Pak. J. Bot., 50(6): 2237-2244, 2018.

EFFECTS OF ELECTROMAGNETIC FIELDS (CREATED BY HIGH TENSION LINES) ON SOME INDIGENOUS PLANT SPECIES–V. BORAGINACEAE Juss., BRASSICACEAE Burnett AND CAESALPINIACEAE R. Br.

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Abstract

The effects of electromagnetic fields (EMFs, created by high tension wires) were studied on 34 plant specimens belonging to 9 species of 3 angiosperm families, i.e., Boraginaceae, Brassicaceae and Caesalpiniaceae. The specimens were collected from their natural ecosystems, growing in and around Karachi, under high tension wires of 132, 220 and 500 kilo volts. The same species were also collected as control specimens from areas free from high tension wires. The test and control specimens were studied for PMC meiosis, meiotic product and pollen fertility. A significantly higher frequency of meiotic abnormalities and pollen sterility was observed in the test over the control specimens. Besides, a considerable increase in the magnetic field strength was observed with the increase in voltage of high tension wires. Significantly higher percentages of meiotic abnormalities occurred in the test specimens compared to controls. These abnormalities included univalents and multivalent formation, stickiness, precocious chromosomes, laggards, bridges, multipolar division etc. The study of meiotic product showed the formation of diads and hypertetrads in addition to the normal tetrads. In addition, the percentage of sterile pollen grains was significantly higher in the high EMF exposed specimens.

Key words: Electromagnetic fields, Boraginaceae, Brassicaceae and Caesalpiniaceae.

Introduction

Nowadays we are continuously exposed to EMFs of different intensities, such as high tension wires, electrical appliances, mobile phones etc. Besides humans other organisms including animals, plants and microorganisms are also constantly exposed to the same intensity of EMFs. In case of plants, the conditions are even more severe because if they are growing under high tension wires, then they must be exposed to these fields throughout their life time and even for generations because they are sedentary and their seeds are often dispersed in close vicinity.

With the increasing exposure to EMFs attempts are made to find out their probable harmful or beneficial effects if any, on living organisms. The first important work was that of Wertheimer & Leeper (1979), who reported that EMFs generated from electrical wiring configurations may cause childhood cancer. After them numerous studies have been conducted to investigate whether these fields are carcinogenic. Some studies support this idea while others do not.

Certain studies focused on the genotoxic potential of these fields. Genotoxic effects of EMFs were observed in humans by Garcia-Sagredo & Monteagudo (1991), Othman *et al*., (2003), and Vijayalaxmi & Obe (2005), according to them EMFs caused an increase in chromosomal aberrations in human lymphocytes. Considerable work has been done in case of plants; Linskens & Smeets (1978), Saxena & Gupta (1987), Runthala & Bhattacharya (1991), Rapley *et al*. (1998), Zaidi & Khatoon (2003, 2012), Hanafy *et al*. (2006), Tkalec *et al*., (2007), Mịhaela (2009), Majd & Shabrangi (2009) and Aksoy *et al.*, (2010), Zaidi *et al.,* (2012, 2013a, 2013b) studied the effects of EMFs, EFs, and MFs on mitosis and meiosis of different plant species and they observed that these fields cause an increase in chromosomal aberrations, mitotic and meiotic index and pollen sterility of these plants. In most of the cases the fields were generated artificially in laboratory conditions whereas in few cases the genotoxic effects were observed under *In vivo* conditions, but in all cases only one or two species were examined. With this background the present study was conducted to investigate the influence of long term EMF exposure on natural flora within their respective ecosystem for many generations under the high tension wires.

The objectives of the study were to examine the changes induced by high electromagnetic fields on 1) PMC meiosis, 2) meiotic products and 3) pollen sterility in a number of plant species belonging to the families Boraginaceae, Brassicaceae and Caesalpiniaceae.

Materials and Methods

The plant material for the study was 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 m around these lines) from different localities of Karachi Division and Thatta and Jamshoro Districts.

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 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 were fixed on the spot in Carnoy's solution (absolute alcohol: glacial acetic

acid, 3:1, V/V). Some fully grown buds and flowers were also fixed in the same solution to study meiotic products and pollen fertility respectively.

For the study of meiotic behavior of chromosomes, temporary slides were prepared from young anthers by the usual squash technique with 1% propionic carmine as the 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 231043.

Slides for meiotic products were prepared from young anthers by squash technique with 1% propionic carmine as stain. Hundred 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 and hypertetrads were taken by Nikon Photomicroscope 231043.

For the study of pollen fertility, slides of anthers from mature flowers were prepared by squash technique. Minimum of one fifty to maximum of one thousand pollen grains were studied to score fertile, sterile, and diploid pollen grains. The pollen grains were also photographed by Nikon Photomicroscope. 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 PMC meiosis are summarized in Table 1. The stages studied during the study of PMC meiosis included diakinesis, metaphase I and II and anaphase I and II. Different abnormalities were observed in test specimens including univalents and multivalent formation and stickiness during diakinesis, stickiness and precocious chromosomes during metaphase I and II; whereas during anaphase I and II stages, stickiness, laggards, precocious chromosomes, bridges and multipolar divisions were observed (Fig. 5a-n). These abnormalities were also observed in control specimens but their percentages were comparatively less than those of test specimens. Stickiness was found to be the most common abnormality observed in each stage. In Diakinesis the highest percentage of abnormal cells was observed in *Heliotropium ophioglossum* (SZ 843, 220 kV), i.e. 80%, this was then followed by *Heliotropium rariflorum* (SZ 747, 132 kV), i.e. 69.57%. In metaphase I and II, highest abnormal cells were recorded in *Heliotropium crispum* (SZ 553, 220 kV), i.e. 87.63% and 54.81% respectively; second highest of metaphase I i.e. 86.27% was observed in *H. rariflorum* (SZ 747, 132 kV) and the second highest of metaphase II i.e. 54.55% was in *H. strigosum* (SZ 824, 500 kV). In case of anaphase I, maximum abnormal percentage (73.54%) was shown by *Senna italica* (SZ 525, 500 kV) and *H. rariflorum* (57.45%) (SZ 748, 132 kV) while in anaphase II stage 43.33% cells were found abnormal in two specimens, *H. ophioglossum* (SZ 843, 220 kV) and *H. rariflorum* (SZ 748, 132 kV). The highest abnormality was exhibited by *H. crispum* (SZ 553, 220 kV) (66.67%), followed by *H. rariflorum* (SZ 747, 132 kV) (59.44%), (SZ 748, 132 kV) (57.14%), *H. crispum* (SZ 318, 132 kV) (50.96%) and *H. ophioglossum* (SZ 843, 220 kV) (50.91%). The comparison of meiotic abnormalities on an overall basis is shown in Fig. 1.

Results of meiotic products and pollen sterility are summarized in Table 2. The study of meiotic product showed that diads (two diplod daughter cells) and hypertetrads (more than 4 daughter cells with some of them very small in size, called micronuclei) are also formed besides normal tetrads (Fig. 5o, p). The diads were observed in *H. rariflorum* (SZ 168, 220 kV) and *H. zeylanicum* (SZ 284, 132 kV) whereas hypertetrads were observed in *H. crispum* (SZ 553, 220 kV), *H. ophioglossum* (SZ 843, 220 kV), and *H. rariflorum* (SZ 747, SZ 748, 132 kV; SZ 168, 220 kV). The hypertetrads were also observed in the control specimens of *H. ophioglossum* (SZ 856) and *H. rariflorum* (SZ 791) but their percentages are comparatively low in control. Highest sterile pollen grains, 50.96% were observed in *H. crispum*(SZ 318, 132 kV), followed by 47% in *H. ophioglossum* (SZ 843, 220 kV) and 33.5% in *H. rariflorum* (SZ 747, 132 kV) (Fig. 5q-t).

The diads may occur due to failure of either anaphase I or anaphase II during meiosis. These diads give rise to diploid pollen grains which in turn produce diploid male gametes. The spontaneous production of diploid gametes in plant ranges from 0% - 0.56% (Levin, 2002); however these test specimens have shown much higher percentages which ranges from 7.29% to 16.56%.

The hypertetrads result in the formation of minute or smaller than the normal pollen grains or micronuclei, which are with less than complete complement of chromosomes and are usually sterile. These micronuclei are presumably formed as a result of certain chromosomal aberrations during meiosis. A probable cause of micronuclei formation is lagging chromosome, either an entire lagging chromosome or a fragment of chromosome which may result in the loss of genetic material (Hanafy *et al.,* 2006). An increase in the number of micronuclei was observed both in plants and humans when exposed to EMF (Simkó *et al.,* 1998; Barnes & Greenebaum, 2007).

From the present study it is evident that high tension wires create EMFs of different intensities, the intensity of these fields' increases with the increase in voltage (Fig. 2), at control the intensity was less than 1 milli Gauss (mG), the mean of this field observed at 132 kV was 6.03 mG, at 220 kV line 24.42 mG and at 500 kV line it was recorded 33.03. Concomitant with the increase in magnetic field intensity an increase in meiotic abnormalities and pollen sterility was also noticed. In control the mean meiotic abnormalities and pollen sterility were 17.58% and 11.51% respectively. At 132 kV the mean meiotic abnormality and mean pollen sterility were 34.8% and 16.52%, at 220 kV 40.7% and 24.43% whereas at 500 kV they were 40.5% and 8.05% respectively. From the results it is evident that with the increase in magnetic field strength the percentages of meiotic abnormalities and pollen sterility also increased but this was not the case at 500 kV. However, this can probably be attributed to small sample size.

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. Boraginaceae								
1	*Heliotropium crispum* Desf.	132 kV, SZ 243	5.1	56.34	38.21	22.5	31.58	…..	35.8
		132 kV, SZ 256	5.1	…..	47.06	0	…..	0	31.07
		132 kV, SZ 318	10.5	0	61.88	…..	23.53	**…..**	50.96
		220 kV, SZ 553	29.7	…..	87.63	54.81	44.44	…..	66.67
		Control, SZ 497	<1	0	36.11	28.89	25	…..	25.2
		Control, SZ 882	<1	0	20.75	21.43	18.18	1.72	14.23
2.	*H. ophioglossum* Stocks ex Boiss.	220 kV, SZ 843	17.3	80	62.38	45.16	24.66	43.33	50.91
		Control, SZ 856	<1	…..	27.59	35	20.69	21.28	25.68
3.	*H. rariflorum* Stocks	132 kV, SZ 747	5.6	69.57	86.27	33.93	…..	46.45	59.44
		132 kV, SZ 748	5.6	…..	72.36	37.21	57.45	43.33	57.14
		220 kV, SZ 168	25	…..	16.67	25	9.09	28.21	22.39
		Control, SZ 791	<1	…..	52.87	27.87	19.35	19.05	36.5
4.	*H. strigosum* Willd.	132 kV, SZ 216	5.1	…..	42.31	21.43	…..	25	25.74
		500 kV, SZ 824	17.9	…..	51.85	54.55	18.92	…..	40.21
		Control, SZ 883	<1	0	17.65	0	0	0	5
5.	*H. zeylanicum* (Burm.f.) Lam.	132 kV, SZ 284	5.1	…..	73.33	50	14.29	22.22	44.53
		220 kV, SZ 433	39.2	…..	33.33	21.88	18.75	8	21.31
		Control, SZ 69	<1	…..	27.95	23.07	27.5	…..	27.32
		Control, SZ 72	<1	14.86	29.33	24.13	16.66	**……**	22.16
6.	*Sericostoma pauciflorum* Stocks	132 kV, SZ 274	5.1	…..	28.57	…..	15.38	…..	23.53
		500 kV, SZ 814	36.8	0	38.89	38.64	16.28	26.19	27.03
		Control, SZ 74	<1	…..	10.71	6.12	…..	…..	6.09
	II. Brassicaceae								
7.	*Farsetia jacquemontii* H. & T.	220 kV, SZ 778	21.3	46.43	57.58	46.62	30.77	32.5	45.79
		500 kV, SZ 818	32.7	43.55	…..	…..	…..	…..	43.55
		Control, SZ 854	<1	0	21.43	18.97	20.09	7.69	14.75
	III. Caesalpiniaceae								
8.	*Senna holosericea* (Fres.) Greuter	132 kV, SZ 217	5.1	0	33.73	19.51	11.69	0	23.78
		132 kV, SZ 244	5.1	32.35	33.96	17.95	10	8.57	21.99
		132 kV, SZ 293	10.8	…..	13.64	50	10.34	12.5	25.64
		Control, SZ 103	<1	2.63	13.72	…..	……	……	9.25
9.	*S. italica* Mill.	132 kV, SZ 225	5.1	…..	21.79	15.79	16.66	9.09	17.9
		132 kV, SZ 245	5.1	52.24	39.12	31.67	24.24	22.86	34.82
		220 kV, SZ 576	14	…..	50	41.39	…..	20.59	37.11
		500 kV, SZ 525	44.7	0	50.54	73.54	…..	…..	51.19
		Control, SZ 292	<1	…..	6.41	10.2	9.09	0	7.19

kV= Kilo Volt, D = Diakinesis, M. I = metaphase I, M. II = Metaphase II, A. I. = Anaphase I, A. II. = Anaphase II, Ab. = Abnormality, F.I. = Field Intensity, mG = Milli Gauss

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

S. #	Family and plant name	Voltage & Voucher No.	F. I. (mG)	Diads %	HT %	DP %	SP %	P. Ster. %
	I. Boraginaceae							
1.	*Heliotropium crispum* Desf.	132 kV, SZ 243	5.1	0	0	0	0	28.6
		132 kV, SZ 256	5.1	…..	0	0	0	23.72
		132 kV, SZ 318	10.5	0	0	0	0	50.96
		220 kV, SZ 553	29.7	0	62.14	8.96	13.94	25.3
		Control, SZ 497	<1	0	0	0	0	18.03
		Control, SZ 882	<1	0	0	0	0	21.05
2.	*H. ophioglossum* Stocks ex Boiss.	220 kV, SZ 843	17.3	0	29.8	9.88	12.5	47
		Control, SZ 856	<1	0	22.8	9.09	10.6	42.86
3.	*H. rariflorum* Stocks	132 kV, SZ 747	5.6	0	37.16	9.17	25	33.5
		132 kV, SZ 748	5.6	0	37.57	9.36	18.71	27.66
		220 kV, SZ 168	25	30.91	32.14	16.56	20.5	19.86
		Control, SZ 791	<1	0	30.07	7.29	15.7	16.71
4.	*H. strigosum* Willd.	132 kV, SZ 216	5.1	0	0	0	0	2.72
		500 kV, SZ 824	17.9	0	0	0	0	10.51
		Control, SZ 883	<1	0	0	0	0	1.64
5.	*H. zeylanicum* (Burm.f.) Lam.	132 kV, SZ 284	5.1	2.48	0	0	0	10.31
		220 kV, SZ 433	39.2	0	0	0	0	13.04
		Control, SZ 69	<1	0	0	0	0	3.59
		Control, SZ 72	<1	0	0	0	0	2.87
6.	*Sericostoma pauciflorum* Stocks	132 kV, SZ 274	5.1	0	0	0	0	4.26
		500 kV, SZ 814	36.8	0	0	0	0	6.25
		Control, SZ 74	<1	0	0	0	0	0
	II. Brassicaceae							
7.	*Farsetia jacquemontii* H. & T.	220 kV, SZ 778	21.3	0	0	0	0	37.03
		500 kV, SZ 818	32.7	0	0	0	0	…..
		Control, SZ 854	<1	0	0	0	0	19.43
	III. Caesalpiniaceae							
8.	*Senna holosericea* (Fres.) Greuter	132 kV, SZ 217	5.1	0	0	0	0	2.6
		132 kV, SZ 244	5.1	0	0	0	0	10.01
		132 kV, SZ 293	10.8	0	0	0	0	11.32
		Control, SZ 103	<1	0	0	0	0	0.48
9.	*S. italica* Mill.	132 kV, SZ 225	5.1	0	0	0	0	2.53
		132 kV, SZ 245	5.1	0	0	0	0	6.54
		220 kV, SZ 576	14	0	0	0	0	4.35
		500 kV, SZ 525	44.7	0	0	0	0	7.52
		Control, SZ 292	<1	0	0	0	0	0

F.I. = Field intensity, mG (milli Gauss), HT = Hypertetrads, DP = Diploid pollens, SP = Small pollens, P. St.= Pollen sterility

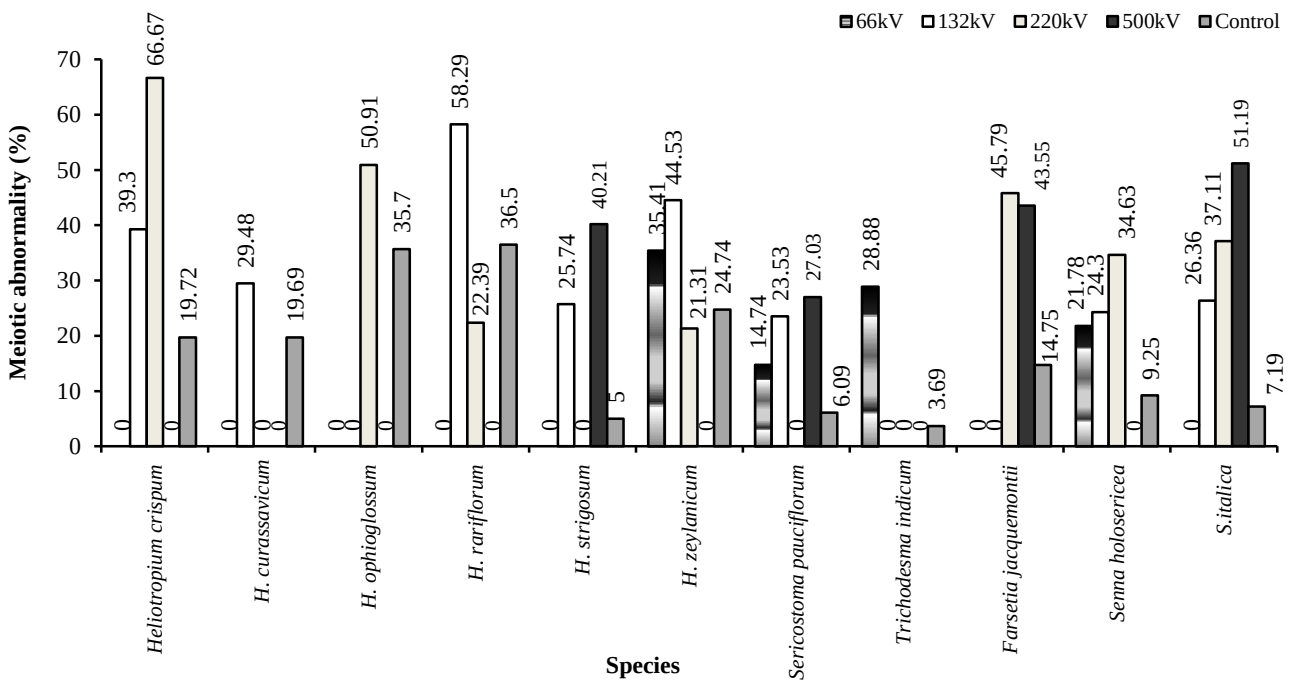

Fig. 1. Bar diagram showing percentages of meiotic abnormalities in test and control specimens.

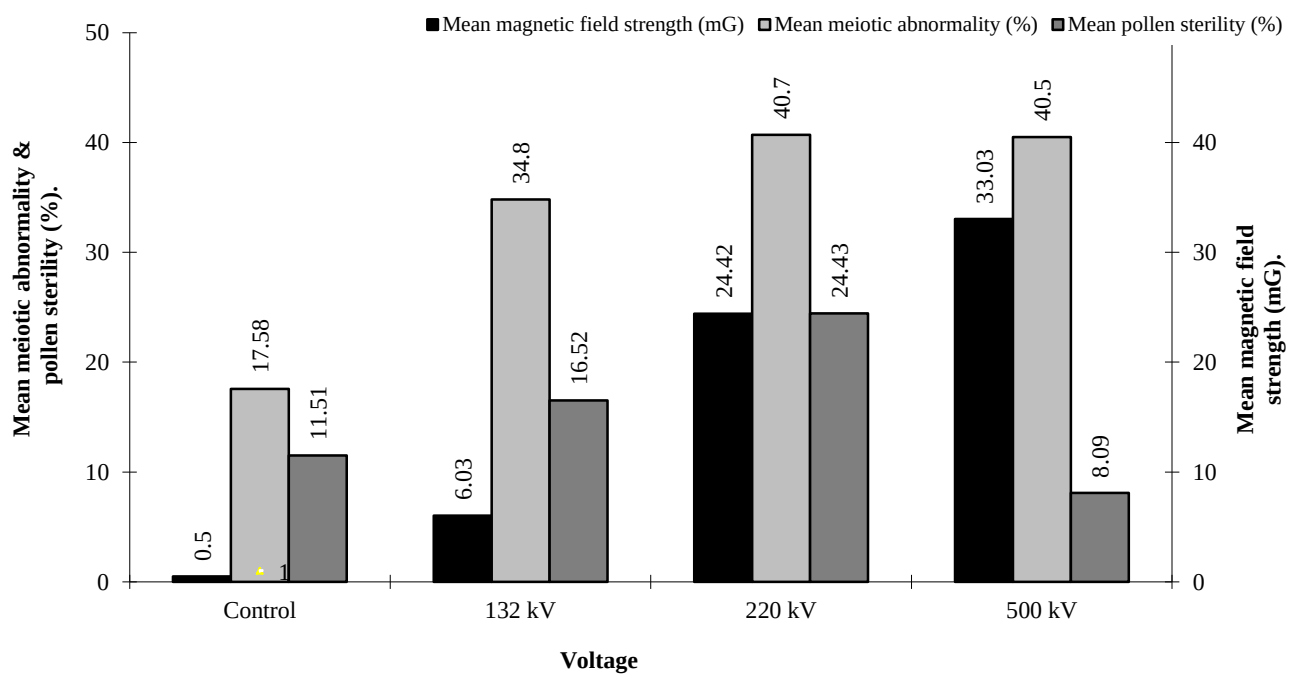

Fig. 2. Comparison of mean meiotic abnormalities, mean pollen sterility and mean magnetic field strength in different voltages.

The statistical analysis of meiotic abnormalities and pollen sterility were summarized in Tables 3 and 4 respectively. It is evident that only in one species at 220 kV no significant difference was found between control and test specimens (Table 3), whereas in 95.65% cases difference was significantly higher in test specimens as compared to control (Figs. 3 and 4). On the other hand 4 three specimens showed non-significant results i.e. only 13.63% specimens whereas in 86.36% cases the difference was highly significant ($p<0.001$) in test specimens as compared to controls (Table 4).

The above mentioned chromosomal aberrations during different meiotic stages ultimately result in the formation of abnormal meiotic products and in the end produce micronuclei (which are usually sterile), diploid pollens and non-reduced gametes which gave rise to polyploids. These chromosomal aberrations can pass to next generations and may result in abnormal individuals which may be sterile and eventually causing local extinction. Present study showed that the EMFs adversely affect the plants, they may also affect other living organisms inhabiting in the close vicinity of the high-tension lines including the human beings.

Table 3. Statistical comparison of meiotic abnormalities using Z-test.

S. #	Family and plant name	Voltage & voucher No.	F. I. (mG)	Z-test value	Z-test status	Level of significance
	I. Boraginaceae					
1.	*H. crispum* Desf.	132 kV, SZ 243	5.1	7.33	S	p<0.001***
		132 kV, SZ 256	5.1	3.6	S	p<0.001***
		132 kV, SZ 318	10.5	10	S	p<0.001***
		220 kV, SZ 553	29.7	17.67	S	p<0.001***
2.	*H. ophioglossum* Stocks ex Boiss.	220 kV, SZ 843	17.3	6.25	S	p<0.001***
3.	*H. rariflorum* Stocks	132 kV, SZ 747	5.6	4.6	S	p<0.001***
		132 kV, SZ 748	5.6	4.29	S	p<0.001***
		220kV, SZ 168	25	2.8	S	p<0.01**
4.	*H. strigosum* Willd.	132kV, SZ 216	5.1	4.2	S	p<0.001***
		500 kV, SZ 824	17.9	4.56	S	p<0.001***
5.	*H. zeylanicum* (Burm.f.) Lam.	132 kV, SZ 284	5.1	5.4	S	p<0.001***
		220 kV, SZ 433	39.2	0.75	N.S	p>0.05
6.	*Sericostoma pauciflorum* Stocks	132 kV, SZ 274	5.1	4	S	p<0.001***
		500 kV, SZ 814	36.8	7	S	p<0.001***
	II. Brassicaceae					
7.	*Farsetia jacquemontii* H. & T.	220 kV, SZ 778	21.3	7.75	S	p<0.001***
		500 kV, SZ 818	37.7	4.33	S	p<0.001***
	III. Caesalpiniaceae					
8.	*Senna holosericea* (Fres.) Greuter	132 kV, SZ 217	5.1	4.54	S	p<0.001***
		132 kV, SZ 293	10.8	3.69	S	p<0.001***
		132 kV, SZ 244	5.1	3.25	S	p<0.01**
9.	*S. italica* Mill.	132 kV, SZ 225	5.1	3.06	S	p<0.01**
		132 kV, SZ 245	5.1	8.75	S	p<0.001***
		220 kV, SZ 576	14	5.77	S	p<0.001***
		500 kV, SZ 525	44.7	10.23	S	p<0.001***

S = Significant, N.S = Non-significant, mG = MilliGauss, V = Volt, kV= Kilo volt

Table 4. Statistical comparison of pollen sterility using Z-test.

S. #	Family and plant name	Voltage & voucher No.	F. I. (mG)	Z-test value	Z-test status	Level of significance
	I. Boraginaceae					
1.	*H. crispum* Desf.	132 kV, SZ 243	5.1	4	S	p<0.001***
		132 kV, SZ 256	5.1	4.17	S	p<0.001***
		132 kV, SZ 318	10.5	15.45	S	p<0.001***
		220 kV, SZ 553	29.7	7.64	S	p<0.001***
2.	*H. ophioglossum* Stocks ex Boiss.	220 kV, SZ 843	17.3	1.08	N.S	p>0.05
3.	*H. rariflorum* Stocks	132 kV, SZ 747	5.6	5.33	S	p<0.001***
		132 kV, SZ 748	5.6	4.23	S	p<0.001***
		220kV, SZ 168	25	0.79	N.S	p>0.05
4.	*H. strigosum* Willd.	132kV, SZ 216	5.1	0.67	N.S	p>0.05
		500 kV, SZ 824	17.9	4.5	S	p<0.001***
5.	*H. zeylanicum* (Burm.f.) Lam.	132 kV, SZ 284	5.1	7	S	p<0.001***
		220 kV, SZ 433	39.2	5	S	p<0.001***
6.	*Sericostoma pauciflorum* Stocks	132 kV, SZ 274	5.1	5	S	p<0.001***
		500 kV, SZ 814	36.8	6	S	p<0.001***
	II. Brassicaceae					
7.	*Farsetiajacquemontii* H. & T.	220 kV, SZ 778	21.3	5.67	S	p<0.001***
	III. Caesalpiniaceae					
8.	*Senna holosericea* (Fres.) Greuter	132 kV, SZ 217	5.1	2.9	S	P<0.01**
		132 kV, SZ 293	10.8	8.33	S	p<0.001***
		132 kV, SZ 244	5.1	6.25	S	p<0.001***
9.	*S. italica* Mill.	132 kV, SZ 225	5.1	5.66	S	p<0.001***
		132 kV, SZ 245	5.1	7	S	p<0.001***
		220 kV, SZ 576	14	4.88	S	p<0.001***
		500 kV, SZ 525	44.7	8.99	S	p<0.001***

S = Significant, N.S = Non-significant, mG = MilliGauss, V = Volt, kV= Kilo volt

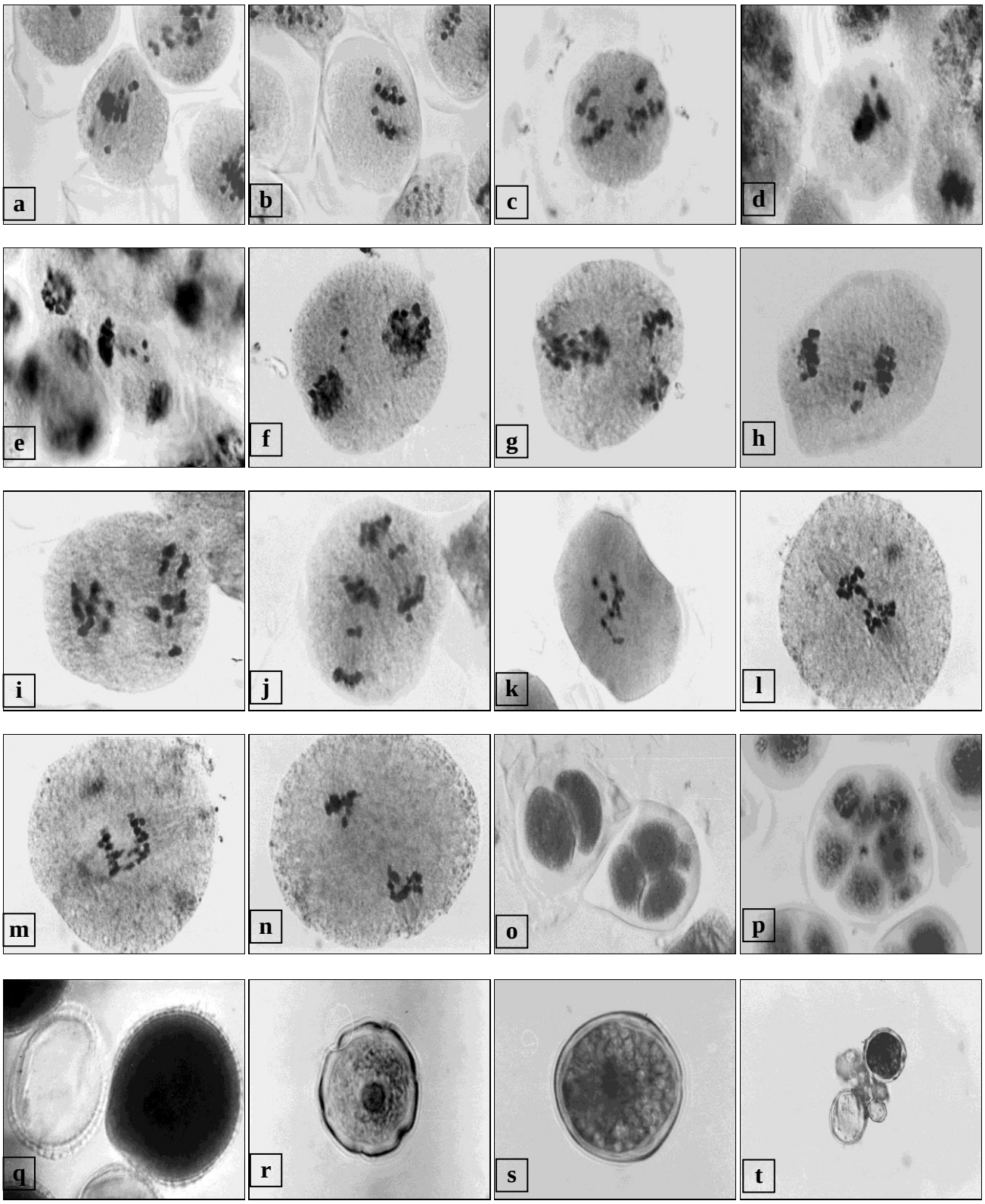

Fig. 5. **a-c:** *Heliotropium crispum* X1000; **a.** Metaphase I with precocious chromosomes, **b.** anaphase I with lagging chromosomes, **c.** Metaphase II with precocious chromosomes and split spindle. **d, e:** *H. ophioglossum* X1000; **d.** Metaphase I with split spindle, stickiness and precocious chromosome, **e.** Metaphase II with stickiness and precocious chromosomes. **f, g:** *H. rariflorum* X1000; **f.** Metaphase II with precocious chromosomes, **g.** Anaphase II with lagging chromosomes. **h-j:** *H. zeylanicum* X1000: **h.** Anaphase I with lagging chromosomes, **i.** Metaphase II with precocious chromosomes, **j.** Anaphase II with lagging chromosomes. **k-m:** *Senna holosericea* X1000: **k, l.** Anaphase I with precocious chromosomes, **m.** Metaphase II with precocious chromosomes. **n.** *S. italica* X1000, Disturbed metaphase I. **o:** *H. crispum* X400: Two cells showing meiotic products, 1 normal tetrad and other with diad. **p**. *H. ophioglossum*, X1000, Hypertetrad. **q.** *H. crispum* X1000, Diplod fertile and haploid sterile pollen grain. **r, s:** *H. ophioglossum* X1000, **r.** Haploid sterile pollen grain, **s.** Haploid fertile pollen grain. **t.** H. *rariflorum* X400, Haploid fertile and sterile pollen grains with small sterile pollen grains.

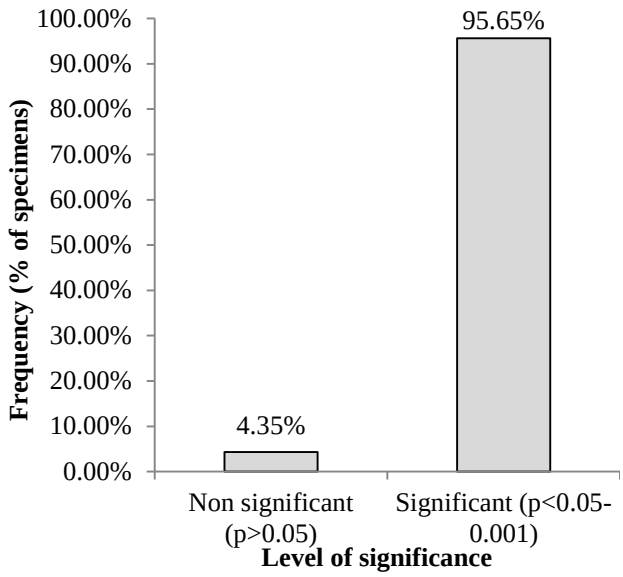

Fig. 3. Comparison of significant and non-significant differences in the meiotic abnormalities of test and control specimen.

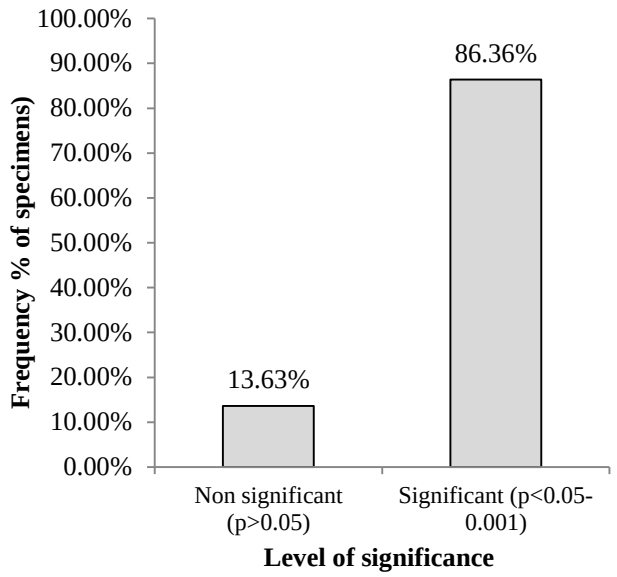

Fig. 4. Comparison of significant and non significant differences in the pollen sterility of test and control specimens.

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(Received for publication 18 February 2017)