INT. J. BIOL. BIOTECH., 10 (4): 483-490, 2013.

EFFECTS OF ELECTROMAGNETIC FIELDS (CREATED BY HIGH TENSION LINES) ON SOME INDIGENOUS SPECIES -VII. MIMOSACEAE, MOLLUGINACEAE, NYCTAGINACEAE AND PAPILIONACEAE

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ABSTRACT

Effects of electromagnetic fields (EMFs) (created by high tension wires) were studied in 33 specimens belonging to 12 species of 4 angiosperm families. In the study genotoxic effects of EMFs were studied on these plants. The aspects covered in the present study include PMC meiosis, meiotic products and pollen viability. The plants were collected from localities having 132, 220 and 500 kilo volt high tension wires and controls were collected from localities free from any type of electric wires. A number of meiotic abnormalities including stickiness, pairing disturbances (univalents and multivalents), precocious chromosomes, laggards, bridges and multipolar divisions were observed both in test and controls; but these abnormalities were found to be significantly higher in test (exposed to EMFs) plants as compared to their controls (unexposed to EMFs).The test plants also showed abnormal meiotic products (dyads and hypertetrads) in some specimens. Besides this the percentages of sterile pollen grains were also significantly higher in test plants. These abnormalities and pollen sterility showed a direct correlation with the increase in voltage i.e. as the voltages increases these abnormalities and pollen sterility also increases.

Key-words: High Tension Lines, indigenous plants, Mimosaceae, Molluginaceae, Nyctaginaceae, Papilionaceae.

INTRODUCTION

The first well known study on the effects of electromagnetic fields (EMFs) was performed by Wertheimer and Leeper (1979), who concluded that these fields cause leukemia in children. After this thousands of studies have been performed on humans, animals, microorganisms and plants to find out the possible harmful effects of these fields.

On plants most of the studies focus upon the growth of plants and the germination of their seeds. According to some studies these fields exerts beneficial effects as it increases rate of growth and early germination of seeds. (Kato, 1988; Magone, 1996; Zhang and Hashinaga, 1997; Carbonell *et al*., 2000; Reina *et al*., 2001; Florez *et al*., 2004; Dardeniz *et al*., 2006; Dao-Liang *et al*., 2009; Cakmak *et al*., 2009). On the other hand some studies suggests harmful effects like inhibition of growth and seed germination (Widacka and Jerzy, 1982; Selga and Selga, 1996; Moon and Chung, 2000; Penuelas *et al*., 2004; Apasheva *et al*., 2006; Ahmad *et al*., 2007)

It is evident from a number of studies that EMFs are genotoxic in nature and causes a number of cellular abnormalities in case of plants. These include stickiness, clumping, ring formation, pairing disturbances, precocious chromosomes, laggards, bridges, multipolar divisions, micronuclei, dyads etc. during mitosis and meiosis (Linskens and Smeets, 1978; Saxena and Gupta, 1987; Runthala and Bhattacharya, 1991; Pavel and Creanga, 2005; Hanafy *et al*., 2006; Zhang *et al*., 2007; Mihaela, 2009; Aksoy *et al*., 2007).

All of the above mentioned studies on plants deal with the study of only one or two species and the experiments were designed in laboratory conditions. With the exception of Zaidi and Khatoon (2003, 2012), Zaidi *et al.* (2012; 2013) and Ahmad *et al* (2007) not a single work is available which deals with the study of the EMFs genotoxic effects on plants in their natural ecosystem.

MATERIALS AND METHODS

The plant material for the study of genotoxic effects electromagnetic fields (created by high tension wires) was collected from different localities in and around Karachi. Theses localities were in the vicinity of high tension wires of different voltages i.e. 132, 220 and 500 kilo Volts (kV). Collection of the same plant material as control was also made from different localities free from any high tension wire or where the intensity was less than 1 mG (milli Gauss). For the measurement of magnetic field intensity Lutron EMF-822A tester was used and the intensity was measured in milli Gauss (unit of magnetic field). The voucher specimens were collected in each case and deposited in Karachi University Herbarium, Department of Botany, University of Karachi, Karachi, Pakistan.

For the study of PMC meiosis, meiotic product and pollen sterility some young buds, some large buds and some fully mature buds respectively were preserved in glass vials containing freshly prepared Carnoy's solution (3:1; Ethanol: glacial acetic acid) on the spot.

To study the meiotic behavior temporary slides of young buds were prepared by squash technique in 1% propionic carmine stain. 50 to 200 pollen mother cells were studied for each available stage and photographs of some of the abnormal cells were taken by Nikon Photomicroscope.

For the study of meiotic product similar procedure was adapted on some larger buds; 100 or more PMCs were observed in each case. The abnormal products showing diads and hypertetrads (more than 4 products from one PMC) were photographed by Nikon Photomicroscope.

Similarly following the same procedure slides for pollen viability were prepared by using mature buds. The temporary slides were left for 15-30 minutes, to take the time for staining and then observed under microscope. The dark stained pollens were counted as fertile whereas light stained or unstained as sterile. 200 to numerous pollens were observed in each case and observations were made as fertile, sterile, diploid, haploid or very small pollen grains. These pollen grains were also photographed by Nikon Photomicroscope. The results were statistically analyzed by Z-test according to Zar (1996).

RESULTS AND DISCUSSION

The observation of PMCs revealed a number of meiotic abnormalities including stickiness and pairing disturbances (formation of univalents and multivalents) during diakinesis, stickiness and precocious chromosomes during metaphase I and II and stickiness, bridges, laggards and multipolar divisions during anaphase I and II (Figs. 4). Stickiness found to be the most common abnormality appears in each stage. The results of PMC meiosis are summarized in Table 1.

Highest abnormal cells (100%) at diakinesis stage were observed in *Tephrosia apollinea*, followed by *Indigofera hochstetterii* (40%). At metaphase I stage highest percentage of abnormal cells (75.5%) was observed in *Tephrosia uniflora*, followed by *Crotalaria medicaginea* (74%) and so on. The highest abnormal percentage of abnormal cells (67%) at anaphase I was observed in *Indigofera argentea*, at metaphase II (57%) in *Commicarpus boissieri* and at anaphase II (36%) in *Glinus lotoides*. It is evident from the results of meiotic abnormalities that with the preceding stages the percentage of abnormal cells increases. As an overall result highest abnormal cells (50%) were recorded in *T. uniflora*, followed by *Prosopis juliflora* (around 50%).

Figure I showed marked difference in the percentages of meiotic abnormalities between control and test plants. It is clear that upon exposure to EMFs the percentage of meiotic abnormalities showed a marked increase as compared to unexposed plants.

The results of meiotic products and pollen sterility are given in Table 2. Abnormal products i.e. dyads and hypertetrads (more than 4 products) (Figs. 4) were observed in three specimens; one belongs to *I. argentea* and two to *I. oblongifolia*. Besides this in case of *I. oblongifolia* diploid pollen grains were also observed and in one specimen micronuclei are also developed. Highest numbers of sterile pollen grains (41%) were also observed in *I. oblongifolia* (Figs. 4).

The results of meiotic abnormalities and pollen sterility are statistically analyzed by Z-test (Zar, 1996) and the results are given in Table 3 and 4 respectively. According to table 3 majority of test specimens showed statistically significant difference when compared to their respective control and there are 65% specimens in which the results are significantly different (Fig. 3). Similarly 80% of test plants showed significant difference in their pollen sterility (Fig. 4).

From the results we conclude that EMFs (created by high tension wires) exhibit meiotic abnormalities in exposed plants. These meiotic abnormalities in some cases also produce abnormal products like dyads and hypertetrads. These dyads in the next step develop in to diploid pollen grains and these diploid pollen grains results in the production of polyploidy. Similarly the hypertetrads produces some micronuclei besides normal products and these micronuclei are usually sterile. If these EMFs effects on plants they can also effect in the same way on other organisms (animals, humans) if they continuously exposed to EMFs. So the human beings are also at risk and any future study needs to focus upon it.

Table 1. Details of meiotic abnormalities showing percentages of different stages of PMC meiosis in test and control plants.

S.#	Family and Plant name	Voltage, Voucher #	F.I (mG)	D %	M.I %	M.II %	A.I %	A.II %	Overall Ab. %
I	**Mimosaceae**								
1	*Prosopis juliflora* (Swartz)	132 kV, SZ 299	10.5	……	29.56	21.81	22.23	26.33	25.86
	DC.	500 kV, SZ 619	39.9	…..	65.28	26.67	37.14	….	49.64
		Control, SR 193	< 1	…..	22.22	11.76	15	16.67	16.66
II	**Molluginaceae**								
2	*Glinus lotoides* L.	220 kV, SZ 446	39.9	7.69	29.41	25	….	35.89	26.04
		Control, SZ 400	< 1	0	21.58	18.52	34.18	31.82	2.32
III	**Nyctaginaceae**								
3	*Commicarpus boissieri*	132 kV, SZ 242	5.1	23.53	38	56.67	10.71	12.5	30.12
	(Heimerl) Cufod.	220 kV, SZ 779	14.2	0	22.58	8.82	20	…..	12.07
		Control, SZ 601	< 1	…..	5.66	7.14	0	…..	4.55
IV	**Papilionaceae**								
4	*Crotalaria burhia* Buch.-	132 kV, SZ 275	5.1	…..	37.86	37.5	24.14	…..	35.82
	Ham.ex Benth.	132 kV, SZ 323	8	0	39.85	54.03	11.09	16.66	37.16
		220 kV, SZ 370	21.7	…..	42.1	49.1	…..	18.57	40.36
		220 kV, SZ 448	39.6	…..	31.58	25.62	28.21	20.75	25.9
		Control, SZ 501	< 1	…..	7.79	12.96	8.33	0	8.02
5	*C. medicaginea* Lamk.	132 kV, SZ 186	4.6	0	16	40	…..	0	18.39
		500 kV, SZ 823	26.3	…..	74.07	38.24	41.38	21.43	43.22
		Control, SZ 887	< 1	0	26.42	8.7	18.18	10	15.34
		Control, SZ 888	< 1	0	26.92	17.65	10	0	15.96
6	*Indigofera argentea* Burm.f.	132 kV, SZ 363	12.8	14.29	29.89	0	66.67	…..	26.47
		Control, SZ 922	< 1	0	21.43	22.22	6.9	0	11.8
7	*I. hochstetteri* Baker	132 kV, SZ 540	25.1	40	69.57	46	0	…..	43.52
		Control, SZ 689	< 1	…..	10.81	3.57	0	…..	5.75
8	*I. oblongifolia* Forssk.	132 kV, SZ 301	10.5	……	30.12	12.5	10	0	20.27
		500 kV, SZ 527	44.7	…..	42.86	28.95	36.11	…..	36.55
		Control, MI 693	< 1	0	29.41	12.96	6.9	4.35	11.68
9	*Melilotus alba* Desr.	500 kV, SZ 624	41.2	0	54.17	40.82	41.38	…..	40.85
		Control, MI 862	< 1	0	21.43	19.05	21.43	8.33	15.25
10	*Tephrosia apollinea* (Delile)	132 kV, SZ 738	5.1	…..	…..	30.77	…..	0	25
	Link	132 kV, SZ 739	5.1	100	30.77	…..	16.67	…..	41.07
		Control, SZ 890	< 1	…..	21.95	12.5	0	0	12.96
11	*T. subtriflora* Baker	220 kV, SZ 569	29.7	14	59.09	55.1	39.29	…..	41.52
		Control, SZ 874	< 1	…..	15.91	15	7.14	0	8.89
12	*T. uniflora* Pers.	132 kV, SZ 194	4.8	0	75.51	48.94	…..	18.52	50.39
		Control, SZ 107	< 1	……	……	15.79	……	……	8.82

Note: F.I. = Field Intensity, mG= Milli Gauss, D = Diakinesis, M I= Metaphase I, M II= Metaphase II, A I= Anaphase I, A II= Anaphase II, Ab. = Abnormality.
SZ, SR, MI= Initials of the names of collectors of plants, Sahar Zaidi, Sadaf Rahimi, Mohammed Imran

Table 2. Details of meiotic products and pollen fertility in test and control plants.

S.#	Family and Plant name	Voltage & Voucher #	Diads %	H. Tet. (%)	Diploid P.G (%)	Small P.G (%)	Pollen Sterility (%)
I	**Mimosaceae**						
1	*Prosopis juliflora* (Swartz)	132 kV, SZ 299	0	0	0	0	2.6
	DC.	500 kV, SZ 619	0	0	0	0	11.76
		Control, SR 193	0	0	0	0	0.9
II	**Molluginaceae**						
2	*Glinus lotoides* L.	220 kV, SZ 446	0	0	0	0	8.88
		Control, SZ 400	0	0	0	0	5.28
III	**Nyctaginaceae**						
3	*Commicarpus boissieri*	132 kV, SZ 242	0	0	0	0	9.89
	(Heimerl) Cufod.	220 kV, SZ 779	0	0	0	0	12.5
		Control, SZ 601	0	0	0	0	1.48
VI	**Papilionaceae**						
4	*Crotalaria burhia* Buch.-	132 kV, SZ 275	0	0	0	0	3.21
	Ham.ex Benth.	132 kV, SZ 323	0	0	0	0	10.14
		220 kV, SZ 370	0	0	0	0	4.03
		220 kV, SZ 448	0	0	0	0	3.94
		Control, SZ 501	0	0	0	0	0.71
5	*C. medicaginea* Lamk.	132 kV, SZ 186	0	0	0	0	8.73
		500 kV, SZ 823	0	0	0	0	17.85
		Control, SZ 887	0	0	0	0	2.26
		Control, SZ 888	0	0	0	0	2.34
6	*Indigofera argentea* Burm.f.	132 kV, SZ 363	0	3.7	0	0	19.61
		Control, SZ 922	0	0	0	0	2.86
7	*I. hochstetteri* Baker	132 kV, SZ 540	0	0	0	0	6.98
		Control, SZ 689	0	0	0	0	1.64
8	*I. oblongifolia* Forssk.	132 kV, SZ 301	4	1.77	16.32	10.2	34.34
		500 kV, SZ 527	2.96	0	3.29	0	40.92
		Control, MI 693	0	0	0	0	6.25
9	*Melilotus alba* Desr.	500 kV, SZ 624	0	0	0	0	10.08
		Control, MI 862	0	0	0	0	1.32
10	*Tephrosia apollinea* (Delile)	132 kV, SZ 738	0	0	0	0	3.23
	Link	132 kV, SZ 739	0	0	0	0	3.85
		Control, SZ 890	0	0	0	0	1.95
11	*T. subtriflora* Baker	220 kV, SZ 569	0	0	0	0	2.91
		Control, SZ 874	0	0	0	0	0.33
12	*T. uniflora* Pers.	132 kV, SZ 194	0	0	0	0	7.69
		Control, SZ 107	0	0	0	0	1.99

Note: H. Tet. = Hypertetrad, PG.= Pollen Grain

Table 3. Statistical analysis of meiotic abnormalities (performed by Z-test).

S.#	Family and Plant name	Voltage & Voucher #	F.I (mG)	Z-Test Value	Z-Test status	Level of Significance
I	**Mimosaceae**					
1	*Prosopis juliflora* (Swartz)	132 kV, SZ 299	10.5	2.9	N.S	p > 0.05
		500 kV, SZ 619	39.9	6.6	S	p < 0.001***
II	**Molluginaceae**					
2	*Glinus lotoides* L.	220 kV, SZ 446	39.8	0.29	N.S	p > 0.05
III	**Nyctaginaceae**					
3	*Commicarpus boisseiri* (Heimerl)	132 kV, SZ 242	5.1	8	S	p < 0.001***
	Cufod	220 kV, SZ 779	14.2	1.25	N.S	p > 0.05
VI	**Papilionaceae**					
4	*Crotalaria burhia* Ham.ex Bentham	132 kV, SZ 275	5.1	5.76	S	p < 0.001***
		132 kV, SZ 323	8	4.14	S	p < 0.01**
		220 kV, SZ 370	21.7	8	S	p < 0.001***
		220 kV, SZ 448	39.6	5.29	S	p < 0.001***
5	*C. medicaginea* Lam.	132 kV, SZ 186	4.6	0.4	N.S	p > 0.05
		500 kV, SZ 823	26.3	5.4	S	p < 0.001***
6	*Indigofera argentea* Burm.f.	132 kV, SZ 363	12.8	3.33	N.S	p > 0.05
7	*I. hochstetteri* Baker	132 kV, SZ 540	25.1	7.03	S	p < 0.001***
8	*I. oblongifolia* Forsk.	132 kV, SZ 301	10.5	2.05	N.S	p > 0.05
		500 kV, SZ 527	44.7	5.21	S	p < 0.001***
9	*Melilotus alba* Desr.	500 kV, SZ 624	41.2	5.2	S	p < 0.001***
10	*Tephrosia appollinea* (Delile) Link	132 kV, SZ 738	5.1	2.03	N.S	p > 0.05
		132 kV, SZ 739	5.1	7.28	S	p < 0.001***
11	*T. subtriflora* Hochst.ex Baker	220 kV, SZ 569	29.7	8.25	S	p < 0.001***
12	*T. uniflora* Pers.	132 kV, SZ194	4.8	7	S	p < 0.001***

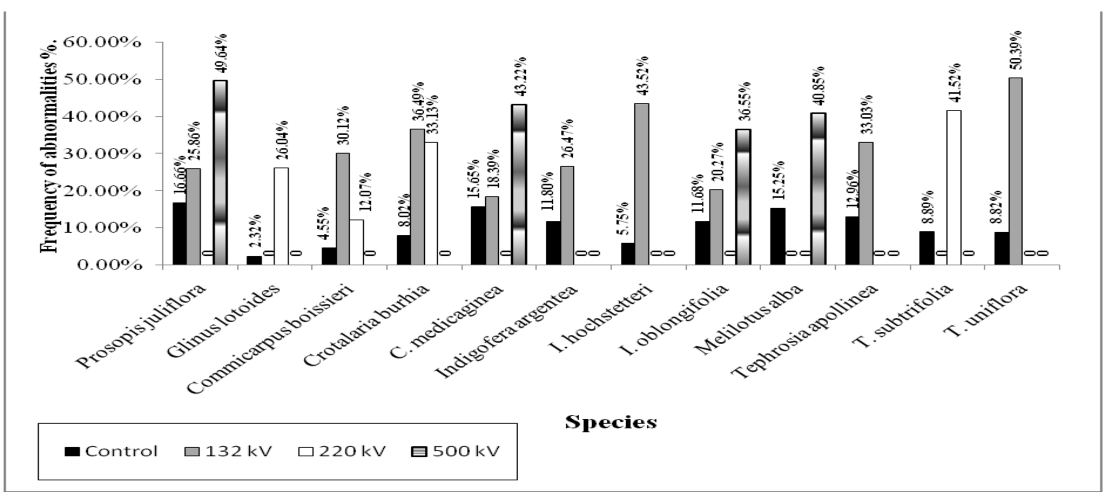


Fig.1. Species wise comparison of meiotic abnormalities in test and control specimens (members of families: Mimosaceae, Molluginaceae, Nyctaginaceae, Papilionaceae).

Table 4. Statistical analysis of pollen sterility (performed by Z-test).

S.#	Family and Plant name	Voltage & Voucher #	F.I (mG)	Z-Test Value	Z-Test status	Level of Significance
I	**Mimosaceae**					
1	*Prosopis juliflora* (Swartz)	132 kV, SZ 299	10.5	2	N.S	p > 0.05
		500 kV, SZ 619	39.9	5.5	S	p < 0.001***
II	**Molluginaceae**					
2	*Glinus lotoides* L.	220 kV, SZ 446	39.8	4	S	p < 0.01**
III	**Nyctaginaceae**					
3	*Commicarpus boisseiri* (Heimerl) Cufod	132 kV, SZ 242	5.1	5.71	S	p < 0.001***
		220 kV, SZ 779	14.2	5	S	p < 0.001***
IV	**Papilionaceae**					
4	*Crotalaria burhia* Ham.ex Bentham	132 kV, SZ 275	5.1	5	S	p < 0.001***
		132 kV, SZ 323	8	9	S	p < 0.001***
		220 kV, SZ 370	21.7	4.41	S	p < 0.01**
		220 kV, SZ 448	39.6	4	S	p < 0.01**
5	*C. medicaginea* Lam.	132 kV, SZ 186	4.6	7	S	p < 0.001***
		500 kV, SZ 823	26.3	9.41	S	p < 0.001***
6	*Indigofera argentea* Burm.f.	132 kV, SZ 363	12.8	8.5	S	p < 0.001***
7	*I. hochstetteri* Baker	132 kV, SZ 540	25.1	3.85	S	p < 0.05*
8	*I. oblongifolia* Forsk.	132 kV, SZ 301	10.5	13.5	S	p < 0.001***
		500 kV, SZ 527	44.7	17	S	p < 0.001***
9	*Melilotus alba* Desr.	500 kV, SZ 624	41.2	7.14	S	p < 0.001***
10	*Tephrosia apollinea* (Delile) Link	132 kV, SZ 738	5.1	2.63	N.S	p > 0.05
		132 kV, SZ 739	5.1	2	N.S	p > 0.05
11	*T. subtriflora* Hochst.ex Baker	220 kV, SZ 569	29.7	2.86	N.S	p > 0.05
12	*T. uniflora* Pers.	132 kV, SZ 194	4.8	6	S	p < 0.001***

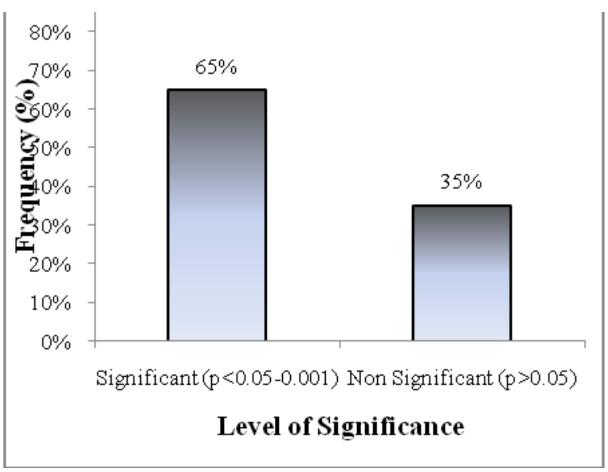


Fig.2. Comparison of significant and nonsignificant differences in the meiotic abnormalities of test and control specimens.

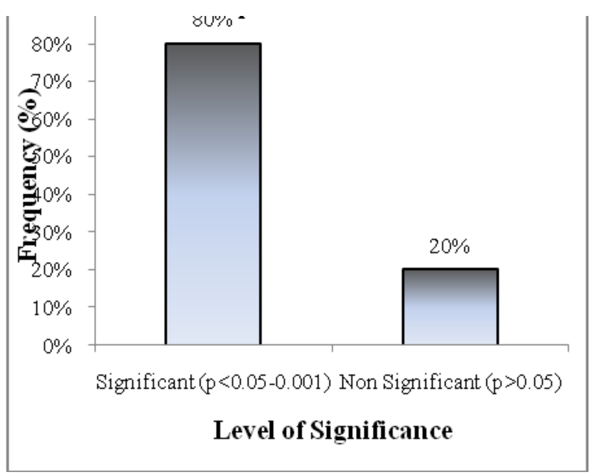
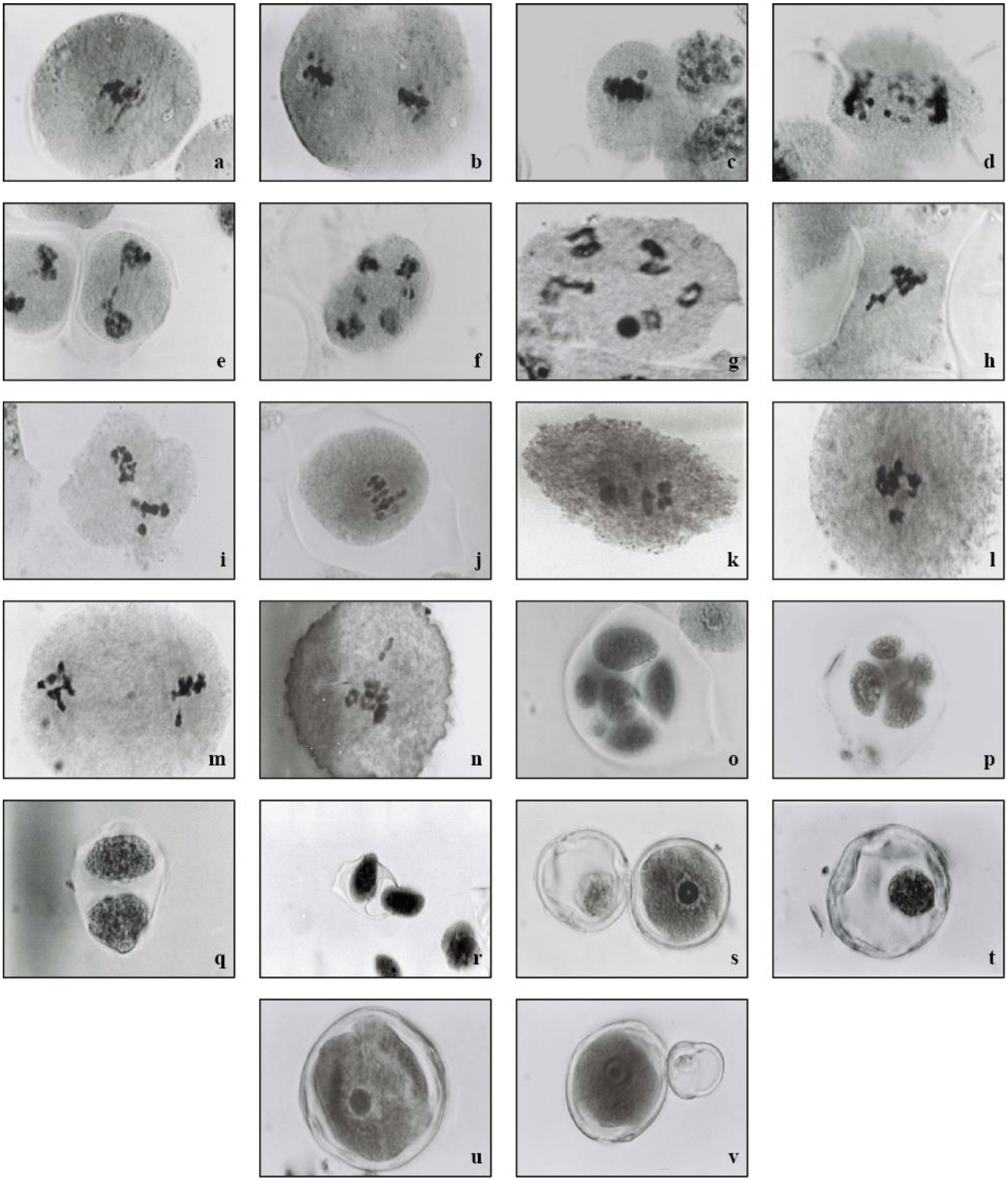


Fig.3. Comparison of significant and nonsignificant differences in the pollen sterility of test and control specimens.



Figs. 4. *Prosopis juliflora* X1000; **a.** Metaphase I with stickiness and precocious chromosome, **b.** Metaphase II with stickiness and some precocious chromosomes. **c-f.** *Glinus lotoides* X1000; **c.** Metaphase I with precocious chromosome, **d, e.** Anaphase I with stickiness and lagging chromosomes, **f.** Anaphase II with lagging chromosomes and stickiness. **g-i.** *Crotalaria burhia* X1000; **g.** Diakinesis with disturbed pairing, **h.** Metaphase I with stickiness and precocious chromosome. **i.** Metaphase II with precocious chromosome. **j.** *Indigofera argentea* X1000, Metaphase I with one precocious bivalent. **k.** *Indigofera hochstetteri* X1000, Metaphase I with precocious chromosome. **l, m.** *Tephrosia uniflora* X1000; **l.** Metaphase I with stickiness and precocious chromosome, **m.** Metaphase II with precocious chromosomes. **n.** *T. subtriflora* X1000, Metaphase I with precocious chromosome. **o.** *I. argentea* X1000, Hypertetrad. **p-q.** *I. oblongifolia* X1000; **p.** Hypertetrad, **q.** Dyad. **r.** *T. uniflora* X400, Dyad. **s-v.** *I. oblongifolia* X1000; **s.** Haploid fertile and sterile pollen grain, **t.** Diploid sterile pollen grain, **u.** Diploid fertile pollen grain **v.** Haploid fertile and minute sterile pollen grain.

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(*Accepted for publication September 2013*)