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# X RAYS EXPOSURE ON LEGUMINOUS SEEDS IN COMBINATION WITH *AERVA JAVANICA* PARTS POWDER FOR THE PROMOTION OF GROWTH AND MANAGEMENT OF ROOT ROT FUNGAL PATHOGENS

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**ABSTRACT:** *Present research investigate the exposure of x-rays on leguminous seeds in combination with Aerva javanica in the management of root rot fungal pathogens and on the growth of crop plants. Seeds of cowpea and mung bean were treated with x-rays for 5, 10 and 20 seconds for 45 and 75 KeV and soil amended with A. javanica leaves powder @1% w/w. Seed treatment with x-rays for 5 and 10 sec. showed significant enhancement in growth parameters and completely control the infection of root rot fungi viz., Fusarium spp., Rhizoctonia solani and Macrophomina phaseolina.*

**KEYWORDS:** x-rays, *Aerva javanica* , soil amendment, leguminous seeds

## INTRODUCTION

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 **(**Ivanov *et al*., 2001, Hyun-pa *et al*., 2006, Sameh *et al*., 2006). 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 & Gullino 2005) chemical compounds which are toxic to fungi (Washington & McGee 2000). X-ray is commonly used to observe and quantify the soil environment including plant root development (Flavel *et al*., 2012, Tracy *et al*., 2012) fungal influence (Kravchenko *et al*., 2011, Martin *et al*., 2012) changes to pore structure (Munkholm *et al*., 2012) and the influence of microbial activity (Nunan *et al*., 2006). X-ray dose influence on plant root growth , fungal or microbial activity. The influence of X-ray dose on plants and animals is under studies (Al-Khayri *et al*., 2012), Stuppy *et al*. (2003) studied that repeated exposure of x-ray was not feasible for living system.

*Aerva javanica* (Burm.f.) Juss. ex Schult. belonging to the family Amaranthaceae, distributed in warm parts of Asia and 20 different species of *Aerva* present in Pakistan and India (Sharif *et al.*, 2011). *A. javanica* locally called Booh is a herb and widely distributed in various parts of the



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world. In traditional, herb is used as diabetic, diuretic, demulcent and kidney stones (Qureshi & Bhatti, 2009). Various species of *Aerva* possess analgesic, anti microbial, anti-inflammatory activities and used as a valuable medicine for sore throat, cough, wounds and in digestion (Imran *et al.,* 2009). Powder of *A. javanica* is applied externally to ulcers in domestic animals. Seeds are used to relieve headache and to remove swelling. *A. javanica* shows antihyperglycaemic (Reddy & Reddy, 2009), cytogenetical (Soliman, 2006), cytotoxic (Al-Fatimi *et al*., 2007) and antiplasmodial activities. Flowers and roots of *A. javanica* possess medicinal properties against kidney problems and rheumatism. Leaves of *A. javanica* are used externally to heal the wounds and inflammation of joints, leaves also used for fodder to goats.

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. (Booth, 1971; Nelson *et al*., 1983). 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 (Sackston, 1981). *Rhizoctonia solani* (Kiihn) and *Fusarium* spp. cause wilting of different crop plants. Wilting is characterized by yellowing of foliage, drooping of apical shoot to ultimate death of whole plant.

Effects of radiation on plants is a broad and complex field and work is being done in many areas on a large number of plants. 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 & Gullino, 2005), chemical compounds which are toxic to fungi (Washington & McGee, 2000). X-ray is commonly used to observe and quantify the soil environment including plant root development **(**Flavel *et al*. 2012; Karahara *et al*., 2012). The influence of X-ray dose on plants and animals is under studies (Yang *et al*. 2011). So present studies carried out to investigate the antifungal activity of *Aerva javanica* with x-rays against root rot fungi like *Fusarium* spp., *R. solani* and *M. phaseolina* .

## MATERIALS AND METHODS

**Collection of material:** *Aerva javanica* 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.

**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, 50, 25 & 15%), pH ranged from 7.5-8.4 with moisture holding capacity (MHC) of 29% (Keen & Raczkowski 1922),



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total nitrogen 0.077-0.099% (Mackenzie & Wallace 1954), 4-5 sclerotia/g of *M. phaseolina* g-1as found by wet sieving technique (Sheikh & 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 & Synder, 1962).

**Experimental setup:** The irradiated and non irradiated seeds were sown in 8 cm diam., plastic pots, 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 *A. javanica* leaves powder @ 1% w/w and leave 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$^{\circ}$C) and after one week colonization of root infecting fungi was recorded.

## RESULTS

### COWPEA

There was significant ($P<0.001$) enhancement in length and fresh weight of shoot and leaf area when seeds irradiated with x-rays (45keV) for 10 sec and soil amended with *A. javanica* leaves powder @ 1%. Root length and weight were increased when leaves of *A. javanica* mixed in soil and seeds irradiated with x-rays (45KeV) for 20 sec. There was significant ($P<0.001$) increase in no. of nodules when seeds irradiated with x-rays for 75 KeV for 10 sec and soil amended with *A. javanica* leaves @ 1%. There was significant ($P<0.001$) reduction in colonization percentage of root rot fungi viz., *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* when seeds irradiated with x-rays and soil mixed with *A. javanica* leaves powder.

### MUNG BEAN

Colonization percentage of root rot fungi viz., *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* were completely suppressed when *A. javanica* leaves powder amended in soil and seeds irradiated with x-rays. Shoot length was significantly ($P<0.001$) increased when seeds of mung bean treated with x-rays (45 KeV) for 5 sec and soil amended with *A. javanica* leaves. There was significant ($P<0.001$) enhancement in root length when seeds irradiated with x-rays (75 KeV) for 20 sec in combination with *A. javanica* leaves. Maximum fresh weight of shoot was observed when soil mixed with *A. javanica* leaves and seeds treated with x-rays (75 KeV) for 5 sec. Fresh weight of roots were significantly ($P<0.001$) increased



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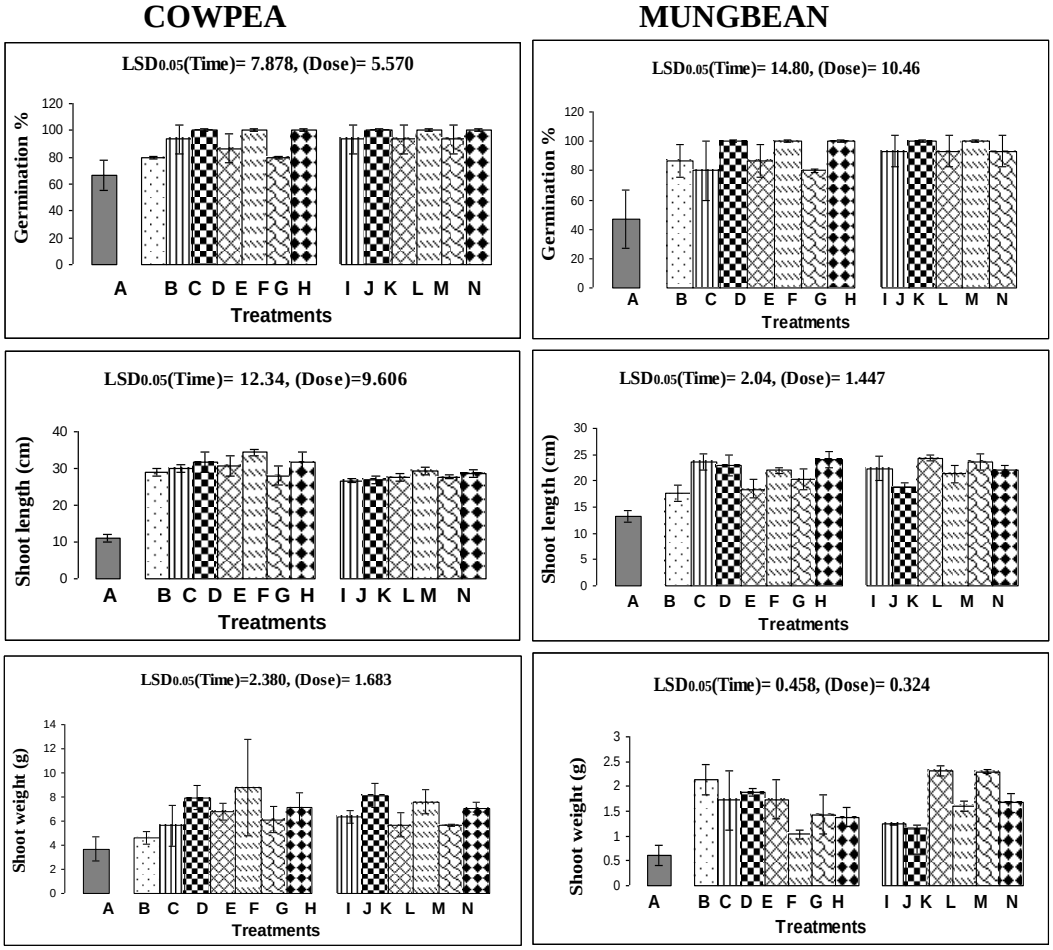
when seeds irradiated with x-rays for 10 sec. After 20 sec of seeds irradiated there was significant ($P<0.05$) enhancement in leaf area. No. of nodules were significantly ($P<0.001$) increased when seeds of mung bean irradiated with x-rays (75 KeV) for 5 sec.

x-rays treated seeds increased the efficacy of wild plant parts for the reduction of root infecting fungi and increased the growth parameters of cowpea and mung bean. Seeds irradiated with x-rays for 45 KeV for 5 sec was best for the control of root rot fungi.



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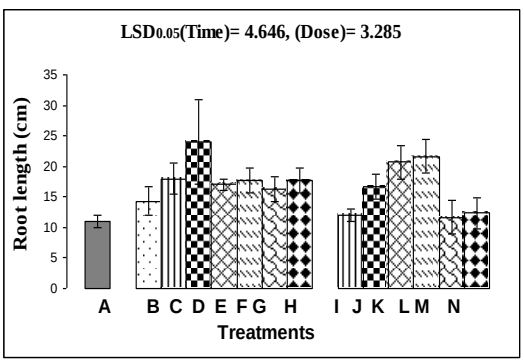


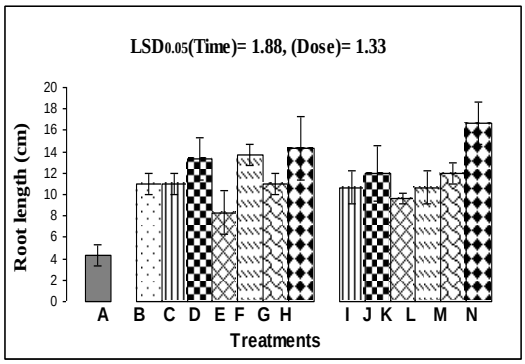
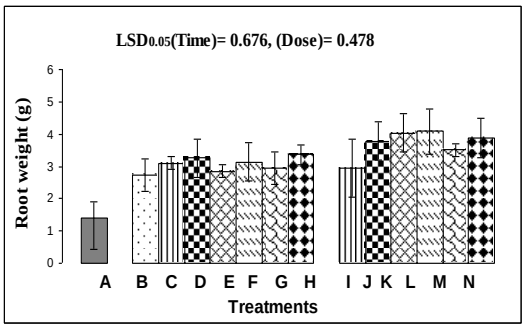
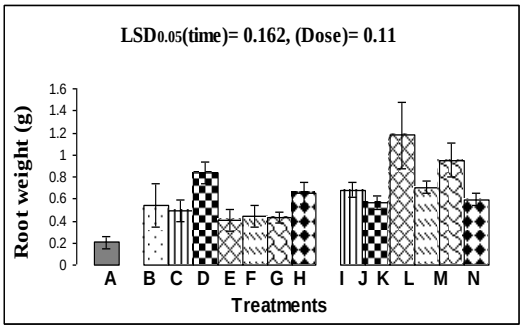
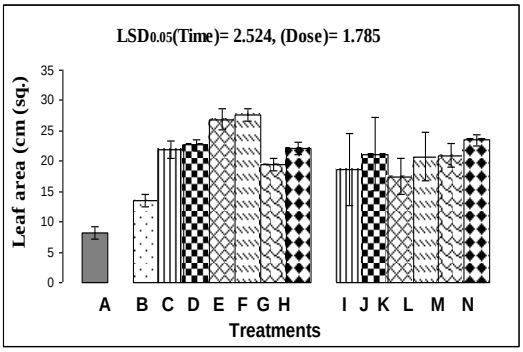



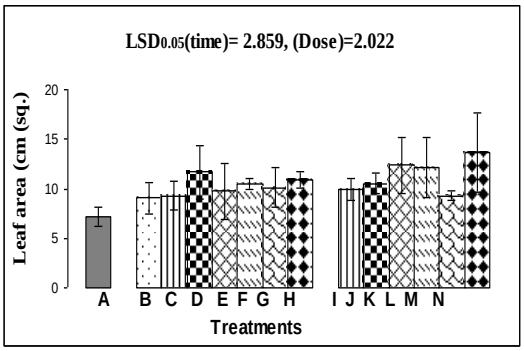

**Fig. Continued…**

**COWPEA** **MUNGBEAN**



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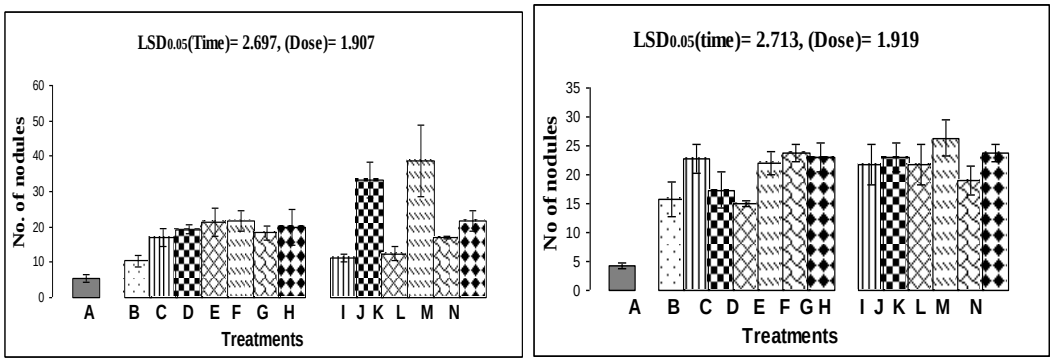
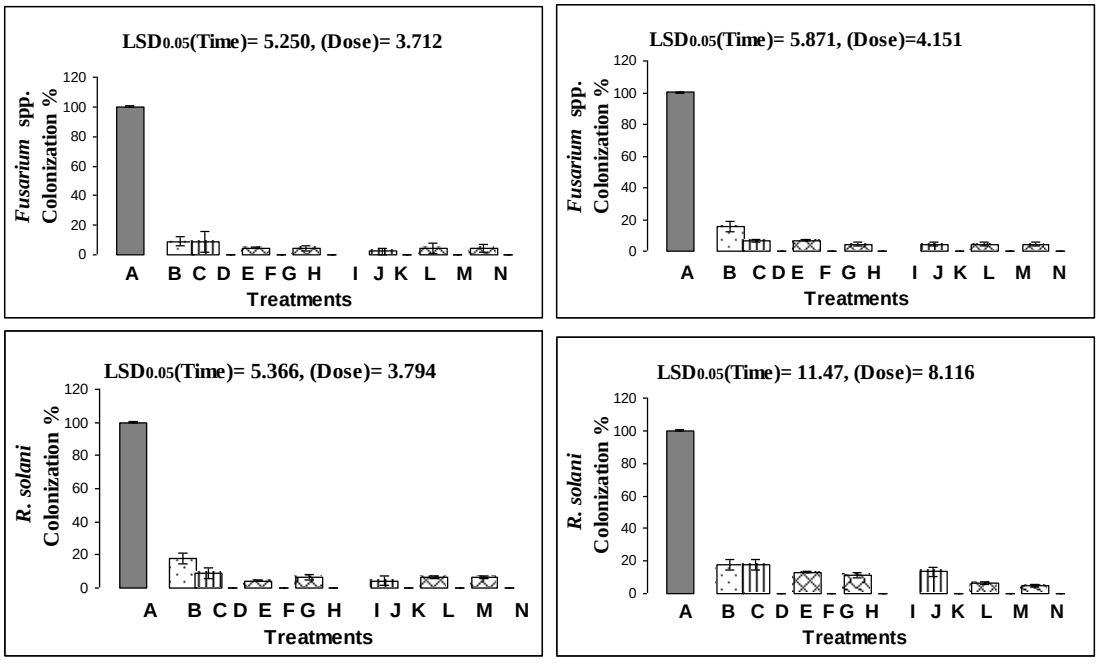



**Fig. 35:** X-rays irradiated seeds in combination with with *Aerva javanica* leaves powder @1% w/w on growth parameters of cowpea and mung bean. A= control, B=*A. javanica* leaves@1%, C= 5sec (45Kev) D=5 Sec (45kv)+ *A. javanica* leaves@1% E=10sec (45kv) F=10 Sec (45kv)+ *A. javanica* leaves@1% G= 20sec (45kv) H= 20 Sec (45kv)+ *A. javanica* leaves@1% I= 5sec (75kv) J=5 Sec (75Kev)+ *A. javanica* leaves@1% K=10sec (75kv) L=10 Sec (75kv)+ *A. javanica* leaves@1% M=20sec (75kv) N=20 Sec (75kv)+ *A. javanica* leaves@1%.

**COWPEA** **MUNGBEAN**



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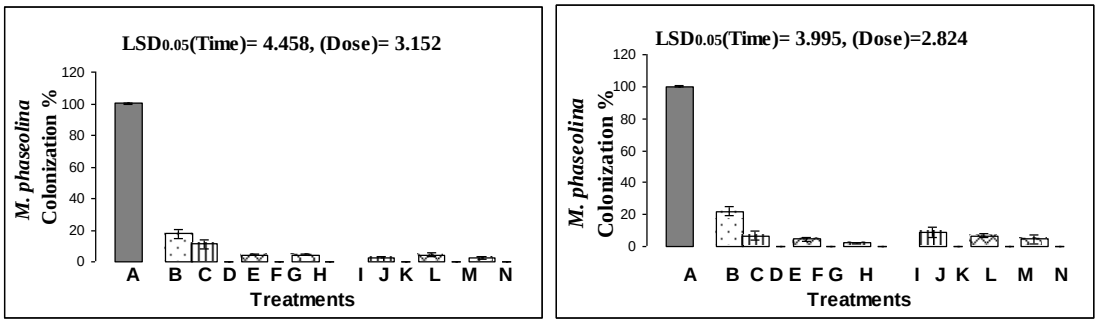


**Fig. 36:** X-rays irradiated seeds in combination with *Aerva javanica* leaves powder @1% w/w in the control of root rot fungi of cowpea and mung bean. A= control, B=*A. javanica* leaves@1%, C= 5sec (45Kev) D=5 Sec (45kv)+ *A. javanica* leaves@1% E=10sec (45kv) F=10 Sec (45kv)+ *A. javanica* leaves@1% G= 20sec (45kv) H= 20 Sec (45kv)+ *A. javanica* leaves@1% I= 5sec (75kv) J=5 Sec (75Kev)+ *A. javanica* leaves@1% K=10sec (75kv) L=10 Sec (75kv)+ *A. javanica* leaves@1% M=20sec (75kv) N=20 Sec (75kv)+ *A. javanica* leaves@1%.

## DISCUSSION

Exposure of mungbean and cowpea seeds treated with x-rays (45kv) for 5 sec. and soil amendment with *A. javanica* leaves powder showed significant enhancement in fresh weight and length of plants. Maherchandani (1957) reported that gamma radiation break the dormancy of *Avena fatua* L. (wild oats) as well as shoot length and dry matter content of seedling raised. 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). Thapa (2004) reported that root, hypocotyl, and epicotyl elongation decreases as the exposure time increases. Present results supported by Thapa (1999) 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. Similarly Dawar *et al*., (2010) 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 root rot fungi.

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 *A. javanica* leaves powder @1%. Our results were supported by Jackson *et al.*, (1967) that fungi are more sensitive to radiation than bacteria, with γ-irradiation doses 10 Gy able to alter fungal populations. Al Khayri *et al*. (2012) found that relatively small X-ray exposures of 0.25 Gy had an influence on biochemical aspects of date



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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*. (2013) 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 work has explored that x-rays can be used to sterilize various foodstuffs to inactivate microbial contamination (Mahmoud *et al*., 2010). 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-rays has provided a useful model for examining the effects of ionizing radiation on survival and repair the genetic damage in microbial systems (Bonura *et al*., 1975; Drieger *et al.*, 1970; Johansen *et al*., 1974; Youngs & Smith, 1976).

## REFERENCES

Al-Khayri J, Al-Enezi N, Al-Bahrany A. 2012. Effect of X-irradiation on date palm seed germination and seedling growth. Emirates Journal of Food and Agriculture 24.

Al-Fatimi, M., M. Wurster, G. Schroder and U. Lindequist. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol*., 111(3): 657-666.

Bonura, T., Youngs, D. A., & Smith, K. C. (1975). R.b.e. of 50 kVp X-rays and 660 keV x-rays (137Cs) with respect to the production of DNA damage, repair and cell-killing in Escherichia coli K-12. International Journal of Radiation Biology, 28,539-548.

Booth, C. 1971. The genus *Fusarium* . Commonwealth Mycological Institute, Kew, Surrey, England, pp. 273.

Driedger, A. A., James, A. P., & Grayston, M. J. (1970). Cell survival and x-ray-induced DNA degradation in Micrococcus radiodurans Radiation Research, 44, 835-845.

Flavel RJ, Guppy CN, Tighe M, Watt M, McNeill A. 2012. Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography. Journal of Experimental Botany: Online present.

Hyun-pa, S, Kim DH, Jo C, Lee CH, Kim KS, Byun MW. 2006. Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. Food Microbiol. 23(4): 372-378.

Ikram, N., S. Dawar, Z. Abbas and M.J. Zaki 2010. Effect of (60 Cobalt) gamma rays on growth and root rot diseases in mungbean ( Vigna radiate L.).Pak. J. Bot.42(3): 2165-2170.

Imran, M, N. Riaz, M. Ibrahim, E. Ahmed, M. Rasool, A. Malik and M. Moazzam. 2009. Further Phytochemical studies on *Aerva persica*. *J. Chem. Soc. Pak*., 31: 126-130.

Ivanov, AS, Ovchinnikov VP, Svinin MP, Tolstun NG, Bogart SL. 2001. 4 MeV highvoltage acelerator with 500 kW electron beam for radiation sterilization. Vacuum. 62: 225- 231.



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Jackson NE, Corey JC, Frederick LR, Picken JC. 1967. Gamma Irradiation and the Microbial Population of Soils at Two Water Contents. Soil Sci Soc Am J 31: 491–494.

Johansen, I., Gulbrandsen, R., & Pettersen, R. (1974). Effectiveness of oxygen in promoting x-ray-induced single-strand breaks in circular phage lambda DNA and killing of radiationsensitive mutants of Escherichia coli. Radiation Research, 58, 384-397

Karahara I, Umemura K, Soga Y, Akai Y, Bando T. (2012) Demonstration of osmotically dependent promotion of aerenchyma formation at different levels in the primary roots of rice using a 'sandwich'method and X-ray computed tomography. Annals of Botany 110: 503–509.

Keen BA, Raczkowski H. 1992. The relation between clay content and certain physical properties of soil. J. Agric. Sci. 11: 441-449.

Kravchenko A, Falconer RE, Grinev D, Otten W. 2011. Fungal colonization in soils with different management histories: modeling growth in three-dimensional pore volumes. Ecological Applications 21: 1202–1210.

Mackanzie, HA, Wallace HS. 1954. The Kjeldahl determination of nitrogen. A critical study of digestion conditions, temperature, catalyst and oxidizing agents. Aust. J. Chem. 7: 55-70.

Martin SL, Mooney SJ, Dickinson MJ, West HM. 2012. The effects of simultaneous root colonisation by three Glomus species on soil pore characteristics. Soil Biology and Biochemistry 49: 167–173.

Mahmoud, B. S. (2010). Effects of x-ray radiation on Escherichia coli O157:H7, Listeria monocytogeneses,Salmonella enterica andShigella flexneri inoculated on shredded iceberg lettuce. Food Microbiology, 27, 109-114.

Mahmoud, B. S., Bachman, G., & Linton, R. H. (2010). Inactiva-tion of Escherichia coli O157:H7, Listeria monocytogeneses Salmonella enteric and Shigella flexneri on spinach leaves by x-ray. Food Microbiology, 27, 24-28.

Maherchandani, N. (1975). Effects of gamma radiation on the dormant seed of *Avena fatua* L. Radiation Botany Volume 15, Issue 4, :439–44.

Munkholm LJ, Heck RJ, Deen B. 2012. Soil pore characteristics assessed from X-ray micro-CT derived images and correlations to soil friability. Geoderma 181–182: 22–29.

Nash, SM, Snyder WC. 1962. Quantitative estimation by plate count of propagules of the bean root rot fungus *Fusarium* in field soils. Phytopathol. *52*: 567-572.

Nelson, P.E., T.E. Toussoun and W. Marasas 1983. Fusarium species, An Illustrated Manual for identification. The Pennsylvania State University Press, University Park and London. pp. 193.

Nunan N, Ritz K, Rivers M, Feeney DS, Young IM. 2006. Investigating microbial micro-habitat structure using X-ray computed tomography. Geoderma 133: 398–407.

Qureshi, R. and G.R. Bhatti, 2009. Folklore Uses of *Amaranthaceae* Family from Nara Desert Pakistan. *Pak. J. Bot*., 41: 1565-1572.

Sackston, W.E. 1981. The sunflower crop and disease progress problems and prospects.



ISSN 2055-8139(Print), ISSN 2055-8147(Online)

Plant Disease , 65: 643-648.

Sameh, ASA, Kumar AP, Rao BS, Singh RP. 2006. Biodegradation of γ-sterilised biomedical polyolefins under composting and fungal culture environments. Poly. Degrad Stab. 91: 1105-1116.

Sharif A., E. Ahmed, A. Malik, Mukhtar-ul-Hassan, A. Munawar and A. Farrukh, 2011. Antimicrobial constituents from *Aerva javanica*. *Journal of Chemistry society Pak*., 33(3): 439-443.

Sheikh, AH, Ghaffar A. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton field. Pak. J. Bot. ***7*:** 13-17.

Spadaro D, Gullino ML. 2005. Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Prot. 24: 601-613.

Stuppy WH, Maisano JA, Colbert MW, Rudall PJ, Rowe TB. 2003. Three-dimensional analysis of plant structure using high-resolution X-ray computed tomography. Trends in Plant Science 8: 2–6.

Thapa, CB. 1999. Effect of acute exposure of gamma rays on seed germination *Pinus kesiya* Gord and P. wallichiana A.B. Jacks. Bot. Orient. J. Pl. Sci. 1 :120-121.

Tracy SR, Black CR, Roberts JA, Sturrock C, Mairhofer S. 2012. Quantifying the impact of soil compaction on root system architecture in tomato (*Solanum lycopersicum*) by X-ray micro-computed tomography. Annals of Botany 110: 511–519.

Washington WS, McGee P. 2000. Dimethomorph soil and seed treated of potted tomatoes for control of damping off and root rot caused by *Phytophthora nicotianae* var. *micotianae. Austr. Pl. Pathol. 29*: 46-51.

Wilhelm S. 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathol. 45*: 180-181.

Yang WN, Xu XC, Duan LF, Luo QM, Chen SB. 2011. High-throughput measurement of rice tillers using a conveyor equipped with x-ray computed tomography. Review of Scientific Instruments 82.

Youngs, D. A., & Smith, K. C. (1976). The yield and repair of x-ray-induced singlestrand breaks in the DNA of Escherichia coli K-12 cells.Radiation Research, 68, 148-154.

Zappala S, Jonathan R, Helliwell, Saoirse R, Tracy, Mairhofer S, Craig J, Sturrock, Tony primore. 2013. Effect of X-rays dose on rhizosphere studies using x-rays computed tomography. Plos one 8: 67250



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