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Effect of Lead Toxicity on Pollen Grains in Matricaria Chamomilla

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***Abstract*—*M. chamomilla* is one of the most important medicinal plants which it grows among 50-80 cm hight. It has the yellow and tubuliform flowers in central of florescence and the white flowers in margin. Heavy metal is an important contamination in environment that they are very toxic, although, their concentration is low. The present study was undertaken to determine the effects of lead on structural characteristics and pollen grain development in *M.chamomilla*. Plants in vegetative stege treated with contaminated hydroponic in 5 treatments (0, 60,120,180,240 µM) with 3 repeat in per treatment for 21 days. Anatomical characteristics of pollen grains affected by lead contamination, also optical microscope studies indicated structural changes in natural shape of pollen grains. Decreased the size of them and decreased the diameter of wall in pollen sac. Maximum changes observed in 180 µM concentration.**

Keyword-Developments, pollen grain, Matricaria chamomilla, lead

I. INTRODUCTION

Matricaria chamomilla is an herb plant and native to Iran (Reichinger, 1943) and Europe that grows as a wild plant (Rai *et al*, 2005). *M.chamomilla* is an important and frequently cultivated medicinal plant belongs to *Asteraceae* family (Salamon, 1992; Khan *et al*, 2005). The flowers are daisy-like and bloom from May to October. Herbal products can have differences in their composition depending on the soil where they grow (Salamon, 2007). Heavy metal pollution of air and soils is one of the most important ecological problems on world scale. According to the environmental protection agency (EPA), Pb is the most common heavy metal contaminant in the environment (Li et al, 2007). Pb not only affects plant growth and productivity but also enters into the food chain causing health hazards to man and animals. Pb tends to accumulate in the surface ground layer and its concentration decreases with soil depth (Pais, 2000). The main sources of Pb pollution are exhaust fumes of automobiles, Industrial dies, and domestic and industrial sewages (Salamon, 1992). The content of Pb in various plant organs tends to decrease in following order: roots>leaves>stem>inflorescence>seeds (Sharma, 2005). Pb contamination in the plant environments known to cause highly toxicity effects on processes such as depression on seed germination, the disturbance in mitosis induction of leaf chlorosis, toxicity of nucleoli , inhibition of root and shoot growth, reduction in photosynthesis transpiration, DNA synthesis and inhibition and activation of enzymatic activities.(Velari, 2009; Li et al, 2007; Sharma, 2005; Hall, 2002).Despite of importance of Pb contamination, it is until unclear that what concentrations of Pb cause to decrease plant growth and toxicity mechanism of lead in plant is unknown (Meo *et al*, 2006; Li *et al*, 2007). Therefore anatomical studies can be useful for understanding the mechanism of lead toxicity in plants.

II. MATERIAL AND METHOD

A. Plant material and growth conditions

Plants of *M.chamomilla* utilized for studies of pollen grains anatomical were transplanted as seedlings (obtained from Isfahan agriculture jihad center) into a plot in Tarbiat Modares University in December 2009. Plants were watered daily. The plants, which initiated vegetative stage by mid-January, were transplanted into hydroponic condition in laboratory greenhouse and placed in a growth chamber at 22^0C day and 16^0C night temperatures and Ph=7. Plants in vegetative stage treated with contaminated hydroponic in 5 treatments (0, 60,120,180,240 µM of Pb) with 3 repeat in per treatment for 21 days. Then flowers at various stages of development were collected.

B. Plant collection and analysis

The parts of flowers separated and fixed in FAA for anatomical studies by Light microscopy and residual parts fixed in ethanol 70% for SEM microscope studies. Samples were observated with Light microscopy and SEM, and then were analyzed parameters with Measurement program and SPSS.

C. Pollen grains structure: SEM

For detailed studies of pollen grains morphology, samples in three distinct developmental stages (mature, bud, and between both of them) were removed from mature flowers of plants and processed for SEM. Tissues were critical-point dried with liquid CO_2, mounted on aluminum stubs with two-sided tape, coated with gold (Edwards S150B Sputter Coater), observed with a Philips SEM 505 at 30kV, and photographed with Polaroid 665 positive/negative film. Negatives and positives were scanned (Epson 3200 Photo)

and images edited using Adobe˘ Photoshop˘ 7.0. Pollen sacs dimensions and the number and developmental stage of the modified pollen grains surface were compared between 5 treatment samples.

D. Pollen grains structure: TEM

Flowers of three distinct developmental stages were collected , tissues were fixed in 1.5% GA in 25mM Na phosphate buffer, pH 6.8, for 0.5h at room temperature, before transferring to 3% GA in buffer for 2h. On ice, samples were rinsed with buffer over 1-12h, post-fixed overnight with 1% OsO_4, then rinsed with distilled water before dehydration in an ethanol series. A gradual substitution of ethanol by propylene oxide preceded sample infiltration and embedding in Supper resin at 60^0C for 48h (Tyler J *et al.,* 2005).

For light microscopy, semi-thin (0.5-1µm) sections of flowers, cut glass knives on Reichert OMU3 ultra microtome, were heat-fixed to microscope slides, stained with toluidine blue, and then mounted in immersion oil under coverslips. Sections were examined with an Olympus microscope with Motic MC2000 (2.0 Megapixel).

Samples were fixed in FAA (formalin: acetic acid: 50% ethanol, 1:1:8 v/v). Flowers of various stages were according to standard methods of paraffin embedding (Johansen, 1940) and sectioned by a rotary microtome. Serial sections of 10-12µm were stained in hematoxylin and eosin.

E. Statical analyses

The design of all experiments was a complete randomized design and treatments consisted of three replications. The data were analyzed with SPSS version 15. Significant differences between means were assessed by a Duncan test at $P<0.0001$.

III. RESULTS

Pollen morphological characters of the *Matricaria chamomilla* L. are investigated. The descriptive photomicrographs are compiled in Fig.1-3. The list of measurement data are showed in Table 1.

A. Characteristic of pollen grains:

Some of the treatment pollen grain *characters of* M.chamomilla *are showed in Table 1.*

a) Size: The size of the pollen grain of M.chamomilla ranges from 38.9/36.4µm. There is a little difference in size of pollen grain in all of treatments but the most variations observed in 240µm (Fig1).

b) Symmetry and shape: The pollen grains are radial symmetry, isopolar and isodiametric, but they have shown asymmetry in some of treatments; the most changes have shown in 180 and 240µm (Fig1;C and D).

c) Spine: The spine length varies from 6.5µm to 7.1µm in M.chamomilla. The number of spone rows between colpi varies 5-9 and 4-8. The pairs of closely placed thin spines appear as bifurcated in all treatments (Fig1 and 2).

d) Ornamentation: Pollen grains were examined through scanning electron microscope. The spines are short and thin in control but in 240µm have shown different feature of spines (Fig3).

e) Appearance: The pollen grains usually have cicular shaape but many of them were deformed. Especially,they were deformed in 180 and 240µm (Fig1).

B. Pollen sac

The pollen sac morphology of *Matricaria chamomilla* is generally circular and its wall ranges from 6.5 to 7µm thickness. This investigation is showed a little difference in ranges of wall thickness. The shape of pollen sac was deformed in many of lead concentration. Especial changes were showed in 240µm. Some of the wall cells were wasted in 180 and 240µm.The most wall thickness changes was reported in 240µm, the wall thickness was decreased in this concentration (Table1).

C. Figures and Tables

1) Pollen grain characters: The characters of pollen grains and pollen sac in *M.chamomilla* are showed in Table I.

TABLE I. THE CHARACTERS OF POLLEN GRAINS AND POLLEN SAC IN TREETMENT SAMPLES

Samples	Appearance of pollen grain	Pollen sac wall thickness	Pollen sac shape	Number of all pollen grain	Number of normal pollen grain	Number of abnormal pollen grain	P/E ratio
Control	Circular	6/6µm	Normal	6-8	6-8	0	1µm
60µM	Circular	6/2µm	Normal	6-8	5-7	0-1	1µm
120µM	Rather circular	5/8µm	Rather normal	6-8	4-6	1-2	1/08µm
180µM	Deforming	5µm	Rather normal	6-8	3-5	2-3	1/16µm
240µM	Deforming	4/3µm	Abnormal	6-8	2-3	3-4	1/2µm

Fig1, 2, 3 and 4: Light micrographs of pollen grains of *M.chamomilla* (X40 and X20) in different concentration of lead.

Fig5: Electron micrographs of pollen grains of *M.chamomilla* (X20 and 50 µm).

Fig6: Effect of Pb on size of pollen grain and pollen sac wall thickness.

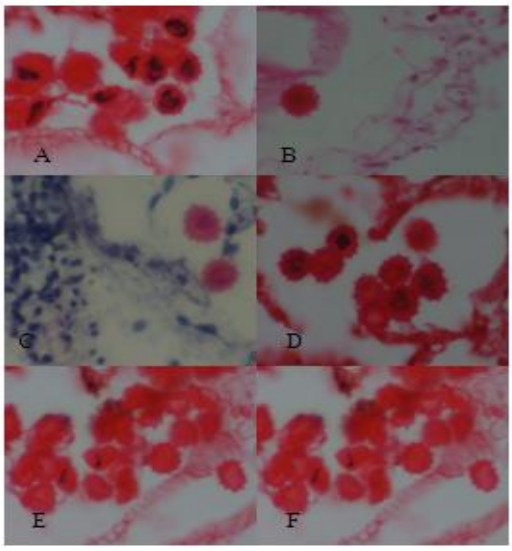

Figure 1. A.60µM; B.120µM; C.180µM; D.240µM;

E. Control.

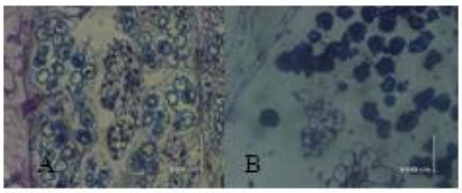

Figure 2. A. Control; B. 240 µM.

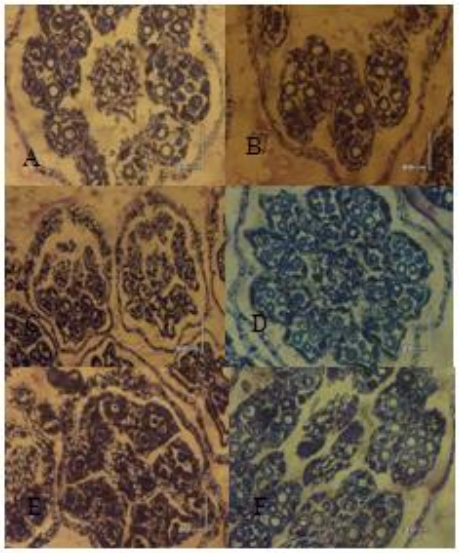

Figure 3. A and F. Control; B. 60 µM; C. 120 µM;

D. 180 µM; E. 240 µM.

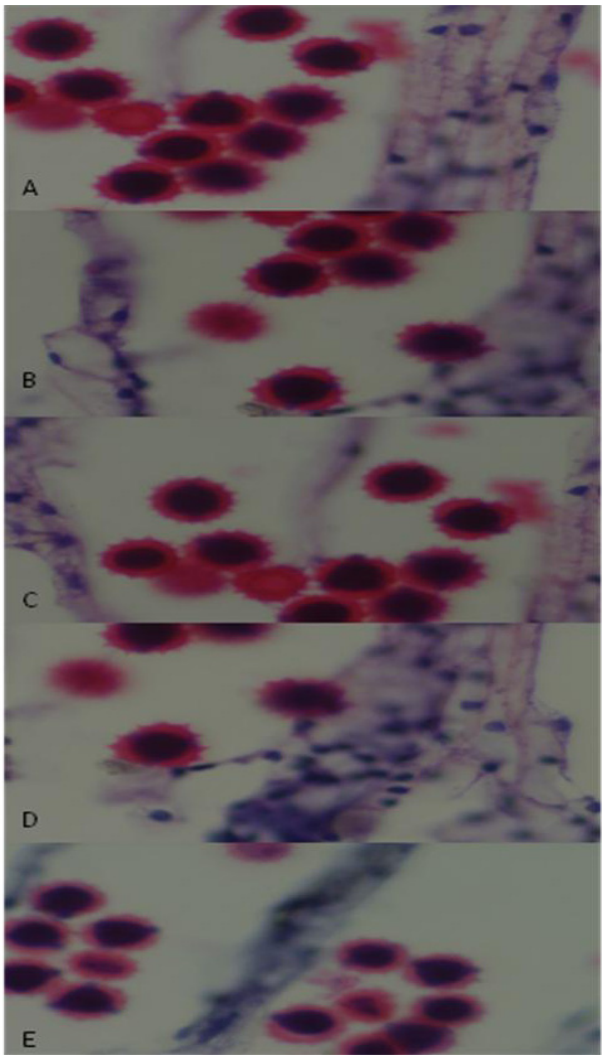

Fig 4. A. Control; B. 60 µM; C. 120 µM; D. 180 µM; E. 240 µM.

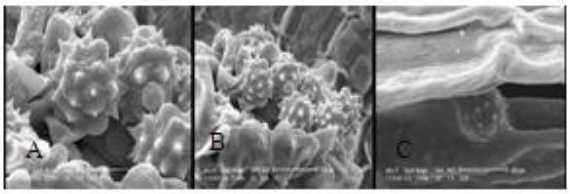

Fig 5. A. 240µM, B. 240µM, C. Control.

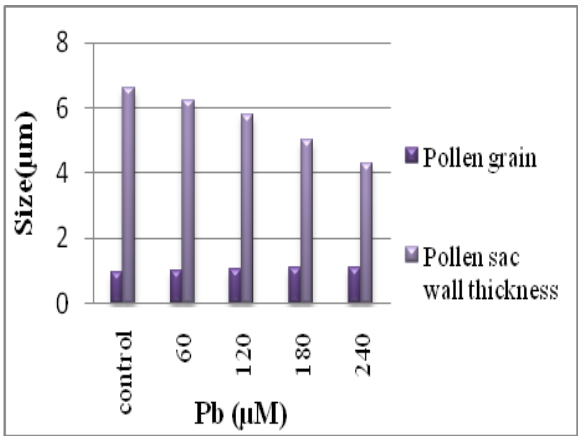

Fig 6. Effect of Pb on size of pollen grain and pollen sac wall thickness.

IV. CONCLUSION

The family *Asteraceae* has been published in Flora of Iran. Pollen morphological characters are considered supplementary to the general plant morphology and play a critical role in taxonomic and evolutionary debate. A thorough knowledge of the toxic effects of this element in plants may contribute to the general understanding of primary toxicity mechanism and of the tolerance trend in living organisms (Alloway,1990).Plants exposed to various stresses may experience an inhibitory effect at various stages of growth .Pollen grains are that part of plant, which is not in direct contact with nutrient medium. Its abnormal morphology can directly affect on the fertilization and reproduction in plants. Various morphological characters like pollen surface area, diameter, and shape of spine are influence of heavy metals. Therefore, the abnormal shape of pollen grains can be affected on fertilization.

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