Pak. J. Bot., 43(4): 2221-2224, 2011.

ROLE OF ULTRA VIOLET (UV-C) RADIATION IN THE CONTROL OF ROOT INFECTING FUNGI ON GROUNDNUT AND MUNG BEAN

ANUM SIDDIQUI, SHAHNAZ DAWAR, M. JAVED ZAKI AND NEELOFAR HAMID

Department of Botany,
University of Karachi, Karachi-75270, Pakistan

Abstract

Seeds of mung bean (*Vigna radiata* (L.) Wilczek and groundnut (*Arachis hypogaea* L.) were treated with ultra violet (UV-C) radiation for 0, 5, 10, 15, 20, 30 and 60 minutes for the estimation of growth parameters and root infecting fungi like *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp. It was observed that both crop plants showed increment in shoot weight, shoot length, root length and root weight, leaf area and number of nodules when seeds of groundnut and mung bean were treated with UV-C for 10, 15, 30 and 60 minutes period. UV-C exposure for 0-60 minutes showed reduction in root infecting fungi whereas 15, 20 and 30 minutes exposure with UV-C gave significant result in increment of total chlorophyll and carbohydrate contents.

Introduction

Ultraviolet (UV) radiations considered as electromagnetic radiation ranges between 10 to 400 nm with energies between 3 to 124 eV. These radiations are invisible to human eye, having many beneficial and damaging effects. It is applicable in the treatment of skin diseases like psoriasis and vitiligo (Dawe *et al.*, 2003). UV-B exposure causes the production of vitamin D in the skin which regulates calcium metabolism, immunity, cell proliferation, insulin secretion and blood pressure (Holick, 2004). The germicidal effects of radiant energy from the sun was first reported by Downs & Blunt (1878). Inactivation of *Escherichia coli* on alfalfa seeds by ultraviolet (UV-C) irradiation was reported by Gonzales-Dector *et al*., (2005). UV C light (UVC; wavelength, 200-280 nm) has a germicidal effect on microorganisms in water, on surfaces, in air and it is used for disinfection both inside and outside hospitals (Sharp, 1939).

Plant pathogens like fungi are considered as the most visible threats to sustainable food production (Cook, 1994; Fravel, 2005).The diseases, have the capacity to cause heavy losses both in volume and to farmer's income (Hafiz, 1986). *M. phaseolina* (Tassi) Goid is considered to be a soil-borne pathogen and is able to infect several plants including groundnut (*Arachis hypogaea*) and mungbean (*Vigna radiata*) etc., (Sinclair, 1982; Ghaffar, 1992). Of the various species of *Fusarium* Patch; *F. oxysporium* Schltdl. and *F. solani* (Mart.) Sacc. are known to cause root rot, stem rot and wilt diseases on wide range of plants (Booth, 1971) and are very common in agricultural fields of Pakistan (Ghaffar, 1992). *R. solani* Kühn exists as active mycelium in the soil, attacks over 2000 species of plants (Parameter, 1970) and has been reported from at least 68 hosts in Pakistan (Mirza & Qureshi, 1978; Ghaffar, 1988; 1991). Root rot of crop plants, may involve attack of more than one pathogen (Dickinson, 1979; Elarosi, 1957). An interaction among the soil borne plant pathogenic fungi can influence the disease intensity and severity on many crop plants (Pieczarke & Abawi, 1978).The association of *M. phaseolina, R. solani* and *F. solani* are known to produce root and stem rot in soybean throughout the world (French & Kennedy, 1963; Sinclair & Gray, 1972).

The main purpose of controlling plant diseases is to improve the growth quality and yield of crops (Stephan *et al.*, 1988). Therefore the present study was carried out on effects of ultraviolet (UV-C <280 nm) light on enhancement of growth parameters and root infecting fungi of mug bean and groundnut plants.

Materials and Methods

Experimental setup: Sandy loam soil was obtained from experimental plot of Department of Botany, University of Karachi having pH 8.0 (Brady, 1990), moisture holding capacity of 40% (Keen & Raczkowski, 1922), total nitrogen 0.079% (Mackenzie & Wallace, 1954), 4 sclerotia/g soil of *M. phaseolina* was found by wet sieving technique (Sheikh & Ghaffar, 1975), 7% of *R. solani* on sorghum seeds used as baits (Wilhelm,1955) and 4000 cfu/g *Fusarium* spp., by soil dilution technique (Nash & Synder, 1962).

Seeds of mung bean (*Vigna radiata* (L.) Wilczek and groundnut (*Arachis hypogaea* L.) were surface sterilized with 1% calcium hypochlorite and air dried under laminar flow hood. These seeds were exposed to ultraviolet (UV-C<280nm) radiations for 0, 5, 10, 15, 20, 30 and 60 minutes period. Five irradiated seeds were sown in 8cm diameter plastic pots, each pot containing 300g soil. The seeds treated with sterilized distilled water served as control. Each treatment was replicated three times and the pots were kept in screen house in randomized complete block design and watered regularly to maintain sufficient moisture required for the growth of plants. The data were recorded after 30 days of germination and the parameters taken into consideration were root length, root weight, shoot length, shoot weight, leaf area, and number of nodules.

Estimation of root colonization by root rot fungi: One cm long root pieces, after washed in running tap water, were surface sterilized with 1% calcium hypochlorite and plated on PDA plates at five pieces per plate, supplemented with antibiotics (penicillin and streptomycin @ 200 mg/L). Petri dishes were incubated at room temperature and after one week, infection on roots by root infecting fungi was recorded.

Estimation of chlorophyll: Fresh leaf material (1 g) was extracted with 80% of acetone, centrifuged at 1000 *g* (3 times) for 5 minutes. Absorbance of extract was recorded at 645 and 663 nm (Maclachlan & Zalik, 1963).

Estimation of carbohydrates: Fresh leaf material (0.5 g) were extracted in distilled water (10 ml) and centrifuged at 500 rpm twice for 5 minutes. Anthrone reagent (5.0 ml) was added in 0.5 ml plant extract, heated for 30 minutes and immediately placed in icecold. Optical density was recorded at 620 nm and amount of carbohydrate were calculated in µg/g by using standard curve (Yemm & Willis, 1954).

Leaf area: Leaf area of largest leaf was measured by product of length (L), width (W) and correction factor (C.F) of mung bean (0.94) and groundnut (0.821) as suggested by Kathirvelan & Kataiselvan (2007) and Mendoza (1986).

Data analysis: Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test and Duncan's multiple range test to compare the treatment means (Gomez & Gomez, 1984).

Result and Discussion

Groundnut and mung bean seeds were treated with UV-C radiations for 0, 5, 10, 15, 20, 30 and 60 minutes improves growth parameters. Significant ($p<0.001$) increase in germination of mung bean was observed when seeds were treated for 30 minutes. However maximum shoot length, shoot weight and root weight were observed when seeds of mung bean were treated with UV-C for 15 minutes while root weight was increased when seeds were treated for 30 minutes ($p<0.01$). Leaf area and number of nodules were maximum when seeds of mung bean were treated with UV-C for 10 and 30 minutes respectively (Table 1). Significant ($p<0.05$) increase in shoot length and shoot weight of groundnut seeds were attained when seeds were treated with UV-C radiation for 60 minutes whereas maximum root length, root weight and number of nodules were observed when seeds were treated with UV-C for 30 minutes (Table 2). Shiozaki *et al.*, (1999) recorded that shoot length and fresh weight of pea plants was enhanced by treatment with UV radiation. Many researchers observed that pre-sowing treatment of seeds with UV was effectively used to increase crop productivity (Jdanova, 1962; Dubrov, 1977; Ghallab & Omar, 1998).

Table 1. Effect of seed treatment with UV-C radiation on growth parameters of mung bean.

Treatment (min.,)	Germination %	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Leaf area (cm^2)	Number of nodule
Control	66.66	13.00	0.36	10.53	0.17	3.57	9
5	66.66	20.38	0.76	15.96	0.29	3.91	13
10	60	14.79	0.75	17.24	0.38	12.03	15
15	60	22.38	1.03	13.31	0.53	6.48	17
20	60	18.56	0.59	14.49	0.27	5.54	17
30	100	21.09	0.74	19.58	0.34	4.98	18
60	73.33	18.64	0.66	14.15	0.24	5.17	9
$LSD_{0.05}$	14.17	2.98	0.26	6.42	0.31	0.67	9.76

Table 2. Effect of seed treatment with UV-C radiation on growth parameters of groundnut.

Treatment (min.,)	Germination %	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Leaf area (cm^2)	Number of nodule
Control	66.66	4.91	1.22	13.62	0.63	2.08	6
5	73.33	6.65	1.86	13.64	0.20	2.98	8
10	66.66	5.93	1.57	14.24	0.91	3.27	11
15	66.00	6.67	1.71	16.34	0.87	3.67	11
20	66.66	6.26	1.56	16.8	0.22	3.99	10
30	53.33	5.9	1.86	18.39	1.38	3.84	11
60	86.66	6.83	2.20	13.81	0.78	3.51	10
$LSD_{0.05}$	19.53	0.95	0.49	7.83	0.89	0.82	1.93

Chlorophyll a content of mung bean seeds showed reduction when seeds were treated with UV-C for 10 and 60 minutes whereas chlorophyll b increased in all treatments. However, maximum increment in chlorophyll content was recorded when seeds were treated with UV-C for 20 and 30 minutes ($p<0.001$). Chlorophyll b of groundnut leaves was more or less equal whereas chlorophyll a showed significant ($p<0.05$) increment when seeds were treated with UV-C for 15 minutes. Similarly carbohydrate content was maximum when seeds of mung bean and groundnut were treated with UV-C for 15 and 30 minutes period ($p<0.001$) (Tables 3, 4). It was noted that chlorophyll a may be degraded first and it was due to stress (Kariola *et al.*, 2005). Therefore it is suggested that radiation of crop plants with UV-C radiation is effective in enhancing growth parameters and reduction of root infecting fungi.

The infection caused by root infecting fungi was reduced in all treatments. However, maximum reduction of *Fusarium* spp., *R. solani* and *M. phaseolina* on mung bean roots were recorded when seeds of mung bean were treated with UV-C for 5, 10, 15 and 20 minutes ($p<0.01$) (Table 5). Groundnut seeds when treated for 20, 30 and 60 minutes intervals showed maximum reduction in infection of *R. solani* and *M. phaseolina* ($p<0.05$) (Table 5). It was noted that UV-C radiations was lethal to bacteria, viruses, mold spores, yeast and algae but it was observed that the doses needed to inactivate these microbial spores vary (Hijnan *et al.*, 2006).

Table 3. Effect of seed treatment with UV-C radiation on chlorophylls and carbohydrates of mung bean.

Treatments	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Chlorophyll a+b (µg/g)	Chlorophyll a/b (µg/g)	Carbohydrates (µg/g)
Control	0.44	0.35	0.8	1.35	5.33
5	0.46	0.48	0.94	0.99	13.66
10	0.41	0.43	0.85	0.98	14.33
15	0.45	0.48	0.94	1.1	14.66
20	0.47	0.58	1.05	0.83	13.66
30	0.46	0.54	1.00	0.91	13.66
60	0.37	0.47	0.84	0.84	10.33
$LSD_{0.05}$	0.189	0.164	0.216	0.647	2.74

Table 4. Effect of seed treatment with UV-C radiation on chlorophylls and carbohydrates of groundnut.

Treatments	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Chlorophyll a+b (µg/g)	Chlorophyll a/b (µg/g)	Carbohydrates (µg/g)
Control	0.558	1.723	2.282	0.317	10.33
5	0.586	1.734	2.319	0.336	14.86
10	0.582	1.735	2.318	0.338	17.66
15	0.598	1.732	2.331	0.345	15.33
20	0.579	1.736	2.315	0.335	17.33
30	0.588	1.737	2.325	0.337	18.66
60	0.567	1.733	2.301	0.325	12.26
$LSD_{0.05}$	0.199	0.160	0.293	0.111	5.54

Table 5. Effect of seed treatment with UV-C radiation on infection % of root rot fungi on groundnut and mung bean.

	Mung bean			Groundnut		
Treatment (min.,)	***Fusarium* spp.**	***R. solani***	***M. phaseolina***	***Fusarium* spp.**	***R. solani***	***M. phaseolina***
Control	100	100	100	100	100	100
5	88.88	22.22	11.11	66.66	66.66	33.33
10	55.55	33.33	22.00	33.33	33.33	22.22
15	88.88	44.44	11.11	11.11	33.33	22.22
20	88.88	11.11	33.33	44.40	22.22	22.22
30	66.88	44.66	33.33	55.50	22.22	11.11
60	88.88	66.66	44.00	33.31	22.22	11.11
$LSD_{0.05}$	59.54	61.60	40.91	56.30	58.60	51.2

References

Booth, C. 1971. The genus *Fusarium*. Common Wealth Mycological Institute, Kew Surrey, England.

Brady, N.C. 1990. *The Nature and Properties of Soils.* 10th ed. Macmillan pub. Company. New York.

Cook, R.J. 1994. Introduction of soil organisms to control root rot diseases. In: *Soil Biota: Management in Sustainable farming system*. (Eds.): C.E. Pankhurst, B.M. Doube, V.V. Gupta and P. Grace. Csiro, East Melbourne, Australia., pp. 13-22.

Dawe, R.S., H. Comeron, S. Yule, I. Man, N.J. Wain Wright, S.H. Ibbotson and J. Ferguson. 2003. A randomized controlled trial of narrow band ultraviolet B vs. bath-psoralen plus ultraviolet A photochemotherapy for psoriasis. *British Journal of Dermatology,* 148(6): 1194-1204.

Dickinson, C.H. 1979. External synergisms among organisms inducing disease. In: *Plant pathology*: An advanced Treatise, (Eds.): J.G. Horsefall and E.B. Cowling. Vol. IV, *How pathogens induce Disease*. Academic Press, New York, pp. 97-111.

Downes, A. and T.P. Blunt. 1878. Research on the effects of light upon bacteria and other organisms. *Proc. Roy. Soc. London,* 26: 488-500.

Dubrov, A.P. 1977. *Physiological and biophysical study of ultraviolet irradiation influence on plants on early stages of development*. Ph.D. Thesis. Leningrad.

Elarosi, H. 1957. Fungal association. 1. Synergistic relation between *Rhizoctonia solani* Kuhn and *Fusarium solani* Synder and Hansen in causing a Potato tuber rot. *Ann. Bot.*, 21(4): 555-567.

Fravel, D. 2005. Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol.,* 43: 337-359.

French, E.R. and B.W. Kennedy. 1963. The role of *Fusarium* in the root rot complex of soybean in Minnesota. *Plant Dis. Rep*., 47: 672-676.

Ghaffar, A. 1988. *Soilborne Diseases Research Centre*. Final Research Report. Deptt. of Botany, University of Karachi, Karachi-75270, Pakistan, pp. 87.

Ghaffar, A. 1991. *Use of rhizobia in the control of soil borne plant diseases caused by sclerotial fungi*. Final Research Report, Department of Botany, University of Karachi, Karachi1-75270, Pakistan, pp. 115.

Ghaffar, A. 1992. *Use of microorganisms in biological control of root rot diseases of crop plants*. NSRDB. Final reaserch Report. Department of Botany, University of Karachi, Karachi-75270, Pakistan, pp. 85.

Ghallab, A.M and M.M. Omar. 1998. Wheat productivity as affected by seed irradiation with laser rays. *Annals of Agricultural Sciences of Moshtohor,* 36(1): 261-274.

Gomez, K.A and A.A. Gomez. 1984. *Statistical procedures for Agricultural Research* 2nd ed. Willey, New York, pp. 680.

Gonzalez-Dector, D., F. San Martin, E. Palou and A., López-Malo. 2005. Inactivation of *Escherichia coli* on alfalfa seeds by ultraviolet (UV-C) irradiation. IFT Annual Meeting, July 15-20 - New Orleans, Louisiana Session.

Hafiz, A. 1986. *Plant diseases*. PARC, Islamabad

Hijnan, W.A.M., E.F. Beerendonk and G.J. Medema. 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: A review. W*ater Research*, 40: 3-22.

Holick, M.F. 2004. Vitamin D. Importance in the prevention of cancers, type 1 diabetes, heart disease and osteoporosis. *Am. J. Clin. Nutr.*, 79: 362-371.

Jdanova, E.B. 1962. About ultraviolet irradiation influence on germination and growth of rye. *Proceedings of Agricultural Academy*. 77:451-455. Kagan VE, Fabisiak Japan.

Kathirvelan, P. and P. Kalaiselvan. 2007. Groundnut (*Arachis hypogaea* L.) leaf area estimation using allometric model. *Researcgh J. of Agric & Biol. Sci.,* 3(1): 59-61.

Kariola, T., G. Brader, J. Li and E.T. Palva. 2005. Chlorophylase 1, a damage control enzyme, affects the balance between defence pathways in plants. *The Plant Cell,* 17: 282-294.

Keen, B.A. and H. Raczkowski. 1922. The relationship between clay content and certain physical properties of soil. *J. Agric. Sci*., 11: 441-449.

Lu, H.Y., C.T. Lu., M.L. Wei, L.F. Chan. 2004. Comparison of different models for nondestructive leaf area estimation in taro. *J. Argo.,* 96: 448-453.

Mackanzie, H.A. and H.S. Wallace. 1954. The kjeldahl determination of nitrogen. A critical study of digestion conditions, temperature, catalyst and oxidizing agents. *Aust. J. Chem*., 7: 55-70.

Maclachlan, S. and S. Zalik. 1963. Extraction and estimation of chlorophyll content. *Journal Botany*., Part ll, 41: 1053.

Mendoza, T.C. 1986. Light interception and total biomass productivity in sugarcane intercropping. *Philipp. J. Crop. Sci.*, 11(3): 181-187.

Mirza, J.H. and M.S.A. Qureshi. 1978. *Fungi of Pakistan*. Dept of Plant Pathology, Univ. of Agric., Faisalabad, Pakistan. 311 pp.

Nash, S.M. and W.C. Synder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot, *Fusarium* in the field soils. *Phytopath.*, 52: 567-572.

Parameter, J.R. 1970. *Rhizoctonia solani* Biology and Pathology. Univ. of California Press, Berkeley Los Angeles and London. pp. 255.

Piecsarka, D.J. and G.G. Abawi. 1978. Effect of interaction between *Fusarium*, *Pythium*, *Rhizoctonia* on severity of bean root rot. *Phytopath.,* 68: 403-408.

Sharp, G. 1939. The lethal action of short ultraviolet rays on several common pathogenic bacteria. *J Bacteriol.,* 37: 447-459.

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

Shiozaki, N., I. Hattori and T. Tezuka. 1999. Activation of growth nodulation in a symbiotic system between pea plants and leguminous bacteria by near UV radiations. *J. Phytochem. Photobiol. B: Biol.,* 50: 33-37.

Sinclair, J.B. 1982. *Compendium of soybean diseases. 2nd Ed.* Amm. Phytopath. Society, Min. USA. pp. 104.

Sinclair, J.B. and L.E. Gray. 1972. Three fungi that can reduce soybean yields. *Illinois Res*., 14: 5.

Stephan, Z.A., I.K. Al-Mamoury and B.G. Antoon. 1988. The efficiency of Nematode, Solar heating and the fungus *Paecilomyces lilacinus* in controlling root knot nematode *M. javanica* in Iraq. *ZANCO*, 6: 69-76.

Wilhelm, S. 1955. Longetivity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology,* 45: 180-181.

Yemm, E.W. and A.J. Willis. 1954. The estimation of carbohydrate in the plant extract by Anthrone method. *Journal of Biochemistry*, 157: 508-514.

(Received for publication 20 March 2010)