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Effect of UVB radiation on antioxidant compounds and Carbohydrate content on medicinally important plant Simarouba glauca. DC

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ABSTRACT

The effect of UVB radiation on carbohydrate metabolism and antioxidant compound content in medicinally important plant *Simarouba glauca* were investigated under UVB chamber. Photosynthetic status determined on the basis of soluble sugar and starch. In present study carbohydrates metabolism were studied. Starch and total sugar content in root and stem tissue were increased significantly according to increasing enhanced UVB radations.The reducing sugar fount to be decreased according to increased enhanced UVB irradiations. Under UVB treatments the antioxidant compounds status has been determined under a UVB stress condition the considerable poline accumulation takes place while Ascorbic acid content were alter significantly. Ascorbic acid content decreased under but in leaf tissue it was enhanced significantly. In our study this result presumes that in future increased the enhanced UVB irradiations will have significant impact on the photosynthetic productivity and defensive mechanism.

Keywords-UVB radiations,Antioxidant compounds,Free Proline, Ascorbic acid,Carbohydrates.

I. INTRODUCTION

On the earth's surface due to depletion of stratospheric ozone layer increased UVB radiation is one of the change in current climate change pattern, because of such technological difficulties ambient and enhanced UVB radiations effects on plants has been recorded. A present research approach was used to integrate the effects of enhanced UVB radiation on oil yielding plant *Simaruba glauca.* In all green plants carbohydrates plays an important role in primary metabolism. Carbon skeletons for several carbon compounds were supplied by carbohydrates which are present in plant tissues in higher plants protective cell wall of cells is a major constituent of sugar polymers like cellulose and pectin. The complete degradation is very difficult of these polysaccharides due to their complex structure of cell wall.

Among the 20 amino acid proline is Pyrrolidine – 2 – carboxylic acid, a five carbon cyclic amino acid which belonging to glutamate family. This amino acid is synthesized from a glulamic acid or arginine (Funk *et al.,* 2008), containing intermediate ornithine through the action of ornithin – d – aminotransferase in seedlings of *Arabidopsis (*Roosens *et al.,* 1998). For proline synthesis energy in the form ATP as well as reducing equivalents is consumed. Furthermore, proline biosynthesis is an expensive process which is highly

energetic.Proline accumulates in leaves under abiotic stress condition. According to Matysik *et al* 2002 proteinogenic amino acid a proline which serves as a role of, Osmolyte, free radical scavenger, electron sink as well as stabilizer of macromolecules and component of cell wall. Proline plays a key role under a osmotic stresses (Voctberg and Sharp, 1991).

Ascorbic acid or vitamin (c) is found in eukaryotes like plants and animals (except human beings) but completely lacking in prokaryote is (except cynobacteria). According to Muller Moule *et al.*, (2004) Ascorbate and glutathione is an antioxidant as water soluble. Along with chloroplasts in all subcellur compartments containing the apoplast ascorbic acid is found (Smirnoff 2000).

In present study it has been provide the observations and conclusions on the besis of existing knowledge on the interactive effects of UV-B on medicinally important plant *Simarouba glauca* particularly focus on the possible implications for plant defensive performance and protection against given UVB stress.

II. Materials and Methods

A.Plant Material-

Simarouba glauca DC. edible oil tree is commonly planted along wastelands or dry land forest areas by Department of forest in Maharashtra, Karnataka and Andhra Pradesh as well as in agricultural Universities of these states. Freshly harvested seeds of *S. glauca* were purchased from Sri Sri Institute of Agriculture, Bangalore.

B. Methods

Supplementary UV-B radiation treatments-

One year old seedlings of *S.glauca* where purchased from social forestry Kagal. Seedlings with plastic bags were kept in polyhouse under minimum and maximum air temperature at 21 to 31°C respectively with relative humidity of air up to 55%.

In early April seedlings were exposed to UV-B treatments. UV-B radiations was artificially supplied by UV-B tubes (Philips TL20 W/16,NV,Holland).The UV-B irradiance was provided for10h(08:00am-18:00pm)for different days (4,8,12 and 16 days) as per the method described by Lydon *et al.* ,(1986). The tubes were installed 15Cm above perpendicular to the seedlings and oriented in an east-west direction. Tubes were wrapped with 13 mm cellulose diacetate (CA) film to remove out UVC radiation shorter than 290 nm. CA paper was changed per week to avoid photo degradation.Control seedlings were exposed to normal day light.

Carbohydrate content-

The sugars were analysed with the help of method described by Nelson (1944). Oven dried 0.250 g powder of root, stem & leaves from UV-B irradiated and control seedlings were homogenized in mortal with pestle by using 80% ethyl alcohol and filtered through Buchner's funnel using Whatman No.1 filter paper. Residue with the filter paper was used for starch analysis. The volume of filtrate was adjusted to 50 ml and was reduced on water bath to about 5 ml and for decolourization to this 2 g lead acetate and potassium oxalate (1:1) were added and by adding 40 ml distilled water and filtered through Buchner's funnel. Finally total volume of filtrate was measured and served as an extract for determination reducing sugers. For quantification of total sugars a 20 ml extract from above filtrate was hydrolyzed for 30 minutes with 4 ml concentrated hydrochloric acid. After cooling filtrate were neutralized with anhydrous Na_2CO_3 and filtered, and served as an extract for analysis of Total sugars.

The insoluble residue along with a filter paper was transferred to the conical flask and used for starch analysis. To this 50 ml distilled water and 5 ml concentrated HCl were mixed and hydrolyzed at 15 Lbs. pressure (1½ hour) and cooled at room temperature. The conical flasks were neutralized by the addition of anhydrous sodium carbonate and filtered. The final volume of filtrate was measured used for determination of starch.

For quantification of reducing sugars, total sugars from each filtrates 0.2 ml. were taken in a separate set of tubes respectively. In other test tubes different grades of glucose (0.1 mg ml^{1}) were taken. For making final volume 1 ml requisite amount of distilled water was added. The blank was prepared with1 ml. distilled water. Somogy's alkaline copper tartarate reagent was prepared by mixing 4 g $CuSo_4$.$5H_2O$, 24 gm unhydrous Na_2Co_3, 16 gm Na-K-tartarate and 180 gm. unhydrous Na_2So_4 in 1 liter distilled water. Nelson's arsenomolybdate reagent prepared by mixing (25 g ammonium molybdate in 450 ml distilled water, 3 gm. sodium arsenate dissolved in 25 ml distilled water, 21 ml concentrated HCl, these ingredients were mixed and allowed to digest for 48 hours at 37° C) 1ml each of there were carefully added. The reaction mixture was further diluted to 10 ml with distilled water. The absorbance of these samples was measured at 560 nm on a double beam spectrophotometer (Shimadzu, 1900).

Antioxidative compounds-

a. Free proline-

According to the method of Bates *et al.*,(1973) free proline content was determined. 0.250 mg of oven dried powder of root, stem and leaves from control and UV-B irradiated plants were taken and homogenized in 10ml 3% sulfosalicylic acid and filtered through Buckner's funnel using Whatman No.1 filter paper. With 3% sulfosalicylic volume of filtrate was adjusted to 20ml. Then 0.5ml filtrate were allowed to react with 2ml glacial acetic acid and 2ml acid ninhydrin reagent (1.25g Ninhydrin dissolved in 30ml glacial acid and 20ml 6 M phosphoric acid by warming and agitation and stored at 4°C) and boiled for 1 h. in a boiling water bath at 100°C. For different concentration of standard proline solution (0.1 mg ml^{-1}) was also followed similar

procedure. The reaction was stopped by transferring the test tubes immediately to ice bath. 2 ml of toluence were added in to this and mixed vigorously for 15-20 seconds. The absorbance of coloured complex in toluene layered was measured at 520 nm on double beam spectrophotometer. Proline content was calculated from calibration curve of standard praline final values are expressed as mg $100g^{-1}$ dry tissue.

b. Ascorbic acid content-

A method described by Dhopte and Phadanwis (1989) was used for determination of ascorbic acid content from root, stem and leaves. One gram of root stem and leaf tissues was taken and crushed in 10ml of 0.4% oxalic acid in mortar with pestle.Through a double layered muslin cloth this extract was filtered and by making equal volume of filtrate, these extracts were centrifuged for 20 min at 10,000rpm. Supernatant was slowly taken out and volume made 15ml with 0.4% oxalic acid.Reduction of dichlorophenol indophenols reagent (in 150ml of distilled water 50 mgs of DCPIP was mixed.On a water bath this solution was heated to dissolve the dye.To this solution 42mgs of NaHCO3 was added and kept for cooling.After cooling volume of this solution was made 200ml. with distilled water) and was measured.For standardization of DCPIP standard ascorbic acid solution(0.1mg/ml in 0.4% oxalic acid solution)was used.Five ml of standard ascorbic acid solution was titrated against DCPIP reagent until the solution becomes pink.With a standeredized indophenols reagent 5ml of root stem and leaves extracts were titrated, in the similar manner and readings were recorded.In plant samples the ascorbic acid content was expressed as µg of ascorbic acid per gram of fresh weight.

III. Result and Discission

Effect of UV-B radiations on carbohydrate content in root,stem and leaves of *Simarouba glauca* is shown in fig.1,2,3.It is noticed from fig. that the starch content(fig.1.) is elevated in root, stem tissue with increasing UV-B irradiations, while in leaf tissue it is slightly increased up to 12day UV-B irradiation and decreased in 16days treated plants.

The total sugar content(fig.3.) of root, stem and leaves is significantly increased with increasing days of exposures to UV-B irradiations and this increase is more significant in case of leaf tissue. The reducing sugar content(fig.2.) of root, stem and leaves is found to be decreased with increasing the exposure of UV-B irradiations and this decrease is more significant in stem tissue at 12 and 16 days of exposure to UV-B irradiations.

In all green plants carbohydrates plays an important role in primary metabolism. Carbon skeletons for several carbon compounds were supplied by carbohydrates which are present in plant tissues in higher plants protective cell wall of cells is a major constituent of sugar polymers like cellulose and pectin. The complete degradation is very difficult of these polysaccharides due to their complex structure of cell wall. Starch and sucrose indicates the main output of steady state photosynthesis furthermore synthesis of starch carried out in plastid while synthesis of sucrose takes place in Cytosol. In higher plants the main product photosynthetic

carbon assimilation is oligosaccharides that are sucrose which serves as non reducing sugars utilized in plants growth and development as energy source (George 1993). Growth of sink tissue is depends on the limiting supply of transport sugar and sucrose (Farrar, 1996).

Sucrose breakdowns in to Glucose and Fructose which is a major reducing sugar in plants generally utilized as substrate for respiration and as substrate for starch synthesis in storage organs like seeds and tubers. Along with respiratory substrate and source for carbon skeletons sugar further plays vital role in osmoregulation.

Between the autotrophic source tissue and number of sink tissues carbohydrate partition is competing a common pool of carbohydrates a processes which is highly peculiar that characterized all stages of growth and development in higher plants (Roitsch *et al.,* 2003). The variable level of different carbohydrates in a plant tissue which is highly characterized by both endogenous and environmental factors. Therefore it is most important to access the overall metabolic status of the tissue level of various carbohydrate fractions.

In the present investigation the elevation in total sugar and starch content was observed in root, stem and leaf tissue of *Simarouba glauca* in response to UV-B irradiation. This increased levels of carbohydrates under stress condition might be helpful for the allocation of carbon for various metabolic activity this will helpful to develop stress tolerance of *Simarouba glauca.*

Antioxidative compounds-

a. Free Proline:

The effect of UV-B radiations on the free proline content of *Simarouba glauca* is shown in fig.4. It is noticed from fig. the free proline content of root, stem and leaf tissue is considerably altered. It is indicated that the proline content of root, stem and leaves tissues is increased with increasing treatments of UV-B irradiations. This increase in proline content under UV-B stress is more significant in leaves and stem tissues.

Among the 20 amino acid proline is Pyrrolidine – 2 – carboxylic acid, a five carbon cyclic amino acid which belonging to glutamate family. This amino acid is synthesized from a glulamic acid or arginine (Funk *et al.,* 2008), containing intermediate ornithine through the action of ornithin – d – aminotransferase in seedlings of *Arabidopsis (*Roosens *et al.,* 1998). For proline synthesis energy in the form ATP as well as reducing equivalents is consumed. Furthermore, proline biosynthesis is an expensive process which is highly energetic.Proline accumulates in leaves under abiotic stress condition. According to Matysik *et al* 2002 proteinogenic amino acid a proline which serves as a role of, Osmolyte, free radical scavenger, electron sink as well as stabilizer of macromolecules and component of cell wall. Proline plays a key role under a osmotic stresses (Voctberg and Sharp, 1991). Thomas (1990) reported that, not only proline plays important role in the protection of enzyme from denaturation in salt accumulation proline shows a hydroxyl radical (OH^-) scavenging activity Smirnoff and Cumbes (1989). Proline helps in stabilization of membrane by interacting with phospholipids (Rudolph *et al.,* 1986). Proline acts as cryoprotectant which protects the tissue from

freezing damage in higher plants (Santarius 1992).It helps in protein salvation (Paleg *et al.*, 1984). In several plants proline is responsible for the stomatal closure (Rajagopal, 1981).

Proline is a less harmful amino acid even it is present in high concentration in cell. Proline causes a least disturbance in metabolic processes at higher concentration thus proline also called as compatible solute (Athawale *et al.*, 2005). Proline increases the bound water under stress condition due to it is highly soluble in water. Due to free proline accumulation osmotic pressure of cell sap gets increased which is most important for water relation of the cell.

Under a stress condition proline provide energy by sparing nitrogen for growth and development for confirming resistance (Stewart *et al., 1966*). Accumulation of proline take place after biosynthesis or proteolysis activator in a radiation treated plants (Agrawal *et al., 1994*).

In the present study considerable accumulation of proline takes place due to UV-B radiation stress. In number of experiments it is indicated that under abiotic stress condition proline may acts as osmoticum or it may protect the tissues from damage as well as it helps in stabilization of membrane. Thus, the elevated level of proline under UV-B stress might be helpful to stabilize the membrane from damage of UV-B radiation stress.

b.Ascorbic acid content -

Effect of UV-B radiation on ascorbic acid content in root, stem, and leaves of *S. glauca* is shown in fig.5. It is noticed from fig that the ascorbic acid content is slightly decreased in root and stem tissue in response to UV-B stress. In leaf tissue the ascorbic acid content is increased by 10 to 15 % over the control in response to UV-B stress.

Ascorbic acid or vitamin (c) is found in eukaryotes like plants and animals (except human beings) but completely lacking in prokaryote is (except cynobacteria). According to Muller Moule *et al.*, (2004) Ascorbate and glutathione is an antioxidant as water soluble. Along with chloroplasts in all subcellularr compartments containing the apoplast ascorbic acid is found (Smirnoff 2000). In plants, several antioxidants are present from these vitamin c or ascorbic acid attracting most attention of stress physiologists. Ascorbic acid is structurally simplest vitamin. Generally in most of cell parts like cytosol, chloroplasts, vacuoles, mitochondria and cell wall ascorbate occurs (Anderson *et al.*, 1983; Rauten kranz *et al.*, 1994). Under stress condition concentration have been reported as high as 50 mm and about 30-40% ascorbate is found in chloroplast of plant cells (foyer and Noctor 2005).

For water soluble antioxidants examination is essential to study Ascorbic acid content. Under UV-B radiation ascorbic acid content alter significantly. Ascorbic acid content decreased under ambient UV-B radiation ($p=0.0002$) but DHA and total ASA content (ASA+OHA) enhances under UV-B radiation ($p<0.0001$ and $p=0.0069$ resp.) Thus, ratio ASA/DHA reduces after a exposure of UV-B radiations ($P<0.0001$) (Xu *et al.*, 2008).

Adedooye *et. al.,* (2008) observed that after 4 hour treatment of UV-B light ascorbic acid concentration reduces but after an 8 hour treatment concentration get increased as compared to control plants. They concluded that initial reduction shows sudden response to stress condition as defense mechanism to encounter stress condition giving stimulus to synthesis the antioxidant as ascorbic acid. Ascorbic acid content increased under a U-B radiation in a starting stage significant growing up to 54.3% under a UV-B 2 (+3.6kj m^{-1}) radiation as compared to control plants. Further stage shows reduction in ascorbic acid content which acts as antioxidant which react with hydroxyl radical, singlet oxygen and superoxide radicals. According to several studies it is clear that increments in ascorbic acid content due to UV-B stress condition (Costa *et. al., 2002*; Nasibi and kalantari, 2005). Under a stress condition reduction in ascorbic acid content due to increment in ascorbate peroxidase activity under a UV-B condition and resulted into higher consumption of ascorbic acid for effective quenching of oxyradicals. It is reported by Agrawal and Rathore (2007) in wheat and mung bean under a supplemental UV-B stress condition ascorbic acid content get reduced.

In the present study the ascorbic acid content in root and stem tissue was decreased while in leaf tissue it was slightly elevated. Thus the water soluble antioxidant ascorbic acid is found to be decreased in root and stem tissue and slight elevation in leaf tissue which might be used for effective quenching of oxiradicals, which might be helpful to maintain a balanced antioxidant system under UV-B stress.

Summary and Conclusion-

The elevation in total sugar and starch content was observed in root, stem and leaf tissue of *S. glauca in* response to UV-B irradiation. This increased level of carbohydrates under stress condition might be helpful for the allocation of carbon for various metabolic activities.

The free proline content of root, stem and leaf tissue was considerably altered. It was indicated that the proline content of root, stem and leaf tissues was increased with increasing treatments of UV-B irradiations. This increase in proline content under UV-B stress was more significant in leaf and stem tissues. In number of experiments it is indicated that under abiotic stress condition proline may acts as osmoticum or it may protect the tissues from damage as well as it helps in stabilization of membrane. Thus, the elevated level of proline under UV-B stress might be helpful to stabilize the membrane from damage of UV-B radiation stress.

The ascorbic acid content was slightly decreased in root and stem tissue in response to UV-B stress. In leaf tissue the ascorbic acid content was increased by 10 to 15 % over the control in response to UV-B stress. Thus the water soluble antioxidant ascorbic acid was found to be decreased in root and stem tissue and slightly elevated in leaf tissue which might be helpful for effective quenching of ROS and helps to, maintain a balanced antioxidant system under UV-B stress.

Table 1 : Effect of UV-B radiation on starch content of root, stem and leaves of *S. glauca*.

Treatments	Root	Stem	Leaves
Control	2.59	2.95	2.09
4 (Days)	2.03 (-21.62)	2.46 (-16.61)	2.04 (-2.39)
8 (Days)	3.99 (+54.05)	3.79 (+28.47)	3.16 (+51.19)
12 (Days)	2.91 (+12.35)	3.42 (+15.93)	3.18 (+52.15)
16 (Days)	2.88 (+11.19)	3.68 (+24.74)	1.82 (-12.91)

Each value is mean of three determinations.
Values are expressed as g 100^{-1}g dry wt.
Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

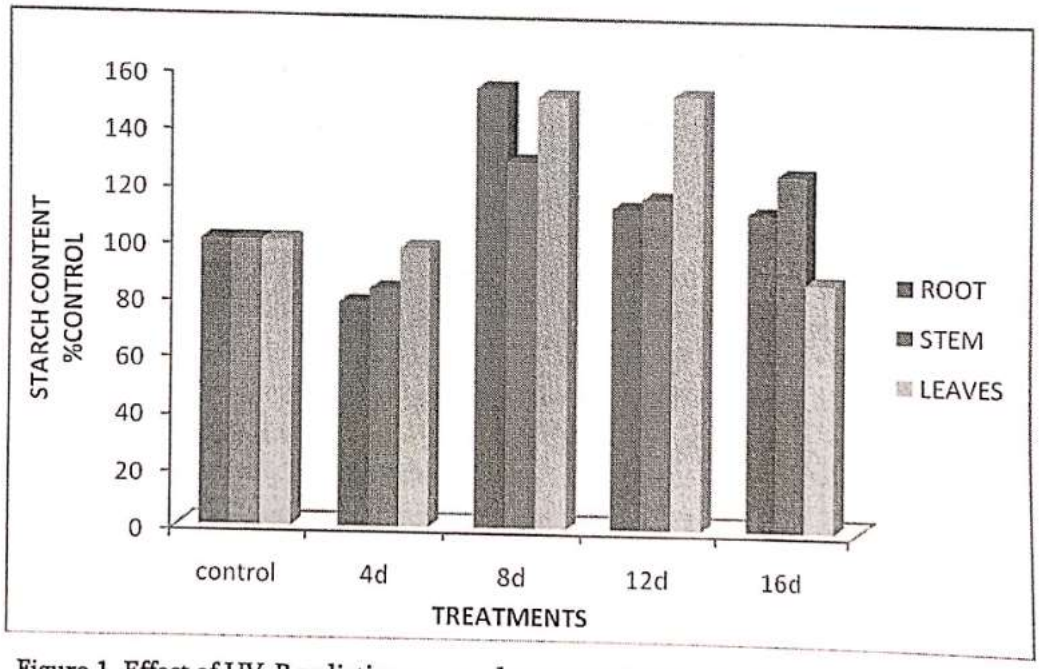

Figure 1. Effect of UV-B radiation on starch content of root, stem and leaves of *S. glauca*.

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Table 2: Effect of UV-B radiation on reducing sugars of root, stem and leaves of *S. glauca.*

Treatments	Root	Stem	Leaves
Control	0.07	0.10	0.41
4 (Days)	0.06 (-10.07)	0.23 (+129.56)	0.45 (-9.8)
8 (Days)	0.13 (+74.93)	0.13 (+33.30)	0.40 (+2.8)
12 (Days)	0.07 (+4.77)	0.07 (-25.93)	0.36 (+13.42)
16 (Days)	0.14 (+94.96)	0.04 (-51.86)	0.38 (+8.93)

Each value is mean of three determinations.

Values are expressed as g 100^{-1}g dry wt.

Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

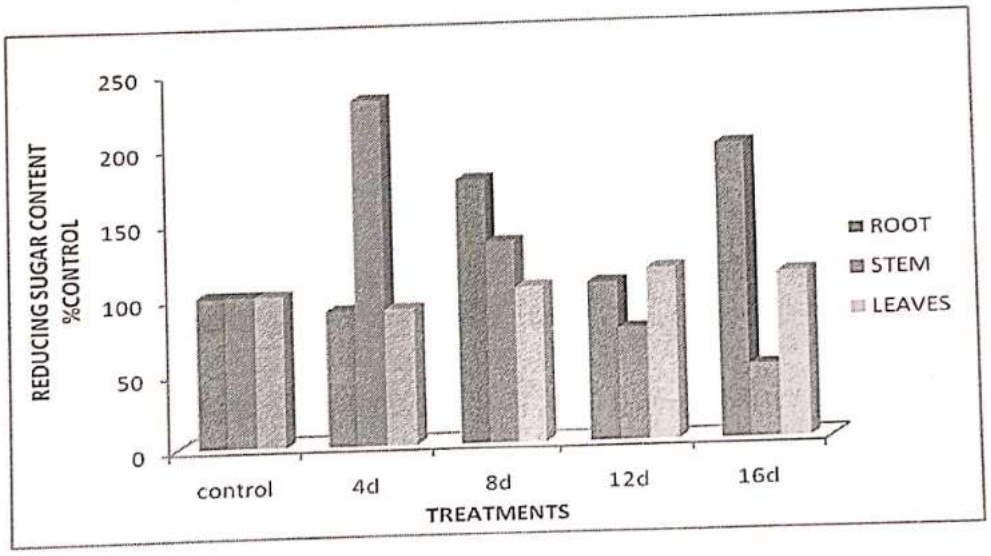

Figure 2: Effect of UV-B radiation on reducing sugars of root, stem and leaves of *S. glauca.*

Table 3: Effect of UV-B radiation on total sugars of root, stem and leaves of *S. glauca*.

Treatments	Root	Stem	Leaves
Control	0.44	0.33	0.56
4 (Days)	0.49 (+11.36)	0.37 (+12.12)	0.58 (-3.44)
8 (Days)	0.48 (+9.09)	0.48 (+45.45)	0.92 (-39.13)
12 (Days)	0.64 (+45.45)	0.39 (+18.18)	0.99 (-43.43)
16 (Days)	0.54 (+22.72)	0.47 (+42.42)	0.79 (-29.11)

Each value is mean of three determinations.

Values are expressed as mg 100 g dry wt..

Values in parenthesis indicate percent increase (+) or decrease (-) over the control.

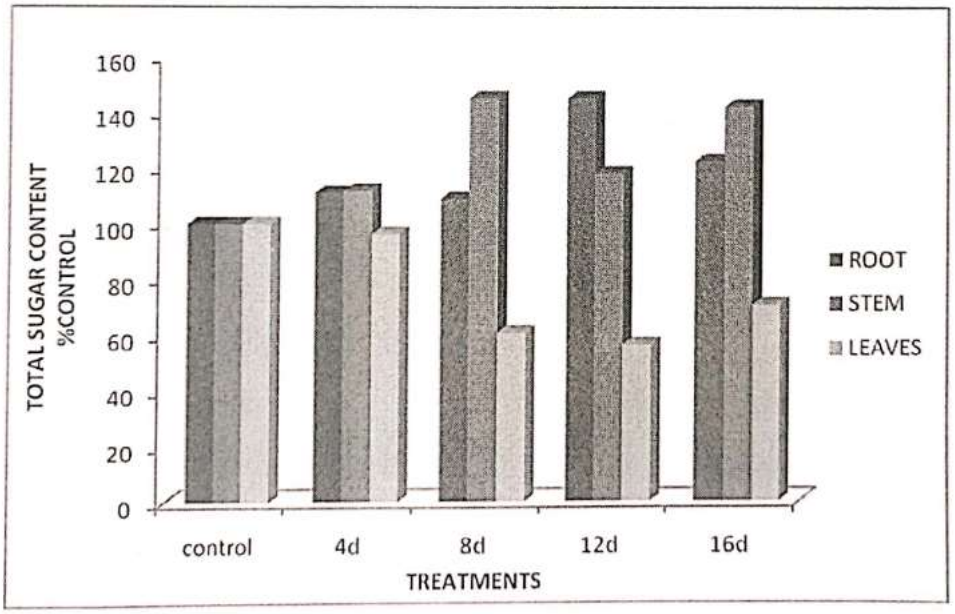

Figure 3: Effect of UV-B radiation on total sugars of root, stem and leaves of *S. glauca*.

Table 4 : Effect of UV-B radiation on free proline content of root, stem and leaves of *S. glauca.*

Treatments	Root	Stem	Leaves
Control	19	25	21.1
4 (Days)	29.1 (+53.15)	56.9 (+127.6)	42.0 (+99.05)
8 (Days)	28 (+47.36)	56.4 (+125.6)	43.7 (+107.10)
12 (Days)	25.8 (+35.78)	106.4 (+325.6)	76.6 (+263.03)
16 (Days)	45.1 (+137.36)	165.4 (+561.6)	98.9 (+368.72)

Each value is mean of three determinations.

Values are expressed as mg 100^{-1} g dry wt.

Values in parenthesis indicate percent increase (+) or decrease (-) over the control

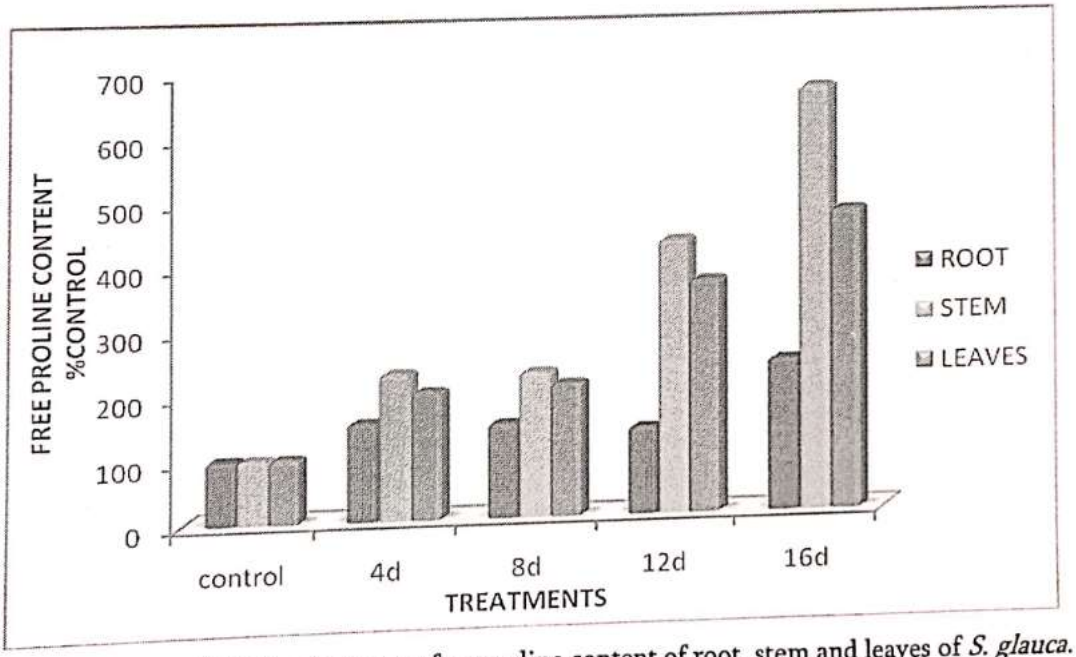

Figure 4: Effect of UV-B radiation on free proline content of root, stem and leaves of *S. glauca.*

Table 5 : Effect of UV-B radiation on Ascorbic acid content of root, stem and leaves of *S. glauca.*

Treatments	Root	Stem	Leaves
Control	0.033	0.07	0.033
4 (Days)	0.026 (-21.21)	0.067 (-4.28)	0.056 (-69.69)
8 (Days)	0.028 (-15.15)	0.038 (-45.71)	0.045 (+36.36)
12 (Days)	0.028 (-15.15)	0.043 (-38.57)	0.039 (+18.18)
16 (Days)	0.032 (-3.03)	0.044 (-37.14)	0.044 (+33.33)

Each value is mean of three determinations.

Values are expressed as mg 100^{-1} g dry wt.

Values in parenthesis indicate percent increase (+) or decrease (-) over the control

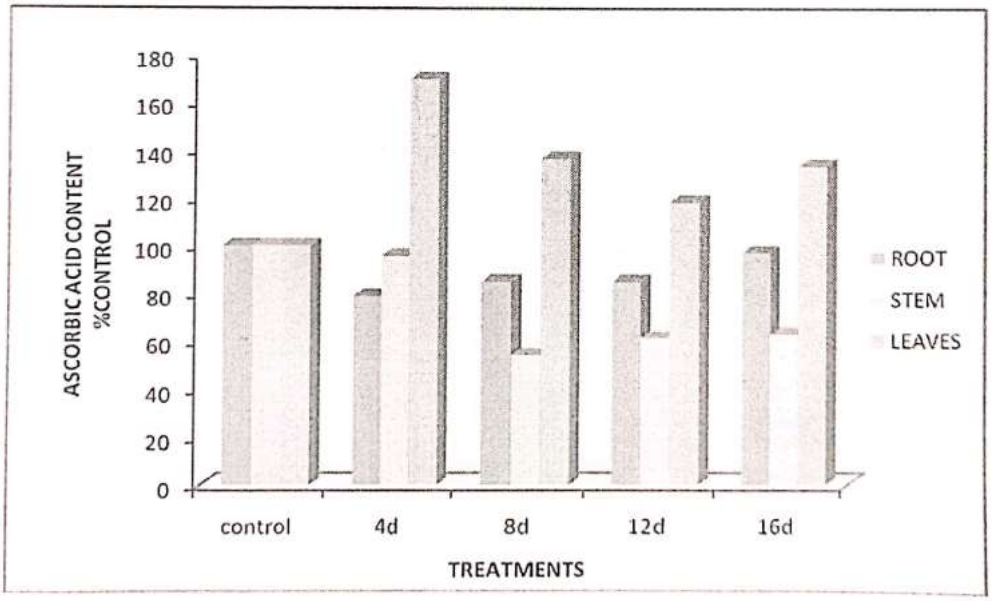

Figure 5 : Effect of UV-B radiation on Ascorbic acid content of root, stem and leaves of *S. glauca.*

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