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EFFECT OF MICROWAVE RADIATION ON PLANTS INFECTED WITH ROOT ROT PATHOGENS

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Abstract

Microwaves are non-ionizing radiations known to stimulate seed germination and growth. A complete randomized design screen house experiment was performed to evaluate the effect of microwave radiation on fungal survival and plant growth.Seeds of mash bean (*Vigna mungo* L.), chick pea (*Cicer arietinum* L.) and sunflower (*Helianthus annuus* L.) were irradiated with microwave radiations for 10, 20, 30 and 40 seconds. We demonstrated that exposure of seeds to microwave radiations for 20 and 30 seconds significantly suppressed root rot fungi like *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (kühn) and *Fusarium* spp. We also showed that by exposing seeds to radiations for 10 and 30 seconds the growth of chickpea, sunflower and mash bean plants was increased. These data collectively demonstrate that microwave radiations can potentially control fungal pathogens and thereby improve plant health.

Key words: Colonization %, Crop plants, Growth parameters, Microwave radiations, Root infecting fungi.

Introduction

Plant pathogenic fungi cause the most severe losses to agricultural crops around the world. For instance, *Fusarium* spp are important pathogens causing plant wilt which affect cell membrane permeability and interrupt plant metabolism (Garces de Granada *et al*., 2001; Pawar & Thaker, 2007). Disease causing soil borne organisms including *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp., attack root, reduce nutrition uptake and induce root rot disease complex and finally cause death of plants. *Rhizoctonia solani* causes foot and root rot which blocks the conduction of water and subsequently leads to mineral and water uptake deficiency (Wallwork, 1996). However, *Macrophomina phaseolina* affects plants when it reaches to its final stage of development, however, when crop reaches to their final stage, while disease seem to appear at any stage of plant growth (Machado, 1987). *Fusarium solani*, *F. oxysporum, Sclerotium rolfsii, Rhizoctonia solani, Alternaria solani, Macrophomina phaseolina* and *Pythium* spp. are the causal agents of the most deleterious fungal diseases like damping-off, root rot and wilt of vegetables (Khanzada *et al*., 2016; Ramamoorthy *et al*., 2002; Steinkellner *et al*., 2008). In order to protect crops it is necessary to control these pathogens which cause great harm to our crops. Although chemical treatments for the control of these pathogens is available, it is not recommended because these chemicals are not safe for the environment. Radiation technology has shown promising effect in the control of plant diseases caused by crop pathogens (Raupach & Kloepper, 1998).

Electromagnetic radiations including ultraviolet, ultrasonic, microwave, radio frequency and optical radiations are non ionizing long wave radiations. Among these, microwave radiation has shown minimum harmful impact on seed germination and vigour as compared to other radiations or thermotherapy methods. Microwave radiations is a form of electromagnetic radiation with frequency ranging between 300 MHz to 300 GHz which are used in microwave ovens, radar technology, spacecraft, television, telephone communications etc. Studies conducted in various laboratories have shown different responses by plants exposed to electromagnetic radiations. Martinez *et al.,* (2003) found that plants become taller and heavier when exposed to magnetic radiations. Similarly, germination of *Cicer arietinum* seeds was enhanced when exposed to moderate magnetic radiations (Vashisth & Nagarajan, 2008). Balmori-Martinez (2003) noted that microwave radiations at frequency of 0.2-30 MHz altered the cellular contents of calcium and sulphur. Microwave radiations suppress pathogens development and growth under optimum temperature and exposure time can be developed for a given host-pathogen interaction (Friesen, 2014). Microwave radiation has been used since mid-twentieth century and is considered to be one of the best non chemical methods to efficiently control plant fungal pathogens (Kalinin *et al.,* 2005). In addition, microwave radiation has been used for the control of pests in food processing, crop storage, and seed production (Bouraoui *et al*., 1993; Cunha *et al*., 1993). Microwave radiation uses heat to kill pathogens (Grondeau *et al*., 1994). It has been suggested that radiation may disrupt the microbial cells directly, although this has not been confirmed (Hankin & Sands, 1977; Yoshida & Kajimoto, 1988). Microwave seed treatment stimulates early germination and at the same time increases vigour (Van Assche & Leuven, 1988; Tylkowska *et al*., 2010). Due to its ability to rapidly generate heat, a short period of radiation exposure is needed for seed or pathogen for treatment with tradition (Adu *et al*., 1995).

Eglitis & Johnson (1970) noted a substantial reduction in damping-off caused by *Trifolium incarnatum* after they treated dry soil with microwave rays.

In the present study, we investigated the effects of microwave radiation on the growth and development of chick pea, sunflower and mash bean seeds exposed to low power microwave radiations (60 Hz). We also examined the impact of microwave radiation on crop plants development, growth and the reduction of root rot pathogens.

Materials and Methods

Exposure of seeds to microwave radiation: Seeds of mash bean (*Vigna mungo*L.), chick pea (*Cicer arietinum* L.) and sunflower (*Helianthus annuus* L.) were surface sterilized using 1% $Ca(OCl)_2$ for 3 minutes, dried under laminar flow hood. Then 20 seeds were separately transferred to a test tube. Then each sample was separately exposed to microwave Panasonic® NN-N740 (120V, 60 Hz, 120 Amps, 1200 W for 10, 20, 30 and 40 seconds. Reduction of seed coat was prevented by placing 150 mL of water into a 250 mL beaker (Reddy *et al*., 2000). Sterilized distilled water was also kept into the oven when sample was introduced to avoid an increase in temperature due to continuous exposure.

Experimental Design: Pot experiment was conducted in a green house and the soil used for planting seeds was obtained from an experimental plot at the Department of Botany, University of Karachi. Physical characteristics of experimental soil was noted as sand (60%), silt (22%) and clay (18%), pH 7.1-7.5, moisture holding capacity of 29% (Keen & Raczkowski, 1922), amount of nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), 2- 3 sclerotia g^{-1} of *M. phaseolina* (Sheikh & Ghaffar, 1975), 5-9% of *R. solani* (Wilhelm, 1955) and *Fusarium* spp., 3500 cfu g^{-1} (Nash & Synder, 1962). Seeds of test crops were exposed to microwave radiation for 10, 20, 30 and 40 seconds and separately planted in pots containing 300 g soil. Initially 5 seeds were planted per pot but after germination two seedlings were removed to allow other seedlings to grow further to reduce the competition between seedlings for nutrients. All pots were placed on screen house bench with complete randomized design (three replicates per treatment) and watered regularly in order to provide sufficient moisture. A set of pots containing seeds treated with sterilized distilled water was also placed to serve as control. After thirty days of germination, growth parameters were observed. Roots were washed with sterilized distilled water, disinfected with sodium hypochloride for 2-3 minutes and placed on Potato Dextrose Agar medium (PDA) for obtaining the colonization of root rot fungi (Rafi *et al*., 2016).

Analysis of data: One way analysis was performed for five treatments of microwave for each crop plants. Least significant difference was also estimated at 5 % significance level (Gomez & Gomez, 1984).

Results

Here, we showed that the exposure of microwave radiation to plant seeds before planting improved the quality of crops as well reduce or eliminate the fungal colonization. It was found that chickpea seeds exposed to microwave radiation for 10 seconds showed enhancement in shoot weight, shoot length (p<0.01) and root length. However, maximum root weight was recorded when seeds treated for 30 seconds (Fig. 1). Colonization of root infecting fungi were significantly reduced after radiation exposures however, 20 seconds exposure of microwave radiation completely inhibited the colonization of *R. solani*and *Fusarium*spp (p<0.05). It was also noted a substantial reduction in the colonization of *M. phaseolina* when seeds were exposed to radiation for 30 seconds (Fig. 1). In case of mash bean, shoot length and root weight were significantly (p<0.05) increased in seeds which exposed to radiation for 40 seconds followed by 10 and 20 seconds respectively (Fig. 1). Seeds of mash bean exposed to radiation for 20 seconds enhanced shoot weight and root length (p<0.05) followed by a 40 seconds exposure. We found a complete reduction of *R. solani*colonization on mash bean plant when the seeds were exposed to radiation for 20 and 30 seconds before planting, however, a 30 seconds exposure significantly (p<0.01) reduced colonization of *Fusarium* spp and *M. phaseolina* (Fig. 2). Sunflower plant length (p<0.01) and weight were increased significantly by using microwave radiation for 30 seconds while root weight was significantly (p<0.05) enhanced when sunflower seeds were exposed for 10 seconds (Fig. 1). Complete reduction (p<0.05) of *Fusarium* spp and *R. solani* were recorded when sunflower seeds were exposed for 20, 30 or 40 seconds while sunflower seeds exposed for 10 seconds reduced (p<0.05) colonization of *M. phaseolina* (Fig. 2). In the present study we have shown that exposure of seeds to microwave radiation for less than a minute improves the quality of plants and protect them from diseases that are caused by root infecting fungi. We also observed difference in the responses by different seeds. For example, chick pea seeds showed promising results with 10 seconds exposures, while mash bean with 40 and 20 seconds and the sunflower seeds with 30 seconds exposure.

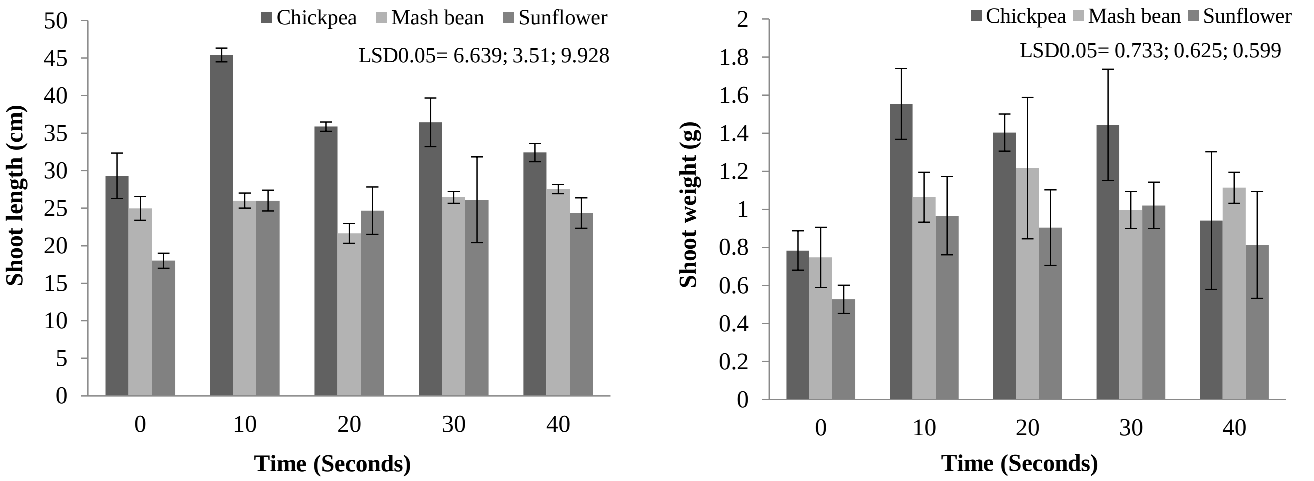

Fig. 1. Effect of microwave treated seeds with different time intervals on chick pea, sunflower and mash bean growth.

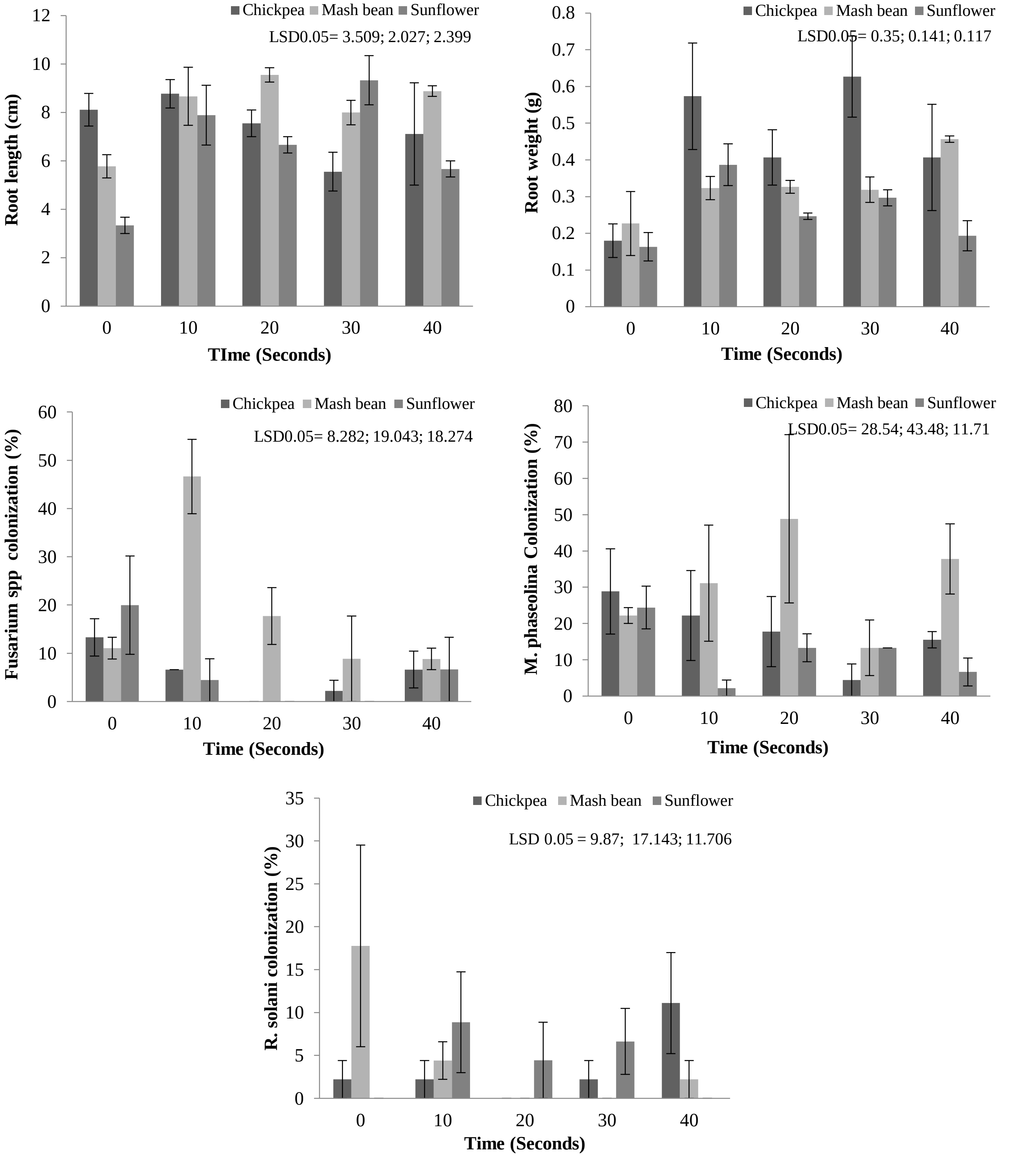

Fig. 2. Effect of microwave treated seeds with different time intervals on colonization % of root rot fungi of chick pea, sunflower and mash bean growth.

Discussion

Microwave radiation is a form of non-ionizing radiation that lies between common radio and infrared frequencies. Its frequency range from 300 MHz to 300 GHz which propagate through the space as time varying electric and magnetic fields (Feng *et al.*, 2012). Distinct characteristics such as faster heating rate and greater penetration in food stuff depth have made microwaves a unique tool for many agricultural and food industries. Datta & Davidson (2000) reported that no pathogen had been found to be microwave resistant. The exposure of long duration with microwave radiation may cause injury by retarding germination as well as destruction of germinative capacity. Therefore, decreases in germination rate or seminal roots length after radiation was due to longer duration of treatment which destructed the pathogen but also injured the plant cell so there was a need to work on a new approach in fungal control research by using less hazardous substances or control methods, were more compatible with environment.

We tested this possibility by exposing seeds to microwave radiation for 30 seconds, and found it was positively effective for sunflower growth and reduction of root rot fungi. However, 20 and 40 seconds improved mash bean growth. According to Bhaskara *et al*., (1998), exposure to microwave radiations for 30 seconds at temperature of 37.2ºC reduced fungal incidence upto 75% and improved the seed quality. No harmful effects on seed physiology were recorded previously, when seeds were exposed to radiation for 10 and 20 seconds (Smith, 2017). Spilde (1989) reported that 20 and 30 seconds of microwave radiation exposure to the navy bean seedsyielded the best results. We found similar results when mash bean and sunflower seeds were exposed to microwave radiations for 20 and 30 seconds. Several studies have reported that microwave radiations show different effects on seed germination in wheat and barley due to their own physical requirements (Marcu *et al*., 2013; Sparrow, 1962; 1965). Gaurilcikiene *et al*., (2013) demonstrated an increased wheat grain yield when seeds were exposed to strong microwave electric field at 2.6 GHz for 20 minutes. It is also reported that plant growth was increased due to low microwave frequency while high frequencies showed little or no effect on plant (Murakarmi *et al*., 2001). In addition, stimulating effect on seed germination and seedling vigour of wheat, Bengal gram, green gram and moth bean was observed at low power microwave radiation (1 KHz) (Ragha *et al*., 2011). Jakubowski (2010) reported that microwave irradiated potato tuber variety Felka Bona at frequency of 2.45 Ghz for 10 seconds increased the weight and growth biomass in potato while highest frequency like 38, 46 and 54 GHz for 20 seconds did not give positive impact on the weight of irradiated seed potato germs and potato tubers crop of variety Felka Bona. Ark & Parry (1940) observed that short term exposure to radiation gave increased impact on germination and vigour of seedlings while long exposure to microwave radiation even usually resulted in death of seed. This may be due to rapid diffusion of moisture through porous materials of plants results in very small rise in temperature of plants (Brodie, 2007).

Mycelial form of pathogens eradicated easily than spore which are more resistant to microwave radiation (Cavalante & Muchovej, 1993). Present work showed that seed treated for 20 and 30 seconds gave complete reduction of *R. solani* and *Fusarium* spp from roots of sunflower, mash bean and chickpea. Our results are similar toGaurilcikiene *et al*., (2013) showing shorter exposure time of 30 seconds demonstrated a higher stimulating effect than longer exposure. Some studies indicate that soil nutrients and pH are not affected due to radiations, however, soil populations (bacteria, fungi) are reduced upto 78 % after 16 minutes of microwave exposure (Cooper & Brodie, 2009). Soriano-Martin and colleague (2006) found reduced colonization of *Fusarium oxysporum*, responsible for *Fusarium* wilt when*cucumismelo* seeds were exposed to radiation (Soriano-Martin *et al*., 2006). Populations of soil microorganisms were reduced due to microwave treatment with increased exposure time. However, if amount of soil or soil water content are increased (between 16 and 37%) then the effect of microwave exposure can be decreased (Gupta & Jangid, 2011). *Phaseolus vulgaris* seeds showed minimum disease symptoms upto 17-23 % in cv. AC Ole (pinto bean) when treated with microwave radiation between 40-50 seconds (Friesen *et al.*, 2014). We found exposure of seeds to microwave radiation as the most convenient method for rapid elimination of soil borne pathogens from soil without excessive detrimental effects.

Conclusion

The use of microwave radiations as alternative control methods is a valuable tool for crop improvement because it is inexpensive, and show minimal harmful impact on the seed and the environment. In the present study, when seeds of test plants irradiated with 20, 30 seconds showed a complete reduction of soil borne pathogenic fungi. The exposure doses applied in this study show positive impact on growth of mash bean, sunflower and chickpea plants. More studies are needed to evaluate the effect of microwave radiation on biochemical and physiological parameters.

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