Our Nature (2010) 8:26-33

Gamma Radiation (^{60}Co) Exposure and Application of Antagonists for the Suppression of Root Rot Diseases in Sunflower (*Helianthus annus* L.) and Mung Bean (*Vigna radiata* (L.) R. Wilczek)

S. Dawar, M.J. Zaki, N. Ikram and M. Tariq

Department of Botany, University of Karachi, Karachi- 75270, Pakistan
*E-mail: *shahnaz_dawar@yahoo.com*

Received: 10.08.2010, Accepted: 29.11.2010

Abstract
An experiment was carried out for the control of root rot fungi such as *Fusarium* spp., *Macrophomina phaseolina* (Tassi) Goid., and *Rhizoctonia solani* Kühn in Sunflower (*Helianthus annus* L.) and Mung bean (*Vigna radiata* (L.) R. Wilczek). Seeds were treated with gamma radiation (^{60}Co) for 0, 2, 8 and 16 minutes intervals and soil was drenched with different antagonists such as *Trichoderma harzianum* Rifai, *Pseudomonas aeruginosa* (Schroeter) Migula and *Rhizobium meliloti* Dangeard. All the antagonists significantly increased germination percentage, shoot length, root length, shoot weight, root weight, leaf area and showed complete suppression of *M. phaseolina* and *R. solani* observed in mung bean and sunflower. In both crops, mostly growth parameters increased as the exposure of gamma rays increased whereas in some cases it decreased. *P. aeruginosa* and *R. meliloti* were observed to be satisfactory biocontrol agents followed by *T. harzianum* whereas all exposure time of irradiation of gamma rays (^{60}Co) to seeds of mung bean and sunflower were found to be best.

***Key words*:** Gamma rays, ^{60}Co, mung bean, sun flower seeds, root infecting fungi, suppression

Introduction

Radiation considered being an excellent tool for sterilization, preservation of food and other different food engineering processes, which gives benefit to the human society (Ivanov *et al*., 2001; Hyun-Pa *et al*., 2006; Sameh *et al*., 2006). The study of the effects of radiation on plants is a broad and complex field and work is being done in many areas on a large number of plant species. It was observed that radiation affect the size and weight of plants. There are variety of control methods used in order to avoid the yield losses due to soil borne pathogens such as use of radiations (Spadaro and Gullino, 2005), chemical compounds which is toxic to fungi (Washington and McGee, 2000). Although these methods have been effective in controlling soil born pathogens but there are some hazardous effect of these methods which turn the research towards environmentally safe methods as biological methods. A large number of studies have been devoted to the identification of microorganisms able to reduce the activity of soil-borne pathogens. The efficient bio-control agent produces inhibitory compounds, competitive for space and nutrients and secretes chitinolytic enzymes (Zimand *et al*., 1996). Among the rhizosphere organisms, *Pseudomonas* is a free-living bacterium which enhances emergence, colonize roots, improves seed germination, root development, mineral nutrition and water utilization, and can also suppress plant diseases and stimulates overall plant growth. The manipulation of crop rhizosphere by inoculation with *Pseudomonas* for biocontrol of plant pathogens has shown considerable promise (Siddiqui and Mahmood, 1999). Similarly, presence of rhizobia in the rhizosphere may also protect the host root from damage caused by pathogens (Siddiqui and Husain 1992). *Trichoderma* species have been reported as effective biological control agents against most of soil born diseases (Ahmed *et al*., 2003; Roberts *et al*., 2005).

Therefore, present work indicates the application of antagonists to soil and seeds of sunflower (*Helianthus annus* L.) and mung bean (*Vigna radiata* (L.) R. Wilczek) treated with gamma rays to improve growth parameters and control root rot diseases.

Materials and methods

Cultures of antagonists like *T. harzianum* Rifai, *P. aeruginosa* (Schroeter) Migula and *R. meliloti* Dangeard were obtained from Karachi University Culture Collection Centre (KUCCC). *T. harzianum* maintained on Potato Dextrose Agar (PDA), *P. aeruginosa* on Nutrient Agar (NA) whereas *R. meliloti* on Yeast Extract Mannitol Agar medium (YEMA).

Seeds of Sunflower (*Helianthus annus* L.) and mung bean (*Vigna radiata* (L.) R. Wilczek) were surface sterilized with 1% calcium hypochloride, dried under laminar flow hood. The seeds were then exposed to radiations with a ^{60}Co emitting gamma-rays with time periods of 0, 2, 8 and 16 mins. The seeds were irradiated at the Department of physics, University of Karachi.

The irradiated seeds were sown in 8 cm diameter, plastic pots, each containing 300 g soil. Soil used was obtained from experimental plot of Department of Botany, University of Karachi. The sandy loam soil containing (sand, silt, clay, 60, 22 and 18%), pH ranged from 7.1-7.5 with moisture holding capacity (MHC) of 29% (Keen and Raczkowski, 1992), total nitrogen 0.077-0.099% (Mackanzie and Wallace, 1954), 3-4 sclerotia/g of *M. phaseolina* g-^{1} as found by wet sieving technique (Sheikh and Ghaffar, 1975), 5-10% of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and *Fusarium* spp., 3500 cfu g^{-1} as assessed by soil dilution technique (Nash and Synder, 1962). Twenty ml of aqueous conidial or cell suspension of antagonists like *T. harzianum* (100×10^{3} cfu g^{-1}), *P. aeruginosa* (133.33×10^{9} cfu g^{-1}) and *R. meliloti* (120×10^{9} cfu g^{-1}) were drenched in each pot. Seeds treated with sterile distilled water and without antagonists suspension were served as control. Pots were watered regularly to maintain sufficient moisture required for the germination of seeds. The pots were kept in screen house in randomized complete block design. Per treatment three replicates were used. Growth observations both on the control and irradiated seedlings were recorded after 30 days of seed germination. The parameters taken into consideration were shoot length, shoot weight, root length, root weight and leaf area.

To determine the incidence of fungi, one cm long root pieces after washing in running tap water were surface sterilized with 1% $Ca(OCl)_2$ and transferred on PDA plates supplemented with Penicillin @ 100,000/liter and streptomycin @ 200 mg/liter at 5 pieces per plate. Petri dishes were incubated at room temperature (28°C) and after one week, infection of roots by root infecting fungi was recorded.

Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test at P=0.05 and Duncan's multiple range test to compare treatment means, using statistica software according to Sokal and Rohlf (1995).

Results

Mung bean and sunflower seeds treated with gamma rays and soil drenched with antagonists were germinated after 4 days of sowing. Results showed that 100% germination was observed when seeds were treated with gamma rays (^{60}Co) at 0, 2, 8 and 16 min., and soil was drenched with all antagonists like *T. harzianum*, *P. aeruginosa* and *R. meliloti* as compared to control in mung bean seeds (Fig. 1). In case of sunflower, seeds irradiated with gamma rays (^{60}Co) and soil drenching with *R. meliloti* showed an increase in germination (87%) (Fig. 1).

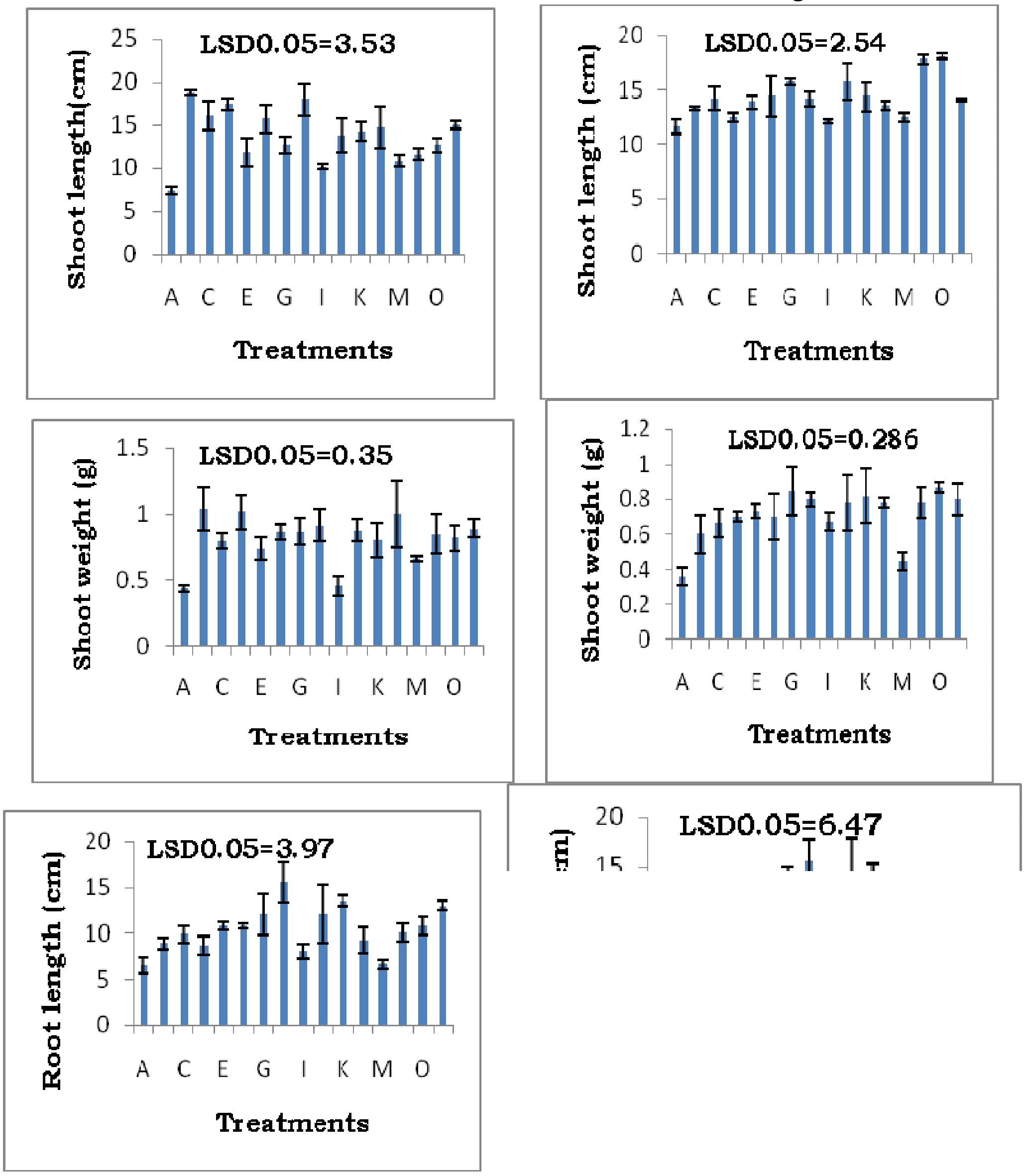
Application of soil with *P. aeruginosa* and seeds of mung bean irradiated with (^{60}Co) gamma rays for 16 min., caused significantly ($P<0.001$) increased in plant length (12.83 cm) and weight (0.67 g)compared to control (11.64 cm, 0.36 g). Root length and root weight were significantly ($P<0.05$) increased when *P. aeruginosa*, *R. meliloti* applied to soil and seeds of sunflower and mung bean were irradiated with 2 and 8 min., exposure of gamma rays (^{60}Co) (Fig. 1). Leaf area of plants was observed to be maximum when *R. meliloti* was drenched in soil and seeds of sunflower (9.74 cm) was irradiated with gamma rays (^{60}Co) for 8 min., ($P<0.01$). Result showed that *P. aeruginosa* and *R. meliloti* were observed to be satisfactory bio-control agents followed by *T. harzianum* whereas all exposure time of irradiation of gamma rays (^{60}Co) to seeds of mung bean and sunflower were found to be best in increasing plant height and weight. Increased growth parameters were observed in both sunflower and mung bean as the time of gamma rays exposure increased.

Effect of gamma rays (^{60}Co) and soil drenching with antagonists caused significant reduction of root infecting fungi like *R. solani, M. phaseolina* and *Fusarium* spp. Exposure of sunflower and mung bean seeds with gamma rays at 16 min., and soil drenching with *T. harzianum, R. meliloti* and *P. aeruginosa* caused significant ($P<0.05$) decrease (22.22%) and complete reduction (0%) of *Fusarium* spp., as compared to control (100%). *R. solani* and *M. phaseolina* were completely suppressed (0%) in almost all treatments except control (100%) when *T. harzianum, R. meliloti* and *P. aeruginosa* drenched in soil and seeds of sunflower and mung bean were exposed with gamma rays (^{60}Co) at 0, 2, 8 and 16 min., interval ($P<0.001$) (Fig. 2).

Discussion

Exposure of sunflower and mung bean seeds with gamma rays (^{60}Co) at 2, 8 and 16 minutes and soil drenching with antagonists like *T. harzianum, P. aeruginosa* and *R. meliloti* showed significant increase in growth parameters such as shoot length, shoot weight, root length, root weight and leaf area. Present results were supported by Hall *et al*. (1996), Glick *et al*. (1997) in which strains of *Pseudomonas putida* and *P. fluorescens* have been reported to increase

Sunflower **Mungbean**



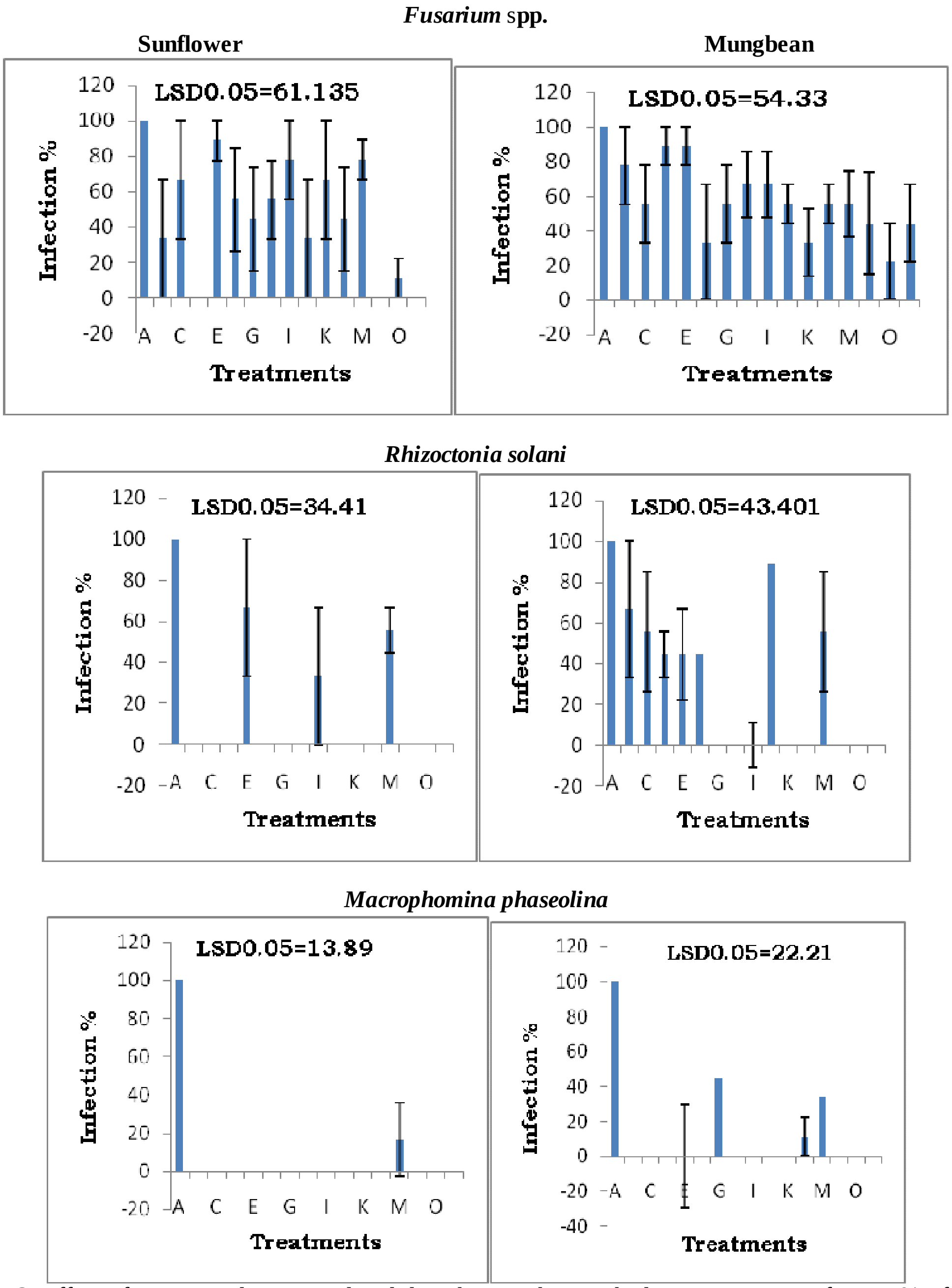


Figure 2. Effect of gamma radiations and soil drenching with microbial antagonists on infection % of mung bean and sunflower. A= 0 min, B=0 min + *T. harzianum*, C=0 min+*P. aeruginosa*, D= 0 min+*R. meliloti*, E= 2 min, F= 2 min + *T. harzianum*, G=2 min+*P. aeruginosa*, H= 2 min+*R. meliloti*, I=8 min, J= 8 min + *T. harzianum*, K=8 min+*P. aeruginosa*, L= 8 min+*R. meliloti*, M=16 min, N=16 min + *T. harzianum*, O=16 min+*P. aeruginosa*, P= 16 min+*R. meliloti*

root and shoot elongation in canola, lettuce and tomato plants. Thapa (2004) reported that root, hypocotyl, and epicotyl elongation decreases as the exposure time increases. A group of bacteria are now referred to plant growth promoting rhizobacteria (PGPR) which participate in many key ecosystem process such as those involve in the biological control of plant pathogen, nutrient cycling and seedling establishment and therefore deserve particular attention for agricultural or foresty purpose (Weller and Thomashow, 1993; Glick, 1995). Presently it was observed that soil drenching with *T. harzianum, R. meliloti* and *P. aeruginosa* and seeds of sunflower, mung bean were exposed with gamma rays (^{60}Co) at 0, 2, 8 and 16 min., of interval completely reduced *R. solani, M. phaseolina. Fusarium* spp., was completely suppressed by *R. meliloti* which is similar to that of Haikal (2007) where it was observed that *Cucumis sativus* seeds when soaked in 15% concentration of aqueous extract of *Azadirachta indica*, *Ziziphus spina-christi* and *Zygophylum coccineum* decreased in incidence of root rot disease when soil artificially infested by *F. solani* or with *F. solani* and *T. harzianum*. Chao (1990) reported that *Rhizobia* strains have tremendous antagonistic activity against fungi. Present research concluded that increased exposure time results in increment of plant growth parameters. Thapa (1999) observed that germination and seedling growth of *Pinus kesiya* Gord and *P. wallichiana* A.B. Jacks were inhibited with the increased in exposure of gamma rays (^{60}Co) whereas in some cases the lower exposure was stimulatory. Irradiation of mung bean seeds with gamma rays (^{60}Co) for 0 and 4 minutes enhance the growth parameters in terms of shoot length, shoot weight, root length, root weight, leaf area and reduce the infection of root infecting fungi (Ikram *et al*., 2010).

Results from the present study suggest that use of antagonists and irradiation with gamma rays induce stimulating effect to the plant height and weight which results in promoting the economy of country. This combination was also helpful in reducing root infecting fungi.

Acknowledgement

The authors are grateful to Mr. Zeeshan Abbass, Department of Physics, University of Karachi for his help in irradiation of sunflower and mung bean seeds by (^{60}Co) gamma radiations.

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