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EFFECTS OF ELECTROMAGNETIC FIELDS (CREATED BY HIGH-TENSION LINES) ON SOME INDIGENOUS PLANT SPECIES IN THE VICINITY OF KARACHI–II. ASTERACEAE

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Abstract

To study the effects of electromagnetic fields of high-tension lines on plants, 13 specimens belonging to 10 species of family Asteraceae were collected from different localities in and around Karachi under high-tension lines of 132 kV. In addition same species were also collected as control from areas free from electromagnetic fields. The test and control specimens were studied for meiosis in pollen mother cells (PMCs), meiotic products and pollen fertility. A marked difference in meiotic abnormalities and magnetic field strength was observed in control and test specimens and localities. PMC meiosis showed certain abnormalities which included stickiness, precocity, laggards, bridges, multipolar divisions etc. The difference between the meiotic abnormalities of test and control specimens was found to be statistically significant. Microspore diads, triads and hypertetrads were observed besides normal microspore tetrads. The pollen fertility also decreased in test specimens; and the difference between test and control specimens was found to be significant.

Introduction

With the modernization and industrialization human beings as well as other organisms (animals and plants) exposes to electromagnetic fields of different intensities. From the past 30 or more years there has been a controversy about the possible harmful or beneficial effects of EMFs on different living organisms and hundreds of studies are available on these organisms (humans, animals, plants and microorganisms) in literature.

The electric fields, magnetic fields and electromagnetic fields are found to cause geno- and cytotoxic affects on different plant species. It is observed from different studies that an increase in the percentage of chromosomal aberrations occurred during mitosis and meiosis, such as disturbed spindle, fragments, bridges, isolated chromosomes, laggards, micronuclei, clumping, stickiness, unequal pairing, multipolar division and a decrease in mitotic and meiotic indices (Linskens & Smeets, 1978; Saxena & Gupta, 1987; Runthala & Bhattacharya, 1991; Zaidi & Khatoon, 2003; Pavel & Creanga, 2005; Hanafy *et al.,* 2006; Zhang *et al.,* 2007).

Some studies focused upon the effects of these fields on growth of plants and germination of seeds. The study of Magone (1996), Moon & Chung (2000), Reina *et al.,* (2001), Aladjadjiyan *et al.,* (2002), Flórez *et al.,* 2004, Apasheva *et al.,* (2006), Dardeniz *et al.,* (2006), Dao-Ling *et al.,* (2009), and Cakmak *et al.,* (2009) showed an increase in the rate of germination of seeds and growth of plants upon exposure to these fields. On the other hand a decrease in growth and germination rate upon exposure to electric and magnetic fields was observe by Selga & Selga (1996) and Ahmad *et al.,* (2007), whereas Rajendra *et al.,* (2005) and Tkalec *et al.,* (2005) did not observed any affect upon exposure.

Most of the above mentioned studies have been conducted in lab conditions and performed upon one or 2 species only. Only little work is available in literature which deals with the study of effect of EMF (created by high-tension transmission lines) on different plant species growing in their natural or semi natural ecosystem. With this background, the present study has been conducted to investigate the effect of long-term EMF exposure on plants growing under high-tension transmission lines. The present work includes studies on PMC meiosis, meiotic products and pollen fertility.

Materials and Methods

To study the effects of electromagnetic fields the material was collected from different plant populations in the vicinity of high-tension lines of 132 kV (just below the lines and the area of approximately 10 Km around these lines) (Table 1).

Table 1. List of localities.

S. No.	District / Div.	Locality
1.	Thatta	Gharo farm house, Gharo
2.	Thatta	Along National Highway, Bhambhore museum turning
3.	Thatta	Goth Gul Hasan Baloch, along National Highway
4.	Thatta	10 km from Ghaggar Phatak, along National Highway
5.	Karachi	On way to Port Bin Qasim, in front of Indus Motor Company
6.	Thatta	Near Gharo, under the bridge, along National Highway
7.	Lasbella	Shahanshah Balochistan Hotel, Opposite Hub Police Station, Hub
8.	Thatta	14 km before Gharo, under the bridge, along National Highway

Collection of the same species was also made as control specimens from areas not exposed to High-tension lines or where intensity of electromagnetic fields is less than 1mG, 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.

To perform cytological studies young buds, mature buds and some fully grown flowers were fixed on the spot in Carnoy's solution (absolute alcohol: glacial acetic acid, 3:1) for PMC meiosis, meiotic products and pollen fertility respectively. For the study of 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. Photographs of PMCs showing meiotic abnormalities with good contrast were taken by Nikon Photomicroscope. Similar procedure was adapted for meiotic products study. 100 or more meiotic products were observed in each case. Photographs 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, anthers from mature flowers were also squashed in 1% propionic carmine, left for 10 to 20 minutes for staining and then observed under the microscope. The dark stained pollens were counted as fertile whereas light stained or unstained as sterile. A minimum of 200 to a maximum of 1000 pollen grains were studied to score fertile, sterile, and diploid pollen grains. The pollen grains were also photographed. 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) with the help of following formula to find out if the difference is statistically significant.

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

$\hat{p}_1$ = % of normal cells in test plants
$\hat{p}_2$ = % of normal cells in control plants
$\hat{q}_1$ = % of abnormal cells in test plants
$\hat{q}_2$ = % of abnormal cells in control plants
n_1 = Total number of cells observed in test plants
n_2 = Total number of cells observed in control plants

Results and Discussions

The stages which were observed during the study of PMC meiosis include diakinesis, Metaphase I, Anaphase I, Metaphase II and Anaphase II. The abnormalities which were observed during these stages include stickiness, univalents and multivalent formation during diakinesis. Stickiness, precocious chromosomes and disturbances were found during metaphase I and II, while the stickiness, laggards, bridges and multipolar divisions were observed during anaphase I and II (Fig. 5"a-l"). The results of PMC meiosis of test and control specimens are summarized in Table 2. Stage wise highest (57%) abnormal percentage of cells at diakinesis was observed in *Pluchea arguta*, At metaphase I (71%) in *Oligochaeta ramosa* and at anaphase I in *Blumea obliqua* (32 %,). In case of metaphase II and anaphase II the percentages were 38% and 37% respectively, observed in *Oligochaeta ramosa.* Overall species wise highest (47%) abnormality was observed in *O. ramosa*, while lowest was observed in *Pulicaria carnosa* (Fig. 1).

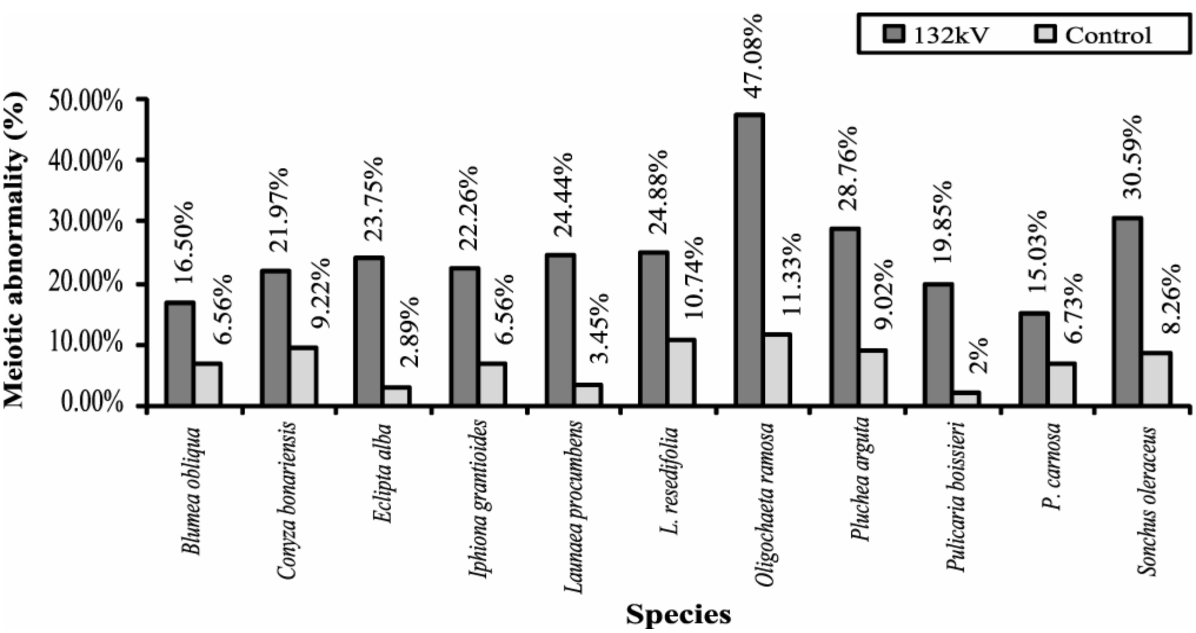

Fig. 1. Species-wise comparison of meiotic abnormalities in test specimens and control specimens.

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

S. #	Family and plant name	Voltage voucher No.	M. F. S (mG)	D %	M I %	M II %	A I %	A II %	Overall abnormality %
1.	*Blumea obliqua* (L.) Druce	220 kV, SZ 373	27.6	0	22.5	21.05	31.85	….	16.5
		Control, SR 537	<1	0	6.52	0	14.52	…..	6.56
2.	*Conyza bonariensis* (L.) Cronquist	132 kV, SZ, 187	4.6	40.9	29.78	16.67	0	0	21.97
		Control, SZ 201	<1	….	18.37	13.33	0	0	9.22
3.	*Eclipta alba* (L.) Hasskl.	132 kV, SZ 646	12.2	…..	29.03	20.41	….	…..	23.75
		Control, SZ 912	<1	0	3.75	4.55	0	0	2.89
4.	*Iphiona grantioides* (Boiss.) Anderb.	132 kV, SZ 319	10.5	18.42	20	….	16	16.22	18.26
		132 kV, SZ 320	9.8	23.59	20.97	…..	……	……	22.42
		132 kV, SZ 330	12.5	19.54	33.78	…..	…..	…..	26.09
		Control, SZ 455	<1	0	12	4.41	…..	8	6.56
5.	*Launaea procumbens* (Roxb.)	132 kV, SZ 229	5.1	…..	21.05	…..	14.29	…..	17.81
	Ramayya & Rajagopal	132 kV, SZ 261	5.1	33.76	43.29	18.18	21.43	6.89	31.06
		Control, SZ 902	<1	0	8.33	7.14	0	0	3.45
		Control, SZ 602	<1	0	7.5	10.14	8	5.13	6.73
6.	*L. resedifolia* (L.) O. Ktze.	132 kV, SZ 361	12.8	13.43	38.14	14.81	…..	…..	24.88
		Control, SZ 904	<1	0	15.38	15.79	12.9	…..	10.74
7.	*Oligochaeta ramosa* (Roxb.) Wagenitz	132 kV, SZ 324	10.5	…..	71.43	38.1	8	36.59	47.08
		Control, Kh 1672	<1	0	11.54	21.98	12.9	8.33	11.33
8.	*Pluchea arguta* Boiss.	132 kV, SZ 339	12.5	56.76	24.66	13.79	0	0	28.76
		Control, SZ 911	<1	…..	21.43	6.9	0	0	9.02
9.	*Pulicaria boissieri* Hook.f.	132 kV, SZ 710	4.9	8.33	36	25	8	4.55	19.85
		Control, SZ 459	<1	0	4.52	3.39	0	0	2
10.	*P. carnosa* (Boiss.) Burkill	132 kV, SZ 417	12.5	0	14.81	20.51	27.27	25	15.03
		Control, SZ 602	<1	0	7.5	10.14	8	5.13	6.73
11.	*Sonchus oleraceus* L.	132 kV, SZ 280	5.1	…..	57.89	15.38	0	0	30.59
		Control, SZ 897	<1	0	13.56	8.33	0	…..	8.26

Note: M.F.S.= Magnetic Field Strength, mG= Milli Gauss, D= Diakinesis, MI= Metaphase I, MII= Metaphase II, AI= Anaphase I, AII= Anaphase II

The study of meiotic product showed abnormal production of diads besides normal tetrads. The diads were observed in *Launaea procumbens* and *Pulicaria carnosa* (Fig. 5"m,n"). Highest percentages 16% of sterile pollens were observed in *Blumea obliqua* (Fig. 5"o"). The results of meiotic products and pollen fertility are summarized in Table 3. In comparison with control the mean magnetic field strength, mean pollen sterility and mean meiotic abnormalities showed a gradual increase in test plants (Fig. 2). In control plants the mean magnetic field strength, mean pollen sterility and mean meiotic abnormalities were 0.5 mGauss, 2.19% and 6.95% respectively, which were 9.08 mGauss, 5.31% and 13% in test plants.

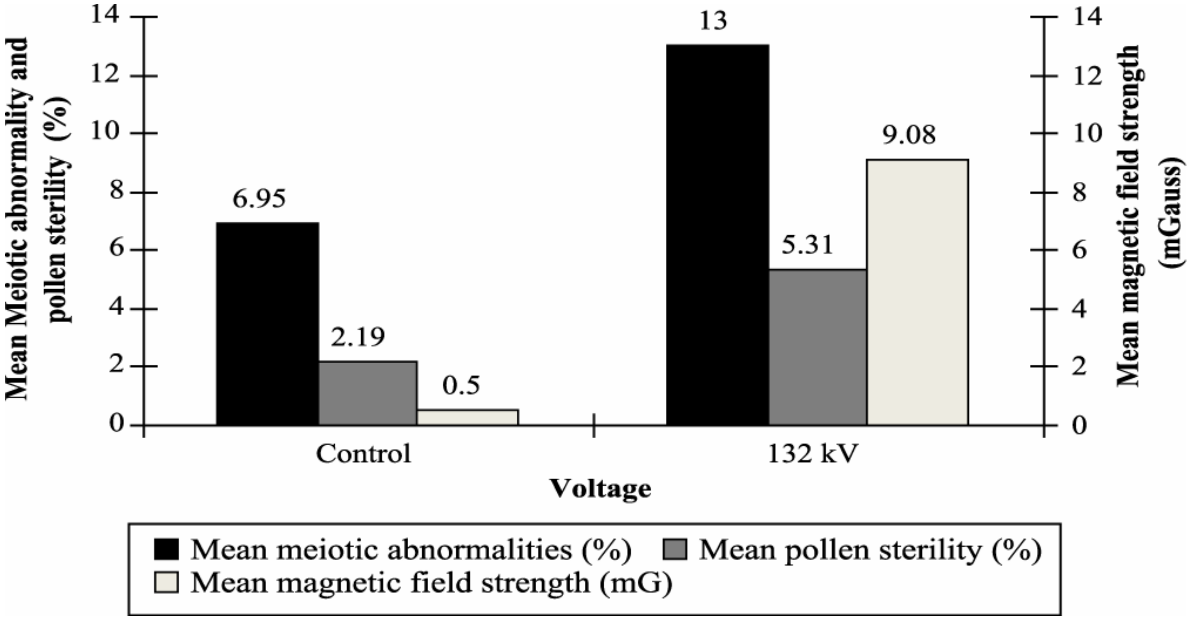

Fig. 2. Bar diagram showing comparison of mean meiotic abnormalities, mean pollen sterility and mean magnetic field strengths at control and 132 kV.

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

S. #	Family and plant name	Voltage, Voucher No.	M. F. S (mG)	Diads %	Diploid pollens (%)	Pollen sterility (%)
1.	*Blumea obliqua* (L.) Druce	220 kV, SZ 373	27.6	0	0	15.96
		Control, SR 537	<1	0	0	2.56
2.	*Conyza bonariensis* (L.) Cronquist	132 kV, SZ 187	4.6	0	0	2.09
		Control, SZ 201	<1	0	0	0.72
3.	*Eclipta alba* (L.) Hasskl.	132 kV, SZ 646	12.2	0	0	5.06
		Control, SZ 912	<1	0	0	2.49
4.	*Iphiona grantioides* (Boiss.)Anderb.	132 kV, SZ 319	10.5	0	0	2.67
		132 kV, SZ 320	9.8	0	0	2.61
		132 kV, SZ 330	12.5	0	0	2.6
		Control, SZ 455	<1	0	0	1.64
5.	*Launaea procumbens* (Roxb.) Ramayya & Rajagopal	132 kV, SZ 229	5.1	2.92	0	10.22
		132 kV, SZ 261	5.1	0	0	8.04
		Control, SZ 902	<1	0	0	0.88
6.	*L. resedifolia* (L.) O. Ktze.	132 kV, SZ 361	12.8	0	0	0
		Control, SZ 904	<1	0	0	0.99
7.	*Oligochaeta ramosa* (Roxb.) Wagenitz	132 kV, SZ 324	10.5	0	0	9.09
		Control, Kh 1672	<1	0	0	2.93
8.	*Pluchea arguta* Boiss.	132 kV, SZ 339	12.5	0	0	11.11
		Control, SZ 911	<1	0	0	9.5
9.	*Pulicaria boissieri* Hook.f.	132 kV, SZ 710	4.9	0	0	2.77
		Control, SZ 459	<1	0	0	0.72
10.	*P. carnosa* (Boiss.) Burkill	132 kV, SZ 417	12.5	0.74	0.9	3.17
		Control, SZ 602	<1	0	0	0.66
11.	*Sonchus oleraceus* L.	132 kV, SZ 280	5.1	0	0	9.57
		Control, SZ 897	<1	0	0	0.99

Note: M.F.S.= Magnetic Field Strength, mG = Milli Gauss

The statistical analysis of meiotic abnormalities in test and control specimens showed that in 93% cases the meiotic abnormalities were significantly higher in test specimens as compared to control specimens (Table 4, Fig. 3). While in case of pollen sterility 57.14% specimens showed significant difference (Table 5, Fig. 4). In case of meiotic products in two cases microspore diads observed which may arise due to the failure of either anaphase I or anaphase II of PMC meiosis. The diads give rise to diploid pollen grains which in turn produce diploid male gametes.

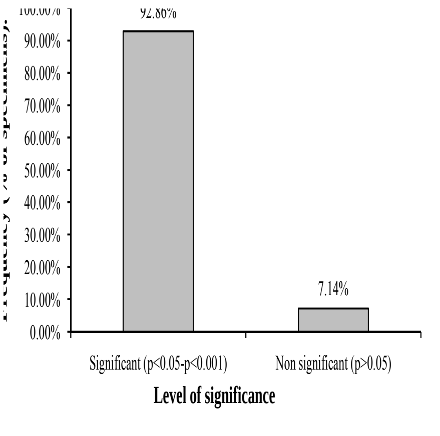

Fig. 3. Comparison of significant and non-significant differences in the meiotic abnormalities of test anf control specimens.

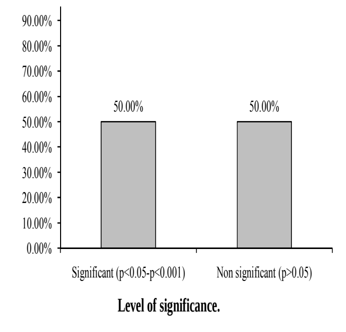

Fig. 4. Comparison of significant and nonsignificant differences in the pollen sterility of test and control specimens.

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
1.	*Blumea obliqua* (L.) Druce	220 kV, SZ 373	3	S	p<0.01**
2.	*Conyza bonariensis* (L.) Cronquist	132 kV, SZ 187	3.02	S	p<0.01**
3.	*Eclipta alba* (L.) Hasskl.	132 kV, SZ 646	4.2	S	p<0.001***
4.	*Iphiona grantioides* (Boiss.) Anderb.	132 kV, SZ 319	3.67	S	p<0.001***
5.	*Launaea procumbens* (Roxb.) Ramayya & Rajagopal	132 kV, SZ 320	5.36	S	p<0.001***
		132 kV, SZ 330	5.14	S	p<0.001***
		132 kV, SZ 229	0.21	N.S	p>0.05
		132 kV, SZ 261	4.67	S	p<0.001***
6.	*L. resedifolia* (L.) O.Ktze.	132 kV, SZ 361	3.5	S	p<0.001***
7.	*Oligochaeta ramosa* (Roxb.) Wagenitz	132 kV, SZ 324	12	S	p<0.001***
8.	*Pluchea arguta* Boiss.	132 kV, SZ 339	7.33	S	p<0.001***
9.	*Pulicaria boissieri* Hook.f.	132 kV, SZ 710	7.04	S	p<0.001***
10.	*P. carnosa* (Boiss.) Burkill	132 kV, SZ 417	2.5	S	p<0.05*
11.	*Sonchus oleraceus* L.	132 kV, SZ 280	3.83	S	p<0.001***

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

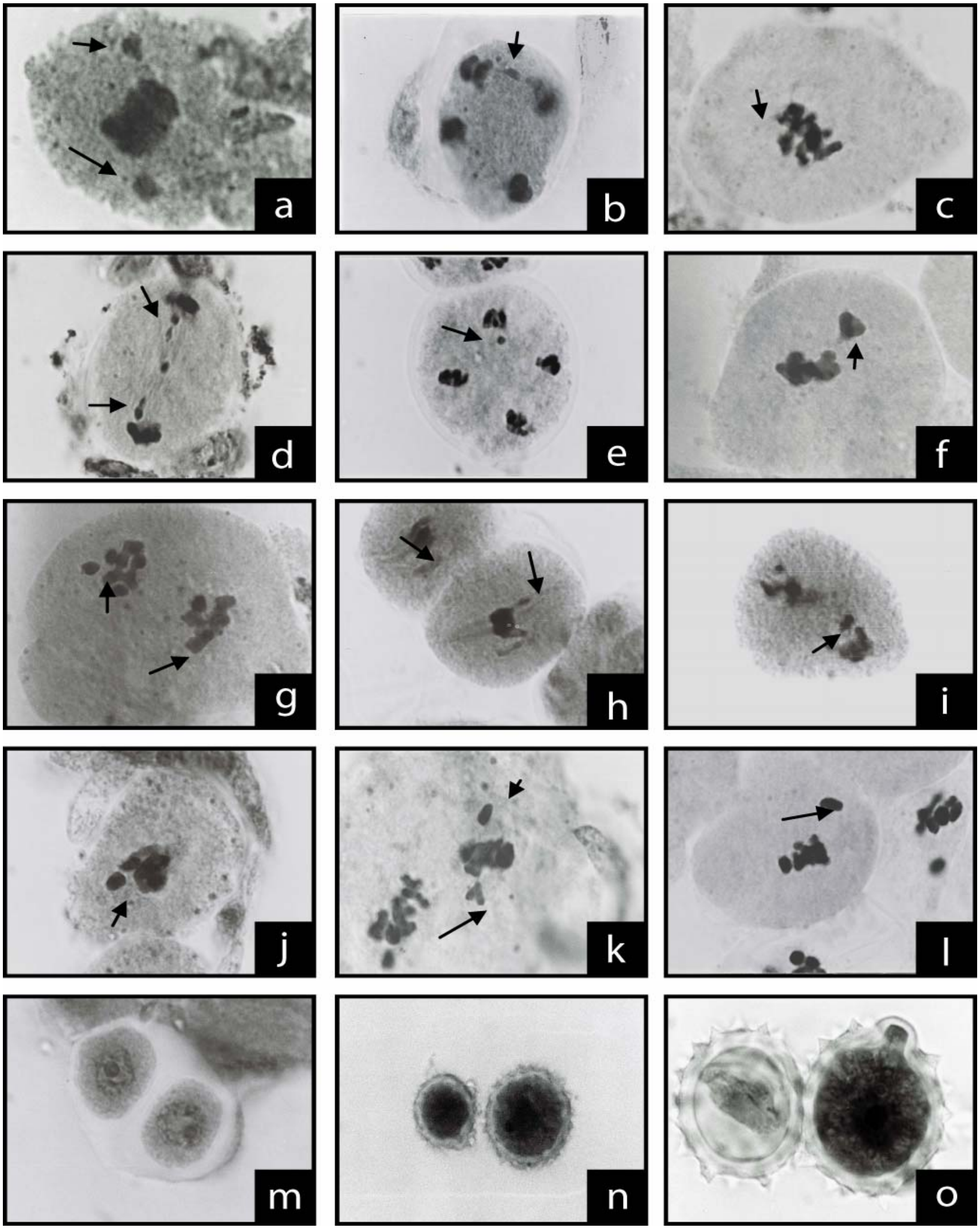

Fig. 5, a, b. *Iphionia grantioides* SZ 445 X1000, a. Metaphase I with stickiness and two precocious bivalents, b. Anaphase II with stickiness and lagging chromosomes. c - e. *Launaea procumbens* SZ 261 X1000, c. Metaphase I showing stickiness, d. Anaphase I with stickiness and lagging chromosomes, e. Anaphase II with stickiness and one lagging chromosome. f, g. *L. resedifolia* SZ 361 X1000, f, Metaphase I with stickiness of bivalenta and splitting of spindle, g. Metaphase II with stickiness and precocious chromosomes. h, i. *Oligochaeta ramosa* SZ 324 X1000, h. Metaphase I showing stickiness, precocious bivalents and splitting of spindle, i. Metaphase II with stickiness and precocious chromosomes. j, k. *Pluchea arguta* SZ 339 X1000, j. Metaphase I with precocious bivalents, k. Metaphase II with precocious chromosomes. l-o. *Pulicaria carnosa* SZ 417, l. Metaphase I with split spindle, X1000, m. Microspore dyad (abnormal) X1000, n. Haploid and diploid fertile pollen grains, X400, o. Haploid and diploid fertile pollen grains, X1000.

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
1.	*Blumea obliqua* (L.) Druce	220 kV, SZ 373	4.48	S	p<0.001***
2.	*Conyza bonariensis* (L.) Cronquist	132 kV, SZ 187	1.59	N.S	p>0.05
3.	*Eclipta alba* (L.) Hasskl.	132 kV, SZ 646	3	S	p<0.01**
4.	*Iphiona grantioides* (Boiss.) Anderb.	132 kV, SZ 319	1.25	N.S	p>0.05
		132 kV, SZ 320	1.41	N.S	p>0.05
		132 kV, SZ 330	1.3	N.S	p>0.05
5.	*Launaea procumbens* (Roxb.) *Ramayya & Rajagopal*	132 kV, SZ 229	7.14	S	p<0.001***
		132 kV, SZ 261	4.66	S	p<0.001***
6.	*L. resedifolia* (L.) O.Ktze.	132 kV, SZ 361	1	N.S	p>0.05
7.	*Oligochaeta ramosa* (Roxb.) Wagenitz	132 kV, SZ 324	4.61	S	p<0.001***
8.	*Pluchea arguta* Boiss.	132 kV, SZ 339	0.71	N.S	p>0.05
9.	*Pulicaria boissieri* Hook.f.	132 kV, SZ 710	3.45	S	p<0.001***
10.	*P. carnosa* (Boiss.) Burkill	132 kV, SZ 417	6.25	S	p<0.001***
11.	*Sonchus oleraceus* L.	132 kV, SZ 280	5.29	S	p<0.001***

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

The present study shows that high tension wires create EMFs of different intensities which cause chromosomal aberrations during different meiotic stages. This ultimately result in the formation of abnormal meiotic product which give rise to diploid pollens and non-reduced gametes which give rise to polyploids. These chromosomal aberrations may pass to next generations resulting formation of abnormal generations which may be sterile. It is anticipated that since these 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 detail investigation including other living organisms should be conducted.

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