8.	Malvaceae				
	Sida tiagii Bhandari	66000V	SZ 40	-----	18.78
	(n = 14)	1,32,000	SZ 59	-----	5.24
		control	SZ 76	-----	3.09
9.	Papilionaceae				
	Tephrosia uniflora Pers.	1,32,000	SZ 64	1.33	5.85
		control	SZ 107	-----	1.99
10.	Solanaceae				
	Lycium edgeworthii Dunal	2,20,000	SZ 131	-----	6.33
	(n = 12)	control	SZ 135	-----	3.93
	Withania somnifera (L.) Dunal	1,32,000	SZ 93	0.19	10.10
		1,32,000	SZ 94	0.22	10.49
		control	SZ 116	-----	4.16
11.	Zygophyllaceae				
	Fagonia indica L.	66,000	SZ 41	-----	1.84
	(n = 9)	1,32,000	SZ 53	-----	7.06
		2,20,000	SZ 152	-----	4.94
		control	SZ 106	-----	2.25
	Tribulus terrestris L.	1,32,000	SZ 189	-----	3.55
		2,20,000	SZ 145	36.10	42.13
		control	SZ 184	-----	0.41

Table 3. Statistical comparison of test plants with their respective control.

S. #	Name of species	Voltage V	Voucher #	Z-test values	Z-test	Probability level
1	Aizoaceae					
	Zaleya pentandra (L.) Jeffray	2,20,000	SZ 132	1.66	Non-significant	$p > 0.05$
2	Amaranthaceae					
	Amaranthus graecizans L.	2,20,000	SZ 124	1.86	Non-significant	$p > 0.05$
	Digera muricata (L.) Mart.	2,20,000	SZ 123	4.13	Significant	$p < 0.001$***
3	Boraginaceae					
	Heliotropium curassavicum L.	1,32,000	SZ 78	3.28	Significant	$p < 0.01$**
	H. zeylanicum (Burm.f.) Lam.	66,000	SZ 33	4.46	Significant	$p < 0.001$***
		66,000	SZ 35	2.91	Significant	$p < 0.01$**
	Sericostoma pauciflorum Stocks	66,000	SZ 114	3	Significant	$p < 0.01$**
	Trichodesma amplexicaule Roth	66000	SZ 115	9.26	Significant	$p < 0.001$***
4	Caesalpiniaceae					
	Senna holosericea Fres.	66,000	SZ 112	4.33	Significant	$p < 0.001$***
		1,32,000	SZ 91	3.82	Significant	$p < 0.001$***
		1,32,000	SZ 92	5	Significant	$p < 0.001$***
		1,32,000	SZ 180	4.72	Significant	$p < 0.001$***
		2,20,000	SZ 121	7.22	Significant	$p < 0.001$***
5	Capparidaceae					
	Cadaba fruticosa (L.) Druce.	2,20,000	SZ 171	4.54	Significant	$p < 0.001$***
	Gynandropsis gynandra (L.) Briq.	2,20,000	SZ 122	3	Significant	$p < 0.01$**
6	Chenopodiaceae					
	Atriplex stocksii Boiss.	1,32,000	SZ 98	2.35	Significant	$p < 0.05$*
7	Convolvulaceae					
	Convolvulus prostratus Forsk.	1,32,000	SZ 65	9.82	Significant	$p < 0.001$***
		2,20,000	SZ 160	7.14	Significant	$p < 0.001$***
8	Malvaceae					
	Sida tiagii Bhandari	66000	SZ 40	1.7	Non-significant	$p > 0.05$
		1,32,000	SZ 59	2	Significant	$p < 0.05$*
9	Papilionaceae					
	Tephrosia uniflora Pers.	1,32,000	SZ 64	5.64	Significant	$p < 0.001$***
10	Solanaceae					
	Lycium edgeworthii Dunal	2,20,000	SZ 131	3.12	Significant	$p < 0.01$**
	Withania somnifera (L.) Dunal	1,32,000	SZ 93	0.91	Non-significant	$p > 0.05$
		1,32,000	SZ 94	3.33	Significant	$p < 0.001$***
11	Zygophyllaceae					
	Fagonia indica L.	66,000	SZ 41	9.2	Significant	$p < 0.001$***
		1,32,000	SZ 53	8	Significant	$p < 0.001$***
		2,20,000	SZ 152	13.66	Significant	$p < 0.001$***
	Tribulus terrestris L.	1,32,000	SZ 189	2.92	Significant	$p < 0.01$**
		2,20,000	SZ 145	10.59	Significant	$p < 0.001$***

In the overall meiotic abnormalities, *T. terrestris* showed the highest percentage, while the family Zygophyllaceae showed the highest percentage of meiotic abnormalities among all the studied families (Tables 1 & 2, Fig. 1).

The present study shows that the EMFs of high-tension wires cause chromosomal abnormalities in plants, which result in meiotic disturbance and pollen sterility. These chromosomal abnormalities such as stickiness in bivalents during diakinesis, precocity and disturbance during metaphases I & II, lagging chromosomes and bridge formation in anaphases I & II, can pass on to next generations. Stronger EMFs also results in the formation of diploid pollen and non-reduced gametes, which may ultimately result in polyploidy. Like plants, other living organisms in the vicinity of high-tension lines may also get affected.

Acknowledgement

We are thankful to Prof. Dr. S. Shahid Shaukat, Department of Botany, University of Karachi, for his help in the statistical analysis.

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