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EFFECT OF ENHANCED UV-B RADIATION, ALLELOPATHY AND THEIR COMBINED STRESS ON GERMINATION, SEEDLING GROWTH, PIGMENTS AND AMYLASE ACTIVITY IN *VIGNA RADIATA*

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ABSTRACT

The study examined the effect of UV-B radiation and allelochemical stress on germination, seedling growth, chlorophyll a and b content and amylase activity of *Vigna radiata*. The individual effect of UV-B radiation and aqueous extract of *Pluchea lanceolata* inhibited germination of all test species. The species showed differential response. Germination was reduced by both the UV-B radiation and aqueous extract of *Pluchea lanceolata* in order: *Lens culinary >Vigna mungo >Vigna radiata*. Combined stress showed decrease in germination as well as root length, shoot length8l. ;and fresh weight of *Vigna radiata*. The individual effect of UV-B radiation increased the chlorophyll a and b while the individual effect of aqueous extract decreased the chlorophyll content whereas combined stress of UV-B and aqueous extract of *Pluchea lanceolata* also decreased the chlorophyll content in *Vigna radiata*. Simulated rain leachate of *Pluchea lanceolata* also inhibited the germination, root length and shoot length of *Vigna radiata*. Decaying *Pluchea lanceolata* in soil at a rate of 5, 10, 20gm/350gm soil substantially inhibited the germination but increased the shoot length and decreased the root length of *Vigna radiata*. UV-B radiation and aqueous extract of *Pluchea lanceolata* alone and in combination decreased the amylase activity of *Vigna radiata*.

Key-words:

INTRODUCTION

Ultraviolet radiation is non-ionizing radiation and comprises approximately 8-9% of the total solar radiation (Coohill,1989) UV radiation is divided into three wavelength ranges, UV-C (200-280nm) ,UV-B (280-320nm) it induce verity of damaging effect on plants; UV-A (320-400nm) it has a less hazardous effect. UV-B radiation is a highly energetic component of sunlight and this has increased due to ozone layer depletion (Strid *et al.*, 1994).

The stratospheric ozone layer has efficiency to filter out shortwave UV radiation but as the ozone level is depleted and led to increased penetration of solar UV-B radiation through the atmosphere to the earth's surface. The depletion of the stratospheric ozone layer is caused by man-made pollutants such as chlorofluorocarbon (CFCs), nitrogen oxide (Harm, 1980; Pang *et al*., 1991; Farland *et al*., 1992; Stapleton, 1992; Stolarski *et al.*, 1992; Hollosy, 2002; Mpoloka, 2008) hydro chlorofluorocarbon, methyl bromide and other industrial products that contain halogens (Kerr, 1988). This pollutant accelerates the depletion of the ozone layer. This may cause potential harm to agriculture and ecosystems. 1% reduction in ozone level can cause an increase of 1.3-1.8% of UV-B radiation reaching the earth's surface. Enhanced UV-B exposure affects plant growth directly and indirectly by altering various physiological process (Caldwell,1971) increased auxiliary branching, and leaf curling and decrease the plant height, plant leaf area and its dry weight (Greenberg, 1997) the relative growth rate and nitrogen productivity were also decrease because of decrease in leaf area productivity and leaf nitrogen productivity enhanced UV-B radiations also cause reduction in grain yield, structure of plant, its pigmentation, susceptibility to decrease and alteration in species competition (Teramura *et al.*,1990; Gao *et al*., 2004) and can cause deleterious effect to genetic material(DNA) and photosynthetic system such as alteration in light penetration into the leaf, changes in stomatal density and chlorophyll (Gao *et al*., 2004). Plant accumulates some UV- screening phenolic compounds antioxidants and other defense mechanisms are induced to prevent the harmful effect of UV-B radiations.

Allelopathy is an important ecological mechanism in both managed and natural ecosystem (Rice, 1984, 1995; Inderjit, 1998). Allelopathic species release secondary metabolites into the environment (Rice, 1984; Rizvi, and Rizvi, 1992) that inhibit the growth and development of other neighbouring species. The effect of allelochemicals of plants is considered as biotic stress which is also known as allelochemicals stress (Singh, 2009). Allelochemicals are of various types such as phenolic acids, terpines, terpenoids, glycosides, alkaloids, flavonoids, steroids and tannins (Inderjit *et al*., 1999 a, b; Mandava, 1985; Blum, 1996; Shaukat and Siddiqi, 2002). These chemicals are released into the soil and atmosphere by various processes such as by means of volatilization, leaching, decomposition of the residue and root diffusates (Singh *et al*., 2009).

The plants show inhibitory effect on germination and growth (Buchholtz, 1971; Bell and Koeppe, 1972; Einhellig and Rasmussen, 1973; Rasmussen and Einhellig, 1975; Ashraf and Sen, 1978; Shaukat *et al*., 1985; Ahmed and Wardle, 1994; Burhan and Shaukat, 1999) These chemicals are synthesized by the process of phenylpropenoid pathway (Jozwiak-Zurek, 2011) The intermediate product of this pathway is Ferulic acid (FA) which is present in the form of CoA. The stress caused by FA are very complex because they inhibit foliar expansion and root elongation, reduce rate of photosynthesis, reduce water utilization, and induce lipid peroxidation not only this but phenylpropenoid pathway is activated by FA by inducing the activity of enzyme such as (PAL) phenylalanine ammonia lyase (Jozwiak-Zurek, 2011; Shaukat *et al*., 2013). Putnam and Weston (1986) listed 90 weeds species while Narwal (1994) listed 129 weed species having allelopathic potential.

The principal allelopathic agent in weeds and other allelopathic plants are phenolic compounds (Inderjit, 1998; Burhan and Shaukat, 2000; Shaukat *et al.,* 2003). Allelopathic compounds cause deleterious effect on neighboring plants. The several molecular targets are known to affect many cellular process viz. stomatal closure cell divisions (Singh, 2009) membrane permeability nutrient uptake plant water balance respiration and other metabolic process. These secondary metabolites also inhibit the growth of beneficial microbes (Alsaadawi and Rice, 1982; Shaukat *et al.,* 2002). *Pluchea lanceolata* is a troublesome perennial weed of cultivated and uncultivated areas of semi-arid regions (Inderjit and Dakshini, 1990). It is a hoary pubescent under shrub with coriaceous sessile, strongly veined leaves, lilac flowers borne in terminal compound corymbs, and prominent subterranean parts. Involvement of *Pluchea lanceolata* in allelopathic interference to crop plants has been suggested under laboratory, greenhouse, and field conditions (Inderjit *et al*., 1996, 1998, 1999a, b).

MATERIAL AND METHODS

The study was carried out at Institute of Environmental Studies University of Karachi during (March-October 2013). Seeds of test plant species were purchased from the local market. *Pluchea lanceolata* (DC.) Oliv. & Hiern, was collected from Karachi University campus, near Genetics Department, University of Karachi, growing on sandy soil.

The plant material was air-dried under shade and chopped into small pieces. Extract of *Pluchea lanceolata* was prepared by soaking 10 g plant material in 100 ml of distilled water for 24h to obtain the stock solution. Using stock solution 100%), four other concentrations were prepared i.e., 20, 40, 60 and 80%. Clean seeds of *Vigna radiata* (L.) Wilczek, *Vigna mungo* (L.) Hepper and *Lens culinaris* Medik were first surface sterilized with 5% sodium hypochlorite solution for 2 minutes, rinsed with distilled water and then soaked in distilled water for 30 minutes. Ten seeds of the test species were placed in 9cm diameter sterile Petri plates fitted with 1 disc of Whatman No. 1 filter paper.

Effect of UV-B radiation: petri plates were subsequently transferred to the radiation chamber and exposed to fluorescent UV-B tube. The chamber was covered with wooden lid for safety reasons. Within the chamber a UV-B fluorescent tube (TL40W/12, Philips, Eindhoven, The Netherland) was installed, which exhibited its emission>280nm to a maximum at 312nm (the actual UV-B range is 280nm-320nm). The Petri plates containing 10 seeds were exposed for 10,20,30,40 and50 minutes to UV-B radiation. Three replicates were kept for each treatment and control. Initially, 5ml sterile distilled water was added to each plate. For germination study Petri plate was kept at 28^0C on a laboratory bench. Daylight was supplemented by light from two fluorescent tubes. Observation on germination was recorded daily. Small amount of distilled water added periodically when the petri plate was beginning to dry out.

Effect of aqueous extract of *Pluchea lanceolata*: Each plate received 5 ml of the extract of four other concentration (20, 40, 60 and 80%.), for controls, distilled water was used. Germination was made daily up to 5 days. At the end root and shoot length, of the seedling and their fresh weight ad dry weight (seedling was dried at 80^0C for 24hr) was recorded. Germination velocity (GV) was measured using the index proposed by Khandakar and Bradbeer (1983), as follows:

GV= [N1/1+N2/2+N3/3+……. Nn/n] x 100/1

Where N1, N2, N3…….. Nn is the proportion of seed that germinated on day1, 2, 3,…… n respectively, the index ranges from 0 (if no seeds germinate on the first day) to 100 (if all the seeds germinate on the first day).

For combine stress of UV-B radiation exposure and aqueous extract of *Pluchea lanceolata* and for further experiment one seed is selected out of three test species which is *Vigna radiata*, the reason of its selection was, it

gives the effective results.

Effect of combined stress of UV-B and aqueous extract of *Pluchea lanceolata:* Seeds of *Vigna radiata* were exposed to UV-B radiation for 60 minutes and then treat with four different concentrations (20, 40, 60 and 80%.) of aqueous extract of *Pluchea lanceolata* using the same method as described above in Germination counts were made daily and shoot, root length and fresh and dry weight of the seedlings were recorded after 5 days.

Effect of UV-B radiation on Chlorophyll a and b: Seeds were exposed to UV-B radiation in 0 (control), 10 ,20,30,40, and 50 minutes and the seedling grown as described above, subsequently at 5 day chlorophyll in the leaf and cotyledons was determined. Chlorophyll a and b were extracted from the irradiated shoots and estimated by the method of Maclachlan and Zalik (1963). For extraction 1 gram of shoots was grounded in 10 ml of 98% (v/v) acetone and centrifuged at 2000rpm for 15 minutes to clear the suspension. Supernatant, which contained soluble pigments, was used for determination of chlorophylls. Absorbance of the extract was recorded in 663 and 645nm on (Beckman Coulter Du 730) spectrophotometer against 98% (v/v) acetone blank. The chlorophyll content was calculated using the formula given below and expressed in mg/g fresh weight.

Chlorophyll a mg/gm=12. 7 A663 – 2.69 A645 ÷ 1000 XW x V
Chlorophyll b mg/gm= 22.9 A645 – 4.68 A663 ÷ 1000 XW x V

Where "V" is the volume of extract in ml and "W" is the fresh weight of leaf sample in grams.

The same procedure is used for the determination of chlorophyll when treated with aqueous extract of *Pluchea lanceolata* alone and in the combined stress of UV-B radiation and aqueous extract *of Pluchea lanceolata.*

Effect of *Pluchea lanceolata* simulated rain leachate on germination and growth of *Vigna radiata:* The leachate were prepared by using 100 grams of dry leaf of *Pluchea lanceolata* , the leaf is transferred into funnel fitted with Whatman No 1 filter paper ,100 ml of distilled water is sprayed over the leaf and the stock leachate is collected in a beaker.100% and 50% solution were used to treat the seeds of *Vigna radiata* following the same procedure as described above.

Effect of decaying *Pluchea lanceolata* on germination and seedling growth of *Vigna radiata*: Dried powdered material of *Pluchea lanceolata* was mixed thoroughly with garden soil in 5, 10 and 15 g/350g of soil. Pots were watered once and soil was left for biodegradation. After one week, 10 seeds of *Vigna radiata* were sown in each pot. Controls and treatments were replicated thrice and pots were randomized on the greenhouse bench. Daily rate of emergence was recorded while shoot and root lengths and fresh and dry weights were measured after fifteen days.

Effect on amylase activity of *Vigna radiata:* The effect of *Pluchea* and UV –B radiation was ascertained at 90 h after the commencement of imbibition and was measured by an agar-gel diffusion method described by Clum (1967) with minor modifications. The starch substrate was prepared by adding 1 g of soluble starch and 20 g Agar –agar to one liter of boiling distilled water. The mixture was stirred for 5 minute and poured into 9 cm Petri plate, at 25 ml / plate. An amylase extract was prepared by macerating 20 germinating seeds (from one Petri plate) in 25 ml ice cold 0.2 N acetate buffer (pH 5.3). The marcerate was centrifuged at 4000 rpm for 10 minutes. The clear supernatant was made up to 49 ml with the buffer and 1 ml chloramphenicol solution (5000 ppm) was added to give the extract a final concentration of 10 ppm chloramphenicol to control the growth of micro- organisms without seriously affecting the metabolism of tissue or tissue extract (Sabota *et al.,* 1968). Four Whatman No. 1 filter paper discs (each 6 mm in diameter) were evenly placed on agar-gel plate. Each disc receives 10 µl of amylase extract. The plates were then placed at 21±1°C for 3 days. After this the disc was removed and plates flooded with 5% I_2 KI solution. The diameters of the clear zones were measured twice at right angles. Each treatments and controls were replicated three times.

Effect of UV-B radiation and aqueous extract of *Pluchea lanceolata* alone and combined stress were recorded on amylase activity of 3-day-old seedlings.

Statistical Analysis: The data were subjected to analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test at p=0.05. For the germination percentage, data were arcsin transformed prior to ANOVA.

RESULTS

Effect of UV-B radiation on germination and growth of *Vigna radiata:* An adverse effect of supplemental UV-B radiation was observed on the root length of *vigna radiata*. Decrease in root length of plant was observed in all 5 exposure time to UV-B radiation. The maximum root length was observed at 10 minute treatment and decrease in shoot length at all the exposure time except at 20 minute exposure to UV-B radiation. UV-B radiation also affects the fresh and dry weight content, fresh weight increased at all the UV-B exposure, the maximum fresh weight observed at 10 minutes exposure. The decrease in dry weight observed at 10 minutes at all the exposure, and germination velocity was decreased at 30minutes and 50 minutes exposure to UV-B radiation as compare to control (Table 1).

Effect of aqueous extract of *Pluchea lanceolata* on germination and growth of *Vigna radiata:* The inhibitory effect increased with the increase in concentration, seedling growth of *Vigna radiata* was adversely affected and growth reduction was greater at higher concentrations. Root and shoot growth of *Vigna radiata* was inhibited to a greater extent generally, shoot growth was reduced to a greater degree than the root growth. Fresh weight and dry weight was also inhibited at various concentrations over the control (Table 2).

Effect of combined stress of UV-B radiation and aqueous extract of *Pluchea lanceolata* on *Vigna radiata*: UV-B treatment was observed to be effective on *Vigna radiata*, the decrease in root length was observed in all 5 exposure times. The combined stress of UV-B radiation exposure and aqueous extract of *Pluchea lanceolata* results in a decrease in root length as compare to aqueous extract treatment of *P. lanceolata* alone. Increase in shoot length was observed both alone and in combined stress as compared to aqueous extract treatment of *P. lanceolata*, but decrease in shoot length as compared to control (Table 3) the fresh weight was increased in the treatment of UV-B radiation and in combined stress as compared to control but decrease in fresh weight after the treatment of aqueous extract of *P. lanceolata*. No changes were observed in dry weight, in combined stress Germination velocity was surprised as the concentration increase in combined stress as compared to controls, but enhance as compared to the individual treatment of aqueous extract of *P. lanceolata* (Table 3).

Effect of UV-B radiation, aqueous extract of *Pluchea lanceolata and their* combined stress on Chlorophyll a and b: The results show that UV-B radiation exposure cause increase in Chlorophyll a and b content at 50 minute exposure time as compared to control. (Table 4) whereas chlorophyll a and b were gradually decreasing as the concentration of extract was increasing as compared to the control. (Table 5), Combine stress also affects chlorophyll content up to a greater extent, both the chlorophyll a and b decreased as compared to control (Table 6).

Effect of rain leachate of *Pluchea lanceolata* on *Vigna radiata*: The germination velocity was observed to decrease more at 100% concentration as compare to control. Whereas root and shoot length was increase at 100% concentration, and no significant changes observed in fresh weight whereas dry weight was observed to be increase both at 50% and 100% concentration (Table 7).

Effect of decaying *Pluchea lanceolata* on *Vigna radiata*: Root length was decreased as the amount of plant material increased in soil whereas shoot length was observed to be increased as a plant material increase in soil, no significant change was observed in fresh weight whereas dry weight was slightly increased as plant material increase (Table 8).

Table 1. Effect of UV-B radiation on *Vigna radiata*.

Time of UV B exposure (min)	Average root length (cm)	Average shoot length (cm)	Average fresh weight (g)	Average dry weight (g)	Germination velocity (%)
Control	8.06±0.5	8.32±0.5	0.12±0.001	0.02±0.0007	100%
10	5.89±0.3	7.55±0.4	0.19±0.003	0.01±0.0004	100%
20	5.12±0.3	9.16±0.4	0.18±0.003	0.01±0.0007	100%
30	5.39±0.3	8.12±0.5	0.17±0.003	0.01±0.0007	96.66%
40	4.60±0.3	6.52±0.4	0.14±0.003	0.01±0.0007	100%
50	5.83±0.4	4.9±0.4	0.12±0.001	0.01±0.0004	98.26%

Table 2. Effect of Aqueous extract of *Pluchea lanceolata* on *Vigna radiata*.

Conc. of plant extract (%)	Average root length (cm)	Average shoot length (cm)	Average fresh weight(g)	Average dry weight(g)	Germination velocity (%)
Control	10.39±0.7	7.32±0.7	0.20±0.03	0.02±0.004	98%
20	5.87±0.8	4.31±0.5	0.16±0.02	0.02±0.004	100%
40	2.72±0.4	2.31±0.4	0.06±0.01	0.01±0.003	88%
60	4.60±0.8	2.17±0.3	0.12±0.02	0.02±0.004	86.3%
80	1.65±0.3	1.34±0.3	0.06±0.01	0.01±0.003	73.1%

Table 3. Effect of Combined stress of UV-B radiation and aqueous extract of *Pluchea lanceolata*.

Time of UV B exposure(60 minute) +conc.	Average root length (cm)	Average shoot length (cm)	Average fresh weight (g)	Average dry weight (g)	Germination velocity (%)
Control	4.25±0.5	7.8±0.56	0.08±0.004	0.02±0.003	100
20	3.15±0.9	9.42±0.37	0.14±0.005	0.02±0.003	100
40	0.96±0.001	5.17±0.52	0.08±0.002	0.02±0.004	93.33
60	0.33±0.005	2.74±0.28	0.09±0.003	0.02±0.005	76.66
80	0.86±0.004	3.25±0.04	0.07±0.005	0.02±0.001	81.61

Table 4. Effect of UV-B Radiation on chlorophyll of V*igna radiata* Chlorophyll.

Time of UV B exposure (min)	Chl-a (mg/g)	Chl-b (mg/g)
10	0.14±0.02	0.05±0.02
20	0.13±0.02	0.04±0.02
30	0.14±0.02	0.05±0.02
40	0.14±0.02	0.07±0.02
50	0.14±0.02	0.06±0.02
60	0.15±0.02	0.08±0.02

Table 5. Effect of aqueous extract of *Pluchea lanceolata* on chlorophyll content of *Vigna radiate.*

Conc. of plant extract (%)	Chl-a (mg/g)	Chl-b (mg/g)
20	0.06±0.005	0.01±0.005
40	0.03±0.005	0.03±0.005
60	0.03±0.005	0.01±0.005
80	0.02±0.005	0.01±0.005

Table 6. Effect of combined stress on chlorophyll of *Vigna radiata* Chlorophyll.

Conc. of plant extract (%)	Chl-a (mg/g)	Chl-b (mg/g)
20%	0.10±0.03	0.01±0.003
40%	0.06±0.008	0.02±0.005
60%	0.06±0.008	0.01±0.003
80%	0.05±0.006	0.01±0.003

Table 7.Effect of rain leachate of *Pluchea lanceolata.*

Conc. of plant extract (%)	Average root length (cm)	Average shoot length (cm)	Average fresh weight (g)	Average dry weight (g)	Germination velocity (%)
Control	2.6±0.26	2.6±0.31	0.1±0.01	0.01±0.001	100%
100%	4.4±0.36	3.9±0.41	0.1±0.02	0.03±0.001	94%
50%	2.5±0.15	1.4±0.14	0.1±0.01	0.03±0.001	96%

Table 8. Effect of decaying *Pluchea lanceolata* on *Vigna radiata*.

Amount of plant material (g)	Average root length (cm)	Average shoot length (cm)	Average fresh weight (g)	Average dry weight (g)
Control	9.0±0.02	9.9±0.02	0.2±0.002	0.02±0.0004
20	6.7±0.06	20.2±0.5	0.2±0.004	0.02±0.0006
40	4.5±0.03	17.4±0.4	0.2±0.005	0.03±0.0005
60	2.0±0.1	14.4±0.2	0.1±0.001	0.08±0.0003

Table 9. Effect of UV-B on amylase activity of *Vigna radiata.*

Time of UV B exposure (min)	Zone (cm)
Ctrl	1.6±0.1
10	1.0±0.07
20	0.9±0.06
30	0.8±0.02
40	0.8±0.05
50	0.8±0.05

Table 10. Effect of aqueous extract of *Pluchea lanceolata* on amylase activity of *Vigna radiata.*

Conc.of plant extract (%)	Zone (cm)
20%	0.8±0.01
40%	1.0±0.06
60%	0.9±0.03
80%	0.9±0.03

Table 11. Effect of combined stress on amylase activity of *Vigna radiata.*

Conc. of plant extract (%)	Zone (cm)
20%	0.1±0.03
40%	0.1±0.04
60%	0.2±0.08
80%	0.2±0.07

Effect of UV-B, aqueous extract of *Pluchea lanceolata* and their combined stress on amylase activity of *Vigna radiata*: Amylase activity is decreasing as increase the time of UV-B radiation exposure as compared to control. The maximum activity recorded at 10 min exposure of UV-B radiation (Table 9), and amylase activity was also decreased due to the treatment of aqueous extract of *Pluchea lanceolata* as compared to control. The maximum activity recorded at 40% concentration of aqueous extract of *P. lanceolata* (Table 10). Amylase activity was greatly reduced due to combine stress of UV-B radiation and aqueous extract of *P. lanceolata.* The maximum activity was

recorded at 40% and 60% concentration of aqueous extract of *P. lanceolata* (Table 11).

DISCUSSION

Several investigations have demonstrated that increasing UV-B radiation exposure induce several morphological (Day and Demchik, 1996; Furness *et al*., 1999) and biochemical/physiological changes in higher plants (Kozlowska *et al.,* 2007). Decline in growth is related with change in cell division, cell elongation and synthesis and transport of growth regulators (Tevini, 1994) reported that UV treatment, in *Crotalaria juncea* L resulted in 50% reduction in shoot length. Earlier it was also reported decrease in height of *Pisum sativum* due to UV-B exposure. Increased leaf epidermal thickness can decrease the penetration of UV-B radiation to more sensitive layer .The UV-B radiation may cause change in membrane integrity due to lipid peroxidation and deterioration on tissues which therefore result in growth reduction Shaukat *et al.* (2011) reported that the UV radiation may altered cell division via transcriptional repression of the genes encoding for a mitotic cycling and protein kinase. Stomatal opening and closing and the rate of transpiration were also altered due to The UV-B treatment. In the present study UV-B exposure decreased the germination velocity, root length, shoot length, fresh weight and dry weight of *Vigna radiata* whereas in *Vigna mungo* and *Lens culinaris* germination velocity was decreased and root length, shoot length, fresh weight and dry weight was observed to be increased as the exposure time of UV-B radiation increased.

Aqueous extract of a various other allelopathic weeds are known to have inhibitory effects on crop seed germination (Shaukat, *et al.,* 1985; Putnam and Weston, 1986; Narwal, 1994; Lydon *et al.,* 1997; Rajbanshi and Inubushi, 1997; Ito *et al.,* 1998; AI Humaid and Warrag, 1998). Aqueous extract of *Pluchea lanceolata* decrease the germination velocity as well as root, shoot length fresh and dry weight of all three test species. Different species showed different response in order of *Lens culinaris>Vigna mungo>Vigna radiata*. *Vigna radiata* was observed to give effective result in both the treatment so *Vigna radiata* was selected for the further observation of different effects.

Combined stress of UV-B radiation and aqueous extract of *P. lanceolata* were applied on *V. radiata* and the result showed that combine stress suppress the germination velocity, growth of root and shoot length and decrease in fresh weight of seedling whereas no significant changes observed in dry weight of *V. radiata*. This result shows that combined stress has measurable impact on root and shoots length of *V. radiata* seedling. The fresh weights of seedling were significantly reduced by the combined stress it may be due to the inhibition of photosynthesis. The suppression of seedling growth could also be reduced due to the accumulation of phenolics compounds synthesized in the plant in response to UV-B stress (Shaukat *et al.,* 2011).

Decaying different amount of *P. lanceolata*in garden soil was affected the germination and growth of seedling of *V. radiata* that should be used during experiment. Toxins released by allelopathic donor plants ultimately get deposited in adjacent soil. The affectivity of toxins in soil, however, depends upon a number of factors including texture, accumulation capability and microbial activity. The toxins must accumulate to a physiological active level for exhibition of allelopathy. It was reported that *Lantana* soil when tested showed no inhibitory effects on the germination and growth of test species. The result coincides with findings of Sahid and Sugau (1993) and Achhireddy and Singh (1984) who reported that *Lantana* affected soil did not influence the crop.

Leaf area could be reduced due to the decreased uptake of water, shrinkage of cell contents and unbalanced nutrition (Ali *et al*., 2004). Reduction in pigment fractions have been reported in a number of crop plants following the UV-B exposure (Ali *et al.,* 2004). It is recorded that the UV-B exposure can cause reduction in 30% total chlorophyll of *Pisum sativum*. A reduction in the amount of chlorophyll (20.5%) and carotenoid (15.4%) contents of *Crotalaria juncea* leaves following the UV-B exposure has also been observed (Shaukat *et al.,* 2011).

Shukla *et al.,* (2008) also reported decrease in chlorophyll a (43%), chlorophyll b (23%), and carotenoid (53%) in *Brassica campestris* seedlings. However, Liu *et al.,* (1995) found no significant differences in level of pigment in barley and other grass species. Shaukat *et al.,* (2011) observed increased in chlorophyll content on high UV-B treatment the photosynthetic pigments may also be destroyed by UV exposure, while chlorophyll a is more affected than the chlorophyll b (Strid *et al.,* 1990). UV radiation can lead to dramatic changes of the fine structure of chloroplasts and mitochondria. The result of current experimental studies showed that exposure to UV-B radiation cause slight increase in chlorophyll a and b content whereas effects of aqueous extract and combined stress was observed which cause decrease in chlorophyll a and b content in *V. radiata*.

In this study effects of simulated rain leachate of *P. lanceolata* were also examined which cause increase in root and shoot length and maximum root and shoot length were recorded at 100% concentration whereas no significant changes was observed in fresh weight while slightly changes were recorded in dry weight. As this rain leachate was freshly prepared by spraying distilled water on the leaves of *P. lanceolata* so it may not contain all phenolic

compounds in it that is why it does not show any adverse effect on seedling germination and on its growth as compared to aqueous extract of *P. lanceolata* which was prepared after soaking 24hour in distilled water. The results may be varying due to climatic/environmental condition and geographical location of the country or may be due to human error.

Starch is quantitatively the most abundant storage material in all seeds including *V. radiata* evidence indicates that in germinating seeds starch is degraded predominantly via amylotic pathway (Dua and Sawhney, 1991). Amylase activities were also observed in this study. Effect of UV-B radiation and aqueous extract of *Pluchea lanceolata* alone and in combination was observed which cause decrease in amylase activity.

Conclusion

The current studies show that UV-B radiation and aqueous extract of *P. lanceolata* alone and combined effect the crop plants, it suppresses the germination and also affects the growth of selected crop plants.

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