

## Electromagnetic Fields Effects on Physiological Indices, Cytogenetic and Pollen Structure of *Cucurbita Maxima* Duchesne

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**Abstract:** Electromagnetic fields is one of the effective factors on the living organisms and therefore in the present study the effects of different treatments of electromagnetic fields on the growth indices, chromosomal state and pollen structures of pumpkin were assayed. Seed samples of pumpkin were treated by 2 mT of electromagnetic fields in 15 and 30 mins, in two different groups named dry and wet. The results showed that the treatment affected some indices in the negative or somehow positive way. The root length and germination percentage however were not affected significantly. The hypocotyl length of 15 min treatment was decreased in comparison with control samples. The shoot length of wet treated samples was significantly reduced compare to controls. Caryologic studies showed that the most important effect of electromagnetic fields, belongs to chromosomal aberrations so that some mitotic phase such as metaphase and anaphase were delayed. SEM microscoping assay showed that more electromagnetic fields causes disorder of pollen surface.

**Key words:** Electromagnetic fields, physiological parameters, chromosomal status, pollen structures, *cucurbita maxima*

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### INTRODUCTION

*Cucurbita maxima* Duchesne, pumpkin is a prostrate, coarse, annual vine growing to a length up to 4m. Fruits of pumpkin are good sources of calcium, phosphorus and iron (Nabizadeh *et al.*, 2014). It also has been found that *Cucurbita maxima* has antidiabetic, hepatoprotective, anthelmintic, antihypertensive, anticancer and antihypercholesterolemic features. The remedial effect of pumpkin seed on prostatic hyperplasia has already shown in some studies (Gossell-Williams *et al.*, 2006; Hong *et al.*, 2009; Jiang *et al.*, 2011). *Cucurbita maxima* seed oil also reduces the size of prostate (Mitra *et al.*, 2009). Usage of different electronic equipments has brought about electromagnetic fields in environment (Shabrangi *et al.*, 2011) and therefore can cause damages. Electromagnetic fields alter the permeability of membrane and cell growth. It also interacts with ions and organic molecules. Electromagnetic fields includes electric and magnetic fields. Variable magnetic field induces electric field into living tissues, so that the effects of these two fields are related to each other. It seems that the magnetic fields are more effective than that of electric one, so that electric fields cross through the cell and the cells act as a

capacitor while the magnetic one influence into the cells and affect much longer (Falistocco *et al.*, 1995). The studies have suggested that the effects of low frequency electromagnetic fields are related to the theirs effects on the cell membrane permeability. The performance of ionic channels is based on the different concentrations of ions and the following voltage difference (Paul *et al.*, 2006). The ions activities in the cells are balanced, so it's interfering by electromagnetic fields can cause resonance which itself brings about cells disorders (Florez *et al.*, 2007). Biotic and abiotic stress alter the capability of pollen's germination and may cause less yield of plants. Some studies have reported different effects of electromagnetic fields on the growth, ontogenic features and other parameters such as germination (Florez *et al.*, 2007; Burtebayeva *et al.*, 2003; Martinez *et al.*, 2000). In the present study therefore, the electromagnetic fields effects on physiological indices, cytogenetic and pollen structure of *Cucurbita maxima* Duchesne were assessed.

### MATERIALS AND METHODS

**Electromagnetic field source:** The source of the magnetic field was means of a pair of Helmholtz coils system which



Fig. 1: Source of electromagnetic field

Table 1: Treatments configuration

Exposure time (min)	State seed	Electromagnetic treatment (mT)
15	Wet	2 mT
	Dry	
30	Wet	2 mT
	Dry	
0 (control)	Wet	0 (control)
	Dry	

creates a uniform magnetic field into rather large space volume. Each Helmholtz coil from exposure device has a diameter by 260 mm and 1000 numbers of turns (Fig. 1).

**Experiment implementation:** Seeds of *Cucurbita maxima* Duchesne were purveyed from Sina Bazr Alvand Co. There were two different treatments based on the length of exposing samples to electromagnetic field so that 15 and 30 min treatments by a magnitude of 2 mT for 5 days, in Petri dishes were accomplished. Moreover, there were two groups of seeds. The first one which called dry and the second one called wet seeds that were soaked for 24 h, before being treated. For both wet and dry treatments, separate groups of control samples were considered so that they were placed in the similar coil which was disconnected to the power (Table 1). For each treatment, there were at least 6 replications. The temperature was adjusted to  $(24 \pm 0.5^\circ\text{C})$ . Photoperiods was 14 light  $10^{-1}$  darkness, (10-13 MJ  $\text{m}^{-2}$  d $^{-1}$ ). After treatments, the seeds were allowed to germinate and then, transferred to jardinières.

**Physiological parameters:** Germination percentage of seeds was measured, 7 days after treatments using formula (germinated seeds/total seeds $\times$ 100) and then compared to the control samples.

Growth parameters such as root length, hypocotyls length and diameter (8 days-old samples), length and

diameter of shoots, numbers of stoma, leaves and nods (25 days-old samples), polar and equatorial diameter and weight of the fruits (75 days-old samples) were assayed using measurement software.

**Cytogenetic assessment:** In order to study the cells in the meiotic state, colchicines, 8-Hydroxycineole, paradichlorobenzene, alfa-bromonaphthalene or mixture of them were used (Sharma and Sharma, 1990). Colchicines treatment also was used to accounting the numbers of chromosomes. 1cm of apical of root was pre treated via colchicines and then, fixed in fixture solution (1:1 of formaldehyde 10% and chromic acid 1%) for 24 h in  $4^\circ\text{C}$ . Sodium hydroxide 1N was used to plasticize the samples for 10 min. Acetic ferric hematoxylin was used for dyeing samples (Arnon, 1949).

**Study of pollen structure using SEM:** Scanning electron microscope was used to study the pollen structures. Several pollens were considered for each treatment. The samples were first fixed in the Aluminum stock and then covered by golden layer. Scanning electron microscope (EM 3200) was used for scanning.

**Statistical analysis:** SPSS ver16. was used for statistical analysis. Means were compared using the duncan test at  $p < 0.05$ , level of significance to distinguish the differences between treatments and control samples. There were three replicates for all experiments data were expressed as the mean $\pm$ SE.

## RESULTS AND DISCUSSION

**Growth parameters:** As it is shown in Table 2, the highest percentage of germination is reported in 15 min-wet treatment and the lowest one goes to control sample. However, there is no any significant difference. There was also no significant difference in speed of seed germination. The numbers of stoma was increased in 15 min-wet treatment while it was decreased in control samples. The polar diameter of fruits also was increased in 15 min-dry treatment. In case of equatorial diameter of fruits, although 30 min-dry treatment showed increasing, no significant difference was seen. The fruit and seed weight were increased in 30 min-wet and control-wet samples, respectively. The least and most diameter of fruits were reported in control-dry and 30 min-wet samples, respectively. The 30 min-dry treatment decreased the length of root but it was not significant. The differences among treatment in case of hypocotyls length, also neither. On the other hand, diameter of hypocotyls

Table 2: The mean of growth parameter in electromagnetic field treatments,  $p \leq 0.005$

Parameter	Germination per.	Germination Sp. (h)	Polar fruit diameter (cm)	Equatorial fruit diameter (cm)	Fruit weight (g)	Weight of	No. of stoma	Biomass(g)
Control D	83.3±16 <sup>a</sup>	72±0.05 <sup>a</sup>	14.23±0.145 <sup>d</sup>	15±0.5 <sup>c</sup>	34.66±5.6 <sup>d</sup>	251.9±0.1 <sup>f</sup>	20.8±1.5 <sup>b</sup>	1.62±0.1 <sup>b</sup>
Control W	91.3±4.3 <sup>a</sup>	72±0.2 <sup>a</sup>	26.16±0.166 <sup>d</sup>	27.16±0.4 <sup>b</sup>	213.6±8 <sup>c</sup>	515.3±1 <sup>a</sup>	24.5±2.1 <sup>ab</sup>	2.59±0.2 <sup>a</sup>
15 min D	84±4.5 <sup>a</sup>	72±0.003 <sup>a</sup>	31.33±0.66 <sup>a</sup>	31.33±0.6 <sup>a</sup>	382.6±1 <sup>a</sup>	436.1±0.05 <sup>b</sup>	25.35±1.4 <sup>ab</sup>	2.64±0.3 <sup>a</sup>
30 min D	87.33±7.8 <sup>a</sup>	73±0.33 <sup>a</sup>	28.16±0.16 <sup>c</sup>	31.66±0.3 <sup>a</sup>	333±1.6 <sup>b</sup>	380.8±0.5 <sup>d</sup>	21.83±1.5 <sup>b</sup>	2.6±0.4 <sup>a</sup>
15 min W	95.6±4.3 <sup>a</sup>	73±0.03 <sup>a</sup>	29.26±0.39 <sup>b</sup>	30.3±0.3 <sup>a</sup>	320±1.2 <sup>b</sup>	360±0.3 <sup>a</sup>	30.8±2.6 <sup>a</sup>	2.02±0.2 <sup>ab</sup>
30 min W	87.3±7.2 <sup>a</sup>	73±0.03 <sup>a</sup>	30.66±0.1 <sup>a</sup>	31.1±0.1 <sup>a</sup>	387±3 <sup>a</sup>	398±0.05 <sup>c</sup>	23.01±3.5 <sup>b</sup>	2.04±0.1 <sup>ab</sup>

Table 3: Number of nodes did not shows significant changes

Parameter	Hypocotyls diameter (cm)	Hypocotyls length (cm)	Root length (cm)	Fruit diameter (cm)	No. of nodes	No. of leaves	Shoot diameter (cm)	Shoot length (cm)
Control D	0.47±0.2 <sup>b</sup>	4.8±0.6 <sup>a</sup>	11.16±0.6 <sup>a</sup>	0.63±0.1 <sup>b</sup>	7±0.57 <sup>abc</sup>	7±0.57 <sup>ab</sup>	5±1 <sup>a</sup>	8.5±0.5 <sup>a</sup>
Control W	0.53±0.03 <sup>ab</sup>	4.33±0.4 <sup>a</sup>	12±1 <sup>a</sup>	1.4±0.2 <sup>a</sup>	6.66±0.33 <sup>bc</sup>	7±0.5 <sup>b</sup>	3.36±0.3 <sup>ab</sup>	4.76±0.5 <sup>b</sup>
15 min D	0.5±0.1 <sup>ab</sup>	3.8±0.2 <sup>ab</sup>	10.26±0.9 <sup>ab</sup>	1.36±0.3 <sup>ab</sup>	8.33±0.33 <sup>a</sup>	8.33±0.3 <sup>a</sup>	4±0.5 <sup>a</sup>	6±1 <sup>b</sup>
30 min D	0.48±0.1 <sup>b</sup>	3.8±0.1 <sup>ab</sup>	10±1.5 <sup>a</sup>	1.83±0.16 <sup>a</sup>	6.33±0.3 <sup>c</sup>	7.33±0.6 <sup>ab</sup>	3±0.28 <sup>b</sup>	4.33±0.8 <sup>b</sup>
15 min W	0.62±0.1 <sup>a</sup>	2.6±0.4 <sup>c</sup>	10.6±0.1 <sup>a</sup>	1.6±0.2 <sup>a</sup>	6.66±0.3 <sup>bc</sup>	7.33±0.6 <sup>ab</sup>	2.66±0.1 <sup>b</sup>	3.83±0.33 <sup>b</sup>
30 min W	0.56±0.1 <sup>ab</sup>	3.9±0.2 <sup>a</sup>	11±1 <sup>a</sup>	1.86±0.06 <sup>a</sup>	8±0.5 <sup>ab</sup>	8±0.5 <sup>ab</sup>	3.1±0.15 <sup>b</sup>	5±0.7 <sup>b</sup>

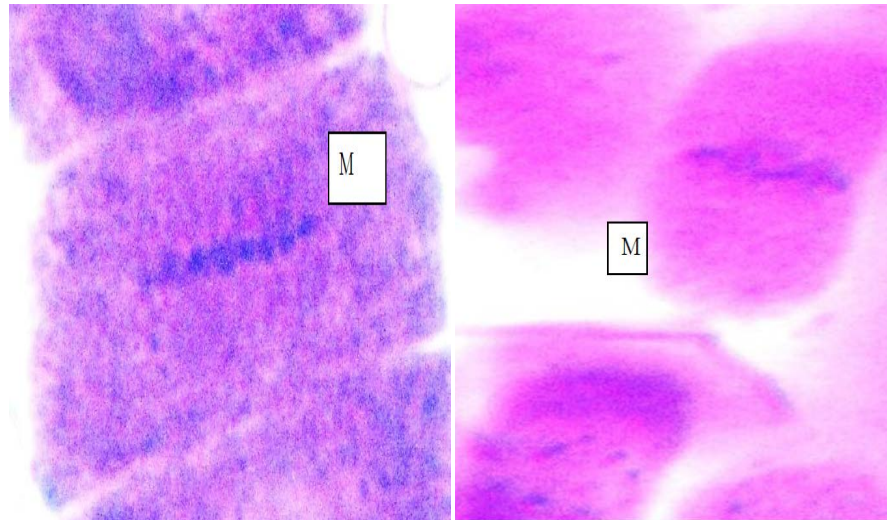


Fig. 2: Chromosomal disorder in metaphase (M) of 15 min-wet (right) and dry (left) treatment

was significantly increased by 15 min-wet treatment. The biomass of samples did not alter significantly. The length of shoot was decreased by 15 min-wet treatment. This treatment also decreased the diameter of shoot but it was not significant. About 15 min-dry treatment caused increasing in number of leaves. The numbers of nodes did not show significant changes (Table 3).

**Cytogenetic:** The results showed chromosomal disorder in metaphase of 15 min wet and dry treatments (Fig. 2). 30 min-wet samples also showed the same features (Fig. 3). In telophase of 15 min-wet treatment chromosomal disorder also reported (Fig. 4).

**Pollen structures:** By electromagnetic fields treatments and through the increasing of exposure to the fields, exine

structure of pollens undergo some changes and disorders especially in dry-treated samples. As it is shown in Fig. 5, along with increasing of time of exposure, more stigmas are visible on the surface of pollens. The same changes was somehow reported in wet-treated samples (Fig. 5).

Despite other studies which have reported increasing of seed germination by electromagnetic fields induction, here in the present study, there is no significant changes in percentage and speed of germination, probably due to the different plant an also dose of treatment. Florez *et al.* (2007), Shabrangi *et al.* (2011) and Alexander and Doijode (1995), all reported increasing of seed germination, induced by electromagnetic fields. These researchers also reported more speed of seed germination.

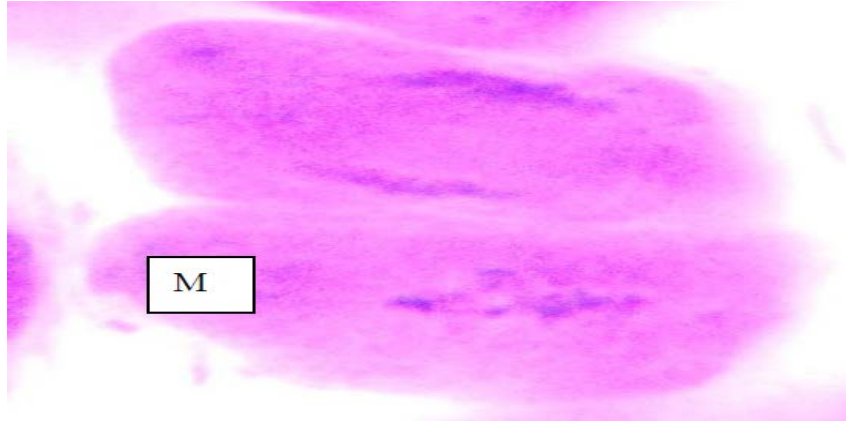


Fig. 3: Chromosomal disorder in metaphase (M) of 30 min -wet samples

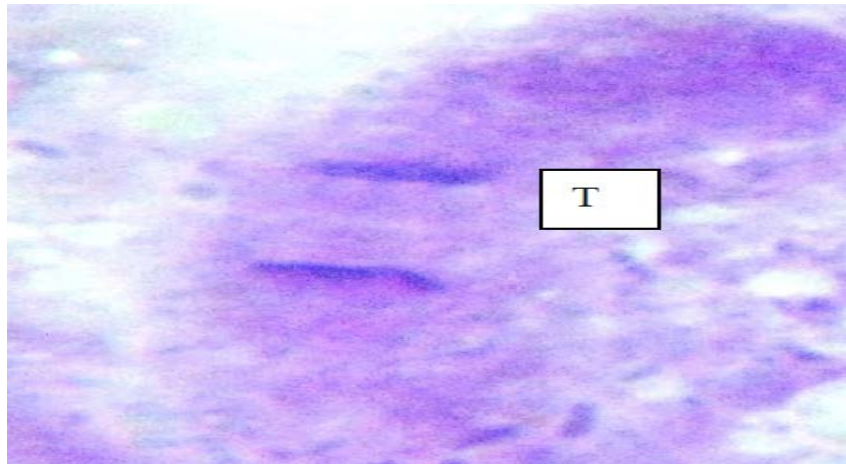


Fig. 4: Chromosomal disorder in telophase (T) of 15 min-wet treatment

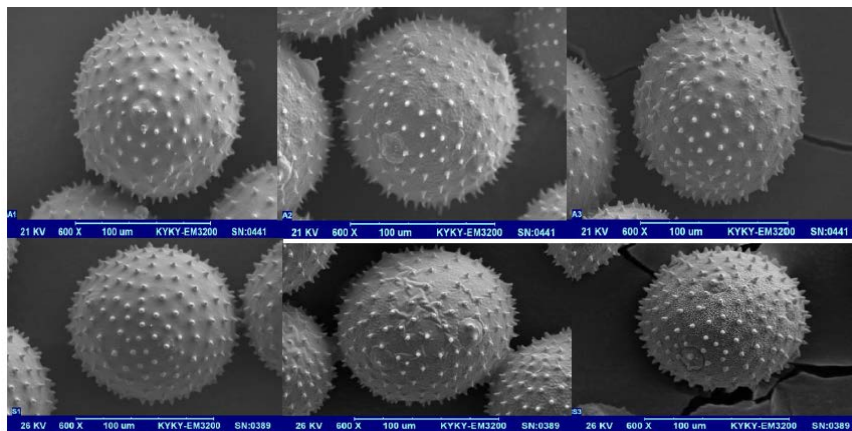


Fig. 5: Pollen structures of electromagnetic fields-treated samples: A1: Control dry, A2: 15 min-dry, A3: 30 min-dry, S1: Control wet, S2: 15 min-wet, S3: 30 min-wet

As mentioned in the result, growth parameters underwent different pattern of changes and in some case, no significant alternation recorded. Ramezani Vishki showed no changes in root length of *Satureja bachtiarica*, by electromagnetic fields treatments. This researcher also reported less shoot length, biomass and area of leaves. In the case of pumpkin, also decreasing of shoot length and diameters reported. It might be due to the activation of oxidises such as IAA oxidise which can bring about less extendibility of cell walls and therefore less growth (Roz and Tevini, 1995). On the other hand, it has been shown that magnetic fields caused more shoot growth. Majd *et al.* (2010, 2012), also reported more biomass of *Satureja hortensis* L. via electromagnetic fields treatments. More biomass of *Raphanus sativus* L. also reported through the electromagnetic fields (Touati *et al.*, 2013).

In the previous studies, chromosomal disorder induced by electromagnetic fields have been reported. Shabrangi *et al.* (2011) showed that <10 mT of electromagnetic fields, caused chromosomes destructions. Other authors reported the same results (Piacentini *et al.*, 2001; Nirmala and Rao, 1996; Ruediger, 2009; Aksoy *et al.*, 2010). Krishnan and Berleage (1986) reported delay of mitotic division due to electromagnetic fields. Arbabian *et al.* (2010), showed disorder of pollen structures by electromagnetic fields treatments.

### CONCLUSION

Total results showed different changes in the growth parameters and chromosomal disorders via electromagnetic fields treatments. Pollen structure also underwent alternation through the electromagnetic fields.

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