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INDIVIDUAL AND COMBINED EFFECT OF UV-B RADIATION AND HEAVY METALS (LEAD AND CADMIUM) ON *CUCUMIS SATIVUS* L. (CUCUMBER)

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ABSTRACT

The present study deals with the individual and combined effect of two stresses *i.e.* UV-B radiation and heavy metals (lead and cadmium) on germination and growth of test crop *Cucumis sativus* L. (cucumber). The experiments were performed in three different ways *i.e* i) individual effect of UV-B radiation, ii) individual effect of heavy metals (lead and cadmium) and iii) combined effect of UV-B radiation and heavy metals. The results showed that a gradual decline in germination and growth occurred with the increase in concentration of heavy metals and enhancement in the time of exposure of UV-B radiation. Individually, the heavy metals affected more as compared to UV-B radiation. Cadmium affected more adversely as compared to lead on crop. In the combined treatment (heavy metals and UV-B radiation) more negative effects observed in comparison to individual treatment whereas, cadmium and UV-B radiation affect more severely as compare to lead and UV-B radiation treatment. Statistically the results were also significant.

Keywords: Seed germination, speed of germination, UV-B radiation, lead chloride, cadmium chloride.

INTRODUCTION

Heavy metals are those metals which are referred as pollutant when occur in larger quantities than their permissible limits and an increase of heavy metals cause environmental pollution. These heavy metals are released in environment by the anthropogenic activities. The heavy metals have great interest for research with respect to toxicological importance to human health, plants and animals (Almeida *et al*., 2007; Jarup 2003). The heavy metals toxicity was known for the inhibition of germination and retardation of many physiological aspects in plants (Morzeck and Funicelli, 1982; Pahlsom, 1989, Khan, 2007). High concentration of lead and cadmium has negative impact on plants germination and growth (Adriano, 1986; Gough *et al.*, 1979). The accumulation of cadmium found to suppress the growth of roots in soya bean plant (Cataldo *et al*., 1983) and caused significant reduction in yield of spinach, pea, carrot, radish and turnip (Singh and Nayar, 1990). Individual and combined effect of lead, cadmium and copper decrease the biomass of *Sesamum indicum* seedlings (Singh *et al.*, 1994). Certain heavy metals effect on the seed germination of Alfa alfa (Aydinalp and Marinova, 2009). The seedling of *Albizia lebbek* showed lowest tolerance against the treatment of lead and cadmium (Farooqi *et al*., 2009). The seed germination and seedlings growth of *Sinapis arvensis* adversely influenced by lead and cadmium (Heidari and Sarani, 2011). The heavy metals caused reduction in the germination of *Arachis hypogea* seeds (Abraham *et al*., 2013).

The UV-B radiation refers to that specific portion of sun energy which is able to damage biological tissues. Many researchers studied the effect of UV-B radiation and its different responses on different living beings including plants (Mackenerness, 2000; Jansen 2002 and Ravindran *et al*., 2008). UV-B radiation is a minor part of solar spectrum but affect the plant morphology, photosynthesis, flowering and pollination (Jordan, 1996; Jansen *et al*., 1998). High level of UV-B radiation causes the reduction in seed germination of *Glycine max* (Sazada *et al*., 2007). UV-B radiations exposure significantly reduces leaf area and biomass of rice plant (Dai *et al*., 1995). Germination, root and shoot growth significantly suppressed by UV-B irradiance in mash bean plants (Shaukat *et al*., 2013). Root and shoot growth and photosynthesis also suppressed in mash bean (Feguerоa *et al*., 2009). UV-B radiations effect on plant growth such as oat and turnip species (Flint and Caldwell, 2003). The growth significantly reduced in *Colobenthus quitensis* and *Daechanpia antarctica* (Day *et al*., 1999). In cucumber, tomato, radish bean and millet, the exposure of UV-B radiations decreased the growth of seedling (Krizek *et al.*, 1975).

The heavy metals and UV-B radiations both causes determinant effects on plants. They both exist in nature causing great damage to plants. Individually both these environmental stresses have been studied by many scientists, but in combination, not a single study is available in literature. Due to this reason, present study is designed to study how these two stresses collectively affect plants.

MATERIALS AND METHODS

The effect of UV-B radiation and two of the heavy metals (lead and cadmium) were studied on seeds germination and seedlings growth of cucumber (*Cucumis sativus* L.). The seeds of *Cucumis sativus* were obtained from National Seed Certification Department Malir Halt Karachi. The seeds were sterilized with 0. 5 % mercuric chloride for 1 minutes then washed with distilled water for many times and then soaked for 10 minutes in distilled water. For the investigation three different series of experiments were performed. For the comparison control was also established with no heavy metal and UV-B radiation.

I). Seeds of cucumber (*Cucumis sativus)* were exposed to UV-B light for different time interval (20 minutes, T1; 40 minutes, T2 and 60 minutes, T3) in UV-B wooden chamber (containing UV-B fluorescent tube TL40W/12, Philips). Some seeds remained unexposed to serve as control (C). Ten seeds were placed for each treatment and control in petri plates containing double layers of Whatman filter paper No. 1. The petri plates were moistened with 2ml of distilled water. These Petri plates were exposed in alternate days to UV-B radiation for 20, 40 and 60 minutes and labelled as T1, T2 and T3 respectively. The germination of seeds was recorded daily up to 5 days and length of roots and shoots were recorded after 15 days and fresh and dry weight of the seedlings was also recorded.

II).Both heavy metals solutions were prepared in different concentrations i.e. 2mg/L, 4mg/L and 6mg/L. These solutions were prepared by adding 2mg in one litre of distilled water for 2ml/litre, similarly remaining concentrations were prepared in the same way, and then sterilized seeds were placed in petri plates. The petri plates were labelled as T4, T5 and T6 for 2mg/L, 4mg/L and 6mg/L respectively for lead and T7, T8 and T9 2mg/L, 4mg/L and 6mg/L respectively for cadmium. The petri plates were moistened with 3mL of their respective aqueous heavy metal concentrations. Three replicates were made for each concentration. Germination of seeds were recorded daily up to 5 days and then length of root and shoot were recorded after 15 days. Fresh and dry weight of the seedlings was also recorded.

III). Sterilized seeds of *Cucumis sativus* placed in petri plates, petri plates were moistened with the concentration of heavy metals. The 2mg/L concentration containing Petri plates of lead and cadmium were exposed with 20min, 40min, and 60mins of UV-B radiation, similarly 4 and 6mg/L concentrations of lead and cadmium were also exposed with 20mins, 40mins, and 60mins in UV-B radiation. The petri plates were labelled with their respective exposed time and concentrations of heavy metals. The germination were recorded daily up to 5 days, root and shoot length were recorded after 15 days. Fresh and dry weights of seedlings were also recorded.

The germination speed was calculated according to following formula (Khandakar and Bradbeer 1983),

‘S’= [N1/1+N2/2+N3/3+……. +Nn/n] ×100.

Where, N1, N2, N3… Nn stand for the seed germinated at day 1, 2, 3…… respectively.

Post hoc test was employed to separate mean by using, DMRT at 5% confidence level (Zar, 1999).

RESULTS

i) Effect of UV-B radiation on germination and growth of *Cucumis sativus* seedlings:

The results are presented in Table 1. The reduction in germination percentage was observed with the enhancement of exposure time of UV-B radiation. Highest germination percentage was recorded in control, while it decreases with increase of exposure of UV-B radiation. The minimum germination percentage was observed in T3 (60 minutes exposure), while the speed of germination also reduced as compared to control. The growth of root and shoot also decrease gradually at maximum exposure and showed highly significant results ($p<0.05$). Root growth showed more sensitivity to UV-B radiation as compare to shoot growth, while the fresh weight and dry weight also decline in T1, T2 and T3 as compared to control.

ii) Effect of Heavy metals (Cadmium & Lead) on germination and growth of *Cucumis sativus* seedlings:

The results are summarized in Table 2. The final germination percentage gradually reduced at 2, 4 & 6mg/L of Lead and Cadmium as compared to control. On the other hand speed of germination increase at 2mg/L and 4mg/L of Lead and then decrease in 6mg/L of lead and also decrease in all concentration of Cadmium. The root length showed a decrease with the increase in the concentration of Lead and Cadmium. The shoot length was found to be more sensitive with the increase in concentration of Cadmium and the decline is significant ($p<0.05$). Fresh weight and dry weight decreased gradually with increase of concentration of heavy metals hence germination and growth decline more by cadmium then lead.

iii) Combined effect of UV-B radiation and Heavy metals (Cadmium & Lead) on germination and growth of *Cucumis sativus* seedlings:

The results are summarized in Table 3 and 4. The final germination percentage and the speed of germination reduced with the gradual increase of UV-B radiation in combination of heavy metals. The speed and final

germination percentage more reduced by 6mg/L in combination of 60 minutes exposure. On the other hand root growth was found to be more sensitive by combine action of lead concentration and UV-B radiation, while shoot growth decline more by combine action of cadmium concentration and UV-B radiation that is highly significant *i.e.* ($p<0.05$). The fresh and dry weight also decreases in both combined factors as compared to control and also compared with individual treatments.

Table 1. Effect of UV-B radiation on germination and growth of *Cucumis sativus* seedlings.

Treatment (UV-B radiation)	Final germination percentage %	Speed of germination %	Root length (cm)	Shoot length (cm)	Fresh wt. (whole plant) (g)	Dry wt. (whole plant) (g)
Control	100±0.00a	100±0.00a	3.03±0.75a	3.17±0.98a	0.332±0.094a	0.097±0.013a
T1	100±0.00a	98.32±0.08a	2.62±0.46b	2.72±1.11b	0.313±0.33a	0.023±0.003b
T2	98.42±0.05a	95.01±0.3b	2.41±1.37b	2.26±0.70c	0.218±0.030b	0.037±0.002a
T3	96.10±0.1b	90.3±0.02b	2.2±0.49b	2.41±0.43c	0.167±0.026c	0.026±0.008b

Key: T1=20 minutes, T2= 40 minutes, T3=60minutes exposure time of UV-B radiation. Means not followed by the same letter are significantly different from each other at $p\leq0.05$. Each mean is of three replicates.

Table 2. Effect of heavy metal on germination and growth of *Cucumis sativus* seedlings.

Treatment	Final germination percentage %	Speed of germination %	Root length (cm)	Shoot length (cm)	Fresh wt (whole plant). (g)	Dry wt. (whole plant) (g)
Control	100±0.00a	100±0.00a	3.03±0.75a	3.17±0.98a	0.332±0.094a	0.097±0.013a
T4	84.3±0.09b	100±0.00a	1.40±0.11b	1.55±0.35c	0.3±0.05a	0.04±0.02b
T5	56.6±0.12d	94.35±0.08b	1.31±0.40b	1.19±0.20c	0.22±0.004b	0.0214±0.003c
T6	53.33±0.01d	73.60±0.02c	0.91±0.23c	1.1±0.33c	0.11±0.02c	0.03±0.04bc
T7	56.66±0.2d	79.11±0.1c	0.9±0.17c	0.97±0.35d	0.167±0.029c	0.011±0.002c
T8	70±0.32c	70±0.1c	0.69±0.07c	0.65±0.16d	0.21±0.04b	0.03±0.03b
T9	51.10±0.04d	62.31±0.09d	0.58±0.16c	0.52±0.19d	0.09±0.02c	0.04±0.01c

Key: T4=2mg/L Pb, T5=4mg/L Pb, T6=6mg/L Pb, T7=2mg/L Cd, T8=4mg/L Cd, T9=6mg/L Cd. Means not followed by the same letter are significantly different from each other at $p\leq0.05$. Each mean is of three replicates.

DISCUSSION

In the present study, the combined and individual effect of UV-B radiation and heavy metals were examined on the seeds of *Cucumis sativus*. UV-B exposure reduced the final germination percentage and the speed of germination on the seeds of cucumber. Our results are found to be correlating with many workers like exposure to UV-B decreased plant height, leaf area and plant dry weight (Greenberg *et al.,* 1997). Root and shoot growth of *Vigna mungo* seedlings and decreased both chlorophylls a and b and the total amount of chlorophyll (Shaukat *et al.,* 2013), similarly in *Lens culinaris* (Khan *et al.,* 2014). Many workers observed the effect of UV-B radiation on number of physiological changes in plants (Jansen, 2002; Warren *et al.,* 2003; Ranjberfordoie *et al.,* 2009 and Ravindran *et al.,* 2008). In concern of growth and biomass of cucumber, our results similar with previous finding like the growth of seedling and biomass reduced with increase of exposure time. Other workers also observed the reduction in growth and biomass in different plants due to exposure of UV-B radiation like plant height in pea plant (Alexieva *et al.,* 2001), reduction of length in seedling of cowpea and shoot height of *Suaeda maritime* (Prem kumar and

Kulandaivelu 1996 and Ravindran *et al*., 2001), and a decrease in plant height in two varieties of soya bean plant (Feng *et al*., 2001). Similar results were found in *Senecio jacobaea* (Tosserams *et al*., 1997) and in *Bromus tectorum* (Dai and Upadhyaya, 2002).

Table 3. Combined effect of Lead concentrations and UV-B radiation on germination and growth of *Cucumis sativus* seedlings.

Treatments	Final germination %	Speed of Germination %	Root length (cm)	Shoot length (cm)	Fresh wt. (whole plant) (g)	Dry wt. (whole plant) (g)
Control	100±0.00a	100±0.00a	3.03±0.75a	3.17±0.98a	0.332±0.094a	0.097±0.013a
T10	70±0.01c	82.6±0.01b	0.62±0.15c	0.63±0.24c	0.294±0.06a	0.09±0.01b
T11	63.3±0.3c	98.2±0.22a	0.39±0.16c	0.55±0.31c	0.25±0.07b	0.08±0.02b
T12	46.6±0.01d	69.32±0.1c	0.28±0.10d	0.49±0.25c	0.21±0.04b	0.06±0.03c
T13	83.33±0.23b	92.1±0.9a	1.10±0.31b	1.12±0.40b	0.30±0.43a	0.05±0.04c
T14	60±0.09c	87.18±0.06b	0.88±0.15c	0.81±0.05c	0.222±0.008b	0.018±0.004d
T15	52.13±0.11c	67.40±0.04c	0.65±0.13c	0.68±0.07c	0.136±0.03c	0.06±0.04b
T16	50±0.06c	70±0.01c	1.25±0.17b	2.2±0.45b	0.123±0.05c	0.05±0.04b
T17	56.6±0.17c	30.44±0.1d	0.80±0.14c	0.85±0.06c	0.116±0.09c	0.020±0.0029d
T18	36.6±0.09d	28.44±0.3d	0.53±0.21c	0.61±0.18c	0.066±0.005d	0.011±0.002d

Key: T10=20 min, 2mg/L Pb, T11=20 min, 4mg/L Pb, T12=20 min, 6mg/L Pb, T13=40 min, 2mg/L Pb, T14=40 min, 2mg/L Pb, T15=40 min, 6mg/L Pb, T16=60 min, 2mg/L Pb, T17=60 min, 2mg/L Pb, T18=60 min, 6mg/L Pb. Means not followed by the same letter are significantly different from each other at $p \leq 0.05$. Each mean is of three replicates.

Table 4. Combined effect of Cadmium concentrations and UV-B radiation on germination and growth of *Cucumis sativus* seedlings.

Treatment	Final germination %	Speed of Germination %	Root length (cm),	Shoot length (cm)	Fresh wt (whole plant) (g)	Dry wt (whole plant) (g)
Control	100±0.00a	100±0.00a	3.03±0.75a	3.17±0.98a	0.332±0.094a	0.047±0.013a
T19	76.6±0.24c	68.18±0.05c	1.23±0.24b	0.53±0.25c	0.18±0.02b	0.04±0.005a
T20	90±0.09a	52.24±0.11d	0.87±0.02c	1.55±0.13b	0.15±0.01b	0.04±0.006a
T21	90±0.11a	69.32±0.01c	0.54±0.31c	0.80±0.02c	0.125±0.015b	0.0091±0.004d
T22	40±0.10d	57.96±0.33d	0.31±0.11c	0.29±0.02c	0.281±0.06a	0.03±0.01b
T23	26.6±0.05e	46.12±0.01d	0.37±0.14c	0.46±0.18c	0.143±0.03b	0.026±0.008b
T24	43.3±0.02d	78.42±0.12c	0.47±0.18c	0.75±0.42c	0.11±0.02b	0.03±0.001b
T25	66.6±0.13c	54.44±0.2d	0.61±0.13c	0.29±0.14c	0.141±0.01b	0.018±0.005c
T26	46.6±0.05d	50.21±0.09d	0.55±0.07c	0.69±0.18c	0.13±0.01b	0.03±0.004b
T27	33.3±0.14e	32.15±0.03e	0.66±0.21c	0.2±0.10c	0.075±0.026c	0.006±0.0012d

Key: T19=20 min, 2mg/L Cd, T20=20 min, 4mg/L Cd, T21=20 min, 6mg/L Cd, T22=40 min, 2mg/L Cd, T23=40 min, 2mg/L Cd, T24=40 min, 6mg/L Cd, T25=60 min, 2mg/L Cd, T26=60 min, 2mg/L Cd, T27=60 min, 6mg/L Cd. Means not followed by the same letter are significantly different from each other at $p \leq 0.05$. Each mean is of three replicates.

The results of present study revealed that cadmium and lead adversely influenced the seed germination, when the concentration increases the growth stunted. Kalimuthu and Sivasubramanian in 1990 also observed similar

results like, seed germination in corn reduced due to the different concentrations of lead acetate. The plant growth of *Dalbergia sisso* showed gradual decline in plant growth with the increase of Cadmium level (Iqbal and Mehmood, 1991). The abraded seeds of *Prosopis juliflora* showed no detrimental effect on their germination up to 400 ppm Cadmium *in vitro*, although germination velocity declined. In 50-day old seedlings raised in pot under Cd influence, the seedling growth was found to be differentially organ-specific. Fifty *per cent* reduction in radicle, epicotyl, shoot (hypocotyl + epicotyl), number of leaves, number of leaflets per seedling, and seedling biomass associated with Cd concentration (in soil) as 129.5, 136, 249.8, 169.2, 104.9 and 180 mg /kg, respectively. The primary leaf developed with delay (Khan, 2007). A reduction in the germination of wheat seeds with the increase of concentrations of lead (Hasnain *et al*., 1995). The seed germination of *Pisum sativum* was effected by cadmium (Chung and Sawhney 1996), and in *Sinapis arvensis* (Heideri and Sarani 2011). A low tolerance of *Cassia siamea* seedling were observed against 100ppm of lead and cadmium (Shafiq and Iqbal 2005). Enhanced concentration of heavy metals inhibited germination and early growth of barley, rice and wheat seedling (Mehmood *et al*., 2007; Ahmad *et al*., 2012). Similarly, other scientists also described that root shoot growth and seedling growth of different plants were sensitive to lead and cadmium (An, 2004, Correa *et al*., 2006 and Ahmed *et al*., 2008).

When lead concentration applied with UV-radiation on the seeds and seedlings of cucumber, the seed germination observed at 20 minute exposure with different concentration of lead, the germination decrease at 2mg/L, then increase at 4mg/L and again decrease at 6mg/L. at 40 minutes exposure the germination percentage increase at 2mg/L and 4mg/L but remain unchanged at 6mg/L, the most pronounced effect were observed at 60 minutes exposure in which all three concentration gradually decline the germination. The speed of germination decrease with the increase of exposure time and increase of concentrations of lead and this reduction in speed is more as compared to individual effect. Similar results were observed in root length, shoot length, fresh weight and dry weight which showed highly significant decline as compared to control.

The combined treatment of cadmium with UV-B radiation also confirmed that when both these stresses unite their effect are found to be more severe as compared to their effect when they apply singly, either it is germination percentage, germination speed, root length, shoot length, fresh weight or dry weight.

Therefore, it is concluded that the effect of individual and combined effect of UV-B radiation and heavy metals on germination and growth parameter (root, shoot growth, fresh and dry weight) are found to be highly significant. Therefore two or more stresses effect more as compared to single stress and this is also found to be statistically significant.

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