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USE OF MICROWAVE RADIATION IN SOIL STERILIZATION AND EFFECTS ON THE BACTERIA, FUNGI AND SOME GROWTH CHARACTERISTICS OF CHICKPEA PLANT (*CICER ARIETINUM* L.)

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Abstract

Two experiments were applied to study effect of sterilization by using microwave oven on germination, soil microorganisms and some growth characteristics of local chickpea planted in sterilized soil. First experiments applied in *vivo* using petri dishes, while the second carried out in *vitro* using pots. Seeds and soil treated by microwave at different exposure times ranged between (0 to 60/ sec) based on two energy levels at 50% and 100%. The *vivo* results showed significant effect of time sterilization on seed germination, that declined after 25 and 30 sec with both of energy level (50 and 100%) compared with non-sterile treatment. While, the experiment of soil microbial in pots showed highest decrease in number of fungi and bacteria in sterilized soil started after 30sec at (50 %) and 40sec at (100%) of levels of energy for bacteria and fungi respectability, compared with non-sterile treatment. Also the results showed that, significant differences between sterilization treatments on plant growth, if recorded treatment of 50 sec/time with 50% energy higher value compared with other sterile and non-sterile treatment of soil. For 100% energy level the result indicated that, non-growth of seedling in most of all treatments.

Keywords: Microwave radiation, Sterilization , Bacteria, Fungi, Chickpeas.

Introduction

In recent years, modern agriculture has faced significant challenges in terms of how to obtain high-quality field crops and vegetables, which are characterized by their beneficial nutrition and human productivity. Therefore, many countries are seeking to developing agricultural production using various means to increase crop production, with the use of environmentally friendly farming methods. Using of pure seeds of impurities and pollutants is one of the important factors that contribute to obtaining good germination, which in turn lead to improved plant growth and increase final production. Several environmental and physiological studies have demonstrated the effect of conditions on seed germination and plant development, also many several biotechnologies have been used to improve seed germination and improve the plant growth in planted soil (Sahin, 2014). As well as to exposed of seeds and seedlings to moderate pressures to stimulate plant responses and stress adaptation. It is known that many agricultural pests in the soil or associated with the seeds not only affect seed germination, but also reduce the production and quality of crops causing significant economic losses (Ashraf and Foolad, 2007; Azooz, 2009). On the other hand, soil is a complex environment because it contains many components, including different types of microorganisms, competing crop plants and weeds (Sahin, 2014).

Therefore, researchers in the field of soil science resort to different methods of sterilization of soil to contribute the elimination of pathogens, especially bacterial, fungal and nematodes, that causing significant losses in plant production (Rahi and Rich, 2008). There are many methods used to sterilizing soil, in most have selective effect on types of soil micro without the other, such fungicides and synthesis chemical (formalin, methyl bromide) which should be considered in the use of collateral damage such as toxicity to the human, residual impact, cost and effort (Almeida *et al.*, 1994). In addition other methods have been used as autoclaves, kama rays and ultraviolet rays which caused general extermination of all types of soil microscopic (Cicatelli *et al.*, 2015).

Harmful effects of the using pesticides and other methods on soil-microbial and environment, encouraged the researchers to more of numerous attempts to develop new strategies such as microwave-based technology (MW) for large-scale seed and soil treatment. The current studies remain inconclusive about using microwave (MW), although they have revolutionized agriculture (Nelson, 1996), such as this practical treatments can be used to purify soil and seeds from bacterial and fungal contaminants and reduce weeds (Brodie *et al.*, 2007). Khan *et al.* (2016), reported that, soil exposure used microwave (MW) (2.45 GHz) for 120 seconds before planting with wheat seeds, led to increase reached to 175%in dry biomass and 96% in grain yield compared with untreated microwave soil. Aim of current research to evaluate using Microwave radiation in soil sterilization, and effect it in seed germination, soil microbial and growth characteristics of Chickpea planted in soil with possibility to useful in agriculture in future research.

Materials and Methods

Effect time of sterilization on seed germination

Vivo experiments tested by glass dishes to determine the effect of sterilization on wave on germination seeds at different time intervals: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 seconds. Ten (10) seeds putted on petri dish between two pieces of sterile gauze with three replicates, then equal amount of normal water was added, then the dished transferred to microwave oven using two levels of energy 50 and 100 %. On the basis of the times mentioned above, seeds were incubated at 30 ° C and followed up after

7 days. Results were recorded on the basis of germination percentage of the total number of seeds in each dish.

Effect time of sterilization on soil microbial

The field experiment tested by collection of mixed soil in plastic pots which are described in Table (1). The local chickpea seeds were planted in pots with 3 seeds for each, then covered with the same soil mixture. Then the samples was irradiated by according to the selected time(0 to 60 sec) and at the two energy levels (50 and 100). After extracting the cultivated seed from the oven, it was irrigated as needed. Counting method was used to calculate the total of bacterial and fungi (UFC/m3) after growth it on Nutrient agar medium and Potato Agar Dextrose medium in the soil according to the method described by Komarova *et al.* (2008). The results of seed germination collected after 10 days of planting in pots, while some growth characteristics (plant height, root weight and dry green) taken after 30 days of planting.

Table 1 : Experimental laboratory and agricultural experiments.

Treatment	Exposure time (seconds)												
	0	5	10	15	20	25	30	35	40	45	50	55	60
50% Level energy (dishes)	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
100 % Level energy (dishes)	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26
50 % Level energy (pots)	T27					T28	T29	T30	T31	T32	T33	T34	T35
100 % Level energy (pots)	T36					T37	T38	T39	T40	T41	T41	T42	T43

Design Experimental

The experiment was designed according to the RCBD design with three replicates. The data analysed using the ANOVA table using SPSS V23. The mean of the experimental parameters was measured using the LSD test at a probability level of 0.05%.

Results and Discussion

Effect Radiation in Germination

Result from Table. 2 and Figure.1 shows the effect of the time of sterilization on seed germination at 50% 100% after 7 days and 10 days in dishes pots respectively. Generally the results using microwave sterilization recorded different percentage of germination rate with exposure time. In petri dishes experiment at level energy 50% the time exposure recorded negative effect with germination percentage reached (0%) starting from 35 until 60 sec. While time exposure with 5 and 10 sec recorded completely germination (100%), then the percentage reduced to (80%) with time 15 and 20 sec follow by time 25 and 30 which recorded percentage ranged (33.33 to 46.66%). Regarding using 100% energy level of microwave sterilization, the results indicated a similar effect of 50% with a slight difference in germination rates depending on time exposure.

Table 2 : Effect of the time of sterilization in the percentage germination of chickpea seeds at 50 and 100% energy levels and different time intervals

Dish experiment				Pot experiment			
50% level energy		100% level energy		50% level energy		100% level energy	
Treat	Germ (%)	Treat	Germ (%)	Treat	Germ (%)	Treat	Germ (%)
T1	100	T14	100	T27	90	T36	90
T2	100	T15	83.33	T28	66.66	T37	100
T3	80	T16	70	T29	66.66	T38	100
T4	80	T17	80	T30	83.33	T39	0
T5	60	T18	60	T31	100	T40	0
T6	46.66	T19	30	T32	83.33	T41	0
T7	33.33	T20	0	T33	66.66	T42	0
T8	0	T21	0	T34	66.66	T43	0
T9	0	T22	0	T35	66.66	T44	0
T10	0	T23	0				
T11	0	T24	0				
T12	0	T25	0				
T13	0	T26	0				

In pots experiment the results showed clear changes in germination rates without dependence on the level of radiation (50%) and exposure time with (100, 90, 83.33 and 66.66%) for time exposure 30 sec, 0 sec, 15 and 25 sec and other times sequentially as shows in table. 2. While there was a clear effect of (100%) energy level on germination ratio with increased exposure time. As recorded control treatment without exposure percentage was (90%) compared with time 5 and 10 sec which reached percentage germination (100%). The Other treatments with different exposure time (15until 60 sec) given fully inhibition of germination (0%).

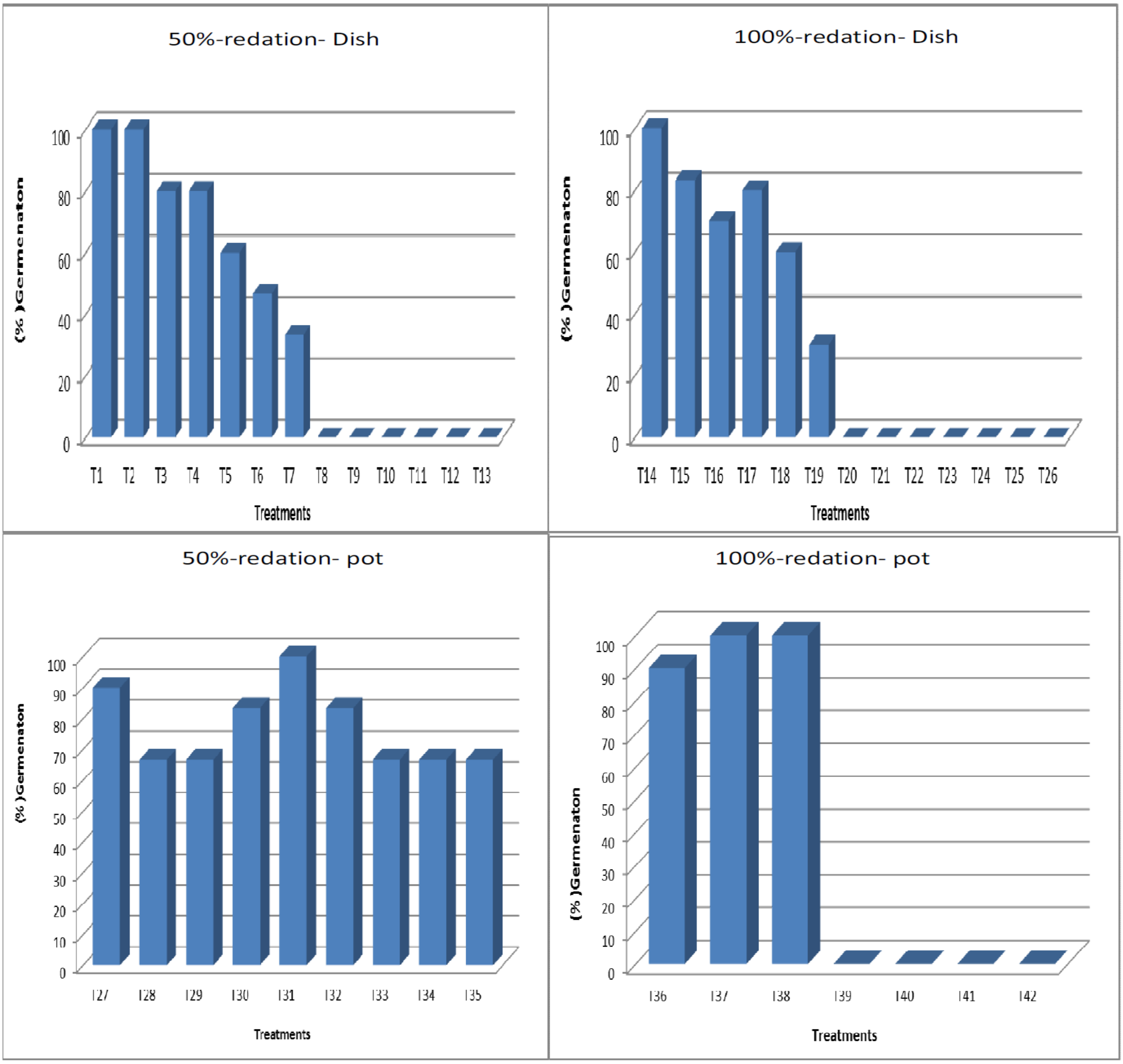

Fig. 1: Effect of different levels energy and time exposure on germination percentage under vivo and vitro conditions (planted in dishes and pots).

Result of current study are agreement with the findings of Magda and Sheena (2016) that, using Wi-Fi radiation with low levels and exposure time did not effect on germination percentage of Broccoli, Pea, Red Clover and Garden Cress . While result study of (Marcu *et al.*, 2013; Miller, 2015) reported that, the exposure time with high levels of radiation, showed negative effect on seeds germinate, and subsequent seedlings are at high-risk of mortality and the damage continued later in the early stages of growth. This effect may be back to genetic mutations that, caused by high risk that led to inhibition seed growth and increased mortality at mostly of radiation levels. Finding of (Komolprasert and Morehouse, 2004; Rashid and Daran's, 2013) reported that, a clear significant impact of radiation on seed germination and seedlings, it has also been shown that radiation affects different types of seeds differently.

Effect of Radiation in Soil Microbial

Results of table (3) and Figure .2 showed that, a significant decrease in the number of bacteria and fungi in treated soil by different time and level radiation. Treatment with different time and level range 50% recorded increase number of fungi and bacteria (3.1×10^5, 2.7×10^5, 0.6×10^5, 0.01×10^5 and 0 /CFU /g) for 5, 10, 15 , 20 and (25 to 60 sec) sequentially compared with control treatment which recorded high value reached 8×10^5 (T27).

Table 3 : Effect of the time radiation (0-60 sec) and level energy of microwave (50-10%) on groups soil microbial planted with chickpea seeds.

50%			100%		
Treatments	**Fungi (CFU) $\times 10^5$**	**Bacteria (CFU) $\times 10^5$**	**Treatments**	**Fungi (CFU) $\times 10^5$**	**Bacteria (CFU) $\times 10^5$**
T27	8	46	T36	8	46
T28	3.1	20	T37	1.8	15.7
T29	2.7	0.3	T38	0.42	0.04
T30	0.6	0.1	T39	0	0
T31	0.01	0.02	T40	0	0
T32	0	0	T41	0	0
T33	0	0	T42	0	0
T34	0	0	T43	0	0
T35	0	0	T44	0	0

Regarding the number of bacteria at same treatments (time and level) there are similarity impact of 50% .As registered control treatment higher value followed by 5 sec after that, strong rise noted in inhibition microbial community with increase time exposure table .2 As noted the 100% level energy gave a significant reduction in microbial community were (1.8×10^5 , 0.42×10^5 and 0) and (15.7×10^5 , 0.04×10^5 and 0) for 5, 10 , 15 and (20-60) sec for fungi and bacteria respectively. While the higher values were registered with control (8×10^5 /fungi and 46×10^5 /bacteria).

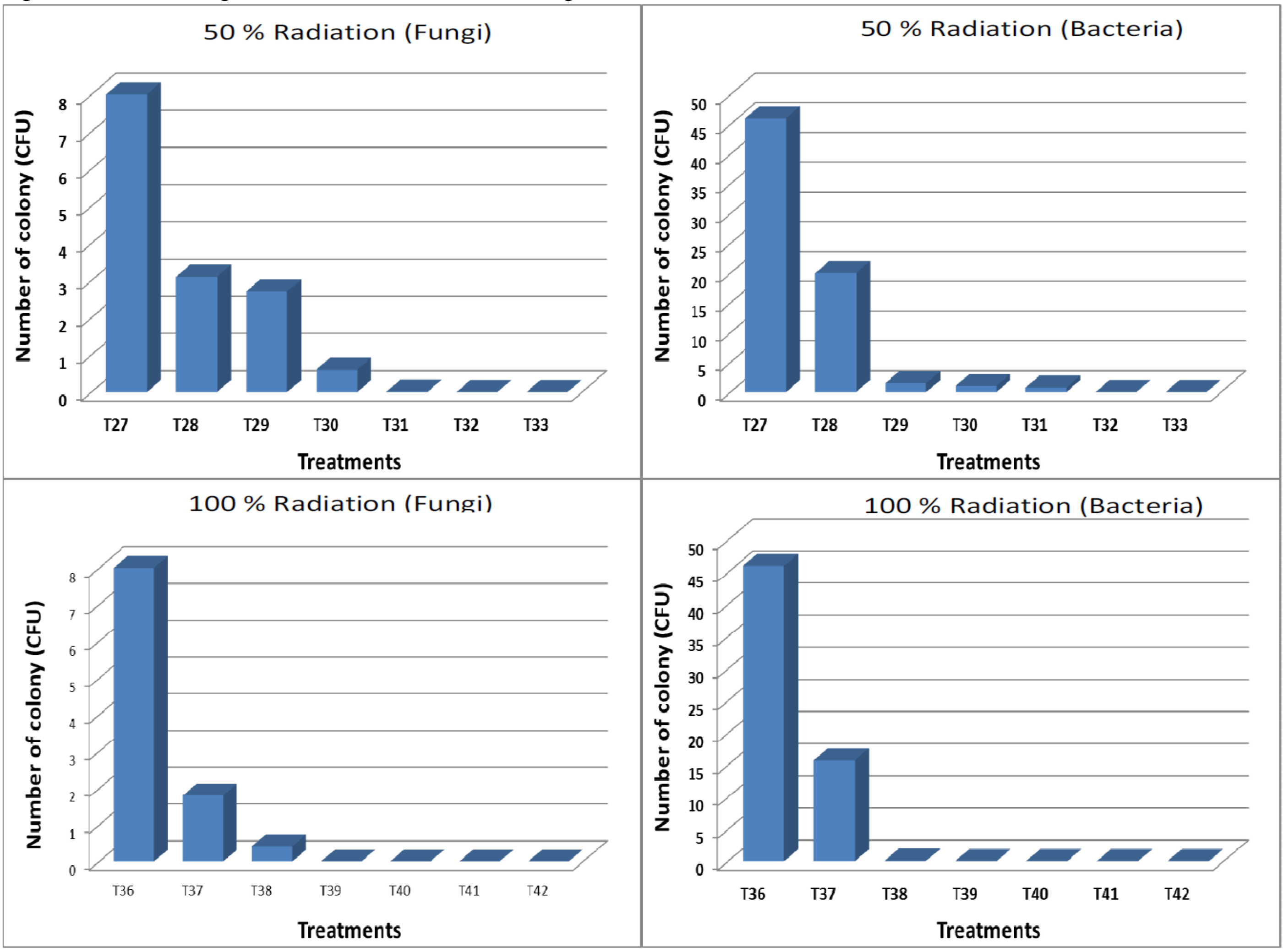

Fig. 2 : Effect of different levels energy and time exposure on number of colony (CFU) of fungi and Bacteria in planted soil.

The effect of microwave radiation on microbial aggregates in soil is associated with many unknown factors (Nelson, 1996). The microwave wave propagation in the soil is influenced by many factors such as soil moisture content, total density, organic matter content and temperature (O'neill and Jackson, 1990). The effect of microwaves depends on the growth of microorganisms primarily on the wave frequency and total energy absorbed by the microorganisms (absorbed dose). Furthermore, when microwave waves are used at high frequency and high energy for a long time, their thermal effect is most likely to be effective in killing (Cooper and Brodie, 2009). Study of Brodie *el al*. (2007) have shown that soil treatment with microwaves leads to the reduction or elimination of weed seeds, nematodes, soil bacteria and pathogenic fungi such as *Fusarium oxysporum* and *Sclerotium rolfsii*.

Effect of Radiation in growth plant

Table (4) shows the effect of different time of sterilization on the plant height, dry vegetative weight and dry root weight at 50% and 100% after 30 days of planting. There are significant differences between the treatment and control treatment at 50%. Treatment T33 significantly exceeded from all other treatments with value reached 27.6cm, 0.39g and 0.25 g for height plant, dry vegetable and dry root weight, while the control treatment (untreated) gave 18.5 cm, 0.07g and 0.05g. Other treatment (100%) radiation with different time exposure shows negative impact on mostly plant growth characterise. As noted high decline in value of means starting with time exposure 10 sec (10.33, 0.02 and 0.01) for plant high, dry weight of shoot plant and root respectively. After that, all the treatments registered fully inhibition of growth plant. While control treatment exceeded of all other treatments with average (18.5cm, 0.07g and 0.05g) for growth characteristics sequentially.

Table 4 : Effect of the time radiation (0-60 sec) and level energy of microwave (50-10%) on growth characteristics of Chickpea plant after 30 days of planting.

Pot experiment							
(level energy) 50%				(level energy) 100%			
Treat	High plant (cm)	Dry weigh (g)	Dry root weight (g)	Treat	High plant (cm)	Dry weigh (g)	Dry root weight (g)
T27	18.5	0.07	0.05	T36	18.5	0.07	0.05
T28	21.83	0.25	0.2	T37	23.33	0.08	0.02
T29	24.58	0.36	0.22	T38	10.33	0.02	0.01
T30	25.5	0.36	0.22	T39	0	0	0
T31	23.67	0.34	0.23	T40	0	0	0
T32	24.67	0.3	0.23	T41	0	0	0
T33	27.6	0.39	0.25	T42	0	0	0
T34	24	0.29	0.21	T43	0	0	0
T35	22.8	0.26	0.19	T44	0	0	0
LSD	1.35	0.008	0.024		6.72	0.01	0.007

The current result regarding plant growth, may be due the increased accumulation of nitrogen and other nutrients in the soil due to the thermal effect of the microwave that, come in agreement with finding of Khan *et al*. (2016), Also finding of current study are consistent with report of Wainwright *et al*. (1980), that reported using microwave radiation sterilize increasing ammonium ions and sulphur oxidation in the soil, which may lead to improve seedling and growth plant. Radiations can be caused damages on inflicted directly on the particular process or on the regulatory molecules involved in that process. Examples of such damage include membrane disruptions, protein conformational change, effect on plant hormones and pigments that ultimately affects the plant growth, yield, development, and numerous cellular processes such as photosynthesis and respiration (Zlatev *et al*., 2012). Choudhary and Agrawal (2014) also demonstrated morphological changes due to UV-B in another study on pea cultivars such as decrease in leaf area, root nodules, and root-to-shoot ratio in field conditions.

Conclusion

Using microwave radiation with low energy level and exposure time can be a safe way in sterilize soil as fast and alternative method compared with other treatments. While high level of energy show negative impact on all characterises under study. The use of microwave as an effective way to sterilize the soil can be effective and alternative with use warnings under high doses in soil sterilize.

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