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Comparative study of phytochemical parameters and antioxidant activity of accumulated *Zea mays* from different regions of Isfahan

Narine Keshishian[1], Leila Amjad[2*], Sima Yahyaabadi[2]

[1]*Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

[2]*Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

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Abstract

It is known that environment pollutions are health-endangering for human and their effects include blood enzyme changes, anemia, hyperactivity, and neurological disorders. A multitude of natural antioxidants have already been isolated from different kinds of plant materials. Plants contain a diverse group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. Therefore, in this research, the effects of industrial and electromagnetic fields pollutions on phytochemical parameters of *Zea mays* were determined. So, plants were collected from east of Isfahan area (surrounding industrial areas, with electromagnetic fields areas and non-pollution area), Iran and the contents of phenolic compounds, total flavonoid and antioxidant activity were investigated. So that , for evaluation of antioxidant activity and total phenolic, flavonoid of methanolic extract these plants were used from DPPH (2,2-diphenyl-1-picrylhydrazyl) model system, Folin-Ciocalteu method and aluminum chloride colorimetric method, respectively. The results of this study were analyzed using different statistical tests including ANOVA and LSD. The results indicated that the amount of phenol, flavonoid and antioxidant activity were decreased in all polluted samples but a non-significant decrease was observed in collected *Zea mays* from electromagnetic pollution areas. These results showed that different responses in polluted plants and non-polluted plants is due to induction oxidative stress and

***Corresponding Author:** Leila Amjad ✉ Amjad.leila@gmail.com

Introduction

Metalions are essential in the preservation and evolution of all life systems, and also mediate all stages of diffusion of genetic information carried in the genetic code. Although the relative toxicity of different metals to plants can differ with plant genotype and experimental conditions, the metals which, when present in extreme amounts, are the most toxic to higher plants and microorganisms are Hg, Cu, Ni, Pb, Co, Cd and possibly also Ag, Be and Sn (Kabata-Pendiasand,1984). Metals like mercury, ead and arsenic maybe increasingly taken up b yplants, especially crops, and transferred urthertothefoodchain (Devkotaand-Schmidt, 2000). Food plants, which tolerate are latively high concentrations of the sepotentially dangerous metals, are likely to create a greater health risk than those which are more sensitive and showed finite symptoms of toxicity (Carbonell-Barrachina *et al.,*1999).

Electromagnetic field is one kind of stress, which can affect directly or indirectly the plant exposed to it. Plant species vary in their sensitivity and response to environmental stresses because they have various capabilities for stress perception, signaling and response (Bohrert *et al.,* 1995). Several researches tried to define the effect of such field on the growth rate of the plant. Electromagnetic field can cause deformation inside grain through compression or tension of particular layers (Sumorek and pietrzyk, 1999). On the other hand, it has been proved that the electromagnetic field inhibited the biologicalproperties of the membrane protein (Germana*et al.,* 2007). Need to create these components, incorporating the applicable criteria that follow. Also, redistributions of membrane proteins by 50 Hz electromagnetic fields is reported by Pazur*et al.* (2006).

Studies on the meristem cells of the plants have shown that magnetic field is an element that affects normal cell metabolisms and also has impacts on the cell division. In numerous experiments, it has been investigated that electromagnetic field's effects on organisms vary depending on the intensity of the magnetic field, frequency, exposure duration to electromagnetic field, genotype of organisms and the biological system (Dardeniz *et al.,* 2006).

Datillo*et al.* (2005) showed that an alternating magnetic field enhanced the anomalies pollen tube in *Actinidiadeliciosa* plant (Datillo*et al.,* 2005). A study has showed that electromagnetic field radiations increased lipid peroxidation and hydrogen peroxide content in *Lemna minor* L. (Tkalec*et al.,* 2007). Sandu *et al.* (2005) demonstrated a decrease in chlorophyll content in leaves of *Robiniapseudoacacia* (Sandu*et al.,* 2005).

Zea mays known in some countries as corn, is a large grain plant domesticated by indigenous peoples in Mesoamerica in prehistoric times. The leafy stalk produces ears which contain the grain, which are seeds called kernels. Maize kernels are often used in cooking as a starch. Maize is a facultative short-day plant (Dong *et al.*, 2001).

Therefore, the present study carried out to evaluate the effect of environmental pollutions on phytochemical parameters and antioxidant activity of *Zea mays* fruits.

Materials and methods

Plant materials

The fruits of *Zea mays* were collected from east of Isfahan area (surrounding industrial areas: industrial pollution area), from north of Isfahan area (surrounding electromagnetic field areas: electromagnetic pollution area) and non-pollution area, province of Isfahan, Iran, in October 2013. The voucher specimen was deposited at the herbarium of the Research-Institute of Isfahan Forests and Rangelands. The fruits were air-dried under shade and ground in to fine powder using electric blender, then, 20 gr of fruits powder were extracted with 200 ml methanol 80% by Maceration method. In this way, a mixture of methanol and fruit powder were placed on the shaker for 72 hours, then this was smoothed by the filter paper. The residue was evaporated at room temperature and the dried extract was stored at 4°cuntil used. The extract (0.05 gr) was dissolved in distilled water (10 ml) and obtain different concentrations of fruits extract.

Total phenol determination

Total phenols were determined by Folin Ciocalteu reagent (Sharafati-Chaleshtori *et al.,* 2011; Sumaya-Martinez *et al.,* 2011). Different concentrations of each plant extract (10, 20, 40, 60, 80, 100 µl) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (1 ml, 1:10 diluted with distilled water) and 7% Na2CO3 (1 ml). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1} solutions of gallic acid in methanol : water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound.

Total flavonoid determination

Aluminum chloride colorimetric method was used for flavonoids determination (Miliauskas *et al.,* 2004; Pourmorad *et al.,* 2006). Each plant extracts (10, 20, 40, 60, 80, 100 µl) in methanol were separately mixed with 1.5 ml of methanol, 1 ml of 2% aluminum chloride, 6 ml of 5% potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 40 min; the absorbance of the reaction mixture was measured at 415 nm for flavonoid assay. The calibration curve was prepared by preparing rutin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol.

Free radical scavenging activity determination

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Jayaprakasha *et al.,* 2001). Different concentrations of each extract (10, 20, 40, 60, 80, 100 µl) were added, at an equal volume, to methanolic solution of DPPH (0.004g per 100 ml) . After 120 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid were used as standard controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Statistical analysis

The experimental design was a split plot in a randomized complete block design with three replications. The presented data included means of three separate experiments ± SD. In order to analyze the data, SPSS software and ANOVA test were used. Thus, the statistical significance between phytochemical activities values of the extracts was evaluated with a LSD test. P values less than 0.05 were considered to be statistically significant.

Results

Analysis of data on total phenol showed that fruits of *Zea mays* of accumulated from polluted areas showed significant reduction in polyphenol content compared to *Zea mays* of accumulated from non-polluted area (P<0.05) (Figure 1).

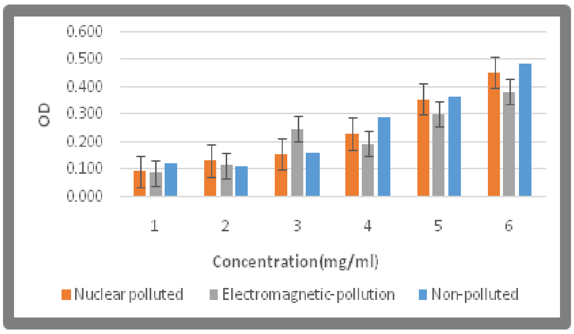

Fig. 1. Significant reduction of phenol content (concentration and absorbance) of fruits of accumulated from polluted areas in compared with fruits of accumulated from non-polluted area. Bars are least significant differences where p < 0.05.

Figure 2 show the content of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 29.85x +0.043, r_2= 0.990). The total phenol varied from 11.39 ± 0.01 to 14.87 ± 0.02 mg g-1GA in the extract powder. Non-polluted *Zea mays* group with total phenol content of 14.87 ± 0.02 mg g-1GA had the highest amount among the phenol in this study.

Analysis of data on flavonoids showed that fruits of *Zea mays* of accumulated from polluted areas showed significant reduction in flavonoid content compared to *Zea mays* of accumulated from non-polluted area ($P<0.05$) (Figure 3).

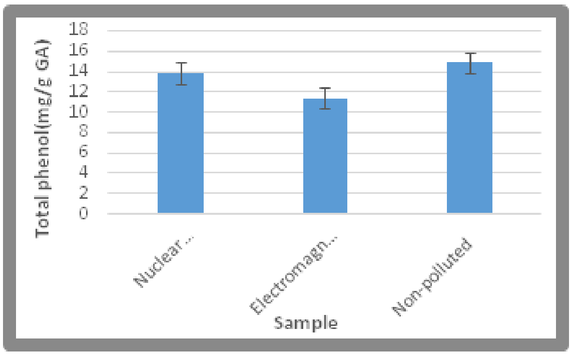

Fig. 2. Phenol content in the studied *Zea mays* fruits Extract.

The flavonoid content of the extracts in terms of rutin equivalent (the standard curve equation: y = 0.0603x + 0.0007, r2 = 0.985) were between 1.40± 0.002 and 1.629 ± 0.01 mg g-1RTA (Figure 4). The flavonoid content in the *Zea mays* of accumulated from electromagnetic polluted area (1.40 ± 0.002 mg g-1RTA) and in the *Zea mays* of accumulated from metal polluted area (1.54 ± 0.01 mg g-1RTA) were lower than that in the *Zea mays* fruits of non-polluted (1.629± 0.01 mg g-1RTA).

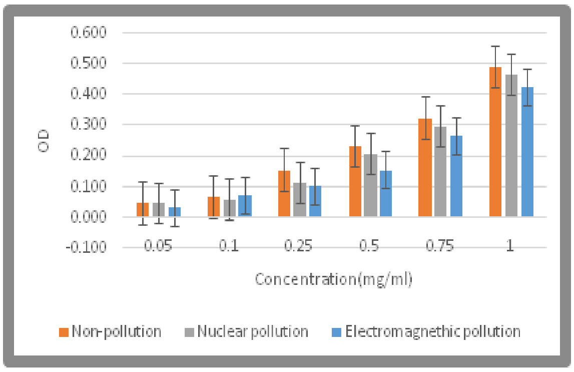

Fig. 3. Significant reduction of flavonoid content (concentration and absorbance) of fruits of accumulated from polluted areas in compared with fruits of accumulated from non-polluted area. Bars are least significant differences where $p < 0.05$.

Analysis of data on antioxidant activity showed that fruits of *Zea mays* of accumulated from polluted areas showed significant reduction in inhibition percent compared to *Zea mays* of accumulated from non-polluted area and control group (Ascorbic acid) ($P<0.05$) (Figure 5).

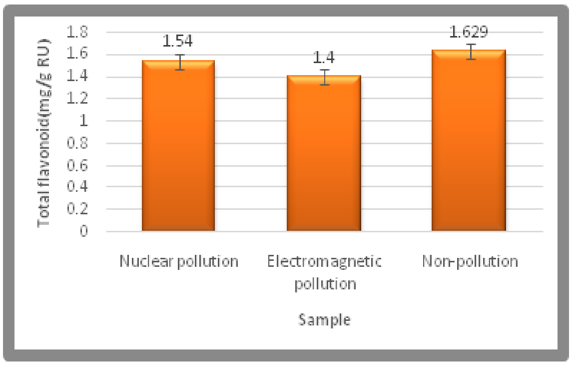

Fig. 4. flavonoid content in the studied *Zea mays* fruitsextract.

Figure 6 shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} of the standard compounds, Ascorbic acid were 514.13 µg/µl. The highest radical scavenging activity was showed by non-polluted *Zea mays* with IC_{50}=580.11 which is nearby of Ascorbic acid (Figure 6).

Discussion

According to the results, in collected *Zea mays* of different areas (electromagnetic polluted, metal polluted and non polluted) in this study, total phenol, flavonoid and antioxidant activity significantly decreased in collected *Zea mays* of electromagnetic polluted and metal polluted as compared to the collected *Zea mays* of non polluted ($P<0.05$).

The flavonoid contents of the extracts in terms of quercetin equivalent (*the standard curve equation:* $y = 0.0603x + 0.0007$, $r^2 = 0.985$) were between 1.40± 0.002 and 1.629 ± 0.01 mg g-1RTA (Figure 4). Table 1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 29085x + 0.043, r^2= 0.9873). The total phenol varied from 11.39 ± 0.01 to 14.87 ± 0.02 mg g-1GA in the extract powder. Non-polluted *Zea mays* with total phenol contents of 14.87 ± 0.02 mg g-1GA had the highest amount among in this study.

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Das and Pereira, 1990; Younes, 1981). According to our study, the high contents of these phytochemicals in non-polluted *Zea mays* can explain its high radical scavenging activity.

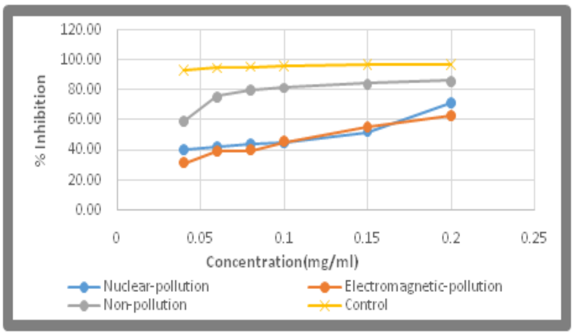

Fig. 5. Significant reduction antioxidant activity of (concentration and inhibition percent) of*Zea mays* fruits of accumulated from polluted areas in compared with fruits of accumulated from non-polluted area and control group (Ascorbic acid).

Free radicals are involved in multitude disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant effect of a special compound or plant extracts (Koleva *et al.*, 2002). The highest radical scavenging activity was showed by non-polluted *Zea mays* with IC_{50}=580.11 µg μl^{-1} which is near that of Ascorbic acid.

This results were similar with results of Yoshimura *et al.* (2000), they reported that high concentrations of heavy metals can decrease antioxidant activity.

Heavy metals Cd, Pb, Al, Zn, and Cu induce oxidative stress in plant species (Malecka *et al.*, 2001; Shah *et al.*, 2001). Arora *et al.* (2000) showed that phenolics (especially flavonoids) are able to alter peroxidation kinetics by changing the lipid packing order (Arora *et al.,* 2000). Antioxidant activity of phenolic compounds is due to their high trend to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind especially heavy metals (Jung *et al.,* 2003).

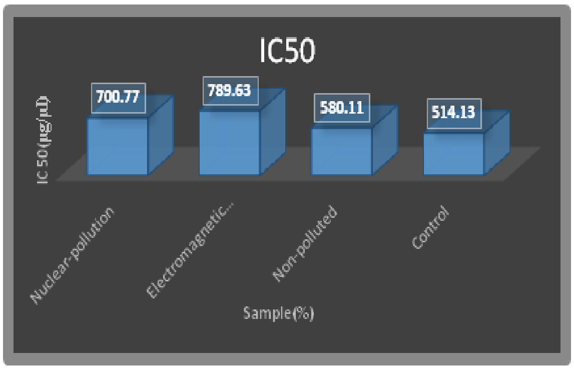

Fig. 6. IC_{50} (µg/μl^{-1}) values of *Zea mays* fruits for free radicalscavenging activity by DPPH radical. Lower IC_{50} value indicateshigher antioxidant activity

One of such theories that would explain the biological effects of electromagnetic fields is based on the possible effects on the formation of free radicals due to electromagnetic field exposure (Piacentini *et al.,* 2001).

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). Germana *et al.* (2003) hypothesised that a electromagnetic field can increase the transport of calcium across the cell membrane and alter pollen germination. 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 (Datillo *et al.,* 2005).

Shabrangi and Majd (2009) showed that seedlings grown from dry treated seeds of *Brassica napus* showed the most signicant increase in developmental growth at 10mT and seedlings grown from wet treated seeds showed the most signicant decrease in developmental growth at 10mT comparing to control ($p<0.05$). They observed an overall stimulating effects of electromagnetic field in *Zea mays* with respect to developmental growth characteristics and the most signicant increase observed at 10mT ($p<0.05$). All experimental data suggested that monocotyledons are more resistant than dicotyledons against electromagnetic field as abiotic stress.

Shabrangi *et al.,* 2011 demonstrated that a significant decrease in protein content of treated samples with electromagnetic 3-10 mT for 4h was observed. They showed that electromagnetic field with the magnitude of 3 and 10mT for 4h caused significant increase in catalase and ascorbate peroxidaes activities in both root and shoot tissues.

Conclusion

Heavy metals are a toxic factor limiting crop production. The present study demonstrated that in plants under environmental pollutions and electromagnetic pollutions amount of phenol, flavonoid compounds and antioxidant activity significantly were decreased. These changes were related to many factors including metal concentration, frequency intensity of electromagnetic field, plant species and plant tissues. It has now become clear that environmental pollutions cause generation of free radicals resulting in oxidative damage to final biomolecules, lipids and proteins. Therefore, the phenolic and flavonoid compounds due to hydroxyl groups existing can provide as a radical scavenger.

Acknowledgements

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