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Comparative effectiveness of ascorbic acid, Salicylic acid and orange juice on soybean cultivar (*Glycine Max* L.) under UV-B (Ultraviolet radiation) stress

Anuja Anil Arsule, Bhavna Nigam and Indra Jeet Chaudhary

Abstract
Increasing composition of gaseous pollutant derived by anthropogenic activity disturbed natural atmospheric gaseous composition and deplete the stratospheric ozone layer. Major consequences of this depletion are increase incidents of harmful UV-B radiations reaching to terrestrial surface. Plants being permanent tool to removed harmful effect of solar radiation. Plants use sunlight as primary energy sources, therefore the small increase in UV-B radiation is recognized as harmful for plants. Many studies have shown that solar UV-B radiation causes significantly effect on morphological, physiological and yield components the plant. Therefore the present study conducted for assessment of relative effectiveness of Ascorbic acid, Salicylic acid and orange juice on soybean cultivar (*Glycine Max.* L.) under temperature acclimated UV radiation stress. Resultants ambient UV-B radiation caused harmful effect to soybean cultivar and reduce plant biomass 36% at juvenile stage and 40% at vegetative stage as compared to UV-B filter treatment. The effectiveness of Ascorbic acid, Salicylic acid and orange juice was following the trends 40% OJ > 20% OJ > 50 ppm AA > 100 ppm AA > 100 ppm SA > 50 ppm SA as compared to control plant. Based on obtained results orange juice and synthetic ascorbic acid will be useful tool for assessment of harmful effect of UV-B radiation on plants.

Keywords: UV-B radiation, *Glycine Max* L., exogenous protectants, morphological, physiological and yield characteristics of plant

1. Introduction
The release of chlorofluorocarbons (CFCs) and other active compounds into the atmosphere promotes ozone breakdown in the stratosphere, resulting in an increase in the flux of ultraviolet-B radiation (UV-B, 280-320 nm) transmitted to the Earth's surface (Wong *et al*., 2015) [41]. Increased global warming, which causes increased cooling in the stratosphere, effects the extent of ozone depletion and, as a result, increases in UV-B incident on the planet's surface (Porfirio *et al*., 2012) [27]. Although this is projected to gradually improve in the coming years (McKenzie *et al*., 2011; Williamson *et al*., 2014) [23, 40], ozone depletion, exacerbated by global warming (Watanabe *et al*., 2011), remains a significant concern.
The stratospheric ozone layer of the Earth is vital for absorbing UV radiation emitted by the sun. In the last thirty years, it has been discovered that stratospheric ozone is depleting as a result of increasing in various human originated ODSs (ozone-depleting substances) (Chipperfield *et al*., 2020) [11]. This depletion in the ozone layer significantly increases the harmful fraction of UV radiation i.e., UV-B (280–320 nm) on the earth's surface. Although ultraviolet radiations subdivide into three parts UV-A (315- 400 nm), UV-B (280-315 nm) and UV-C (200-280 nm), UV-C radiation is completely absorbed by the atmosphere and UV-A fully transmitted to the earth's surface cannot be absorbed by the ozone layer and less harmful than the other UV radiation. However, some astronomical parameters, such as solar zenith angle, as well as physical characteristics of the earth's surface, like altitude, albedo and meteorological conditions, also affect the transmission of UV-B (Porfirio *et al*., 2012) [27], transmission of UV-B is mainly controlled by ozone (Bais *et al*., 2019) [3]. Gases such as CFCs (CFC-11, CFC-12, and CFC-113) with a high potential to deplete ozone having a half-life ranging from 50 to 150 years (Bais *et al*., 2019) [3]. Egorova *et al*. (2023) [15] reported that 1% decrease in ozone results in an increase of 1.3–1.8% in UV-B radiation reaching the biosphere. Though the Montreal Protocol is working, the recovery of the O_3 layer is not expected before

2070 (Egorova *et al*., 2023) [15].

Therefore, UV-B radiation reaching to the earth's surface poses a significant threat to all living organisms.

Ascorbic acid, often known as vitamin C, has various functions in plants. Here are a few examples: Ascorbic acid is a potent antioxidant that protects plant cells from damage caused by reactive oxygen species (ROS) produced during metabolic processes or in reaction to stress. Ascorbic acid's antioxidant property aids in the prevention of premature ageing and senescence in plants (Akram *et al*., 2017) [2]. Ascorbic acid also functions as an enzyme cofactor for numerous enzymes engaged in several metabolic pathways in plants. It is essential, for example, for the manufacture of several key plant hormones such as ethylene and abscisic acid (Paciolla *et al*., 2019) [25]. Ascorbic acid is involved in the regulation of photosynthesis in plants. It contributes to the efficiency of the photosynthetic apparatus.

Salicylic acid (SA) is a plant hormone that helps plants grow, develop, and respond to biotic and abiotic stressors. Salicylic acid has a role in plant defence against pathogens such as bacteria, fungus, and viruses. It causes the synthesis of pathogenesis-related (PR) proteins, which serve as a defence mechanism against pathogens (Lefevere *et al*., 2020) [20]. Salicylic acid aids in the induction of systemic acquired resistance (SAR) in plants. Tolerance to abiotic stress Salicylic acid is also involved in plant tolerance to abiotic stress, such as drought, excessive salt, and harsh temperatures. Most studies have examined the responses of plants to UV-B grown under unrealistic UV-B radiation in growth chambers and greenhouses, or under balanced UV-B radiation in the field (Kataria *et al*., 2014; Rai *et al*., 2021) [18, 28]. Studies on the effect of ultraviolet-B under realistic environment is scare. Moreover, studies were conducted for the plant response to UV-B on individual species however, plants response to UV-B varies not only at species level but also at cultivars of similar species. Therefore, this study was conducted under ambient condition with using different exogenous protectants to identify the plants response to UV-B and effectiveness of applied protectants. We hypothesized that the present solar UV-B radiation has a potential impact on plant growth and metabolism. Moreover, we also hypothesized that the role of different exogenous protectants in specific cultivar and the sensitivity of plants to UV-B is antioxidant dependent.

2. Material and Methods

2.1 Study materials and experimental design

A soybean cultivar (*Glycine Max*, L.) was used for the present study. Seeds of *soybean* cultivar was collected from agricultural land of Satara. A pot experiment was conducted at terrace of Department of Environmental Science, Savitribai Phule Pune University Campus for assessment of ambient UV-B radiation on soybean plants and relative effectiveness of orange juice, synthetic ascorbic acid and salicylic acid. The experiment was carried out during February to April (2023). Average day temperature range during the experiment period was 25 °C to 40 °C.

Eight different treatments were set for experiment

- (T_1) The UV-B filter frames (4’ length × 2’ width × 4’ height) each comprised iron stands covered by a Plexiglas acrylic sheet, to ensure that plants received photosynthetically active radiation, but not UV-B radiation. The acrylic sheet absorbs 98% of total-UV radiation. Each acrylic sheet was 3-mm thick.
- (T_2) Ambient UV-B radiation open field without any filter exposed to natural solar radiation.
- (T_3) Ambient UV-B radiation + 50 ppm SSA(Synthetic salicylic acid)
- (T_4) Ambient UV-B radiation + 50 ppm SAA (Synthetic ascorbic acid)
- (T_5) Ambient UV-B radiation + 100 ppm SSA (Synthetic salicylic acid)
- (T_6) Ambient UV-B radiation + 100 ppm SAA (Synthetic ascorbic acid)
- (T_7) Ambient UV-B radiation + 20% OJ (orange juice)
- (T_8) Ambient UV-B radiation + 40% OJ (orange juice)

2.2 UV-B Radiation measurement

UV Index of daily solar spectrum was obtained from weather online data (AccuWeather, 2023) [17] (Fig. 1). Current UV radiation was measure with the help of UV meter. UV index 2 indicates UV radiation level is low UV radiation was moderate amount of time without experiencing any harmful effects UV Index 3 can show both positive and negative impact on plant depending upon length of exposure. UV Index 4 high and moderate level shows negative impact on plants high exposure can cause damage to plants DNA impairing ability to carry out photosynthesis and lead reduces its growth.

2.3 Application and preparation of exogenous protectants

The solution of Ascorbic acid and salicylic acid were prepared by dissolving in ethanol then added drop wise to water (ethanol/water: 1/1000 v/v). SA and AA at concentration of 100 ppm were sprayed at morning hours on every 10th days from germination to till last sampling. The dose of SA and AA was selected bases on earlier findings (El-Esawi *et al*., 2017; Chaudhary *et al*., 2023) [7, 16]. A constant volume (100ml pot^{-1}) of salicylic acid and ascorbic acid was manually sprayed on each pot at morning hours on every 10th days from germination to last maturity.

Before using, the content of ascorbic acid in 20 and 40% of Orange Juice was estimated to be 20.7 and 38.6 mg/L using Keller & Schwager's process, (1977) [19]. In addition to ascorbic acid, the OJ produces a mixture of inorganic and organic nutrients (Chanson-Rolle *et al*., 2016) [4]. In distilled water, various solutions were prepared, including 20 and 40% OJ; 50 and 100 ppm synthetic salicylic acid and ascorbic acid. Plant sample was collected for growth & physiology analysis after 20 DAS, 40 DAS and final harvesting.

2.4 Measurement of plant growth & biomass

For leaf area graphical method was used**.** The length of root and shoot was determined regularly on a meter scale and were averaged. Physical growth parameters like number of leaves and roots were counted at regular averaged time intervals. The fresh and dry weight of different plant parts was recorded at a regular interval using single pan electric balance. Distilled water was used for washing different plants parts and excessive water was removed after placing them in two layers of filter paper before weighing. Dry weight of the same tissue was recorded after drying it in hot air electric oven 80 degree centigrade till the constant weight achieved.

2.5 Relative water content (RWC)

A young fresh leaf was taken from each plant per treatment and its fresh weight measured. Then, it was dipped in distilled water and kept it for three hours at room temperature. Thereafter, its turgid weight was measured. The leaves were then kept in an oven and their dry weights measured. The percentage of RWC was determined using following formula.

RWC (%) = [(Fw-Dw)/ (Tw-Dw)] x 100

2.6 Membrane permeability (MP)

Fresh leaves were taken from each pot, and cut the circular shape pieces (1 cm^2) and 20 pieces transferred to beaker containing 10 mL of distilled water. The beaker were kept at room temperature for 2 h and electrical conductivity was determined.

2.7 Photosynthetic pigments

2.7.1 Chlorophyll and carotenoids contents

Photosynthetic pigment was analyses by Machlachlan & Zalik, (1963) [22] and Duxbury & Yentsh, (1956) [13] methodology. A leaf sample (100mg) was chopped and added to a 10 mL of 80% acetone solution. The samples were kept overnight at 4 °C. It was then homogenized and centrifuged at 6000xg for 15 minutes. The optical densities of the supernatant were measured at 645 and 663 nm wavelengths. The contents of chlorophyll *a* and *b* and carotenoid were calculated by using the following formulae:

$$\text{Chl. } a \text{ (mg g}^{-1}\text{ dry leaf)} = \frac{(12.3 \text{ X } D_{663}) - (0.86 \text{ X } D_{645})}{\text{d X 1000 X W}} \text{ X V}$$

$$\text{Chl. } b \text{ (mg g}^{-1}\text{ dry leaf)} = \frac{(19.3 \text{ X } D_{645}) - (3.6 \text{ X } D_{663})}{\text{d X 1000 X W}} \text{ X V}$$

$$\text{Carotenoid (mg g}^{-1}\text{ dry leaf)} = \frac{(7.6 \text{ X } D_{663}) - (1.49 \text{ X } D_{645})}{\text{d X 1000 X W}} \text{ X V}$$

Where,
V = volume of chlorophyll extract (ml)
d = length of light path (cm)
W = weight of leaf taken (g)
Total chlorophyll = chl. *a* + chl. *b*

2.8. Ascorbic acid

The method of Keller and Schwager, (1977) [19] was used for the extraction and determination of ascorbic acid contents. 500 mg fresh leaf sample was crushed in an ice bath with 20 ml of extracting solution. The homogenate was centrifuged at 6000 xg for 15 minutes 1 ml of the supernatant and 5 ml of 2, 6-dichlorophenol-indophenol solution was mixed with constant shaking and the O.D. of the pink solution (Es) was determined at 520 nm wavelength. Then one drop of ascorbic acid solution was added in order to bleach the pink colour of the dye completely and O.D. of the obtained turbid solution (Et) was measured at the same wavelength. For blank (Eo), 1 ml extracting solution and 5 ml DCPIP solution was mixed together and O.D. was measured as mentioned above.

A calibration curve was prepared by using 1% aqueous ascorbic acid solution. The total amount of ascorbic acid was calculated by using the following formula.

Ascorbic acid (mg g^{-1} fresh leaf) = [{Eo – (Es – Et)} x V] / (v xW x 1000)

Where,
W = weight of leaf taken (g)
V = total volume of the mixture (ml)
v = supernatant taken for analysis (ml)
Value of {Eo-(Es – Et)] is estimated by the standard curve.

2.9. Statistical Analysis

The study involved a fully randomized two-factor UV stress and exogenous ascorbic acid treatment. Using the HPSS software, the data collected for each parameter was subjected to Duncan's Multiple Range Test analysis. To estimate the significant differences among the mean values, the least significant difference was estimated at the 0.05 percent likelihood stage. Using Origin 2023b software, PCA was used to describe the homogeneous characteristics of a soybean cultivar as well as the association between each vector tested under different treatments at two sampling dates (2023).

3. Results

Present study found the detrimental effect of temperature acclimated UV radiation on morphological, biochemical and defense system of the experimental soybean cultivar. The degree of UV damage was varies at the age of cultivar.

3. 1. Visible injury

Plant grown under direct exposure to sun light showed visible injury due to the UV-B portion of solar spectrum. Cultivars of *Glycine Max* showed curling of leaves at the margin and chlorotic spreading (Fig. 2). Chlorosis can caused by various factors including nutrient deficiencies environmental factors. Plants exposed to extreme temperature this leads to synthesis of chlorophyll heat stress can cause stomata leaves to close on plants leaves to close reducing transpiration and photosynthesis rates reducing chlorophyll synthesis results in chlorosis.

3. 2 Plants morphological characteristics

Temperature acclimated UV radiation caused harmful effect to selected cultivar (*Glycine Max*. L.) at all growth stages and higher reduction of leaf area was found at vegetative stage (-39.53%) than juvenile stage (-15.25%) as compared to control plants (Fig.3A). Application of exogenous applied natural and synthetic protectant play a protective role in leaf area and higher increased of leaf area was seen in treatment T_8 (40% applied orange juice) as compared to control plants. While effectiveness of protectants at both stages based on leaf area increment were $T_8 > T_7 > T_6 > T_5 > T_4 >$ and T_3. As compared to control higher increased of leaf area was seen in orange juice (40%) applied treatment (60.82%) at juvenile stage (Fig.3A).

Root, shoot, and total plant height were observed to decrease in the presence of UV ambient conditions (T_2) compared to plants under UV filter conditions (T_1) (Fig.3B,C,D). However, when exogenous protectants were applied, the plant height showed an increase. The most significant increase in root length was observed at the juvenile stage in treatment T_8 (50.72%), whereas treatment T_3 exhibited the least increase in root length (1.49%) during the vegetative stage (Fig.3B. Across all treatments, root length consistently increased with plant age. The trend of root height increment followed the sequence $T_3 < T_4 < T_5 < T_6 < T_7 < T_8$.

Shoot length exhibited a similar trend to root height concerning both age and treatments (Fig.3C). The presence of ambient UV radiation had a significant impact on the shoot length of the selected plants, resulting in a higher reduction in treatment T_2 (-33.09%) at the juvenile stage and (-26.48%) at the vegetative stage compared to the control treatment. Among the treatments, the maximum increase in shoot length was observed in treatment T_8 (56.46%) at the juvenile stage, followed by T_7 (28.98%) and T_6 (28.29%) at the vegetative stage. The effectiveness of the protectants in terms of shoot length increment followed a specific pattern at both stages. The order of effectiveness, from highest to lowest, was observed as $T_8 > T_7 > T_6 > T_5 > T_4 > T_3$.

The presence of ambient UV radiation had a detrimental effect on the plant height of the selected plants. The maximum reduction in plant height was observed in treatment T_2 (-35.87%) at the juvenile stage and (-27.66%) at the vegetative stage compared to the control treatment (Fig.3D). On the other hand, when compared to the control treatment, the highest increase in plant height was noted in treatment T_8 (51.42%) at the juvenile stage, followed by T_7 and T_6 (37.28% and 24.58% respectively) at the vegetative stage.

3.3 Changes in biomass characteristics

3.3.1 Leaf fresh and dry biomass

The exposure of the selected cultivar (*Glycine Max.* L.) to temperature-acclimated UV radiation resulted in harmful effects at all growth stages. The most significant reduction in leaf fresh weight was observed in treatment T_2 at the juvenile stage (-23.86%) compared to the control plants. However, the application of exogenous natural and synthetic protectants demonstrated a protective role (Fig. 4A). The maximum increase in leaf fresh weight was observed in treatment T_8, followed by T_7 and T_6 (69.31%, 19.31%, 2.27%, respectively).

Leaf dry weight (LDW) exhibited different outcomes based on treatment and age factors. The most significant reduction in LDW was noted in T_2 treatment (-61.64%) at the juvenile stage compared to the control treatment (Fig. 4B). However, the application of exogenous natural and synthetic protectants improved LDW, with the highest increase observed in treatment T_8 (259.39%, respectively).

3.3.2 Root, shoot and total plants biomass

The presence of UV ambient conditions (T_2) resulted in decreased shoot and root dry weight compared to plants under UV filter conditions (T_1). The most significant reduction in shoot and root dry weight was observed in T_2 treatment at the juvenile stage, with a decrease of -108.53% and -6.28% respectively, compared to the control treatment (Fig. 4C,D). However, the application of exogenous protectants showed positive effects, leading to increased shoot and root dry weight. The maximum enhancement in shoot and root dry weight was recorded in T_8 treatment, followed by T_7 and T_6 (67.74% and 45.09%, 61.75% and 36.68%, 56.61% and 35.47%, respectively) at the juvenile stage (Fig. 4C, D).

The root-shoot ratio of the plants exhibited a negative percentage reduction in the T_2 treatment at the juvenile stage compared to the control plants. However, the application of exogenous natural and synthetic protectants resulted in positive percentage increments, particularly in the T_8 treatment at the juvenile stage (Fig. 4E). The presence of ambient UV radiation can have detrimental effects on plant development and biomass growth. Particularly during the juvenile and vegetative stage, when UV temperatures range between 34 and 38°C, high ambient UV radiation can lead to lower biomass production at the vegetative stage. Compared to the control treatment (T_1), total biomass was reduced by -10.91% at the juvenile stage and by -13.37% at the vegetative stage (Fig. 4F). However, the application of exogenous natural and synthetic protectants showed positive results in improving total plant biomass. The maximum increase in plant biomass was observed in treatment T_8 (40.35%) at the juvenile stage.

3.4 Photosynthetic Pigment

The exclusion of ambient solar ultraviolet radiations resulted in a significant decrease in chlorophyll a, b, and total chlorophyll content compared to the control group (Fig. 5A, B, C). However, when exogenous natural and synthetic protectants were applied to stress plants, there was an increase in chlorophyll a, b, and total chlorophyll content. The maximum increase in chlorophyll a, b, and total chlorophyll was observed in treatment T_8 (50.19%, 72.79%, and 54.16%, respectively). Among the treatments, the increasing trends of chlorophyll a, b, and total chlorophyll were observed in the order of $T_8 > T_7 > T_6$.

3.5 Carotenoids and ascorbic acid contents

Carotenoids are essential compounds that work in conjunction with chlorophylls to facilitate photosynthesis and provide photoprotection in photosynthetic bacteria, algae, and plants. They play a crucial role in capturing light energy and transferring it to chlorophylls through singlet-singlet excitation transfer. In the experiment, it was observed that the carotenoid content increased by 49.30% at the juvenile stage and 36.08% at the vegetative stage after 20-40 days of sampling in treatment T_8. Conversely, a decrease in carotenoid content was observed by -295% at the juvenile stage and -5.70% at the vegetative stage in treatment T_2, as depicted in (5D).

Ascorbic acid (AA) plays a crucial role in regulating cell division, cell elongation, and cell differentiation. In the juvenile stage, the content of ascorbic acid decreased by -22.65% and in the vegetative stage by -71.53% in the T_2 treatment (Fig. 5E). However, the application of exogenous natural and synthetic protectants showed positive effects by increasing the content of ascorbic acid. The maximum content of ascorbic acid (AA) was recorded in treatment T_8, followed by T_7 and T_6 (78.86%, 67.52%, and 61.68%, respectively). The flavonoids contents was following the same trends as ascorbic acid contents with treatments and age (Fig.5F).

3. 6 Relative water content and membrane permeability

The relative water content of the cultivar experienced a decrease due to high temperatures in the months of March and April. However, when treated with T_8 (40% orange juice), the relative water content increased by 54.23% at the juvenile stage. On the other hand, the presence of UV radiation resulted in a higher reduction of relative water content at the juvenile stage in the T_2 treatment (-13.72%). Overall, the effectiveness of treatments in increasing relative water content followed the sequence $T_8 > T_7 > T_6 > T_5 > T_4 > T_3$ at both stages (Fig 5G).

Membrane permeability following the similar trends as relative water contents. Higher loss of relative water content was seen in treatment T_2 therefore higher membrane permeability was found in treatment T_2. Exogenous applied protectants reduce the water loss in plant cell and also reduce the membrane permeability (Fig. 5H).

3.7 Principle component analysis

PCAs analysis shows that the application of protectant positively correlated with each parameters (Fig.6). Total percentage variance of cultivar was found 95.89% and 3.25% at PC1 and PC2 with Eigenvalue 88.68 and 3.00. Percentage variation at PC3 was noted 0.48% and eigenvalue 0.44. Flavonoid and carotenoids showed strong correlation while plant biomass slightly correlated with each other's. Leaf area and ascorbic acid content was also slightly correlated with each other's while plant height shows no correlation with higher loading value (0.89). Treatment wise highest positive score value of cultivar was noted in T_8 (2.65) at vegetative stage and minimum score was shown in treatment T_2 (-1.45) at juvenile stage. Antioxidants defence showed positive values at both PCs. Therefore PCA analysis data was confirmed that the application of 40%OJ is more effective than 20% OJ > 100 ppm AA>50 ppm AA >100 ppm SA < 50ppm SA as compared to UV filter plants and elevated UV caused negative effect on cultivar.

4. Discussion

Understanding of relationship between crop and environment has substantially improved during the last few decades of the 20th century. Most of the studies conducted on the UV-B effect on plants were at variable experimental setup (greenhouse, sunlit chambers, and field) hence, the degree of damage was contingent on experimental conditions (Yoshida *et al*., 2021) [43]. Moreover, most of these studies were conducted with fairly high UV-B radiation levels (>15 kJm^{-2} per day) that are likely to be unusual in the future climates as current levels of UV-B during the cropping season vary anywhere between 2 and 12 kJm^{-2} per day on the Earth's surface, which includes an increase of 6–14% of UV-B radiation (UNEP, 2012). Another important concern for UV-B dosimetry studies are the unequal change in intensity of wavelengths in UV-B spectra (280–325 nm).

Morphological effect of solar UV-B seen as the visible injury on most of the cultivar studied (Rodríguez-Calzada *et al*., 2019) [31]. However, injury was species specific and both the cultivars of similar species showed almost similar damage that was quite different from other species symptoms. Result of present study shows soybean plant direct exposer to solar UV-B radiation shows bronze or brown spots appear on the leaf surface that later converted into necrosis, and desiccation of the leaves. Rodríguez-Calzada *et al.* (2019) [31] also found injury on the leaves of chili pepper plants. Yoshida *et al*., (2021) [43] explain the foliar injury as the decrease in leaf chlorophyll on exposure to UV-B.

Decrease in plant height was observed in to solar UV-B radiation exposed plants. Similar result reported in different species in earlier study such as reduction of plant height under UV-B stress (Poorter *et al*., 2019; Sheridan *et al*., 2022) [26, 33]. Decreased in plant height was mainly due to shorter internodes rather than fewer nodes, Whereas reduction of plant growth under UV-B stress as a result of alteration of the rate and duration of cell division and elongation, perhaps due to inhibition of Indole acetic acid (IAA), a key regulator of plant growth (Kataria *et al*., 2014; Poorter *et al*., 2019) [18, 26]. However, in this study we observed that the root length of the plants grown under solar UV-B spectrum was increased. No reports on the root length increase were seen in the literature due to UV-B radiation on any plant. This increase can be explained as the plants growing in the pots under high solar spectrum had higher water scarcity due to less water availability in soil and high evaporation rate. Plant increases the root length in search of water in the soil.

Biomass loss was observed in all plants exposed to solar UV-B radiation; loss of biomass varies from species to species suggested that loss of biomass was species specific. Similar study also reported that reduction in biomass accumulation due to UV-B exposure in several crop species (Liu *et al*., 2014) [21]. Weraduwage *et al*. (2015) [39] reported that the rate of biomass production is directly proportional to the rate of photosynthesis, leaf area index and light intercepting efficiency. Exposure of mung bean cultivars to supplemental UV-B caused reduction in total biomass. Change in biomass accumulation is an important measure to assess UV-B sensitivity.

Increase in membrane permeability found in all plant exposed to solar UV-B radiation. UV-B radiation induces overproduction of reactive oxygen species (ROS) and results in oxidation of lipids in various plant tissues (Tan *et al*., 2023) [35]. Oxidation of polyunsaturated lipid lead to the formation of MDA. Thus, it could be concluded that MDA accumulation directly link to the membrane leakage (Chaudhary & Rathore, 2020, 2021b) [5-8]. UV-B radiation increases cell membrane permeability in *nannochloropsis* (Sobrino *et al*., 2014) [34].

Solar UV-B radiation negatively influence the Photosynthetic pigments. In terms of interspecific variation it was observed that all the species of *Triticum aestivum* shows more sensitivity towards solar UV-B radiation. Mainly UV-B Radiation affects the photosynthetic pigments, either by inhibiting pigment synthesis or by affecting on the enzymes involved in the chlorophyll (Chl) biosynthetic pathway. The destruction in the photosynthetic pigments directly leads to the decrease in the photosynthetic capacity of plant. Wu *et al*. (2023) [42] found a significant decrease in total chlorophyll content of potato leaves under UV-B stress. Agrawal & Rathore (2007) [1] observe significant decrees in total chlorophyll contents of wheat and mung bean cultivars after exposing to UV-B irradiation. Kataria *et al*. (2014) [18] reported that UV (254, 302 and 365nm) radiation resulted in reduction in the amount of Chl '*a*' as compared to Chl '*b*'and suggested that UV-B may cause selective destruction of Chl '*a*' biosynthesis or degradation of its precursors. A significant reduction of carotenoids was determined in *barley* after UV-B exposure. Carotenoids protect chlorophyll from photooxidative destruction, so a reduction in carotenoids could have serious consequences for the effect of UV-B radiation on Chl pigments (Kataria *et al*., 2014) [18].

Plant cells also contain an important array of non-enzymatic antioxidants such as ascorbic acid and phenolic compounds, for mitigating the toxic effects of ROS. Present study observed that plant in exposure to solar UV-B radiation under polythene filter and open control increase the ascorbic acid content in all species. Similar study reported by Dwivedi *et al*. (2015) [14] that ascorbic acid content in *V. acontifolia* leaves increase after UV-B exposure. Similar finding also reported by Dwivedi *et al*. (2015) [14] observed that ascorbic acid increase on UV-B exposure was observed in Vigna species plant. Increase of foliar ascorbic acid concentration in UV-B exposed plants suggested a defense strategy of plant to UV-B stress. Ascorbic acid is one of the most powerful antioxidants that scavenges the H_2O_2 and other ROS profound (Dwivedi *et al*., 2015) [14]. Tolerance of plant towards UV-B is response of antioxidative enzymes against ROS production and lipid peroxidation. Similar finding also reported in *Oryza sativa* plant species (Sen *et al*., 2021) [32].

Worldwide agricultural productivity is the major issue for growing economy of the countries. Because of agricultural loss due to various biotic and abiotic stress. Therefore minimization of agricultural loss due to stress is the main task for researcher. In the present investigation, ambient UV radiation caused harmful effect to soybean plant. Recently various study reported that abiotic stress such as ozone, salt stress, dust pollution and UV radiation caused harmful effect to plants (Rathore & Chaudhary 2019; Chaudhary & Rathore 2021a, b, c; Nigam *et al*., 2021) [5-10, 30]. While the application of exogenous protectants such as orange Juice, salicylic acid and ascorbic acid improve the plant growth and development of soybean cultivar. The higher effectiveness was found in the natural ascorbic acid (40% OJ) than 20%OJ> 100ppm synthetic ascorbic acid and s salicylic acid > 50ppm ascorbic acid and salicylic acid. Its means that 40% of orange juice enriched with AA will have a useful tool for agricultural sustainability against ozone stress. Data was analyze by PCAs and application of OJ, SAA and SSA showed strong relation with plant physiological characters its means applications of selected protectants is the used full tool for agricultural productivity under UV radiation prone region.

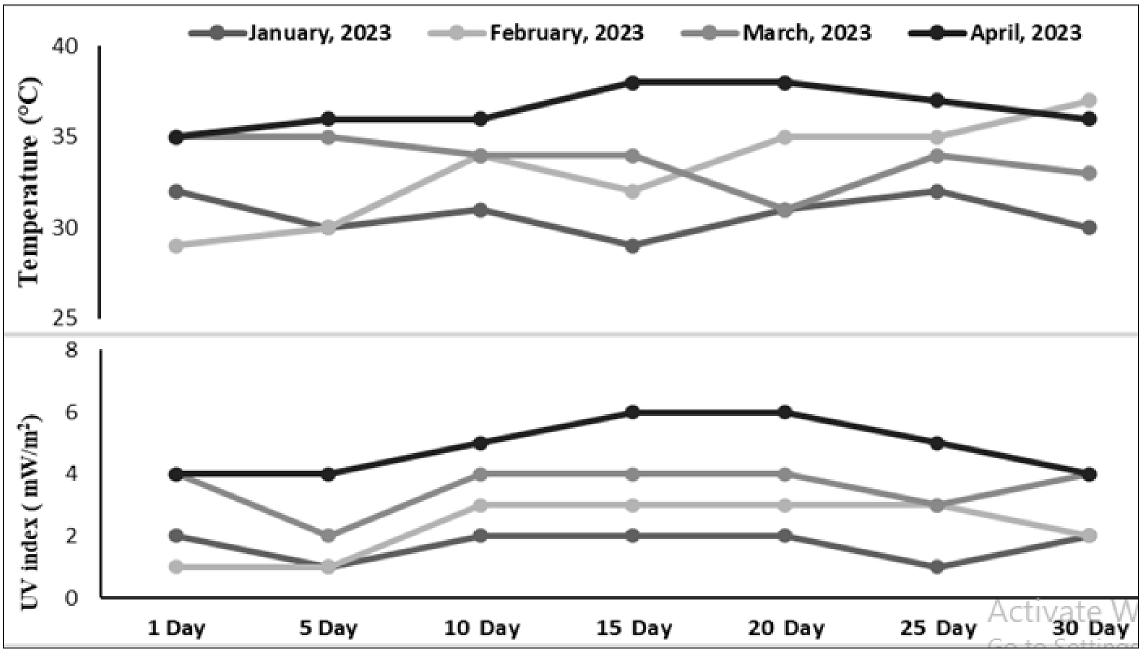


Fig 1: Ambient UV-index and temperature during experimental period

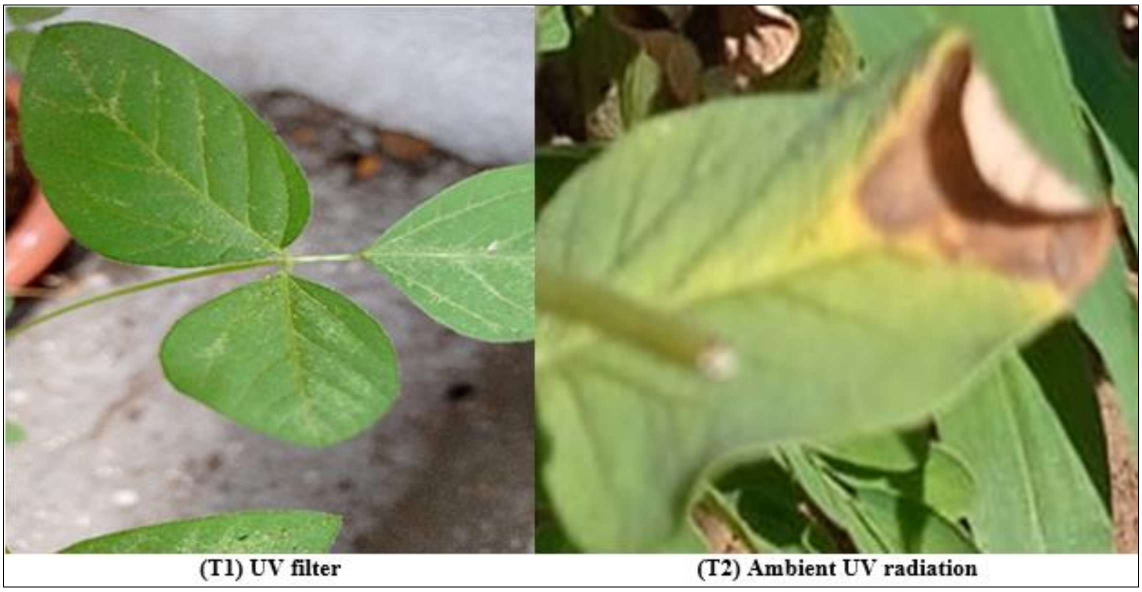
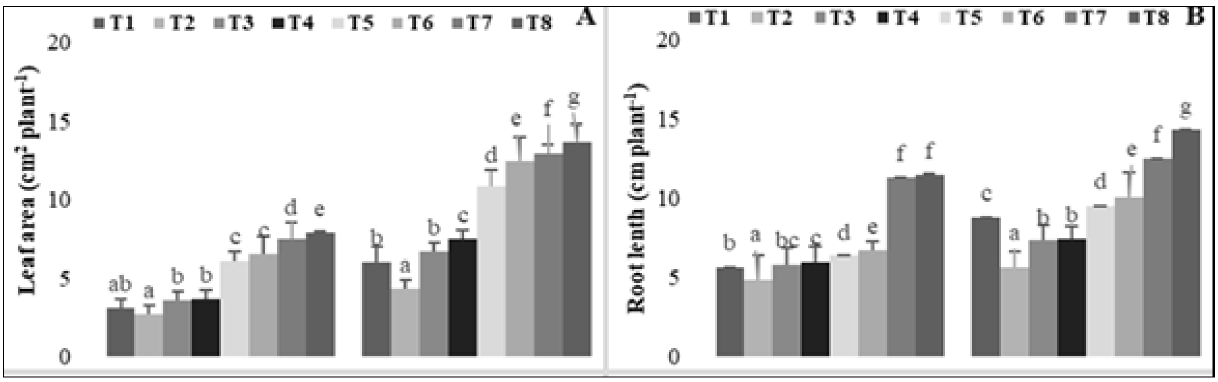


Fig 2: Visible injury due to ambient UV-B radiation on selected cultivar



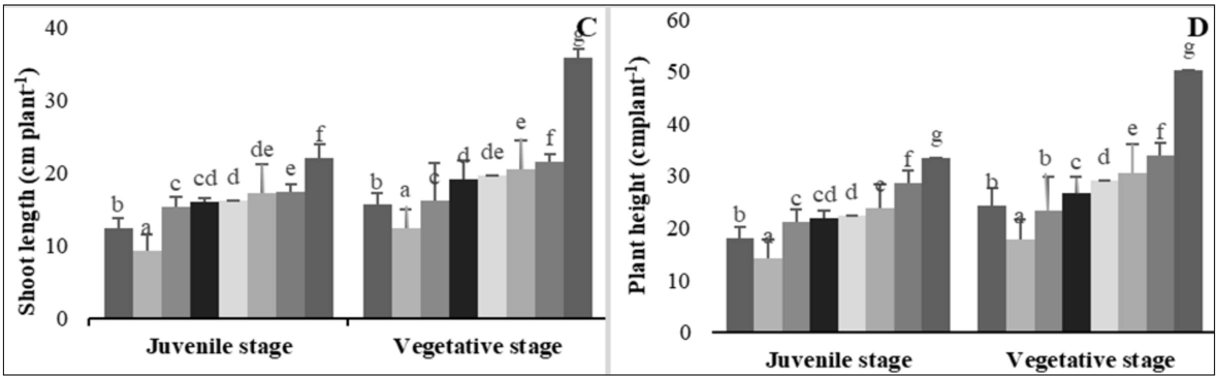


Fig 3: Effect of UV-B radiation and effectiveness of selected protectants on (A) leaf area (cm^2 $plant^{-1}$), (B) root length (cm $plant^{-1}$), (C) shoot length (cm $plant^{-1}$) and (D) plant height (cm $plant^{-1}$) of soybean cultivar (Mean ± standard deviation of three replicates, Value within each column followed by the same letter are not significantly different ($p< 0.05$) using Duncan's Multiple Range Test)

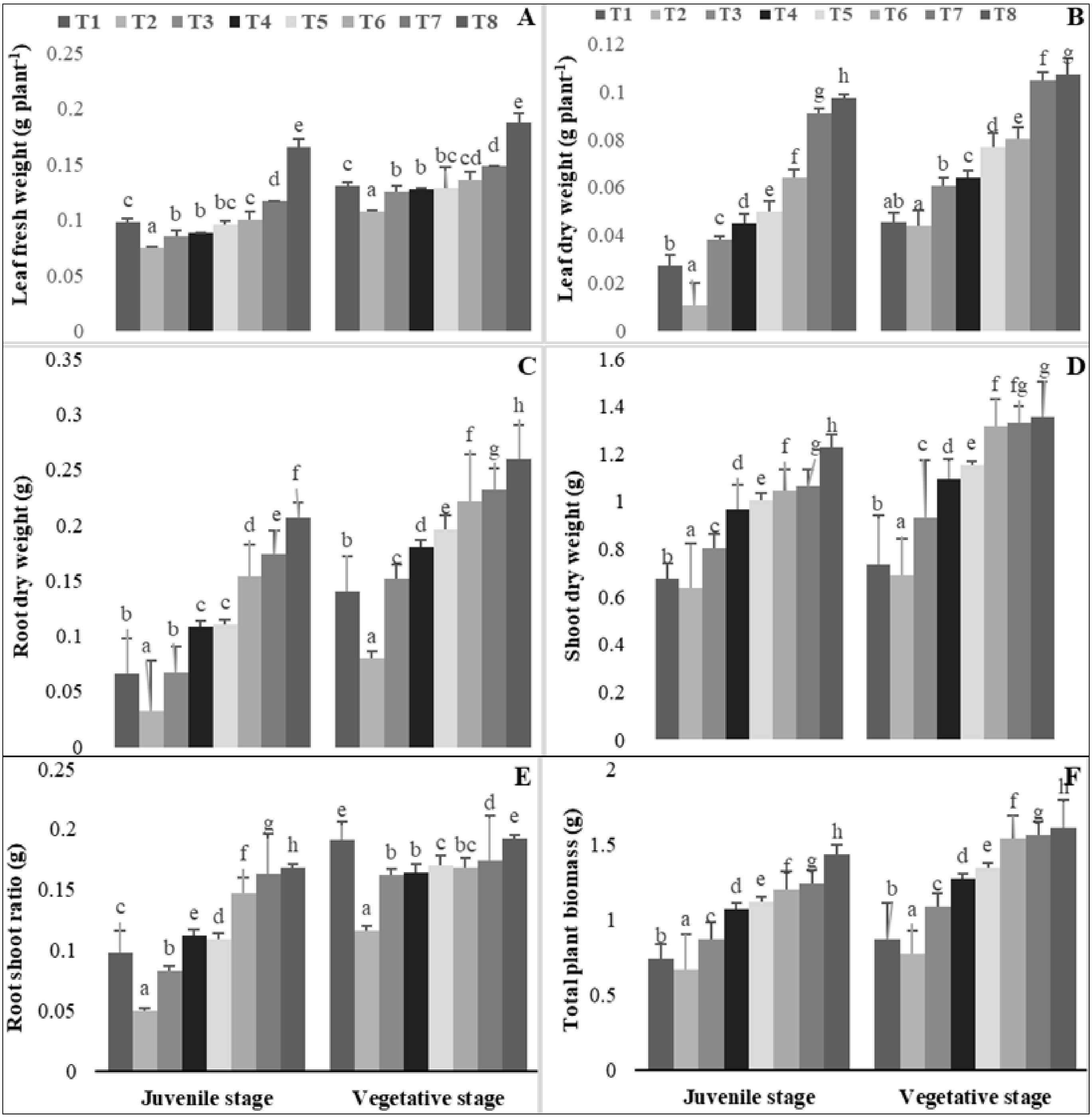


Fig 4: Effect of UV-B radiation and effectiveness of selected protectants on (A) leaf fresh weight (g $plant^{-1}$), (B) leaf dry weight (g $plant^{-1}$), (C) root dry weight (g $plant^{-1}$), (D) shoot dry weight (g $plant^{-1}$), (E) root shoot ratio (g $plant^{-1}$) and (F) total plant biomass (g $plant^{-1}$) of soybean cultivar (Mean ± standard deviation of three replicates, Value within each column followed by the same letter are not significantly different ($p< 0.05$) using Duncan's Multiple Range Test)

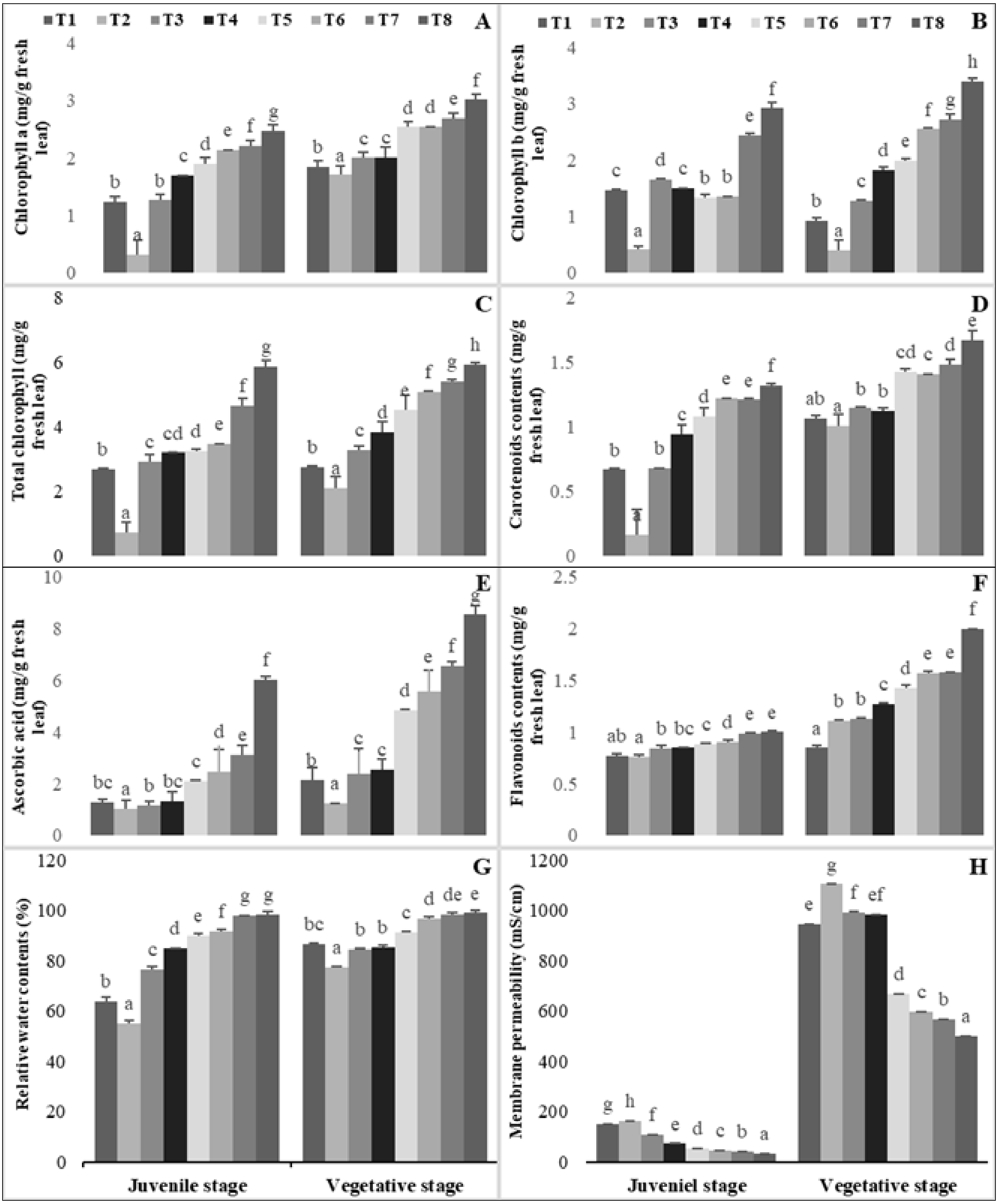


Fig 5: Effect of UV-B radiation and effectiveness of selected protectants on (A) chlorophyll a (mg/g fresh leaf), (B) chlorophyll *b* (mg/g fresh leaf), (C) total chlorophyll (mg/g fresh leaf), (D) Carotenoids contents (mg/g fresh leaf), (E) ascorbic acid contents (mg/ g fresh leaf), (F) flavonoids content (mg/g fresh leaf), (G) relative water content (%) and (H) membrane permeability (mS/cm) of soybean cultivar (Mean ± standard deviation of three replicates, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test)

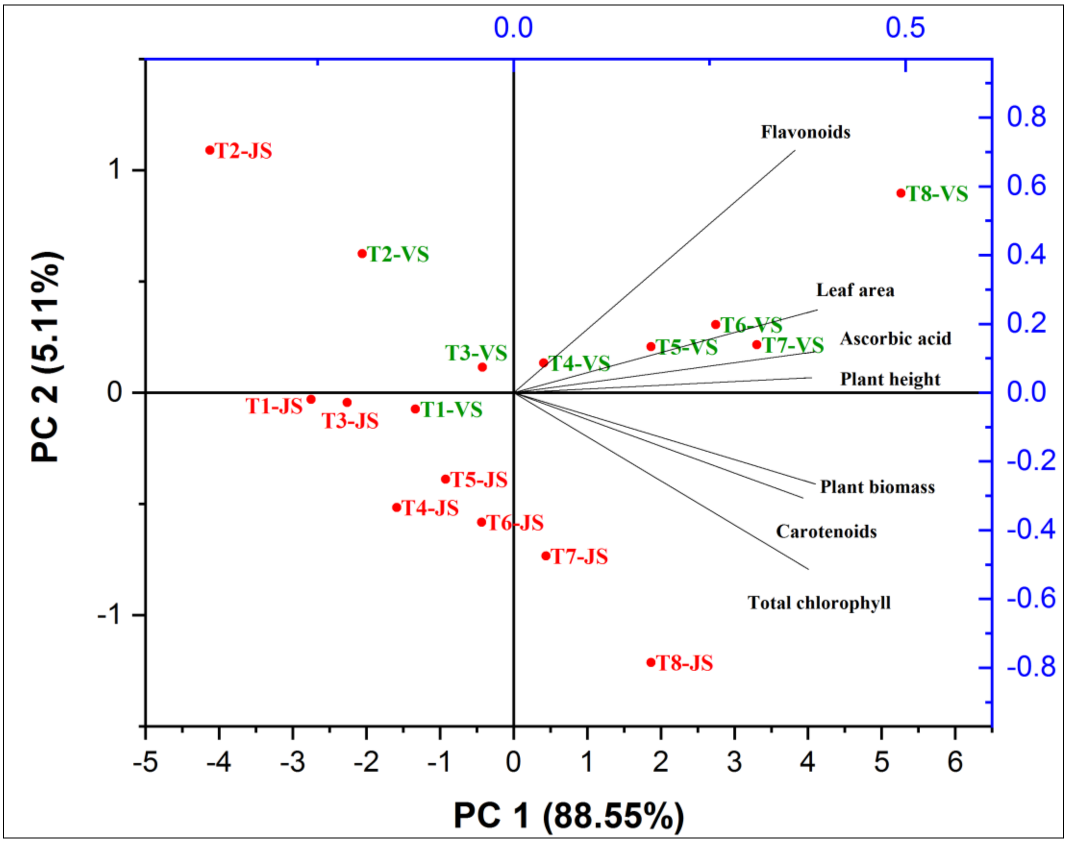


Fig 6: Principle component analysis (PCA) correlation bi-plot of growth, biomass and biochemical responses to UV stress. Symbol represent the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the UV stress and role of protectants on soybean cultivar. Vector coordinates represent the correlations between standardized variables and principle components (PCs)

5. Conclusion

Increase in human originated ozone depletion substances (ODSs) led to disturbance in the natural atmospheric gaseous composition and deplete the stratospheric ozone layer. Major consequences of this depletion are increase incidents of harmful UV radiations reaching to terrestrial surface. Plants being immobile are more prone to solar radiation and ultimately UV-B, thus it affects the growth and crop productivity. The present study indicated that the UV spectral range of solar radiation is an important environmental factor, which has a significant potential to damage the crop plants and eventually reduced growth and biomass. Solar UV-B radiations induce change in biomass and biochemical characteristic of plant. Antioxidative defence mechanism of cultivar defines its response towards UV radiation. Relative effectiveness of protectant is 40%OJ > 20%OJ > 50ppm AA > 100ppm AA > 100ppm SA > 50ppm SA as compared to control plant. Orange juice and peel enriched with ascorbic acid and micronutrients will be affordable and useful tool for assessment of UV-B radiation impact on plants. The concentration of protectant was also play an effective role for growth and development of plants. In presented study 100 ppm synthetic ascorbic acid and salicylic acid play more effective role than 50 ppm. Same trends was also following in natural ascorbic acid (40% OJ > 20% OJ). The present study helps to identify the most effective protectants against UV-B radiation as well as elucidate the molecular mechanism involved in protection of photosynthesis to develop crop models to combat the predicted level of UV-B radiation.

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Declarations

Data availability

Not applicable.

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Conflict of Interest

This work does not include any human subject. This manuscript has not been published and is not under consideration for publication elsewhere. Authors have no conflicts of interest to disclose.

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