

Cell Phone Radiations Affect Early Growth of *Vigna radiata* (Mung Bean) through Biochemical Alterations

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The indiscriminate use of wireless technologies, particularly of cell phones, has increased the health risks among living organisms including plants. We investigated the impact of cell phone electromagnetic field (EMF) radiations (power density, $8.55 \mu\text{W cm}^{-2}$) on germination, early growth, proteins and carbohydrate contents, and activities of some enzymes in *Vigna radiata*. Cell phone EMF radiations significantly reduced the seedling length and dry weight of *V. radiata* after exposure for 0.5, 1, 2, and 4 h. Furthermore, the contents of proteins and carbohydrates were reduced in EMF-exposed plants. However, the activities of proteases, α -amylases, β -amylases, polyphenol oxidases, and peroxidases were enhanced in EMF-exposed radicles indicating their role in providing protection against EMF-induced stress. The study concludes that cell phone EMFs impair early growth of *V. radiata* seedlings by inducing biochemical changes.

Key words: Cell Phone Radiations, Seedling Growth, Biochemical Changes

Introduction

Accelerated and widespread use of different wireless technologies in the past few years has enhanced the exposure of living organisms to electromagnetic fields (EMFs). These technologies are continuously emitting a wide range of radiations (300 MHz–300 GHz) and include extremely low-frequency sources such as power lines and appliances, as well as high-frequency sources like radio, television, and more recently cell phones and their antennas (Elwood, 2003). Among these, mobile phones or cell phones are used indiscriminately and have become an integral part of modern telecommunications as they provide a continuous communication without any hindrance to movement of people. Therefore, the hazardous effects of EMFs on living systems are increasing (Berg, 1995; Goodman *et al.*, 1995). It has been documented that EMFs affect the cell protein (Kwee *et al.*, 2001), change the cell membrane characteristics (Goltsov, 1999), and alter the enzyme activity (Paulraj and Behari, 2002; Barteri *et al.*, 2004) and gene expression (Lee *et al.*, 2005) in animals. Additionally, radiofrequency EMFs induce lipid peroxidation and heat shock proteins, and elicit antioxidant response in hu-

man cells (Kwee *et al.*, 2001; Moustafa *et al.*, 2001; Leszczynski *et al.*, 2002).

The various effects of EMFs have been extensively studied on animals, humans and microorganisms, but very little work has been carried out on plants. Hart and Marino (1977) surveyed plant life near high-voltage transmission lines and observed a change in vegetation patterns. However, Tambiev and Kirikova (2000) noticed better growth and photosynthesis in the blue-green alga *Spirulina platensis* after treatment with radiofrequency EMFs. Likewise, the growth of rice plants was enhanced with an electric field of 28.5 kV m^{-2} compared to that without an electric field (Rotcharen *et al.*, 2003), whereas a reduction in wheat and corn yield was observed in fields near high-tension lines (Soja *et al.*, 2003). Tafforeau *et al.* (2002) showed that exposure to 900 MHz for 2 h resulted in induction of epidermal meristems and reduction in Ca^{2+} , Na^{+} , and K^{+} contents; however, there was no change in the divalent to monovalent cation ratios. Atak *et al.* (2003) reported that exposure to magnetic fields stimulated the root and shoot regeneration, and increased the fresh weight and total chlorophyll content in regenerated seedlings of soybean. Tkalec *et al.* (2005) re-

ported that the growth of *Lemna minor* was inhibited when exposed to 900 MHz for 2 h, however, no such change was observed with 400 MHz. Later, it was observed that exposure of *Lemna minor* to radiofrequency EMFs (400 and 900 MHz) for 2 h induced significant changes in lipid peroxidation, H_2O_2 content and activities of antioxidative enzymes, thus indicating that EMFs induce oxidative stress in plants (Tkalec *et al.*, 2007).

Though effects of EMFs have drawn the attention of biologists and environmentalists, still there is lack of literature regarding the effect of EMFs, particularly of mobile phones, on early growth and associated biochemical changes in plants. Therefore, a systematic and extensive study is necessary to explore the mechanism of action of EMFs in plants. Thus, a study was conducted to determine the effects of cell phone EMFs on germination and growth of mung bean (*Vigna radiata*). Furthermore, the changes in biomolecules and some vital enzyme activities were investigated in response to cell phone radiations so as to better understand the mechanism of action of mobile phones on early growth of plants.

Material and Methods

EMF treatment

Exposure to EMFs from cell phones was carried out in a closed shielded chamber (47.5 cm × 26 cm × 17.5 cm) that acts as a Faraday cage on the pattern of the mode stirred reverberation chamber (Sharma *et al.*, 2009). It was to assure equal distribution of the EMF to all the plant material placed inside the chamber without any outside interference. Two commercial GSM (global systems for mobile communication, 900-MHz band) cell phones were used in the present study. The radiated variable EMF – power density – was measured with the help of RF Power Density Meter (Orgone Biophysical Research Laboratory, Inc., USA). The average EMF power density was $8.55 \mu W cm^{-2}$. During exposure, cell phones were used in the conversation mode (listen + talk) attached with a voice recorder and vials containing seed were equidistantly kept between the cell phones for 0.5, 1, 2, or 4 h (at a distance of ~8 cm) (Sharma *et al.*, 2009). A set of seeds was placed in another chamber without cell phone EMF to serve as parallel control. All other mobile phones used inside and outside the exposure laboratory were eliminated during exposure

sessions. The chambers were maintained at a temperature of 25 °C.

Germination and growth studies

Healthy and certified seeds of mung bean [*Vigna radiata* (L.) Wilczek cv. ML-5] purchased locally from the market were surface-sterilized with sodium hypochlorite (NaOCl, 0.1%, w/v) and washed under running tap water followed by distilled water. Seeds of *V. radiata* were soaked in distilled water for 8 h (5 sets of 50 seeds each). After soaking, four of the test sets were exposed to the cell phone EMF for 0.5, 1, 2, or 4 h. The fifth set was unexposed and used as control. The highest temperature while exposing the test samples did not exceed 32 °C, which is in the temperature range considered favourable for the growth of *V. radiata*. The treated seeds were equidistantly placed in Petri dishes (Ø 15 cm; 10 seeds per dish, 5 dishes per treatment) and lined with a thin layer of wet cotton over Whatman #1 filter paper. They were allowed to germinate and grow for 7 d in an environmentally controlled growth chamber maintained at $(28/18 \pm 2) ^\circ C$, a 16 h light photoperiod of a photon flux density of about $240 \mu mol m^{-2} s^{-1}$, and relative humidity of $(75 \pm 2)\%$. On the seventh day, the number of seeds that germinated was counted, radicle and plumule length, and dry weight of emerged seedlings were determined. The roots of *V. radiata* seedlings were cut with a fresh blade and used for further biochemical and enzymatic assays.

Biochemical estimations

Estimation of total proteins and carbohydrates

Root tissue (200 mg) was homogenized in distilled water (10 ml). The mixture was centrifuged at $15,000 \times g$ for 45 min. The supernatant was used for the estimation of protein and carbohydrate contents. The protein content was estimated using the Folin-Ciocalteu reagent against bovine serum albumin as standard (Lowry *et al.*, 1951), while anthrone was used for the estimation of carbohydrates (Loewus, 1952).

Assays of enzyme activities

The activities of enzymes [proteases, EC 3.4.4.1; α -amylases, EC 3.2.1.1; β -amylases, EC 3.2.1.2; polyphenol oxidases (PPO) EC 1.14.18.1; and peroxidases (POX) EC 1.11.1.7] were assayed as per Batish *et al.* (2006). For this, the root tis-

sue (200 mg) was homogenized in 10 ml phosphate buffer (0.1 M, pH 7.0) in a pre-chilled pestle and mortar. Homogenates were centrifuged at $18,000 \times g$ for 45 min. The supernatant thus obtained was used for the enzyme assays. Proteases were estimated using casein (1%, w/v, in 0.1 M phosphate buffer, pH 7.0) as a substrate (Basha and Beever, 1975). The specific activity of α -amylases was determined using starch as a substrate (Muentz, 1977). The activity of β -amylases was assayed following Bernfeld (1951) and Dure (1960). Pyrocatechol (0.01 M in 0.1 M phosphate buffer, pH 6.0) was used for measuring the activity of PPO (Van Lelyveld and Pretorius, 1973), whereas hydrogen peroxide (0.2 M) was used for measuring the activity of POX (Batish *et al.*, 2006).

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) with five replications, each comprising of a single Petri dish containing 10 seeds. For the enzyme assay, there were five replicated, independent (tissue) samples. The experiments were repeated and data presented is the mean of two. The data were analyzed by one-way ANOVA followed by comparison of mean values using *post hoc* Tukey's test at $P \leq 0.05$.

Results and Discussion

Exposure of *V. radiata* to cell phone EMFs affected their germination and growth depending upon the time of exposure (Table I, Fig. 1). There was no change in seed germination when *V. radiata* seeds were treated for ≤ 2 h. However, exposure to an EMF for 4 h reduced the germination by 50% (Table I). Not only germination, but

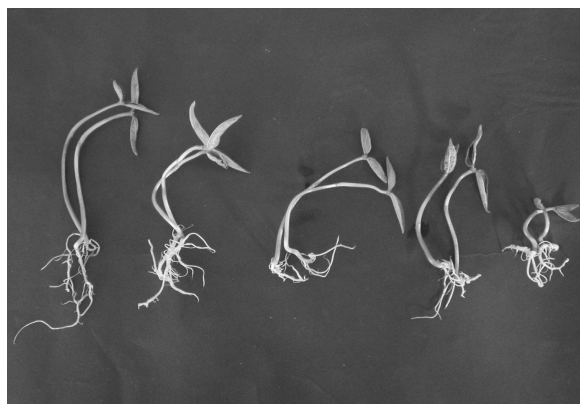


Fig. 1. Photograph showing the effect of cell phone EMF radiations on seedling growth of *Vigna radiata* (mung bean). From left to right: Seedlings emerged from seeds exposed for 0 (control), 0.5, 1, 2, and 4 h.

also seedling growth (in terms of radicle and plumule length) and seedling dry weight of *V. radiata* were adversely affected. Exposure to cell phone EMF for ≥ 1 h significantly (at $P \leq 0.05$) reduced the radicle and plumule length by ~ 11 –59% and 11–47%, respectively (Table I). The radicle length was declined by nearly 59% over control when *V. radiata* seeds were exposed to cell phone radiations for 4 h. On the other hand, 47% inhibition was observed in plumule length at exposure for 4 h (Table I). Likewise, the seedling dry weight of *V. radiata* was appreciably reduced by about 6–43% (significant at $P \leq 0.05$) in response to cell phone radiations exposure. In general, the inhibitory effect was more on radicle than on plumule length or dry weight (Table I, Fig. 1).

These observations are parallel to earlier studies of Apasheva *et al.* (2006) who observed a reduction in the germination capacity of winter

Table I. Effect of cell phone EMF radiations on germination and early growth of *Vigna radiata* measured one week after exposure.

Exposure time [h]	Germination (%)	Radicle length [cm]	Plumule length [cm]	Seedling dry weight [mg]
0 (Control)	100 \pm 0.0 a	7.8 \pm 0.12 a	5.8 \pm 0.04 a	12.5 \pm 2.06 a
0.5	100 \pm 0.0 a (0)	7.6 \pm 0.09 a (10.6)	5.2 \pm 0.06 b (10.5)	11.7 \pm 1.67 a (6.3)
1	100 \pm 0.0 a (0)	6.2 \pm 0.07 b (20.6)	5.0 \pm 0.07 b (14.7)	9.7 \pm 1.39 b (21.5)
2	100 \pm 0.0 a (0)	4.6 \pm 0.16 c (41.9)	3.6 \pm 0.08 c (39.1)	7.9 \pm 0.89 c (37.0)
4	50 \pm 1.78 b (50.0)	3.2 \pm 0.13 d (58.8)	3.1 \pm 0.06 d (47.1)	7.1 \pm 1.02 c (43.1)

Values in parentheses represent the percent decrease over control. Means with different letters in a column represent significant difference at $P \leq 0.05$, applying Tukey's test.

wheat and purple amaranth (*Amaranthus blitum* L.) upon exposure to an EMF of 0.3–0.7 mT for ≥ 1 h, whereas at lesser exposure times there was stimulation. Tkalec *et al.* (2005) observed that *Lemna minor* L. exposed to an electric field of frequency 900 MHz for 2 h significantly reduced the growth over control. Earlier, Soja *et al.* (2003) reported a reduction in yield and straw production of wheat and corn under high tension transmission lines with an EMF in the range of 0.4–4.5 mT. Pazur *et al.* (2006) reported that barley plants exposed to static magnetic and 50-Hz electromagnetic fields matching the Ca^{2+} cyclotron conditions (ICR) grew shorter (10–12%), with reduced plant weight and total pigment contents compared to a control without EMF. These workers concluded that an EMF affects the available *in vivo* Ca^{2+} levels and thus the regulatory processes (Pazur *et al.*, 2006). Sandu *et al.* (2005) demonstrated that exposure of black locust (*Robinia pseudoacacia* L.) seedlings to an ultra-high-frequency field (400 MHz) for > 2 h caused a logarithmic decrease in the ratio of chlorophyll *a* and *b*, and the content declined further with increase of the exposure time. In the present study, the inhibitory effect of EMFs was greater on radicle growth than on plumule growth. This indicates that cell phone EMFs affect the cell division resulting in reduced/impaired growth. In fact, the cell phone EMF radiations have been found to reduce the mitotic activity in an onion root tip bioassay (data not presented). These observations are further strengthened by a recent study of Tkalec *et al.* (2009) in which exposure of cells of onion to an EMF of 900 and 1400 MHz at field strengths of 41 and 120 V m^{-1} induced mitotic abnormalities including lagging chromosomes, vagrants, disturbed anaphases and chromosome stickiness and impairment of mitotic spindle. Aksenov *et al.* (2007)

opined that inhibition in germination and germination ability due to EMF exposure may be due to the desynchronization of the germination process by stimulation of the release of membrane-bound proteins and inhibition of the synthesis of complex structures during cell division.

Furthermore, to explore the reason for growth inhibition in young seedlings of *V. radiata* in response to cell phone radiations, some biochemical estimations were also undertaken. Exposure to cell phone EMF radiations caused a significant (at $P \leq 0.05$) reduction in total protein and carbohydrate contents in the roots of 7-day-old seedlings of *V. radiata*. The reduction in the carbohydrate content was greater compared to that in the protein content. Upon exposure to cell phone EMFs for 0.5 h, there was over 55% reduction in the carbohydrate content in *V. radiata* roots compared to ~8% loss in the protein content (Table II). The content of proteins and carbohydrates declined with increase of the exposure time in a dose-dependent manner. Upon an exposure for 4 h, the protein content declined by nearly 58%, whereas the carbohydrate content was reduced by nearly 76% (Table II).

In the present study, the reduction in the content of macromolecules (proteins and carbohydrates) is not surprising since a weak EMF is reported to generate free radicals and affect the status of biochemicals and thus the physiological processes in plants (Monselise *et al.*, 2003). It is accompanied by a number of endo- and exogenous factors including signal molecules (Monselise *et al.*, 2003). The interaction of cell phone EMF radiations with growth and physiology of plants seems to involve signal pathways, as has been suggested for animal cells exposed to high-frequency radiations (de Pomerai *et al.*, 2002). The reduced content of proteins and carbohydrates in roots of *V. radiata*

Table II. Effect of cell phone EMF radiations on total content of proteins and carbohydrates in roots of *Vigna radiata* measured one week after exposure.

Exposure time [h]	Proteins [mg g ⁻¹ DW]	Carbohydrates [mg g ⁻¹ DW]
0 (Control)	61.8 \pm 2.25 a	48.0 \pm 2.32 a
0.5	56.8 \pm 1.27 b (8.1)	21.3 \pm 1.29 b (55.6)
1	33.3 \pm 2.05 c (46.1)	17.1 \pm 1.46 c (64.4)
2	31.8 \pm 1.73 c (48.5)	13.5 \pm 0.69 d (71.9)
4	26.1 \pm 1.24 d (57.8)	11.5 \pm 0.72 e (76.0)

Values in parentheses represent the percent decrease over control. Means with different letters in a column represent significant difference at $P \leq 0.05$, applying Tukey's test.

indicates their rapid hydrolysis to overcome the stress caused by cell phone radiations. These results are in agreement with an earlier study which showed that increased synthesis and disintegration of proteins occurs in plant roots in response to weak magnetic fields (Belyavskaya, 2004).

The decrease in the protein content upon exposure to cell phone EMFs was accompanied by a significant (at $P \leq 0.05$) increase in the specific activity of proteases – the protein hydrolyzing enzymes. The protease activity increased by ~2.6-times after 0.5 h of exposure to a cell phone EMF and it further increased with the exposure time (Table III). Upon an exposure for 4 h, the protease activity was nearly 11.9-times higher than that of the control thus indicating an enhanced protein hydrolysis. Like proteases, the activities of α - and β -amylases also enhanced significantly in *V. radiata* roots exposed to cell phone radiations. A greater increase was observed in the activity of β -amylases (2.08- to 15.55-times over control) compared to that of α -amylases (1.02- to 2.44-times over control) (Table III). With an exposure to cell phone EMFs for 4 h, the activity of α -amylases was nearly 2.4-times compared to the control. On the other hand, the β -amylase activity increased to a much higher level and was ~15.55-times higher compared to the control. Besides hydrolytic enzymes, the activities of PPO and POX (oxido-reductases), another important class of enzymes, also increased significantly ($P \leq 0.05$) in response to cell phone radiations in a time-dependent manner (Table III). The PPO activity increased in the range of 1.4-times (at 0.5 h exposure) to 8.5-times (after 4 h of exposure). In contrast, the POX activity increased in the range of 1.57- to 6.07-times upon exposure to cell phone EMF (from 0.5–4 h) compared to

the control. Upon exposure to cell phone radiations for 4 h, the PPO activity was 8.5-times more over control, whereas the POX activity was over 6-times higher than in that of control *V. radiata* roots (Table III).

The enhanced activity of hydrolytic enzyme proteases in response to cell phone radiations indicates an increased breakdown of proteins. The high activity of amylases (α - and β -) in *V. radiata* roots indicates a greater hydrolysis of reserved polysaccharides resulting in an increased generation of sugars. Possibly, this increased supply of sugars is required to meet the enhanced energy demands in cell phone EMF-exposed roots of *V. radiata* compared to the control (unexposed). It was strengthened from an earlier study that the activity of β -amylases enhances during stress induction (Kaplan and Guy, 2004). Of late, the studies have demonstrated that EMF radiations induce oxidative stress in the plant tissue which correlates to inhibition in germination and radicle growth (Monselise *et al.*, 2003; Tkalec *et al.*, 2007). Recently, Rochalska and Grabowska (2007) reported an increased activity of glutathione *S*-transferases in wheat seedlings exposed to low-frequency magnetic fields thereby indicating higher resistance levels towards stress caused by magnetic fields.

In fact, the enhanced activities of PPO and POX in the present study, in EMF-treated seedlings, indicate the induction of stress upon EMF exposure – a type of abiotic stress. These enzymes play an important role in encountering the stress, and provide resistance against a variety of abiotic stresses to plants (Alscher and Hess, 1993). The POX activity was enhanced (41%) in duck weed (*Lemna minor* L.) after exposure to an electric field of 900 MHz for 2 h (Tkalec *et al.*, 2005). Lat-

Table III. Effect of cell phone EMF radiations on specific activities of proteases, α - and β -amylases, polyphenol oxidases (PPO), and peroxidases (POX) in roots of *Vigna radiata* measured one week after exposure.

Exposure time [h]	Proteases [$\mu\text{g h}^{-1} \text{mg}^{-1}$ protein]	α -Amylases [$\mu\text{g min}^{-1} \text{mg}^{-1}$ protein]	β -Amylases [$\mu\text{g min}^{-1} \text{mg}^{-1}$ protein]	PPO [$\text{kat s}^{-1} \text{mg}^{-1}$ protein]	POX [$\text{kat s}^{-1} \text{mg}^{-1}$ protein]
0 (Control)	18.8 \pm 0.51 a	13.1 \pm 0.02 a	6.4 \pm 0.14 a	0.10 \pm 0.01 a	0.14 \pm 0.02 a
0.5	48.2 \pm 0.92 b	13.4 \pm 0.03 a	13.3 \pm 0.21 b	0.14 \pm 0.01 a	0.22 \pm 0.01 b
1	68.2 \pm 1.06 c	15.2 \pm 0.04 b	26.4 \pm 1.21 c	0.22 \pm 0.01 b	0.29 \pm 0.02 c
2	116.6 \pm 2.84 d	19.9 \pm 0.10 c	49.5 \pm 1.04 d	0.41 \pm 0.02 c	0.41 \pm 0.01 d
4	224.1 \pm 2.14 e	31.9 \pm 0.24 d	99.5 \pm 2.01 e	0.85 \pm 0.02 d	0.85 \pm 0.01 e

Values in parentheses represent the percent decrease over control. Means with different letters in a column represent significant difference at $P \leq 0.05$, applying Tukey's test.

er, it was observed that exposure to a non-thermal EMF of 400 and 900 MHz induced oxidative stress in duck weed and significantly increased the MDA and H₂O₂ content, and enhanced the activities of catalases (Tkalec *et al.*, 2007). However, it depends upon frequency, field strength, modulation, and exposure time to an EMF (Tkalec *et al.*, 2007). Recently, Roux *et al.* (2008) reported that exposure to a 900-MHz EMF caused reduction in the levels of ATP that acts as a signal molecule triggering the production of reactive oxygen species. Earlier, Monselise *et al.* (2003) found that alanine is accumulated in duck weed upon exposure to low-intensity variable magnetic fields (of 60 and 100 Hz). Similar to heat shock proteins in animals, alanine acts as a stress signal in plants,

and is produced due to the generation of free radicals by EMF exposure (Monselise *et al.*, 2003). Parola *et al.* (2005) demonstrated that treatment of *Spirodela oligorrhiza* (an aquatic plant) with a variable magnetic field induces metabolic stress linked to free radical generation.

In conclusion, the present study depicts that cell phone radiations retard the germination and growth of seedlings in plants. The inhibition in growth is accompanied by changes in macromolecules and enzyme activities. However, whether the observed inhibition of germination and radicle growth and changes in biochemicals in *V. radiata* roots in relation to cell phone EMFs is due to the induction of oxidative stress was not evaluated in the present study.

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