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MICROWAVE RADIATIONS MODULATES GROWTH, PHOTOSYNTHETIC PIGMENTS AND COLONIZATION OF ROOT INFECTING FUNGI ON GUAR, OKRA AND LUFFA

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ABSTRACT

Seeds of Guar (*Cyamopsi tetragonoloba* L.) Taub), Okra (*Abelmoschus esculentus* (L.) Moench.) and Luffa (*Luffa cylindrica* (L.) M. Roem.) were treated with microwave radiations (2540 MHz) for 10, 20, 25, 30, 35, 40, 60, 90 seconds. The germination was recorded for 7 days *in vitro*. We noted a reduced seeds germination after 30 to 45 second exposure, while either 60 or 90 seconds of microwave exposure of seeds inhibited their germination. Further, another batch of seeds were exposed to microwave radiations for 0, 10, 20 and 30 second and planted them in pots in greenhouse conditions. Then we assessed the effects of radiation on plant growth, photosynthetic pigments and colonization of root infecting fungi. We noticed a significant increase in shoot weights and leaf area of okra plants when seeds were exposed for 30 seconds. The chlorophyll "a" content varied in different crops upon exposure to microwave radiations. For instance, Chlorophyll "b" content was increased with time of guar and okra seed exposure, whereas luffa showed no effect on chlorophyll "b" contents upon microwave exposure. Carotenoid content remained unaffected with increasing time of seed exposure. We observed a reduce colonization % of *R. solani* and *Fusarium* species with increase in the time of seed exposure but colonization % of *M. phaseolina* increased with the time of exposure.

Key-words: Microwave radiations, root-infecting fungi, Okra, Luffa, Guar.

INTRODUCTION

There are variety of methods such as chemical, cultural practices and biological that have been widely used to reduce the severity of soil borne plant pathogens (Singleton *et al*., 1992). Seed pretreatment including chemical and physical treatments are widely used to improve plant growth. Recently, microwaves have received attention because of its far-reaching effect on animals, plants and microorganisms. Microwaves are electromagnetic waves with frequencies ranges between 300 MHz and 300 GHz. Microwaves are known to affect plant growth and development by reducing seed germination, however, the microwaves weak intensity does not affect plant growth but at high exposure time it influences plant growth by slowing seed germination (Oprica, 2008).

We selected three plant species, guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a drought resistant annual legume plant and a common agricultural crop of India and Pakistan, okra (*Abelmoschus esculentus* (L.) Moench.) is a vegetable crop, grown in tropical and subtropical regions and luffa (*Luffa cylindrica* (L.) M. Roem.) a vegetable is known as sponge gourd that is widely cultivated in Asia, South America and USA (Demir *et al*., 2008; Laidani *et al*., 2011, Oboh and Aluyor, 2009) to investigate the effect of microwaves on plant growth and their pathogens.

Guar plants are attacked by number of pathogens including soil-borne pathogens *Rhizoctonia solani, Sclerotium rolfsii, Fusarium equiseti* and *F. coeruleum* (Arab, 1983; Morsy *et al*., 1978; Vir *et al.*, 1972), causing root rot and wilt diseases. Okra plants are also attacked by various soil borne fungi including *Macrophomina phaseolina, Rhizoctonia solani, Fusarium* spp., and root-knot nematode *Meloidogyne* species (Ehteshamul-Haque *et al*., 1996; Parveen *et al*., 1994; Sultana *et al*., 2005). Okra crop losses due to root-infecting fungi usually ranged from negligible to 50% (Dawar *et al*. 2008; Safiuddin and Shahab, 2012). According to Seebold (2010) common fungal disease affecting cucurbits are belly rot (*Rhizoctonia solani*), *Choanephora* rot (*Choanephora cucurbitarum)*, cottony leak (*Pythium* sp.), *Fusarium* fruit rot and Southern blight *(Sclerotium rolfsii*).

There have been few reports on the use of microwaves in agricultural crops and very little attention has been paid in its effects on plant growth and pathogens (Naeem *et al*., 2013). Therefore, in the present study we focus our investigation on the effects of microwaves on guar, okra and luffa seed germination, and plant growth. We also investigate the effect of root-rot diseases on microwave treated seeds of these plant species.

MATERIALS AND METHODS

Seed treatments for *in vitro* germination

Seeds of Guar (*Cyamopsis teteragonoloba* (L.) Taub.), Okra (*Abelmoschus esculentus* (L.) Moench) and Luffa (*Luffa cylindrica* (L.) M. Roem.) were washed with sterilized water, dried and treated with microwave radiation (2540MHz) for 10, 20, 25 30, 35,40,45,60 and 90 second. Seeds were placed on blotting paper that was soaked in sterilized water, kept in Petri dishes (9 cm diameter). Petri dishes were placed at room temperature (25-35 °C) under dark and seed germination was recorded after 7 days of incubation.

Soil

Sandy loam soil (Sand: Silt: Clay. 70:19:11) was obtained from an experimental field (pH 7.7-8.2) with 40%of moisture holding capacity. Population of soil fungi like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* species was determined.

Green house experiment

Seeds of guar, okra and luffa were first washed with sterilized water and after drying them in seeds were treated with microwave radiation (2540 MHz) for 10, 20 and 30 seconds. Untreated seeds served as control. Each treatment was replicated three times. Five seeds were planted in each pot (8cm diameter) containing 300g of soil. The pots were arranged in a completely randomized design (considering the effect of light) in green house for 30 days and watered daily (about 20-40 mL/ pot) to keep soil moist. After 30 days, all plants were uprooted, and data were collected on shoot lengths, fresh shoot weight, dry shoot weight, leaf area and photosynthetic pigments. Ten leaves of various sizes from Guar (*Cyamopsis tetragonoloba* L.), Okra *(Abelmoschus esculentus*(L.) *Moench),* Luffa *(Luffa cylindrical*) were collected and their linear measurements for the length of lamina (L) and lamina breadth (B) were recorded. To determine true leaf area, the leaf outline was carefully drawn on graph paper and area determined with all possible precision and accuracy. The multiplication factor (K) was calculated by employing the formula, $K = Area_{measured} / (L \times B)$. Employing average value of the multiplication factor k, leaf areas were calculated as $Area_{computed} = K$ (length x breadth) for comparison with the observed areas of the leaves. Bivariate linear and power relationships of leaf area with measured linear dimensions of the leaf were variously computed. In addition to it the regression coefficients were also determined by employing multiple regression method fitting in the allometric model, $Y = a + b1X1 + b2\ X2 \pm SE$. The arithmetic and allometric methods were compared for their precision and suitability. One cm long root pieces were washed, and surface sterilized with 1% of sodium hypochlorite and transferred onto PDA plates supplemented with antibiotics. Petri dishes were incubated at room temperature and colonization of root-infecting fungi was recorded at one week of incubation. Colonization % of root-infecting fungi was calculated according to the following formula:

$$\text{Colonization \%} = \frac{\text{Number of root pieces colonized by a fungus}}{\text{Total number of root pieces used}} \times 100$$

Determination of Photosynthetic Pigments

Leave sample (0.1g) was extracted in 5mL chilled Acetone (80%) and centrifuged for 10 minutes at 4000 X *g*. Supernatant was collected and absorbance was recorded at 645 and 663nm for chlorophylls and 480 and 510nm for carotenoids. Photosynthetic pigments were estimated as described by Arnon (1949) and Duxbury and Yentsch (1956), respectively, and expressed as $\mu g.\ g^{-1}$ fresh weight.

Analysis of Data

Data were analyzed by analysis of variance (ANOVA) to compare the treatments followed by Fishers' least significant difference (LSD) and Duncan's multiple range test (Gomez and Gomez, 1984).

RESULTS

Seed Germination

We noticed that germination % were unaffected by 0, 10, 20 or 25 seconds of microwave exposure. However, a decrease in germination % was observed from 30 to 45 second of microwave exposure. Whereas, luffa seeds showed a decline in % germination by 25 seconds seed exposure. We did not notice any seed germination that was exposed to microwave radiation for 60 and 90 seconds (Fig. 1).

Growth Parameters

There was no significant effect of radiations on shoot length. However, luffa plant showed higher shoot length in treated plants compared to control (Fig. 2). In general plant shoot weight appear to be increased with the exposure of time. In guar plant, highest fresh shoot weight was achieved at 10 seconds of treatment. However, in okra and luffa plants the maximum fresh shoot weight were achieved at 30 second of microwave exposure (1.771 and 1.709 g, respectively) as compared to unexposed control plants (1.260 g) (Fig. 3, A). Dry shoot weight showed variable response, in guar and okra dry weight declined at 20 second and improved at 30 seconds of radiation exposure. However, luffa plants were still unaffected by radiation exposure even by the increase in exposure time (Fig. 3, B).

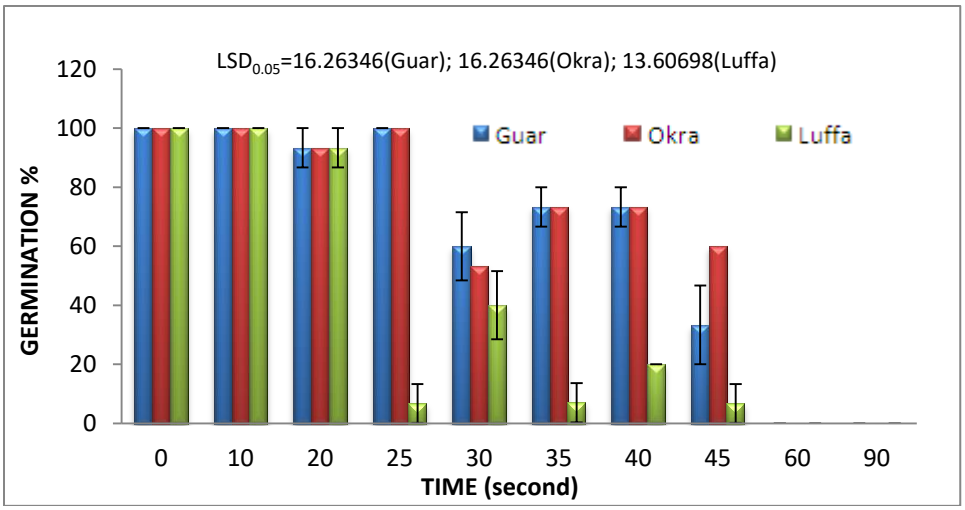


Fig. 1. Effect of microwave radiation on seed germination in guar, okra and luffa plants.

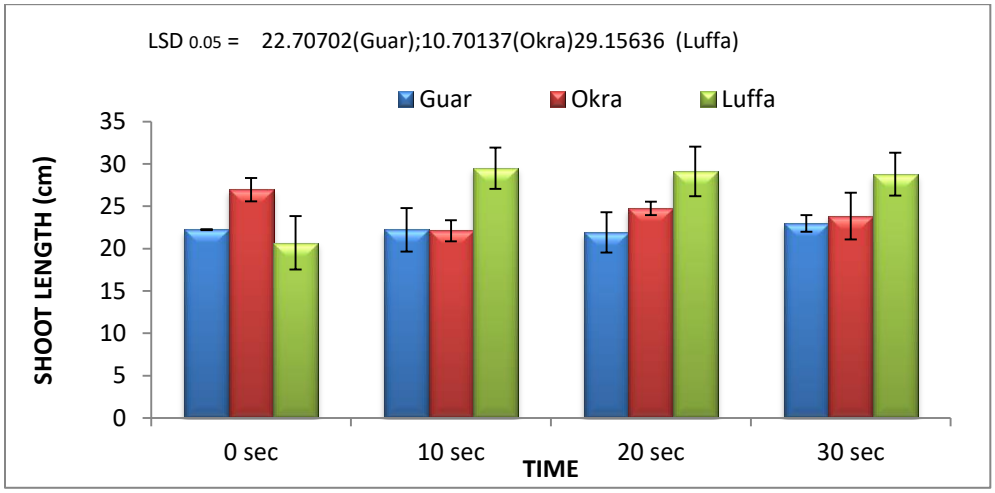


Fig. 2. Effect of microwave radiation on shoot length of guar, okra and luffa plants.

Leaf Area

In all crops, the leaf area of largest leaf increased with the increase in time of radiation exposure; the highest leaf area was recorded at 30 second of exposure of microwave radiation compared to unexposed control. However, this increase appeared to be more prominent in okra and luffa but not in guar plants. The leaf area of smallest leaf showed similar trend; improved leaf area at high (30 seconds) exposure time. Increased in leaf area with microwave radiations is more prominent in small leaves as compared to large leaves in all tested crops (Fig. 4, A and B).

Effect of radiation on Photosynthetic pigments

Microwave radiation produced variable amount of chlorophyll "a" content in different crop plants. For example, in guar and luffa plants the chlorophyll "a" content declined with the increase in radiation exposure time. However, in okra chlorophyll "a" improved by exposure time increase, and the highest chlorophyll "a" content was observed

at 30 seconds of microwave exposure. Chlorophyll "b" contents appeared to increase with time of exposure in guar and okra, where, maximum increase in chlorophyll "b" contents were observed at 30 seconds of microwave exposure. However, in luffa plants chlorophyll "b" contents remained unaffected at all radiation exposure regime. . On the other hand, the carotenoid contents reduced at 20 seconds of exposure in okra and luffa plants as compared to other exposure regiments. However, carotenoid contents decreased in guar plant only after 10 seconds of radiation exposure (Fig. 5).

Effect of microwave radiation on Root colonization by root infecting fungi

The % colonization of *R. solani* decreased with an increase in the time of exposure to microwave radiation (Fig.6). However, this reduction was more prominent in guar plant compared to other crops. In luffa plant the colonization increased at 10 and 20 seconds of exposure to microwave radiation then decreased at 30 seconds of exposure. Maximum % colonization was noted in Luffa and okra in plants exposed to radiation for 30 second in microwave. Similarly, % colonization of *M. phaseolina* increased with exposure time in all tested crops (Fig.7). Percent colonization of *Fusarium* species declined at 10 and 20 seconds of exposure to microwave radiations in all radiated crops. However, at 30 seconds *Fusarium* species showed higher % colonization (Fig.8).

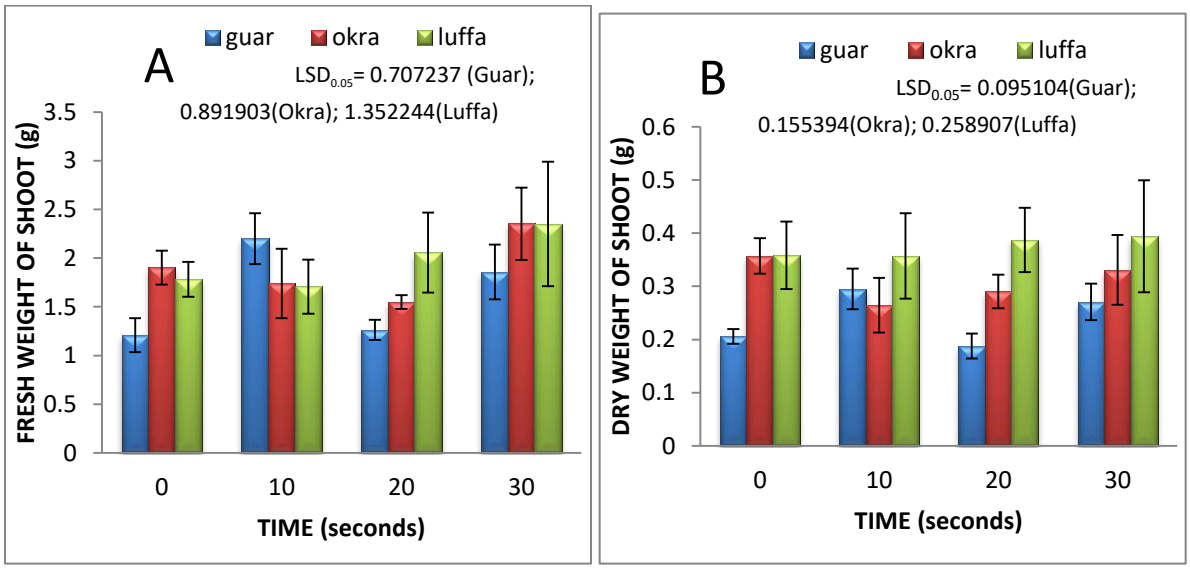


Fig. 3. Effect of microwave radiation on fresh (A) and dry (B) weights of shoots of guar, okra and luffa plants.

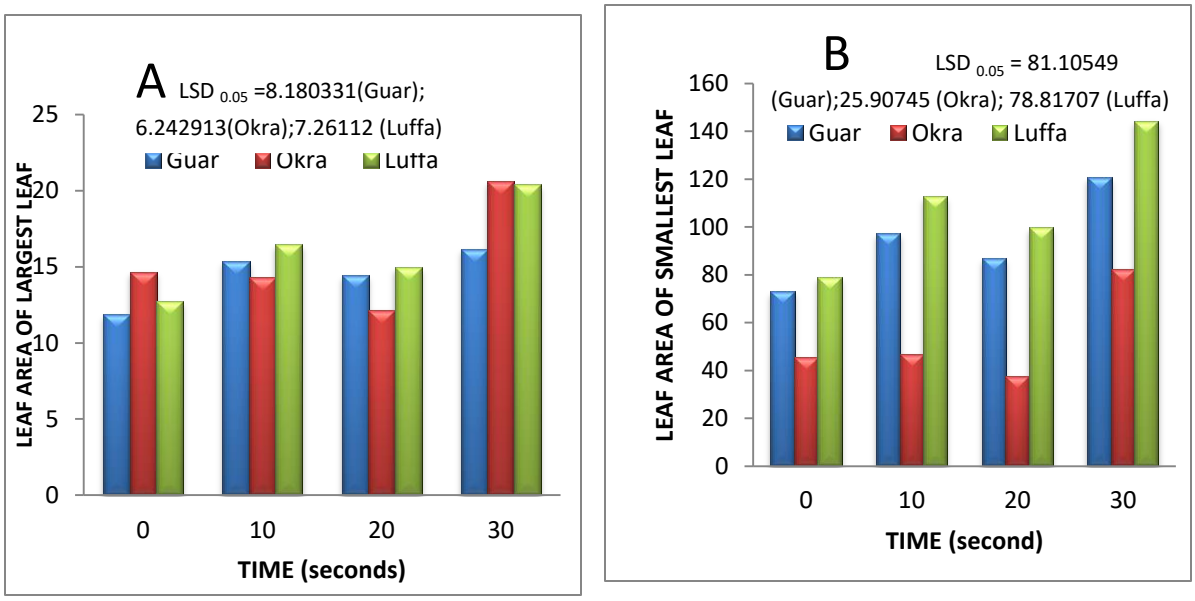
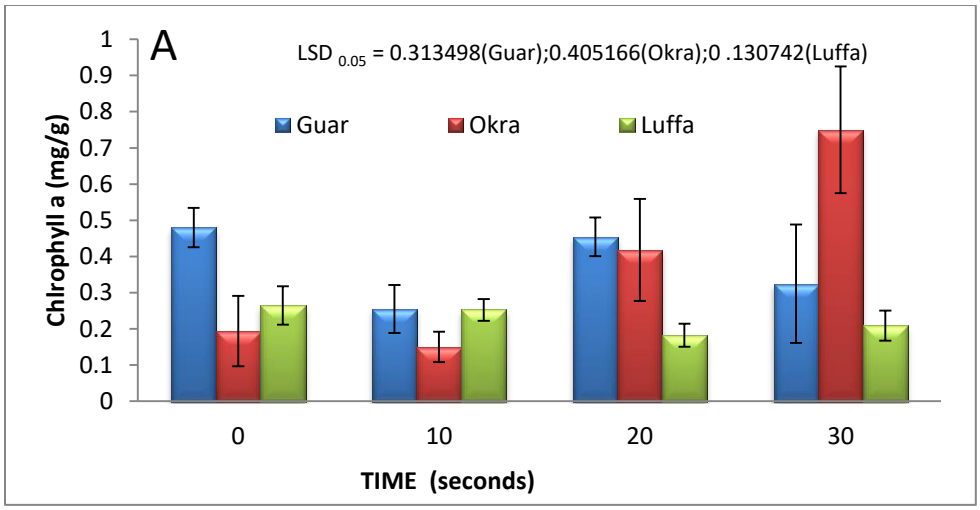
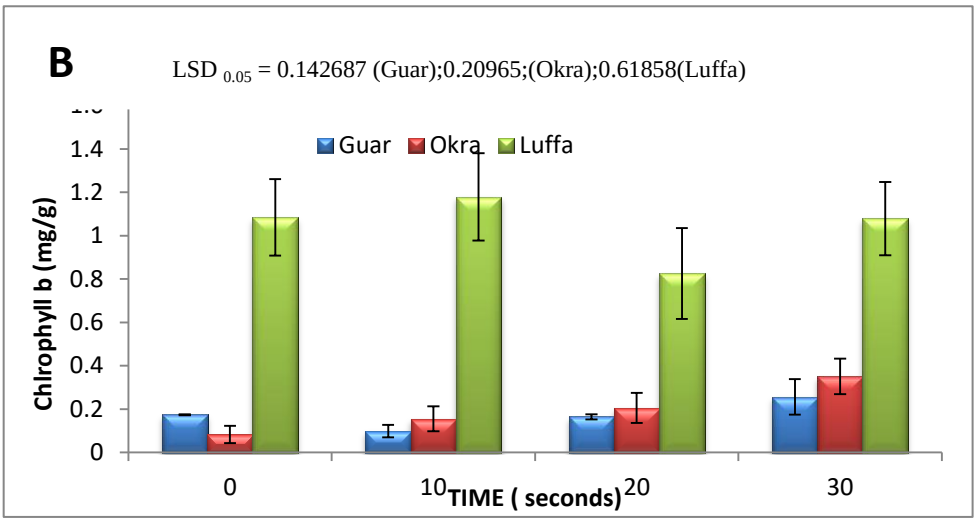


Fig. 4. Effect of microwave radiation on leaf area of guar, okra and luffa plants.





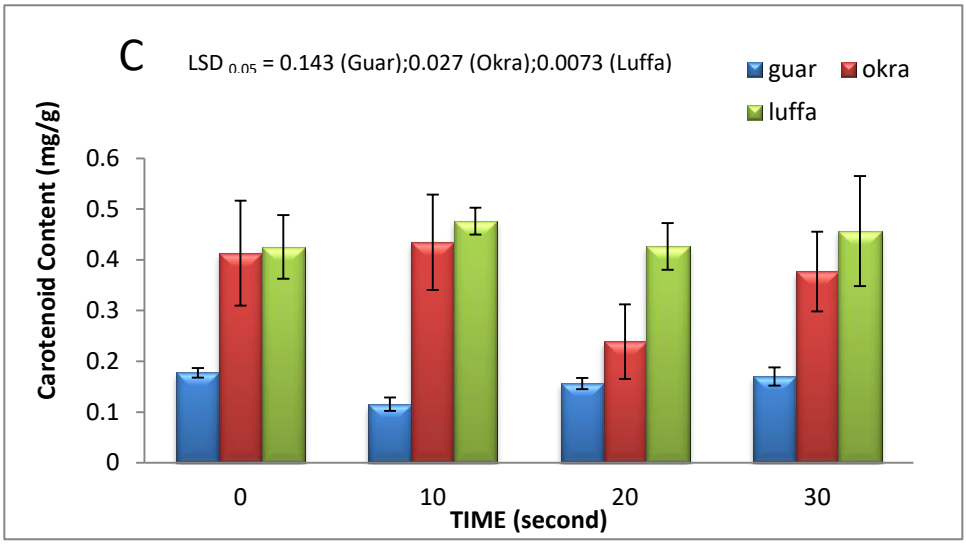


Fig. 5. Effect of microwave radiation on photosynthetic pigments in guar, okra and luffa plants.

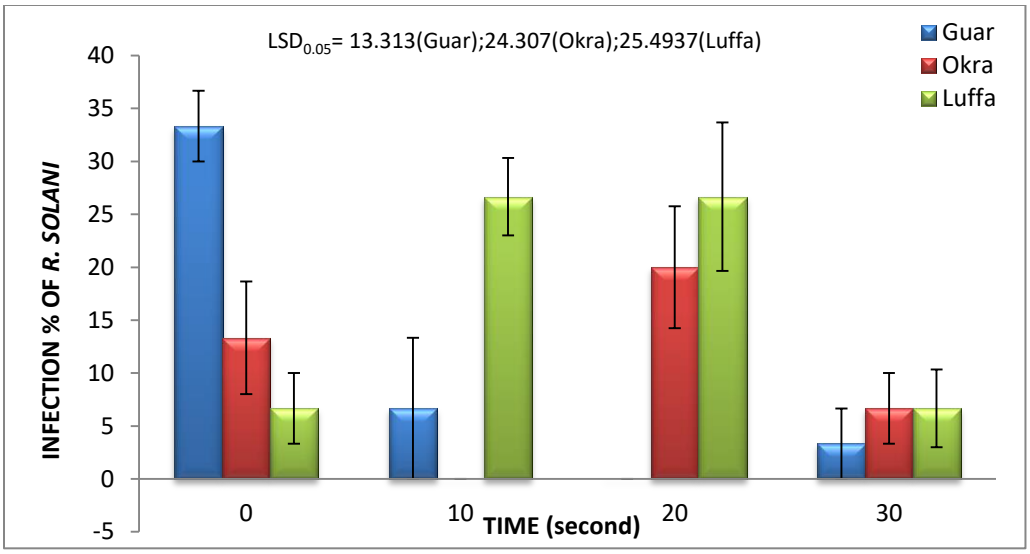


Fig. 6. Effect of microwave radiation on colonization of *R. solani* on guar, okra and luffa plants.

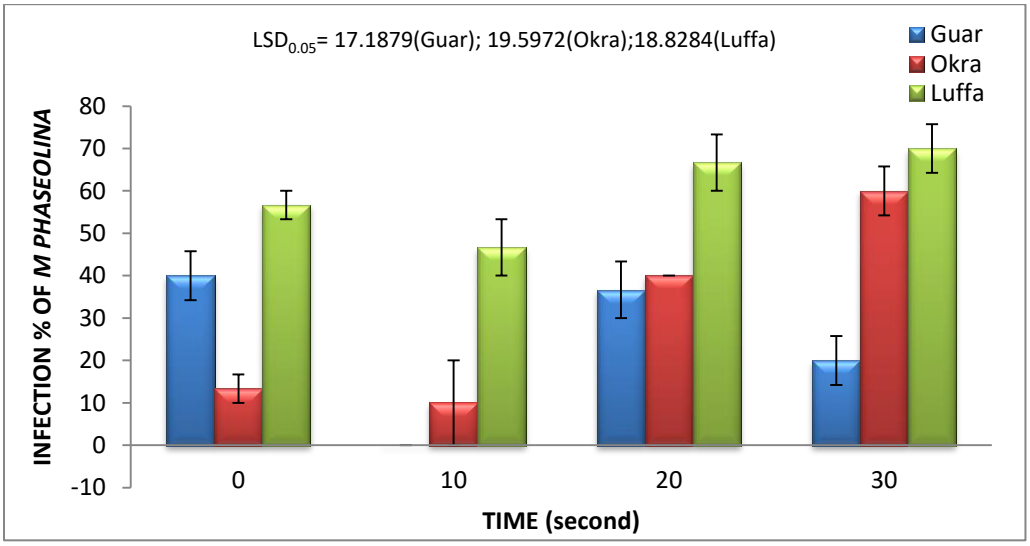


Fig. 7. Effect of microwave radiation on colonization of *M. phaseolina* on guar, okra and luffa plants.

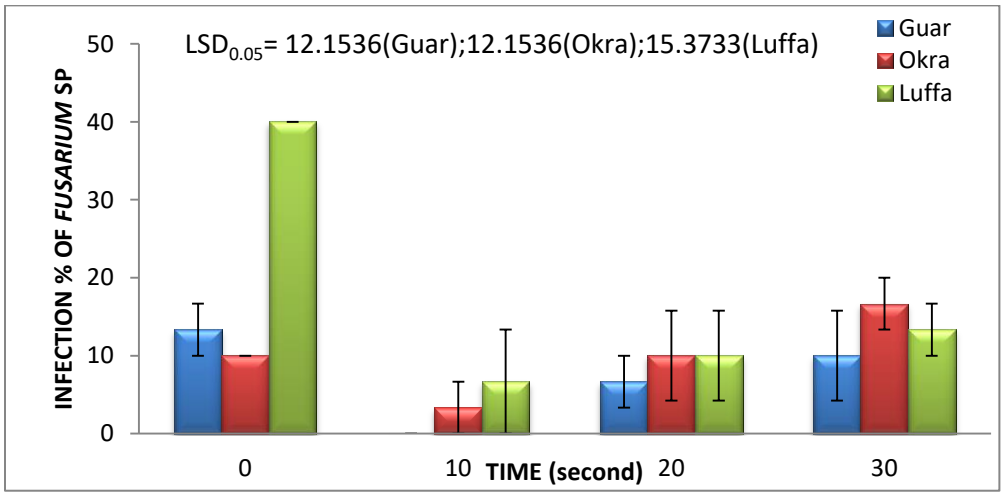


Fig. 8. Effect of microwave radiation on colonization of *Fusarium* species on guar, okra and luffa plants.

DISSCUSSION

Microwaves are electromagnetic waves with frequencies ranges between 300 MHz and 300 GHz. Microwaves are known to affect plant growth and development by reducing seed germination, however, the microwaves weak intensity does not affect plant growth but at high it influences plant growth by slowing seed germination (Oprica, 2008). The present study deals the effects of microwave radiation on okra, luffa and guar seed germination and plant growth. We noted a significant reduction in seed germination of okra, luffa and guar plants with an increase in the time of exposure to microwave radiation. It has been shown that exposure of seeds to high levels of radiation led to slow growth in seedlings, reduce plant fertility and aberrations in chromosomes (Belyavskaya, 2001; Martínez *et al*., 2003; Nikulin *et al*., 2009; Soja *et al*., 2003). Wheat seeds exposed to microwave radiation (6.74–7.44 GHz) affected wheat seed germination and development. Similarly, Lakshmappa *et al.* (2011) observed decrease in seed germination with the increase in microwave radiation exposure time from 12 to 28 minute

In this study, we noted a substantial increase in shoot length of guar and luffa plants when their seeds were exposed to microwave radiation at various time (10 to 30 seconds) than unexposed seeds. Similar result was also obtained by Duran *et al.* (2013). Duran noted that a single dose of microwave radiation to plant seeds increased the root lengths and shoot heights of the plants compared to unexposed control group. In the present study, we found that an exposure of test plant seeds to microwave radiation for short duration may enhance the root and shoot lengths of guar, okra and luffa plants.

Photosynthetic pigments are extremely sensitive to environmental changes. Variability in the photosynthetic contents may be used as an evidence of stress in plant (Salama *et al.*, 2011; Samuolienė *et al*., 2012). In the present study, we found a decrease in carotenoids contents when seeds were exposed to microwave radiation in contrast to Rock *et al.* (1998) who found an increase in carotenoid contents after one minute of radiation exposure. This difference may be the differences in both protocols or time of seed exposure to radiation (one-minute vs few seconds). Hamada (2007) showed increase in photosynthetic pigments in wheat plants after wheat seeds exposed to microwave radiation for 75 minutes.

Microwave irradiance declined the colonization of *Rhizoctonia solani* and *Fusarium* species. However, *Macrophomina phaseolina* remains unaffected with microwave radiations. A maximum exposure time 40–50 seconds with Microwave radiation decreased the *Colletotrichum lindemuthianum* symptoms by 17–23% in *Phaseolus vulgaris* (Friesen *et al*., 2014). Colonization % of *Fusarium* sp. in okra and Luffa plants were reduced when seeds were exposed to microwave radiation at different interval of time. Reddy *et al*. (1998) proved that microwave irradiation at 2.45 GHz effectively reduce infection of *F. graminearum* in wheat. Microwave radiation to soil is an effective method to eliminate the soil borne fungi and nematodes (Ferriss, 1984).

In conclusions, we suggest that the microwave radiations exposure to plant seeds is an effective method to reduce fungal colonization and for healthy plant growth. However, this response varies based on exposure time and test plant species. Research is underway in our laboratory to determine an optimum microwave radiation exposure to seeds to obtain better results.

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