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RESEARCH ARTICLE

FOLIAR APPLICATION OF BRASSINOSTEROIDS ENHANCES GROWTH, PIGMENT LEVEL AND BIOCHEMICAL CONSTITUENTS OF *VIGNA UNGUICULATA* UNDER UV-B RADIATION.

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Abstract

The impact of short-term UV-B radiation on growth, pigment composition and photosynthetic activities was studied in *Vigna unguiculata*. UV-B exposure for 3 days/ 15 min at 5 W.m⁻² resulted in decreases the changes on the morphology as well as physiological characteristics. Brassinosteroid (BR) is the endogenous plant growth regulator involved in various physiological processes of plant growth and development. In the present experiment, an attempt has been made to understand the BR induced responses in *Vigna* seedlings. Application of BR at 1µM and 1.5µM significantly enhanced the rate of morphology, photosynthetic and anti-oxidant enzyme activities to a level of 15% to 25% respectively. The UVB treated seedlings were treated with foliar application of BR at 1.0 µM concentration was ameliorative the UVB effects in different growth stages of *Vigna* seedlings.

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Introduction:-

During the last 60 years, stratospheric ozone has decreased by about 5%, mainly due to the release of ozone destroying anthropogenic pollutants such as chlorofluorocarbons resulting in higher levels of UV-B (280–320 nm) radiation at the Earth's surface (UNEP, 2002). Depletion of stratospheric ozone leads to enhanced level of incoming solar UV-B. Current global terrestrial UV-B radiation range between 2 and 12 kJm⁻² d⁻¹ on a given day with near equator and mid-latitudes receiving higher doses with an increase of 6–14% since 1980s. Solar UV-B flux is not uniform throughout the earth's surface but vary gradually from high to low latitude regions. The biological effects of enhanced solar UV-B include reduction in plant growth, photosynthetic activity and biomass. Several investigations of the effects of UV-B on plants have been carried out in response to concern over decreasing global stratospheric ozone and a concomitant increase in tropospheric UV-B, (Bornman, and Teramura, 1993). Inhibitions of growth, photosynthesis and phytomass accumulation are some of the UV-sensitive basic responses. Phytohormone is the growth regulator. It is an organic compound which in small amounts or low concentration promotes, inhibits or qualitatively modifies growth and development. Brassinosteroid is one of the recent known growth hormone. Mitchell *et al.* (1970) initiated screening of pollen for the presence of new plant hormones at the USDA Agricultural Research Center in Beltsville, Maryland, as pollen is a rich source of plant growth regulating substances. BR regulate various aspects of plant growth and development including cell expansion and organ formation, promotion of cell division, regulation of vascular differentiation, promotion of senescence, regulation of fertility, modulation of biotic and abiotic stresses, skotomorphogenesis and photomorphogenesis, timing of flowering and regulation of gene expression (Rao *et al.*, 2002; Sasse, 2003; Ashraf *et al.*, 2010. BR can induce the resistance to wide range of abiotic and biotic stresses such as temperature, salt, drought and fungal infections (Rao *et*

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al., 2002; Krishna, 2003; Bajguz and Hayat, 2009, Vardhini *et al.*, 2010). BR treatment reduced chilling injury in rice, maize and cucumber by enhancing chlorophyll synthesis, osmoregulation and membrane stability in cells (He *et al.*, 1991). Epibrassinolide treatment caused thermotolerance with increased heat shock protein synthesis and accumulation, as well as increased expression of translational machinery in *B.napus* L. seedlings (Dhaubhadel *et al.*, 2002).

Material and Methods:-

Healthy and viable seeds of *Vigna unguiculata* (L.) were purchased from Agricultural Research Institute, Kovilpatti. The percentage of seed germination was found to be 85%. The seeds were sown in pots containing mixture of red soil, black soil, and sand mixed in the ratio of 2: 2: 1.

Foliar spray Treatment:-

BR was obtained from Mount Biosciences technology and solutions (Hyderabad) and initially dissolved in 100 μ l of methanol and concentrations of 0.5×10^{-6} M to 2.0×10^{-6} M were made up using distilled water. The seedlings were sprayed with solutions using an atomic sprayer. Each seedling was sprayed with 5ml of BR solution. The foliar spray was given during the early hours of the day and continued for three days. The analyses were carried out only in 5, 10, 15 and 20 days old seedlings.

UV-B treatment:-

Seedlings (days after emergence) were exposed to short-term UV-B irradiation. The square-wave supplementation system was used to provide desired UV-B radiation, delivered at a constant rate from 0.5 m above the plant canopy for 15min/d for 3 days at 1W.m^{-2} by four fluorescent UV-313 lamps (Q Panel, Cleveland, U.S.A.) driven by 40W dimming ballasts. To filter out the UV-C radiation (<280 nm), the lamps were wrapped with pre-solarized 0.07 mm cellulose diacetate (CA) film and replaced with fresh ones every 4 days to avoid photo ageing. The simulated O_3 depletion was 6-24%. UV-B radiation at the plant surface was $400 \text{mW.m}^{-2}.\text{s}^{-1}$. The amount of energy delivered at the top of the plant canopy was measured with a Spectroradiometer (IL-700, International Lights, U.S.A) equipped with a broadband light sensor (type SEE 400). Although the instrument is outdated, the radiation measurement procedure remained the same as reported by Lingakumar and Kulandaivelu (1998).

Estimation of pigments and anti-oxidant enzymes activity:-

The procedure of extracting of photosynthetic pigments from freshly harvested leaves was the same as that of Lingakumar and Kulandaivelu (1996). To assay the catalase activity (Kar and Mishra, 1976), peroxidase activity (Addy and Good man, 1972), Superoxide Dismutase activity (Bowler *et al.* 1992), Glutathione reductase activity (Foyer and Halliwell 1976), lipid peroxidation (Cakmak and Horst, 1991), Phenylalanine ammonia lyase activity (Zuckerb, 1969).

Result:-

The influence of the hormone over ultraviolet-B (UVB) induced changes in terms of plant morphology, photosynthetic pigments and anti-oxidant enzyme activities was analyzed in cowpea seedlings were selected for the study and were exposed to short-term UVB alone at $400 \text{mW.m}^{-2}.\text{s}^{-1}$.

Effect of 24-eBRL foliar spray on UV-B induced changes in morphological parameters:-

Typical changes in morphology such as shoot length, root length, shoot fresh weight, shoot dry weight and leaf area of *V.unguiculata* seedlings exposed to short-term UVB radiation are shown in Fig.1. With respect to *V.unguiculata*, there was 16-25% decrease in shoot length, observed throughout the growth period under UVB treatment (Fig.1a). Pre-treatment with 24-eBRL at $1.0 \mu\text{M}$ concentration, only 10-18% reduction in shoot length was observed when exposed to UVB (Fig.1b). A 6-8% resistance in shoot length was observed in cow pea seedling. The changes in root length of cowpea exposed to UVB alone and combined treatment with 24-eBRL+UVB are shown in Fig.1c & 1d. UVB treatment alone had caused an 11-19% decrease in root length whereas hormonal pre-treatment caused 8-11% decrease in UVB exposed seedlings. A significant reduction in shoot fresh weight was observed under short-term UVB treatment. The shoot fresh weight of cowpea seedlings exhibited significant decrease amounting to 25-30% throughout stages of growth under UVB treatment (Fig.1e) whereas pre-treatment with 24-eBRL had caused only 20-23% decrease (Fig.1f). The resistance to UVB induced inhibition was nearly 5-7% in cowpea seedlings. The shoot dry weight of the UVB exposed cowpea seedlings decreased to 20-30% throughout the stages of growth (Fig.1g). Pre-treatment with 24-eBRL had caused a significant decrease to 10-16% in shoot dry weight (Fig.1h). A

10-14% resistance was observed due to hormone pre-treatment. The changes in leaf area, exposed to UVB alone and 24-eBRL+UVB are shown in Fig.1i & 1j. UVB treatment alone had caused 13-20% decrease in leaf area (Fig.1i). Pre-treatment with 24-eBRL had caused only 9-15% decrease in leaf area (Fig.1j) indicating nearly 4-5% resistance to UVB action.

Effect of 24-eBRL foliar spray on UV-B induced changes on photosynthetic pigment composition:-

The quantitative changes in photosynthetic pigments such as Chl *a*, Chl *b*, Chl *a+b*, ratio of Chl *a* to *b* and carotenoids of *V.unguiculata* seedlings exposed to short-term UVB radiation are shown in Figs.2. With respect to *V.unguiculata*, there was 10-19% decrease in Chl *a* throughout the growth period under UVB treatment (Fig.2a). Pre-treatment with 24-eBRL at various concentrations induced differential responses in photosynthetic pigments. At 1.0 μ M concentration of 24-eBRL, there was only 5-10% reduction in chl *a* when exposed to UVB (Fig.2b). A 5-9% resistance was observed due to hormone pre-treatment. The changes in chl *b* exposed to UVB alone and 24-eBRL+UVB of cowpea are shown in Fig.2.c&2d. UVB treatment alone had caused 12-15% decrease in chl *b* whereas hormonal pre-treatment caused a decrease in 5, 7, 6 and 9% after 5, 10, 15 and 20 days of plant growth in UVB exposed. A 5-7% resistance was observed due to hormone pre-treatment. The changes in total chl of UVB exposed cowpea seedlings exhibited significant decrease amounting to 13-16% throughout stages of growth (Fig.2e). UVB treatment alone had caused 20-25% decrease in carotenoids content (Fig.2f). Pre-treatment with 24-eBRL had caused only 12-15% decrease in carotenoids content (Fig.2g). Nearly, 8-10% resistance to UVB induced inhibition was observed in 1.0 μ M 24-eBRL pre-treated seedlings.

Effect of 24-eBRL foliar spray on UV-B induced changes in anti-oxidant enzyme activity:-

Changes in catalase activity of *V.unguiculata* seedlings varied with exposure to short-term UVB treatment that caused 30, 33, 35 and 37% increase after 5, 10, 15 and 20 days of plant growth (Fig.3a). At 1.0 μ M concentration of 24-eBRL, there was only 15-22% reduction in catalase activity when exposed to UVB. A 15% resistance was observed due to hormone pre-treatment. The changes in peroxidase activity of *V.unguiculata* seedlings that was treated in exposed to short-term UVB treatment. UV-B treatment caused 15, 17, 20 and 23% increased after 5, 10, 15 and 20 days of plant growth (Fig.3b). At 1.0 μ M concentration of 24-eBRL, there was only 8-13% reduction in peroxidase when exposed to UVB. A 7-10% resistance was observed due to hormone pre-treatment (Fig.3c&d). SOD activity of *V.unguiculata* seedlings that was treated in exposed to short-term UVB treatment. UV-B treatment caused 22-25% increased after 5, 10, 15 and 20 days of plant growth (Fig.3e). Pre-treatment with 24-eBRL, proved to be effective in relieving the UV-B inhibition. At 1.0 μ M of 24-eBRL, there was only 8-12% reduction in SOD activity when exposed to UVB. A 14% resistance was observed due to hormone pre-treatment (Fig.3f). MDA content of *V.unguiculata* seedlings increased to 23, 29, 43 and 50% after 5, 10, 15 and 20 days of plant growth exposed to short-term UVB treatment (Fig.3g). At 1.0 μ M of 24-eBRL, there was only 15-23% reduction in MDA content when exposed to UVB. A 8-27% resistance was observed due to hormone pre-treatment was observed due to hormone pre-treatment (Fig.3h). PAL activity in UVB treated *V.unguiculata* seedlings increased to 43, 47, 51 and 53% after 5, 10, 15 and 20 days of plant growth (Fig.3i). Upon epibrassinolide hormone treatment, (1.0 μ M of 24-eBRL), there was only 20-33% reduction in PAL activity in UVB treated seedlings. The hormone pre-treatment had conferred nearly 23% resistance in the enzyme activity (Fig.3j)

Elemental analysis of leaf samples by AAS:-

The leaf samples of UVB and hormone treated seedlings were subjected to AAS for the analysis of important minerals like magnesium and calcium. AAS analysis of 20 days old seedlings showed that both magnesium and calcium level increased in 24-eBRL pre-treated *Vigna* seedling (Table 1).

Discussion:-

24-eBRL induced ameliorative changes in morphological parameters:-

24-eBRL was given as foliar spray to control (-UV) and short-term UV-B irradiated *Vigna* seedlings. In the present study, it was observed that different concentrations such as 0.5 to 2.0 μ M of hormonal pre-treatment of 24-eBRL enhanced the vegetative growth in terms of shoot, root length, shoot fresh, dry weight and leaf area. The shoot, root length, shoot fresh and dry weight ameliorative at 1.0 μ M of 24-eBRL treated at 5, 10, 15 and 20 days growth seedlings. 24-eBRL significantly increases the fresh masses of shoots and roots in mung bean under an AI stress (Ali *et al.*, 2008a). The plants treated with BR possessed more surface area which could mainly be an expression of induced cell division and cellular enlargement, increases in leaf area in response to hormonal treatment (Sairam, 1994). Kumar *et al.* (2012) reports that *B.juncea* seedlings showed susceptibility to temperature stress. After

temperature stress treatment to seedlings, growth (e.g. shoot and root length) was inhibited. 24-eBRL treatment helped to mitigate stress and increased the morphological growth.

24-eBRL induced ameliorative changes in photosynthetic pigment content:-

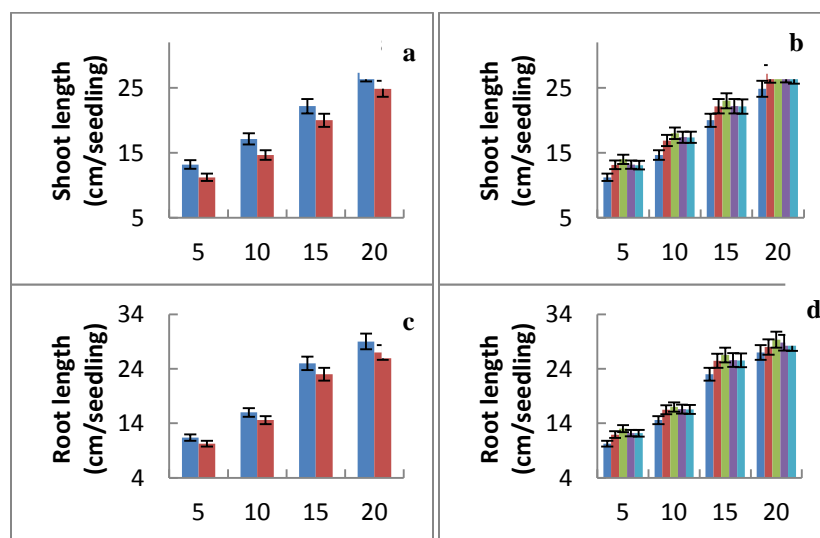
The content of photosynthetic pigments such as chl *a*, chl *b*, chl *a+b*, and carotenoids are ameliorated at 1.0 μ M of 24-eBRL concentration and it has ameliorated the UVB impacts. Li *et al.* (2012) demonstrated that the leaves of soyabean drought stressed plants, a BR treatment increases the maximum quantum yield of photosystem II, the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase. Allen and Ort, (2001) found that chilling stress has direct impacts on the photosynthetic apparatus, essentially by disrupting the thylakoid electron transport, carbon reduction cycle and stomatal control of CO₂ supply, the eBR treated alleviated the chilling stress.

24-eBRL induced ameliorative changes in anti-oxidant enzymes activities:-

The activity of CAT is largely determined by photon flux density, concentration of CO₂, availability of Zn (Tiwari *et al.*, 2005) and gene expression (Kim *e al.*, 1994). On the other hand, BRs enhance the activity of CAT and reduce the activities of POX and ascorbic acid oxidase under osmotic stress in sorghum (Vardhini and Rao, 2003) and regulate secondary metabolism in tomato which may enhance tolerance to phenanthrene (Ahmed *et al.*, 2013). Ameliorative properties of 28-HBL were deliberated in *R.sativus* L. under chromium toxicity mediated by regulating the peroxidase and help in mitigating the toxic effects of chromium (Sharma and Bhardwaj, 2007; Sharma *et al.*, 2011). Yusuf *et al.* (2011) indicates that protective role of 28-HBL in cultivars of *T.aestivum* in Ni stressed plants. SOD is a key enzyme in the detoxification of superoxide radicals which might mitigate the toxic free-radical concentration to decisive levels to protect plant from its detrimental effects of plants (Mazorra *et al.*, 2002). Sirhindi *et al.*, (2009) reported that homobrassinolide at 10⁻¹⁰ M concentration ameliorated SOD activity significantly as compared to other BRs and control seedlings. Sharma and Bhardwaj (2007) reported maximum increase in SOD activity in seedlings treated with 10⁻⁷ M 28-HBL under Cu metal stresses. Formation of MDA and other aldehydes in plants exposed to UVB stress is a reliable indicator of free radical formation in the tissue and is currently used as indicator of lipid peroxidation (Meirs *et al.*, 1992; Borsanio *et al.*, 2001). Sgherri *et al.* (2000) found that the MDA content increased in 3 genotypes of wheat under drought stress. BR pretreated plants that were thereafter put under drought stress has much less H₂O₂, MDA content than drought stressed plants (Behnamnia *et al.*, 2009).

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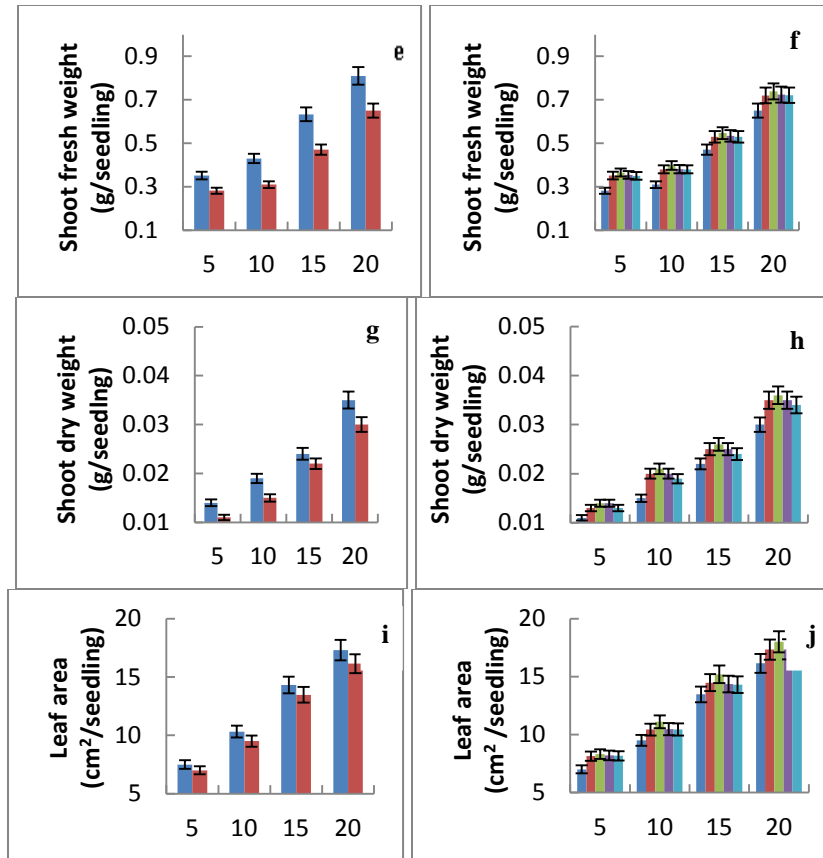
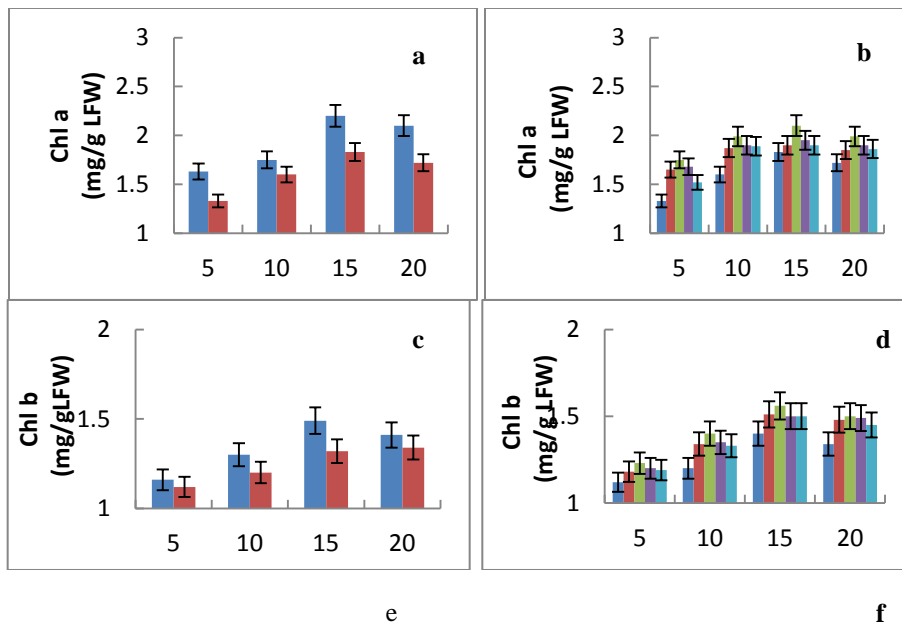


Fig.1:-Profile of morphological parameters in short-term UVB exposed and epibrassinolide treated *V.unguiculata* seedlings grown in ambient daylight conditions. The seedlings were exposed to UVB at 1W.m⁻² for 15 min/d for 3 days and eBRL at 0.5 μM, 1.0 μM, 1.5 μM and 2.0 μM. The values are an average of three independent measurements and significantly different at $p < 0.05$. Con: control; 0.5eBRL +UVB, 1.0μM+UVB, 1.5μM+UVB and 2.0μM+UVB.



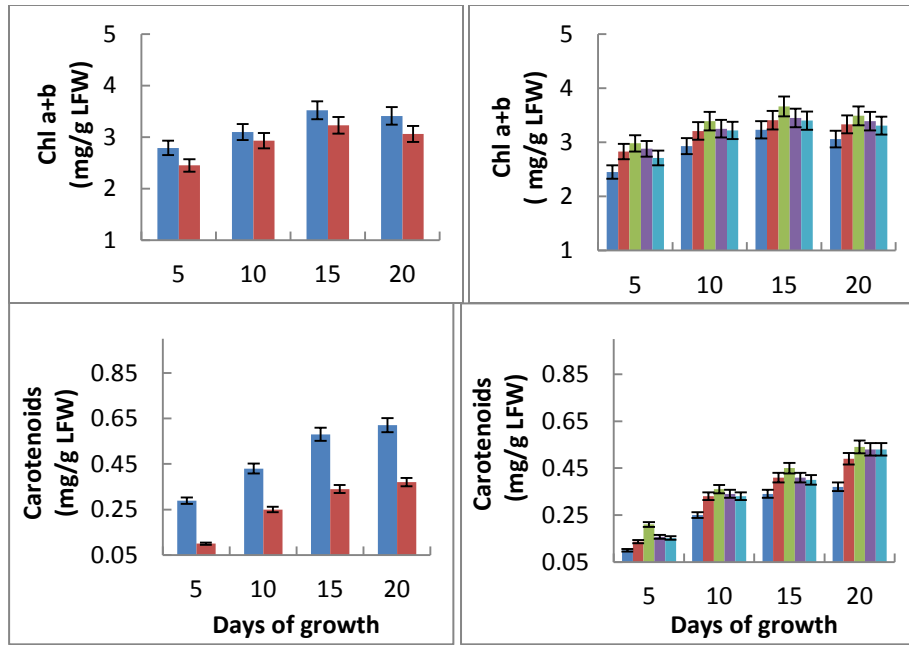
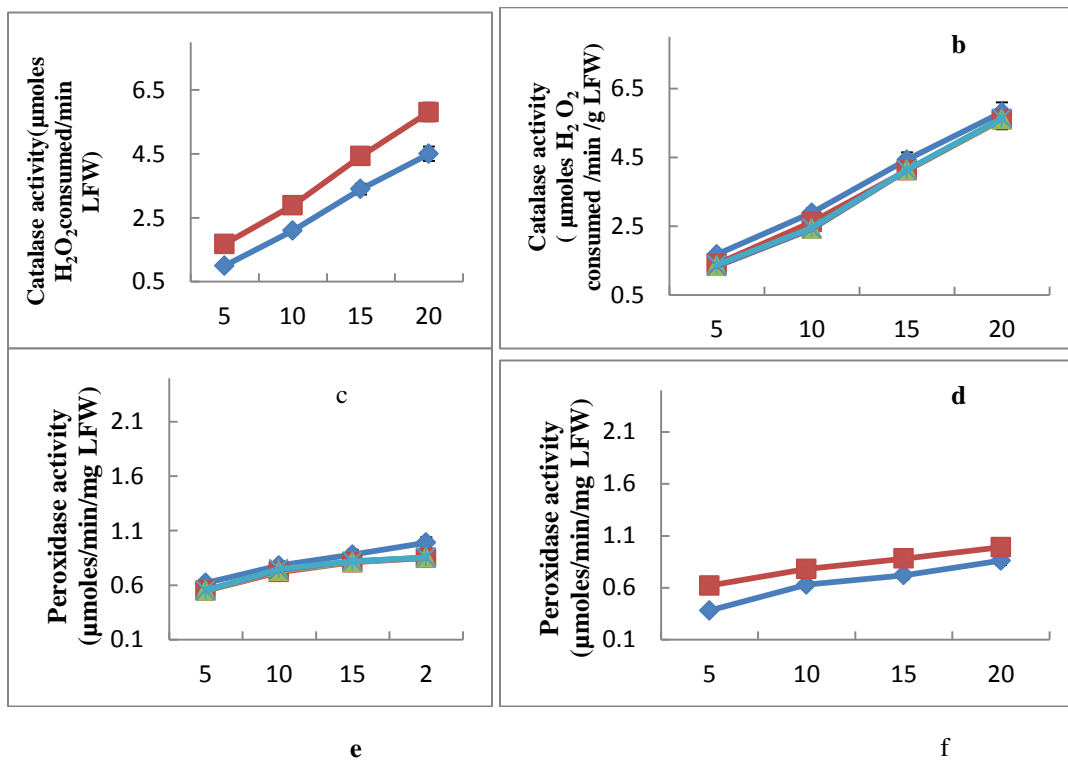


Fig.2:-Profile of photosynthetic pigments in short-term UVB exposed and epibrassinolide treated *V.unguiculata* seedlings grown in ambient daylight conditions. The seedlings were exposed to UVB at $1W.m^{-2}$ for 15 min/d for 3 days and eBRL at 0.5 μM , 1.0 μM , 1.5 μM and 2.0 μM . The values are an average of three independent measurements and significantly different at $p < 0.05$. con: control; UVB: Ultraviolet-B; 0.5 μM +UVB, 1.0 μM +UVB, 1.5 μM +UVB and 2.0 μM +UVB.



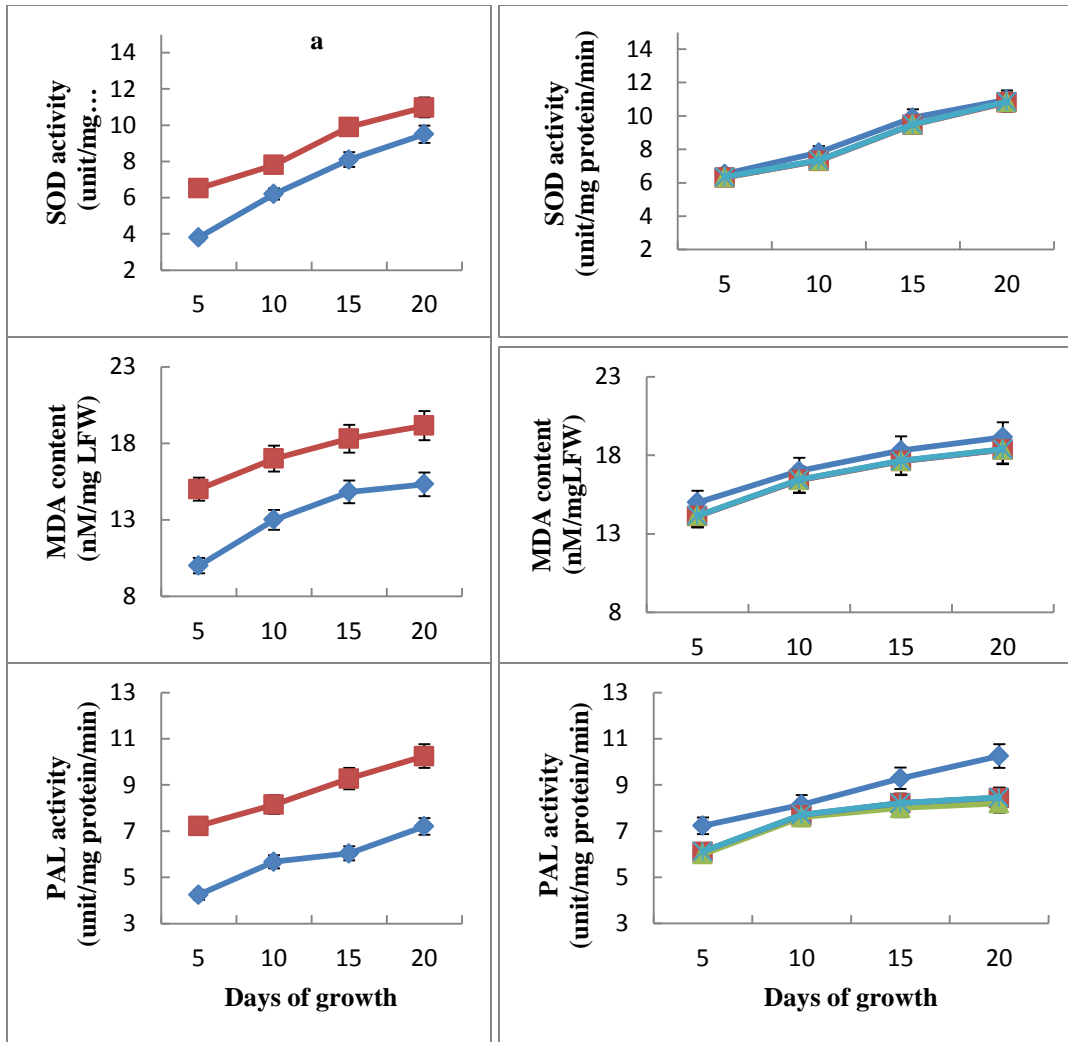


Fig. 3:-Profile of anti-oxidant enzyme activities in short-term UVB exposed and epibrassinolide treated *V. unguiculata* seedlings grown in ambient daylight conditions. The seedlings were exposed to UVB at 1W.m⁻² for 15 min/d for 3 days and eBRL at 0.5 μM, 1.0 μM, 1.5 μM and 2.0 μM. The values are an average of three independent measurements and significantly different at *p*< 0.05. con: control; UVB: Ultraviolet-B; 0.5μM +UVB, 1.0μM+UVB, 1.5μM+UVB and 2.0μM +UVB.

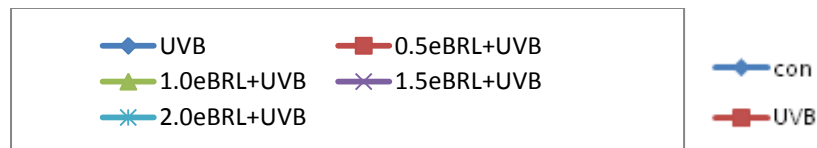


Table. 1:-The effect of UVB and 24-eBRL foliar spray (1.0μM) on Mg and Ca content in cowpea

Cowpea	Calcium (ppm/gLFW)	Calcium (ppm/gLFW)
Control	150.55±0.04	31.33±0.05
+UVB	89.45±0.02 (59%)	26.47±0.03 (84%)
24-eBRL (1.0μM)+UVB	128.07±0.01 (+43%)	30.38±0.02 (+14%)

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