# RESPONSE OF MAIZE SEEDLINGS TO MICROWAVES AT 945 MHz

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*Abstract*. The objective of the present study was to investigate the changes of germination, growth rate and absorbance efficiency of photosynthetic pigments of maize grains after exposed to microwaves (MW). Experiment was carried out by exposing maize plants to continuous microwaves energy at (935.2–960.2 MHz with intensities 0.07–0.15 mW/cm$^2$). The test plants and the control plants were subjected to the same environment during four weeks. The hypothesis was that plants exposed to MW would be different from those plants not exposed. The present experiment showed that germinating grains, growth rate (g dry wt./week and cm$^2$/week) of exposed maize seedling and absorbance efficiency ($\eta$) significantly increased compared to the control. Also, photosynthetic pigments, total soluble sugar and total carbohydrates were positively affected by MW exposure. Microwaves altered the anatomical features of maize leaves by increasing midrib vascular bundle length, wing and assimilating layer thickness. In all measured variables differences between the control and the microwave treatment plants occurred.

*Key words*: microwave (MW), *Zea mays*, germination, growth, metabolic biosynthesis, absorbance efficiency, anatomy.

## INTRODUCTION

The use of wireless telecommunications devices, notably cellular phones, has increased dramatically in Egypt over the past decades. This increased demand for wireless communication has been accompanied by the installation of a network of base stations across Egypt to receive and send communication signals. The cellular phone station in their receiving and sending mostly used microwave with 945 MHz [2].

Microwaves are used in communications as telephone links course, in racking ships, aircraft, rockets and satellites as radar [9]. Banik *et al*. [3] used the microwave to activate the biomethanation of *Methanosarcina barkeri* DSM-804

Received: September 2008;
in final form February 2009.

bacteria. Oza *et al*. [16] used the microwave in estimation of crop area, growth and phonological information crop condition and productivity assessment.

Cellular phones and base stations emit RF radiation, microwave fields penetrate the living bodies to a distance that decreases with increasing frequency [25]. Microwave has thermal effects because of rising in temperature produced by the energy absorbed from oscillating electric fields. The energy quanta of radiation at 0.9 GHz equal 4 μeV; this value is extremely small compared with the energy of about 1 eV which needed to break the weakest chemical bonds in nucleic acid molecules (DNA). When the electromagnetic microwave radiation is absorbed in tissues, it provokes ionic movement, rotation of dipoles and electron orbit distortion, which leads to heating [25].

Few studies on the effect of microwave radiations on plants have been achieved. These studies were summarized as follows:

Jonas [13] reported that microwave radiations caused burns along the vascular system of *Zea mays* seedlings, damage to the photosynthetic system and significant increase in carotenes and anthocyanins production.

Magone [14] observed an increase in vegetative reproduction rates of duckweed (*Spirodela polyrhiza*) grown in flasks outdoors some 2 km from a radio station transmitter. The frequency and intensity of the radiation used were 156–162 MHz and 0.1–2.6 $\mu W/cm^2$, respectively.

Picazo *et al*. [17] found that thistles (*Cynara cardunculus*) decreased in both weight and length during the 3 week exposure period to microwave as compared to a control group. Stem length of lentils (*Lens culinaris*) subjected to electromagnetic fields increased over the course of the 3 weeks, the plants had fewer branches, lower stem, and total weight than the control.

Murakami *et al*. [15] observed slight growth acceleration in *Brassica campestris* plants at the lowest intensity, this is may be due to a slight increase in soil temperature. But high intensity caused wilting to plants, this wilting may be due to soil warming or drought. On the other hand, Skiles [22] found no difference between the control and exposed alfalfa plants to microwave radiation.

Some of the above-mentioned studies supported the harmful effect hypotheses [26], others [10, 17, 22] showed that the microwave radiation has no effect on plants, while, Magone [14] and Murakami *et al*. [15] found that microwave radiation enhanced plants growth. Generally, the low microwave radiation frequencies lead to growth acceleration, while high frequencies reduced plant growth or have no effect.

The present study aimed to assess the effect of microwave radiation at 945 MHz (commonly used by Egyptian cellular phone station-towers) on the growth rate and absorbance efficiency of photosynthetic pigments of *Zea mays* L. seedlings sown in the vicinity to installed microwave base station near the agriculture fields.

## MATERIALS AND METHODS

### PLANT MATERIALS

The plant species used was maize (*Zea mays* L.) var. Giza 66, an agronomically important plant and one representative of a number of monocotyledonous plant species obtained from the Agronomy Department, Agriculture Research Center. This plant was chosen, as it is easy to grow in pots and an important crop in Egypt for human and animal food.

### TREATMENTS

Uniform grains were selected, sterilized for 10 min by 0.05% $HgCl_2$ and washed for 30 min by running water, and then they were sown in plastic pots. Approximately 10 grains per pot were added to the upper 2 cm of soil in each pot. Each 13 cm pot contained roughly 600 g of loamy soil (22% sand, 45% silt and 33% clay) introduced from farms closed to Benha city. No addition of nutrients was done, and all plants were watered as needed so there was no moisture stress. After 10 days from sowing, the seedlings were thinned to six seedlings/pot.

Five treatments were applied as follows:

Plants grown away from the effect of mobile station (control)

Plants grown under the mobile station at 3 m from the station base (D1)

Plants grown under the mobile station at 6 m from the station base (D2)

Plants grown under the mobile station at 9 m from the station base (D3)

Plants grown under the mobile station at 12 m from the station base (D4)

Microwave intensities within the exposure area were determined with electromagnetic survey meter model 8718B (Narda, microwave communication company, Hauppauge, New York, USA). The meter uses an isotropic electric field probe model 8760D attached to a power density meter. The device as configured for this study has a frequency range of between 300 KHz and 3 GHz in the near field. The measurements confirmed that the mobile station transmitter was emitting microwave radiation at frequency band 935.2 – 960.2 MHz and test plants received intensities between 0.07 – and 0.15 $mW/cm^2$ within the exposure area.

### Germination percentage

Germination percentage of *Zea mays* grains was calculated after 4, 6, and 8 days from sowing for the control and the microwave exposed grains.

### Growth-related aspects

The heights of ten seedlings of 2, 3, and 4 weeks old were measured, The area of all leaves of the seedlings were estimated according to equation 1 [20]. The weights of 10 fresh seedlings were determined and then oven dried at 80 ºC until constant weight and weighted using electronic scale as dry biomass (g/plant).

$$\text{Leaf area} = [(L_{cm} \times W_{cm}) \times 0.785] \quad (1)$$

where $L$ is the length and $W$ is the broadest width of the leaves.

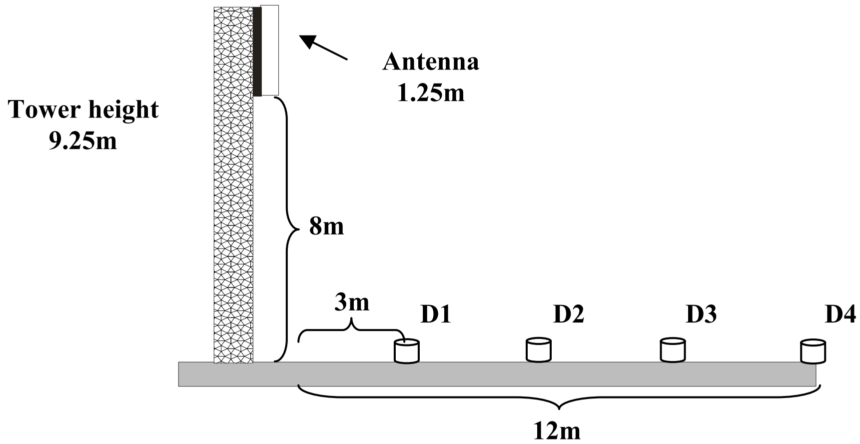



Fig. 1. Cellular phone station diagram and experiment design.

**Anatomical features**

Third leaf of *Zea mays* plants was sectioned as described by Johanson [12]. The sections were photographed by using light microscope (Olympus) with digital camera (Canon Power Shot S80) connected to computer; the photographs were taken by Zoom Browser Ex program. The dimensions of leaf sections were measured by using Corel Draw program ver. 11.

**Photosynthetic pigments**

Fresh leaves of *Zea mays* were ground in 80% acetone, then filtered and diluted to a known volume. Photosynthetic pigments content (chlorophylls a and b and carotenoids) was determined according to VonWettstein [28] by using the following equations:

$$\text{Chl.a} = 9.78 \times E_{662} - 0.99 \times E_{644} \text{ (mg/g fresh wt.)} \quad (2)$$

$$\text{Chl.b} = 21.426 \times E_{644} - 4.65 \times E_{662} \text{ (mg/g fresh wt.)} \quad (3)$$

$$\text{Total chl.} = \text{Chl.a} + \text{Chl.b} \text{ (mg/g fresh wt.)} \quad (4)$$

$$\text{Carot.} = 4.695 \times E_{440.5} - 0.286 \times (\text{Chl.a} + \text{Chl.b}) \text{ (mg/g fresh wt.)} \quad (5)$$

Absorption coefficient (α) of photosynthetic pigments was registered spectrophotometrically at 440.5, 644, and 662 nm (Jenway spectrophotometer model 6300). These measurements taken for control, 2, 3, and 4 weeks after plant exposure to mobile phone station at different distances (D1, D2, D3,, and D4). The absorption efficiency ($\eta$) was calculated at minimum absorption coefficient for all weeks at different distances by the relation:

$$\eta = 1 - 10^{-\alpha d} \quad (6)$$

where $d$ is the thickness of plant extract, $d = 1$ cm.

**Carbohydrates**

Total soluble sugars: 200 mg of *Zea mays* shoots powder were macerated in 80% warm ethanol and then filtered. The filtrate was cleared using basic lead acetate. Excess of basic lead acetate precipitated by sodium monohydrogen phosphate, the mixture was filtered and the filtrate was completed to known volume (Chaplin and Kennedy [5]). Total soluble sugars were estimated in filtrate colorimetrically by phenol sulfuric acid method as described by Dubois *et al*. [8]. Total soluble sugars were calculated as g glucose/100 g fresh wt.

Total carbohydrates: 200 mg of plant shoot powder was hydrolyzed in 2.5 ml of 2 M HCl in sealed tubes. The sealed tubes were heated at 100 ºC for a period of 3 hours, the mixture was filtered and cleared as described above. The hydrolyzed reducing sugars were estimated colorimetrically by the phenol sulfuric acid method as described by Dubois *et al*. [8]. Total carbohydrates were calculated as g glucose/100 g dry wt.

**Statistical analysis**

Standard deviations of the studied parameters were calculated and subjected to one-way analysis of variance (ANOVA) to examine the effect of microwave radiation at four distances on maize grain germination, growth, leaf anatomy, photosynthetic pigments and carbohydrates. Means were compared with the least significant difference test (LSD) following Snedecor and Cochran [23] using SPSS package ver. 15.

## RESULTS AND DISCUSSION

### GRAINS GERMINATION AND SEEDLINGS GROWTH

Table 1 showed that germination of *Zea mays* L. grains was activated when germinated under microwave station at different distances comparing to non-exposed grains. The grains away from the cellular phone station by 6 m recorded a higher germination percentage after 4 and 6 days (50 and 80%, respectively) from sowing than that of the control (30 and 60%, respectively) and that of the grains at other distances, while after 8 days from sowing the germination recorded 100% for all treatments.

*Table 1*

Germination percentage of *Zea mays* L. plants growing at different distances under cellular phone station (± SD)

| Treatment (days) | Germination (% ) | | |
|---|---|---|---|
| | 4 | 6 | 8 |
| Control | 0 | 30d ± 1.5 | 98 ± 0.1 |
| D1 | 30b ± 2.4 | 60c ± 5.4 | 100 ± 0.0 |
| D2 | 50a ± 3.8 | 80a ± 3.7 | 100 ± 0.0 |
| D3 | 30b ± 3.3 | 70b ± 5.5 | 100 ± 0.0 |
| D4 | 30b ± 4.5 | 70b ± 2.8 | 100 ± 0.0 |
| LSD at 5% | 3.5 | 7.8 | N.S |

Means within a column flagged by the same letter (a–d) are not significantly different at $p < 0.05$.

The data which are recorded in Table 2 illustrated that the growth of maize seedlings was significantly activated when exposed to microwave radiation. It was noticed that seedlings grown away from the cellular phone station-tower by 3 m recorded the highest height, leaf area and fresh and dry weights as compared to those of the other treatments. Generally, water content of maize seedlings slightly increased because of microwave radiation (Table 2), the maximum significant increase was recorded by 3 weeks aged plants especially at D1 (89%), while the lowest water content was recorded by 2 weeks aged plants at D2 and D3 (75.6 and 78.2%) compared to control plants (78.8%).

Growth rate of maize plants was calculated as $cm^2$/week and g dry wt./week and it was illustrated in Fig. 2. It is obvious that the growth rate of maize plants increased with increasing plant age. Microwave radiation accelerated expansion rate of leaf area and accumulation of dry biomass by rates higher than that of control plants. Plants growing at 6 m away from the tower recorded the highest growth rates especially after 3 and 4 weeks from sowing (11.8 and 16.0 $cm^2$/week, respectively) in comparison to control plants which recorded the lowest growth rate (3.7, 4.0 and 6.4 $cm^2$/week) after 2, 3 and 4 weeks from sowing, respectively.

The present results agree with that of Magone [14] who found that exposure of duckweed plant to microwave radiation with frequencies ranged between 156–162 MHz increased the vegetative reproduction rates. In addition, Murakami *et al*. [15] observed a slight growth acceleration for *Brassica campestris* when exposed to microwave radiation with frequency 2.45 GHz. Also Picazo *et al*. [17] found that stem length of lentils (3 weeks old) subjected to microwave radiation increased over the control ones. These results may be attributed to a slight increase in plant or soil temperature. However, Schmutz *et al*. [20] found that microwave radiation at 2450 MHz increased the temperature of sucrose solution ~4 ºC and they reported that the temperature with sucrose solution indicated that young spruce and beech trees which received 100–300 $W/m^2$ were considerably warmed by the incident microwave radiation. In addition, Brown *et al*. [4] reported that a temperature increase within certain limits could produce an increase in germination. Chen *et al*.

[6] found that *Isatis indogotica* plants pretreated with laser and microwave at 2450 MHz significantly changed the thermodynamic parameters during the seeds germination and these changes led to promotion of seed germination. In addition, the growth and development of seedlings were accelerated, also the leaves area and biomass were significantly increased as compared with the control.

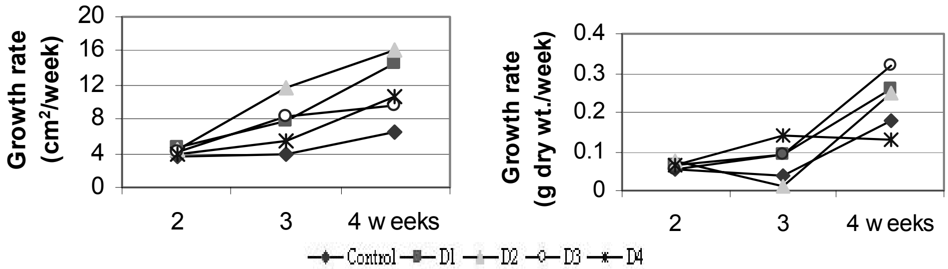



Fig. 2. Early growth rates of *Zea mays* L. plants growing at different distances under cellular phone station.

LEAF ANATOMY

Generally, exposure of maize plants to microwave significantly increased midrib thickness, midrib vascular length, midrib assimilating layer thickness, wing thickness, assimilating layer thickness and number of small bundles (Table 3, Fig. 3), while it significantly reduced midrib parenchyma layer thickness of the plants. It was grown away from the station by 6 and 9 m.

The present findings did not support the findings of Jonas [13], who showed that the electromagnetic microwave radiations lead to burns along the vascular system and damage to the photosynthetic system appeared. However, microwave used in this investigation leads to improvement of the anatomical features of maize leaves by: (i) increasing the thickness of the assimilating layer (improving the photosynthetic processes). (ii) decreasing midrib parenchyma thickness (decreased leaf water content to reduce the thermal effect of microwave, however, Velázquez-Marti *et al*. [27] found the thermal effect of microwave magnified in the presence of water, and (iii) increasing small vascular bundles (improving the hydraulic conductance).

*Table 3*

Leaf dimensions of *Zea mays* L. plants growing at different distances under cellular phone station

| | | Cont. | D1 | D2 | D3 | D4 | LSD at 5% |
|---|---|---|---|---|---|---|---|
| Midrib thickness (th.) | µm | 2611d | 3907a | 2122e | 2700c | 2909b | 125 |
| M R upper epidermis th. | | 189a | 93bc | 186a | 105b | 82c | 14 |
| M R lower epidermis th. | | 116a | 119a | 95b | 77c | 122a | 11 |

*Table 3 (continued)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| M R vascular bundle length | | 549d | 830b | 724c | 870a | 744c | 35 |
| M R assimilating layer th. | | 253c | 290a | 273b | 276b | 273b | 12 |
| M R parenchyma th. | | 1912c | 2841a | 1461e | 1791d | 2114b | 45 |
| Hinge cells th. | | 301b | 270c | 292b | 332a | 298b | 22 |
| Wing th. | | 641b | 694a | 694a | 693a | 667a | 28 |
| Wing assimilating layer th. | | 205c | 226b | 272a | 226b | 272a | 18 |
| No. of main midrib vascular bundles | | 5b | 7a | 5b | 5b | 3c | 1 |
| No. of small midrib vascular bundles | | 14d | 23b | 18c | 25b | 29a | 3 |
| No. of wing V. Bs between 1st and 2nd main vascular bundles | | 10c | 10c | 12b | 14a | 10c | 2 |

Means within a row flagged by the same letter (a–d) are not significantly different at $p < 0.05$.

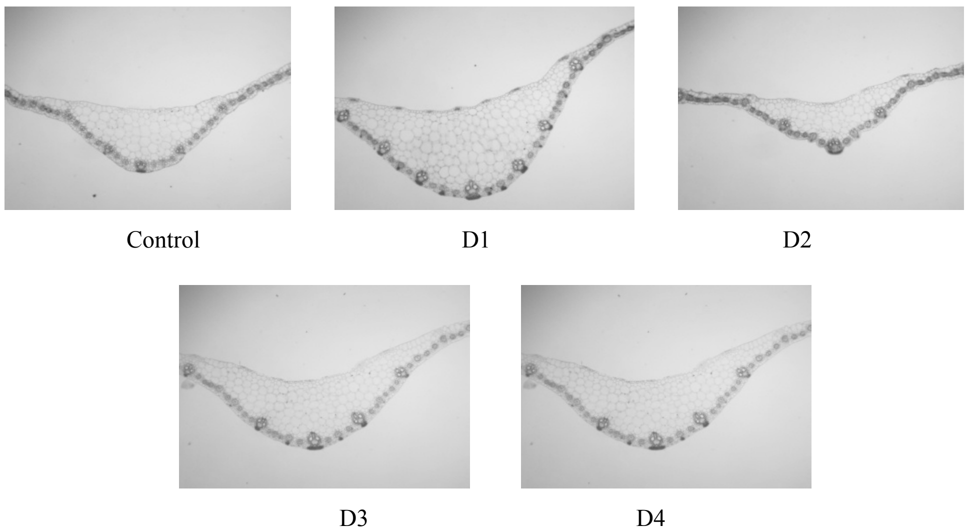



Fig. 3. Transversal sections in *Zea mays* L. leaves growing at different distances under cellular phone station (× 140).

METABOLIC BIOSYNTHESIS

**Photosynthetic pigments**

Continuous exposure of maize seedling to microwave radiation for two weeks resulted in significant increase in chlorophyll a but reduction in chlorophyll b and carotenoids content (Table 4). Maize seedlings (2 weeks old) grown at 12 m away from the cellular phone station contained the highest amount of chl. a, chl. b, total chlorophyll and carotenoids (13.2, 8.7, 22.5, and 2.8 mg/g fresh wt., respectively) as compared to those of the control (8.7, 7.3, 15.8, and 2.7 mg/g fresh wt., respectively) and those other seedlings exposed to microwave irradiation. With increasing the exposure time to 3 weeks, the photosynthetic pigments of the seedlings grown away from the cellular phone station by 3, 6 and 9 m significantly increased, while those of seedlings grown at 12 m away from the station decreased. Table 4 shows that the microwave radiation still stimulated biosynthesis of chl. a and chl. b in maize seedlings (4 weeks old) growing under the cellular phone station at D1, D2 and D3 distances, while it inhibited their production in the plants grown at D4 but stimulated carotenoids production at D2 and D4 distances.

Increase in chl. a and chl. b production in the present study indicated that maize plants survive normally, in addition MW activated the plants for chlorophyll biosynthesis and production. There is a positive correlation between plant growth and photosynthetic pigment contents. Increase in carotenoids biosynthesis, without negative effect on chlorophyll a and b content, indicated that the plants subjected to mild stress may be caused by the thermal effect of MW. However, carotenoids have a critical role as photoprotective compounds by quenching triplet chlorophyll and singlet oxygen derived from excess light energy, thus limiting membrane damage (7, 18). Thus increase in carotenoids content can be considered as a defense mechanism for mild thermal stress.

*Table 4*

Photosynthetic pigments of *Zea mays* L. plants growing at different distances under cellular phone station (± SD)

| Treat. (weeks) | Photosynthetic pigments (mg/g fresh wt.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | | 3 | | | | 4 | | | |
| | chl a | chl b | chl a+b | Carot. | chl a | chl b | a+b | Carot. | chl a | chl b | chl a+b | Carot. |
| Cont. | 8.7d ±1.08 | 7.3b ±0.96 | 15.8c ± 0.77 | 2.7a ±0.40 | 10.8d ±0.82 | 3.1c ± 0.57 | 13.8c ± 1.41 | 4.0b ± 0.27 | 5.27d ± 0.18 | 1.5b ± 0.19 | 6.8c ± 0.33 | 1.9c ± 0.39 |
| D1 | 10.7c ± 0.22 | 6.2c ±0.35 | 16.8bc ±0.40 | 2.1b ±0.40 | 12.9c ±0.52 | 4.1b ± 0.23 | 15. 7bc ± 2.14 | 4.1b ± 1.22 | 8.78a ± 0.14 | 1.2c ± 0.22 | 10.0a ± 0.13 | 1.3d ± 0.25 |
| D2 | 9.8cd ±1.15 | 5.4d ±0.27 | 16.0c ±0.38 | 1..9b ±0.3 | 13.5bc ±0.68 | 4.4ab ± 0.24 | 17.5ab ± 0.31 | 5.2a ± 0.2 | 5.75c ± 0.23 | 1.7ab ± 0.25 | 7.4b ± 0.48 | 2.3b ± 0.26 |
| D3 | 11.1b ±0.76 | 6.4cb ±0.36 | 17.5b ±1.02 | 2.4a ±0.2 | 14.5a± 0.48 | 4.8a ± 0.38 | 19.3a ± 0.77 | 4.6ab ± 0.31 | 7..95b ± 0.12 | 1.8ab ± 0.27 | 9.7a ± 0.36 | 1.2d ± 0.12 |
| D4 | 13.2a ±1.15 | 8.7a ±0.29 | 22.5a ±0.55 | 2.8a ±0.1 | 5.1e ±0.44 | 1. 7d ± 0.15 | 10.9b ± 6.74 | 2.2c ± 0.23 | 3.87e ± 0.32 | 0.2a ± 0.03 | 4.0d ± 0.30 | 2.7a ± 0.13 |
| LSD at 5% | 1.51 | 0.94 | 1.19 | 0.59 | 1.29 | 0.56 | 3.05 | 0.87 | 0.24 | 0.39 | 0.47 | 0.39 |

Means within a column flagged by the same letter (a–d) are not significantly different at $p < 0.05$.

Skiles [22] found the long-term of microwave at 2.45 GHz had no effect on the chlorophyll content of alfalfa plants. On the other hand, Chen *et al*., [6] proved that laser radiations have a thermal effect as microwave radiation, and found that pretreated *Isatis indogotica* plants with laser increase the concentration of chlorophyll a, chlorophyll b and total chlorophyll. In addition, Jonas [13] found that the production of carotenes and anthocyanins was significantly increased in microwave irradiated maize plants.

The findings of Randhir and Shetty [19] indicate that perhaps microwaves create an intense burst of oxidative stress in the cells. This oxidative stress induced by microwaves could be the signal for the plants to sense the heat stress and induce the biochemical pathways that confer survival strategies.

The absorption coefficient, α, of plant extraction at different concentrations was calculated at different values of wavelengths (440.5, 644 and 662 nm). These results were represented in Figure 5 for different weeks at different distances, noting that the minimum absorption was at 644 nm. At this wavelength, calculating the absorption efficiency (η) which is represented in figure (6) depends on weeks; it was found that the efficiency (η) was higher in the second week.

The measurement of absorption coefficient (α) of plant through all weeks suggested that the minimum absorption of light was at 644 nm. The low value may be due to the low motion of ions or that the pigment molecules do not have resonance in this range of frequency.

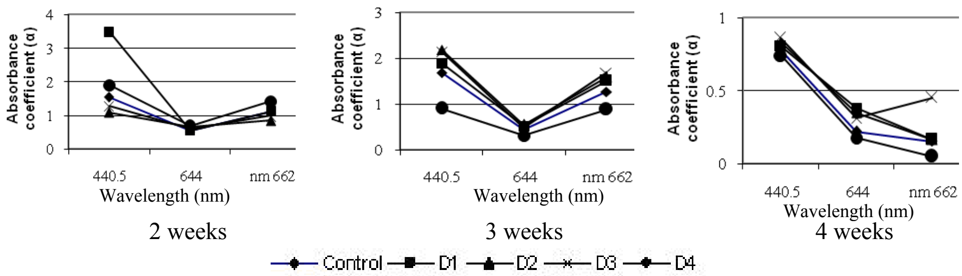



Fig. 4. Relation between absorbance coefficient (α) and wavelengths at different distances through different weeks (2, 3, and 4 weeks).

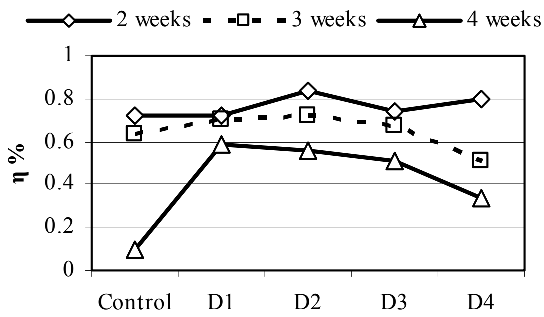


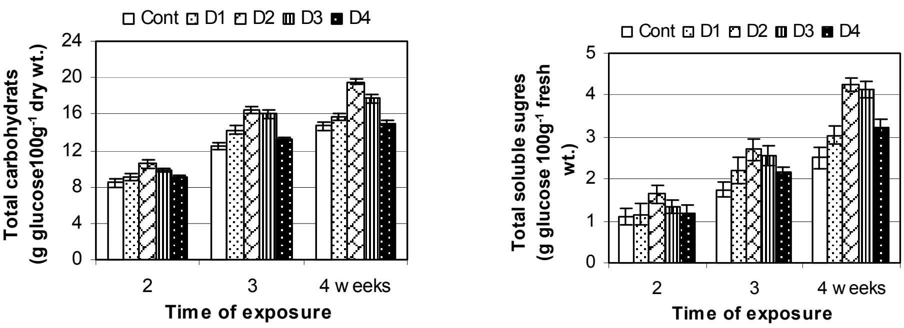

Fig. 5. The relation between efficiency (η) and different distances through three weeks.

The efficiency coefficient (η) was calculated at minimum absorption for all weeks. These values suggested that, the efficiency was higher than the control value at all distances for all weeks while the second week had higher value than other weeks. These results according with the growth rate cm/week and due to the large amount of protein are required to achieve the photosynthetic rates necessary to support high productivities in C3 crops [28].

**Carbohydrates**

Exposure of maize seedlings to microwave radiation caused a significant increase in their total soluble sugars and total carbohydrate content during the growth periods (2, 3 and 4 weeks). The highest carbohydrates content was recorded in the seedlings grown at 6 and 9 m away from the cellular phone station.

The present results indicated a positive correlation among plant growth, photosynthetic pigment contents and carbohydrate concentration in maize tissues. However, increasing in photosynthetic pigments content leads to increase in photosynthetic rate, so the biosynthesis of sugars increased and accumulated in plant tissues to increase their biomass. Presence of high concentrations of soluble sugars in maize tissues may be due to high photosynthetic rate or to the defense mechanism against the thermal effect of MW.




LSD at 5%→ 0.18 0.54 0.95 LSD at 5%→ 0.86 1.12 1.65

Fig. 6. Total carbohydrates content and total soluble sugars of *Zea mays* L. plants growing at different distances under cellular phone station (±SD).

The present study supports the findings of Chen *et al*. [6] when they pretreated *Isatis indogotica* plants to laser and microwave. They found that the biochemistry and physiology metabolisms of the plants pretreated with laser were accelerated by increasing photosynthesis parameters of 24–25 days seedlings, e.g. net photosynthetic, stomatal conductance and water utilization efficiency. In addition, increase in chlorophyll content indicates acceleration in photosynthetic processes, increase in carbohydrate production and promotion of seedlings growth.

Armond *et al*. [1] and Gounaris *et al*. [11] demonstrated that within photosynthetic apparatus, PSII is the most heat-sensitive complex. Increasing temperature led to a blockage of PSII reaction centers and then to dissociation of antennae pigment-protein complexes from the central core of the PSII light-harvesting complex. The present results proved that the heat produced from the used microwave was too lower to cause blockage of PSII reaction centers.

## CONCLUSION

The microwave radiation used in the present study promoted grains germination, accelerated seedling development and growth, enhanced metabolic biosynthesis by increasing photosynthetic pigments and carbohydrate biosynthesis and improving the hydraulic conductance by increase of small vascular bundles.

Literature in this field of study and the present study indicated that microwave radiation had a positive effect on some plants and had an adverse effect on others; thus we can suggest that microwave radiation effects may depend on: radiation frequency, exposure period and the environmental conditions.

To prove the positive effect of microwave radiation on plants, intensive studies deal with the effect of the microwave radiation on the ultrastructure of cell organelles, enzymes activity, genetic changes and yield quality of different plants must be investigated.

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