

Effect of Mobile Phone Radiations on Morphological and Biochemical Parameters of Mung Bean (*Vigna radiata*) and Wheat (*Triticum aestivum*) Seedlings

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Abstract: Over the last two decades an enormous escalation in wireless equipments has been made our lives easy. The uses of wireless accessories including mobile phone have been enabled instant our communication resources advance and fast, on the other hand along with their advantages there is great increase in radiations, an unprecedented type of pollution, in our environment. Current study was an attempt to observe the morphological and biochemical changes induced by cell phone radiations on Mung bean (*Vigna radiata*) and Wheat (*Triticum aestivum*) seedlings. The effect of cell phone Electromagnetic Radiations (EMR) were determined by measuring membrane damage in terms of malondialdehyde [MDA] contents and changes in the levels of antioxidant enzymes like Guaiacol Peroxidase (GPX), Ascorbate Peroxidase (APX), and Catalase (CAT). Our results showed that cell phone EMR caused significant reduction in growth, fresh weight, dry weight, and relative water contents. Where as MDA contents and antioxidant enzymes were increased in stressed seedlings as compared to unstressed seedlings. We concluded that radiations emitted by mobile phone can induce oxidative stress which results in reduced growth and increase in the activity of antioxidant enzymes in mung bean and wheat seedlings.

Key words: Antioxidant enzymes, ascorbate peroxidase, catalase, electromagnetic radiations (EMR), guaiacol peroxidase, malondialdehyde, mobile phone, mung bean, wheat

INTRODUCTION

Escalation of wireless electronic equipments like mobile phone has been raised the risk of the tissue level damage for all living organisms not only for human and animals but plants as well. Cell Phone Electromagnetic Radiations (EMR) inhibit root growth of mung bean by inducing ROS-generated oxidative stress despite increased activities of antioxidant enzymes (Sharma *et al.*, 2009, 2010). Mobile phone radiation exposure can cause cell damage by reactive oxygen species formation, and cell death (Oral *et al.*, 2006; Sokolovic *et al.*, 2008). The effect of electromagnetic radiation from the Skrunda Radio Location Station was studied on the vegetative growth and morphology of the duckweed *Spirodela polyrhiza* (L.) Schleiden plant in the next generation and found that plants developed completely to daughter fronds under exposure from the electromagnetic field had a shorter life-span (Magone, 1996). Enhanced MDA and H₂O₂ content accompanied by diminished antioxidative enzymes activity caused by exposure EMR, especially at 900MHz, indicate that oxidative stress could partly be due to changed activities of antioxidant enzymes (Tkalec *et al.*, 2007). A modulated field at 900 MHz strongly inhibited the growth of and, to some extent, peroxidase activity of Duckweed (*Lemna minor* L.) (Tkalec *et al.*, 2005).

MATERIALS AND METHODS

Seeds of mung bean (*Vigna radiata*; genotype NM19-19) and Wheat (*Triticum aestivum* genotype; AS-2000) were obtained from National Agricultural Research Centre (NARC), Islamabad. Current study was performed in the Lab of Department of Genetics, University of Karachi in 2010. Seeds were imbibed in distilled water (d/w) for 8 h, placed in 8×4 inch air tight plastic boxes, lined with filter paper moistened with d/w. A commercially available GSM mobile phone (900 MHz band with modulated voice and low frequency signals;



Fig. 1: Effect of mobile phone radiations on mung bean seedlings

Karachi 2000) was used for the exposure of EMR for current study. Cell phone was placed in box with 25-30 seeds around it, during exposure cell phone was placed on talking mode in vibration and phone battery was kept charged (Fig. 1). For control treatment 25-30 seeds were kept in same sized box without cell phone. Both boxes were placed at room temperature of $28^{\circ}\text{C}\pm 2$ for 72 h, away from instruments or machines in order to avoid any kind of unwanted EMR. Seedlings were then harvested for morphological and biochemical analysis.

Morphological analysis: After 72 h, germination percentage, seedling length and fresh weight were recorded. Seedlings of equal fresh weight were allowed to dry at 70°C for 24 h to record the dry weight. Relative water content was calculated using the formula $\text{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$.

Biochemical analysis:

Estimation of lipid peroxidation: Lipid peroxidation is estimated by measuring the amount of malondialdehyde (MDA) content, which is a product of lipid peroxidation. Approximately 0.2 g of seedlings were homogenized by addition of 1 ml of 5% TCA solution using cold pestle and mortar. Homogenate was centrifuged at 12000 rpm for 15 min. at room temperature. Supernatant was saved for the estimation of Malondialdehyde.

Estimation of MDA was performed by the method of Dhindsa *et al.* (1981) where 1 mL of supernatant and 4 mL of 0.5% Thiobarbituric Acid [TBA] in 20% TCA solution were added into a test tube and incubated at 96°C for 30 min. The tubes were immediately kept in ice bath and then centrifuged at 2000 rpm for 10 min. Absorbance was recorded at 532 nm and corrected for non specific turbidity by subtracting the absorbance at 600 nm [0.5% TBA in 20% TCA solution was use as blank]. MDA content was determined by using the extinction coefficient of 155 m/M/cm.

Extraction of protein and antioxidant enzymes:

Antioxidant enzymes were extracted by the method of Jiang and Huang (2001). Seedlings of 2.0 g were homogenized in 4 mL of 0.05 M Phosphate buffer (pH 7.0), containing ascorbic acid and polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 rpm for 30 min at 4°C . Supernatant was saved for the estimation of proteins by the method of Lowry *et al.* (1951) and antioxidant enzymes.

Estimation of Ascorbate Peroxidase (APX): APX was assayed as decrease in absorbance at 290 nm due to oxidation of ascorbic acid (Nakano and Asada, 1981). The reaction mixture contained 0.05 M ascorbic acid, 0.05M sodium phosphate buffer (pH, 7.0), 0.0001 M EDTA, and 150 μL of enzyme extract. Hydrogen peroxide (0.0001M) was added in the last and immediately absorbance was recorded for 2 min with intervals of 15 sec. Ascorbate peroxidase was determined by using the extinction coefficient of 2.8 m/Mol/cm.

Estimation of Guaiacol Peroxidase (GPX): GPX activity was determined in term of increase in absorbance at 470 nm due to oxidation of guaiacol (Everse *et al.*, 1994). Reaction mixture contained 50 μL sample, sodium phosphate buffer [0.15M, pH 5.6], 0.176 M hydrogen peroxide, and 0.1 M guaiacol and then reaction mixture was incubated for 8 min, absorbance was recorded at 470 nm. Oxidized guaiacol peroxidase was determined by extinction coefficient of 26.6 m/Mol/cm

Estimation of Catalase (CAT): CAT activity was determined as the rate of disappearance of hydrogen peroxide at 240 nm (Aebi, 1984). A reaction mixture contain 0.05M sodium phosphate buffer (pH7.0), supernatant and at end hydrogen peroxide (0.125 M) was added into reaction mixture. Immediately absorbance was started to record at 240 nm after intervals of 15 sec till 2 min. Catalase activity was determined by using extinction coefficient of 40 m/Mol/cm.

Statistical analysis: Experiment was conducted in two factorial as Complete Randomize Design (CRD) with 3 replications. Analysis of variance for morphological and biochemical parameters were performed by a computer program SPSS version 11.

RESULTS

Effect of cell phone radiation morphological parameter of seedlings:

Table 1 showed that there was highly significant difference for genotype with non significant difference in treatment as well as interaction as far as germination percentage was concerned. Current results showed that there was 100% germination in unstressed as well as stressed mung bean. Whereas, wheat genotype showed 77.3 mean germination percentage for

Table 1: Mean square of various morphological and biochemical parameters of 72 h old *Vigna radiata* and *Triticum aestivum* seedlings after exposure of mobile radiations

Sources of variations	df	MS									
		Germination	length	F.W	D.W	R.W.C	Protein	MDA	GPX	APX	CAT
Genotype(G)	1	1728.72**	16.40**	0.00028 ^{ns}	0.0094**	1487.85**	7500**	22.54 ^{ns}	6951.8**	5.46 **	0.000019 ^{ns}
Treatment (T)	1	5.109 ^{ns}	60.79**	0.0015*	0.00012 ^{ns}	111.26**	12033*	2339.72**	633.78 ^{ns}	6.148**	0.0152*
G X T	1	23.64 ^{ns}	8.89*	0.00000033 ^{ns}	0.000032 ^{ns}	12.20 ^{ns}	4033*	0.483 ^{ns}	616.77*	2.22*	0.00024 ^{ns}
Error	8	23.64	1.43	0.00214	0.000098	7.61	597.22	20.493	77.73	0.16	0.00039

F.W = Fresh Weight, D.W = Dry Weight, R.W.C= Relative Water Content, MDA = Malondialdehyde Contents, GPX= Guaiacol Peroxidase, APX= Ascorbate Peroxidase, CAT = catalase, **: Significant at 0.01, *: Significant and ns= non significant at 0.05

Table 2: Mean values of various morphological and biochemical parameters of mung bean and wheat seedlings, harvested after stressed and unstressed conditions

Genotype	Mung bean		Wheat	
	Control	Treatment	Control	Treatment
Germination	100	100	77.3	74.69
Length	13.08	10.3	12.46	6.24
F.W	0.256	0.233	0.246	0.223
D.W	0.031	0.021	0.084	0.081
R.W.C	92.6	88.52	72.34	64.24
Protein	283.3	236.6	340	250
MDA	30.78	59.77	33.86	61.45
GPX	0.557	0.753	34.35	63.2
APX	1.92	4.21	4.13	4.7
CAT	0.026	0.106	0.033	0.095

unstressed seedlings which reduced to 74.69% in stressed seedlings (Table 2).

Mean squares for seedling length possessed highly significant difference for genotype as well as treatment with significant interaction. There was non significant differences for genotypes as well as interaction with significant difference for temperature for F.W but for D.W the differences for treatments and interaction were non significant, however genotypes possessed significant difference. Similarly genotypes and treatments showed significant differences with non significant interaction for R.W.C. (Table 1). Seedlings length in both genotypes was reduced under stressed condition with more reduction in wheat seedlings as compared to mung bean. Similarly F.W, D.W and R.W.C were reduced in treated plants in comparison with control (Table 2).

Effects of mobile phone radiations on protein and antioxidant enzymes: Table of ANOVA (Table 1) showed significant differences in protein contents for genotypes, treatments as well as interaction. It was further observed that EMR caused reduction in protein, which was high in wheat (Table 2).

MDA contents possessed non significant differences for genotypes and interaction while treatment exhibited significant difference (Table 1). The MDA content was high in stressed samples as compared to unstressed ones of both genotypes (Table 2).

GPX activity exhibited significant differences for genotypes and interaction with non significant difference for temperature. Mean square values for APX showed that there were significant differences for genotypes, treatment and interaction between them. For CAT, the mean square values were non significant for genotypes and interaction, however treatment possessed significant differences. (Table 1). The GPX, APX and CAT activity were increased when mobile phone radiation was given to mung and wheat seedlings as compared to unstressed seedlings (Table 2).

DISCUSSION

In present research we found that EMR was unable to show any reduction on germination of mung but slightly reduced for wheat seedlings. There was reduction in mean seedling length, F.W, D.W and R.W.C of both genotypes under stress. Current results are in agreement with the findings of Sharma *et al.* (2009, 2010), reported that the mobile phone radiations significantly reduced the seedling length and dry weight of *V. radiata* after exposure for 0.5, 1, 2, and 4 h. Similarly these findings are in agreement with Singh and Prakash (2011) who reported that, Cell phone EMR inhibits Brassica root growth by inducing ROS-generated oxidative stress. Experiments conducted by many researchers reported that reduction in growth by mobile phone or simulated mobile phone radiation exposure can cause cell damage and reactive oxygen species formation which are the primary cause of DNA strand breaks and cell death (Oktem *et al.*, 2005; Oral *et al.*, 2006; Sokolovic *et al.*, 2008) It was also observed that when seedlings of different plant species placed in weak magnetic field, shown that the growth of their primary roots was inhibited during early germination stages in comparison with control as the cell reproductive cycle slows down due to the expansion of G₁ phase in many. (Barteri *et al.*, 2005).

Our results showed the inhibitory effect on various morphological parameters, with altered biochemical response. The contents of protein was reduced with enhanced MDA and various enzymes activities in our experiment after treatment of EMR-exposure in mung bean and wheat seedlings. Similar results were observed by Sharma *et al.* (2010), reported that the contents of proteins was reduced in EMR-exposed plants. However, the activities of proteases, alpha-amylases, beta-amylases, polyphenol oxidases, and peroxidases were enhanced in EMR-exposed radicles indicating their role in providing protection against EMR-induced stress. However the microwave pre treatment in wheat seedlings induces the enzyme activities of catalase and peroxidase, (Chen *et al.*, 2008)

Increased lipid per oxidation in term of melondialdehyde was also observed by Singh and Prakash (2011) and Tkalec *et al.* (2007). We observed increase in MDA content, these findings are in agreement with Ozguner *et al.* (2005) stated that electromagnetic radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems including human by increasing free radicals, which appear mainly to enhance lipid per oxidation, and by changing the antioxidant defense systems of human tissues.

CONCLUSION

From the current results, it is concluded that cell phones can negatively effect germination, relative water

content, fresh and dry weight of two entirely different plant genotypes. This reduction is associated with decrease in protein synthesis and increased membrane damage and antioxidant enzymes activity.

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