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COMBINED EFFECT OF UV-B RADIATION AND ALLELOPATHY ON GERMINATION, SEEDLING GROWTH AND PHYSIOLOGICAL RESPONSES OF *VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

This study examines the effect of supplemental UV-B radiation and allelochemical stress alone and in combination on growth and biochemical responses of cowpea (*Vigna unguiculata*). UV-B irradiation alone (20-40 min. exposure) suppressed the final percentage germination as well as speed of germination. Both root and shoot growth of cowpea were also retarded. The effect of allelopathy was tested using the allelochemical gallic acid which at 40-80ppm inhibited final germination percentage as well as speed of germination, but root and shoot growth were reduced at 20-40ppm concentrations.. Greater final germination percentage and speed of germination were reduced when both allelochemical and UV-B irradiation were applied together. Likewise, root and shoot growth in the combined stress were suppressed to a greater extent compared to the influence of either stress applied alone. UV-B alone or in combination with gallic acid treatment resulted in accumulation of total soluble phenols and greater accumulation was observed in the combined stress. Likewise, phenyl ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) activities were remarkably increased when both the stresses were applied simultaneously compared to UV-B stress alone. The implications and mechanisms of biochemical responses to UV-B and allelopathy together are discussed.

Key-words: UV-B radiation, allelopathy, growth parameters, germination, cowpea.

INTRODUCTION

Two major threats to life on earth are damage of stratospheric ozone layer and global climatic changes. The ozone layer exists at an altitude of 10-30 Km around the earth. In the past fifty years or so, the concentration of ozone has decreased to about 5%, primarily due to release of anthropogenic pollutants such as chlorofluorocarbons and other industrial products containing halogens (Kerr, 1988; Pyle, 1997).The thinning of stratospheric ozone layer has resulted in increased penetration of solar UV- B (280-320 nm) radiation through the atmosphere, reaching earth's surface (McKenzie *et al.,* 1999). The resulting enhanced levels of UV radiation can be harmful for all life forms, plants, animals and even microorganisms. Madronich *et al.,* (1998) calculated that 2% biologically effective UV radiation can be increased by 1 % depletion of ozone layer. Exposure to high levels of UV-B radiation can easily result in damage of DNA (Landry *et al.,* 1997), proteins (Strid *et al.,* 1994), cell membranes and the chloroplasts and its associated thylakoid system and pigment (Day and Vogelman, 1995; Greenberg *et al.,* 1997) in many plants. In humans, UV-light may cause cataracts, skin cancer, herpes suppression of immune system etc. (Brian and Taylor, 2001).

A number of studies have demonstrated that UV-B can induce some general stress responses and other physiological and photomorphogenic responses (Mackerness, 2000; Jansen, 2002; Ravindran *et al.,* 2008). Numerous workers have found significant impact of enhanced UV-B radiation on growth, development, biomass accumulation, yield and metabolism of plants (Rozema *et al.,* 1997; Gao *et al.,* 2003; Ravindran *et al.,* 2008). Some studies have also shown the inhibition of stem growth thereby altering the shoot morphology (Kim *et al.,* 1998; Kobzar *et al.,* 1998). Mechanisms such as increased leaf thickness alterations in cuticle and increased production of UV-B protective pigments have been investigated in different plant species (Gwynn-Jones, 2001). Enhanced UV-B radiation due to 5% simulated ozone resulted in the depletion of biomass and leaf area (Barnes *et al.,* 1993). Greenberg *et al.,* (1997) stated that UV- B absorbing compounds and chlorophylls (physiological parameters) have been found to be useful indicators of UV-B sensitivity and tolerance. If protective mechanism fails to protect the genome and photosynthetic machinery against UV-B, repair mechanisms are relied upon (Takeuchi *et al.,* 1998). One protective mechanism which seems to be common under stress conditions is the increase in the phenol content (Kozlowska *et al.,* 2007; Ravindran *et al.,* 2008). Exposure to near ambient UV-B results in increase in leaf phenolic content in soybean plants (Zavala *et al.,* 2001).

L-Phenylalanine ammonia lyase (PAL) which catalyses the formation of trans-cinnamate from L-phenylalanine by non-oxidative deamination occurs in most plants and in some fungi (Kalghatgi and Subba-Rao, 1975). Consequently, the importance of PAL is that it catalyses the first committed step in the biosynthesis of defense

related phenylpropanoids. Thus stress conditions generally result in increased PAL activity of plant tissue (Zucker, 1965; Pegg and Sequeria, 1968; Chmielowski *et al.*, 2008). Lavola *et al.*, (2008) demonstrated that UV-B radiation significantly increased PAL activity in birch seedlings. The products of PAL and TAL (tyrosine ammonia lyase) are modified through phenylpropanoid metabolism, including, lignin, flavonoids and pigments and phytoalexins that play a key role in a range of diseases and stresses (Morrison and Buxton, 1993).

Allelopathy includes process involving secondary metabolites produced by plants and microorganisms that influence the growth and development of agricultural and biological systems (excluding animals) (Torres *et al*., 1996; Lara-Nunez *et al.,* 2006). The chemicals released by the donor plant to the environment which influence the other biological systems are called as allelochemicals. Allelopathy is an important ecological mechanism that can play a vital role in intra- and interspecific competition (Rice, 1984). The allelochemicals released by the plants are responsible for replacing susceptible species with resistant ones or invading species that take over during succession (Rice, 1984; Kruse *et aI*., 2000). The allelochemicals include a variety of chemical compounds (alkaloids, aldehydes, organic acids, glycosides, phenolics, etc.) but more often they are phenolic in nature which are synthesized via the phenylpropanoid pathway (Blokker *et al.*, 2006; Li *et al*., 2010). Phenolics are the secondary metabolites that accumulate in soils causing inhibition of both germination and early seedling growth of many plant species (Inderjit, 1998; Reigosa *et al*., 2006; Li *et al*., 2010). Many other workers have also reported the presence of phenolic compounds in soils (Blum, 1996; Kobayashi *et al*., 1996; Regnier and Macheix, 1996; Janovicek *et al.*, 1997). These are accumulated as a result of death and decay of allelopathic plants such as many weeds. Putnam and Weston (1986) listed 80 weed species while Narwal (1999) listed 128 weed species having allelopathic potential.

In general, most workers have focused on plant responses to individual stress conditions. In natural environment, however, plants are exposed to multiple stresses simultaneously, e.g., two or more stresses like UV-B radiation, allelpathy, excessive heat or cold, electromagnetic waves, heavy metals, pathogens, etc. Various stresses competing with supplemental UV-B radiations have been demonstrated to modify the effects of UV-B (Conner and Zangori, 1998; Sanderman, 2004; Jozwiak-Zurek *et al.*, 2011)

Despite several studies on the physiological responses of plants to UV-B radiation and allelopathic potential of plants, the literature on combined effect of UV-B and allelopathic stresses is scant. Furness *et al.*, (2008) examined the allelopathic influence of houndstongue (*Cyanoglossum officinale*) and its modification by UV-B radiation. Li *et al.,* (2008) studied the effect of UV-B irradiance on the allelopathic potential of *Zanthoxylum bungeanum*. Jozwia-Zurek *et al.,* (2011) investigated the combined effect of UV-B radiation and allelopathic stress on the PAL (phenyl ammonia lyase) activity of two genotypes of cucumber (*Cucumis sativus* L.). Cowpea *Vigna unguiculata* (L.)Walp. an important bean crop in the Indo-Pakistan sub-continent. This study focuses on germination, seedling growth and development, and biochemical responses of cow-pea (*Vigna unguiculata*) to supplemental UV-B radiation and allelopathy. alone and in combination.

MATERIALS AND METHODS

Germination conditions and UV-B exposure

The seeds of cowpea (*Vigna unguiculata* (L.) Walp.) var. Elite used in the current study were obtained from Pakistan Agricultural Research Council, Karachi. Cowpea is an important pulse (bean) crop that is rich in proteins and cultivated widely in Asia and Africa. Clean seeds of *Vigna unguiculata* were first surface sterilized with 0.5 percent sodium hypochlorite for 2 min., rinsed and soaked in distilled water for 2 h and then 20 seeds were placed in 9 cm diameter sterile Petri plates fitted with two discs of Whatman No.1 filter paper, subsequently transferred to radiation chamber and exposed to fluorescent UV-B tube. The chamber was covered by wooden lid for safety reasons. Within the chamber a UV-B fluorescent tube (TL40W/12, Philips, Eindhoven, The Netherlands) was installed, which exhibited its emission >280nm to a maximum at 312 nm (the actual UV-B range is 280-320nm). Acetate paper was fitted above the Petri plates that cuts of any radiations below 280nm. The Petri plates containing 20 cowpea seeds, moistened with distilled water for 0.5 h, were exposed for 10, 20, 30, 40 and 50 minutes to UV-B radiation. Five replicates were kept for each treatment and control. Five ml sterile distilled water was added to each Petri plates for germination study Petri plates were kept at 28° C and 50 % humidity on a laboratory bench. Day light was supplemented by light from two fluorescent tubes. Observations on germination were recorded daily. Small amounts of distilled water were added periodically when Petri plates were beginning to dry out. Germination was recorded daily up to 10 days. At the end root and shoot lengths of the seedlings were recorded. Germination velocity (GV) was measured using the index proposed by Khandakar and Bradbeer (1983), as follows:

$$GV= [N_1/1 + N_2/2 + N_3/3 + \ldots.+ N_n/n] \text{ X } 100/1$$

Where N_1, N_2, N_3,…N_n are the proportion of seeds that germinated on day 1,2 3, … n respectively.

Allelopathic effects

To examine the allelopathic effect and Combined UV-B and allelopathy we used gallic acid (3,4,5-trihydroxybezoic acid) , a phenolic (allelopathic) compound which is widely distributed in plants in a free state (Ishikura *et al.,* 1984) and is known to cause inhibition of germination and seedling growth (*Reigosa et al, 1999).* Gallic acid (GA), a key intermediate in the synthesis of plant hydrolysable tannins, is also a primary anti-inflammatory agent found in wine, tea, and cocoa in addition to numerous other plant species. Although it has long been recognized that plants, bacteria, and fungi synthesize and accumulate GA, the pathway leading to its synthesis was not clear and three different pathways were proposed for its synthesis (Ishikura *et al.,* 1984). Musil (1995) provided evidence that shikimate dehydrogenase (SDH), a shikimate pathway enzyme essential for aromatic amino acid synthesis, is also required for GA production. Thus, we used 10, 20, 40, 60 and 80 ppm gallic acid to examine the allelopathic effect on *V. unguiculata* in Petri plates *in vitro*. The experimental setup was the same as described above. 5ml of gallic acid solutions were added to each of the Petri plates. Treatments and controls were replicated 4 times each.

Combined effect of UV-B and allelopathy

Twenty surface sterilized seeds of *Vigna unguiculata*, moistened for half an hour, placed on two layers of Whatman No. 1 filter paper and exposed for 30 minutes to UV-B radiation. Unexposed seeds in Petri plates served as controls. Subsequently, the seeds were treated. with 5 ml of either 0 (control), 40 or 60 ppm gallic acid. Germination was counted daily and shoot and root length measured at 7 days. At 7^{th} day soluble phenols and PAL and TAL activities were ascertained.

Soluble Phenols

Treatments and cultural conditions of sunflower seedlings are described above. Soluble phenol phenol contents were ascertained in the root of *V. unguiculata* seedlings. Total soluble phenols were determined following the method of Gonzalez *et al*., (2003) with minor modifications. Root tissues (500 mg) were taken from seedlings in each Petri dish and homogenized in an ice bath with 2 ml cold 80% ethanol (v/v). The homogenate was centrifuged at 6000 g for 3 min. One hundred µl of the supernatant was added to 0.5 ml Folin-Ciocalteau reagent and 1 ml of 20 percent sodium carbonate. Finally, distilled water was added to make a final volume of 10 ml. The mixture was incubated at 40º C for 30 min and the absorbance of the developed blue color was read at 750 nm using a Shimadzu UV-1201 spectrophotometer. Gallic acid was used as standard. The amount of soluble phenols was expressed as µg mg^{-1} fresh weight.

Phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) activities

Fresh root tissue were homogenized with chilled 50 mmol Tris-HCl (pH 8.8, 1/10 w/v) supplemented with 0.5 mmol EDTA and 1% polyviny pyrrolidone. The homogenized suspension was obtained by centrifuging at 12000 rpm for 10 min at 4° C. The supernatant was used for the assay of PAL and TAL activity. PAL activity was measured as the rate of conversion of L- phenylalanine to trans-cinamic acid in accordance with Dickerson *et al.,* (1984). The enzyme extract 0.4 ml was incubated at 37° C in 0.5 ml of 0.1 M borate buffer (pH 8.8) to which was also added 0.5 ml of 12 mM L-phenylalanine. The reaction was terminated with 0.3 ml of 6N HCl and the absorbance was recorded at 290nm. The extraction and incubation procedures for tyrosine ammonia lyase (TAL) were the same as described above. TAL activity was measured using L-tyrosine as the substrate (Beaudoin-Eagan and Thorpe, 1985). The product, p-coumaric acid was measured spectrophotometrically recording absorbance at 333nm.

Statistical analysis

The data were subjected to appropriate statistical analysis which included the analysis of variance (ANOVA) and post-hoc tests namely, Duncan's multiple range test (DMRT) and Fisher's least significant (LSD) test following Zar (2009). Computer programs for all the statistical analyses were developed by the first author (S.S.S.) in C++ and are available on request at a nominal cost.

RESULTS

Effect of UV-B radiation alone on germination and seedling growth

The final germination percentage was significantly (P at the most 0.05) reduced at 20, 30, 40, 50 min exposures to UV-B radiation (Table 1).However, germination velocity (GV) increased substantially at 10 and 20 min exposure but it was markedly retarded at 30 and 40 min exposure.

Root growth was significantly retarded in all the treatments (10,20,30, and 40 min UV-B exposure periods) (P at the most 0.05) (Table 2). Shoot growth was suppressed at 20 min exposure period and onwards. Likewise, root and shoot fresh weights were also decreased significantly at 30 min or more exposure to UV-B radiation. At higher exposure periods curling, twisting and distortion of seedlings was also observed.

Effect of gallic acid (allelopathy) on germination and seedling growth

The final germination percentage was significantly increased ($p < 0.05$) over the controls at 10 ppm gallic acid (Table 3). There was no significant influence at 20 ppm . However, at 40, 60 and 80 ppm germination percentage was significantly ($P < 0.01$) suppressed by gallic acid compared to controls. Germination velocity was retarded at 20-80 ppm gallic acid (Table 3). Root length was significantly increased over the controls ($P<0.05$) at 10 ppm gallic acid. However, both root and shoot lengths were suppressed significantly (P at the most 0.05) at 20-80ppm gallic acid. In addition, both root fresh weight and shoot fresh weights declined compared to controls (P at the most 0.05) at higher (40-80 ppm) gallic acid.

Table 1. Final germination percentage and germination velocity (GV) of *Vigna unguiculata* wet seeds exposed to various durations (min) of UV-B radiation. Mean ± standard error.

Treatments UV-B exposure	**Final germination percentage**	**Germination velocity**
0 (control)	96.0 ± 1.2 a	25.26
10 min	92.5 ± 2.4 a	30.37
20 min	84.6 ± 2.8 b	28.42
30 min	72.5 ± 3.5 c	19.38
40 min	68.8 ± 2.7 c	17.62
50 min	70.4 ± 3.6 c	18.19

Means not sharing the same letter are significantly different at $P <0.05$

Table 2. Effect of UV-B radiation exposure for different duration (min) on root and shoot growth of *V. unguiculata*. Means ± standard error.

UV-B exposure (min)	**Root Length (cm)**	**Shoot Length (cm)**	**Root Fresh wt. (g)**	**Shoot Fresh wt. (g)**
0 (Cont.)	5.14 ± 0.33a	3.68 ± 0.43a	0.66 ±0.02a	0.32 ± 0.07a
10	5.26 ± 0.28a	3.82 ± 0.48a	0.67 ± 0.14a	0.35 ± 0.03a
20	4.36 ± 0.30b	2.50 ± 0.36b	0.52 ± 0.16a	0.37 ± 0.08a
30	3.28 ± 0.35c	2.24± 0.23bc	0.45 ± 0.09b	0.22 ± 0.2b
40	3.41 ± 0.28c	2.35 ± 0.28b	0.44 ± 0.06b	0.25 ± 0.06b
50	3.47 ± 0.36c	2.11 ± 0.25c	0.33 ±0.05c	0.26 ±0.08b

Means not sharing the same letter in a column are significantly different at $P<0.05$

Table 3. Effect of gallic acid (allelochemical) on final germination percentage and germination velocity of *Vigna unguiculata*. Mean ± standard error.

Treatments Gallic acid (ppm)	**Final germination percentage**	**Germination velocity**
0 (control)	94.0 ± 2.0 a	27.42
10	98.2 ± 1.6 b	32.28
20	90.5 ± 3.8 a	26.772
40	83.0 ± 3.2 c	21.62
60	76.8 ± 4.5cd	19.54
80	72.4 ± 3.2 d	19.65

Means not sharing the same letter are significantly different at $P <0.05$

Table 4. Effect of different concentrations of gallic acid on root and shoot growth of *Vigna unguculata*. Means ± standard error.

Gallic acid (ppm)	Root length (cm)	Shoot length (cm)	Root fresh wt. (g)	Shoot fresh wt. (g)
0 (Cont.)	5.27 ± 0.22a	3.45 ± 0.27a	0.67 ± 0.05 a	0.35 ± 0.03a
10	5.66 ± 0.18a	3.73 ± 0.18a	0.69 ± 0.09a	0.32 ± 0.08a
20	4.74 ± 0.33b	2.44 ± 0.20ab	0.48 ± 0.08b	0.35 ± 0.02a
40	4.54 ± 0.37b	3.01 ± 0.17c	0.47 ± 0.11b	0.27 ± 0.04b
60	3.92 ± 0.21c	2.85 ± 0.28d	0.41 ± 0.06bc	0.23 ± 0.05b
80	3.76 ± 0.26c	2.74 ± 0.27d	0.35 ± 0.04d	0.21 ±0.03c

Means not sharing the same letter in a column are significantly different at P <0.05

Table 5. Combined effect of UV-B 30-min exposure and gallic acid (allelochemical) on final germination percentage and germination velocity on *Vigna unguiculata.* Means ± standard error.

Treatments Gallic acid (ppm)	Final germination percentage	Germination velocity
0 (control)	86.4 ± 2.5 a	26.62
10	88.5 ± 4.3 a	25.48
20	78.4± 3.9 b	25.72
40	50.5 ± 4.3 c	17.34
60	52.4 ± 3.1 c	18.55
80	48.7 ± 3.0 c	15.25

Means not sharing the same letter are significantly different at P <0.05

Table 6. Combined effect of UV-B 30 min exposure and different concentrations of gallic acid (allelochemical) on root and shoot growth of *Vigna unguiculata*. Means ± standard error.

Gallic Acid (ppm)	Root length (cm)	Shoot length (cm)	Root wt. g (fresh)	Shoot wt. g (fresh)
0 (Cont)	4.45 ± 0.16a	2.86 ± 0.26a	0.45 ± 0.06 ab	0.27 ± 0.05a
10	4.40 ± 0.12a	2.77 ± 0.18a	0.49 ± 0.08a	0.28 ± 0.06a
20	4.23 ±0.27ab	2.49 ± 0.15b	0.41 ± 0.09ab	0.23 ± 0.07a
40	4.04 ± 0. 20b	2.34.± 0.19b	0.33 ± 0.04b	0.21 ± 0.03a
60	3.64 ± 0.18c	2.16 ± 0.22cd	0.30 ± 0.08bc	0.18 ± 0.08b
80	3.18 ± 0.15 d	1.89 ± 0.18d	0.31 ± 0.06bc	0.16 ± 0.05b

Means not sharing the same letter are significantly different at P <0.05

***Combined effect of UV-B and gallic acid (allelochemical) on germination and seedling growth*:**

UV-B (30 min exposure) in conjunction with gallic acid at 30-80 ppm significantly reduced the final germination percentage over the positive controls (30 min UV-B exposure only) as well as the germination velocity. The shoot and root growth of the seedlings was also inhibited by UV-B (30 min) in combination with 40-80 ppm gallic acid. The fresh and dry weights likewise, declined over the positive control (UV-B 30 min exposure) .

***Combined effect of UV-B and allelochemical on phenol contents, PAL and TAL activities of cowpea seedlings*:**

UV-B exposure together with gallic acid at 20ppm or more concentration significantly (P at the most 0.05) enhanced soluble phenol content over positive control (30 min. UV-B exposure + 0 ppm gallic acid). Likewise phenyl ammonia lyase (PAL) activity was significantly (P at the most 0.01) increased over the positive controls at UV-B exposured in conjunction with 20-80 ppm gallic acid. Moreover, tyrosine ammonia lyase activity was enhanced significantly (P at the most 0.05) at all the combinations of UV-B and gallic acid.

Table 7. Combined effect of UV-B 30 min. exposure and different concentrations of gallic acid (allelochemical) on phenolic contents, PAL and TAL activities of *Vigna unguiculata* seedlings. Mean ± SE.

Gallic acid (ppm)	Total soluble phenol (µg/g FW)	PAL activity (µmol/h/g FW)	TAL activity (µmol/h/g FW)
0 (no UV-B)	52.4 ± 42 a	0.9 ± 0.07a	0.5 ± 0.04a
0 (+UV-B)	71.6 ± 4.5 b	1.4 ± 0.10 b	0.8 ± 0.05 b
10	74.8 ± 4.2 b	1.6 ± 0.21b	1.2 ± 0.08 b
20	83.4 ± 5.6 c	1.8 ± 0.14 b	1.0 ± 0.13 b
40	82.2 ± 6.1 cd	2.4 ± 0.18 bc	1.7 ± 0.11 cd
60	88.0 ± 7.8 cd	2.2 ± 0.17 c	1.6 ± 0.14 cd
80	87.1 ± 6.3 cd	2.7 ± 0.23 bd	1.9 ± 0.10 d

Means not sharing the same letter in a column are significantly different at P <0.05; FW= fresh weight

DISCUSSION

The effect of supplemental UV-B radiation was o examined on germination and early seedling growth of cowpea (*Vigna unguiculata*). The results of the experiment clearly demonstrated the deleterious effects of UV-B radiation on the cowpea seedlings in terms of the resulting physical and chemical damage. UV-B radiation not only caused decrease in shoots and root growth but also resulted in the curling of roots and to some extent shoots. These results corroborate the findings of earlier studies of Barnes *et al.*, (1988, 1990) Greenberg *et al*., (1997) Furness *et al*., (1999), Shaukat and Shah (2011) and Shaukat *et al*. (2011) who reported marked changes in the morphological traits such as reduction in plant height, decreased leaf area, curling of leaves, etc. However, the response to UV-B radiation varies among species (Barnes *et al.,* 1990; Musil, 1995; Cybulski and Peterjohn, 1999) and even in different species of the same genus (Johanson *et al*., 1995). The differences among species, though not examined here, can be attributed to the mechanism whereby the plants reduce or tolerate the damage inflicted by UV-B radiation. The presence of leaf hairs (Karabourniotis *et al.*, 1992), a high content of UV-B screening compounds (Day, 1993; Lois and Buchanan, 1994; Day *et al*., 1999) or the production of thick leaves that reduce the penetration of UV-B radiation (Sullivan and Teramura, 1990; Sullivan *et al*., 1994). In addition, the effect on perennial plants is cumulative and in long term studies the magnitude of effect varies from year to year (Sullivan and Teramura, 1990; Johanson *et al*., 1995). In a comparative study (Furness *et al*., 1999) of the effect of UV-B radiation on three weeds (*Cynoglossum officinale, Centauria diffusa* and *Tragopogon pratensis*), the UV-B radiation decreased the growth and leaf area in all three weeds while most susceptible was *Cynoglossum officinale*. The results of the current experiment show that the level of UV-B radiation used has measurable suppressive effects on root and shoot growth of cowpea seedlings. The dry weights of shoots were reduced significantly by the UV-B radiation which was presumably due to inhibition of photosynthesis and disruption of photosynthetic pigments. A similar response to these radiations has been reported previously for other species (Rozema *et al*., 1997; Deckmyn and Impens, 1999; Gonzalez *et al.*, 1996; Gonzalez *et al*., 1998; Shaukat and Shah, 2011).

Treatment of seeds with gallic acid resulted in marked suppression of final seed germination percentage, germination velocity as well as seedling growth at 40-80 ppm concentrations. Gallic acid is a phenolic (allelopathic) compound which is widely distributed in plants in a free state (Ishikura *et al*., 1984; Sasikumar *et al*., 2001; Li *et al*., 2010; Bichra *et al*., 2012; Gawron-Gzella, 2012) and is known to cause inhibition of germination and seedling growth (Reigosa *et al*, 1999; Sasikumar *et al*., 2001).

The combined stress of UV-B and allelopathic compound (gallic acid) caused a greater reduction in final germination percentage as well as greater suppression of root and shoot growth. The inhibition of growth could be the result of cell elongation as has been demonstrated for some other phenolics (Dos Santos *et al*., 2008) and ultraviolet radiation (Kumari *et al*., 2000).

Exposure of cowpea seedlings to UV-B radiation resulted in accumulation of soluble phenols. Accumulation of phenols as a result of exposure of plants to UV-B radiation has also been reported by Ambasht and Agarwal (1998), Kozlowska *et al*., (2007) and Ravindran *et al*., (2008) which provides a protection against UV-B radiation. It has been established that phenol metabolism is activated in plants as a reaction to abiotic stress (Abreu and Mazzafera, 2005; Olenchenko and Zagoskina, 2005; Ganeva and Zozikova, 2007). Shaukat *et al*., (1999, 2010) demonstrated that the exposure of plants to heavy metals such as Cd, Cr, Pb and Zn results in the accumulation of soluble phenols. Plant phenolics have been regarded as defences against pathogens and herbivores (Dixon and Paiva, 1995; Shaukat *et al*., 2009) and provide protective mechanism against a variety of abiotic stresses including stress due to heavy metals. Our results provide additional support to this conjecture. Simultaneous application of two stresses, i.e., UV-B and allelopathy (gallic acid) resulted in a drastic increase in total soluble phenols. Secondary metabolic pathway

is physiologically important as it provides the means of channeling and storing carbon compounds, accumulated from photosynthesis during periods when nitrogen is limiting and whenever leaf growth or cotyledons are suppressed. In this connection it is noteworthy that the cotyledons and first leaf growth was suppressed by the UV-B radiation alone and in conjunction with gallic acid.. The protective role of phenolics may be due to structural stabilization of cell wall through condensation-polymerization of phenols and quinines. Secondly, they can provide photoprotective mechanism *i.e*., by altering the absorbance of visible and UV-radiation. Thirdly, they act as powerful antioxidant and antiradical agents (Harborne, 2007; Edreva *et al*., 2008).

The response of single stress (UV-B), including PAL and TAL activation, was enhanced by simultaneous application of two stresses namely UV-B and allelochemical (gallic acid). Phenyl ammonia lyase (PAL) and tyrosine ammonia lyase TAL) , are key enzymes of the phenylpropanoid pathway and could be involved in the protection mechanism against UV- radiation stress as flavonoids are known to be important UV-screening pigments (Lavola *et al*., 2008; Kumari *et al*., 2000). Some reports exists which suggest that derivatives of cinnamic acid inhibit the PAL activity (MacDonald and D'Cunh, 2007). On the other hand, enhanced PAL activity due to exogenous application of phenolic acid has been demonstrated (Politycka and Mielcarz, 2006). An increase in PAL or TAL activity is symptomatic of plant tissue subjected to some kind of stress (heavy metals, disease wounding, heat shock, UV-B radiation, etc) (Jiang and Joyce, 2003; Chmielowski *et al.*, 2008; Chakraborty and Som, 2010). However, TAL activity remains at a lower level than PAL activity but both gradually increase in response to stress (Khan *et al*., 2003). The results of the present study corroborate the findings of Jozwiak-Zurek *et al*., (2011). Who demonstrated enhanced PAL activity by combined stress of UV-B and allelopathy (ferulic acid)..

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