Ultrastructure, Germination And Viability In Pollens Of Achillea Wilhelmsii C.Koch Exposed To Electromagnetic Fields

Ahmad Majd, Leila Amjad, Azadeh Ghadirianmarnani

Abstract: - Electromagnetic field is an inevitable environmental factor for living beings which many investigations have been conducted to consider its effect. In this research the effects of electromagnetic field on pollen ultrastructure, pollen germination and viability of *Achillea wilhelmsii* were studied. *Achillea wilhelmsii* C. Koch was collected from around area of Isfahan city (Iran). In case of pollen treatments, the pollens were exposed to electromagnetic field by a magnitude of 2,4 mT at 10 min and 2,4 mT at 20 min in the electromagnetic field generator apparatus. Untreated pollens were used as control under similar condition. Pollens were studied by Scanning electron microscopy. The effect of electromagnetic fields on pollen germination was evaluated. Germination was quatified as the percentage of germinated pollen grains per 100 evaluated. Pollen grains for study of viability, were stained with Evans blue. The results were analyzed using ANOVA statistical to analyses of the treatments. Treatment under pollens with electromagnetic fields 2mT-10,20min have few changes, but treatment under pollens with electromagnetic fields 4mT-20min become abnormal, shrinkage and fragile. the percentage of pollen germination and viability decreased that was significantly($p< 0.05$). electromagnetic field have effects on the structure of some organs and developmental characteristic of them in *Achillea wilhelmsii*. Therefore, a direct influence of environmental stresses is discussed affecting Ca^{2+} levels via the ion cyclotron resonance mechanism.

Index Terms: - electromagnetic field, germination, pollen, viability.

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1 Introduction

PLANTS are exposed to various environmental stresses and show a wide spectrum of developmental and biochemical responses contributing to stress adaptation [1]. These stresses produce intracellular signals, such as calcium transients, that lead to modifications of growth or morphogenesis of the all plant [2]. Abiotic stress results in the formation of ROS in plants which creates a case called oxidative stress that can harm cellular components [3]. Oxidative stress arise when there is a main imbalance between the production of ROS and antioxidative defence. Exposure to electromagnetic field can product ROS and lead to cell death as a result of increase in free oxygen radicals and DNA damage [3]. Pollen grains are generated by meiosis of microspore mother cells that are located along the inner border of the anther sacs. Fertility decrease under kind stresses due to the direct and indirect effects on sexual apparatus. The effects of atmosphere pollution on anther and pollen grains are possible via the electromagnetic field radiations. Therefore, reduced fertility leads to the production of fewer, smaller pollen grains and an increased number of shapeless pollen grains compared to plants of the same species growing in non polluted areas [4], [5]. Datillo et al. 2005 demonstrated that an alternating magnetic field enhanced the anomalies pollen tube in *Actinidia deliciosa* plant [4]. a study has showed that electromagnetic field radiations increased lipid peroxidation and hydrogen peroxide content in *Lemna minor* L. [6]. Sandu et al. (2005) showed a decrease in chlorophyll content in leaves of *Robinia pseudoacacia* [7]. Achillea is one of the most important genera of the Compositae family and comprises more than120 spices [8]. *Achillea wilhelmsii* C. Koch (Asteraceae) is widely found in different parts of Iran. Thus, The present work was investigated the effects of EMFr on germination , viability and ultrastructural of *Achillea wilhelmsii* pollen grains at 10, 20min after EMFr (2mT, 4mT) exposure.

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2 Materials and Methods

Achillea wilhelmsii C. Koch was collected from around area of Isfahan city (Iran) in the May of 2011. The voucher specimen was deposited at the herbarium of Research Institute of Isfahan Forests and Rangelands. Fresh pollen grains were purified by passage through mesh with 30-µm- diameter pores [9]. Exposure to EMF was performed using a locally designed EMF generator. The magnetic field was provided by a parallel pair of identical circular coils spaced one radius apart and wound so that the current electrical flow through both coils in the same direction. Magnetic field exposure arrangement is produced the low frequency uniform and homogeneous form experiments over a known strength volume. This system consisted of one handmade coil, cylindrical in form, made of 20cm in diameter and 300 roll of winding. To ward production of field with intensity of 2-4 mT, was transmitted 0.1 amper electrical flow between the coils. The coil was not shielded for electrical field and the pollens were exposed to both magnetic and electric fields generated by the coils. The winding results in a very uniform magnetic field between the coils with the primary component parallel to the axes of the two coils. The samples placed in the middle of a horizontally fixed coil and were exposed. The temperature was measured with a thermometer to be 22°C [3]. Three replicates were used in the experiment with 20 pollens in each treatment. In case of pollen treatments, the pollens were spread on the filter papers in Petri dishes and then placed in the middle of a horizontally fixed coil and were exposed to EMF by a magnitude of 2 mT at 10 min (T.1), 2 mT at 20 min (T.2), 4 mT at 10 min (T.3) and 4 mT at 20 min (T.4) in the EMF generator apparatus. Untreated pollens were used as control under similar condition. It means they were placed in the similar coil but not connected to the power. Pollens were studied by Scanning electron microscopy (SEM). Control and treatment under samples were coated with gold; these samples were analyzed using a Scanning electron microscope (Model SEM – x 130, Philips, Netherland) [5]. The effect of electromagnetic fields on pollen germination was evaluated. The experiment was block in time with three replications following a randomized complete block design. Tissue culture plates containing optimal germination medium (0.1gr boric acid, 10gr sucrose, 0.3gr calcium nitrate, 0.1gr potassium nitrate) in 100ml distilled water and 0.5gr agar were prepared and stored at 4°c until needed. Pollens germination evaluated with a light microscope at 40× magnification. Germination was quatified as the percentage of germinated pollen grains per 100 evaluated [10], [11]. Pollen grains for study of viability, were placed with a brush in petri dishes and stained with four or five drops of Evans blue and examined after 10-20 min under 2-4 mT electromagnetic field radiation [12]. Means of triplicate evaluation and standard errors were determined for each sample. The results were analyzed using one-way ANOVA statistical to analyses (significance $P<0.05$) of the treatments.

3 RESULTS

The study of pollen ultrastructure by SEM showed that the outline of pollen grains seen in polar view is circular, triangular and equatorial with an elliptic outline. Pollens shape could be recognized prolate. The pollens aperture are tricolporate. The colpi are usually wide at the equator and narrow near the poles. The colpus membrane is often covered with granular elements. The colpi are not fused at the poles. Exine ornamentation is echinate with microgranula (Fig.1). Treatment under pollens with electromagnetic fields 2mT-10,20min have few changes (Fig.2,3), but treatment under pollens with electromagnetic fields 4mT-10min have the most changes among colpi unroll and exine surface echins in this pollen grains are short (Fig.4). Treatment under pollens with electromagnetic fields 4mT-20min become abnormal, shrinkage, destroy, defective and fragile (Fig.5).

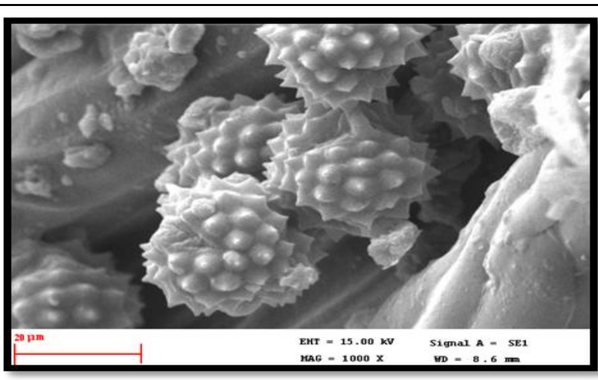

Fig. 1. Scanning electron micrographs of pollen grains of plant grown under condition of control. (The pollen grains and exine are circular and triangular)

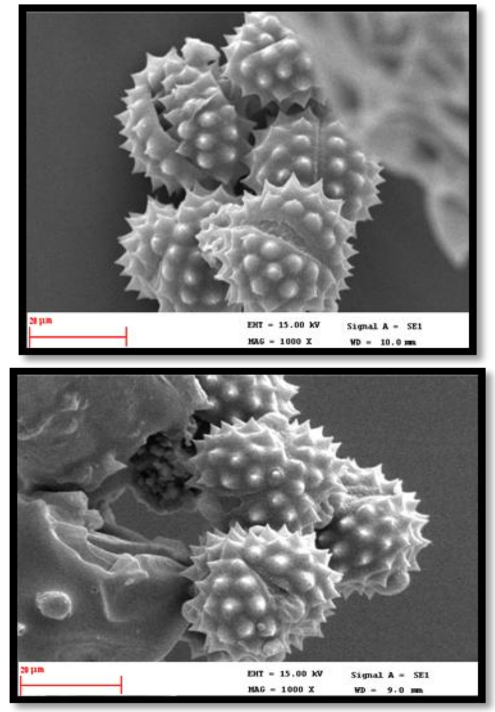

Fig. 2, 3 Scanning electron micrographs of pollen grains after contamination with electromagnetic fields (2mT, 10,20min). (The pollen grains are with few changes)

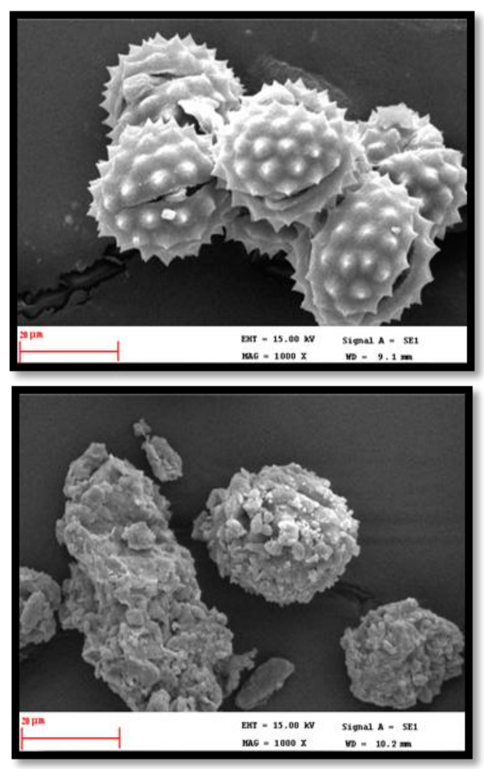

Fig. 4,5. Scanning electron micrographs of pollen grains after contamination with electromagnetic fields (4mT, 10,20min). (The pollen grains are abnormal, degeneration of exine surface)

Observations in our study showed that, according to Table 1 in the radiation samples in comparision to control, the percentage of pollen germination decreased that was significantly ($p<0.05$). In control pollens , pollen germination and pollen tube growth is normal (after 48-72h of pollen culture) (Fig.6). Treatment under pollens with electromagnetic fields 2mT-10,20 min have shorter and folded pollen tube (Fig. 7,8), but treatment under pollens with electromagnetic fields 4mT-10min have very short pollen tube (Fig.9) and treatment under pollens with electromagnetic fields 4mT-20min have not germination (Fig. 10). In *Achillea wilhelmsii* plants electromagnetic field exposure caused significant decrease in pollen viability ($p<0.05$) (Table 1). The decreasing in the viability of pollens are considered as an important response of electromagnetic field radiation.

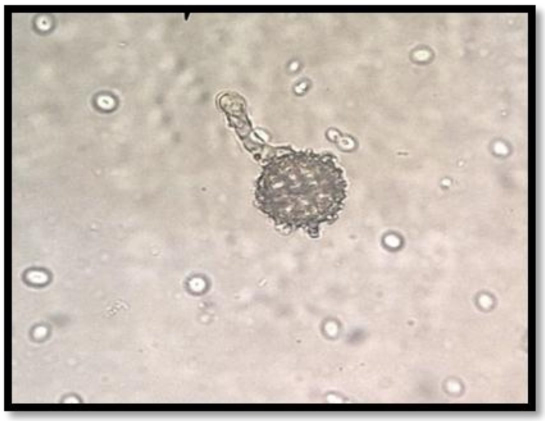

Fig. 6. Pollen grains with normal germination and normal pollen tube growth

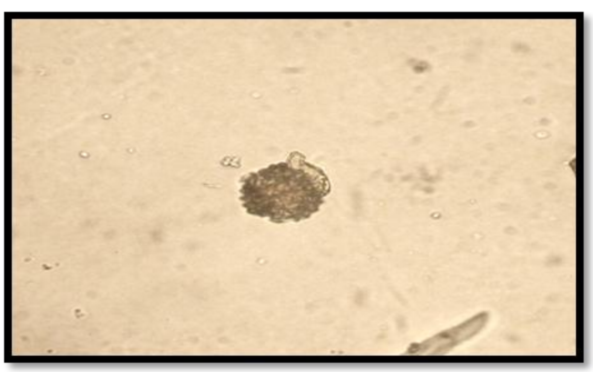

Fig.8. Light micrographs of pollen grains growth after contamination with electromagnetic fields(2mT, 20min). (The pollen tube is shorter and folded compared to control sample)

Fig 9. Light micrographs of pollen grains growth after contamination with electromagnetic fields(4mT, 10min). (The pollen tube is very short compared to control sample)

4 DISCUSSION

In recent years, very research has focused on the mechanisms with which plants sense and respond to EMF. The physicists and biophysicists have proposed several hypotheses about physical aspects of interaction between biomolecules/cellular components and EMF. Basic studies to determine whether the reduced growth of plants in electromagnetic field was due to the changes in the cell division have been carried out with *Achillea wilhemsii.* Nanushyan and Murashov (2001) have shown that the cells number of *Allium cepa* L. root and shoot meristems is decreased by electromagnetic fields [13]. Thus, the root meristems of *Pisum sativum* L. and Lens culinaris L. are increased in the cell cycle duration [14]. In this plant, alterations in condensed chromatin distribution became apparent, so that the reduction in volume of granular nucleolus component and an appearance of nucleolus vacuoles in EMF-exposed cells might indicate a decrease in activities of rRNA synthesis in some nucleoli [14]. Ca^{2+} ions are in particular essential regulatory components of all organisms. Being a second messenger, Ca^{2+} is involved in regulation at all stages of plant growth and development, including growth and differentiation, photomorphogenesis and embryogenesis, the self-incompatibility responses in pollen-pistil interactions, perception of symbiotic signals, hypersensitive responses induced by pathogens and elicitors, gravitropism and phototropism, assembling and disassembling of cytoskeleton elements, perception of red and blue light, cyclosis and movement of stomatal cells [15]. Accordingly Ca^{2+} is the most investigated ion for the stress [16]. Ca^{2+} signaling has been involved in plant responses to a number of abiotic stresses including low temperature, osmotic stress, heat, oxidative stress, anoxia, and mechanical disorder, which has been studied by Knight (2000) [17]. Zonia et al. (2001) showed that, there is no direct information on the aim components of the electromagnetic field, changes in cytoplasmic components, such as Cl^- ion flux, can produce deformation of the pollen apex [18]. Germana et al. (2003) hypothesised that a electromagnetic field can increase the transport of calcium across the cell membrane and alter pollen germination [19]. It is well known that Ca^{2+} is fundamental in the regulation of the cell cycle and it has been recently suggested that its fast oscillation is necessary for centrosome duplication [4]. Chiabrera et al. (1984) showed that Ca^{2+} changes the cytoplasm stimulate the depolarization of tubulin, which is the basic protein of the microtubules [20]. If the electromagnetic fields affects the transport of Ca^{2+}, a variation in the pollen tube cytoskeleton system may occur. We observed a high number of changes in *Achillea wilhelmsii* pollen tubes, thus confirming similar observations reported for other plants.

TABLE 1
PERCENTAGE GERMINATION AND VIABILITY IN THE CONTROL AND ELECTROMAGNETIC TREATMENT POLLENS

Sample	Germination(%)	Viability (%)
Control	53.3	51.16
Treatment 2-10	45	46.8
Treatment 2-20	41.17	30.3
Treatment 4-10	31.25	21.87
Treatment 4-10	23.33	12.16

5 CONCLUSION

The environment changes interact with the pollen of plants causing alteration of morphology, germination and viability of pollens. These changes to pollens ultrastructure can affect proteins of pollens. In most cases EMF suppress the growth stages, cell division and differentiation, induce significant changes at the cellular and subcellular level, alter the Ca^{2+} balance, enzyme activitie and different metabolic stages.

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