

To Investigate the Effect of Electromagnetic Radiations on Flavonoids of Lettuce Species

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Escalation in use of wireless electronic equipments such as mobile phone, Wi-Fi routers and microwave ovens has rapidly increased the risk of damage in tissue level for all living organisms not only for humans and animals but plants as well. The cell phone is in increasing day by day so we are investigating the positive or negative impact of cell phone radiations on secondary metabolites and morphological parameters of plants. In this current study we are focusing on the effect of electromagnetic radiations on flavonoids of lettuce species at 2G and 3G frequencies for different exposure times. The seeds of lettuce were exposed to cell phone radiations for half hour, 2 hours, 4 hours and 6 hours and later were compared with control. It was concluded that there was reduction in germinating percentage, shoot length, root length and leaf area while fresh weight, dry weight increased. The concentration of flavonoids increased with the increase in the exposure time. As the flavonoids are antioxidant and anti-inflammatory compounds, the use of electromagnetic radiations will help in increased production of such substances and will become the good herbal antioxidant source.

Keywords: Electromagnetic radiations, wireless, electronic equipments.

The readily increase in the rate of cell phones, diverted all the researchers to detect the effect of radio waves on living organisms not only on humans but also on plants. Many studies suggested adverse health symptoms which are caused by mobile phone radiations. Exposure to these mobile phone radiations can cause ill effects; cell damage by formation of reactive oxygen species and ultimately cell death. These radiations are of two types and that are- ionizing and non-ionizing radiations and both of them affect the environment, human and plants. These radiations

can be harmful to organisms and result in changes to the natural environment (Hoong, 2003⁸). The foremost increasing use of mobile phones has affected life of all the organisms and troubled the environment. By using cell phones, the radiations are transferred to the body which causes health problems and they are well known to affect the neurons as they interfere in connectivity of two neurons and that causes many health issues in humans. They cause high blood pressure, fatigue, deafness, migraines and headaches (K Bhargavi, 2013⁹).

Now a days there are various types of cell phone technologies are available for consumers that are 1G, 2G, 3G and 4G but out of them mostly used ones are 2G and 3G. The frequency range of 2G is 900-1800 MHz. Radiations that are emitted by

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the mobile phones are harmful for both plants and animals (Roosli, 2008¹⁶). 3G operates at the frequency of 2100 MHz. A cell phone has a specific SAR value that is Specific absorption rate (K Bhargavi, 2013⁹).

Lettuce (*Lactuca sativa*) is an annual herb that belongs to daisy family Asteraceae. Lettuce (*Lactuca sativa L.*) is one of the most popular among the salad vegetable crops (Whitaker 1969²⁰). Nitrogen is required at a very high rate for the growth and development of lettuce. Lettuce is regarded as the first cultivated crop that is being commercialized internationally (Rayyan A et al, 2004¹⁴). Some compounds that belong to the flavonoids, alkaloids are used as drugs or taken in diet which helps in curing or preventing diseases (Raskin et al, 2002¹³).

All flavonoids have the same basic structure that is a three-ringed molecule. Different flavonoids in a group differ from each other by the number and position of substituents (the hydroxy, methoxy, or sugar groups) (Kumar and Pandey, 2013¹⁰). These flavonoids possess remarkable biochemical actions like anti-inflammatory, antioxidant, antiallergic, antiviral and anticarcinogenic activities (Hohl et al, 2001⁷; Ren et al, 2003¹⁵).

We are focusing on the effect of cell phone radiations on Lettuce species which will help us in detecting the effect of 2G and 3G radiations on germination percentage, morphological parameters and in the variation of flavonoid content of control and irradiated seedlings of lettuce species.

MATERIALS AND METHODS

Material required

2G and 3G mobile phones, Soxhlet apparatus, Rotary vacuum evaporator, UV-spectrophotometer.

Chemicals required

Methanol, distilled water, Ferric chloride, Sodium hydroxide, Hydrochloric acid, Lead acetate, Magnesium fillings, Silica gel.

Plant material

The seeds of Lettuce (*Lactuca sativa*) were collected from licensed seed store of Phagwara, Punjab. The radiation exposure was given to seeds at different frequencies and different

time duration. The first seeds set and second set were exposed to 2G and 3G frequencies for time intervals of 30 minutes, 2 hours, 4 hours and 6 hours respectively (Fig 1). Then the irradiated seeds were sown in pots along with the non-irradiated seeds which were used as control so that we can compare the irradiated and non-irradiated plants.

Morphological analysis

Five seeds were germinated in petriplates according to the time of exposure for analyzing germination percentage, fresh weight, dry weight; seedling length and for morphological analysis; shoot length, root length and leaf length was measured in 2G and 3G irradiated plants and that was compared with the control ones. After 40-45 days of sowing, lettuce plants were observed for morphological analysis. Washed under tap water then shade dried them for 2-3 days and further drying was done with the help of hot air oven and then grinded to powder form by mechanical grinding. The powdered plant material was used for the extraction of flavonoids which was done with the help of soxhlet apparatus.

Biochemical analysis

Tests for flavonoids: Some tests were done for the tests of flavonoids and that are given below: (Mahesh et al, 2013¹, Agarwal et al, 2012³).

Shinoda Test

To the sample, add 5ml of 95% ethanol and then few pieces (0.5 g) of magnesium turnings were added to this solution followed by few drops of concentrated hydrochloric acid. Observance of pink coloration confirms the presence of flavonoids.

Lead Acetate Test

To the sample, add lead acetate solution which causes formation of yellow precipitate that shows the presence of flavonoid.

Sodium Hydroxide Test

The addition of an increasing amount of sodium hydroxide, the sample containing flavonoids showed yellow coloration which rapidly increased and this decolorized after addition of acid.

Ferric chloride test

To the sample, few drops of ferric chloride solution were added by which formation of blackish red color indicated presence of flavonoids.

Thin layer chromatography

Silica gel was applied on TLC Plates and

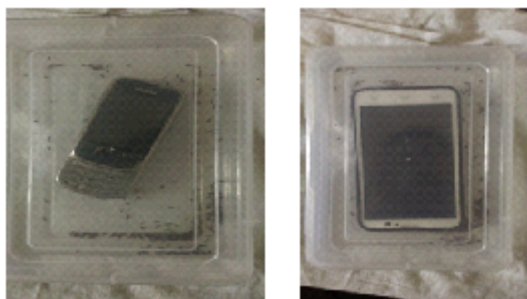


Fig. 1. Radiation exposure to seeds by 2G and 3G cell phones

was kept in hot air oven for 3 hours. Chloroform: Methanol with ratio 9:1 was used as the mobile phase. The TLC plates were marked with the sample spot. The solvent front, sample spot was marked, and the plate was finally allowed to dry. The bands that formed were separated by detecting in UV light of 254nm, 366nm and the Colorless components were detected by using visualizing agent, Anisaldehyde spray (A. R Mahesh et al, 2013¹).

Spectrophotometric analysis

For determination of total flavonoid content spectrophotometer was used. Quercetin

Table 1. Morphological parameters of *lettuce seedlings* irradiated at 2G cell phone radiations.

Time exposure	Germination percentage		Seedling length (in Cm)		Fresh weight (in grams)		Dry weight (in grams)	
	R1	R2	R1	R2	R1	R2	R1	R2
Control	100 %	100 %	4.88	4.86	0.07	0.07	0.001	0.001
30 MINS.	60%	80%	4.89	4.92	0.06	0.07	0.001	0.001
2 HOURS	80%	60%	4.96	5.25	0.07	0.06	0.002	0.002
4 HOURS	80%	60%	5.41	5.93	0.07	0.08	0.002	0.002
6 HOURS	60%	60%	6.01	5.35	0.08	0.06	0.003	0.004

Table 2. Standard Deviation Analysis for Morphological Parameters of *lettuce seedlings* irradiated at 2G cell phone radiations.

Exposure time	Germination percentage	Seedling length	Fresh weight
CONTROL	100±0	4.87±0.014	0.07±0
30 MINS.	70±14.142	4.905±0.021	0.065±0.007
2 HOURS	70±14.142	5.105±0.205	0.065±0.007
4 HOURS	70±14.142	5.67±0.367	0.075±0.007
6 HOURS	60±0	5.68±0.466	0.07±0.014

Table 3. Morphological parameters of *lettuce seedlings* irradiated at 3G cell phone radiations

Time exposure	Germination percentage		Seedling length (in Cm)		Fresh weight (in grams)		Dry weight (in grams)	
	R1	R2	R1	R2	R1	R2	R1	R2
Control	100 %	100 %	4.88	4.86	0.07	0.07	0.001	0.001
30 MINS.	80%	60%	4.92	4.95	0.07	0.06	0.002	0.002
2 HOURS	60%	80%	5.30	5.15	0.08	0.07	0.002	0.001
4 HOURS	60%	60%	5.24	5.94	0.07	0.08	0.003	0.002
6 HOURS	60%	40%	6.31	6.22	0.08	0.06	0.003	0.004

was taken as standard and was prepared by mixing 1 milligram in 1 milliliter of methanol and the optical density was measured at 415 nm by using UV spectrophotometer. The standard curve and the optical density of extracts were compared (Agarwal et al, 2012⁴).

RESULTS AND DISCUSSION

Morphological analysis

The table 1 shows that the germination percentage decreased in 2G irradiated seeds as compare to control ones whereas there was increase in the seedling length, fresh weight and dry weight.

The table 3 shows that the germination

Table 4. Standard Deviation Analysis for Morphological Parameters of *lettuce seedlings* irradiated at 3G cell phone radiations.

Exposure time	Germination percentage	Seedling length	Fresh weight
CONTROL	100±0	4.87±0.014	0.07±0
30 MINS.	70±14.142	4.935±0.021	0.065±0.007
2 HOURS	70±14.142	5.225±0.106	0.075±0.007
4 HOURS	60±0	5.59±0.494	0.075±0.007
6 HOURS	50±14.142	6.265±0.636	0.07±0.014

Table 5. Morphological parameters of *lettuce plant* emerging from seeds irradiated at 2G cell phone radiations

Exposure time	Shoot length (in cm)	Root length (in cm)	Leaf length (in cm)
CONTROL	4.5	7.5	16.3
30 MINS.	4.5	7.4	16
2 HOURS	4.1	7.0	15.7
4 HOURS	3.9	6.6	15.5
6 HOURS	3.5	6.2	15.4

percentage decreased in 3G irradiated seeds as compared to control ones whereas there was increase in seedling length, fresh weight and dry weight

The reduction in germination percentage was observed more in 3G irradiated seeds as compared to 2G where as there was increase in seedling length, fresh weight, dry weight in 3G irradiated seeds as compared to 2G irradiated seeds.

Morphology of plant

The plant morphology can be observed by measuring root length, shoot length and leaf length.

There was decrease in the shoot length, root length and leaf length in plants emerging from 2G irradiated seeds as compared with those of controlled plants (Table 5).

There was decrease in the shoot length, root length and leaf length in plants emerging from 3G irradiated seeds as compared with those of controlled plants and also there was more decrease in shoot length, root length and leaf length in plants emerging from 3G irradiated seeds as compared to 2G irradiated ones.

Biochemical analysis

The table 7 shows the intensity of flavonoids present with respect to change of colour in each test.

Table 6. Morphological parameters of *lettuce plant* emerging from seeds irradiated at 3G cell phone radiations

Exposure time	Shoot length (in cm)	Root length (in cm)	Leaf length (in cm)
CONTROL	4.5	7.5	16.3
30 MINS.	4.3	7.0	13
2 HOURS	4.0	6.5	12.7
4 HOURS	3.8	6.0	12.2
6 HOURS	3.0	5.7	11.8

Table 7. Phytochemical screening

Test	control	2G	2G	2G	2G	3G	3G	3G	3G
		½ Hour	2 Hour	4 Hour	6 Hour	½ Hour	2 Hour	4 Hour	6 Hour
Shinoda test	+	+	+	++	++	+	++	+++	+++
Lead acetate test	+	+	+	++	++	+	++	+++	+++
Sodium hydroxide test	+	+	+	++	++	+	++	+++	+++
Ferric chloride test	+	+	+	++	++	+	++	+++	+++

Spectrophotometric analysis

Fig 2 shows the standard curve of Quercetin which was taken as standard compound.

The above table and graph shows that the absorbance increased in 2G and 3G samples as compared to the control ones. This shows that the concentration of flavonoids is increasing with the increase in exposure time of radiations.

DISCUSSION

The effects of radiations on the morphological parameters and secondary metabolites have been discussed above and that showed the increase in the seedling length, fresh

weight and dry weight in the seeds that were exposed to radiations as compared to the control ones.

Much work has already accomplished by various workers on different aspects of Lettuce (Boroujerdnia and Ansari, 2007⁴; Burda and Oleszek, 2001⁵; Coelho et al, 2005⁶). As discussed by Rana and Parihar, 2014¹²; that the radiations cause reduction in the germination percentage, seedling length, fresh weight and dry weight of seedlings. But in current study there was a reduction in germination percentage while other morphological parameters of seedlings. In the previous study the biochemical parameters of the plant increased and in current study there was also an increase in the content of secondary metabolites.

Ragha et al 2011¹¹ concluded that the plant height and root length was decreased where as biomass percentage was increased with the increase in the frequency, the current study also showed decrease in the root length. Similar results were reported by Sharma and Parihar, 2014¹⁷ while checking the effects of mobile radiations on nodule formation in the leguminous plants.

Afzal and Mansoor, 2012² examined the effect of cell phone radiations on morphology of Mung bean (*Vigna radiata*) and Wheat (*Triticum*

Table 8. Rf value of different samples

Exposure Time	Rf Value	
Standard Quercetin	0.93	0.93
	2g	3g
Control	0.87	0.87
30 Minutes	0.87	0.89
2 Hours	0.88	0.90
4 Hours	0.90	0.90
6 Hours	0.91	0.92

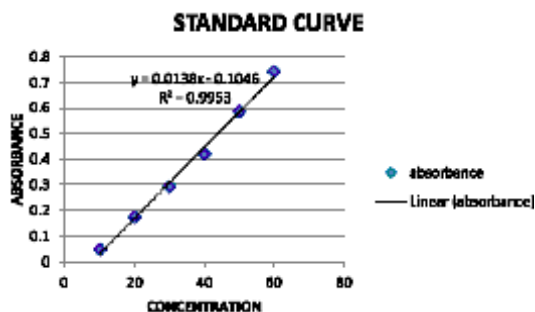


Fig. 2. Standard curve of Quercetin at 415 nm

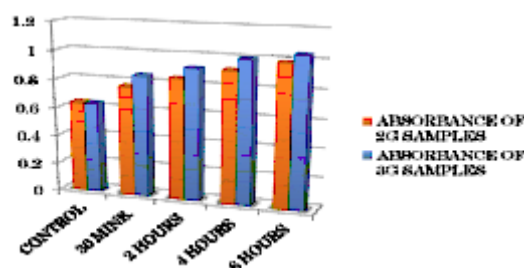


Fig. 3. Absorbance of 2G and 3G samples

Table 9. Absorbance Of Samples

Exposure Time	Absorbance At 415nm		Concentration In µg/MI	
	2g	3g	2g	3g
Control	0.632	0.632	38.217	38.217
30 Minutes	0.765	0.845	47.855	53.652
2 Hours	0.842	0.916	53.434	58.797
4 Hours	0.915	0.994	58.724	62.347
6 Hours	0.984	1.04	63.724	67.782

aestivum) seedlings and concluded that cell phone radiations induce the oxidative stress and caused reduction in growth but increased the antioxidant enzymes activity and like the present study that also showed the reduction in growth whereas increased the flavonoid content in romaine lettuce. Similarly mung bean roots were checked for mobile phone radiation by Sharma et al, 2011¹⁸ whereas Sheikh, 2008¹⁹ checked the resistance of *Aloe vera* against microwaves.

CONCLUSION

The present research can be concluded that the cell phones radiations affects the morphological parameters in romaine lettuce and cause increase in the seedling length, fresh weight and dry weight whereas it causes reduction in the germination rate, shoot length, root length and leaf length (Table 1-6). The Rf (retention factor) value was compared with the control and that increased in irradiated samples at different exposure time (Table 8). It also resulted in the increase in concentration of flavonoids which were evaluated by the spectrophotometric analysis and it was observed that the absorbance rate was high in the irradiated samples as compare to the control samples (Fig 3 and Table 9). It can be noted that by exposure to the cell phone radiations there was increase in the concentration of flavonoids (Table 7). This shows that by the electromagnetic radiations the flavonoid content increased and so this indicates cell phones radiations has positive effect on the flavonoid content of romaine lettuce.

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