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X Rays Treated Leguminous Seeds in Combination with Wild Plant Powder for the Promotion of Growth and Control of Root Rot Fungi

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Abstract

Present research work was designed to investigate the x rays treated leguminous seeds in combination with *Prosopis juliflora* Swartz in the suppression of root rot fungi. Seeds of cowpea and mungbean were treated with x-rays at 45 and 75 kiloelectron volt (keV) for 5, 10 and 20sec and soil was amended with *Prosopis juliflora* leaves powder @1%. Both leguminous crops showed significant increase in all growth parameters and reduction in infection of root rot fungi viz., *Fusarium spp*., *Rhizoctonia solani* and *Macrophomina phaseolina*. Complete reduction of *Rhizoctonia solani* and *Macrophomina phaseolina* was observed when seeds were treated with 45 keV for 5, 10 and 20 sec. and soil was amended with *Prosopis juliflora* leaves powder @1%. There was significant increase in all growth parameters when seeds of both leguminous crops exposed with x-rays for 5sec (45 keV).

Keywords: X-rays; *Prosopis* leaves powder; Soil amendment; Leguminous seeds

Introduction

X-rays are a form of electromagnetic radiation, just like light waves and radio waves. Because X-rays have higher energy than light waves, they can pass through the body. X-rays were first discovered over 100 years ago and were quickly applied to medical diagnostic use. Today x-rays remain a valuable tool in diagnosis and treatment of many injuries and diseases. Radiation considered being an excellent tool for sterilization, preservation of food and other different food engineering processes, which gives benefit to the human society [1-3]. Effects of radiation on plants are a broad and complex field and work is being done in many areas on a large number of plant species. Radiation affects the size and weight of plants. There is variety of control methods used in order to avoid the yield losses due to soil borne pathogens such as use of radiations [4], chemical compounds which are toxic to fungi [5]. X-ray is commonly used to observe and quantify the soil environment including plant root development [6-8] fungal influence [9,10], changes to pore structure [11] and the influence of microbial activity [12].

X-ray dose influence on plant root growth [6,13], fungal [9] or microbial activity [14]. The influence of X-ray dose on plants and animals is under studies [15], Stuppy et al. [16] studied that repeated exposure of x-ray was not feasible for living system. Dutilleul et al. stated that repeated exposure of x-ray is not suitable for living organisms and particularly plants. Dose is the quantity of energy absorbed by an object after exposure to radiation [13,17]. Many x-ray studies provide insufficient information on impact of x-ray dose on plant growth and microbial activity [18].

Irradiation of soil could influence microbial communities through the direct ionization of cells causing DNA mutation, and the indirect radiolysis of cell water creating damaging free radicals within extra- and intra-cellular fluids [19]. Free radicals can cause single or double stranded DNA breaks [20], damaging future cell and plant development. The impact of X-ray radiation on soil constituents, much of the focus being based on γ-rays due to its application in soil sterilization procedures. Responses to radiation continually change as enzymatic activity in soils aid recovery from acute doses, although sensitivity is dependent on a large range of physiological factors such as metabolic activity, organism size and complexity, and life-cycle stage [20]. A dose of 10 Gy of X-ray radiations is equivalent to 10 Gy of γ-ray radiation since X-rays and γ-rays have the same radiation weighting factor, formerly known as quality factor, which is a measure of the expected biological impact of ionizing radiation often used in radiation protection [21]. *Rhizoctonia solani* (Kiihn) and *Fusarium* spp. cause wilting of different crop plants. Wilt has become a major disease causing significant reduction in yield.

Root rot fungi viz., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (Kiihn) and *Fusarium spp*., cause wilting of different plants. Wilting is characterized by yellowing of foliage, drooping of apical shoot to ultimate death of whole plant. The pathogen is a soil inhabiting fungus and forms in the senescing tissues of the diseased plant and may survive in the soil for many years. There are many methods which are presently being used to control various plant pathogens including wilt pathogens. Disease causing organisms which adversely affect the crop productivity like charcoal rot fungus *Macrophomina phaseolina* has a very wide host range and attacks the root and basal stem [22]. Mostly disease causing organisms are soil borne pathogens viz., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (Kiihn) and *Fusarium spp*., attack roots limiting nutrition uptake resulting in the death of plants [23,24].

Prosopis juliflora is a shrub belonging to the *Mimosaceae* family. *P. juliflora* has astringent, antiseptic, antibacterial and antifungal properties [25,26]. The extracts of *P. juliflora* seeds and leaves were well studied for several *in vitro* pharmacological effects such as antibacterial [27], antifungal [28,29] and anti-inflammatory properties [28,30]. These properties have been attributed to piperidine alkaloids [31]. The main alkaloids including the juliflorine and julifloricine of *P. juliflora* were isolated by Ahmad et al. [32] and their antibacterial and antifungal activities have also been reported [33,34]. The flavonoid patulitrin isolated from its flowers and fruits showed significant activity

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against lung carcinoma in vivo [35]. Ikram and Dawar [36] reported that *Prosopis juliflora* stem and leaves powder @ 0.1 and 1% were found effective for the enhancement of plant height and weight of cowpea and mungbean.

The aim of present research work was to find out the effect of x-rays treated seeds and *P. juliflora* leaves powder in the control of root rot fungi and on growth of cowpea and mungbean.

Methodology

Collection of material

Prosopis juliflora leaves were collected from University of Karachi campus air dried and ground in a grinder.

Exposure of seeds to radiation

Seeds of cowpea (*Vigna unguiculata)* mung bean (*Vigna radiata L.*) were exposed to x-rays of 45 and 75 keV at time periods of 5, 10 and 20 sec. The seeds were irradiated at the Pakistan laboratory Karachi, Pakistan.

Physical properties of 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 & 18%), pH ranged from 7.1-7.5 with moisture holding capacity (MHC) of 29% [37], total nitrogen 0.077-0.099% [38], 3-4 sclerotia/g of *M. phaseolina* g-1as found by wet sieving technique [39], 5-10% of *R. solani* on sorghum seeds used as baits [40] and *Fusarium* spp.,3500 cfu g-1 as assessed by soil dilution technique [41].

Experimental setup

The irradiated and non-irradiated seeds were sown in 8 cm diam., plastic pots, and each containing 300g soil and watered regularly to maintain sufficient moisture required for the growth of plants. The pots were kept in screen house in randomized complete block design with three replicates per treatment. Non treated seeds served as control. Soil amendment with *P. juliflora* leaves powder @ 1% w/w and leaves it for one week to decompose. Growth parameter of control and irradiated seedlings were recorded after 30 days of seed germination.

Determination of root infecting fungi

To determine the incidence of root rot 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 @ 200 mg/L and streptomycin @ 200mg/ L. Petri dishes were incubated at room temperature (28^0C) and after one week colonization of root infecting fungi was recorded.

Statistical analysis

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 statistics software according to Sokal & Rohlf (1995).

Results

Mungbean

There was significant (P<0.01) increase in germination, shoot weight and root length when seeds of mungbean treated with x-rays for 5 sec at 45keV as compared to 10 and 20 sec. Leaf area and no. of nodules significantly (P<0.001) increased when seeds were treated with x-rays for 20 sec at 45keV as compared with control. 100 % germination (P<0.001) was observed when mungbean seeds were treated with x-rays (45 and 75keV) for 5, 10 and 20 sec. and soil was with *P. juliflora* leaves powder @1%. Mungbean plants showed significant (P<0.001) increased in length, fresh weight of root, leaf area and no. of nodules when seeds of mungbean exposed on x-rays (45 keV) for 5 sec and soil amended with *P. juliflora* leaves powder @1%. Shoot weight was significantly (P<0.01) increased when seeds of mungbean treated with x-rays (45Kev) for 20sec and was soil amended with P. *juliflora* leaves powder @1%. Root length was significantly increased (P<0.05) when seeds irradiated with x-rays (45Kev) for 5sec and soil amended with leaves powder (Figure 1).

Effect of x-rays and soil amendment with *P. juliflora* leaves powder @1% caused significant reduction (P<0.001) in the infection of root rot fungi like, *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina.* Colonization of all root infecting fungi completely suppressed when seeds were treated with x-rays at 5, 10 and 20 sec and soil amendment with *P. juliflora* leaves (Figure 2).

Cowpea

After 30 days of germination, cowpea roots showed significant (P<0.001) reduction in colonization of root rot fungi. Colonization of *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* were completely suppressed when cowpea seeds treated with x-rays at 5, 10 and 20 sec and soil was amendment with *P. juliflora* leaves and germination, leaf area and no. of nodules significantly (P<0.001) increased when cowpea seeds exposed to x-rays (45keV) for 5 and 10 sec than control and amended soil. Fresh weight of shoot and root significantly (P<0.01) increased when seeds were exposed to x-rays for 5 sec (45keV) (Figure 2).

There was significant increase (P<0.01) in growth when seeds of cowpea were treated with x-rays and soil was amended with *P. juliflora* leaves powder @1%. Shoot length increased (34.66cm) when seeds were treated with x-rays (45 keV) for 5sec and soil amended with *P. juliflora* leaves as compared to control (11cm). Shoot and root weight significantly increased (P<0.01) when seeds exposed to x-rays (45 keV) for 10 sec. and soil mix with *P. juliflora* leaves @1%. There was significant increase (P<0.001) in rot length, leaf area and no. of nodules when seeds were treated with x-rays (45 keV) for 5 sec. in combination with amended soil (Figure 3).

Discussion

Exposure of mungbean and cowpea seeds treated with x-rays (45kv) for 5 sec. and soil amendment with *P. juliflora* leaves powder showed significant increase in all growth parameters such as plant length and weight leaf area and no of nodules. Similarly Dawar et al., [42] 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., interval completely reduced the infection of *R. solani, M. phaseolina* and *Fusarium spp*. Thapa [43] reported that root, hypocotyl, and epicotyl elongation decreases as the exposure time increases. Present results supported by Thapa [44] 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 mungbean 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 [45].

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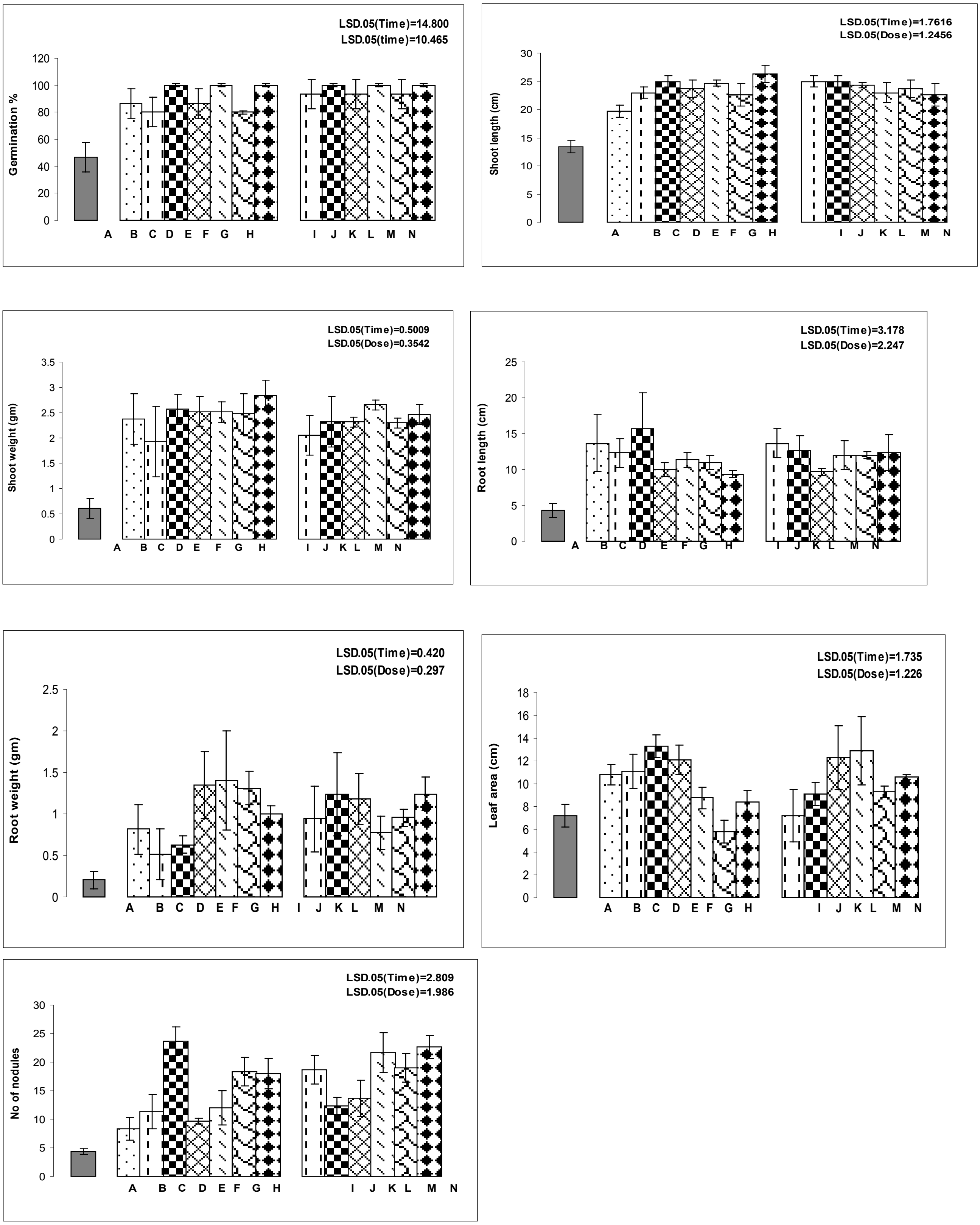

Figure 1: Effect of X-rays in combination with *Prosopis juliflora* leaves powder @1% w/w on growth parameters of mung bean. A=control, B=*Prosopis* leaves@1%, C=5sec (45Kev) D=5 Sec (45 keV)+*Prosopis* leaves@1% E=10sec (45 keV) F=10 Sec (45 keV)+*Prosopis* leaves@1% G=20sec (45 Kev) H=20 Sec (45 Kev)+*Prosopis* leaves@1% I=5sec (75 keV) J=5 Sec (75Kev)+*Prosopis* leaves@1% K=10sec (75 keV) L=10 Sec (75 keV)+*Prosopis* leaves@1% M=20sec (75 keV) N=20 Sec (75 keV)+*Prosopis* leaves@1%.

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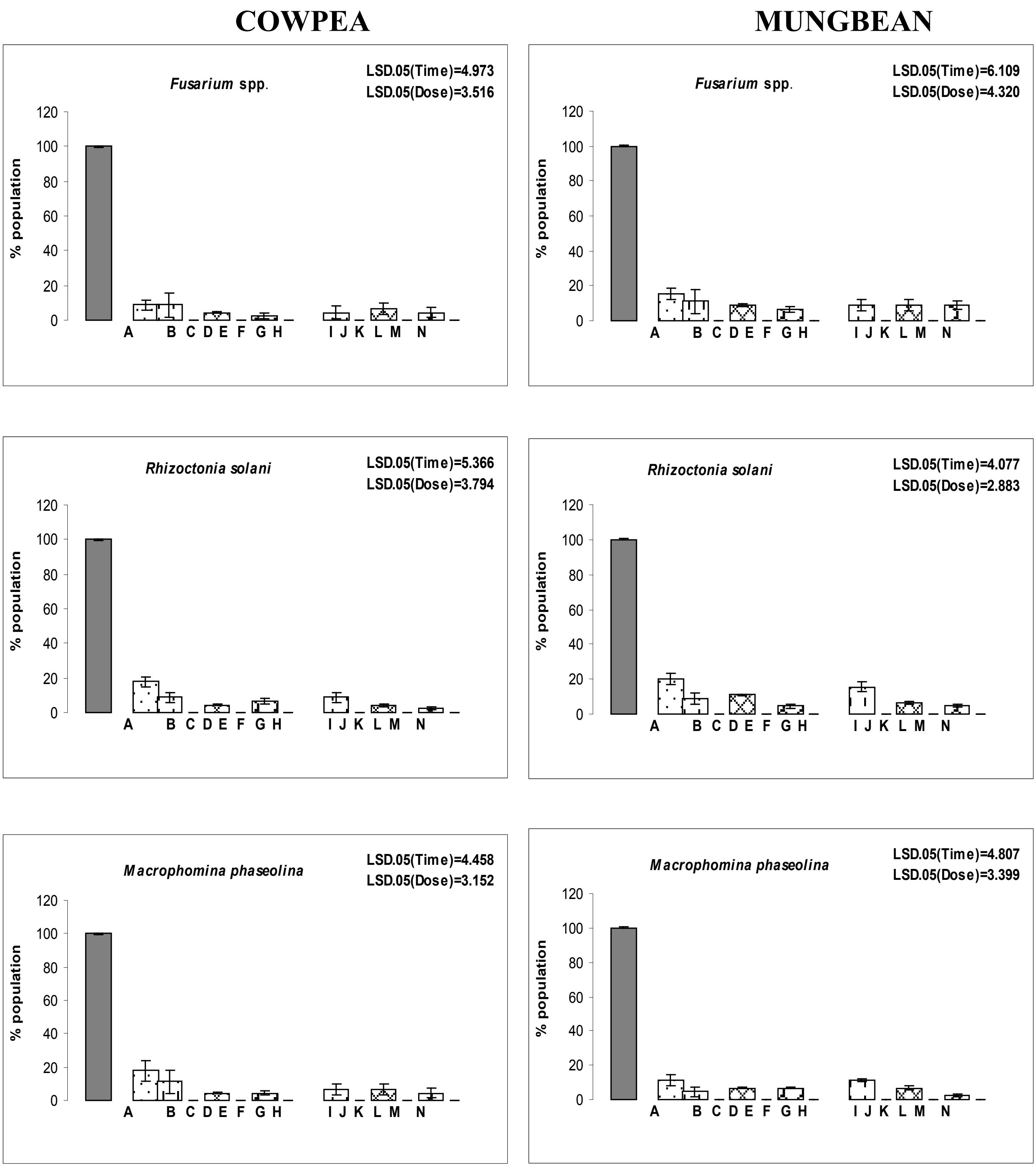

Figure 2: Effect of X-rays in combination with *Prosopis juliflora* leaves powder @1% w/w on root rot fungi of cowpea and mung bean. A=control, B=*Prosopis* leaves@1%, C=5sec (45Kev) D=5 Sec (45 keV)+*Prosopis* leaves@1% E=10sec (45 keV) F=10 Sec (45 keV)+*Prosopis* leaves@1% G=20sec (45 Kev) H=20 Sec (45 Kev)+*Prosopis* leaves@1% I=5sec (75 keV) J=5 Sec (75Kev)+*Prosopis* leaves@1% K=10sec (75 keV) L=10 Sec (75 keV)+*Prosopis* leaves@1% M=20sec (75 keV) N=20 Sec (75 keV)+*Prosopis* leaves@1%.

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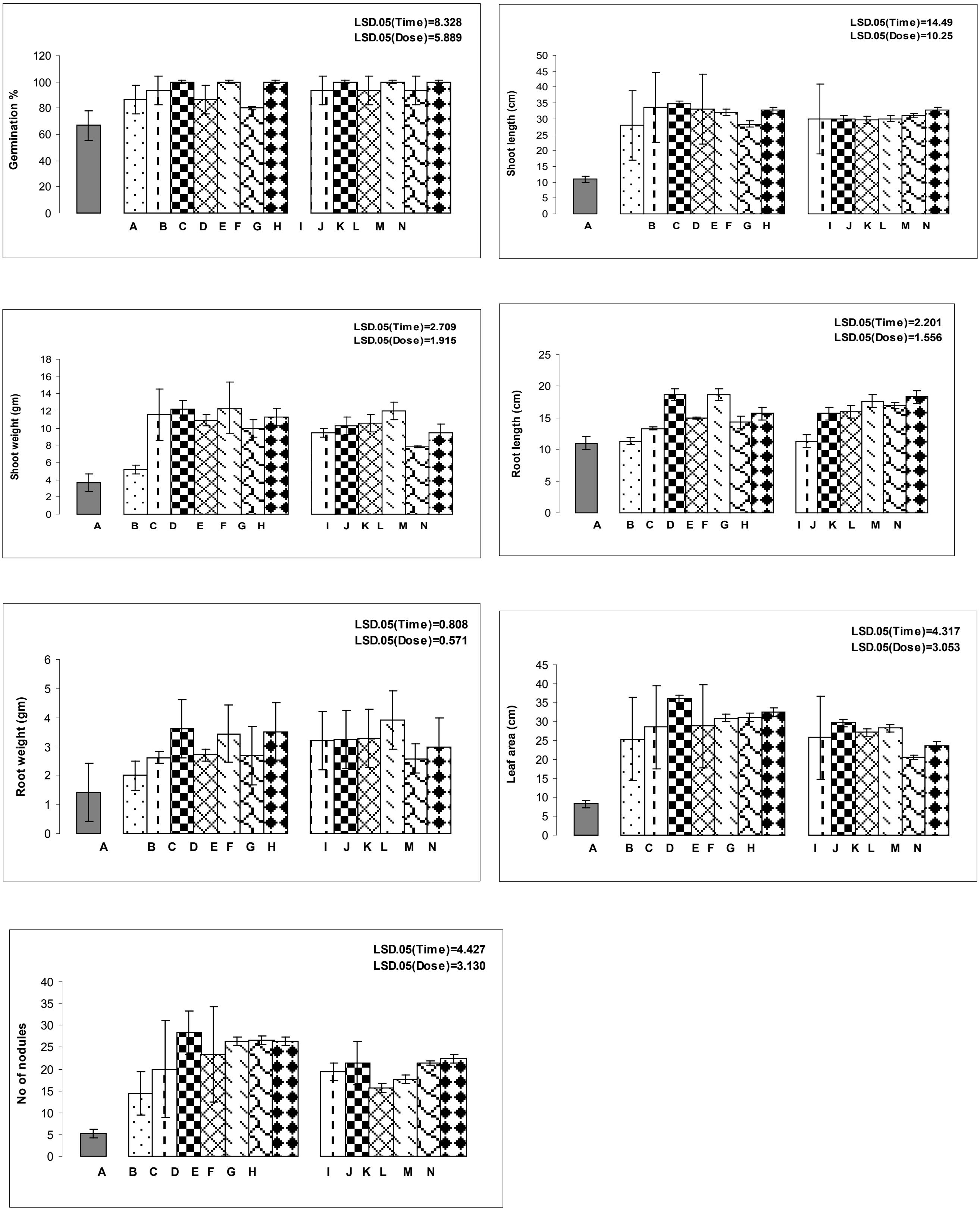

Figure 3: Effect of X-rays in combination with *Prosopis juliflora* leaves powder @1% w/w on growth parameters of cowpea. A=control, B=*Prosopis* leaves@1%, C=5sec (45Kev) D=5 Sec (45 keV)+*Prosopis* leaves@1% E=10sec (45 keV) F=10 Sec (45 keV)+*Prosopis* leaves@1% G=20sec (45kv) H=20 Sec (45 Kev)+*Prosopis* leaves@1% I=5sec (75 keV) J=5 Sec (75Kev)+*Prosopis* leaves@1% K=10sec (75 keV) L=10 Sec (75 keV)+*Prosopis* leaves@1% M=20sec (75 keV) N=20 Sec (75 keV)+*Prosopis* leaves@1%.

There was complete suppression of root rot fungi when seeds were treated with x-rays for 5, 10 and 20 sec. and soil was amended with *P. juliflora* leaves powder @1%. Present results were supported by Jackson et al., [19] demonstrated that fungi are more sensitive to radiation than bacteria, with γ-irradiation doses 10 Gy able to alter fungal populations. Al Khayri et al. [46] found relatively small X-ray exposures of 0.25 Gy had an influence on biochemical aspects of date palm (*Phoenix dactylifera L.*) development i.e. DNA and pigment synthesis.

There was significant increase in all growth parameters when seeds of both leguminous crops exposed with x-rays for 5sec (45 keV). Whereas Zappala et al. [47] reported that X-ray CT does not impact on plant growth and soil microbial populations when employing a low level of dose (<30 Gy).

Recently several works has explored that x-rays can be used to sterilize various foodstuffs to inactivate microbial contamination [48,49].

Many biomedical research facilities handling pathogenic agents assist in the identification of possible safety threats and reduce the cross-border traffic in dangerous materials with the help of x-irradiation. X-irradiation has provided a useful model for examining the effects of ionizing radiation on survival and repairs the genetic damage in microbial systems [50-57].

Conclusion

Present result suggested that low dose of x-rays treated and amended soil with *P. juliflora* leaves powder induce stimulated effect on plants and reduced the root rot fungi. So, experiment would be carried out on large scale for obtaining good yield of crops.

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