Pak. J. Bot., 54(2): 717-722, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-2(9)

EFFECTS OF MICROBIAL ANTAGONISTS AND MAGNETIC RADIATIONS ON SUNFLOWER

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Abstract

Environmental damage and increase in human population showed that the global food production may soon become insufficient to feed the world's people. It is essential to improve the agricultural productivity by adopting more simple, sustainable and biologically friendly approach. For this purpose, radiations (gamma rays for 2 minutes, microwave rays for 30 seconds, Uv-c rays for 40 minutes and X-rays (45 Kev) for 10 seconds) and some biocontrol agents (*Trichoderma harzianum, Paecilomyces lilacinus, Bacillus thuringiensis* and *Pseudomonas fluorescens*) combinely used to observe influences on crop growth and on pathogenic fungi. Research showed that UV rays with *T. harzianum* and *P. lilacinus* gave maximum length and weight of sunflower plant while weight and number of seeds was improved due to combination between microwave rays and *P. fluorescens. T. harzianum* with x-rays and UV with *B. thuringiensis* showed reduction of root infecting fungi like *M. phaseolina* and *Fusarium* species. However, Fv/fm Quantum yield ratio and photosynthetic performance activity showed elevated results with the treatment having gamma exposed seeds in combination with *B. thuringiensis* which leads to increase the P values in OJIP curve.

Key words: Seed irradiations, Root rot fungi, Plant growth parameters and Physiological parameters.

Introduction

Over the course of recent century, demands for more food and fiber was increasing and agriculturists produce more food for growing human population but this goal was threatened by various factors like climate change, plant diseases etc. resulting in loss of billion dollars of farm productivity of affected areas (Beddington *et al*., 2012; Lobell *et al*., 2008). Different technologies were applied without disturbing equilibrium of useful and harmful composition of environment and ecosystem. This includes mainly chemicals but research showed that usage of chemicals produce adverse effect on environment as well as on beneficial microorganisms present in air and soil (Kumar & Gupta, 2012; Alori & Fawole, 2017; Baker *et al*., 2002). Some other ways to overcome crop losses problems includes use of fungicide, microbial antagonists, crop rotation, resistant varieties, soil amendment, soil drenching, hydropriming, biopriming, radiotherapy, thermotherapy etc (Babalola & Glick, 2012; Suyal *et al*., 2016). Another chemical free method is seed treatment with radiations used to destroy seed borne microorganisms has been applied since the mid- 20th century (Seaman & Wallen, 1967; Hankin & Sands, 1977). Exposure with ionizing radiation affects number of chemical, physiological, morphological and physical steps between initial absorption of energy and final biological injury (Kim *et al*., 2004). During this process the most important target is water molecule which is present in every organism. During chain reaction, radicals like H^+ and OH^- are produced becoming trapped, resulting in damage and modification of important components of cells finally affects physiological and biochemical processes which are vital for organism survival (Esnault *et al*., 2010).

Radiations with different wavelengths gave harmful as well as beneficial effect (Jeannie, 2001). Higher doses of gamma radiation were inhibitory whereas lower doses may be stimulatory (Radhadevi & Nayar, 1996). It was reported that gamma rays with low dose stimulates cell division, cell proliferation, germination, cell growth, enzyme activity, stress resistance, and crop yields (Baek *et al*., 2005; Chakravarty & Sen, 2001; Jan *et al*., 2012). Although an essential explanation for the stimulatory effects of gamma rays has not been available yet, but several studies is in support of this mechanism include changes in the enzyme activities and an increase in the antioxidant capacity of cells (Kim *et al*., 2004; Jan *et al*., 2012). Ultra violet (UV) comprising of wavelengths between 100 to 400 nm which is further subdivided into 3 bands namely UVA (315-400nm), UVB (280-315 nm) and UVC (100-280 nm). UVC radiations are highly energetic wavelengths which are not present in the sunlight at earth's surface. UV radiations effect plants in many ways like development of defence compounds, their structures, prevention against insects and fungal attack and DNA damage (Nielsen, 2018). High doses of UVC causes burning effect on plants while too low have no effect. However, appropriate dosage will not only increases branching and number of flowers but also total chlorophyll and carbohydrate contents of seeds were also increased due to exposure with UV-C for 20 and 30 minutes (Siddiqui *et al*., 2011). On the other hand, microwave radiations at 300 MHz to 300 GHz (non ionizing radiations of electromagnetic spectrum) affects cell membrane's permeability, cell growth rate and interference with ions and organic molecules especially proteins (Ungureanu, 2009). Moreover, exposure level of about 90 w for 30 and 60 seconds was helpful in growth of plants (Gupta *et al*., 2013; Ghiyasi *et al*., 2011; Kanwal *et al*., 2018; Ragha *et al*., 2011). X-rays have both destructive as well as simulative effects on plants depend upon the amount of doses given. Several researchers tested on x-rays by keeping dose very low on older plants as they aware about doses may affect plant growth (Perret *et al*., 2007; Kaestner *et al*., 2006). Blaser *et al*., (2018) uses x-ray CT of less than 8 Gy on two hosts, *Vicia faba* and *Hordeum vulgare* and recorded that in *Vicia faba* reduces root length, leaf growth, number and length of first, second order laterals while *Hordeum vulgare* donot show any negative impact of x-ray dose on root parameters.

Performance index, dark adapted quantum yield and chlorophyll fluorescence are the important parameters to find out the physiological effects of radiations. Induction by ionizing radiation plays an important role in intracellular signal transduction and activation of enzymatic antioxidant defence system (Wrzaczek *et al*., 2013). Microbial inoculants comprises of beneficial microorganisms are environmentally friendly, an alternative to chemical fertilizers, pesticides and also helpful in management of many crop diseases by exhibiting mycoparasitism against a wide range of plant pathogens (Babalola & Glick, 2012; Kumar *et al*., 2014; Sharma *et al*., 2014).

In view of these, this paper aims to explore the beneficial biological resource along with low doses of radiations for sustainable maintenance of plant health and ameliorating the harmful effect of root rot pathogens.

Materials and Methods

Microbial antagonists: Fungal antagonists including *Paecilomyces lilacinus* Thom Samson (Pl-6), *Trichoderma harzianum* Rifai (Th-60) were obtained from the Karachi University Culture Collection (KUCC) and were maintained on Potato Dextrose Agar (PDA) supplemented with appropriate amount of antibiotics. Bacterial cultures (*Bacillus thuringiensis* (Bt-9) and *Pseudomonas inflorescence*) were obtained from Department of Microbiology, University of Karachi and maintained on Nutrient Agar (NA) or Yeast Extract Mannitol Agar Medium (YEMA).

Seeds irradiation: Four radiations like UVC (<280 nm), microwave (Panasonic® NN-N740, 120V, 60 Hz, 120 Amps, 1200 W), x-rays (45 keV) and gamma rays (*TC*-99M) were used in this study. Sunflower (*Helianthus annuus* L.) seeds were sterilized using sodium hypochlorite solution (5 minutes) and air dried. These sterilized seeds were divided into four sets and each sets were treated with radiations namely gamma rays (2 minutes), microwave rays (30 seconds), UVC (40 minutes) and x-rays (45 keV for 10 seconds). These doses were selected as they gave promising results in improvement of crops growth (Kanwal *et al*., 2017; 2018).

Experimental setup: Field was prepared at Department of Botany, University of Karachi, Pakistan during September to November, 2019. Each plot per treatment sized 0.5 m x 0.5 m (total 25 treatments), prepared in randomized complete block design with three replications. Ten treated seeds were sown in each plot which after seedling emergence 5 plants was maintained. After emergence of seedling, 50 mL aqueous conidial or cell suspension of antagonists like *T. harzianum* (170×10^6 cfu g^{-1}), *P. lilacinus* (179×10^7 cfu g^{-1}), *B. thuringiensis* (120×10^9 cfu g^{-1}) and *Pseudomonas florescence* (220×10^9 cfu g-^{1}) were drenched in each plot. A control plot is also maintained in which seeds were treated with sterilized distilled water without any antagonistic microorganisms and radiations. Plants were germinated upto yield.

Growth and colonization of root rot fungi: After harvesting of plants, data on root length and fresh weight, shoot length and fresh shoot weight, number of pods, number and weight of seeds were recorded. Fresh roots were washed with sterilized distilled water and then with sodium hypochlorite (1.0%). These roots were placed on Petri plates containing Potato dextrose Agar (PDA) after cutting into small (1 cm long) pieces for the isolation of root infecting fungi (Kanwal *et al*., 2018).

Chlorophyll fluorescence: Between 2 to 4 pm the chlorophyll fluorescence was analyzed using the fresh sunflower leaf. Chlorophyll fluorescence meter (OS-30p+, Opti-Science, USA) was used to conduct the parameter. Leaves were covered with the clips for 20 minutes to perform the dark adapted test and dark adaptive quantum yield (fv/fm) ratios. By which the working efficiency of PSII and photosynthetic performance index (PIabs) on absorption basis were recorded (Maxwell & Johnson, 2000).

Results

Plant growth and control of root pathogenic fungi: Radiations alone or in combination with antagonistic microorganisms improves plant length and weight as compared to control. UV rays in combination with *T. harzianum* enhances plant length while combination between UV rays and *P. lilacinus* gave maximum plant weight ($p<0.001$; Table 1). Weight of flower, number and weight of sunflower seeds markedly elevated due to combination between microwave and *P. lilacinus* followed by microwave radiations and *P. fluorescens.* Radiations, antagonistic agents and their combinations markedly reduces infection in sunflower roots. However, *R. solani* was completely reduced by the combination between X-rays and *P. fluorescens* followed by UV rays combinely with *P. fluorescens* (Table 1). *T. harzianum* alone and used in combination with X-rays showed maximum reduction of *Fusarium* species in contrast to control. Another root infecting fungi *M. phaseolina,* greatly reduced by the combination between UV rays and *B. thuringensis* followed by microwave radiation with *T. harzianum* (Table 1).

Effect on physiological parameters: Efficiency of some physiological parameters have been described by the chlorophyll fluorescence test in which work had been made on different areas such as, Fv/fm Quantum yield ratio or the ratio of variable fluorescence and maximum fluorescence, performance index (PI) and OJIP curve. Photosynthesis is very important mechanism of plants and it is necessary for plant growth and survival. These parameters have been used as an indicator of photosynthetic activities.

Table 1. Field application of irradiated seeds with different radiations in combination with microbial antagonists for the control of root rot fungi of sunflower.

Treatments	Shoot length (cm) ± SD	Root length (cm) ± SD	Root weight (g) ± SD	Shoot weight (g) ± SD	Weight of flower (gm) ± SD	No of seeds ± SD	Weight of seeds (gm) ± SD	*M. phaseolina* % ± SD	*Fusarium* spp % ± SD	*R. solani* % ± SD
Control	74±9.93	11.33±2.35	5.8±1.72	1.95±0.79	5.59±3.45	56±25.66	4.13±2.10	53.33±14.40	60±0.00	44.44±17.49
Gamma rays (2mins)	115.3±11.11	11.93±2.25	9.1±1.67	14.53±17.30	6.12±1.47	67.33±21.31	6.88±1.87	26.66±14.40	15.55±21.99	24.44±8.31
Microwave rays (30secs)	110.66±15.5	5.13±1.97	21.13±7.18	4.4±1.34	7.33±0.47	97.33±4.10	7.4±1.23	28.88±6.28	6.66±0.00	2.22±3.13
Uv-C (40 mins)	108±6.63	5.66±2.62	9.33±6.12	4.4±22.64	4.43±0.80	61±27.58	6.83±0.23	28.88±6.28	28.88±31.89	2.22±3.13
X-rays (45kev, 10 secs)	76.66±36.71	11.8±7.98	5.46±2.56	3.39±2.57	8.36±2.21	51.7±36.2	5.9±0.82	35.55±13.69	20±0.00	4.44±6.28
B. thuringiensis drenching	91±3.55	4±0.81	13.33±2.05	1.72±0.49	14.11±1.99	65.33±4.10	7.03±2.23	37.77±12.56	46.66±18.85	26.66±28.80
P. fluorescens drenching	103±28.99	4.25±1.95	20.33±9.97	5.73±1.26	12.53±1.28	73.33±14.72	7.66±1.24	48.88±3.14	15.55±8.31	8.88±6.28
P. lilacinus drenching	87.66±36.5	26.9±12.3	25.33±24.56	4.85±2.94	13±4.54	86±9.93	11±4.96	19.99±14.39	19.99±10.88	17.77±3.14
T. harzianum drenching	83.33±16.99	3.2±1.27	27.33±5.24	6.33±2.05	6±2.16	76.33±4.92	8.33±0.47	28.88±6.28	4.44±6.28	13.32±9.42
Gamma & *B. thuringiensis*	70.33±8.17	9.5±1.63	6.33±0.47	1.55±0.31	6.1±2.09	38.33±16.49	4.66±0.94	37.77±3.14	19.99±5.44	22.22±13.70
Gammarays & *P. fluorescens*	88.33±4.64	2.88±5.07	6.33±0.97	1.26±0.59	6.23±2.09	66.66±27.35	5.2±1.88	44.44±20.6	17.77±3.14	13.33±18.85
Gammarays & *P. lilacinus*	99.66±8.05	15.30±9.93	7.33±2.86	2.94±1.20	9.86±7.12	118±52.94	10.4±2.26	24.44±3.13	13.33±0.00	6.66±9.42
Gammarays & *T. harzianum*	62.33±8.80	12.90±3.69	6.66±0.47	1.18±0.15	7.66±0.94	31.66±25.00	5.73±1.31	26.66±5.44	35.55±22.66	11.11±15.71
Mv rays & *B. thuringiensis*	101±10.67	6.43±2.8	32.33±10.87	5.03±2.84	23.33±8.99	129.3±50.39	13.33±7.54	35.55±29.97	13.32±9.42	20±0.00
Mv rays & *P. fluorescens*	100±0.00	8.4±0.99	30.33±13.76	7.57±1.79	31.24±3.68	171±20.99	19.43±4.22	33.33±0.00	7.77±13.69	17.77±13.69
Mv rays & *P. lilacinus*	92±16.57	5.16±1.92	45.66±14.52	9.62±1.31	39.21±20.57	205.6±72.09	21.73±7.84	24.44±8.31	6.66±9.42	13.33±10.88
Mv rays & *T. harzianum*	92±18.83	5±1.63	29.66±5.43	4.87±1.96	29.1±3.64	131.3±21.06	16±4.32	11.10±11.32	6.66±5.44	11.11±15.71
Uv rays & *B. thuringiensis*	81±4.24	6.8±1.88	22.33±7.40	5.2±1.52	14.06±3.65	66.3±21.23	5.54±1.83	8.88±6.28	24.44±34.56	24.44±8.31
Uv rays rays & *P. fluorescens*	70±35.59	6±0.81	3.03±0.83	25.66±12.39	13.66±2.05	83.33±9.42	6.83±2.24	13.33±0.00	17.77±12.56	2.22±3.13
Uv rays & *P. lilacinus*	102±10.80	4.66±2.05	6±2.51	34.66±10.87	15.33±7.58	59±15.25	5.13±1.31	22.22±3.13	35.55±12.57	4.44±6.28
Uv rays & *T. harzianum*	116.6±5.73	6.33±0.47	68.33±0.47	20.33±6.94	12±2.44	70.68±23.67	7.66±0.94	28.88±6.28	11.08±3.17	13.33±9.44
X- rays & *B. thuringiensis*	107.6±8.8	14.46±2.16	4.33±0.47	3.74±2.61	18.93±10.71	192.6±146.6	11.88±5.70	37.77±16.62	39.44±19.62	11.11±15.71
X-rays & *P. fluorescens*	82.3±16.9	14.46±7.09	5±1.41	1.87±0.49	6±2.20	49.33±19.48	4.73±2.64	24.44±6.28	15.55±3.14	0.00±0.00
X- rays & *P. lilacinus*	95.3±13.59	13.33±1.88	7.66±0.94	1.88±0.29	10.53±3.3	54.33±28.24	13±6.68	24.44±34.56	25.11±16.56	6.66±9.42
X- rays & *T. harzianum*	80±13.14	11.36±3.00	6.1±2.32	2.19±0.96	7.88±3.71	47.33±33.87	4.3±2.09	19.99±9.42	4.44±3.13	11.11±15.71
$Lsd_{0.05}$=	34.970	8.894	15.297	12.187	12.285	80.376	13.307	27.092	30.107	23.575

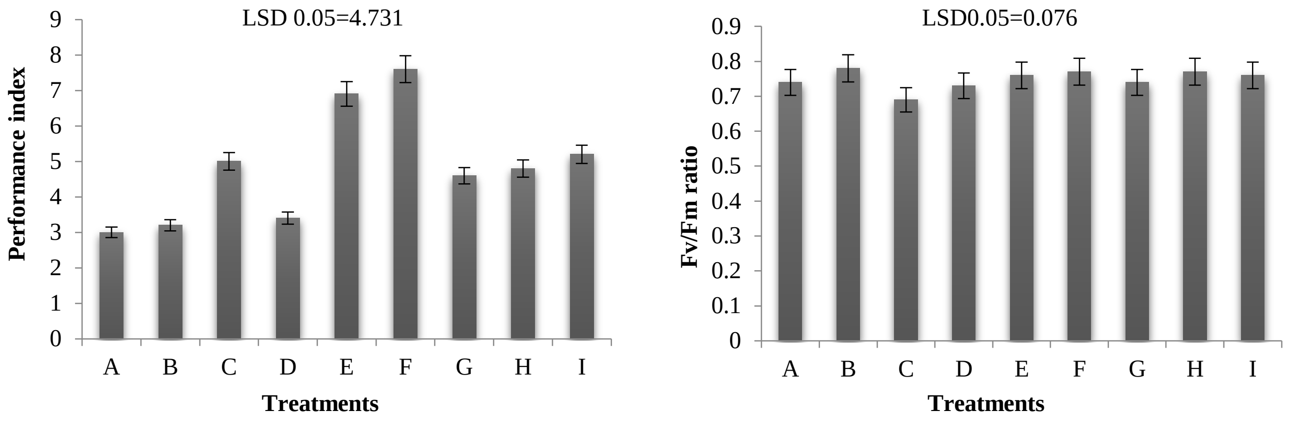

Fig. 1. Effect of seed treatment with effective doses of radiations in combination with microbial antagonistic soil drenching on performance index and Fv/Fm ratio.
A= control; B = gamma rays; C = Mv rays; D = *B. thuringiensis;* E = *P. lilacinus;* F = gamma + *B. thuringiensis*; G = gamma + *P. lilacinus;* H = Mv rays + *B. thuringiensis;* I = Mv rays + *P. lilacinus.*

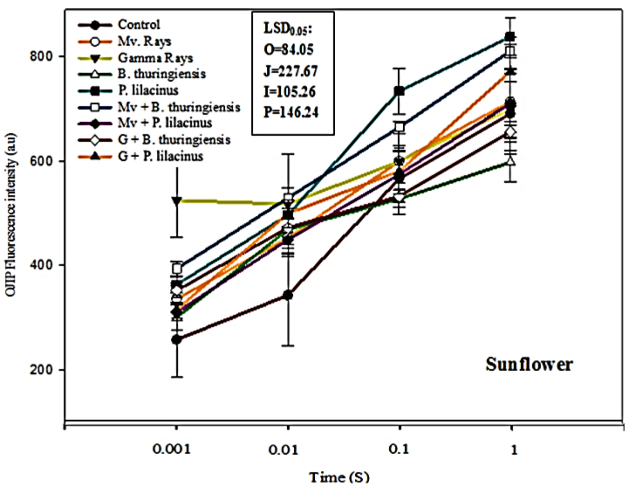

Fig. 2. Effect of seeds treated with effective doses of radiations in combination with microbial antagonistic soil drenching on OJIP induction curve.

Photosynthesis performance index leads to the enhancement of biomass production and antioxidant enzymes activity. Treatment having *B. thuringiensis* drenched soil and gamma exposed seeds for 2 minutes, showed elevated results in terms of photosynthetic performance activity in sunflower crop, followed by the treatment having *P. lilacinus* drenched soil (Fig. 1). In our studies it has been estimated that the ratio of variable fluorescence and maximum fluorescence Fv/fm of sunflower plants increases as compared to the control with all treatments (Fig. 1). Fv/fm Quantum yield ratio increased significantly (p<0.05), in the treatment having gamma exposed seeds of sunflower sown in *B. thuringiensis* drenched soil. The same result of enhancement of Fv/fm ratio was also followed by microwave rays at 30 seconds time interval in combination of *P. lilacinus* drenched soil for sunflower plants. It leads to the better activity of PSll reaction centre and automatically hints to the significant CO_2 fixation. Environmental condition and treatments which are going to be favourable for plant affect the efficiency of PSl and PSll markedly. Sunflower gamma irradiated seeds along with the *B. thuringiensis* drenched soil leads to increase the P values in OJIP curve (Fig. 2).

Discussion

Present experiment showed that the selective doses of different rays in combination of microbial antagonists gave significant results in terms of growth parameters due to improving the crop physiology and yield productivity. Our results were in the harmony of Mehmood (2003) that chickpea crop growth increases in terms of yield and field productivity has been investigated in the treatment having seed irradiation with *T. harzianum* and *P. fluorescens* prominently. *P. fluorescens* reported to be potential antagonist for the control the root rot fungi. Plant growth was enhanced prominently due to *P. fluorescence* treatments because they produce mineralizing phosphate which is gibberellins like hormones and act as a growth promoter (Sharma *et al*., 2013). Biocontrol agents have high competitive ability against the pathogen to enhance the growth of treated plants. Microbial antagonists produce phytoharmones, organic acids and show other defensive mechanisms such as nitrogen fixation, synthesized fungal cell wall lyses enzymes which degrades the pathogen cell wall and reduce them (Nelson, 2004). Paul & Sharma (2006) reported that pyoluteorin and pyrrolnitrin produced by *P. fluorescens*. These antifungal metabolites leads to the inhibition of fungal activity. *Fusarium oxysporum* colonization in the roots of cotton significantly reduced due to the seed treatment with *T. hazianum* and *Bacillus subtilis* (Zhang *et al*., 1996). Host defensive machanisms have been stimulated in host when treated with some *Trichoderma* spp and *Pseudomonas* spp., (Harman *et al*., 2004). In the control of several diseases such as angular leaf spot, bacterial wilt and *Fusarium* wilt, inoculation of (PGPR) plant growth promoting rhizobacteria found to be effective (Ryu, *et al*., 2004). Tomato seed treatment with *B. subtilis*, proved to be significant in terms of elevation of growth parameters and automatically yield production. Biomass production of plants had been stimulated in the host plant treated with the various strains of *Trichoderma* (Shoresh *et al*., 2010).

One of the most important biological processes in plants to survive is photosynthesis (Blankenship, 2014). CO_2 is taken up by plants and converted into carbohydrates stable compound and energy rich compounds energy rich compounds with the release of O_2 (Eaton-Rye *et al*., 2012). CO_2 assimilation not only consists of an enzyme but also on four major protein complexes. Photosystem II catalyses the splitting of water molecules and oxidation reduction process continuously which catalyses the final stages of this reaction, which is the production of NADPH and ATP. For plant breeder it was a hectic challenge to increases the biomass production which automatically lead to photosynthesis (Evans, 2013). In the present study work have been done on Fv/Fm ratio, OJIP and PIabs parameters of chlorophyll fluorescence. FV/Fm ratio was calculated to find out maximum quantum yield of PSII and chlorophyll fluorescence induction OJIP. Three phases OJ, JI and IP have been used for work out. It has been reported that OJ rise represent the photo chemical phase depend on reduction of QA in PSII active centers, (Stirbet & Govindjee, 2012). It revealed that OJ rise lead to the photochemical rate which depend on photo reduction of QA in PSII centers. High IP phase represent that reduction of PQ pool (Oxidized) so, lead to the rise in florescence (Prasil *et al*., 1996). IP phase is the distribution of energy between PSII and PSI where low fluorescence state converted into high state. In our studies IP phase was raised due to treatments of irradiation and drenching, better increase in the curve at the point of IP phase, has been investigated. In sunflower plants, rise in OJIP curve markedly observed when seed irradiated with microwave along with *B. thuringiensis* whereas gamma irradiated seeds responsible for the rise in OJ phase. Value of PIabs of sunflower has been increased significantly in the treatment of gamma rays along with the *P. lilacinus* and *B. thuringiensis* and followed by *P. lilacinus* individually. In our study the ratio of Fv/Fm increased which would be the indicator of greater photosynthesis activity. Various growth promoting hormones reported to be elevated significantly in the gamma radiated treatment of plant material. It has be reported by Geras'kin *et al.*, (2017) that gamma rays at low doses effectively enhanced the Glucose-6-phosphate dehydrogenase and pyruvate kinase level in barley treated seeds which automatically leads to the larger respiration and larger energy production water splitting activity represented by J-I fluorescence phase whereas OJ phase from OJIP induction curves represent the reduction of primary electron acceptors of PSII such as NADPH production indicator is IP phase. So, highest IP leads to the highest NADPH production.

Haldimann *et al.*, (1996) published a report that plant stress's environmental conditions lead to photo inhibition in maize leaves. Through Fv/Fm ratio, photochemical efficiency of PS-II and photosynthetic performance index estimated. It has been reported that Fv/Fm ratio has been declined when sunflower plant faces the salt stress which was noticed as an indicator of stress condition and activity of photosynthesis also become negatively affected (Umar & Siddiqui, 2017). In our results, the values of Fv/Fm ratio, significantly increased in sunflower when the seeds irradiated with gamma rays for 2 minutes in combination with *B.thuringiensis* followed by *P.lilacinus.*

In our studies, better and dominant rise in the values of PIabs was noticed when sunflower seeds were exposed with gamma rays in combination with *B. thuringiensis* suspension drenched soil it is followed by the treatment having the suspension of *P.lilacinus* in drenched form. Fracheboud *et al.*, (1999) suggested that PSII (photosystem II) activity has been used as a marker of photosynthesis rate. In sunflower crop the activity of photosynthesis increased due to our treatment which leads to biomass production. Photosynthesis is the good indicator of adaptation of plants to their environment so for environmental research chlorophyll fluorescence is used as an attractive and effective tool. However, seeds radiation with different electromagnetic rays is a useful method for improvement of crop productivity and tolerance level of root diseases caused by rot pathogens.

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(Received for publication 21 June 2020)