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EFFECT OF MOBILE PHONE EMF ON BIOCHEMICAL CHANGES IN EMERGING SEEDLINGS OF *PHASEOLUS AUREUS* ROXB.

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ABSTRACT

During the last decade or so, there has been a widespread increase in the usage of mobile phones resulting in increased EMF radiations in the environment. A study was planned to explore the effects of EMF radiations from mobile phones on the early growth and biochemical changes in emerging seedlings of *Phaseolus aureus*. It was observed the radiations from mobile phone reduced the radicle and plumule growth (after 48 h) and decreased the amount of proteins and carbohydrates in radicles. The activities of hydrolytic enzyme -proteases and amylases- and the stress related enzyme peroxidases increased upon exposure to mobile phone radiations. The study concludes that radiations from mobile phone interfere with the biochemical processes in radicles and affect their elongation.

KEY WORDS
Mobile phone radiations
Radicle growth
Hydrolytic enzymes
Peroxidases, Protein content

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INTRODUCTION

During the last decade or so, there has been a widespread increase in the usage of cell phones resulting in increased electromagnetic radiations in the environment, sometimes referred to as electromagnetic smog. An enhanced level of radiations in the environment has further increased the exposure risks to living organisms. Most of the studies pertaining to ill-effects or risk-analysis of cell phone radiations have been conducted on animals, including humans or microorganisms (Busljeta *et al.*, 2004; Meral *et al.*, 2007). It has been reported that in animals electromagnetic radiations affect cell proliferation, enzyme activity, cell-membrane permeability, and even reproductive capacity (Busljeta *et al.*, 2004; Dimitris *et al.*, 2004; Meral *et al.*, 2007). In spite of these reports indicating ill-effects of mobile phone radiations on physiology and biochemistry in animal systems, little work has been done in plant systems. Ssawastin (1930) was first who observed the effects of magnetic field on plant growth. Further, most of the available reports pertain largely to the effect of electromagnetic fields generated from electrical installations or radio frequency that is lower than mobile phone frequency (Hart and Marino, 1977; Johnson *et al.*, 1979; Soja *et al.*,2003). Nevertheless, there have been some studies with simulated systems emitting same frequency / wavelength of radiations as that of mobile phones. For example, Tkalec *et al.*, (2005) reported that electromagnetic radiations of 900 MHz inhibit growth of Lemna minor after 2 h of exposure. With this background in mind, we conducted a study to explore the effect of electromagnetic radiations on radicle elongation and changes in amounts of proteins, and carbohydrates, and activities of proteases, amylases, and peroxidases in *Phaseolus aureus*.

MATERIALS AND METHODS

Exposure to mobile phone electromagnetic radiations

It was done in an insulated closed shielded chamber with no radiation interference from outside. Pre-imbibed (for 8 h) seeds of *Phaseolus aureus* purchased from market were taken in glass vials and placed in between two mobile phones (GSM technology). The phones were kept in talk mode and seeds (100 seeds per treatment) were exposed for 1, 2, and 4 h. For control, a parallel set of seeds was maintained in another chamber but without mobile phones. After exposure, seeds were used for growth bioassay. The entire process of exposure was done at temperature of 25ºC under controlled conditions.

Growth bioassay

After exposure, the seeds were placed in 15 cm diameter Petri dishes each containing 10 seeds. There were ten replicates (as Petri dishes) for each treatment including control. Petri dishes were placed in a randomized block design in a seed germinator (Caltan make, Narang Scientific Works, New Delhi) under temperature of 30 (±2)°C, and a 16 h light photoperiod of photon flux density of ~250 μmol $m^{-2}s^{-1}$. After 48 h, radicle emergence was noted and the radicle and plumule length were measured. Roots were excised and stored in a freezer for further biochemical analysis.

Biochemical analyses

For estimation of total proteins and carbohydrates content, ~250 mg of roots were homogenized in 10mL of distilled water, centrifuged at 15,000 × g for 45 min and the supernatant collected was stored at 4°C for further analysis. The protein content was estimated as per Lowry *et al.*, (1951), whereas carbohydrates were estimated as per Loewus (1952).

For enzymatic analysis, roots were homogenized in 0.1M phosphate buffer (pH 7.0), centrifuged at 18,000 × g for 30 min. The supernatant was used for assaying activities of hydrolytic enzymes –proteases and amylases, and stress marker enzyme peroxidases. Activity of proteases was assessed using 1% (w/v) casein as a substrate (Basha and Beevers 1975). α-Amylases was determined using starch as per Muentz (1977), β-amylases as per Dure (1960), and peroxidases activity was determined as per Batish *et al.*, (2006).

Statistical analysis

For growth bioassay, there were ten independent Petri dish replicates. Each biochemical analysis involved at least five independent tissue samples. The data were analyzed by one-way analysis of variance and then by Tukey's test (SPSS,2002).

RESULTS

The radicle growth was significantly reduced in the seedlings emerging from mobile phone electromagnetic radiation exposed *P. aureus* seeds (Table 1). Radicle length decreased by ~17% in seedlings emerging from seeds exposed to mobile phone radiations for 1 h compared to unexposed control. The reduction in radicle length was greater in seeds exposed for higher duration to mobile phones. Parallel to radicle length, plumule length also decreased significantly (Table 1). In general, the inhibition was greater in radicle than in plumule. We therefore assessed the changes in the associated biochemical aspects during early growth of radicle.

Table 1: Radicle and plumule length of *P. aureus* seedlings measured 48 h after emergence from mobile phone radiation exposed seeds

Period of exposure (h)	Radicle length (cm)	Plumule length (cm)
0 (Control)	2.64 ± 0.10 a	2.70 ± 0.09 a
1	2.20 ± 0.05 b	2.02 ± 0.09 b
2	1.94 ± 0.20 c	1.80 ± 0.04 c
4	1.48 ± 0.07 d	1.64 ± 0.08 c

Different alphabets in a column represent significant difference at $p<0.05$ applying Tukey's test.

The contents of water-soluble proteins and carbohydrates were lesser in radicles emerged from mobile phone radiation exposed seeds (Table 2). In general, the level of reduction was greater in protein content than of carbohydrates. Total protein content decreased by ~ 18%, 38% and 72% in radicles emerged from seeds exposed to mobile phone radiations for 1, 2, and 4 h, respectively. In contrast, the reduction in carbohydrate content was only 6%, 34%, and 49%, respectively (Table 2).

Table 2: Changes in contents of total proteins and carbohydrates in roots of *P. aureus* seedlings measured 48 h after emergence from mobile phone radiation exposed seeds

Period of exposure (h)	Total protein content (mg g^{-1} f.w.)	Water-soluble carbohydrates (mg g^{-1} f.w.)
0 (Control)	32.1 ±1.37 a	8.2 ±0.15 a
1	26.4 ±1.05 b	7.7 ±0.09 b
2	12.3 ±1.27 c	5.4 ±0.08 c
4	9.2 ±1.28 d	4.2 ±0.11 d

Different alphabets in a column represent significant difference at $p<0.05$ applying Tukey's test. f.w.: Fresh weight

Contrary to the decrease in the content of macromolecules (proteins and carbohydrates), the activities of three hydrolytic enzymes (proteases, α-amylases, β-amylases) and peroxidases increased sharply in radicles upon mobile phone radiation exposure (Table 3). Activity of proteases almost doubled in seedlings emerging from 1 h exposed seeds. It increased further and was 6- and 9-folds higher than unexposed control after 2 and 4 h exposure (Table 3). Parallel to proteases activities of both α-and β-amylases increased in seedlings from mobile phone exposed seeds (Table 3). In general, these increased by 1.5- to 9.7-folds over that of unexposed seedlings (Table 3). Likewise, the activity of stress-related enzyme peroxidases was also higher in mobile phone exposed seedlings. In seedlings emerging from 4 h exposed seed, peroxidases activity was >9.0-folds higher than of unexposed control (Table 3).

DISCUSSION

The present study depicts that electromagnetic radiations

Table 3: Changes in specific activities of proteases, amylases and peroxidases in roots of *P. aureus* seedlings measured 48 h after emergence from mobile phone radiation exposed seeds

Exposure time (h)	Proteases (μg $h^{-1}mg^{-1}$ protein)	α-Amylases (μg $min^{-1}mg^{-1}$ protein)	`β-Amylases (μg $min^{-1}mg^{-1}$ protein)	Peroxidases (kat $sec^{-1}mg^{-1}$ protein)
0 (Control)	57.4 ± 2.51 [a]	56.1 ± 2.85 [a]	5.3 ± 0.42 [a]	4.5 ± 0.03 [a]
1	113.6 ± 7.62 [b]	122.2 ± 8.67 [b]	8.0 ± 1.01 [a]	10.2 ± 0.59 [b]
2	348.0 ± 12.67 [c]	303.3 ± 16.91 [c]	22.5± 2.34 [b]	34.5 ± 1.15 [c]
4	509.1 ± 21.53 [d]	497.2 ± 12.08 [d]	51.3 ± 2.68 [c]	41.4 ± 0.67 [d]

Different alphabets in a column represent significant difference at $p<0.05$ applying Tukey's test.

from mobile phones inhibit the radicle and plumule growth in growing seedlings. Such an inhibitory effect of electromagnetic radiation is in agreement with few earlier studies. For example, exposure of *Lemna minor* to electric field (900 MHz) for 2 h reduced seedling growth (Tkalec *et al.*, 2005). Parallel to our observation, an inhibition in root growth of pea, barley and millet was measured after 48 h exposure to weak magnetic forces (Kursevich and Travkin, 1973). Not only the seedling growth, even the seedling biomass has been reported to decrease in response to electromagnetic radiations (Lebedev *et al.*, 1977). Though these studies point to the inhibitory activity of electromagnetic radiations, yet their mechanism of action is unknown. Nevertheless, a few studies have reported changes in the physiological parameters like chlorophyll pigments (Pazur *et al.*, 2006) and decrease in mitotic activity (Tkalec *et al.*, 2009). The greater inhibitory activity of mobile phone radiations upon the radicle length observed in present study also point to its interference with cell division in the growing root tips. In this direction, a few studies have reported that electromagnetic radiation of 900 MHz inhibit mitotic activity in onion root tips and induce abnormalities (Selga and Selga, 1996; Tkalec *et al.*, 2009).

In the present study a reduction in the proteins and carbohydrates content and increase in activities of hydrolytic enzymes was observed after exposure to mobile phone electromagnetic radiations. Possibly, the hydrolysis of the macromolecules was required to provide extra energy to overcome the stress induced by mobile phone radiations. It is strengthened by the earlier reports that electromagnetic radiations induce generation of reactive oxygen species (Monselise *et al.*, 2003) and cause oxidative damage (Tkalec *et al.*, 2007). The greater hydrolysis of proteins and carbohydrates was paralleled to increased activities of hydrolytic enzymes observed in present study. Of late, Roux *et al.* (2008) reported that electromagnetic field induce stress-related transcripts indicating oxidative stress and altered enzyme activity as one of the modes of their action. Earlier studies have reported that activity of stress related enzymes increases significantly during exposure to electromagnetic fields indicating their role in providing protection against induced stress (Rochalska and Grabowska, 2007; Tkalec *et al.*, 2007). Earlier, Tkalec *et al.*, (2007) reported that activity of stress-marker enzyme peroxidases increased significantly in response to 900 MHz electromagnetic fields and is parallel to the observations made in the present study.

Thus based on the results obtained in present study, we conclude that mobile phone electromagnetic radiations inhibit radicle emergence through interference with associated biochemical changes.

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