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COMBINED EFFECT OF ALLEOPATHY AND UV-B RADIATION ON *LENS CULINARIS* MEDIK

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Abstract

The effect of UV-B radiation and alleopathic chemicals of *Prosopis juliflora* were studied on *Lens culinaris* individually and in combination. The UV-B radiation suppressed the germination percentage and germination velocity at 10, 20 and 30 minutes exposures. Similarly the allelochemicals of *Prosopis juliflora* at different concentration (25%, 50%, 75% and 100%) also inhibit the final germination percentage and germination velocity. The final germination percentage and germination velocity were shown more reduction when both these stresses that is *P. juliflora* extract and UV-B radiations for 30 minutes applied simultaneously. Both growth of shoot and root and fresh and dry weights were also reduced in all treatments, and the reduction increases with the increase in time of exposure and concentration of extract. It is obvious that the combined effect of UV-B radiation and allelopathy influence more *Lens culinaris* as compared to individual effect.

Introduction

Ultraviolet radiation (UV radiation) is a part of sun's electromagnetic rays reaching to earth. The invisible light (UV-B radiation) can greatly cause biological damage on earth crust. (Blaustein *et al.,* 2003). A number of scientists studied the UV-B radiation and its different responses (Mackenerness, 2000; Jansen 1998, 2002; Ravindran *et al.,* 2008). The UV-radiation is being increased which is harmful for all organism like humans, animals, plants and microorganisms present on earth (Madronich *et al.,* 1998). Exposure of high level UV-radiation can cause the damage of DNA (Landry *et al.,* 1997) some workers stated that the UV-B radiation damage the cell membrane, chloroplast, thylakoid and pigment of plants (Day and vogelman, 1995; Greenberg *et al* 1997)

Molish (1937), first time used the term "allelopathy" as an interaction among the plants and the microorganisms. Alleopathy is the direct or indirect effect by one plant, including micro-organisms, on another through the production of chemical compounds that different into the environment and subsequently influence the growth and development of neighboring plant (Rice,1974). Although some stated that allelopathy is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth (Harper, 1977; May & Ash, 1990). Toxic compound emitted from allelopathic plant not only interfere with the normal growth of associated crop but also influence on associated microorganism especially those that are known to cause root disease (Narwal 1994; Shaukat and Siddiqui., 2001). Some scientist stated that allelopathy is the direct or indirect effect of plants on other plants through the release of chemicals (Kohli *et al*; 1998 and Singh *et al;* 2001).

Most of the workers have focused on single stress condition on the test plant. In nature environment due to damage of stratospheric ozone layer and global warming climatic changes are created which affect the environmental plants directly or indirectly. Shaukat and Shah (2011) focused on the effect of UV-B radiation on *vigna radiata* as a test plants. The present study was conduct to investigate the allelopathic chemical of *Prosopis juliflora* and supplement UV-B radiation effect alone and in combination on lentil (*Lens culinaris*) for the first time in Pakistan.

Materials and Methods

The seeds of lentil (*Lens culinaris* Medik) were collected from Federal Seed certification department Malir Halt Karachi. The seeds were first surface sterilize with 0.5 % Sodium hypochlorite for 2 minutes and then soaked in distilled water for 2 hours.

For the study three different series of experiments were performed.

I. **Treatment with UV-B Radiation:** Two hours imbibed seeds of lentils were exposed to UV-B light in UV-B chamber (containing UV-B fluorescent tube TL40W/12, Philips) for 10 (T1), 20 (T2) and 30 (T3) minutes. The chamber was made up of wood for safety reason. Some seeds were not exposed to UV-B radiation to serve as control (C). Ten seeds in each case were placed in petri plates containing two discs of Whatman filter paper number 1. Three replicates were made in each case. Five ml distilled water was added to each petri plate. The Petri plates were kept at room temperature (28 C˚) and 50 % humidity for ten days on a laboratory bench where light was provided for 14 hours. Germination of seeds and length of root and shoot were recorded daily up to 10 days. At the end fresh and dry weight of the seedlings were recorded.

II. Treatment with Allelochemicals: To examine the alleopathic effect *Prosopis juliflora* shoot sample of plant was collected. The plant was kept in green house for 2-3 week to completely dry. After that a fine powder was made by grinding the dried plant material in villimill. The plant extract (stock) was prepared by adding 10 gm of powder in 100 ml of distilled water. From this stock 25% (T1), 50% (T2), 75% (T3) and 100% (T4) extract were made. In case of control (C) distilled water was added Ten seeds of lentil were placed in Petri plate. Three replicates were made for each extract and 5 ml of extract was applied to Petri plates containing double discs of Whatman filter paper number 1, however in control distilled water was added. Similarly the germination of lentil seeds was observed up to 10 days along with its root and shoots length and at the end fresh and dry weights were recorded.

III. Treatment with UV-B radiation and allelochemicals: The surface sterilize seeds were placed in Petri plates, exposed to UV-B radiations for 30 minutes under UV-B chamber and then these petriplates were moistened with *Prosopis julifora* extract (25% (T1), 50% (T2), 75% (T3) and 100% (T4)) however in control (C) Petri plates distilled water was added. Three replicates were taken in each case. The observations were taken for ten days, the germination rate and root and shoot length was observed daily and at the end the fresh and dry weight of whole plants were measured.

Germination velocity (GV) of all three experiments was recorded by using the index proposed by Khandakar and Bradbeer (1983), as follow:

$$GV= [N1/1+N2/2+N3/3+\ldots\ldots.+Nn/n]\times 100/1$$

Where N1, N2, N3,….Nn are the proportion of seeds that germinated on day 1, 2, 3…… respectively.
Seedling mean and standard error of roots shoot length, fresh and dry weight was calculated with the help of computer program Germ- Sp and VARMEAN (developed in C++ by Prof. Dr. S.S. Shaukat (Department of Environmental Sciences, University of Karachi).

Results

The UV-B radiation and allelopathic effects were studied individually and in combination for the final germination percentage, germination velocity, root and shoot length and fresh and dry weight.

I. Effect of UV-B radiation on germination, seedling growth, fresh and dry weight of *Lens culinaris:* The results are summarized in Table 1. The final germination percentage was significantly (p at the most 0.05) reduced with the increase in exposure time of UV-B radiation. The control specimens showed highest germination speed and minimum germination speed was observed in T3 specimens. The same effect was observed on the germination velocity at 10, 20 and 30 minutes UV-B exposures. The root and shoot length were also retarded as compared to control. The fresh and dry weight also decline due to UV-B radiation. At higher exposure of UV-B curling, Twisting and drying of seedling were recorded whereas the control plants did not show such effects.

II. Effect of *Prosopis juliflora* (allelochemicals) on germination, seedling growth, fresh and dry weight of seedlings: The results are summarized in Table 2. The final germination percentage and velocity showed a marked decline with the increase in percentage of extract of Prosopis juliflora and highest decrease was observed at 100 % aqueous extract. Similarly the root and shoot growth also showed a decline with the increase in concentration of extract. The fresh weight of the treatment seedlings decline significantly as compared to control.

III. Combined effect of UV-B radiation and allelochemicals on germination, seedling growth and fresh and dry weight of seedlings: The results are summarized in Table 3. The speed of germination reduced in all the treatments as compared to control. The root and shoot length and fresh and dry weights also decrease with the increase in concentrations. The decline observed is much more as compared to single factor effect.

Table 1. Germination percentage, germination velocity (Gv), Root length, shoot length, fresh weight and dry weight of Control and treatments (UV-B exposed seeds) of lentil seedlings.

S. No.	Treatment (UV-B radiation)	Final germination percentage	Germination velocity	Root length (cm) mean±S.E	Shoot length (cm) mean±S.E	Fresh wt (whole plant) mean±S.E	Dry wt (whole plant) mean±S.E
1	0 min (control)	100±0.0	98.33	3.21±0.13	2.73±0.16	0.4±0.06	0.09±0.01
2	10 min (T1)	98.1±1.9	97.22	2.27±0.11	1.82±0.25	0.3±0.07	0.08±0.02
3	20 min (T2)	96.66±3.3	91.95	1.84±0.08	1.75±0.08	0.21±0.04	0.06±0.03
4	30 min (T3)	94.61±5.3	84.44	1.17±0.09	1.03±0.13	0.11±0.02	0.05±0.04

Table 2. Germination percentages, germination velocity (Gv), Root length, shoot length, fresh weight and dry weight of Control and treatments (Treated with allelopathic extract) of lentil seedlings.

S. No.	Treatment (shoot extract)	Final germination percentage	Germination velocity	Root length (cm) mean±S.E	Shoot length (cm) mean±S.E	Fresh wt (whole plant) mean±S.E	Dry wt (whole plant) mean±S.E
1	0 %(control)	100±0.0	98.33	3.21±0.13	2.73±0.16	0.4±0.06	0.09±0.01
2	25% (T1)	95.2±2.9	80.50	3.2±0.16	2.5±0.25	0.3±0.05	0.09±0.02
3	50% (T2)	88.3±1.7	78.75	3.06±0.14	2.4±0.1	0.21±0.04	0.07±0.03
4	75% (T3)	75.1±2.9	75.18	2.91±0.09	2.0±0.09	0.11±0.02	0.05±0.04
5	100% (T4)	65.2±2.8	62.51	2.47±0.04	1.73±0.1	0.09±0.02	0.04±0.01

Table 3. Combined effect of aqueous extract of *Prosopis juliflora* and UV-B radiation (30 mints) on germination percentage, germination velocity (Gv), root length, shoot length, fresh weight and dry weight of *Lens culinaris* seedlings.

S. No.	Treatment (UV-B exposure + shoot extract)	Final germination percentage	Germination velocity	Root length (cm), mean±S.E	Shoot length (cm) mean±S.E	Fresh wt (whole plant) mean±S.E	Dry wt (whole plant) mean±S.E
1	0 min, 0 %(control)	100±0.0	98.33	3.21±0.13	2.73±0.16	0.4±0.06	0.09±0.01
2	30 min, 25% (T1)	94.5±2.8	69.38	2.72±0.28	2.3±0.14	0.18±0.02	0.04±0.005
3	30 min, 50% (T2)	62.21±3.3	54.44	2.27±0.26	1.7±0.32	0.15±0.01	0.04±0.006
4	30 min, 75% (T3)	42.22±5.3	31.67	1.91±0.09	1.12±0.14	0.13±0.01	0.03±0.004
5	30 min, 100% (T4)	33.33±7.7	30.44	1.07±0.06	0.41±0.16	0.11±0.02	0.03±0.001

Discussion

Present study is designed to find out how two different stresses (UV-B radiation and allelopathy) affect either collectively or individually on *Lens culinaris* (lentil) seedlings. Individually both these stresses affected negatively i.e. they caused a decrease in germination speed and velocity, retarded root and shoot growth and a decline in fresh and dry weights of the seedlings. These affects showed a gradual increase with the increase in time of UV-B radiations exposure and with more concentrated allelopathic extract. These stresses not only affect the seedling growth but also resulted in the curling, twisting and distortion of the seedlings. The same result was observed by previous worker on different crops (Barnes *et al.*, 1988, 1990) however some reported change in morphology of test plants (Shaukat and Shah., 2011; Shaukat and Siddiqui., 2011, and Greenberg *et al.*,1997). The UV-B radiation response is different on different plants (Barnes., 1990; Musil 1995 and Cybulski and Peterjohn, 1999). It is also observed by previous worker that different species of the same genus also showed different response to UV-B radiation (Johanson *et al.,* 1995). The same effect of *Prosopis juliflora* were observed by some other worker on different crops (Gatechew *et al*., 2012, Al-Humaid and Warrag., 1998; Noor *et al*., 1995; Khan *et al*., 2005; Khan and Shaukat; 2006)

When both these stresses were applied simultaneously, they showed relatively more severe effects and these effects were higher than individual stress effect. The combined stress of UV-B and different concentration (25%, 50%, 75% and 100%) aqueous extracts of *Prosopis juliflora* showed a greater reduction in final germination percentage, germination velocity, root and shoot length and fresh and dry weight as compared to control of lentils crop. The combined effect of UV-B radiation were also observed by early worker (Shaukat *et al.,* 2013) and the same result were observed; however combined effect of UV-B radiation and aqueous extracts of *Prosopis juliflora* were not studied on *Lens Culinaris* Medik.

In conclusions we can say that the combined stress affected more seriously as compared to a single stress under natural condition crops are usually facing multiple stresses including the stress of different weeds and UV-B radiations from sun light. So we can say that these stresses affect the productivity of crops to a greater extent in conjunction with each other.

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