CUMULATIVE INTERACTION OF BRASSINOSTEROIDS AND SILICON IN MITIGATING LEAD METAL TOXICITY IN *TRIGONELLA FOENUM-GRAECUM* L.

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Botany

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LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

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DECLARATION

I, hereby declare that the presented work in the thesis entitled “Cumulative Interaction of Brassinosteroids and Silicon in Mitigating Lead Metal Toxicity in *Trigonella foenum-graecum* L.**”** in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision Dr Neeta Raj Sharma, working as a Professor, in the Department of Botany/School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled “Cumulative Interaction of Brassinosteroids and Silicon in Mitigating Lead Metal Stress in *Trigonella foenum-graecum* L.” submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Botany, School of Bioengineering and Biosciences, is a research work carried out by Dhriti Sharma, 41900305, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

Lead (Pb) toxicity affects the quality and productivity of crop plants all over the map. Taking this into consideration, the present experimental work was accomplished to investigate the ameliorative efficiency of 24-EBL and Si in combating Pb induced stress in fenugreek plants. To begin with, a pre-treatment of surface sterilized fenugreek seeds with a) 10^{-7}M 24-EBL b) distilled water was carried out for 8 hour duration. Lead metal was supplied in three distinct concentrations. Supplementation of Si was performed at a concentration of 2 mM in both the types of treatment stages i.e. *in vitro* and *in vivo*. Further, *In vivo* harvesting of fenugreek plants was carried through 15, 30 and 45 days old stages to assess different traits pertaining to morphology, biochemistry and molecular biology.

Measurement of discrete morphological characters along with ascertainment of photosynthetic pigments was performed. Additionally, content of metabolites plus indicators of oxidative harm were evaluated. Membranal and nuclear impairments in *In vitro* grown seedlings were ascertained with confocal microscope. Endogenous measures of Pb metal, carbohydrates, osmolytes with that of proteins were assessed. Antioxidant system of defense plus the expression levels of stress-linked genes was also put to evaluation.

Lead stress brought about a decline in the attributes related to plant growth, photosynthetic efficiency together with quantities of different metabolites. Furthermore, the toxicity induced by Pb scaled up the production levels of oxidative harm indicators leading to impairment of membranal plus nuclear components. Measures of Pb metal, carbohydrates, osmolytes, proteins along with constituents of antioxidative defense, exhibited significant alterations in response to Pb induced stress. However, ameliorative i.e. EBL and Si treated fenugreek plants were recorded to have enhanced growth parameters, photosynthetic efficiency, together with contents of osmolytes, carbohydrates, and proteins. Diminution of ROS synthesis plus upregulation of activity of diverse antioxidants under stressful environments was also noticed. Escalation in expression extent of stress-linked genes (SOD and CAT) was recorded in EBL and Si supplemented stressed plants.

Comprehensively, it was deduced from this experimental work that cumulative application of 24-EBL and Si could be viably utilized as a useful mitigation strategy to combat Pb

mediated negative influence in *Trigonella foenum-graecum* through improvement of different attributes concerned with its morphology, physiology, biochemistry and molecular nature.

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“Have thine own way, My lord,

Have thine own way………

Thou are the potter and

I am the clay…….”

Dhriti

(Dhriti Sharma)

Abbreviations

DNPH	2,4-Dinitrophenylhydrazine
DAPI	4,6-diamino-2-phenylindole
$AlCl_3$	Aluminum chloride
As	Arsenic
APX	Ascorbate peroxidase
AAS	Atomic absorption spectrophotometer
BRs	Brassinosteroids
Cd	Cadmium
CO	Carbon monoxide
CAT	Catalase
Co	Cobalt
Cu	Copper
Cm	Centimeter
Cr	Chromium
DHAR	Dehydroascorbate reductase
DCF	Dichlorofluroscein
DDW	Double distilled water
24-EBL	24-Epibrassinolide
EDTA	Ethylenediamine tetraacetic acid
POD	Guaiacol peroxidase
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
OH-	Hydroxyl
Fe	Iron
Hg	Mercury
µg	Microgram

μM	Micromolar
Mg	Milligram
mM	Millimole
Pb	Lead
Lsi1	Low silicon rice 1
MDA	Malondialdehyde
Mn	Manganese
MDHAR	Monodehydroascorbate reductase
MMA	Monomethylarsonic acid
Ni	Nickel
HNO_3	Nitric acid
NO	Nitric oxide
NBT	Nitroblue tetrazolium
$HClO_4$	Perchloric acid
PS II	Photosystem II
PPO	Polyphenol oxidase
PBG	Porphobilinogen
PCA	Principal component analysis
PI	Propidium iodide
P5CS1	Pyrroline-5-carboxylate synthetase
ROS	Reactive oxygen species
H_4SiO_4	Silicic acid
Si	Silicon
NaOH	Sodium hydroxide
$NaNO_2$	Sodium nitrite
SNP	Sodium nitroprusside
O^{2-}	Superoxide

SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
TMAsO	Trimethylarsine oxide
WUE	Water use efficiency
Zn	Zinc
GABA	γ-aminobutyric acid

List of Legends

Abbreviations	**Treatment**	**Legend**
CN	Control	□
T_1	Pb I	▨
T_2	Pb II	░
T_3	Pb III	▩
T_4	24-EBL	🟦
T_5	24-EBL + Pb I	▨
T_6	24-EBL+ Pb II	░
T_7	24-EBL+ Pb III	▩
T_8	Si	🟧
T_9	Si + Pb I	▨
T_{10}	Si+ Pb II	░
T_{11}	Si + Pb III	▩
T_{12}	24-EBL+Si	🟩
T_{13}	24-EBL+Si+Pb I	▨
T_{14}	24-EBL+Si+Pb II	░

T_{15}	24-EBL+Si+Pb III	▩

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Chapter 1
Introduction

Growth of plants gets restrained by abiotic stresses of diverse types ranging from extremes of salt, temperature, water and heavy metal etc., which negatively influence the different facets of plants' life cycle (Dutta et al., 2018; Khan et al., 2019a). Abiotic stresses adversely affect crop productivity via causing a decline in plant biomass. With accelerating rates of modernization, developmental activities and usage of agrochemicals, metal pollution becomes a grave concern among all the other environmental constraints (Zhai et al., 2018). Frequency of heavy metal contamination of the environment is quite high nowadays which gives rise to various health concerns (Arif et al., 2019). Food security has been greatly threatened on account of heavy metal toxicity as it restricts the process of developing new cultivars (Javaid et al., 2020). Therefore, expansion of existing system of growing crops by about 70- 100% becomes utmost important for catering to the requirements of our ever soaring world population (Zhou et al., 2020). In this context, removing the pernicious elements or immobilizing them would really serve the purpose of reducing their levels in degraded farmlands. Meddling with the environment by releasing toxic wastes of industries, disposal of sewage, usage of pesticides and insecticides etc. have contributed in adding noxious metals and metalloids like arsenic (As), lead (Pb), mercury (Hg), copper (Cu), cadmium (Cd), nickel, (Ni) and manganese (Mn) and etc. into the soil which either accumulate or leach them into groundwater resources (Aydinalp & Marinova, 2009; Basheer, 2018)

Persistence and non-biodegradability of these destructive elements make their natural removal from the environment quite difficult. Some of these metals are readily absorbed by plant roots because of their mobile nature while some metals are simply immobile and do not leave their place of accumulation in the soil (Alharbi et al., 2018b; Burakova et al., 2018). Because of their requirement in minute quantities, a handful of these elements do qualify as essential micronutrients e.g. Cu, Ni, Zn etc. but others such as Pb, Cd prove toxic beyond permissible limit and not confer any kind of benefit to the plants (Ali et al., 2017). Some plants are reported to stock up and extract these noxious elements via the process termed as phytoremediation and are recognized as bio accumulators (Ali et al., 2013a).

Heavy metal lead (Pb) is placed at second position after arsenic (As) in the "substance priority list, 2022" of ATSDR (Agency for Toxic Substances and Disease Registry) considering its frequency, toxicity potential and capability of affecting human health.

Activities like making of ammunition, bangles, batteries, metallic water pipes, coal fuelled thermal power plants, mining, smelting, soldering, smoking etc. in addition to Pb being quite substantially used in paints and petrol have contributed in elevating the natural levels of this metal in the environment. The Pb measures in river Yamuna get as far as 55.7 ppm post-monsoon (Rajankar et al., 2014). In 2010, water samples from rivers Ganga and Brahmani were recorded to have 120µg/l and 12.08µg/l of Pb (Aktar et al. 2010; Reza and Singh 2010). Pollution of soil and water resources by Pb eventually gets transferred to the food. As per a report published in Hindustan Times (26 July, 2019), vegetables growing in floodplains of river Yamuna had Pb in the range of 14.1 mg/kg well beyond the safe limit i.e. 2.5 mg/kg. In Uttar Pradesh, samples of noodles with Pb contamination (17.2 ppm) were collected (Garg, 2015).

Additionally, this metal along with its compounds are graded as potentially capable human carcinogens (NTP, 2016). Pb is further reported to negatively harm the neurological and renal system. Likelihood of lung cancer enhances at blood Pb level as low as 10µg/dL (ATSDR, 2019). However, recommended limit of Pb in the blood of children has been now set at 5µg/dL by Centre for Disease Control and Prevention (CDC), which was at 10µg/dL way back in 2012. Pb toxicity in children lowers down their IQ, attention span and academic performance. Effects of Pb toxicity have not only been observed in human beings but also in crop plants whereby their growth and productivity suffers.

Atmospheric Pb being present in the form of particulate matter is perceived by the soil with the precipitation. It especially contaminates the topmost layer of soil but gravitational settling and percolation pollute the deeper layers as well. With groundwater receiving Pb in minimal quantities, soil and deposits of atmospheric fallout are the major source of Pb for the plants. Inside the plants, this heavy metal interferes with various physiological processes giving rise to stressful conditions of destruction of chlorophyll, necrotic lesions, reduction in photosynthesis, impaired water and nutrient uptake, inhibition of enzymatic activities, apoptosis, hampering of antioxidative defense system and alterations in gene expression, all leading to underdevelopment (Hadi and Aziz, 2015; Pinho and Ladeiro, 2012).

For carrying out deactivation of enzymes, Pb alters their tertiary structure and blocks catalytic sites via coupling of proteins with carboxylic acid groups (Gupta et al., 2010).

Externally, Pb toxicity is majorly indicated by stunted growth, contorted and swollen nature of secondary roots (Kopittke et al., 2007). It further hampers uptake plus sequestration of essential nutrients (Ca, Fe, Mg, Mn and P etc.) by making them non-available through obstructing their entry or binding them to other ion carriers (Xiong, 1998). Water status is also deranged in plant cells upon getting subjected to Pb stress (Singh et al., 2010). Additionally, Pb toxicity induces excessive generation of harmful free radicals (Reactive oxygen species) which subsequently are scavenged by triggering the innate defense system comprised of antioxidants of enzymatic (Catalase, reductase, peroxidise, dismutase, transferase etc.) and non-enzymatic nature (ascorbic acid/Vit. C, glutathione/GSH) (Sharma et al., 2011; Gupta et al., 2010). Scavenging of ROS by these enzymes takes places inside cellular compartments (Islam et al., 2008).

Another point to consider is that plants combat heavy metal induced stress by regulating the measures of osmoprotectants, phenolics, compatible solutes and the compounds capable of metal chelation. Sulfur mechanisms also become operational for protecting the plants from abiotic stresses. Chelating procedures reduce the accessibility of metal ions by converting them into sulfur comprising compounds like thiols (proteinaceous and non-proteinaceous), metallothionines (MTs) and phytochelatins (PCs) (Misra et al., 2009). All these defensive activities on the part of the plants turn inefficient if the metal stress become prolonged and severe.

A number of effective strategies are in practice to battle out Pb generated stress in economically significant crop cultivars, which involves developing the genotypes with Pb-tolerance (Zhang et al., 2020; Gupta et al., 2013) by employing diversified conventional procedures like selection, hybridisation, breeding and even genetic engineering. However, these traditional breeding mechanisms are tedious and time- consuming (Ahmed et al., 2021; Mourad et al., 2019; Salem and Sallam, 2016). Therefore, more effective and ecologically safe strategies are the need of the hour to ensure better crop productivity and thereby food security. With regard to this, supplementing the plants exposed to Pb stress with the steroidal phytohormone, 24- Epibrassinolide (24-EBL) and a mineral metalloid Silicon (Si) is a favourable approach to lower down the absorption of Pb ions by the plant roots for improvement in growth and yield. Applying such phytoprotectants cumulatively could emerge as an efficient stress ameliorative strategy against the negative aftermaths of Pb contamination.

Brassinolide and other compounds derived from it, are together termed as brassinosteroids/BRs (Mandava, 1988). They are a novel class of growth regulators, first extracted in the seventies from pollens of rapeseed plant (*Brassica napus* L.) by Mitchell et al. Since their isolation, over 80 other naturally occurring free molecules or conjugates of this group, all belonging to botanical origin, have been recorded so far together with more than 130 structural or functional analogues (Kvasnica et al. 2019; Liu et al. 2017). In conjugated form, molecules of BRs combine with hexose sugars or fatty acids. Chemically, BRs are steroids (campesterol derivatives) and exclusive to the plants only (Kanwar et al., 2013). Taking into account the numbers of carbons forming the steroid molecule, BRs have three major classes- a) $C_{27;}$ b) C_{28} and c) C_{29} (Fujioko and Yokota, 2003). All of them, differ from each other in having different basic skeleton of 5α-cholestane in case of C_{27}; 5α-ergostane in C_{28}; with that of 5α-stigmastane in C_{29}. This variance is due to dissimilarity in type and alignment of oxygenated functional moeties of its A & B rings along with number plus position of various functional groups on the side chains. All these variations arise because of redox reactions, which took place during their biosynthesis. Additionally, structure wise, they do resemble other steroidal hormones of animal origin as the likes of corticoids, ecdysteroids, estrogens and androgens.

Further, they are reported to occur in the entire spectrum of plant diversity encompassing lower to higher plants, especially the angiospermic ones and also inside every plant organ ranging from roots, stems to leaves, flowers, pollens and seeds (Zullo and Bajguz, 2019; Yokota et al., 2017). Highly dynamic BRs include Brassinolide (BL), 24-epibrassinolide (24-EBL) along with 28- homobrassinolide (28-HBL) and are extensively utilized in various experimental works (Vardhini et al., 2006). They are principally formed inside growing tissues and are involved in almost every phase of growth and development in plants. For instance, these have a say over division, elongation and differentiation of cells, photomorphogenesis, acceleration in enzymatic activities, development of vasculature, lengthening of pollen tubes, process of senescence and resistance against stress (Baghel et al., 2019; Furbank et al., 2015; Bhardwaj et al., 2014; Bari and Jones, 2009; Yu et al., 2004).

A wide range of structural and functional responses are elicited by BRs to enable the plants for battling out stresses of both the types i. e. biotic and abiotic (Nolan et al, 2020; Ahanger et al., 2018; Siddiqui et al., 2018; Wei and Li, 2016; Bajguz and Piotrowska-Niczyporuk, 2014). In conjunction with Gibberellic acid (GA), BRs exhibit similar responses in terms of promoting seed germination and neutralizing ABA inhibition, however, separate pathways are adopted by them for promoting seed germination. BRs cast their influence in stimulating embryo by being independent of GA (Leubner-Metzger, 2003). Comprehension the generation of brassinolides and its various analogues facilitated in elucidating its functional prospects on individual basis as well as in cumulative manner along with other phytoprotectants

Applying BRs exogenously to combat stress conditions has become a promising strategy to realize the goal of enhancing the crop yield together with protecting the plants ecologically (Shahzad et al., 2018). Functional efficacy of BRs in influencing the different aspects of growth has been recorded against diverse array of stressful conditions as created by salinity (Larré et al., 2015), temperature extremes (Gornik and Lahuta, 2017), water deficit/excess (Shahana et al., 2015; Mahesh et al., 2013), and heavy metals- Al (Madhan et al., 2014; Abdullahi et al., 2002), As (Raghu et al., 2014), Cd (Piacentini et al., 2022; Anusha et al., 2015; Hasan et al., 2008; Janeczko et al., 2005), Cr (Sharma et al., 2011), Cu (Yadav et al., 2016), Ni (Lukatkin et al., 2013), and Pb (Rao & Raghu, 2016; Anuradha and Rao, 2011).

Heavy metal stress exposed plants when exogenously supplied with BRs, exhibit activation of anti-oxidative defense mechanisms while reducing the concentrations of these toxic elements. The capability of BRs to modulate the membranal permeability and getting linked to the membranal proteins could be possibly operational behind this (Sharma et al., 2008). To accelerate the process of detoxification, enhancement in metabolic activities, especially the enzymatic ones, have been noticed. The favourable impacts of brassinosteroids over the biochemical as well as physiological facets of plants' growth in reference to heavy metal toxicity are mirrored in the form of better morphological parameters: up-scaling of germination, shoot plus root length, dry biomass, together with the productivity (Shahzad et al., 2018; Vardhini et al., 2010).

Silicon (Si), a metalloid of group 14 in periodic table having atomic no.-14 and atomic weight-28.085 u which ranks second in terms of abundance after oxygen in earth's crust. It brings about various favourable outcomes in plants, hence drawing greater attention of modern day agriculturists (Deshmukh et al., 2017). Existing in two different allotropic forms, its other physical properties include a specific gravity of 2.42 and a melting temperature of 1420 °C (Sommer et al., 2006). With commonly found in an oxidation state of +4, its other two oxidation states are −4 and +2. Additionally, it has 4 isotopes with Si-28, 29 and 30 as stable ones and Si-32 as radiogenic in nature (Tubaña & Heckman, 2015; de Groot, 2004). Major compounds of Si which normally gets accumulated in soil are silica (silicon dioxide) besides its modified forms i.e. silica gel and silicates. Silica gel is basically non-crystalline and porous silica with nanoscale channels while silicate is a salt of silicic acid, occurring in a range of rocky materials and earth's crust (Ganokar et al, 2018; Farooq & Dietz, 2015). Soil content of Si is reported to occur in a range of 23-35% by its weight with 28.8% as the mean value (Prychid et al., 2003).

On account of insolubility of its compounds, phytoavailability of Si in the soil solution is quite low contrary to its abundance (Richmond & Sussman, 2003). Concentration of silicon normally falls in the range of 0.1 - 0.6 mM L^{-1} which is exceptionally low (Sommer et al., 2006). Predominant form of Si in the soil is silicic acid which shows solubility at basic pH (Currie & Perry, 2007).

Plants are considerably benefitted from the role of Si in ameliorating stresses of both biotic and abiotic types as it strengthens their innate immune system. To achieve the goal of sustainable agriculture while improving the productivity of crops worldwide, Si supplementation possibly acts as an appropriate approach for shielding cultivars (Souri et al., 2021; Guntzer et al., 2012). Silicon makes use of numerous strategies to modulate develpmental activities in plants through regulating morphological parameters, photosynthetic responses, antioxidative defense, nutrient absorption, and also constitute a barrier of cell-wall by polymerizing $Si(OH)_4$ (Soundararajan et al., 2014). Silicon-mediated amelioration of stress can be linked to several functional elements like that of translocation and complexation along with chelation, decrease in uptake of heavy metals, modulation of synthesis of antioxidants and expression of genes etc. (Kleiber, 2018).

Silicon displays multifaceted influence in upscaling the plant growth, yield and subsequently the crop quality via boosting mechanical strengthening, intercepting more light and providing better endurance as opposed to constraints of various types, therefore recognized as "quasi-essential" nutrient (Vulavala et al., 2016). Si alleviates baneful effect of metals via rectifying pH of the soil, compartmentalizing, co-precipitating or chelating the metals inside the plant tissues, altering speciation of metals, and modifying the cultivars structurally (Debona et al., 2017; Xiao et al., 2014).

Silicon executes its role both exogenously and endogenously to efficiently safeguard the plants from stressful circumstances (Sahebi et al., 2015). Giving rise to precipitates of metal-silicates or simply declining the phyto-availability of metals by the treatment with Si compounds fall under the category of Si-mediated exogenous mechanisms (Zhang et al., 2008). Though Si can mitigate metal stress in monocots as well as dicots, yet the monocots are benefitted largely owing to their involvement in its hyper-accumulation (Greger et al., 2018). Immobilizing the toxic metals via Si mediation is another key mechanism to lessen the metal-generated deleterious effects inside the plants.

Altering the metal speciation or upscaling the value of soil pH has been accredited with this task of Si-induced metal immobilization via synthesizing silicate complexes. Improvement in various growth parameters, gas exchange attributes, photosynthetic efficiency, nutrient uptake, and elevated measures of phytohormones and upgradation of antioxidant defense line, were recorded in a diverse array of plant species through the addition of Si for ameliorating the pernicious effects of heavy metal (Malhotra and Kapoor, 2019).

Trigonella foenum-graecum L., is an annual, self-pollinating, herbaceous crop and member of the family fabaceae, which is believed to be originated in Mediterranean region but now cultivated and consumed as a spice worldover (Gu et al., 2017; Bahmani et al., 2015). Its leaves and seeds possess tremendous health potential and their consumption could lower down the measures of glucose together with cholesterol in the blood, so used for preventing and treating diabetes and congestive heart ailments (Visuvanathan et al., 2022). Almost every part of this plant is multifariously used as food, forage, therapeutics and cosmetics. Among medicinal plants, it is one of the oldest ones as documented in written historical records and utilized in curing ailments extending from dyspepsia to baldness. Production of fenugreek majorly takes place in India, Argentina, China, Egypt, France, Morocco, Spain and Turkey. India is its global prime producer with 80% share and states like Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttaranchal and West Bengal, contribute the most (Rasheed et al., 2015). In some regions of Asia, the young plant parts are utilized in the form of "pot herbs" i.e. used either as food or as seasoning while the seeds are employed as a herbal remedy or a spice.

Plants of *Trigonella foenum-graecum* thrive nicely in full sunlight of winter season inside rich and well-drained soil (Kakani et al., 2009). Its annual habit together with ability to readily adapt to drier land conditions, make it qualified enough for being incorporated into the process of crop rotation. Further, nodulation takes place in its roots where N_2 fixing bacteria reside symbiotically which also turns it into a good rotation crop plant especially with that of cotton. Among all its traditional applications of being used as a spice and condiment, it stands out as a flavouring agent (in maple syrup, tobacco products and hydrolysable vegetable proteins), as an ingredient of bread making, a perfume base and yielding sapogenin (a steroid) which is used in manufacturing drugs (Mehrafarin et al., 2010).

Chapter 2

Literature overview

Heavy metals (like Cd, Co, Pb, Pt, Ni, Fe, Zn, As, etc.) reside naturally in the soil (mainly in rocks) and water in greater proportions than in the atmosphere. However, anthropogenic interventions like urbanization and industrialization with modernization of agricultural practices have escalated their levels in the entire biosphere. A small number of heavy metals are considered essential for plant metabolism in minimal concentrations as they take part in redox reactions and enzymatic activation, and quite aptly termed as micronutrients in plant nutrition (Wani et al. 2018; Moghaddam et al. 2020). Nevertheless, they turn toxic in excess, thereby contributing in the defiling of environment plus negatively influencing the wellness of plants and human beings (Monni *et al.*, 2000; Kohzadi et al. 2019). Moreover, heavy metals possess non-biodegradability and highly persistent nature, which contributes towards making them readily, interspersed in the rhizospheric arena (Cuypers et al. 2013, Hou et al. 2019). An unplanned over usage of chemicals in the form of manures, fertilizers and pesticides for the purpose of enhancing the crop productivity coupled with improper management of wastewater and other aspects of agri-business have been the key reasons for elevating the otherwise normal levels of heavy metal ions in the agricultural fields (Asgari et al. 2017, 2019). However, the scenario has worsened due to additional discharge of heavy metals into the edaphic domain on account of increase in natural and anthropogenic interferences (Ye et al. 2017).

Soil-ecosystem relationship is negatively affected as the elevated concentrations of these heavy metals pollute the environment in general and food chain in particular either via a direct entry or indirectly along with other contaminants (Achary et al. 2017, Kibet et al. 2019). The piling up of metals in aquatic environments over a time period consequently lead

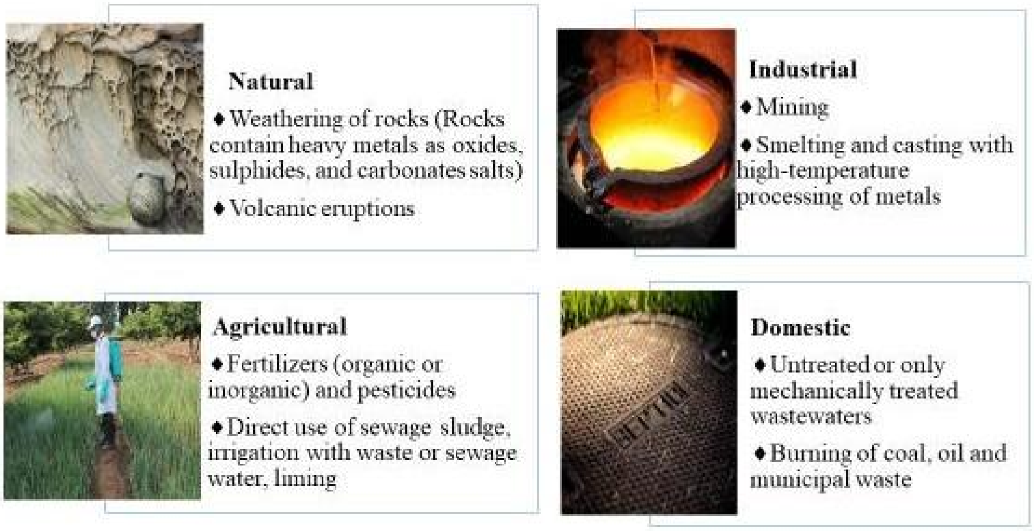


Fig. 2.1: Depicting Major Sources of Contamination of Heavy metals in the Environment

to serious afflictions in human bodies like that of cancer, hepatic damage, pulmonary congestion, renal and reproductive organ failure (Luo et al., 2020; Shaban et al., 2018). Plants, in particular, face the heavy metal toxicity when their roots uptake these metals from the contaminated soil or the metal ions simply get deposited onto their leaves. Out of various types of stressors, heavy metals are a major contributor in a way that well-being of various edible and medicinal plants is greatly affected (Karahan et al. 2019; Street 2012). Stress incites different kinds of responses at morphological, biochemical and functional level inside the plants, which directly cast an impact on crop yield (Ghori et al., 2019). Severe agricultural losses are incurred by a country's economy if crop plants, specifically, get subjected to this heavy metal contamination.

Some of these heavy metals namely As, Cd, Hg and Pb etc. do not have any functional importance in the life of plants and are way more toxic than other metals (Gill et al., 2013). Inhibition of seed germination, destruction of photosynthetic pigments, upsetting of water-mineral uptake, wilting and low biomass measures which ultimately prove to be fatal to the very existence of the plants, are some of the grave implications of their affliction (DalCorso et al. 2013). Anomalies in normal growth and developmental processes of plants are brought about because of the heavy metal toxicity. Reasons include inception of ROS (Reactive oxygen species) which oxidatively damage the plants and alter the structural organization of internal cellular parts thereof (Ishtiyaq et al., 2018; Ashraf et al., 2017).

2.1 Lead: Nature, Sources and Usage

Lead (Pb), a malleable heavy metal with silvery-white hue occurs in solid state under general ecological conditions. It has soft plus dense texture and possesses corrosion resistance, ductility and relatively poor conductivity as opposed to other metals. Chemically, this metal is presented as Pb, which is an abbreviated form of word plumbum of latin origin with a literal meaning of "soft metal" (Pinho & Ladeiro, 2012).

Natural occurrence of this metal in its native form is quite rare but it forms varied and interesting combinations with others elements. Most prevalent mineral ore of Pb is Galena, which changes its colour to dull gray from blue-white upon getting an air exposure (Boldyrev et al., 2018).

Weathering of rocks is the natural source of its addition in toxic levels into the environment (Qu et al. 2020). On the anthropogenic front, there are numerous sources of Pb release in the surroundings such as anti- knock additives, eye cosmetics, fertilizers, paints, pesticides besides the activities involving mining, metal plating, finishing, improper disposal of municipal wastes etc. but plants get exposed to Pb compounds mainly via automobile exhausts as their higher concentrations and adverse effects are more evident in roadside plants (Njati and Maguta 2019; Bhagat and Thawait, 2018; Farooq *et al*., 2008; Paivoke, 2002). While the water soluble nature of this metal assists it to enter into human bodies via drinking water (Gan et al. 2019, Papagiannis et al. 2019). In other words, this persistent and ubiquitously present heavy metal influences the well-being of flora together with fauna (Yan et al. 2019, Zhou et al. 2019).

Moreover, Pb has been documented to universally pollute the cultivable soils especially near the industrial areas (Yan et al. 2019). This metal contaminant exhibits high water solubility and can readily adhere to the soil particles with its highest concentration being found in uppermost layer of soil (Datko-Williams et al., 2014). Physiochemical attributes of soil, particularly its pH, also plays a major factor in deciding the percolation of heavy metals into the soil profile via influencing metal's solubility and adsorptive potential for colloids. For instance, Pb displays maximum mobility and solubility in the soils which are acidic by nature (Kaur et al. 2019; Rezza et al. 2018). Upon being deposited in the soil, Pb is highly persistent, especially inside the organic soils where it firmly combines with the molecules of carbon (Egendorf et al. 2018; Greipsson et al. 2013). Moreover, this metal could be retained by the soils for 150 to 5000 years where its measures have been reported to remain high during the first 150 years (Aslam et al., 2021).

Archaeological findings report its human usage for different purposes way beyond 5,000 years. In fact, such studies also reveal its major applications as in for glazing prehistoric ceramics, therapeutically effective cosmetic kohl plus eyeliner and pigment based paints by Egyptians and for water transportation pipes by Romans (Rehren, 2008; Retief & Cilliers, 2005).

In modern times, this metal is widely put to use in the form of leaded glass, paints, batteries (lead oxide), coolant, protective coatings against radiations, anti-knock agent (tetraethyl lead) in automobiles and as aviation fuel in aircrafts (Prengaman, 2003). The US health agency which registers noxious substances and their ill effects, ATSDR, has recorded over a thousand-fold escalation in environmental measures of Pb since last 3 centuries owing to different human activities. The highest jump was observed from 1950-2000 on account of enhancement in its usage as leaded gasoline worldover. Presently, 332 mines are operational on global level producing 4.42 million metric tonnes of Pb with China leading in terms of both production and consumption (Global Data's mines and projects database, 2022).

Additionally, its demand is further expected to soar globally due to expanding automobile market, especially in China (Roberts, 2003). In the form of a contaminant, this metal pollutes ecosystems of both terrestrial and aquatic nature to a greater extent (Amari et al., 2017; Nazir et al., 2015). However, this elemental pollutant is hard to decompose and remove from the ecosystem.

2.2 Lead uptake and phytotoxicity

2.2.1 Uptake

Lead holds no importance in the life of plants in terms of being essential or beneficial, yet gets readily imbibed and amassed in their different parts (Sharma and Dubey, 2005). For its uptake by the higher plants, factors such as its own concentration and bioavailability with that of soil’s (i) pH, (ii) content of organic matter, (iii) temperature and (iv) occurrence of other elements, contribute together (Grosell et al. 2006, Adejumo et al. 2018, Chaoua et al. 2019). Moreover, some soil microorganisms also possess the potential to aid in translocation of this metal inside the aerial regions of the plants (Khan et al. 2020a; Asilian et al. 2018; Babaeian et al. 2016, Khan et al. 2016, Punamiya et al. 2010). There is no uniformity in absorption of Pb because of its dependency on concentration gradient across the root apex and therefore maximum concentration as high as 95% is observed inside roots only (Chandra et al., 2018; Mahdavian et al. 2017; Pourrut et al. 2013**)**

Reason behind this could be the thin walls and young condition of cells of root apex along with low pH making the absorption of soluble Pb quite easier (Seregin et al. 2004, Huskey et al. 2018) or it could possibly happen by means of strong complexation of Pb ions with the chemical constituents of root cell wall such as glucuronic acid (Inoue et al., 2013). However, further translocation of Pb from roots and its accumulation in aerial parts is dependent upon a number of factors which include immobilization within the cell wall; precipitation inside intercellular spaces, and piling in the cell membrane or vacuolar region of both cortex and rhizodermis (Mitra et al. 2020; SalasMoreno and Marrugo-Negrete 2020; Jiang et al. 2019; Du et al. 2018). For measuring the amount of Pb being translocated from the soil into plants, “transfer or translocation factor” is used which is dependent upon physiochemical attributes of both the plant species and soil (Liu et al. 2010; Arshad et al. 2008). Those with a transfer factor higher than one are referred to as hyper-accumulators of Pb while the ones having its value below one are regarded as non-accumulators (Arshad et al. 2008).

Besides transfer factor, bio-concentration factor too plays a major role in accumulating and trans-locating Pb inside the plants. Lead metal after getting absorbed onto the root tissues (mostly to the exchangeable ionic sites) has to cross partial barrier of endodermis for further translocation. From the cortical region of roots, Pb ions enter apoplastic regions via ascent of sap operated by transpirational pull. However, access to symplastic places like where lateral root inception is taking place or young roots are growing, is also achieved by the same system of xylem loading via transpiration.

Transporters of xylem parenchyma or phloem's companion cells are mainly responsible for assisting the translocation of Pb from rhizal parts to all the aerial regions (Dong et al., 2009). Every transporter protein is basically a membrane protein in nature and primarily responsible for maintaining Pb homeostasis (Romanowska et al., 2012). Toxicity caused by Pb is reduced by its efflux either through transporters of P-type or through its sequestration inside a particular organelle (Jiang et al., 2017). Earlier works have hinted at the possible role of transporters like LRR (leucine-rich repeat), ATM1 (ATP binding cassette transporter of mitochondria 1), and PDR12 (Pleiotrpic drug resistance 12) in cellular extrusion of Pb (Zhu et al., 2013)

2.2.2 Phytotoxicity

Plants exhibit prominent symptoms of toxicity when subjected to a direct exposure of this metal in extreme concentrations (Kushwaha et al. 2018). During the past few decades, man-made contributions to escalate the measures of Pb inside plants have become quite significant (Rahman and Singh, 2019). In its toxic concentration, this metal is regarded as a potent protoplasmic poison having cumulative, ingenious and slow-acting properties.

Sharma & Dubey (2005), enlisted major signs which indicate Pb toxicity in plants like tarnishing and inhibitory effects on root growth, destruction of chlorophyll, inhibition of photosynthesis, reduced enzymatic activities and imbalance between mineral nutrients and water; all contributing towards making the plants underdeveloped as depicted in fig. 2. Seregin and Ivanov (2001) investigated the effects of excessive measures of Pb in plant cells and found that it impairs membranal permeability and may also prove fatal to their survival.

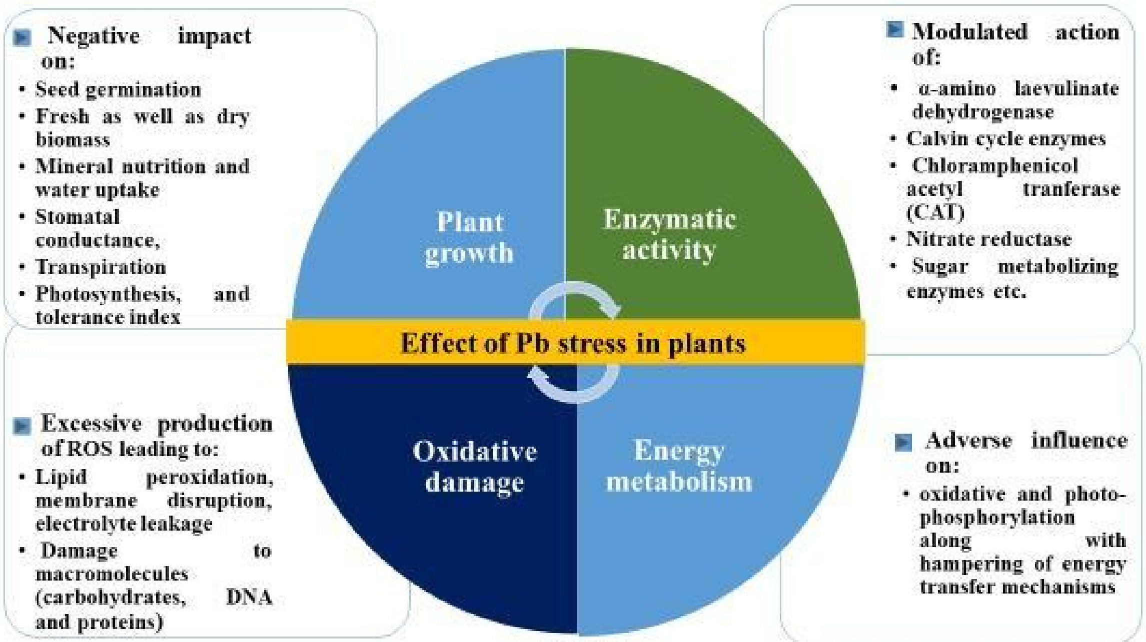


Fig. 2.2

Furthermore, excess of Pb modulates activities of key enzymes like, α-amino laevulinate dehydrogenase, Calvin cycle enzymes, Chloramphenicol acetyl tranferase (CAT), etc. catalyzing principal metabolic processes such as chlorophyll biosynthesis, thermo-chemical phase of photosynthesis, degradation of reactive oxygen species, nitrogen and sugar assimilation (Chen et al. 2017; Ashraf et al. 2015; Pourrut et al. 2013; Kumar et al. 2012; Verma & Dubey, 2003). Activity of carboxylation enzyme is inhibited by the presence of Pb resulting in the stoppage of photosynthesis (Huang et al. 2019). Enzymes contributing for seed germination like amylases, proteases etc. were inhibited by Pb toxicity which in turn did not allow seeds to germinate (Chen et al. 2018).

Activity of the very first enzyme of nitrogen assimilation i.e. nitrate reductase involved in catalyzing conversion of nitrate into nitrite inside the cytoplasm is also hampered by Pb toxicity as reported in *Oryza sativa* (Rice) seedlings and *Triticum aestivum* (wheat) (Alamri et al. 2018; Tariq and Rashid 2013). Equilibrium between inorganic compounds is disturbed by Pb via interrupting either the nitrate uptake or inhibiting the activity of nitrate reductase (Sengar et al. 2009). As the analyzed concentration of Pb exceeds toxic levels in plants, inhibition of nitrate reductase consequently leads to curbing of the process of nitrate assimilation (Dotaniya et al. 2020; Xiong et al. 2006).

Altered enzyme activities are linked to binding of Pb with sulphydryl (-SH) groups of enzymes which hampers the structural stability and catalytic action of these enzymes. Lead metal makes other physiological processes like respiration, ATP and protein synthesis to bear the brunt of its toxicity. Respiration (especially the part dealing with ATP synthesis/oxidative phosphorylation) is adversely affected by Pb toxicity. Lead escalates the rate of mitochondrial respiration without affecting C_2 cycle (photorespiration), thereby contributing towards an enhancement in respiratory activity. In presence of Pb, escalation in respiratory activity has a direct link with ATP formation inside mitochondria as this energy helps in enduring the Pb induced toxicity.

Upon subjection to Pb toxicity, both C_3 and C_4 type plants exhibit 50% elevation in respiration activity along with complete oxidation of substrates inside the mitochondria. Pb further upsets the reactions in ETC (Electron transport chain) on account of its binding with that of mitochondrial membrane resulting in decoupling of the process of phosphorylation (Ashraf and Tang, 2017). Pb toxicity brings about vacuolization of ER and Golgi complex together with mitochondrial swelling and harm to the cristae. Pb toxicity hampers the activity of guard cells, elasticity of cell wall, development of chlorophyll and even the entire leaf, resulting in anomalies of physiology and growth of plants (Kurtyka et al., 2018)

2.3 Impact on plant growth

Alterations in morphological plus functional nature of plants being the resultant of Pb toxicity completely upset the inclusive growth of the plants (Ashraf et al. 2017). Lead affects major metabolic processes of vital importance inside the plants which consequently impede the otherwise normal process of biomass production and growth (Ashraf and Tang 2017; Zulfiqar et al. 2019). The parameters regulating overall growth of plant parts (root, shoot and leaves) with respect to fresh as well as dry biomass, mineral nutrition, water uptake, growth response and tolerance index, are perniciously affected by Pb toxicity as studied by Kevresan *et al*. (2001) in *Pisum sativum*; Malkowski *et al*. (2002) and Cimrin *et al.* (2007) in *Zea mays*; Shua *et al.* (2002) in *Cynodon dactylon*; Jaja and Odoemena (2004) in *Solanum lycopersicum*; Haider *et al.* (2006) in *Lens culinaris* and *Phaseolis vulgaris*; Arias *et al*. (2010) in *Prosopis* sp.; Zhang et al. (2020) in *Festuca arundinacea* and Amjad et al. (2022)

in *Chenopodium quinoa.* Elzbieta and Chwil (2005) found that excess of Pb induces anatomical changes in leaf tissues, thinning of leaf lamina, poor development of vascular bundles including reduction in size of vessels in *Glycine max* whereas Opeolu *et al*. (2010) observed a correlation between Pb toxicity and decrease in number of flowers in a plant.

Ultrastructure of cellular components of leaf tissues as the likes of plastidial thylakoids, mitochondrial membranes and stomata gets negatively affected on account of Pb exposure (Mitra et al. 2020). The toxic nature of Pb constrains elongation of roots, which is affected by the ionic composition, pH and concentration of the surrounding medium (Deng et al. 2020). Length of both roots and shoots along with leaf area expansion, all are hampered by the toxicity of Pb in plants like *Eclipta prostrata, Phyllanthus niruri and Scoparia dulcis* (Chandrasekhar and Ray, 2019).

The principal reason, which can account for the inhibition of growth plus development is the Pb mediated oxidation of auxin (Indole-3-acetic acid), a major plant growth regulator (Agami and Mohamed, 2013). Inhibition of growth is more conspicuous in case of roots, which might be due to higher accumulation of Pb inside them (Liu et al., 2009). Even the micro molar levels of Pb are reported to affect germination and growth of plants (Kopittke et al., 2008). All-inclusive reduction in potential of sugar metabolizing enzymes under Pb stress in *Brassica juncea* is reported to cause retarded emergence of radicle during seed germination (Singh *et al*., 2011).

The negative aftereffects of Pb stress on diverse growth parameters has been tabularised in table 2.1.

Table 2.1 Consequences of Pb toxicity on growth related attributes in various plant species

S. no.	Plant species	Pb dosage	Effect/s	Reference
1.	*Sorghum bicolor* Cultivars: S1, S2 and S3	a) 100 mg kg^{-1} b) 200 mg kg^{-1} c) 400 mg kg^{-1} d) 800 mg kg^{-1}	A significant decline in the length of radical and plumule, germination+ vigor + tolerance index in each of the cultivardue to Pb toxicity	Osman et al., 2023
2.	*Rhus chinensis*	1000 mg kg^{-1}	Morphological attributes of roots, leaf area and biomass recorded a reduction	Shi et al., 2023

			in response to Pb toxicity	
3.	*Basella alba*	a) 4 mM b) 8 mM c) 15 mM d) 20 mM	Germination rates, seedling growth and root length declined drastically due to Pb stress	Gupta et al., 2022
4.	*Zea mays*	2.5 mM	Induction of water stress and reduction in nutrient uptake result in the inhibition of growth	Zanganeh et al., 2021
5.	*Tetraena qataranse*	a) 25 mg L^{-1} b) 50 mg L^{-1} c) 100 mg L^{-1}	Presence of Pb (100 mg/L) inside the growth media at harvest brought about a disruption in healthy growth of the plants especially the development of their roots	Usman et al., 2020
6.	*Zea mays* Two cultivars: -Syngenta 8711 -33H-25	a) 50 ppm b) 100 ppm	Fresh + dry weight of shoots recorded a decline along with the relative water conten (RWC) due to Pb stress	Rasool et al., 2020
7.	*Brassica juncea*	a) 0.25 mM b) 0.50 mM c) 0.75 mM	Significant reduction in dry weight plus tolerance index	Kohli et al., 2018
8.	*Nicotiana tabaccum*	a) 100 µM b) 250 µM c) 500 µM	Significant reduction in length of roots and shoots with that of weight in fresh as well as dried form	Maodzeka *et al.*, 2017
9.	*Lactuca sativa*	20 mg L^{-1}	Pb exposure retarded the germination rates	Silva *et al.*, 2017
10.	*Medicago sativa*	a) 10 µM b) 100 µM	Notable inhibition in growth of roots and shoots	Hattab *et al.*, 2016
11.	*Triticum aestivum*	100 µM	Significant reduction in length of roots and shoots with that of weight in fresh as well as dried form	Tripathi *et al.*, 2016
12.	*Conyza Canadensis*	a) 25 µM b) 50 µM c) 100 µM d) 200 µM	Retarded Growth with regard to height of plant plus wet weights	Li *et al.*, 2016
13.	*Pelargonium graveolans*	2 mM	Drastic decrease in growth parameters like height of plant, leaf area and weight in fresh plus dried form	Rao and Raghu, 2016
14.	*Brassica juncea*	a) 25 µM b) 50 µM c) 100 µM	Growth (percentage germination, length of root plus shoot, weight in dried and fresh form) was reduced notably	Pratima and Pratima, 2016

15.	*Gossypium hirsutum*	a) 25 µM b) 50 µM c) 100 µM	Notable decline in biomass of stem, leaf plus root expressed as their dry weight	Bharwana *et al.*, 2016
16.	*Arachis hypogeal* -Cultivar K6 -Cultivar K9	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Length of root plus shoot was recorded to decline in both the cultivars, however, biomass of k6 cultivar was reduced more than K9	Naresh kumar *et al.*, 2015
17.	*Vigna unguiculata*	200 mg Kg^{-1}	Growth with regard to biomass and number of legume pods and seeds/plant was significantly lowered	Ojwang *et al.*, 2015
18.	*Pisum sativum*	0.25 mg L^{-1}	Reduction in height of plant, length of leaves and number of tendrillar branches was observed though leaf width and number exhibited an increase	Ghani *et al.*, 2015
19.	*Vigna radiata*	a) 0.05 mM b) 0.3 mM	Length of seeds and their germination percentage declined drastically	Hassan and Mansoor, 2014
20.	*Gossypium* sp	a) 50 µM b) 100 µM	Reduction in height of plant, length of roots, per plant number and area of leaves were observed	Bharwana *et al.*, 2014
21.	-*Triticum aestivum* -*Spinacia oleracea*	a) 1.5 mM b) 3 mM c) 15 mM	Dosage dependent decline in growth and weight in fresh and dried form was observed	Lamhamdi *et al.*, 2013
22.	*Zea mays*	a) 1 mM b) 25 mM c) 50 mM d) 100 mM e) 200 mM f) 500 mM	Percentage of germination was lowered down. Growth of seedlings with regard to their root + shoot length was significantly reduced. Similar decline was observed in case of weight (fresh + dry) of seedlings	Hussain *et al.*, 2013
23.	*Triticum aestivum*	a) 40 ppm b) 60 ppm	Remarkable decline in parameters of growth (root + shoot length; fresh + dry weight; number of tendrils)	Bhatti *et al.*, 2013
24.	*Gossypium hirsutum*	a) 50 µM b) 100 µM	Notable decline in height of plant, length of roots, number of leaves plus their area. Similarly, weight of root + shoot + leaf in dried and fresh form was reduced	Bharwana *et al.*, 2013

25.	*Brassica juncea* var. Aravali	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Seed germination plus survival were lowered down. Number of branches and leaves, length of root + shoot and weight (dry + fresh form), all were reduced drastically	Kaur *et al.*, 2013
26.	*Brassica napus* i) Var. Hayola 308 ii) RG5003	a) 100 µM b) 200 µM c) 400 µM	Decline in root length plus tolerance index was recorded in both the varieties	Mosavian and Chaab, 2012
27.	*Sesbania exaltata*	a) 0.1 µM b) 1 µM c) 5 µM d) 10 µM e) 20 µM	Treatment of plants with Pb (1 µM and 5 µM dosage) brought about reduction in biomass together with in length of both roots and shoots	McComb *et al.*, 2012
28.	*Triticum aestivum*	a) 0.05 g L^{-1} b) 0.1 g L^{-1} c) 0.5 g L^{-1} d) 1 g L^{-1}	Negative influence on biomass, percentage germination and stem elongation was noticed	Lamhamdi *et al.*, 2011
29.	*Raphanus sativus*	2.5 mM	Drastic decrease in height of plant plus its weight in fresh and dried form was observed	Anuradha and Rao, 2011
30.	*Zea mays*	a) 10 ppm b) 20 ppm c) 30 ppm	Remarkable inhibition of growth in roots and shoots was observed	Ghani, 2010
31.	*Albizia lebbeck*	a) 10 µmol L^{-1} b) 30 µmol L^{-1} c) 50 µmol L^{-1} d) 70 µmol L^{-1}	Percentage of seed germination, length of root + shoot and their ratio, dry weight plus tolerance index exhibited decline in them	Farooqi *et al.*, 2009
32.	*Lacuta sativa*	500 µM .	Significant lowering down in weight (fresh + dry) of plants	Durvedic *et al.*, 2008
33.	*Vigna mungo* Inbred-i) PU-35 and ii) T-9	a) 9 mg L^{-1} b) 10 mg L^{-1} c) 11 mg L^{-1}	Conspicuous decline in weight (fresh + dry) and height of plants	Gupta *et al.,* 2006

34.	*Sinapsis alba*	100 mg L^{-1}	Root elongation was noted to be inhibited	Fargasova, 2004
35.	*Oryza sativa*	a) 500 µM b) 1000 µM	Decline in root + shoot length and fresh weight in seedling stage	Verma and Dubey, 2003

2.4 Impact on physiochemical attributes

2.4.1 Photosynthetic system

Out of all the physiological processes, photosynthesis stands out in being vital for carrying out a number of other metabolic reactions. (Taiz and Zeiger, 2010; Ashraf and Harris, 2013). A major consequence of heavy metal toxicity is a drastic and invariable reduction in photosynthesis with ultrastructural alterations in photosynthetic machinery and its efficiency (Garg and Aggarwal, 2011). Phytotoxicity of Pb has a prominent negative effect on photosynthesis (Cenkci et al. 2010, Liu et al. 2008, Singh et al. 2010) and it is well documented in plant species like *Anthyllis vulneraria, Calendula officinalis, Festuca arundinacea, Helianthus annuus, Rhus chinensis, Triticuma estivum etc.* (Doncheva et al. 2018, Piwowarczyk et al. 2018, Zhong et al. 2020, Saleh et al. 2020; Barzin & Firozabadi, 2023). Toxicity induced by Pb alters the composition of glycolipids especially monogalactosyldiacylglycerol, the one which influences permeability of chloroplast membranes (Rizwan et al., 2018). Additionally, stomatal conductance, transpiration rates, chlorophyll measures and photosynthetic efficiency, all exhibit a drastic reduction with the increase in Pb uptake in plants (Rasool et al., 2020).

Destruction of chlorophyll causing leaf chlorosis is significant symptom of Pb stress (Srivastava et al. 2014). Chlorophyll precursor i.e. porphobilinogen requires the enzymatic activity of 5-aminolevulinic acid dehydratase which is inactivated by Pb via interacting with sulfahydryl (SH) groups of active sites of this enzyme (Sharma and Dubey 2005). Pb even replaces the Mg^{+2} ion present at the central position of porphyrin head region of chlorophyll, altering its structure. This results in impaired functioning of this molecule together with inhibition of its biosynthesis (Harpaz-Saad et al. 2007). A decreased content of chlorophyll is subsequently followed by reduction in measures of carotenoid pigments (Kaviani et al. 2017).

The possible reasons substantiating the decline in photosynthetic efficiency on account of Pb toxicity could be:

1. Pb ions possess strong affinity for binding nitrogen and sulphur ligands inciting damage to the ultrastructure of photosynthetic apparatus (Islam et al. 2007);
2. Pb ions substitute and inhibit metallic nutrients as the likes of Cu, Fe and Mg (Kupper et al. 1996, Kastori et al. 1998);
3. Degradation of Chlorophyll enhances the enzymatic action of chlorophyllase (Liu et al. 2008);
4. Derangements in electron transport system (Qufei and Fashui 2009);
5. Stomatal closure mediated drop in carbon dioxide concentration (Romanowska et al. 2006);
6. Decreased functional efficiency of Fd-NADP$^+$ reductase and δ-aminolevulinic acid dehydratase enzymes (Gupta et al. 2009);
7. Carotenoids along with plastoquinones are scaled down in their measures (Jing et al. 2007; Liu et al. 2008).
8. Suppression of enzymes which catalyze the reactions in C_3 cycle of thermochemical phase (Liu et al. 2008).

Exposure of Pb in its toxic concentrations results in lowering down the number of granal stacks, measures of starch grains along with the amount of ground substance i.e. stroma. Composition of lipids in the membranes of thylakoids undergoes alterations in response to Pb stress (Stefanov et al. 1995). Xiong and Wang in 2005 reported that Pb toxicity affects the measures of Chl b way more than Chl a. A noteworthy reduction in the contents of Chl a, Chl b and even the total chl hinting at reduction in size of photosynthetic machinery has been recorded in *Anthyllis vulneraria* when subjected to different concentrations of Pb (Piwowarczyk et al., 2018).

Higher intake of Pb results in damaging the secondary form of PSII, hampering the energy transfer amongst amino acids, and reducing the visible light absorption. High accumulation of Pb in plant initiates the activity of NAD^+-malate dehydrogenase enzyme which impedes hill reaction, and interrupts ATP synthesis i.e. photophosphorylation (cyclic as well as non-cyclic) (Romanowska et al., 2002). Negative impact of metal Pb on photosynthetic machinery and its functioning has been tabularised in table 2.2.

Table 2.2 Consequences of Pb toxicity on photosynthetic machinery in various plant species

S. no.	Plant Species	Pb dosage	Impact on photosynthetic parameters	Reference/s
1.	*Rhus chinensis*	1000 mg kg^{-1}	Pb stress reduced the measures of chlorophyll, however the carotenoid content recorded an increase	Shi et al., 2023
2.	*Zea mays*	2.5 mM	Pb toxicity lower down the chlorophyll content in maize plants	Zanganeh et al., 2021
3.	*Zea mays* Two cultivars: - Syngenta 8711 -33H-25	a) 50 ppm b) 100 ppm	SPAD value of chlorophyll content was noticed to decrease, thereby reducing the photosynthetic efficiency	Rasool et al., 2020
4.	*Eruca sativa*	a) 1000 mg kg^{-1} b) 1500 mg kg^{-1} c) 2000 mg kg^{-1}	Chlorophyll content along with photosynthetic activity, both recorded a drastic reduction due to Pb stress	Yildrim et al., 2019
5.	*Brassica juncea*	a) 0.25 mM b) 0.50 mM c) 0.75 mM	Significant reduction in dry weight plus tolerance index	Kohli et al., 2018
6.	*Oryza sativa*	a) 400 ppm b) 800 ppm c) 1200 ppm	Notable reduction in measures of photosynthetic pigments (Chl a, Chl b plus total chlorophyll and carotenoids) was recorded	Ashraf *et al.*, 2017

7.	*Nicotiana tabaccum*	a) 10 µM b) 250 µM c) 500 µM	Drastic decrease in SPAD value, Chl a + Chl b along with measures of total chlorophyll was noticed in all the seven genotypes	Maodzeka *et al.*, 2017
8.	*Salvinia minima*	40 µM	Reduction in Photosynthetic pigments (Chl a + Chl b + carotenoids) leading to decline in rate of photosynthesis	Leal-Alvarado *et al.*, 2016
9.	*Triticum aestivum*	100 µM	qP value and Fv/Fm ratio was reduced	Tripathi *et al.*, 2016
10.	*Pelargonium graveolens*	2 mM	Measures of Chl a + Chl b and total chlorophyll with that of carotenoids were noted to be declined	Rao and Raghu, 2016
11.	*Brassica juncea*	a) 10 mM b) 20 mM c) 30 mM	Measures of total chlorophyll plus carotene were lowered when compared	Pratima and Pratima, 2016
12.	*Gossypium hirsutum*	a) 25 µM b) 50 µM c) 100 µM	Value of SPAD and total chlorophyll were remarkably lowered	Bharwana *et al.*, 2016
13.	*Gossypium plants*	a) 50 µM b) 100 µM	Reduction in total chlorophyll, Chl a + Chl b, carotenoid plus SPAD values was observed along with a gradual decline in net rate of transpiration and photosynthesis and efficiency of water usage	Bharwana *et al.*, 2014
14.	*Spinacia oleracea* and *Triticum aestivum*	a) 1.5 mM b) 3 mM c) 15 mM	Evident decline in total chlorophyll and Chl a + Chl b	Lamhamdi *et al.*, 2013
15.	*Brassica juncea* var. Arawali	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Remarkable reduction in Chl a:Chl b ratio plus amount of chlorophyll was noticed	Kaur *et al.*, 2013
16.	*Gossypium hirsutum*	a) 50 µM b) 100 µM	Drastic drop in total chlorophyll, Chl a + Chl b, carotenoid plus SPAD values was noted along with a gradual decline in net rate of transpiration and photosynthesis and efficiency of water usage	Bharwana *et al.*, 2013
17.	*Triticum aestivum*	a) 40 ppm b) 60 ppm	Diminutive impact on total chlorophyll + chl a + chl b was observed with an enhancement in carotene content	Bhatti *et al.*, 2013

18.	*Raphanus sativus*	2.4 mM	Measures of total chlorophyll plus net rate of photosynthesis were remarkably reduced	Anuradha and Rao, 2011
19.	*Zea mays* var. Neelam and Desi	a) 10 ppm b) 20 ppm c) 100 ppm	Notable reduction in measures of total chlorophyll was recorded	Ghani, 2010
20.	*Lactuca sativa*	500 µM	Depletion in Chl a + Chl b and carotenoid content was noticed	Durvedic *et al.*, 2008
21.	*Vigna mungo* Inbreds- a) PU-35 b) T-9	a) 9 mg L^{-1} b) 10 mg L^{-1} c) 11 mg L^{-1}	Both chlorophyll plus carotenoid content got declined	Gupta *et al.*, 2006
22.	*Sinapis alba*	100 mg L^{-1}	Decrease in quantities of Chl a + Chl b, total chlorophyll, Chl a:b ratio, Chl a+b: carotenoid ratio together with measures of carotenoid was observed	Fargasova, 2004

2.4.2 ROS (Reactive oxygen species)

Plants are capable of responding and adapting to a varied range of stresses of biotic and abiotic nature. When happen to grow on heavy metal defiled soils, plants get subjected to a cumulative stress of nutritional deficiency together with chemical toxicity (Rajkumar et al. 2013). As a result of this, excessive generation of ROS takes place, causing oxidative stress in the cells. However, this oxidative harm can be mitigated by the integral defense system operated by anti-oxidants (Gill and Tuteja 2010, Kohli et al. 2017). ROS are comprised of free radicals such as O^{2-} (superoxide), OH^{-} (hydroxyl with that of non-radicals like H_2O_2 (hydrogen peroxide), ${}_1O^2$ (singlet oxygen) etc. (Pourrut et al. 2008, Yadav 2010). An exposure to high energy or reactions involving electron transfer results in ROS generation in plants via reducing the molecular oxygen (O^2) in a stepwise manner.

In higher concentrations, ROS brings about the damage to macromolecules of importance (polysachharides, DNA and proteins) along with peroxidation of membrane constituting lipids bringing about the membrane disruption and electrolyte leakage thereof (Miller et al. 2018). Pb toxicity also induces this oxidative damage in plants via generating ROS in excess and upsets the redox equilibrium (Yadav et al. 2010, Shahid et al. 2015, Mallhi et al., 2019, Mobeen et al., 2021). Detoxification and scavenging of increased ROS levels are accomplished by an effective antioxidative system, including non-enzymatic plus enzymatic anti-oxidants (He et al. 2017). Influence of Pb metal toxicity on the production of ROS and oxidative damage indicated by MDA and H_2O_2 have been tabularized in table 2.3.

Table 2.3 Consequences of Pb toxicity on ROS genesis and oxidative stress markers in various plant species

S. no.	Plant Species	Pb dosage	Effect/s	Reference
1.	*Rhus chinensis*	1000 mg kg^{-1}	Pb stress brought about an enhancement in $O_2^{\cdot}-$, MDA and H_2O_2 concentrations	Shi et al., 2023
2.	*Oryza sativa*	800 mg kg^{-1}	Results divulged that Pb toxicity induced oxidative harm in terms of elevated levels of MDA, electrolyte leakage (EL), and H_2O_2	Ashraf et al., 2022
3.	*Salvia officinalis*	a) 100 µM b) 200 µM c) 400 µM	Pb toxicity escalated the contents of MDA and H_2O_2 in the stressed plants	El-shora et al., 2021
4.	*Glycine max*	a) 25 mg L^{-1} b) 50 mg L^{-1} c) 75 mg L^{-1} d) 100 mg L^{-1}	Pb exposure notably enhanced measures of MDA.	Kulaz et al., 2021
5.	*Sorghum*	a) 200 mg kg^{-1} b) 400 mg kg^{-1} c) 800 mg kg^{-1} d) 1600 mg kg^{-1} e) 3200 mg kg^{-1}	Enhanced measures of both MDA and H_2O_2 were recorded in response to Pb stress	Candido et al., 2020
6.	*Triticum aestivum*	a) 0.5 mM b) 10 mM	Pb treatment enhanced content of H_2O_2 and extent of lipid peroxidation in wheat seedlings	Hasanuzzaman et al., 2018
7.	*Oryza sativa*	a) 400 ppm b) 800 ppm c) 1200 ppm	H_2O_2 and MDA measures were elevated when rice plants were exposed to Pb stress	Ashraf *et al.*, 2017
8.	*Nicotiana tabacum*	a) 10 µM b) 250 µM c) 500 µM	Escalation in MDA levels was noticed in all the seven genotypes of the study in a dosage dependent manner	Maodzeka *et al.*, 2017
9.	*Salvinia minima*	40 µM	Disruption of membrane was enhanced under Pb stress	Leal-Alvarado *et al.*, 2016
10.	*Medicago sativa*	a) 10 µM b) 100 µM	TBARS quantities inside roots + shoots were made more pronounced by Pb exposure	Hattab *et al.*, 2016
11.	*Triticum aestivum*	100 µM	Shoot plus root content of H_2O_2 and MDA were escalated by Pb stress	Tripathi *et al.*, 2016

12.	*Gossypium hirsutum*	a) 25 µM b) 10 µM c) 100 µM	Levels of H_2O_2 with that of MDA in reaction to Pb applications was noticed	Bharwana *et al.*, 2016
13.	*Arachis hypogea* cultivar a) K6 b) K9	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Histological and biochemical evaluation revealed enhancement in $O^{\cdot -}$ localization along with H_2O_2. Further, elevated measures of MDA inside the roots + leaves of each cultivar were recorded	Nareshkumar *et al.*, 2015
14.	*Vicia faba*	5 µM	Measures of H_2O_2 and TBARS were enhanced	Shahid *et al.*, 2015
15.	*Gossypium* Plants	a) 50 µM b) 100 µM	Extent of electrolyte leakage with H_2O_2 levels were enhanced hinting at more peroxidation of membranal lipids and disruption of membranes thereof	Bharwana *et al.*, 2014
16.	*Gossypium hirsutum*	a) 50 µM b) 100 µM	Electrolyte leakage and measures of MDA + H_2O_2 were observed to be enhanced	Bharwana *et al.*, 2013
17.	*Brassica napus* varities: a) Hayola308 b) RG5003	a) 100 µM L^{-1} b) 200 µM L^{-1} c) 400 µM L^{-1}	Notable enhancement in the quantities of MDA was observed in aerial regions of variety Hayola	Mosavian and Chaab, 2012
18.	*Raphanus sativus*	2.5 mM	MDA measures were elevated drastically	Anuradha and Rao, 2011
19.	*Triticum aestivum*	a) 0.05 g L^{-1} b) 0.1 g L^{-1} c) 0.5 g L^{-1} d) 1 g L^{-1}	MDA measures were recorded to enhance drastically with the elevation in Pb dosage	Lamhamdi *et al.*, 2011
20.	*Najus indica*	a) 1 µM b) 10 µM c) 50 µM d) 100 µM	Enhancement in MDA + H_2O_2 measures was recorded	Singh *et al.*, 2010
21.	*Raphanus sativus*	0.5 mM	Higher measures of TBARS inside foliar and hypocotylar regions were recorded	Teklic *et al.*, 2008
22.	*Oryza sativa*	a) 500 µM b) 1000 µM	Elevation in measures of TBARS with that of superoxide anions was noticed	Verma and Dubey, 2003

2.4.3 Protein content

Pb toxicity interferes with the quantities and composition of nutrients together with conformation of proteins, particularly the regulatory and transporter proteins (Pirzadah et al., 2020). Higher dosages of Pb were reported to lower down the levels of cytoplasmic protein pool (Mishra *et al.*, 2006; Piotrowska *et al.*, 2009). This reduction in cellular protein measures because of Pb stress might be due to elevated production of ROS and oxidative damage thereof; escalation in the enzymatic action of ribonucleases; over usage of proteins for detoxifying Pb and alterations in the FAA (free amino acid) measures (Gupta *et al.*, 2009; Gopal and Rizvi, 2008). In some research findings, Pb stress has been reported to enhance the levels of a few amino acids like proline (Qureshi *et al.*, 2007). This metal in its low as well as non-lethal doses results in enhancement of protein measures, which could be related to plants' innate defense mechanism against Pb toxicity (Gupta *et al.*, 2010; Mishra *et al.*, 2006).

Palma *et al.* (2002) and Tiwari *et al.* (2013) reported reduced protein content in *Brassica juncea* and carbohydrate plus protein quantities in *Triticum aestivum* due to Pb toxicity, which is reasoned to be due to enhanced activity of protein degrading enzymes.

Subjection to Pb toxicity negatively affects the endogenous measures of proteins in various plant species as given in table 2.4.

Table 2.4 Consequences of Pb toxicity on protein measures in various plant species

Sr. no.	Plant Species	Pb dosages	Effect/s	Reference
1.	*Sorghum bicolor* Cultivars: S1, S2 and S3	a) 100 mg kg^{-1} b) 200 mg kg^{-1} c) 400 mg kg^{-1} d) 800 mg kg^{-1}	Content of total protein declined drastically as Pb stress lowers down absorption of nitrogen- a main constituent of amino acids	Osman et al., 2023
2.	*Triticum aestivum* var. a) Punjab-2011 b) Anaj-2017	0.5 Mm	Free amino acids plus total measures of soluble protein were enhanced by Pb exposure	Perveen et al., 2022
3.	*Oryza sativa* Cultivars: -Amber Barka	a) 0.6 mM b) 1.2 mM	A notable decline in the content of protein was recorded in every rice cultivar and at every Pb dosage	Khan et al., 2021

	- Ilmi - Mashkab - Tunnae - Yasmen			
4.	*Anethum graveolans*	a) 50 mg L^{-1} b) 100 mg L^{-1} c) 150 mg L^{-1}	Significant reduction in protein concentrations under Pb stress	Al-Kazzaz, 2020
5.	*Gossypium hirsutum*	5 µM	Protein measures were significantly lowered	Bharwana *et al.*, 2016
6.	*Vigna radiata*	a) 0.05 mM b) 0.3 mM	At 0.05 mM, escalation in soluble protein content was observed while at 0.3 mM Pb treatment, measures of soluble protein were lowered	Hassan and Mansoor, 2014
7.	*Gossypium plants*	a) 50 µM b) 100 µM	Remarkable reduction in content of soluble protein inside roots and foliar regions	Bharwana *et al.*, 2014
8.	*Brassica juncea* Arawali	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Dose dependent reduction in the content value of soluble protein was recorded	Kaur *et al.*, 2013
9.	*Gossypium hirsutum*	a) 500 µM b) 100 µM	Measures of soluble protein were remarkably lowered	Bharwana *et al.*, 2013
10.	*-Spinacia oleracea* *-Triticum aestivum*	a) 1.5 mM b) 3 mM c) 15 mM	Both the plants exhibited escalation in the levels of protein content	Lamhamdi *et al.*, 2013
11.	*Zea mays*	a) 1 mM b) 25 mM c) 50 mM d) 100 mM e) 200 mM f) 500 mM	Protein content was recorded to be lowered in stressed plants and this reduction was way more in roots than in shoots	Hussain *et al.*, 2013
12.	*Jatropha curcas*	a) 0.1 mM Kg^{-1} b) 2 mM Kg^{-1} c) 3 mM Kg^{-1} d) 4 mM Kg^{-1}	Protein measures were lowered	Shu *et al.*, 2011
13.	*Triticum aestivum*	a) 0.05 g L^{-1} b) 0.1 g L^{-1} c) 0.5 g L^{-1} d) 1 g L^{-1}	Soluble protein measures were significantly escalated upon subjection to Pb treatment	Lamhamdi *et al.*, 2011
14.	*Najus indica*	a) 1 µM b) 10 µM c) 50 µM d) 100 µM	Elevation in protein measures was recorded	Singh *et al.*, 2010

15.	*Vigna mungo* Inbreds: -PU-35 -T-9	a) 9 mg L^{-1} b) 10 mg L^{-1} c) 11 mg L^{-1}	Notable reduction in protein levels was observed	Gupta *et al.*, 2006

2.4.3. Secondary metabolites

The plants generate a diverse array of secondary metabolites, which mainly include anthocyanins, flavanoids and phenolics etc. These biochemicals have been reported to modulate various physiological responses especially during stressful conditions. Their mode of action resembles with that of stress-induced antioxidants, probably on account of their electron donating behaviour (Michalak, 2006). Under the influence of heavy metal generated stress, the biosynthesis of these metabolites exhibit either positive or negative alterations in their expression levels for the alleviation of stress which is reported in wheat, maize and common bean plants subjected to stress of Ni, Al and Cd respectively (Dai and Mumper *et al.*, 2010; Winkel-Shirley, 2002). Phenolic compounds have been recorded to chelate with Pb, Cr and Hg ions in plants of *Nymphaea alba* (Lavid *et al.*, 2001). Phenolics show an enhancement in their levels in a dose dependent manner as found in case of *Phaseolus vulgaris* when concentration was enhanced from 25 to 100 ppm (Hamid et al., 2010).

Additionally, measures of total phenols, anthocyanins plus flavanoids were found to be elevated on account of Pb stress in *Brassica juncea* plants (Kohli et al., 2018). On the other hand, a drastic decline in the measures of phenolic compounds was noticed in Pb stress (100 µM) exposed wheat plants (Tripathi *et al.*, 2016). Further, in *Brassica juncea*, an initial enhancement in phenolic compounds was observed, which got diminished with the rise in Pb stress levels from 10 to 40 mM (Pratima and Pratima, 2016). In Cu/ Pb stressed winter wheat varieties namely Hyvento, Hyking and Hyacinth, maximum accumulation of secondary metabolites was recorded in cv. “Hyvento” in response to Cu stress and in cv. “Hyacinth” and “Hyking” in case of Pb stress (Jańczak-Pieniążek et al., 2022).

2.4.5 Osmolytes (Soluble sugars, proline and glycine betaine)

To prevent the harm incited by heavy metal exposure, plants resort to osmo-regulation by which the measures of osmolytes namely prolines, betaines and sugars etc. are regulated for maintaining the osmotic balance. These bio-molecules assist in maintaining the cellular turgor pressure optimum whenever plant is subjected to stressful environment (Misra and Saxena, 2009). The molecular weight of these non-toxic osmoprotectants is very low and they are indispensable for regulating the osmotic gradient inside plants (Slama *et al.*, 2015). In certain cases, the osmolytes have been reported to be applied exogenously for the mitigation of different stressful conditions (Farooq *et al.*, 2013). Osmolytes tend to enhance the osmotic pressure, which regulate water uptake and maintain the cellular turgor pressure, all of which contribute towards the sustenance of cytosolic driving gradient (Serraj and Sinclair, 2002).

Besides carrying out osmotic adustments, these osmoprotective biomolecules could scavenge ROS in stressful environments (Serraj and Sinclair, 2002). Various sugars were recorded to escalate in the plants experiencing abiotic stresses. A protective role is played by these biomolecules in stressed plants via maintaining the osmotic equilibrium, membrane stability along with various other regulatory mechanisms (Lokhande and Suprasanna, 2012). Osmoprotectants are also imperatively involved in safeguarding the plants from damage incited by injuries and dehydration (Sharma and Dietz, 2006; Ashraf and Fooland, 2007). Major osmolytes involve amino acids namely alanine betaine, polyamines, proline and proline betaine etc. and sugars like fructose, glucose, raffinose, sucrose, trehalose etc. (Mudgal *et al.*, 2010). Glycine betaine or GB, being an important compatible solute, has been reported to effectively manage the osmotic pressure in response to metal, osmotic and salinity stresses (Khan *et al.*, 2015; Slama *et al.*, 2015; Bharwana *et al.*, 2014).

Alterations in measures of osmolytes on account of Pb toxicity have been tabularized in Table 2.5.

Table 2.5 Consequences of Pb toxicity on measures of osmolytes in various plant species

Sr. no.	Pb concentrations	Plant Species	Effect/s	Reference

1.	*Triticum sativum* var. a) Punjab-2011 b) Anaj-2017	0.5 mM	Significant escalation in the content of glycine betaine and proline was noticed and it was slightly higher in Punjab-2011 variety	Perveen et al., 2022
2.	*Oryza sativa* Cultivars: -Amber Barka - Ilmi - Mashkab - Tunnae - Yasmen	a) 0.6 mM b) 1.2 mM	Enhancement in the sucrose content along with extent of proline accumulation was noticed when the Pb concentration was increased. Out of all the analyzed soluble sugars, abundancy of sucrose was maximum under Pb stress	Khan et al., 2021
3.	*Oryza sativa*	a) 150 µmolL^{-1} b) 300 µmolL^{-1}	Notable enhancement in the measures of proline by the upregulation of biosynthetic genes	Salavati et al., 2021
4.	*Glycine max*	a) 25 mgL^{-1} b) 50 mgL^{-1} c) 75 mgL^{-1} d) 100 mgL^{-1}	Pb exposure significantly enhanced the measures of proline, relative to control plants.	Kulaz et al., 2021
5.	*Zea mays*	2.5 mM	Subjection to Pb stress resulted in enhanced measures of arginine, glycine betaine, methionine and proline	Zanganeh et al., 2018
6.	*Medicago sativa*	a) 10 µM b) 100 µM	Notable rise in proline measures was observed in Pb stressed plants	Pratima and Pratima, 2016
7.	*Arachis hypogea* (cultivar K6 and K9)	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Escalation in the accumulation of proline was recorded in each cultivar	Nareshkumar *et al.*, 2015
8.	*-Triticum aestivum* *-Spinacia oleracea*	a) 5 mM b) 3 mM c) 15 mM	Increments in measures of proline was recorded in leaves of both the plants	Lamhamdi *et al.*, 2013
9.	*Brassica juncea* var. Arawali	a) 100 ppm b) 200 ppm c) 400 ppm d) 800 ppm	Enhancement in proline + Total sugar content was observed	Kaur *et al.*, 2013
10.	*Brassica napus* (Two variety: -Hayola308 - RG5003)	a) 100 µM L^{-1} b) 200 µM L^{-1} c) 400 µM L^{-1}	Roots + aerial parts of each variety were recorded to have enhanced measures of proline	Mosavian and Chaab, 2012
11.	*Triticum aestivum*	a) 0.05 g L^{-1} b) 0.1 g L^{-1} c) 0.5 g L^{-1} d) 1 g L^{-1}	Enhancement in Pb dosage brought about a notable elevation in proline content	Lamhamdi *et al.*, 2011

12.	*Raphanus sativus*	2.5 mM	Measures of reducing sugars plus that of total sugars were reduced drastically in Pb treated plants	Anuradha and Rao, 2011
13.	*Najus indica*	a) 1 µM b) 10 µM c) 50 µM d) 100 µM	Proline measures were escalated initially with a subsequent reduction	Singh *et al.*, 2010
14.	*Raphanus sativus*	0.5 mM	Significant enhancement in measures of proline was recorded in foliar parts	Teklic *et al.*, 2008

2.4.6. Antioxidant defense mechanism

Enzymatic plus non- enzymatic antioxidants effectively deal with toxicity generated by free radicals of ROS (Kasote et al., 2015). Plants subjected to heavy metal toxicity exhibit prominent responses from anti-oxidative defense. However, the nature of these responses are reported to be dependent on type of metal used, plant species, tissue examined along with severity of stress (Ashraf et al. 2018). Antioxidant potential of CAT, POD and SOD in *Rhus chinensis* and that of APX, CAT, POX and SOD in *Triticum aestivum* was recorded to be enhanced upon subjection to Pb stress (Murtaza et al., 2020, Shi et al., 2023). The elevation of H_2O_2 measures may be associated with the enhanced activity of SOD after the plants are given Pb treatment. Excessive H_2O_2 content is toxic and therefore needed to be quenched by converting it into H_2O in subsequent steps. A number of antioxidative enzymes spring into action to save the Pb stressed plant from harmful impacts of H_2O_2. For instance, enzymatic activity of antioxidants like APX, CAT and POX were escalated in *Eichhornia crassipes* upon Pb exposure (Malar et al., 2016). Additionally, improved measures of antioxidants was documented in the foliar parts of *Citrus aurantium* L. plants when subjected to Pb and Cu toxicity (Giannakoula et al., 2021). However, in some crop plants, a reduction in the enzymatic activities of above mentioned antioxidants at higher concentrations of Pb has also been reported. Table 2.6 summarizes the impact of enzymatic antioxidants in a range of plant species exposed to Pb stress.

Table 2.6 Consequences of Pb toxicity on antioxidant defense mechanisms of various plant species

Name of antioxidant	**Plant species**	**Pb dosage/s**	**Effect on activity**	**Reference/s**
a) Enzymatic antioxidants				
Superoxide dismutase (SOD)	*Oryza sativa*	0, 10, 50 µM	Enhanced	Thakur et al., 2017
	Lymantria dispar	1500 mg kg^{-1}	Decreased	Jiang et al. 2020
	Hordeum vulgare	10 and 15 mM	Enhanced	Dogru, 2020
	Vicia faba	0, 500, and 1000 mg kg^{-1}	Decreased	Abusalima et al., 2020
	Sorghum	0, 200, 400, 800, 1600 and 3200 mg kg^{-1}	Enhanced	Candido et al., 2020
	Solanum lycopersicum	0, 50, and 100 mg kg^{-1}	Enhanced	5 Ma et al., 2022
	Rhus chinensis	1000 mg kg^{-1}	Enhanced	Shi et al., 2023
Catalase (CAT)	*Arabidopsis thaliana*	150 µM	Decreased	Corpas and Barroso 2017
	Oryza sativa	0, 10, 50 mM	Decreased	Thakur et al., 2017
	Triticum aestivum	1.5 mM	Decreased	Turk et al., 2018
	Festuca arundinacea	1000 mg kg^{-1}	Enhanced	Hasanuzzaman et al. 2019

	Sorghum	0, 200, 400, 800, 1600 and 3200 mg kg^{-1}	Enhanced	Candido et al., 2020
	Brassica campestris	0.25, 0.50, 1.00 mM	Decreased	Zhong et al., 2020
	Cordyline fruticosa	250 mg kg^{-1}, 375 mg kg^{-1}	Enhanced	Herlina et al., 2021
	Rhus chinensis	1000 mg kg^{-1}	Enhanced	Shi et al., 2023
Ascorbate peroxidise (APX)	*Brassica napus*	50 and 100 µM	Decreased	Kanwal et al. 2014
	Dimocarpus longan	0, 100, 200, 400, 600, 800 and 1000 mg L^{-1}	Enhanced when Pb levels are low but at higher stress of Pb exhibited a decrease	Wang et al. 2016
	Sorghum	0, 200, 400, 800, 1600 and 3200 mg kg^{-1}	Enhanced	Candido et al., 2020
	Triticum aestivum	0, 15, 30 and 45 mg kg^{-1}	Enhanced	Navabpour et al. 2020
	Glycine max	0, 25, 50 and 75 mg L^{-1}	Enhanced	Kulaz et al., 2021
	Solanum lycopersicum	0, 50, and 100 mg kg^{-1}	Enhanced	Ma et al., 2022
Glutathione reductase (GR)	*Medicago sativa*	0, 10 and 100 µM	Enhanced	Hattab et al. 2016
	Oryza sativa	0, 10, 50 µM	Enhanced	Thakur et al. 2017

	Triticum aestivum	2 mM	Enhanced	Alamri et al. 2018
	Helianthus annuus	300, 600 and 900 mg kg^{-1}	Enhanced	Saleem et al. 2018
	Pisum sativum	10, 100 and 500 mg kg^{-1}	Enhanced	Dias et al. 2019
	Brassica campestris	0.25, 0.50, 1.00 mM	Enhanced	Hasanuzzaman et al. 2019
	Salvia officinalis	100, 200, 400 μM	Enhanced	El-shora et al., 2021
Dehydroascorbate reductase (DHAR)	*Triticum aestivum*	0.5, 1.00 mM	Decreased	Hasanuzzaman et al. 2018
	Zea mays	0, 16, 40, 80 mg L^{-1}	Enhanced	Kaur et al., 2015
	Brassica campestris	0.25, 0.50, 1.00 mM	Enhanced	Hasanuzzaman et al. 2019
	Brassica chinensis	300, 600, 900 mg kg^{-1}	Decreased	Tan et al., 2022
Monodehydroascorbate reductase (MDHAR)	*Triticum aestivum*	0.5, 1.00 mM	Decreased	Hasanuzzaman et al. 2018
	Zea mays	0, 16, 40, 80 mg L^{-1}	Enhanced	Kaur et al., 2015
	Brassica campestris	0.25, 0.50, 1.00 mM	Enhanced	Hasanuzzaman et al. 2019
Guaiacol peroxidase (POD)	*Rhus chinensis*	1000 mg kg^{-1}	Enhanced	Shi et al., 2023
b) Non-enzymatic antioxidants				
Ascorbic acid	*Zea mays*	2.5 mM	Decreased	Zanganeh et al. 2019

	Triticum aestivum	1.5 mM	Decreased	Turk et al., 2018
	Solanum melongena	0, 15, 20, and 25 mg L^{-1}	Decreased	Javed et al. 2019
	Capsicum annuum	0.1mM	Enhanced	Kaya et al. 2019
	Lymantria dispar	1500 mg kg^{-1}	Decreased	Jiang et al. 2020a
Glutathione	*Brassica campestris*	0.25, 0.50, 1.00 mM	Enhanced	Hasanuzza man et al., 2019
	Triticum aestivum	1.5 mM	Decreased	Turk et al., 2018
	Capsicum annuum	0.1mM	Enhanced	Kaya et al. 2019
	Sasa argenteostriata	0, 300, 600, and 900 mg L^{-1}	Enhanced	Jiang et al. 2020b
	Vicia faba	0, 500, and 1000 mg kg^{-1} soil	Decreased	Abuslima et al. 2020

2.5 Gene expression

Noxious metals have been documented to modify the expression levels of vital enzymes catalyzing key metabolic processes via altering profiles of genes and proteins (Singh et al., 2016). Presently, multifarious research activities are focusing on deciphering the response of Pb metal exposure in plants on molecular level. For instance, genes encoding enzymatic antioxidants (APX, CAT and POD) were recorded to express differentially in fenugreek plants when exposed to Pb stress besides Cd and Cr (Alaraidh et al., 2018) which leads to variations in the tolerance potential. In roots of radish plants, identification of Pb stress responsive differentially expressed (DE) genes has been performed inclusive of MAPKs (mitogen-activated protein kinases), Ca^{+2} ion binding proteins, metal transporters and regulators of glutathione metabolism (Wang et al., 2013). Furthermore, same study revealed that a) Pb- exposed and b) untreated roots of radish were found to differ in the expression of gene families namely *DREB, ERF, MYB and WRKY*. Expression levels of *ZmACS6* along with *ZmSAMD* were declined inside the shoots of maize plants while getting enhanced in the root region *(*Zanganeh et al., 2018).

In Pb stressed maize roots, 1832 genes were recorded to be down regulated with that of up-regulation of 2379 genes in contrast to control plants (Shen et al., 2013). In total, 262 transcription factors (TFs) of four different classes having a differential expression i.e. DETFs have been recorded in maize plants that could respond to Pb stress (Zhang et al., 2017). Furthermore, polymorphism in the transcript levels of two TFs of Bzip family namely a) *ZmbZIP54* plus b) *ZmbZIP107* have been reported to assist in the development of tolerance against Pb toxicity in different cultivars of maize. In *Trigonella foenum-graecum* plants, Pb noxiousness has been reported to down-regulate the activity levels of SOD plus CAT genes, thereby hampering the functioning of anti-oxidant defense machinery (Sharma et al., 2023). These studies hinted at the underlying response plus defense mechanism being operational in plants to combat Pb stress, which however fall short of its efficacy if the Pb dosage and exposure time become prolonged.

2.6 Mitigatory influence of BRs on heavy metals

Plant hormones perform a key role in regulating various processes of cellular and physiological nature via maintaining redox balance. Metals when present in exceeding levels beyond a certain limit, regardless of their essentiality, cause severe toxicity in plants (Robert *et al.*, 2008). BRs are the phytohormones having a steroidal nature and capability to alter several morpho-physiological processes while upgrading the tolerance potential of plants in opposition to stresses of both biotic as well as abiotic nature (Bajguz and Hayat, 2009; Vardhini, 2016). BRs further exhibit a crucial involvement in developmental phases and can modulate antioxidative defense mechanisms in stressed plants. On account of this, BRs are recognized as a remarkable tool for enhancing the capability of plants to combat stresses and bring about better development of agri-crops (Bhardwaj *et al.*, 2007; Anuradha and Rao, 2009).

Heavy metal induced phytotoxicity leads towards obstructing the functional groups, key enzymes and vital biomolecules like nucleotides plus essential nutrients together with deranging these nutrient ions from their site of action, denaturation and disruption of some of the cellular components such as chloroplast and vacuoles etc. (Smirnoff, 1996). BRs are largely employed for their capability to lower down the absorption of metals while altering processes of functional importance (Sharma and Bhardwaj, 2007; Kohli *et al.*, 2017), thus participating in amelioration of metal toxicity in the following ways:

2.6.1. Plant growth

Metals are detoxified mainly on cell wall and its surface (Romheld and Awad, 2000). Metal toxicity restrains cell elongation leading to underdevelopment in plants. This slowdown of plant growth could be attributed to interruptions in various cellular processes as metal toxicity upsets the nutrient balance in plants (Schopfer, 1996). BRs, on the other hand are extensively acknowledged for their potential of being growth enhancers (Davies, 2010). BRs promote growth by stimulating cell elongation, progression of cell cycle, together with expressing the genes responsible for encoding xyloglucans of cell wall and its expansion in the plants exposed to metal stress (Gonzalez-Garcia *et al.*, 2011; Zhang *et al.*, 2009).

BRs are also disclosed to modulate the membranal permeability consequently leading to more elongation plus expansion of plant cell (Fariduddin *et al.*, 2009). They further perform a key role in controlling multifarious physiological responses of plants, including growth that can be calibrated by percentage germination, rate of flowering, reduction of cell necrosis, pollen tube lengthening and photo-morphogenetic development (Bajguz, 2010). Furthermore, it was suggested that cascades of BR signaling, modulates loosening or stiffening up of components of cell walls for ensuring its properties with regard to stressful conditions (Rao and Dixon, 2017). Some research outcomes regarding the role of BRs in ameliorating the Pb stress induced growth alterations have been tabularized in Table 2.7.

Table 2.7 Influence of BRs treatment on growth attributes in various metal stressed plant species

Sr. no.	Heavy Metal	Concentration of heavy metal	Plant Species	Type of BR and its concentration		Effect/s	Reference
1.	Zn	a) 1 mM b) 2 mM c) 3 mM	*Medicago sativa*	24-EBL	100 nM	Biomass of roots and shoots was improved and growth damage was mitigated by 24-EBL	Ren et al., 2023
2.	Polymetallic stress ($Al^{3+,}$ Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Pb^{2+}	Al^{3+}--20 µM Mn^{2+}- 50 µM Cd^{2+}- 2.8 µM Cu^{2+}- 2 µM	*Hordeum vulgare*	Homobras-sinolide (HBL)-0.1 and 10nM and Homocastasterone (HCS)		Enhancement in leaf area, total weight, root plus shoot length in response to both low (0.1 nM) and high (10 nM) HCS whereas in case of	Zlobin et al., 2023

		Ni^{2+}- 16 µM Zn^{2+}- 40 µM Pb^{2+}- 30 µM				HBL, both the concentrations increased leaf area but for shoot length, only high concenration of HBL (10 nM) showed prominent positive results	
3.	Cd	a) 300 µM b) 600 µM	*Spinacea oleracea*	Brassinolide	0.5 µM 0.75 µM	Improved the growth attributes by the application of brassinolide alone as well as in combination with salicylic acid	Maghsoudi et al., 2021
4.	Cr (VI)	10 mg kg^{-1}	*Lycopersicum esculeutum*	24-Epibrassinolide (24-EBL)	10^{-7} M	EBL treatment proved to be capable of improving growth attributes like root+shoot length, dry+fresh weight in control and Cr-stress subjected plants	Jan et al., 2020
5.	Mn	a) 30 mg kg^{-1} b) 150 mg kg^{-1}	*Brassica juncea*	24- EBL	10^{-8} M	Growth parameters otherwise reduced on account of Cd stress got renormalized and enhanced further	Hussain et al., 2019b
6.	Cd	2.5 mM	*Cucumis sativus*	24- EBL	5 µM	Length of root + shoot weight in fresh + dry, number and water content of leaves exhibited an improvement upon EBL supplementation	Shah et al., 2019
7.	Cu	120 µM	*Vitis vinifera*	24-EBL	0.5 mg L^{-1} 0.10 mg L^{-1} 0.20 mg L^{-1}	Various parameters of root morphology inclusive of its length, surface area, diameter, volume and number of tips exhibited significant enhancement due to EBL supplementation	Zhao et al., 2018
8.	Cd	0.6 mM	*Brassica juncea*	Castasterone (CS)	100 mM 1mM 0.01mM	Coupled treatment of Cd with that of CS resulted in betterment of growth attributes i.e. root+shoot length, dry+fresh weight	Kaur *et al.*, 2017
9.		a) 0.25 mM	*Brassica*		10^{-7} M	Root + shoot length, dry + fresh weight along	Poonam *et al.*, 2015

	Cu	b) 0.50 mM c) 0.75 mM	*juncea*	CS	10^{-9} M 10^{-11}M	with content of dry matter were improved in stressed plants supplied with CS of 10^{-11}M	
10.	Cu	100 mg Kg^{-1}	*Cucumis sativus*	24-EBL	0.01 µM	Pre-sowing 24-EBL treatment of seeds enhanced dry mass, root+shoot length, and leaf area	Fariduddin *et al.*, 2013
11.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11}M	Enhancement in Water content RWC(relative water content) as well as dry matter content took place	Kohli *et al.*, 2013
12.	Cd, Pb	100 µM (Cd) and 200 µM (Pb)	*Raphanus sativus*	24-EBL	2 µM	EBL enhanced Shoot length+root length, dry weight along with leaf area	Osman and Rady, 2012
13.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11}M	Length of seedlings, roots and shoots, all were improved along with dry weight of plant	Sharma *et al.*, 2011
14.	Cr	1.2 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11}M	Shoot length + root length along with fresh weight of plant were recorded to enhance by 24-EBL treatment	Choudhary *et al.*, 2011
15.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6}M	Improvement in root +shoot length as well as number of leaves hinted at betterment of growth and developmental process	Arora *et al.*, 2011
16.	Zn	a) 0.25 mM b) 0.50 mM c) 0.75 mM d) 2 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6}M	Plants at 30 days stage exhibited increase in number of leaves and length of shoot	Arora *et al.*, 2010a
17.	Zn	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-HBL	10^{-4} M 10^{-6} M	Number of leaves plus length of both root and shoot got enhanced	Arora *et al.*, 2010b

		d) 2 mM			10^{-8}M		
18.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11}M	EBL made fresh weight plus length of both root and shoot better	Choudhary *et al.*, 2010
19.	Cu, Pb and Cd	10^{-6} M (Cu), 10^{-5} M (Pb) and 10^{-6} M (Cd)	*Chorella vulgaris*	Brassinolide (BL)	10^{-8}M	Amelioration of decline in growth attributes of metal stressed alga was reported	Bajguz, 2010
20.	Cu	a) 50 mg kg^{-1} b) 100 mg kg^{-1} c) 150 mg kg^{-1}	*Brassica juncea*	24-HBL	10^{-10} M 10^{-8} M 10^{-6}M	RWC, leaf area, dry weight plus length of both root and shoot recorded to be enhanced.	Fariduddin *et al.*, 2009
21.	Zn	a) 0.25 mM b) 0.50 mM c) 0.75 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4}M 10^{-5} M 10^{-6}M	Length and fresh weight of both root and shoot reported to be elevated by HBL treatment	Arora *et al.*, 2008a
22.	Ni	150 mM	*Brassica juncea*	24-EBL	1 µM	Noteworthy increment in length of both root and shoot, leaf area plus dry weight was recorded however, RWC declined	Ali *et al.*, 2008a
23.	Al	a) 1 mM b) 10mM	*Vigna radiata*	24-EBL and 28-HBL	10^{-8} M	Growth with regard to length of both root and shoot along with weight in fresh and dried form was enhanced by treating metal stressed plants with EBL plus HBL.	Ali *et al.*, 2008b
24.	Cd	a) 50 µM b) 100 µM c) 150 µM	*Cicer arietinum*	28-HBL	0.01 µM	Stressed plants showed partial recovery in fresh weight when given HBL treatment. Further, extent of nodulation plus weight of nodules in fresh and dried form got improved upon supplementation with HBL	Hasan *et al.*, 2008
25.	Ni	a) 0.25 mM b) 0.50 mM c) 0.75 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M	HBL treatment improved seedlings growth with that of length of both roots and shoots	Bhardwaj *et al.*, 2007

					10^{-6} M 10^{-8}M		
26.	Cu	a) 25 mg kg^{-1} b) 50 mg kg^{-1} c) 100 mg kg^{-1}	*Brassica juncea*	24-EBL	10^{-7} M	Pre-sowing application of 24-EBL to the stressed seedling improved length of both roots and shoots along with weight in dried and fresh form	Sharma and Bhardwaj, 2007

2.6.2 Photosynthetic efficiency

Multiple of studies have recognized the importance of BRs in influencing the photosynthetic potential of plants in response to varied stresses. Mitigatory action of BRs in battling out stresses of both abiotic and biotic nature has been widely reported (Bajguz and Hayat, 2009; Krishna, 2003). Generally, photosynthetic parameters are among those major attributes to be affected first by adversities in environmental conditions. Changes in photosynthetic characteristics, therefore, help in identifying the stress quite early in the plants. BRs are further noticed to resist the aftermaths of heavy metal induced stress in plants. For instance, the leaves of *Brassica juncea*, *Cicer arietinum* and *Raphanus sativus* got their chlorophyll content along with activity of carbonic anhydrase replenished when exogenously treated with HBL under Cd stress (Anuradha and Rao, 2009; Hasan *et al.*, 2008). BRs also maintain the optimal functioning of electron transport system and rectify the disorganization of photo- system II (PS II) and its RC (Reaction center) in winter rape (*Brassica napus* var. *napus*) plants under Cd toxicity (Janeczko *et al.*, 2005). Even the net rate of photosynthesis is made better by the supplementation of BRs in Cd stressed Indian mustard (*Brassica juncea)* and Radish (*Raphanus sativus)* (Anuradha and Rao, 2009). Some reports of improvement in photosynthetic potential by applying BRs in response to metal stress have been tabularized in table 2.8 as follows:

Table 2.8 Influence of BRs treatment on photosynthetic machinery in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration		Effect/s	Reference
1.	Zn		*Citrullus lanatus*			Pre-treatment with 24-EBL made an	Liu et

		5 mM		24-EBL	0.05μM	impressive betterment in the levels of chlotrophyll	al., 2023
2.	Cd	1 mM	*Vitis vinifera*	24-EBL	0.05 mg L^{-1} 0.1 mg L^{-1} 0.2 mg L^{-1}	EBL ameliorated the Cd toxicity symptoms, enhanced the optimal (Fv/Fm) and actual photochemical efficiency (Φ) of PS II, along with the quenching coefficient (qP)	Li et al., 2022
3.	Pb	200 μM	*Lycopersicum esculentum*	24-EBL	100 nM	Protection and betterment of photosynthetic apparatus plus enhancement in the content of photosynthetic pigments, rate of electron transport, along with more quantum yield by PS II leading to better net rate of photosynthesis and in more accumulation of biomass	Maia et al., 2022
4.	Fe	a) 250 μM b) 6250 μM	*Oryza sativa*	24-EBL	10 nM	EBL induced elevation in electron transport plus stomatal density resulting in enhancement in net rate of photosynthesis plus carboxylation efficiency	Tadaiesky et al., 2021
5.	Pb	1000 mg Kg^{-1}	*Festuca arundinacea*	24-EBL	0.2 mg L^{-1}	EBL treatment elevated the content of total chlorophyll with that of carotenoids	Zhong et al., 2020
6.	Cd	2.5 mM	*Cucumis sativus*	24- EBL	5 μM	Escalation in photosynthetic rate was recorded by EBL application to mitigate Cd stress	Shah et al., 2019
7.	Ni	100 mg Kg^{-1}	*Solanum lycopersicum*	24- EBL	10^{-8} M	Content of chlorophyll, beta-carotenoids and net rate of photosynthesis exhibited escalation upon getting supplemented with EBL under Ni stress	Nazir et al., 2019
8.	Cd	500 μM	*Vigna unguiculata*	24-EBL	0 nM 50 nM 100 nM	Parameters of gas exchange, content of photosynthetic pigments plus chlorophyll fluorescence are negatively affected by Cd exposure which were re-normalized by a pre-treatment with EBL	Santos et al., 2018
9.	Cd	a) 3 mg Kg^{-1} b) 9 mg Kg^{-1}	*Solanum lycopersicum*	HBL	10^{-8} M	HBL application alleviated Cd mediated negative impacts on	Singh and

						photosynthetic apparatus especially the photochemistry of photo-system II in seedlings of tomato	Prasad 2017
10.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7} M 10^{-9} M 10^{-11} M	Improvement in measures of total chlorophyll content, chl a, b and carotenoid. Parameters of gaseous exchange as in net rate of photosynthesis, transpiration rate, intercellular CO_2 plus stomatal conductance also exhibited enhancement	Yadav *et al.*, 2016
11.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Measures of total chlorophyll and carotenoids were escalated by EBL treatment. Net rate of photosynthesis, transpiration rate, intercellular CO_2 plus stomatal conductance also exhibited enhancement	Bali *et al.*, 2016
12.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Enhancement in gaseous exchange parameters along with levels of photosynthetic pigments (chl a, chl b, total chlorophyll and carotenoid) was observed	Poonam *et al.*, 2015
13.	Cd+ Hg	0.25 mM Cd + 0.25 mM Hg	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Levels of photosynthetic pigments (chl a, total chlorophyll and carotenoid) was observed though Chl b content did not exhibit significant increase	Kapoor *et al.*, 2014
14.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Photosynthetic pigments i.e. Total Chlorophyll along with carotenoids exhibited enhancement in their levels	Kohli *et al.*, 2013
15.	Cu	100 mg Kg^{-1}	*Cucumis sativus*	24-EBL	0.01 µM	SPAD chlorophyll, net rate of photosynthesis, stomatal conductance, internal CO_2 concentration along with water use efficiency got enhanced. Yield of Photo System II expressed as Fv/Fm was also increased	Fraiduddin *et al.*, 2013
16.	Cr	a) 0.5 mM				EBL supplementation enhanced levels	Sharma *et*

		b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	of chl a and chl b along with that of total Chlorophyll content	*al.*, 2011
17.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Enhanced levels of chl a and chl b along with that of carotenoid content were observed	Choudhary *et al.*, 2011
18.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Levels of chl a and chl b along with that of carotenoid content were elevated significantly	Choudhary *et al.*, 2010
19.	Cu	a) 50 mg Kg^{-1} b) 100 mg Kg^{-1} c) 150 mg Kg^{-1}	*Brassica juncea*	HBL	10^{-10} M 10^{-8} M 10^{-6}M	Chlorophyll, net rate of photosynthesis, stomatal conductance, internal CO_2 concentration along with water use efficiency got enhanced.	Fariduddin *et al.*, 2009
20.	Ni	150 mM	*Brassica juncea*	24-EBL	1 µM	Total chlorophyll as well as carotenoid measures were elevated. Efficiency of water use, conductance of stomata and internal CO_2 concentration got significantly enhanced	Ali *et al.*, 2008a
21.	Al	a) 1 mM b) 10 mM	*Vigna radiata*	28-HBL and 24-EBL	10^{-8} M	Intercellular concentration of CO_2 and stomatal conductance were elevated by the treatment of both the hormones. Chlorophyll measures were also enhanced however EBL was way more efficient as opposed to HBL	Ali *et al.*, 2008b
22.	Cd	a) 50 µM b) 100 µM c) 150 µM	*Cicer arietinum*	28-HBL	0.01 µM	HBL application resulted in enhancement in the measures of total chlorophyll	Hasan *et al.*, 2008

2.6.3. Metal uptake

Reduction of metal uptake has been largely linked to the augmentation of BRs in plants exposed to metal contamination (Kohli *et al.*, 2013; Sharma and Bhardwaj, 2007). This decline in metal uptake could be due to BR mediated stimulation of antioxidative defense mechanism, abatement of oxidative stress and enhancement in growth (Sharma and Bhardwaj, 2007; Yadav *et al.*, 2016). Supplementation of crops with BRs affects the absorption and stocking up of heavy metals in plants as observed in agricultural crops like barley, radish, sugar beet and tomato in response to metal toxicities of Cd, Cu, Pb and Zn. Similar outcomes were documented in case of uptake of Ni which was reduced due to 24-EBL treatment where activation of antioxidative defense was suggested to operate behind it (Kanwar *et al.*, (2012). Brassinolide was reported to lower down the accumulation of Al in plants while enhancing the growth and developmental process in *Phaseolus aureus* (Mung bean) (Janeczko *et al.*, 2005).

Decline in uptake of Cu and reduction in BCF (bio-concentration factor) was noticed in Cu stressed Indian mustard *Brassica juncea* plants upon getting supplemented with 24-EBL (Sharma and Bhardwaj, 2007). On the similar lines, a six-fold reduction in uptake of Cr metal was noticed in *Raphanus sativus* upon EBL application (Sharma *et al.*, 2011). Other findings of similar nature have also suggested reduction in Cu and Ni metal uptake by the addition of EBL in stressed plants of *Brassica juncea* (Kohli *et al.*, 2013; Kanwar *et al.*, 2012). In case of tomato plants exposed to Pb stress, the metal content was lowered down by 55% in roots, 65% in stem and 45% in leaves when supplied with 24-EBL (Maia et al., 2022).

2.6.4. Oxidative stress

Some research findings suggested at the vital role played by BRs in ameliorating the oxidative damage via lowering down MDA measures and production of ROS (Ramakrishna and Rao, 2015). This can be substantiated with the help of following examples tabularized in table 2.9.

Table 2.9 Influence of BRs treatment on ROS genesis and oxidative stress markers in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration	Effect/s	Reference

1.	Zn	5 mM	*Citrullus lanatus*	24-EBL	0.05µM	Prior application of 24-EBL lowered down the accumulation of ROS along with measures of MDA	Liu et al., 2023

2.	Cd	1 mM	*Vitis vinifera*	24-EBL	0.05 mg L^{-1} 0.1 mg L^{-1} 0.2 mg L^{-1}	Anions of superoxide along with measures of H_2O_2 and MDA were noticed to decrease by applying EBL to Cd stressed plants	Li et al., 2022
3.	Fe	a) 250 µM b) 6250 µM	*Oryza sativa*	24-EBL	0 M 10 nM	Amelioration of oxidative stress via ROS scavenging	Tadaiesky et al., 2021
4.	Cd	a) 0 and b) 200 µM	*Brassica juncea*	24-EBL	0 M 10^{-7} M 10^{-5} M	The measures of ROS, MDA plus extent of electrolyte leakage exhibited reduction in response to EBL supplementation	Alam et al., 2020
5.	As	a) 0 µM b) 50 µM c) 100 µM	*Triticum aestivum*	24-EBL	0 µM 0.5µM 0.75 µM	Significant reduction in the measures of MDA and H2O2 along with electrolyte leakage was observed by the application of EBL in combination with Si and Salicylic acid	Maghsoudi et al., 2020
6.	Pb	1000 mg kg^{-1}	*Festuca arundinacea*	24-EBL	0.2 mg L^{-1}	EBL treatment reduced the levels of both MDA and H_2O_2	Zhong et al., 2020
7.	Ni	100 mg kg^{-1}	*Solanum lycopersicum*	24- EBL	10^{-8} M	EBL treatment reduced percentage of electrolyte leakage under Ni stress	Nazir et al., 2019
8.	Cd	2.5 mM	*Cucumis sativus*	24- EBL	5 µM	Measures of H_2O_2 and MDA along with extent of electrolyte leakage exhibited reduction upon EBL supplementation	Shah et al., 2019
9.	Cu	120 µM	*Vitis vinifera*	24-EBL	0.5 mg L^{-1} 0.10 mg L^{-1} 0.20 mg L^{-}	Significant decline in measures of superoxide ions, MDA and H_2O_2, inside root and foliar region	Zhao et al., 2018

					1		
10.	Cd	0.6 mM	*Brassica juncea*	CS	100 mM 1 mM 0.01mM	Treatment of CS reduced the levels of H_2O_2 as well as MDA levels. Similar reduction was observed in NO content which indicated mitigation of oxidative stress and better growth	Kaur *et al.*, 2017
11.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7} M 10^{-9} M 10^{-11} M	Anions of Superoxide along with H_2O_2 measures were noticed to decrease by applying CS to Cu stressed plants	Yadav *et al.*, 2016
12.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Drastic reduction in MDA measures was drastically lowered. Levels of H_2O_2 was recorded to be slightly elevated	Choudhary *et al.*, 2011
13.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Content of MDA was observed to be reduced, suggesting decline in lipid peroxidation by 24-EBL supplementation	Sharma *et al.*, 2011
14.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6} M	MDA measures were recorded to be reduced upon EBL application	Arora *et al.*, 2010a
15.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M	EBL treatment brought about a drastic decline in MDA levels	Choudhary *et al.*, 2010

					10^{-9} M 10^{-11} M		
16.	Cu	a) 50 mg Kg^{-1} b) 100 mg Kg^{-1} c) 150 mg Kg^{-1}	*Brassica juncea*	24-HBL	10^{-10} M 10^{-8} M 10^{-6} M	HBL application lowered down the H_2O_2 measures in response to Cu distress	Fariduddin *et al.*, 2009
17.	Cu	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Notable decline in measures of MDA was observed	Arora *et al.*, 2008b
18.	Ni	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	HBL (10^{-6} M of concentration) most effectively reduced the MDA measures	Bhardwaj *et al.*, 2007
19.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Drastic reduction in MDA measures was noticed by 28-HBL application	Sharma and Bhardwaj, 2007

2.6.5. Protein Content

Besides reducing lipid peroxidation, metal stress induced damage to membranal integrity and oxidation of proteins can also be prevented by EBL supplementation (Ramakrishna and Rao, 2015). Additionally, BRs can bring about induction of cellular growth and expansion with the help of activation of proteins and fresh production of nucleic acid. This not only enhances the stability of cell wall but also its permeability (Cao *et al.*, 2005). Table 2.10 depicts the major alterations in protein content in metal stressed plants subjected to BRs treatments.

Table 2.10 Influence of BRs treatment on measures of protein in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration/s	Plant Species	Nature of BRs and concentration/s		Effect/s	Reference
1.	Pb	a) 0.5 mM b) 0.7 mM c) 0.9 mM	*Trigonella foenum-graecum*	24-EBL	10^{-7} M	Significant enhancement in the content of protein was recorded upon EBL supplementation in Pb stressed plants	Sharma et al., 2023
2.	Pb	1000 mg kg^{-1}	*Festuca arundinacea*	24-EBL	0.2 mg L $^{-1}$	EBL supplementation elevated the proteincontents	Zhong et al., 2020
3.	Ni	100 mg kg^{-1}	*Solanum lycopersicum*	24- EBL	10^{-8} M	EBL supplementation elevated the protein contents	Nazir et al., 2019
4.	Pb	2 mM	*Brassica juncea*	24- EBL	10^{-8} M	Toxicity of Pb is alleviated by enhancing the protein measures	Dalyan et al., 2018
5.	Pb	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	EBL	10^{-7} M	Notable enhancement in protein measures was noticed upon EBL treatment	Kohli et al., 2018
6.	Cu	120 µM	*Vitis vinifera*	24-EBL	0.5 mg L^{-1}	Measures of soluble protein were elevated by EBL supplementation	Zhao et al., 2018
					0.10 mg L^{-1}		
					0.20 mg L^{-1}		
		a) 0.25 mM			10^{-7} M	EBL supplementation elevated the protein content	Poonam et al., 2015

7.	Cu	b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-9} M 10^{-11} M		
8.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Total Soluble protein or TSP content got significantly enhanced	Choudhary et al., 2011
9.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Elevation in total protein content	Sharma et al., 2011
10.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6} M	EBL treatment elevated the protein content	Arora et al., 2011
11.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL plus 28-HBL	10^{-11} M 10^{-9} M 10^{-7} M	Protein measures of root and shoot system got elevated by each of these hormones	Randhawa et al., 2010
12..	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Mild betterment in protein content brought about by EBL treatment	Choudhary et al., 2010

13.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6} M	Total measures of soluble protein were improved by EBL supplementation	Arora et al., 2010a
14.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Total measures of soluble protein were remarkably improved by HBL supplementation	Arora et al., 2010b
15..	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Escalation in measures of soluble protein was noticed	Choudhary et al., 2009
16.	Cu	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Escalation in measures of soluble protein was noticed	Arora et al., 2008b
17..	Cd	a) 50 µM b) 100 µM c) 150 µM	*Cicer arietinum*	28-HBL	0.01 µM	HBL application resulted in notable enhancement in the levels of proteins	Hasan et al., 2008
18.	Ni	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Remarkable improvement in measures of soluble proteins by HBL	Bhardwaj et al., 2007
19.	Zn				10^{-4} M	Elevation in levels of soluble protein	Sharma and Bhardwaj,

		a) 0.5 Mm b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-6} M		2007
					10^{-8} M		

2.6.6 Secondary metabolites

Secondary metabolites protect plants from both abiotic and biotic stresses. Secondary metabolites with the functional group phenol are an important category of stress manager (Jung *et al.*, 2003). It was suggested by Xu *et al.*, (2015), that there exists a direct link in-between antoxidative potential of plants and enhanced levels of phenolic compounds. Ahammed *et al.*, (2013b) suggested that enhanced level of phenolics by BR application is possibly due to change in levels of polyphenols, which results in enhanced antioxidative defense ability of plants (Yadav *et al.*, 2016). Several reports demonstrate the employment of phytohormones to increase level of phenolic compounds in plants such as enhanced content of anthocyanin in carrot (Naryan *et al.*, 2005). Few reports of alteration in phenolic compounds in different plant species in response to BRs application has been tabulated in Table 2.11.

Table 2.11 Influence of BRs treatment on secondary metabolites in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration		Effect/s	Reference
1.	Pb	a) 0.5 mM b) 0.7 mM c) 0.9 mM	*Trigonella foenum-graecum*	24-EBL	10^{-7} M	Measures of secondary metabolites exhibited a significant promotion when Pb stressed plants were given EBL treatment	Sharma et al., 2023
2.	Cr	10 mg Kg^{-1}	*Solanum lycopersicon* cv. K-21	24-EBL	10^{-7} M	Chromium stress was mitigated by EBL supplementation and expressed in the form of elevated measures of flavanoids	Jan et al., 2020
3.	Cd	250 and 500 µM	*Brassica napus*	24-EBL	0.02 and 0.5 µM	EBL supplementation with that of thiamine enhanced the measures of total phenolics and flavanoids in Cd stressed canola plants	Sanjari et al., 2019
4.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7} M	supplementation with CS escalated the measures of anthocyanin, total phenolics and flavonoids. Besides this, content of polyphenols especially ellagic acid, catechin etc. was escalated significantly	Yadav et al., 2016
					10^{-9} M		
					10^{-11} M		

5.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M	Cu stressed plants exhibited enhancement in the measures of Anthocyanin, xanthophylls and flavonoids upon EBL supplementation	Bali et al., 2016
6.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Cu stressed plants (0.75 mM) had maximum development of anthocyanin pigment when supplemented with CS. Total phenolic content also got significantly enhanced	Poonam et al., 2015
7.	Cd+ Hg	0.25 mM Cd + 0.25 mM Hg	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL treatment elevated measures of anthocyanin along with flavonoid though flavanoids were enhanced insignificantly.	Kapoor et al., 2014
8.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Total measures of flavanoids, xanthophylls and anthocyanin were elevated upon treatment with EBL	Kohli et al., 2013
9.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Notable enhancement in measures of total phenolics	Choudhary et al., 2011
10.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL concentrations of 10-9 M with that of 10^{-11} M elevated the content of total phenolics. Treatment with 10^{-7} M showed no significant effects	Choudhary et al., 2010

2.6.7 Osmolyte Content

Plants bring to play the synthesis and accumulation of various osmoprotectants upon getting exposed to abiotic stresses, which play a crucial role in managing the stressful conditions (Miura and Tada *et al.*, 2014). In 2013, it was noticed by Xi *et al.* that elevation in levels of osmolytes led to escalation in the activities of antioxidative defense mechanism of berry plants. Large number of studies of modulation of osmolyte content in response to BR application has been tabulated in Table 2.12.

Table 2.12 Influence of BRs treatment on osmolytes in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration		Effect/s	Reference
1.	Ni	--------------------	*Raphanus sativus*	28-HBL	$1\ \mu mol\ L^{-1}$ $5\ \mu mol\ L^{-1}$ $10\ \mu mol\ L^{-1}$	Priming of seedlings with 28-HBL led to a reduction in Ni levels together with an enhancement in proline content	Ahmed & Sardar, 2023
2.	Pb	$1000\ mg\ kg^{-1}$	*Festuca arundinacea*	24-EBL	$0.2\ mg\ L^{-1}$	Proline content exhibited elevation upon EBL treatment	Zhong et al., 2020
3.	Ni	$100\ mg\ kg^{-1}$	*Solanum lycopersicum*	24- EBL	10^{-8} M	Measures of proline exhibited an escalation upon getting treated with EBL under Ni stress	Nazir et al., 2019
4.	Pb	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M	Treatment with 24 EBL in combination with Salicylic acid enhanced the contents of glycine betaine and proline along with trehalose	Kohli et al., 2018
5.	Cd	$0.6\ mmol\ kg^{-1}$	*Brassica juncea*	CS	0.01 nM 100 nM	Along with Citric acid (100 nM), CS improved the levels of proline along with secondary metabolites-flavanoids, anthocyanin etc.	Kaur et al. 2017
6.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7} M 10^{-9} M 10^{-11} M	Enhancement in content of total sugars was observed when plants were given CS treatment	Yadav *et al.*, 2016
7.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M	Reducing sugars were observed to get enhanced in their content. Presence of sugar alcohol (inositol and mannitol), pentoses and hexoses (xylose, glucose and fructose) and disachharides (galactose) was noticed	Bali *et al.*, 2016

8.	Cu	100 mg Kg^{-1}	*Cucumis sativus*	24-EBL	0.01 µM	EBL treatment made the levels of proline high in Cu stressed plants	Fariduddin *et al.*, 2013
9.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL supplementation enhanced the content of glucose, fructose, reducing sugars and total sugars	Kohli *et al.*, 2013
10.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL supplementation remarkably enhanced the proline content	Sharma *et al.*, 2011
11.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Different dosages of EBL elevated the measures of proline and glycine betaine	Choudhary *et al.*, 2011
12.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL application to Cu stressed plants resulted in elevation of proline content	Choudhary *et al.*, 2010
13.	Cu	a) 50 mg kg^{-1} b) 100 mg kg^{-1} c) 150 mg kg^{-1}	*Brassica juncea*	24-HBL	10^{-10} M 10^{-8} M 10^{-6} M	In HBL supplemented metal stressed plants, enzymatic action of CAT, POD and SOD observed to be upscaled.	Fariduddin et al., 2009
14.	Cd	a) 50 µM b) 100 µM c) 150 µM	*Cicer arietinum*	28-HBL	0.01 µM	Remarkable escalation in content of total carbohydrates was noticed in nodules while measures of proline got elevated in plants	Hasan *et al.*, 2008
15.	Ni	150 mM	*Brassica juncea*	24-EBL	1 µM	EBL treatment elevated proline levels	Ali *et al.*, 2008a

2.6.8 Antioxidative defense mechanism

The initial symptoms of metal toxicity inside plants include oxidative burst because of excessive synthesis of ROS, which subsequently damages different components of plants (Krishna, 2003). This makes the membranes unstable and modulates the measures of antioxidants along with antioxidative enzymes (Panda *et al.*, 2003). Ali *et al.* in 2008 suggested that the innate antioxidant defense mechanisms in plants can be activated by both –heavy metal stress as well as application of BRs Extensive studies have been made on the ability of BRs to induce the cascading of antioxidative defense for inhibiting ROS production and lipid degradation (Soares *et al.*, 2016). Activities of antioxidants (Ascorbate, glutathione and tocopherol) of non-enzymatic character with that of enzymatic ones (APOX, CAT, GR, GST, POD, SOD etc.) are modulated by BRs addition to the plants exposed to heavy metal toxicity. This can be substantiated by multifarious reports on protective influence of BRs whereby the antioxidative defense line of action gets upregulated in plants (Arora *et al.*, 2008a,b;

Kagale *et al.*, 2007; Janeczko *et al.*, 2005) which have been tabularized in table 2.13 as follows:

Table 2.13 Influence of BRs treatment on antioxidant defense mechanisms in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration		Effect/s	Reference
1.	Zn	5 mM	*Citrullus lanatus*	24-EBL	0.05µM	Pre-treatment with 24-EBL upregulated the enzymatic action of APX, CAT, GR, POD and SOD with an increase in the levels of AsA plus GSH in response to Zn toxicity	Liu et al., 2023
2.	Cd	1mM	*Vitis vinifera*	24-EBL	0.05 mg L^{-1}	Upregulation of expression of APX, DHAR, GR, MDHAR, POD and SOD in EBL treated seedlings when exposed to Cd stress.	Li et al., 2022
					0.1 mg L^{-1}		
					0.2 mg L^{-1}		
4.	Fe	a) 250 µM b) 6250 µM	*Oryza sativa*	24-EBL	10 nM	A noticeable boost in the enzymatic performance of APX, CAT, POD and SOD in EBL treated stressed plants	Tadaiesky et al., 2021
5	Pb	200 µM	*Oryza sativa*	24-EBL	100 nM	Significant elevation in APX, CAT, POD and SOD due to EBL supplementation	Guedes et al., 2021
6.	Pb	1000 mg kg^{-1}	*Festuca arundinacea*	24-EBL	0.2 mg L^{-1}	EBL treatment increased the contents of CAT and SOD whereas that of APX declined	Zhong et al., 2020
7.	Ni	100 mg kg-^{1}	*Solanum lycopersicum*	24- EBL	10^{-8} M	Notable escalation in CAT and SOD in EBL treated Ni stressed plants	Nazir et al., 2019
8.	Cu	120 µM	*Vitis vinifera*	24-EBL	0.05 mg L^{-1}	Elevation in the enzymatic efficiency of CAT, POD and SOD together with components of ASc-GSH cycle (APX, GR, MDHAR, DHAR in EBL supplied stressed plants. However, glutathione content in its normal and oxidized form was recorded to be decreased	Zhou et al., 2018
					0.10 mg L^{-1}		
					0.20 mg L^{-1}		
9.	Cd	0.6 mM	*Brassica juncea*	CS	100 mM	Elevation in the enzymatic activities of APOX, SOD, DHAR, POD, CAT, GST, GR, GPOX, PPO etc. along with an enhancement in measures of GSH, WSA as well as	Kaur *et al.*, 2017
					10^{-9} M		

					10 -11 M	LSA	
10.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7} M 10^{-9} M 10^{-11} M	CS supplementation elevated APOX, CAT, PPO, POD and SOD enzymatic activities	Yadav *et al.*, 2016
11.	Cu	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Remarkable enhancement in action of APOX, CAT, GR, MDHAR, POD and SOD was noticed due to EBL supplementation	Poonam *et al.*, 2014
12.	Cd+ Hg	0.25 mM Cd + 0.25 mM Hg	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Enzyme action of APOX, POD and SOD was upscaled. Similar outcomes were observed in case of MDHAR, DHAR, PPO. GR, and GPOX enzymes	Kapoor *et al.*, 2014
13.	Cu	100 mg Kg^{-1}	*Cucumis sativus*	24-EBL	0.01 μM	Upgradation in functioning of SOD, POD and CAT enzymes	Fariduddin *et al.*, 2013
14.	Cd , Pb	100 (Cd) + 200 μM (Pb)	*Lycopersicon esculentum*	24-EBL	2 μM	Elevated levels of APOX, SOD, GR and CAT were exhibited. GSH got lowered while ascorbate measures were noticed to be improved	Osman and Rady, 2012
15.	Cr	1.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Both GSH as well as ascorbate measures were recroded to be elevated by 31%. Enzymatic antioxidants levels including CAT, GR, APOX and SOD were elevated. Whereas, no effect on GPOX activity was recorded	Choudhary *et al.*, 2011
16.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Enzymatic action of APOX , CAT, GR, DHAR, MDHAR and SOD was elevated. No notable effect was detected in enzymatic action of POD	Sharma *et al.*, 2011

17.	Cr	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{6} M	Enzymatic action of APOX , CAT, GR, POD and SOD was elevated. No notable effect was detected in enzymatic actions of DHAR as well as MDHAR	Arora *et al.*, 2011
18.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	Enzymatic action of CAT, GR, GPOX and SOD was enhanced. Similar rise in measures of ascorbate was observed	Choudhary *et al.*, 2010
19.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	24-EBL	10^{-10} M 10^{-8} M 10^{-6} M	Significant elevation in GSH measures along with upgradation of enzymatic action of APOX, CAT, DHAR GR, MDHAR, POD and SOD took place by EBL application	Arora *et al.*, 2010a
20.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM	*Brassica juncea*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Enzymatic action of APOX, CAT, DHAR, GR, MDHAR, and SOD reported to be enhanced	Arora *et al.*, 2010b
21.	Cr	a) 0.5 mM b) 1 mM	*Raphanus sativus*	24-EBL, 28-HBL	10^{-11} M 10^{-9} M 10^{-7} M	Hormonal supplementation excalated PPO's enzymatic action inside roots and leaves but EBL was way more effective. No notable change in the action of GPOX upon HBL application was reported	Randhawa *et al.*, 2010
22.	Cu, ,Pb, Cd	a) 10^{-6} (Cu) b) 10^{-5} M(Pb) c) 10^{-6} M (Cd)	*Chorella vulgaris*	Brassinolide (BL)	10^{-8} M	Elevation in enzymatic action of APOX, CAT, GR and SOD was observed. Content of antioxidants of non- enzymatic nature (ascorbate plus GSH got enhanced	Bajguz, 2010
23.	Cu	0.2 mM	*Raphanus sativus*	24-EBL	10^{-7} M 10^{-9} M 10^{-11} M	EBL supplementation escalated measures of CAT and SOD, however, no influence on GPOX was noticed	Choudhary *et al.*, 2009
24.	Cu	a) 50 mg kg^{-1} b) 100 mg kg^{-1} c) 150 mg kg^{-1}	*Brassica juncea*	24-HBL	10^{-10} M 10^{-8} M 10^{6} M	In HBL supplemented metal stressed plants, enzymatic action of CAT,POD and SOD observed to be upscaled.	Fariduddin et al., 2009
25.	Cd	a) 50 µM b) 100 µM c) 150 µM	*Cicer arietinum*	28-HBL	0.01 µM	In HBL supplemented metal stressed plants, enzymatic action of CAT, POD and SOD was reported to be upscaled.	Hasan *et al.*, 2008
26.	Ni	150 mM	*Brassica juncea*	24-EBL	1 µM	Enzymatic action of CAT, GR, POD and SOD was reported to be escalated by EBL dosages	Ali *et al.*, 2008a

27.	Al	a) 1 mM b) 10 mM	*Vigna radiata*	28-HBL + 24-EBL	10^{-8} M	Enzymatic action of CAT, POD and SOD was noticed to be escalated by dosages of both HBL and EBL dosages. However, efficiency of HBL in the activation of antioxidative defense mechanism was a bit higher	Ali *et al.*, 2008b
28.		a) 0.5 mM				In HBL supplemented metal stressed	Arora *et*

	Cu	b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	plants, enzymatic action of APOX, CAT, GR, POD and SOD observed to be escalated	*al.*, 2008b
29.	Ni	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Treatment with HBL, enhanced the enzymatic action of APOX, CAT, GR and POD was enhanced without causing any change in the activity of SOD	Bhardwaj *et al.*, 2007
30.	Zn	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	*Zea mays*	28-HBL	10^{-4} M 10^{-6} M 10^{-8} M	Enzymatic action of APOX, CAT, GR, POD and SOD was observed to be escalated when supplemented with 10^{-8} mM HBL	Sharma and Bhardwaj, 2007

2.6.9 Gene expression

Application of BRs modulates the expression levels of various stress responsive genes, which is summarized as below in Table 2.14.

Table 2.14 Influence of BRs treatment on stress responsive genes in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal concentration	Plant Species	Nature of BRs and concentration		Effect/s	Reference
1.	Pb	a) 0.5 M b) 0.7 M c) 0.9 M	*Trigonella foenum-graecum*	24- EBL	10^{-7} M	Downregulated expression of CAT plus SOD genes in Pb exposed fenugreek plants was recorded to be mitigated upon supplementation with EBL alone and in combination with Si	Sharma et al., 2023
2.	Zn	5 mM	*Citrullus lanatus*	24-EBL	0.05µM	supplementation with EBL upregulated the extent of expression for the genes coding anti- oxidative enzymes namely APX, CAT, GR andCu/Zn SOD in Zn stressed plants	Liu et al., 2023
	Polymetallic stress (Al^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Pb^{2+}	Al^{3+}--20 µM Mn^{2+}- 50µM Cd^{2+}- 2.8 µM Cu^{2+}- 2 µM Ni^{2+}- 16 µM Zn^{2+}- 40 µM	*Hordeum vulgare*	-Homobrassinolide (HBL)-0.1 and 10nM -Homocastasterone (HCS)		HBL affected the transcript levels of all the genes of metal tolerance inside shoots without significantly affecting those of roots. However, HCS application down regulated the expression of all such genes inside both root + shoots	Zlobin et al., 2023

		Pb^{2+}- 30 µM					
3.	Mn	a) 0.5 mM b) 1.0 mM	*Arabidopsis thaliana*	24- EBL	1 µM	Expression levels of genes namely Cu/Zn SD3 (superoxide dismutase), CAT2 (Catalase) and two FSD2 & 3 (iron dismutase) were up-scaled when metal stressed plants were supplemented with 24-EBL	Surgun Acar & Zemheri-Navruz, 2022
4.	Cr	100 µM	*Oryza sativa* Cultivars: a) CY- 927 b) YLY-689	24-EBL	0.01 µM	Both the cultivars exhibited upregulation of transcription level of SOD plus POD gene and in case of APX plus CAT genes, the promotion in their expression extent was noticed only in cv. CY-927	Basit et al., 2021
5.	Cd	200 µM	*Brassica juncea*	24- EBL	10^{-5} M 10^{-7} M	Increment in the enzymatic action of CAT, DHAR, GR, GST, POD and SOD was noticed, suggesting at possible role of elevation in expression levels of the respective genes when Cd stressed plants were supplied with EBL	Alam et al., 2020
6	Pb	a) 0.25 mM b) 0.50 mM c) 0.75 mM	*Brassica juncea*	EBL	10^{-7} M	Supplementation of Pb stressed plants with EBL upregulated the transcript levels of CAT, GST, DHAR, GR and POD genes	Kohli et al., 2018
7.	Cd, Cr	Cd-0.5 mM Cr- 1.0 mM	*Raphanus sativus* L. *var. Pusa Chetki*	EBL or HBL	10^{-7} M	Pre-soaking treatments with HBL promoted the expression extent of *Cat1* + *Cat2*+ *Cat3*, *Cu/ZnSod* plus *MnSod* while EBL stimulated expression of *FeSOD* in Cd stressed radish seedlings. On the other hand, Cr stress upregulated the expression of *SOD* plus *CAT* genes with an exception of *Cat2*	Sharma et al., 2018
8.	Cu	a) 0.25 mM b) 0.5 mM c) 0.75 mM	*Brassica juncea*	CS	10^{-7}M 10^{-9} M 10^{-11} M	Elevation in the transcript levels of antioxidant genes namely GST-1, GR, GSH-S and DHAR was recorded when given a pre-treatment with CS.	Yadav et al., 2018
9.	Zn	10 mM	*Solanum melongena*	24-EBL	0.1 µM	Upscaling of the enzymatic action of antioxidative machinery might be existing on account of elevation in the expression extent of the antioxidative genes upon EBL supplementation	He et al., 2016
10.	Cr	0.5 mM	*Oryza sativa*	24-EBL	0.1mM 0.01mM 0.1 nM	External supplementation with EBL promoted the expression extent in the genes coding various antioxidative enzymes namely SOD, APX, CAT and GR under Cr stress	Sharma et al., 2016

11.	Zn	10 mM	*Solanum melongena*	24-EBL	0.1 µM	Exogenous supplementation with EBL elevated the expression levels of glutathione reductase (GR)+ glutathione synthetase (GS) + dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) in the foliar regions of Zn exposed Solanum melongena	Wu et al., 2016
12.	Cd	100 µM	*Solanum lycopersicum*	24-EBL	0.1 µM	Elevation in the enzymatic action as well as transcript levels was observed in antioxidants when the stressed plants were given EBL treatment	Ahammed et al., 2013b

2.7 Mitigatory influence of Silicon on heavy metals

2.7.1 Absorption, distribution, and accumulation in plants

Silicon being present in most of the plants exhibits a diverse range in its concentration making the plants either, low, medium or high accumulators. In the plants with high Si accumulation potential, maximum accumulation occurs inside the leaves having more than 1.5% of their dry weight (Hu et al., 2020). Plants absorb and translocate Si in the form of silicic acid (H_4SiO_4). Transport of silicic acid to the cell walls and extracellular spaces takes place through transpirational flow via irreversibly precipitating Si in the form of amorphous silica. The Si content in diverse plant species is dependent upon absorption and loading capacity of the plants from their roots to xylem elements (Ma et al., 2001). Inside the soil, deposition of phytoavailable form of Si i.e. H_4SiO_4 (a monomeric molecule of neutral nature) occurs as amorphous silica i.e. ($SiO_2.nH_2O$) (Coskun et al., 2019; Epstein, 2001).

Various Si transporters carrying out both its influx and efflux have been reported to be responsible for distributing Si inside the various parts of the plants (Chaiwong et al., 2020; Ma & Yamaji, 2015). *Lsi1* (influx) and *Lsi2* (efflux) were identified and characterized as the very first Si-transporters in *Oryza sativa* (Rice). *Lsi1* belongs to NIP protein family, whereas *Lsi2* is recognized as an anion transporter and transfer of Si from the soil into the roots has been synergistically brought about via these two proteinaceous transporters in rice (Ma et al., 2006).

Lsi1 exhibits passive transport and transits Si inside the cellular membranes down the concentration gradient from apoplastic regions to the symplast. On the other hand, *Lsi2* resorts to an opposite manner for its passage i.e. by getting actively coupled with a proton-ATP antiport mechanism. *Lsi1* motions Si towards the living elements of the endodermal region at the rear end, while *Lsi2* extrudes it to the stelar regions at the proximal part (Fig. 2.3). This peculiar localization of these Si transporters accounts for making Si uptake to be unidirectional inside the plants (Ma et al., 2006).

Every plant has its own group of Si transporters for absorbing this element in various regions e.g. *OsLsi, AtLsi, ZmLsi*, etc. In *Cucumis sativus* (Cucumber), *Oryza sativa* (Rice) and *Lycopersicum esculentum* (Tomato), Si movement occurs from rhizosphere →root→cortical cells→xylem vessels with an increase at every step from the previous one (Mitani & Ma, 2005). However, absorption and transport potential of these three plants vary from each other, being highest in rice plants (Ma &Yamaji, 2008, 2006). Quantity wise, symplastic areas possess more Si when contrasted against the rhizospheric region in the soil.

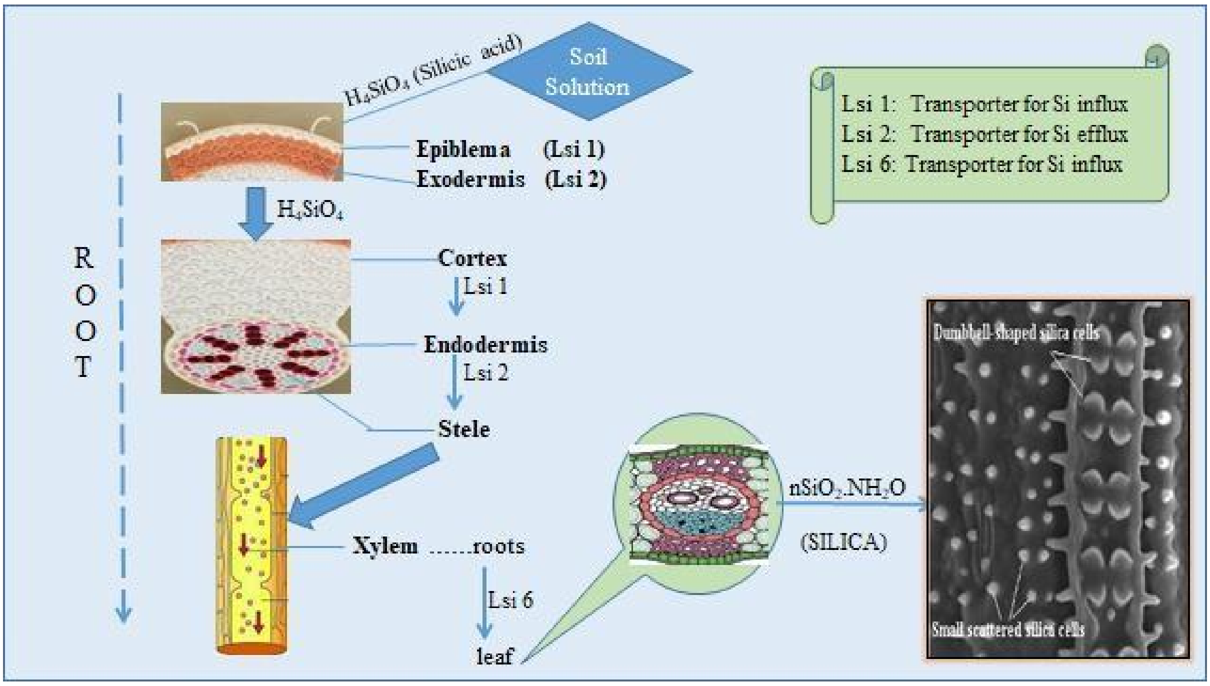


Fig. 2.3 Diagrammatic depiction of absorption and translocation of Silicon inside the plants (modified after Khan et al. 2019b).

Mediation of radial transport is performed in these plant species by a Si transporter to the maximum in rice followed by cucumber and tomato (Mitani and Ma, 2005; Ma and Yamaji, 2006).

Energy dependent mechanism of Si absorption continues to operate even under low temperature circumstances (Mitani & Ma, 2005). Transporter genes play a major role in translocating Si in rice plants while in cucumber and tomato, diffusion is responsible for transporting Si (Ma & Yamaji, 2006). Si transporters in the stems of rice plants are accountable for accumulating Si in greater measures inside them. Absence or impaired transport mechanisms favouring xylem loading could be a reason for stocking up of Si in lower amounts in cucumber and tomatoes (Ma et al., 2004; Ma & Yamaji, 2006). Silicon is transited from the cells of cortex to the elements of xylem after its absorption from the exogenous media via a process termed as xylem loading whereby it enters shoot from roots. Furthermore, plants readily absorb silicic acid but transform it into silica gel at concentrations greater than 2 mM as observed in both rice and wheat plants (Ma & Yamaji, 2006). Once the Si gets transported into the shoot system, it piles up in the upper aerial parts in the hydrated polymeric form of silica gel (Ma et al., 2006). The process of transpiration enhances the concentration of silicic acid in the aerial regions where its polymerization into silica gel takes place (Ma & Takahashi, 2002).

In rice plants, concentrations of silica gel are above 90% in stems. Rate of transpiration determines distribution as well as accumulation of Si inside the shoots. In the cuticular region (< 0.1 mm), deposition of Si gives rise to a two layered thick structure (Ma & Takahashi, 2002). Foliar region of rice possesses silicified cells of two types namely a) silica motor and b) silica cells. The shapes of these cells change after getting accumulated inside specific regions. For instance, formation of dumb-bell like structures by the assembly of silica cells occurs inside the vascular parts (Ma & Yamaji, 2008). Further, a distinct outer organization in the epidermal region makes some of the silicified cells to be termed as bulliform cells.

2.7.2 Plant growth

The maize seedlings exposed to Cd toxicity when supplemented with that of Si amplifies the elongation of roots and production of biomass (Dresler et al., 2015). On the similar lines, different parameters of growth were upscaled in rice plants suffering from Cd toxicity when supplemented with Si (Huang et al., 2021). Treatment with Si made the plant biomass better in hydroponically grown Zn stressed maize cultivars (Bokor et al., 2014a, 2014b). An upsurge in the biomass of rice seedlings have been observed on account of Si application which not only mitigated As stress but also diminished the Zn content. Additionally, in the plants like maize, cotton etc., a remarkable suppression of Zn accumulation was achieved via Si treatment (Anwaar et al., 2015; Patrícia et al., 2008). Further, the uptake plus translocation of Cd from the region of roots to that of shoots were slowed down in Si treated rice plants (Shi et al., 2005). Table 2.15 depicts the impact of Si on different parameters of growth in various plant species exposed to heavy metal stress.

Table 2.15 Influence of Si treatment on growth attributes in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	Cd	20 mg kg^{-1}	*Zea mays*	200 mg kg^{-1}	Treatment of metal stressed plants with Si remarkably improved length plus dry weight of roots along with the height of the plant	An et al., 2022

2.	Ni	100 mM	*Zea mays*	2.5 mM	A notable increase in root length plus biomass was recorded in metal stressed cultivars	Fiala et al., 2021
3.	Cd	50 µmol L^{-1} 200 µmol L^{-1}	*Triticum aestivum*	a) 1 mmol L^{-1} b) 3 mmol L^{-1}	Si made the root's morphological characters by strengthening the antioxidative defense mechanisms and by hampering ROS generation inside tissues of roots being cultured on growth media incorporated with different concentrations of Cd toxicity	Rahman et al., 2021
4.	Pb	500 mg kg^{-1} 1000 mg kg^{-1} 1500 mg kg^{-1}	*Coriandrum sativum*	1.5 mM	Si (NPs) boosted the resistance potential of the plant against Pb toxicity which was expressed in terms of improved leaf area + root length + biomass + plant height when supplemented together with Pb resistant microorganisms	Fatemi et al., 2020
5.	Cd	7.67 mg kg^{-1}	*Triticum aestivum*	a) 25 mg kg^{-1} b) 50 mg kg^{-1} c) 100 mg kg^{-1}	Alleviation of Cd stress was achieved by Si supplementation which resulted in enhancement of plant height, biomass and length of inflorescence	Khan et al. 2020b
6.	Cd	5 µM	*Triticum aestivum*	1 mM	Drastic decline in dry weight of roots + shoots under long term subjection to Cd stress was mitigated by Si application	Wu et al., 2019
7.	Al	10 mM	*Zea mays*	2 mM	Length of seedlings, their germination percentage with that of vigour index displayed augmentation to mitigate Al stress via Si supplementation	Delavar et al., 2017
8.	As	150 µM 300 µM	*Oryza sativa*	a) 0.25 mM b) 1 mM c) 2 mM d) 3 mM	Silicon made the biomass plus other growth attributes much better	Zia et al., 2017
9.	As	150 mM	*Brassica juncea*	1.5 mM	Growth parameters with regard to roots' morphology were negatively hampered by As toxicity. However, Si supplementation enhanced the production of lateral roots, ratio of root length as well as root biomass together with fineness of roots and density of root tissue	Pandey et al., 2016
10.	Cd	0.5 µM 5.0 µM	*Triticum turgidum* Cultivar: Claudio	1 mM	Inhibitory effects of Cd on growth parameters were alleviated by Si application	Rizwan et al., 2016

		50 µM				
11.	Zn	25 µM 50 µM	*Gossypium hirsutum*	1 mM	Silicon mediated suppression in accumulation of Zn in different plant parts resulted in promotion of dry matter content along with other growth parameters	Anwaar et al., 2015
12.	Al	160 mg L^{-1}	*Arachis hypogea*	80 mg L^{-1}	Dry matter of root + shoot along with root/shoot ratio exhibited an improvement when treated with Si	Shen et al., 2014
13.	Pb	100 50 µM	*Gossypium hirsutum*	1 mM	Pb stressed cotton plants when exogenously treated with Si exhibited improvement in different growth parameters including biomass	Bharwana et al., 2013
14.	Cd	1 mM 5 mM	*Gossypium hirsutum*	1 mM	Silicon supplementation in Cd exposedplants of cotton resulted in improvement in different parameters of morphological development.	Farooq et al., 2013
15.	As	10 M 25 M	*Oryza sativa*	a) 0.5 mM b) 1 mM	Tolerant as well as sensitive rice varieties exhibited improvement in shoot length plus biomass under As toxicity by Si supplementation	Tripathi et al., 2013
16.	Cd	50 µmol·L^{-1}	*Oryza sativa*	10 µmol L^{-1}	Cadmium stress induced chlorosis and made the newly emergent leaves to curl. However, the foliar application of Si promoted growth characteristics in rice	Tripathi et al., 2012
17.	Pb	800 mg kg^{-1}	*Musa paradisiaca*	800 mg kg^{-1}	Pb stress affected biomass of root + shoot was recorded to improve by Si treatment	Li et al., 2012
18.	Al	50 mM	*Oryza sativa*	10 µM	Frequency and length of root hairs was significantly enhanced by Si application	Singh et al., 2011
19.	Cd	0.5 mg L^{-1} 5 mg L^{-1}	*Brassica chinensis* cv. Shanghaiqing cv. Hangyoudong	1.5 mM	Each cultivar exhibited an elevation in biomass of root +shoot in response to both the levels of Cd stress	Song et al., 2009
20.	Cd	5 µM	*Zea mays*	35 mM	Silicon application to Cd stressed plants ameliorated the inhibitory effect of Cd on different parameters of growth	Vaculik et al., 2009

2.7.3 Photosynthetic system

Si application brings about several beneficial effects related to photosynthetic potential and chlorophyll biosynthesis in metal stressed plants. Exogenously treating the Zn stressed maize plants with Si, leads to an enhancement in the measures of Chl a. This could be attributed to the acceleration in the process of chlorophyll production through increasing the content of iron in maize plants (Kaya et al., 2009). Photosynthetic pigments were improved in their quantities in Cd stressed rice plants and wheat cultivars upon receiving Si treatment (Hussain et al., 2015; Rizwan et al., 2012; Nwugo & Huerta, 2008). In plants like rice *being* exposed to As and Al stress (Sanglard et al., 2014; Singh et al., 2011) plus wheat and barley exposed to Cr toxicity (Tripathi et al., 2015; Ali et al., 2013b), supplementation with Si brings about improvement in chlorophyll content.

Hydroponically grown cotton and rice plants displayed betterment in parameters of gaseous exchange when augmented with Si to mitigate Cd toxicity (Nwugo and Huerta, 2008; Feng et al., 2010). Similar improvement was achieved with Si application in barley and groundnut plants subjected to Cr and Al toxicity, respectively (Shen et al., 2014; Ali et al., 2013b) and in cotton plants in response to Pb stress (Bharwana et al., 2013). Arsenic toxicity symptoms got alleviated in rice plants upon receiving Si treatment via improving photosynthetic efficiency and curbing stomatal closure (Hu et al., 2013). Arsenic mediated noxious effects on photosynthetic efficiency and gas exchange attributes were mitigated quite remarkably by Si application in the cultivars of rice through enhancing the fixation of carbon (Sanglard et al., 2014). Activity of photosynthetic system together with fluorescence of chlorophyll molecules were made better in Cr stressed wheat and barley plants upon Si application (Tripathi et al., 2015; Ali et al., 2013b). Table 2.16 depicts the impact of Si upon the photosynthetic mechanism of different plant species.

Table 2.16 Influence of Si treatment on photosynthetic machinery in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	As	0.3 mM 0.5 mM 0.7 mM	*Raphanus sativus*	2 mM	Si application mitigated the As stress induced negative effects on various photostnthetic attributes in radish plants	Bhardwaj et al., 2023
2.	Fe	25 µM 5 mM	*Oryza sativa*	2 mM	Si improved net rate of electron transport and that of photosynthesis along with stomatal conductance	Dos santos et al., 2020

3.	Cd	25 mg kg^{-1}	*Triticum aestivum* Cultivars: -Inqalab-91 -Sahar-2006	3 mM	In both the Cd stressed cultivars, a significant elevation in the measures of photosynthetic pigments plus net photosynthetic rate took place by the Si supplementation	Thind et al., 2020
4.	Cd	100 µM	*Triticum aestivum*	1200 mg L^{-1}	Enhanced rates of transpiration and gs along with measures of chl a + chl b +carotenoids resulting in better photosynthetic efficiency were the outcomes of Si application under Cd stress	Hussain et al., 2019a
5.	Cd	100 µM	*Zea mays*	10 µM	Silicon treatment brought about a significant alleviation of Cd stress by enhancing the measures of photosynthetic pigments along with parameters of chlorophyll fluorescence individually plus in combination with salicylic acid.	Singh et al., 2019
6.	Cd, Zn	Cd-50 mg kg^{-1} Zn-200 mg kg^{-1}	*Oryza sativa*	42 mg kg^{-1}	Silicon elevated the parameters of fluorescence in response to metal stress	Huang et al., 2018
7.	Cr	100 µM	*Brassica juncea* cv. Varuna	500 µM and 700 µM	Si application mitigated the inhibitory influence of Cr stress on the photosynthetic activity via enhancing the content of chlorophyll + carotenoid and net rate of photosynthetic process thereof	Asfaque et al., 2017
8.	Ni	50 µM 100 µM	*Gossypium hirsutum*	1 mM	Silicon supplementation caused an elevation in contents of photosynthetic pigments (Chl a + Chl b + Total Chl + Carotenoids) together with an improvement in gaseous exchange characteristics in Ni stressed cotton plants	Khaliq et al. 2016
9.	Al	1.6 mmol L^{-1}	*Eucalyptus platyphylla*	2 mmol L^{-1}	Si treated Al stressed plants exhibited enhancement in levels of total chlorophyll along with improvement in gaseous exchange attributes	Lima et al. 2016

10.	Cd	0.5 µM 5 µM 50 µM	*Triticum turgidum* Cultivar: Claudio	1 mM	Inhibitory effects of Cd on photosynthetic attributes were alleviated by Si application	Rizwan et al., 2016
11.	Zn	25 µM 50 µM	*Gossypium hirsutum*	1 mM	Si treatment causes reduction in accumulation of Zn, thereby enhances the various attributes of photosynthetic process	Anwaar et al., 2015
12.	Mn	6.7 µM 2 mM	*Oryza sativa*	1.5 mM	Si remarkably enhances the tolerance against Mn stress by elevating chlorophyll and ATP concentration along with efficiency of light usage, CO_2 assimilation plus constitution of PS I	Li et al., 2015
13.	Cd	5 µM 50 µM	*Zea mays*	5 mM	Si supplementation in maize plants enhanced the measures of chl plus carotenoids, net rate of photosynthetic process and quantum yield of PS (Photo system) II i.e. ΦPSII in response to Cd toxicity	Vaculík et al., 2015
14.	Cd	5 and 50 µM	*Zea mays*	5 mM	Silicon application notably ameliorated the negative effects of Cd toxicity in maize through escalation in the measures of chl and carotenoids	Malčovská et al., 2014
15.	Zn	2 mM	*Oryza sativa*	1.5 mM	Attributes of gaseous exchange plus chlorophyll measures were observed to be enhanced in Si supplemented rice	Song et al., 2014
16.	Cd	1 µM 5 µM	*Gossypium hirsutum*	1 mM	Stomatal conductance, efficiency of water usage, rates of transpiration plus photosynthesis exhibited enhancement upon Si supplementation	Farooq et al. 2013

17.	Cr	100 µM	*Hordeum vulgare*	1 and 2 mM	Exogenously supplemented Si in Cr stressed barley plants brought about improvement in photosynthetic attributes like SPAD value, parameters of gaseous exchange and Fv/Fm i.e. efficiency of chlorophyll fluorescence	Ali et al., 2013b
18.	Cd	10 µM	*Oryza sativa*	0.6 mM	Efficiencies of a) water and light usage plus carboxylation of Rubisco exhibited elevation upon Si supplementation in Cd stressed rice plants	Nwugo and Huerta, 2011
19.	Cd	100 µM	*Cucumis sativus*	1 mM	Remarkable augmentation in the content of total chlorophyll + carotenoid together with parameters of gaseous exchange in the leaves of cucumber subjected to Cd stress	Feng et al., 2010
20.	Mn	600 µM	*Cucumis sativus*	1 mM	Augmentation of photosynthetic parameters was brought about by Si supplementation to enhance the tolerance potential of Mn stress exposed cucumber plants	Feng et al., 2009

2.7.4 Metal uptake

Silicon can immobilize toxic heavy metals inside the soil which is one of the most remarkable and beneficial outcome of its application. Supplementing the soil with higher quantities of Si in the form of sodium metasilicate incites the increase in soil pH through the synthesis of silicates which in turn hamper the phytoavailability of heavy metals (Rizwan et al., 2012). Silicon addition lowers down the uptake of Pb by banana plants when cultivated in Pb contaminated soil which could be attributed to considerable elevation in soil pH along with decrease in measures of Pb in exchangeable form (Li et al., 2012). Silicon could also bring about the reduction in toxicity of heavy metals in the soil via forming metal silicate complexes, which are relatively non-toxic in nature (Putwattana et al., 2010). Silicon denigrated the mobility of Pb metal in soil solution by making Pb-silicate complexes of non-pernicious nature (Shim et al., 2014) and diminished the bioavailability of Cr in the soil by reducing the measures of Cr in its exchangeable form (Zhang et al., 2013). Further, Ding et al.(2013) showed that silicon application resulted in notable reduction in the levels of Cr metal (exchangeable form) via enhancing the precipitation of Cr together with the organic matter. Table 2.17 depicts the impact of Si on the uptake of metal (loids) in different plant cultivars.

Table 2.17 Influence of Si treatment on metal uptake in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	As	0.3 mM 0.5 mM 0.7 mM	*Raphanus sativus*	2 mM	Individual treatment with Si and in combination with NO reduced the accumulation of As in both roots and foliar regions	Bhardwaj et al., 2023
2.	Cu	100 µM 200 µM	*Linum usitatissimum* Genotypes -Sakha 1 -Sakha 2	1.5 mM	Treatment with Si diminished the accumulation of Cu inside the roots as well as shoots.	El-Beltagi et al., 2020
3.	Cd	35 mg kg^{-1}	*Zea mays*	3293.3 kg ha^{-1}	Uptake plus accumulation of metal was recorded to be reduced by applying Si individually and cumulatively with NO	Liu et al., 2020
4.	Cd	100 µM	*Triticum aestivum*	1 µM	Individual plus coupled treatment of Si and Si + NO brought about a notable reduction in Cd content.	Singh et al., 2020
5.	Cu	35 µM	*Nicotiana tabacum*	1.0 mM	Silicon supplementation resulted in reduction of Cu concentration inside the roots.	Flora et al., 2019
6.	Cr	100 µM	*Brassica juncea* cv. Varuna	a) 500µM b) 700 µM	Chromium measures were recorded to be declined in Si treated rice cultivars.	Ashfaque et al., 2017
7.	Ni	50 µM 100 µM	*Gossypium hirsutum*	1 mM	Silicon supplementation significantly reduced the uptake of Ni by cotton plants	Khaliq et al., 2016

8.	As	150mM	*Brassica juncea*	1.5 mM	Silicon supplementation enhanced the As concentration inside Brassica plants	Pandey et al., 2016
9.	Cd	50 µM	*Zea mays*	a) 0.1 mM b) 0.5 mM c) 1.5 mM d) 3 mM e) 5 mM	Cadmium content notably declined in Si supplemented plant roots with no major impact inside shoot tissues	Dresler et al., 2015
10.	Fe	10 mg L^{-1} 50 mg L^{-1} 100 mg L^{-1} 250 mg L^{-1}	*Oryza sativa*	1.5 mM	Silicon treatment remarkably declined Fe measures below 250 mg L^{-1} Fe toxicity	Chalmardi et al., 2014
11.	Sb	10mgL^{-1} 50mgL^{-1}	*Zea mays*	5mM	At higher levels of Sb toxicity, Si elevated the stocking up of Sb	Vaculíková et al., 2014
12.	Cr	100 µM	*Hordeum vulgare*	a) 1 mM b) 2mM	Silicon augmentation markedly diminished the Cr measures in barley	Ali et al., 2013b
13.	Pb	100 µM 50 µM	*Gossypium hirsutum*	1 mM	Silicon supplementation declined the measures of Pb in different regions of plant	Bharwana et al., 2013
14.	Cd	100 µM	*Solanum nigrum*	1 mM	Drastic decline in Cd concentration was recorded in Si supplemented cultivars.	Liu et al., 2013
15.	Cd	---------------	*Triticum turgidum* cv. Claudio	a) 1 ton ha^{-1} b) 10 ton ha^{-1} c) 15 ton ha^{-1}	Cadmium uptake and translocation were negatively hampered by Si treatment	Rizwan et al., 2012
16.	As	10 mM 25 mM	*Oryza sativa* Cv. -Triguna -IET-4786	a) 0.5 mM b) 1 mM	Arsenic levels were recorded to be declined by Si treatment in both the cultivars, however the reduction in accumulation was more in Triguna	Tripathi et al. 2012
17.	Zn	0.15µM 2mM	*Oryza sativa*	1.5 mM	Silicon supplementation enhanced the measures of Zn in vacuolar regions of rice plants	Song et al. 2011
18.	Zn	0.05 mM 0.5 mM	*Zea mays*	1 mM	Addition of Si declined the Zn concentration inside roots + leaves	Kaya et al., 2009

2.7.5 Oxidative stress

Metal generated oxidative harm in plants is lowered down by supplementing Silicon which is represented in the following table 2.18.

Table 2.18 Influence of Si treatment on ROS genesis and oxidative stress markers in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	Cd	500 µM	*Zea mays* Cultivars: - Sadaf -EV-20	6 mM	Silicon supplementation ameliorated oxidative stress by lowering down the levels of MDA + H_2O_2	Saleem et al., 2022
2.	Cd	25 mg kg^{-1} 50 mg kg^{-1}	*Ocimum basilicum*	a) 1 mM b) 2 mM	Extent of electrolyte leakage plus measures of MDA were escalated by Cd toxicity but Si treatment remarkably lowered them down	Gheshlaghpour et al. 2021
3.	As	4 mg kg^{-1} 8 mg kg^{-1} 12 mg kg^{-1}	*Zea mays*	a) 50 mg kg^{-1} b) 100 mg kg^{-1}	Exogenously supplemented Si reduced the measures of MDA + H_2O_2 which were otherwise elevated due to As toxicity	Kashif et al. 2021
4.	Cd	25 mg kg^{-1}	*Triticum aestivum* Cultivars: -Inqalab-91 -Sahar-2006	a) 1.5 mM b) 3 mM	Each of the Cd stressed cultivar showed higher measures of EL, H_2O_2 and MDA which were lowered down significantly by Si treatment	Thind et al., 2021
5.	As	50 µM 100 µM	*Triticum aestivum*	a) 6 mM b) 12 mM	Si treatment diminishes the burst of ROS in As stressed wheat plants	Magshoudi et al., 2020
6.	As	150 µM	*Brassica juncea*	1.5 mM	Silicon brought about the reduction in the content of MDA plus H_2O_2 in response to As stress.	Praveen et al. 2020
7.	Cd	100 µM	*Triticum aestivum*	1200 mg/L	Silicon (NPs) priming of seeds mitigated the oxidative damage in Cd stressed wheat plants	Hussain et al., 2019a
8.	Cd	1 mg kg^{-1} 5 mg kg^{-1}	*Nicotiana tabacum*	a) 1 mg kg^{-1} b) 4 mg kg^{-1}	Application of Si significantly declined the measures of MDA, thereby enhancing the tolerance potential against Cd	Lu et al., 2018
9.	Cd	50 mg kg^{-1}	*Gladiolus grandiflora*	200 mg L^{-1}	Silicon application curtailed the ROS production in response to Cd stress	Zaheer et al., 2018

10.	Ni	50 µM 100 µM	*Gossypium hirsutum*	1 mM	Silicon treatment brought about a decline in the content of MDA + H_2O_2, with that of EC in Ni stressed plants.	Khaliq et al., 2016
11.	Zn	25 µM 50 µM	*Gossypium hirsutum*	1 mM	Treatment of Si resulted in the significant reduction of the measures of MDA + H_2O_2 along with the extent of electrolyte leakage, hinting at imperative role of Si in the mitigation of oxidative damage	Anwaar et al., 2015
12.	Al	160 mg L^{-1}	*Arachis hypogea*	80 mg L^{-1}	The malondialdehyde, an indicator of membrane disintegration exhibited a significant decline inside Al stressed leaves + roots when supplemented with silicon	Shen et al., 2014
13.	Cd	1 µM 5 µM	*Gossypium hirsutum*	1 mM	A significant reduction in MDA plus H_2O_2measures was recorded upon Si addition	Farooq et al., 2013
14.	Mn	6.7 µM 2.0 mM	*Oryza sativa* cv. Xinxiangyou 640 (XXY) cv. Zhuliangyou 99 (ZLY)	1.5 mM	Significant counteraction of Mn induced higher production of MDA + and H_2O_2 was exhibited by Si application	Li et al., 2012
15.	Mn	0.5 µM 100 µM	*Cucumis sativus*	1.5 mM	Silicon declined the measures of H_2O_2 with that of free Mn2 + in the apoplastic regions in Mn exposed cucumber	Dragisic Maksimovic et al., 2012
16.	Al	20 mM 40 mM 60 mM	*Borago officinalis*	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	MDA content diminished in Si supplemented Al stressed plants	Gagoonani et al., 2011
17.	Zn	0.15 µM 2 mM	*Oryza sativa* cultivars: -TY-167 -FYY-326	1.5 mM	Si supplementation to both the Zn sensitive and tolerant cultivars resulted in a significant decline in the measures of MDA + H_2O_2	Song et al. 2011
18.	Cd	0.5 mg L^{-1} 5 mg L^{-1}	*Brassica chinensis* cv. Shanghaiqing cv. Hangyoudong	1.5 mM	Higher measures of MDA + H_2O_2 were lowered down upon Si supplementation	Song et al., 2009

2.7.6 Protein content

Table 2.19 represents the outcomes of supplementing the plants with Si on their total carbohydrate and protein levels in different plant species.

Table 2.19 Influence of Si treatment on protein measures in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	As	0.3 mM 0.5 mM 0.7 mM	*Raphanus sativus*	2 mM	Arsenic stress induced reduction in protein measures were reported to be enhanced by individual treatment with Si and in combination with NO	Bhardwaj et al., 2023
2.	Cd	15 µM 30 µM	*Zea mays*	a) 100ppm b) 200ppm c) 300ppm d) 400ppm	The results revealed the role of Si NPs in enhancing the protein concentrations while reducing the content of Cd	Ahmed et al., 2023
3.	Cd	50 mg kg^{-1} 100 mg kg^{-1}	*Pisum sativum*	a) 100 ppm b) 200 ppm c) 300 ppm	Total protein measures were reported to be higher in Si treated pea plants under Cd stress	El-Okkiah et al., 2022
4.	As/ Cd	600 µM each	*Isatis cappadocica*	1 mM	Measures of total soluble protein were elevated by Si augmentation under As/ Cd toxicity	Azam et al., 2021
5.	Pb	50 µM 250 µM 500 µM 1000 µM 1500 µM	*Pleioblastus pygmaeus*	a) 100 µM b) 500 µM	Silicon markedly augmented the amount of protein in response to metal stressed conditions	Emamverdian et al., 2020
6.	As	50 µM 100 µM	*Triticum aestivum*	a) 6 mM b) 12 mM	Measures of soluble proteins were observed to be escalated under As toxicity	Maghsoudi et al., 2020
7.	Cd	100 µM	*Triticum aestivum*	1 µM	Individual and coupled treatment of Si alone and with NO promoted the measures of soluble protein in Cd stressed wheat seedlings	Singh et al., 2020
8.	Cd	100 µM	*Zea mays*	10 µM	Total measures of soluble protein were enhanced in Si applied plants	Singh et al., 2019
9.	As	50 µM	*Triticum aestivum*	1 mM	Supplementation of Si in As stressed plants remarkably elevated the protein levels in both the root +	Hossain et al., 2018

			var. Vinjett		shoot tissues	
10.	Cd	50 mg kg^{-1}	*Gladiolus grandiflora*	200 mg L^{-1}	Elevation in levels of protein was noticed in Si-augmented cultivars under stressful conditions	Zaheer et al., 2018
11.	Zn	25 µM	*Gossypium hirsutum*	1 mM	Silicon application elevated the protein measures which were comparatively higher in leaves as compared to roots	Anwaar et al., 2015
		50 µM				
12.	Pb	50 µM	*Gossypium plants*	1 mM	The reduction in the content of soluble protein was remarkably mitigated when Si was applied exogenously to Pb stress exposed plants	Bharwana et al., 2013
		100 µM				
13.	Al	20 mM	*Borago officinalis*	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	Augmentation in amount of protein was noticed in the plants supplemented with lower levels of Si i.e. 0.5 & 1 mM whereas in higher concentrations I,e, 1.5 & 2 mM, protein measures were reduced	Gagoonani et al., 2011
		40 mM				
		60 mM				

2.7.7 Secondary metabolites

The strategies adopted by Si for the amelioration of heavy metal toxicity could possibly be due to its potential to chelate secondary metabolites like phenolics and flavonoids etc. with heavy metals. For instance, Si alleviated Al stress in maize plants, which might be related to enhancement in the Si-induced production of phenolic compounds. Two such phenolics i.e. a) catechin and b) quercetin possess high chelating capacities which were believed to operate behind this Si- mediated mitigation of Al noxiousness in maize (Wang et al., 2004; Kidd et al., 2001). An upsurge in the measures of secondary metabolites was recorded when *Trigonella foenum-graecum* and *Raphanus sativus* plants exposed to Pb and As stress respectively were supplemented with Si alone or in combination with other mitigants (Bhardwaj et al, 2023; Sharma et al., 2023).

These metabolites act as antioxidants for combating oxidative harm as reported in Cd stressed *Triticum aestivum* plants where anthocyanin content was enhanced upon Si supplementation (Thind et al., 2021). Further, Al stress Scarlett cultivar of *Triticum aestivum* exhibited enhancement in the levels of phenolics when given Si treatment (Vega et al., 2019). However, in *Echium amoenum*, Cd exposure elevated the levels of flavanoids together with anthocyanin, which was lowered down by the application of Si (Amiri et al., 2012). Silicon has been reported to form complexes with polyphenols to mitigate abiotic stresses and therefore its supplementation enhanced the extent of phenol synthesis as seen in Mn exposed *Cucumis sativus* (Maksimovik et al., 2007).

2.7.7 Osmolytes

Decline in osmotic potential is brought about by a varied range of environmental stresses, which consequently decreases the cell turgor leading to inhibition of plants' growth. Plants combat these stresses by synthesizing various osmolytes. Osmolytes being hydrophilic in nature, performs stabilization of different cell components in response to unfavourable circumstances without altering cells' constitution or functionality. Osmolyte proline causes decline in osmotic stress via removal of hydroxyl ions. Leaf turgor and osmotic improvements have been observed to increase in Si supplemented cultivars via accumulating osmolytes (Kafi et al., 2021). Table 2.20 depicts the impact of Si application on different osmolytes in various plant species.

Table 2.20 Influence of Si treatment on osmolytes in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	Pb	0.5 mM 0.7 mM 0.9 mM	*Trigonella foenum-graecum*	2 mM	Upregulated levels of osmolytes were recorded in Pb stressed fenugreek plants when treated with Si	Sharma et al., 2023
2.	Cd	20 mg kg^{-1}	*Triticum aestivum*	a) 1.50 mM b) 3.00 mM c) 4.00 mM	This investigation recorded enhancement in the content of proline and sugars upon Si application	Heile et al., 2021
3.	Pb	500 mg kg^{-1} 1000 mg kg^{-1} 1500 mg kg^{-1}	*Coriandrum sativum*	1.5 mM	The coupled treatment of Silicon NPs with that of biological methods led to remarkable decline in proline measures when exposed to Pb stress	Fatemi and Esmaielpour, 2020
4.	As	25 µM 50 µM 100 µM	*Triticum aestivum*	5 mM	Silicon addition to wheat improved the content of reducing and non-reducing sugars.	Sil et al., 2019
5.	Cd	2 mM	*Triticum aestivum*	a) 2 mM b) 4 mM c) 6 mM	Levels of proline got upscaled in Si supplemented plants which were subjected to Cd stress	Alzahrani et al., 2018

6.	Cd, Zn	Cd- i) 25 mg kg^{-1} ii) 50 mg kg^{-1} Zn- i) 600 mg kg^{-1} ii)1000 mg kg^{-1}	*Cajanus cajan*	300 mg kg^{-1}	Endogenous measures of proline were enhanced by Si addition under heavy metal stress	Garg and Singh, 2018
7.	Cd	2 mM	*Triticum aestivum*	3 mM	Content of soluble sugars plus proline was recorded to be enhanced by Si supplementation under Cd stress	Howladar et al., 2018
8.	Cd	150 mg L^{-1}	*Pisum sativum*	2 mM	In Cd-stressed seedlings of pea, proline plus GB measures exhibited remarkable enhancement in response to Si application without or with 24-EBL	Jan et al., 2018
9.	Cr	100 µM	*Brassica juncea* cv. Varuna	a) 500 µM b) 700 µM	Supplementing stressed plants with Si resulted in enhancing the levels of proline which augmented photosynthesis plus plant growth	Ashfaque et al., 2017
10.	Cd	100 M	*Zea mays*	a) 50 mM b) 100 mM c) 150 mM	Si application made the proline content better in response to stressful conditions.	Mohsenzadeh et al., 2012
11.	Al	20 mM 40 mM 60 mM	*Borago officinalis*	a) 0.5 mM b) 1 mM c) 1.5 mM d) 2 mM	Augmentation in amount of Proline was recorded in Si supplemented plants under stressful conditions.	Gagoonani et al., 2011
12.	Zn	0.05 mM 0.5 mM	*Zea mays*	1 mM	Zn induced enhancement in membrane permeability plus proline levels was significantly declined by Si addition	Kaya et al., 2009

2.7.8 Antioxidative enzymes and non-enzymatic antioxidants

Physiological role played by enzymatic antioxidants namely APOX, CAT, GPOX and SOD etc. got triggered by the supplementation of wheat plants with Si (Naeem et al., 2018). Additionally, induction of CAT, POD and SOD in variable ranges was brought about by Si treatment in rice cultivars of three types, which were subjected to organoarsenic toxicity (Geng et al., 2018). Several research findings divulged that Si could incite antioxiadative defense mechanisms variably both within and among different plant species. Applying Si exogenously to the Cd stressed wheat plants resulted in the enhancement of enzymatic action of SOD, CAT and POD (Hussain et al., 2019a). A decline in the measures of APX, CAT, SOD and POD was noticed in the foliar parts of *Solanum nigrum* (Blackberry nightshade plant) (Liu et al., 2013). Similarly, levels of POD and SOD exhibited a sharp decline in cultivars of sorghum (Masarovič et al., 2012) in response to Cd plus Zn toxicity, respectively through Si addition. Enzymatic action of CAT, GPOX and APX was diminished via Si supplementation in roots of maize subject to antimony (Sb) (Vaculíková et al., 2014).

Measures of ascorbate/ (Vit. C) along with glutathione have been reported to diminish by applying Si in response to As toxicity in rice, bringing about an inhibition in enzymatic action of GPX, GR and APX (Das et al., 2018). Silicon mediated upscaling of the physiological action of CAT enzyme was recorded in Cd exposed cultivars of cucumber (Khodarahmi et al., 2012). Cadmium toxicity leads to enhancing the measures of CAT and POD enzymes whereas Si supplementation incited a decline in the same in rice plants (Wang et al., 2014b). Further, endogenous levels of POD and SOD together with CAT were diminished when roots of maize and groundnut plants were contaminated with Zn and Al. (Bokor et al., 2014a; Shen et al., 2014). Functional aspect of POD enzyme suffered adversely in cultivars of cucumber subjected to Mn stress (Dragisic Maksimović et al., 2007). The effect of Si application on the plant's antioxidant defense system is shown in table 2.21.

Table 2.21 Influence of Si treatment on antioxidant defense mechanism in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	Al	2 mM	*Phoenix dactylifera*	1.0 mM	Silicon stimulated enzymatic action of antioxidants i.e. APX, CAT, POD and PPO	Bilal et al., 2022
2.	Al	0.2 mM	*Fagopyrum esculentum*	0.5 and 1 mM	Improvement in enzymatic activity of PPO was noticed in Al stressed buckwheat by Si treatment results additionally illustrated that the application of Si further increased.	Dar et al., 2022
		0.4 mM				
3.	Cd	500 µM	*Zea mays* Cultivars: -Sadaf -EV-20	6 mM	Enzymatic action of antioxidants (CAT, POD and SOD) was upregulated in each cultivar of maize by Si application under Cd stress	Saleem et al., 2022
4.	Al	100 µM	*Glycyrrhiza glabra*	a) 0.5 mM b) 1.50 mM	Measures of SOD displayed a reduction while the contents of GPOX plus POD were amplified upon Si supplementation	Yazdani et al. 2021
		250 µM				
		400 µM				

5.	B	15 mg L^{-1}	*Pisum sativum* cultivars: -Lapar 83 -BRS Forrageira	2 mmol L^{-1}	Functional efficacy of APX, CAT, GPOX, GR and SOD was markedly augmented when boron exposed pea plants were given Si treatment	Oliviera et al., 2020
6.	Cd	25 mg kg^{-1}	*Triticum aestivum* cultivars: -Inqalab-91 -Sahar-2006	3 mM	Elevation in the action of enzymatic antioxidants (APX, CAT, POD and SOD) and non-enzymatic antioxidants (AsC and GSH) was brought about by Si in order to mitigate Cd- induced oxidative stress	Thind et al., 2020
7.	Ni	150 µM	*Brassica juncea*	10^{-5} M	Escalation in the action of enzymatic antioxidants (APX, DHAR, CAT, GR POD, MDHAR and SOD) and non-enzymatic antioxidants (AsC and GSH) was brought about by Si treatment	Abd-Allah et al., 2019
8.	Al	12 mg kg^{-1} 25 mg kg^{-1}	*Zea mays*	4 mg kg^{-1}	Cumulative application i.e. Si NPs + Al brought about an activity upsurge to the enzymatic antioxidants APX, CAT, POX, GPX and SOD along with an increase in the endogenous measures of non-enzymatic components of antioxidative defense	De-sousa et al., 2019
9.	Ni	0.25 mM 0.5mM	*Oryza sativa*	0.50 mM	Exogenous supplementation of Si remarkably upregulated the enzymatic action of APX, CAT, DHAR, GST, GR and SOD	Hasaanuzzaman et al., 2019
10.	Cd	150 mg L^{-1}	*Pisum sativum*	2 mM	Enzymatic pursuit of APX, CAT, MDHAR, GST, GR and SOD plus the endogenous measures of non-enzymatic components of antioxidative defense exhibited elevation upon Si supplementation	Jan et al., 2018
11.	Cd	0.5 mM 1.0 mM	*Brassica napus*	1.0 mM	Cadmium stress exposed Rapeseed cultivars exhibited enhancement in the functioning of APX, CAT, GR, DHAR and MDHAR enzymes when supplemented with silicon	Hasaanuzzaman et al., 2017
12.	Cd	0.5 mM 1.0 mM	*Lolium perenne*	a) 0.5 mM b) 2.0 mM	Physiological efficacy of SOD displayed remarkable decline while measures of APX, CAT plus POD exhibited an upsurge in response to Si + Al supplementation	Pontigo et al., 2017

13.	Cd	5 mg L^{-1}	*Boehmeria nivea*	1 mmol L^{-1}	Si mitigated Cd stress via upregulation of the enzymatic action of antioxidants (APX, POD and SOD) with that of measures of non-enzymatic antioxidants (GSH) which significantly ameliorated the oxidative damage.	Tang et al., 2015
14.	Al	160 mg L^{-1}	*Arachis hypogaea*	80 mg L^{-1}	Silicon supplementation remarkably led to an augmentation in the amount of SOD while lessening the content of CAT as well as POD enzyme	Shen et al., 2014
15.	Pb	800 mg kg^{-1}	*Musa paradisiaca*	800 mg kg^{-1}	The treatment of Si escalated the action of CAT, POD and SOD enzymes in Pb stressed banana plants	Li et al., 2012
16.	Zn	0.15 µM 2 mM	*Oryza sativa* Cultivars: - TY-167 -FYY-326	1.5 mM	Si supplementation to both the Zn sensitive and tolerant cultivars resulted in a significant increase of enzymatic action of antioxidants (APX, CAT and SOD) , whereas malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels were scaled down in Si-fortified plants of both sensitive and resistant rice cultivars exposed to Zn stress.	Song et al. 2011
17.	Cd	200 µM	*Arachis hypogea* -Luhua 11 -Luzi 101	1.8 mM	CAT, POD and SOD activity inside leaves + roots of Luzi 101 were recorded to be enhanced without any significant change in that of Luhua 11	Shi et al., 2010
18.	Cd	0.5 mg L^{-1} 5 mg L^{-1}	*Brassica chinensis*	1.5 mM	Upregulation of the action of antioxidative enzymes (APX, CAT and SOD) and non-enzymes (Ascorbate and glutathione) was brought about by Si supplementation	Song et al., 2009
19.	B	5 mg L^{-1}	*Spinacia oleracea*	150 mg kg^{-1}	Action of ascorbate along with APX, CAT and SOD was diminished by Si application which was otherwise elevated by B toxicity	Gunes et al., 2007

2.7.9 Gene expression

The impact of Si supplementation on expression levels of genes in various metal stressed plant species is depicted in table 2.22

Table 2.22 Influence of Si treatment on stress responsive genes in various metal stressed plant species

Sr. no.	Heavy Metal	Heavy Metal dosage	Plant Species	Si concentration	Effect/s	Reference/s
1.	Al	2 mM	*Phoenix dactylifera*	1.0 mM	Silicon supplementation upregulated the expression of Lsi2 (a Si-related transporter) plus CAT and SOD genes and resulted in amelioration of oxidative damage	Bilal et al., 2022
2.	Cu	200 µM	*Salvia officinalis*	1.0 mM	Expression of RAS, TAT and PAL genes was further upregulated by Si treatment in Cu stress subjected plants which enhances the production of secondary metabolites	Pirooz et al., 2022
3.	Cu	35 µM	*Nicotiana tabacum*	1.0 mM	Upregulation in expression of genes responsible for ethylene biosynthesis (ACO, ACS plus SAMS2) with that of PCS1 was observed due to Si supplementation to the Cu subjected plants	Flora et al., 2019
4.	Cd	2 mM	*Triticum aestivum*	3 mM	Upregulated expression of genes responsible for polyamine synthesis was one of the major outcomes of Si supplementation	Howladar et al., 2018
5.	As	150 µM	*Oryza sativa*	5 mM	Genes responsible for transporting As+3 (Lsi1, Lsi2 plus Lsi6) were reported to have more expression levels in As stressed rice plants. Additionally, this enhancement in gene expression was higher in roots + shoots of 15 days old growth stage as compared to 7 days old growth stage	Khan & Gupta, 2018
6.	Cd	10 mM 20 mM 40 mM	*Oryza sativa*	1 mM	Genes encoding for the transport proteins of Cd namely OsLCT1 plus OsNramp5 exhibited significant decline in their expression levels when supplemented with SiNPs	Cui et al., 2017
7.	Cd	1 µM	*Oryza sativa*	1 mM	Silicon diminishes the enhancement in expression levels of OsLCT1 by cadmium	Gregor et al., 2016
8.	Zn	800 µM	*Zea mays*	5 mM	Si supplementation brought about downregulation of the expression of genes coding for zinc (Zn) transporters in Zn stressed maize roots	Bokor et al., 2015
9.	Mn	6.7 µM	*Oryza sativa* cv. Xinxiangyou	1.5 mM	Exogenously supplemented Si in Mn exposed rice plants promoted the gene expression levels of genes	Li et al., 2015

		2 mM	640 (XXY) cv. Zhuliangyou 99 (ZLY)		namely Lhcb3, PsbP plus HemD which encode for photosynthesis	
10.	Cd	10 µM 20 µM 30 µM	*Oryza sativa*	1.0 mM	Exogenously supplemented Si led to the upregulation of aquaporin OsLsi1 gene expression in Cd stressed rice plants	Ma et al., 2015
11.	Cd/Cu	100 µM each	*Oryza sativa*	1 mM	Genes OsLsi1 and OsLsi2 were reported to have Si- induced upregulated expression under metal stress	Kim et al., 2014
12.	Zn	2 mM	*Oryza sativa*	1.5 mM	The gene coding for polyprotein part of PS II i.e. PsbY (Os08g02530) exhibited enhancement in its transcription levels when Zn stressed plants were given Si treatment	Song et al., 2014
13.	Cu	0.12 µM 30 µM	*Arabidopsis thaliana*	a) 0.1 mM b) 1.5 mM	Stimulation in the expression of PCS1 along with inhibition in that of metallothionein gene were observed in Si supplemented metal stressed plants	Khandekar and Leisner, 2011

Chapter 3

Hypothesis

Different activities centered around industrialization and urbanization greatly contribute towards enhancing the magnitude of heavy metal contamination in the soil. Lead (Pb), a potent heavy metal pollutant has no physiological significance and does not qualify the criteria of essentiality in plants but its intake and accumulation inside plant parts occur quite readily (Nas & Ali, 2018). This naturally reported heavy metal has observed an upsurge in its ecological presence on account of anthropogenic interventions namely recycling, mining, refining, smelting, battery and ammunition manufacturing (Dongre et al., 2020). Intake of Pb by the plant roots is dependent upon a) various parameters of soil namely pH, cation exchange competence and particle size; b) nature of root exudates and c) physiochemical status of the plant. The major symptoms of Pb metal phytotoxicity include tarnishing of roots, destruction of chlorophyll, inhibition of enzymatic action, negative alterations in mineral, water and hormonal status along with anomalies in photosynthesis and other key metabolic processes, thereby bringing about severe retardation in plant growth (Sharma & Dubey, 2005). Upon accessing an entry into the food chain, it becomes a potential threat to human health and has been found to be hepatotoxic, neurotoxic and nephrotoxic causing a damage to liver, brain and kidney respectively (Chen et al., 2022). Its ubiquitous occurrence, wide spread usage, toxic plus persistent nature and bio-accumulative potential makes it a great ecological and occupational concern. All these attributes make either its removal or reduction in levels far more essential, especially in plants to ensure food safety.

Additionally, the cumulative influence of EBL + Si has never been evaluated yet in *Trigonella foenum- graecum*, thereby upscaling the significance of the present work as a novel strategy in ameliorating the Pb stress in plants. Therefore, the present research work is an investigative study to provide insight into the morphological and physiochemical attributes together with molecular aspects of Pb stress exposed *Trigonella foenum- graecum* plants.

Chapter 4

Objectives

The absorption, translocation and even the stocking up of Pb from rhizospheric arena to fenugreek plants have become a prime concern and needed to be addressed for ensuring better growth, productivity and food safety. For enhancing the Pb metal tolerance, an entire range of varied techniques is available which could be put to use to curb or reduce Pb uptake by the plant roots followed by its transfer to the aerial regions. Chemical, electro-kinetic and thermal remediation strategies with that of various other physical methods prove to be less viable on account of not being quite economical, ecologically safe and efficient. Therefore, to deal with Pb toxicity, an urgent need of searching more viable, cost-efficient and safe substances arises. Hence, usage of 24-EBL with Si might prove an advantageous mitigation technique for combating this metal induced stress (Jan et al., 2018).

Further, BRs have been reported to be efficiently involved in battling out the metal toxicity by upscaling the functioning of cellular machinery governing photosynthesis, carbon plus nitrogen metabolism and ROS scavenging (Choudhary et al., 2012). The BR with most scientific applications is 24-EBL and it is receiving a huge consideration for regulating multiple of biological progressions in variously stressed cultivars inclusive of mitigation of Pb metal toxicity. Bio-fortification with Si has been reported to ameliorate oxidative harm while strengthening the membranal structure in response to metal stresses (Rahman et al., 2017). The competence of plants in Si intake and translocation for alleviating Pb metal toxicity differ greatly. Silicon has been recorded to attenuate the Pb induced toxic impacts in various genotypes.

Research Objectives:

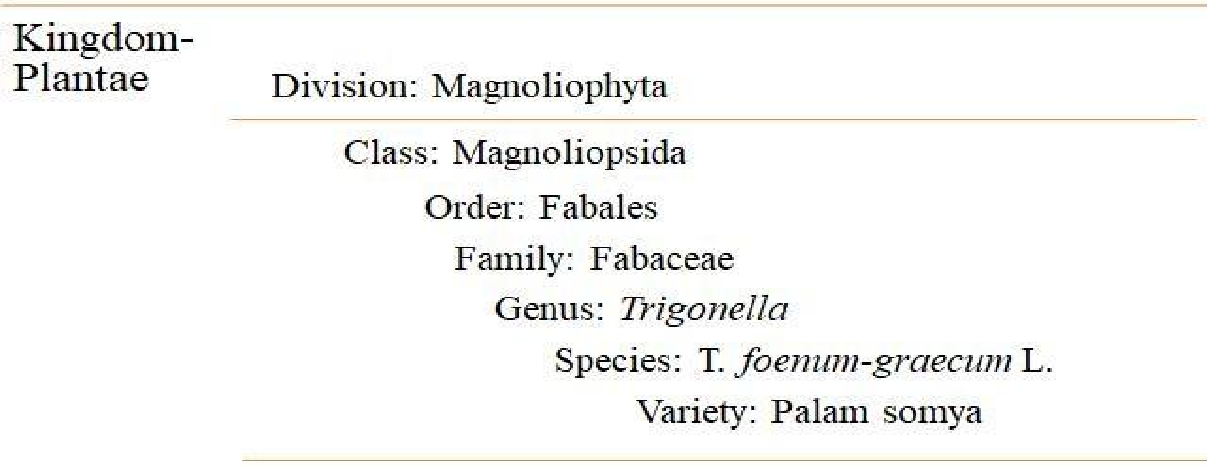
- *In vitro* study of *Trigonella foenum-graecum* L. seedlings exposed to Pb stress along with the supplementation of BRs and Si.
- Monitoring the effects of Pb induced stress in *Trigonella foenum-graecum* L. plants.
- Analysis of potency of BRs and Si in mitigating Pb toxicity stress in *Trigonella foenum-graecum* L. plants.
- Comparative study of gene expression of Pb stress associated genes in *Trigonella foenum-graecum* L. in response to the BRs and Si.

Chapter 5

Materials
+
Methods

Seeds of *Trigonella foenum-graecum* var. Palam somya were sourced from CSKVV, Palampur, Himachal Pradesh. Experimental work was carried out in the labs of Sidharth Govt. College, Nadaun, Hamirpur (Himachal Pradesh) and Lovely ProfessionalUniversity, Jalandhar (Punjab).

5.1.1 Fenugreek: Systematic position, habit and habitat



Kingdom-Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: *Trigonella*
Species: T. *foenum-graecum* L.
Variety: Palam somya

Trigonella foenum-graecum L., an important herbaceous member of the family Fabaceae, is a traditional spice, vegetable and forage crop being distributed and cultivated world over. It has eastern Mediterranean region as its place of origin and India tops the chart in term of both its production and consumption. This multipurpose dicotyledonous crop plant is annual, self-pollinated and diploid by nature with a chromosome number of 2n=16. The etymological meaning of generic name *Trigonella* is ‘little triangle’ which represents the triangular outline of its leaflets while the specific part *foenum-graecum* means ‘Greek hay’ in context to its inceptive roots of introgression in Greece (Acharya et al., 2008).

The plants are semi-erect and moderately branched bearing pinnately trifoliate, light green leaves and papilionaceous flowers of two types i.e. a) closed type (cleistogamous); b) open type (aneictogamous). With white/yellowish white hue and racemose inflorescence, its flowers are pedicellate, hermaphrodite with five petals (1 standard/banner+ 2 alae/wings + 1 keel/carina). Fruits are pods or legumes, having small seeds with deep furrows. Production wise, leading Indian states are- Rajasthan, Gujarat, Madhya Pradesh, Haryana, Uttaranchal and West Bengal whereas U.S.A., U.K., Y.A.R., U.A.E., Saudi Arabia, Sudan, Sri Lanka, Korea and Japan are the main importers of Indian Fenugreek. This spice crop can withstand both temperate and tropical climates, however, it prefers cool climate with no temperature extremes, so mostly grown as a Rabi crop except in southern regions of India, where it is cultivated during the rainy season. Further, It thrives well in low or moderate rainfall but unable to do so in response to heavy downpour (Lal, 2014).

5.1.2 Plant Chemistry

This plant is chemically constituted by various volatile and non-volatile compounds namely steroidal saponins, alkaloids, amino acids, fibres, vitamins, oils, carbohydrates, proteins, minerals and mucilage (Snehlata & Payal, 2012) as depicted in fig. 5.1. Such an entire spectrum of key phytochemicals confers this plant with impressive benefits-nutritionally as well as medicinally. For instance, steroidal saponin-diosgenin and amino acid-4-hydroxy isoleucine render fenugreek plants with anti-diabetic potential while alkaloid-trigonelline provides anti-microbial, anti-diabetic, anti-tumorous and neuroprotective properties (Zhou et al., 2012).

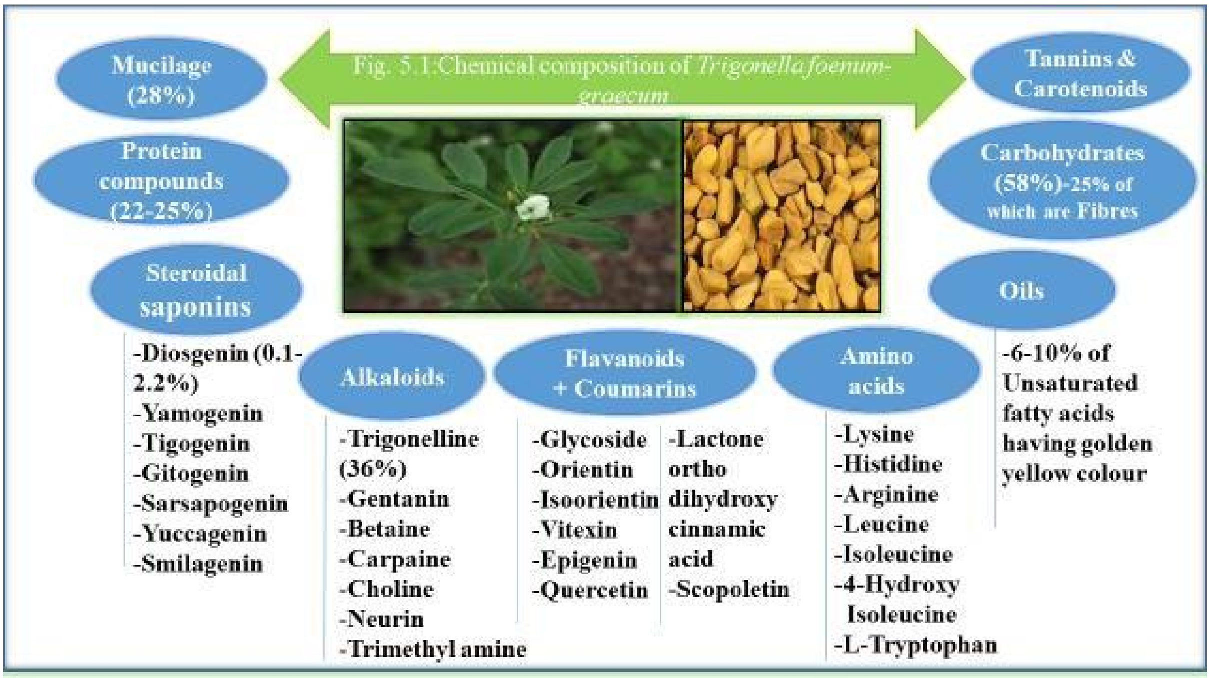
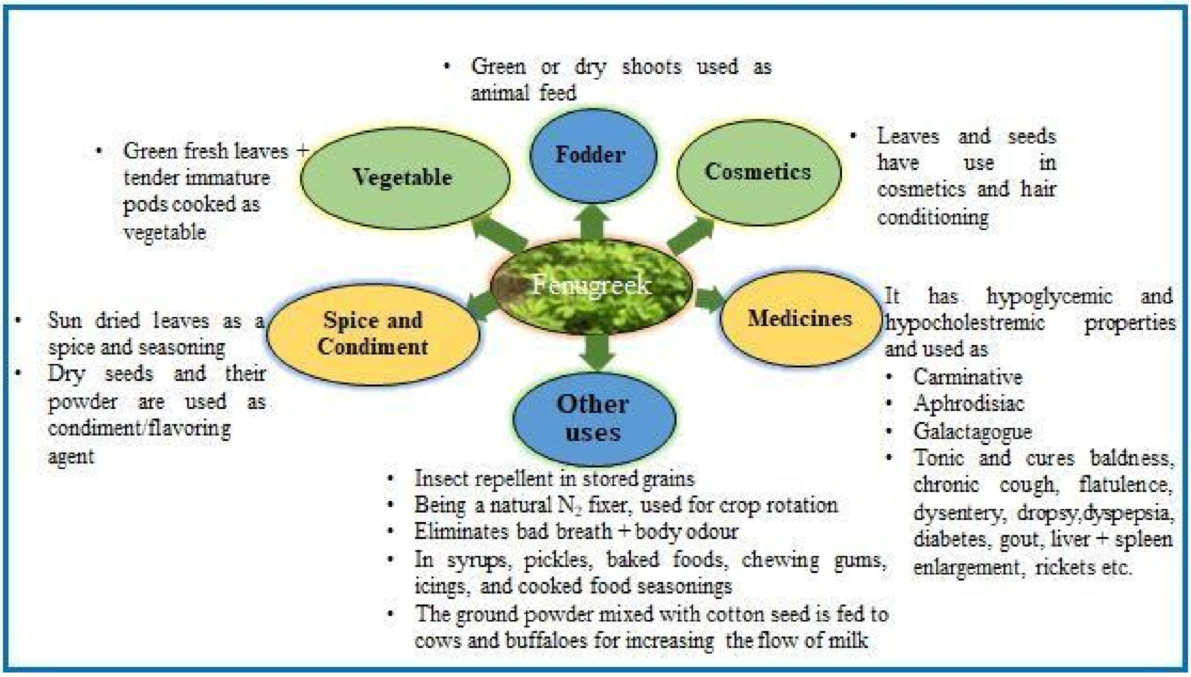


Fig. 5.1:Chemical composition of *Trigonella foenum-graecum*

5.1.3 Uses

Fenugreek is one of the major constituent of spice blends and also used to flavour tobacco, varied food items and beverages. It can be developed as an adhesive, emulsifying agent and food stabilizer. Moreover, its extracts or powdered form find applicability in preparing bakery products. Traditional systems of medicines namely Indian, Chinese and Tibetan have been utilizing this plant multifariously since aeons. Additionally, modern research findings also substantiate its usage for keeping the measures of blood sugar plus cholesterol under check (Acharya et al., 2006). Additionally, it has antioxidant, anti-cancerous, antidiabetic, laxative and immunological properties. An entire range of diverse phytochemicals is responsible for conferring this plant with the vast medicinal potential, out of which diosgenin, fenugreekine, 4-hydroxy isoleucine, galactomannans and trigonelline are a few major ones to be named (Zandi et al., 2015). Diosgenin, a steroidal chemical, supplements various pharmaceutical preparations (Mehrafarin et al., 2010). Multifarious applications of this spice crop have been summarized in fig.5.2.



- Green or dry shoots used as animal feed

- Green fresh leaves + tender immature pods cooked as vegetable

- Leaves and seeds have use in cosmetics and hair conditioning

- Sun dried leaves as a spice and seasoning
- Dry seeds and their powder are used as condiment/flavoring agent

It has hypoglycemic and hypocholestremic properties and used as
- Carminative
- Aphrodisiac
- Galactagogue
- Tonic and cures baldness, chronic cough, flatulence, dysentery, dropsy,dyspepsia, diabetes, gout, liver + spleen enlargement, rickets etc.

- Insect repellent in stored grains
- Being a natural N_2 fixer, used for crop rotation
- Eliminates bad breath + body odour
- In syrups, pickles, baked foods, chewing gums, icings, and cooked food seasonings
- The ground powder mixed with cotton seed is fed to cows and buffaloes for increasing the flow of milk

Fig. 5.2: Diverse range of uses in *Trigonella foenu-graecum* (Fenugreek) plants

The global demand of this crop is quite high owing to its widespread usage in functional food, nutraceutical and pharmaceutical industries.

5.2 Layout of treatments

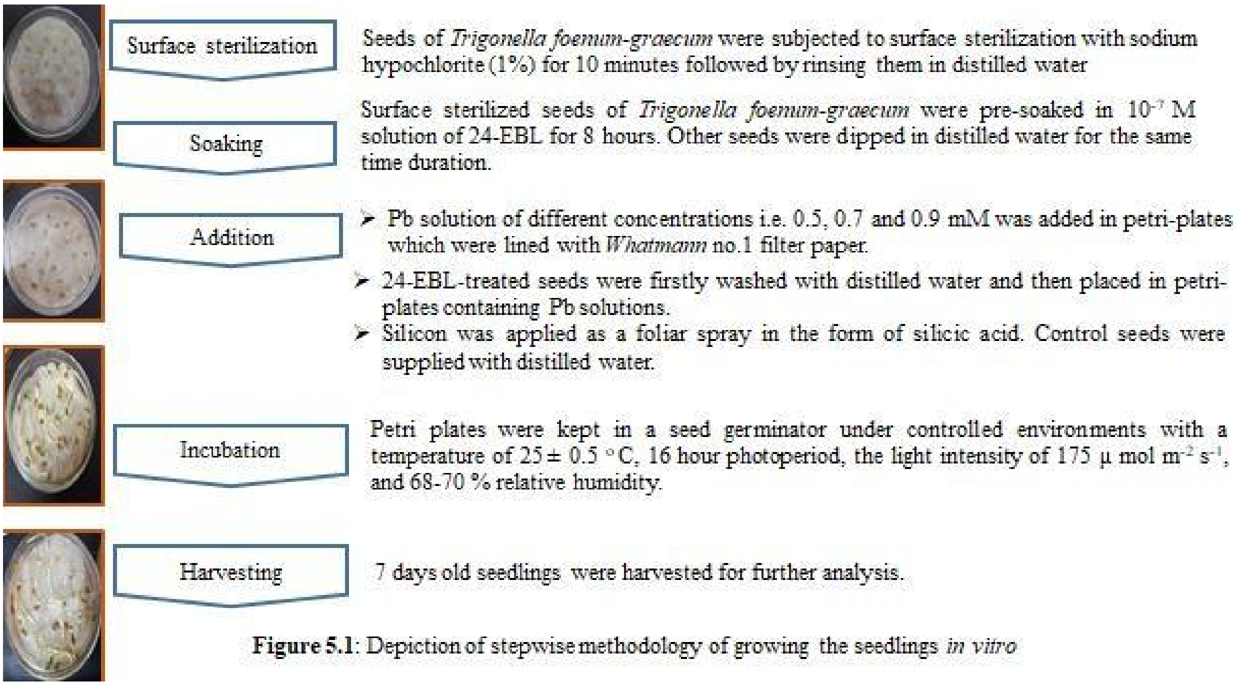
Different treatment combinations used in the current experimental study are specified in table 5.1.

Different treatments selected for the experiment

S.No.	Treatment	Lead (Pb) in mM	24- Epibrassinolide (24-EBL) in M	Silicon (Si) in mM
1	CN	0	0	0
2	Pb I	0.5	0	0
3	Pb II	0.7	0	0
4	Pb III	0.9	0	0
5	24-EBL	0	10^{-7}	0
6	24-EBL+Pb I	0.5	10^{-7}	0
7	24-EBL+Pb II	0.7	10^{-7}	0
8	24-EBL+Pb III	0.9	10^{-7}	0
9	Si	0	0	2
10	Si+Pb I	0.5	0	2
11	Si+Pb II	0.7	0	2
12	Si+Pb III	0.9	0	2
13	24-EBL + Si	0	10^{-7}	2
14	24-EBL+ Si+Pb I	0.5	10^{-7}	2
15	24-EBL+ Si+Pb II	0.7	10^{-7}	2
16	24-EBL+ Si + Pb III	0.9	10^{-7}	2

5.3 Cultivating the seedlings and plants

5.3.2 Growing seedlings *in vitro*



Seeds of *Trigonella foenum-graecum* were subjected to surface sterilization with sodium hypochlorite (1%) for 10 minutes followed by rinsing them in distilled water

Surface sterilized seeds of *Trigonella foenum-graecum* were pre-soaked in 10^{-7} M solution of 24-EBL for 8 hours. Other seeds were dipped in distilled water for the same time duration.

- Pb solution of different concentrations i.e. 0.5, 0.7 and 0.9 mM was added in petri-plates which were lined with *Whatmann* no.1 filter paper.
- 24-EBL-treated seeds were firstly washed with distilled water and then placed in petri-plates containing Pb solutions.
- Silicon was applied as a foliar spray in the form of silicic acid. Control seeds were supplied with distilled water.

Petri plates were kept in a seed germinator under controlled environments with a temperature of 25 ± 0.5 °C, 16 hour photoperiod, the light intensity of 175 µ mol m^{-2} s^{-1}, and 68-70 % relative humidity.

7 days old seedlings were harvested for further analysis.

Figure 5.1: Depiction of stepwise methodology of growing the seedlings *in vitro*

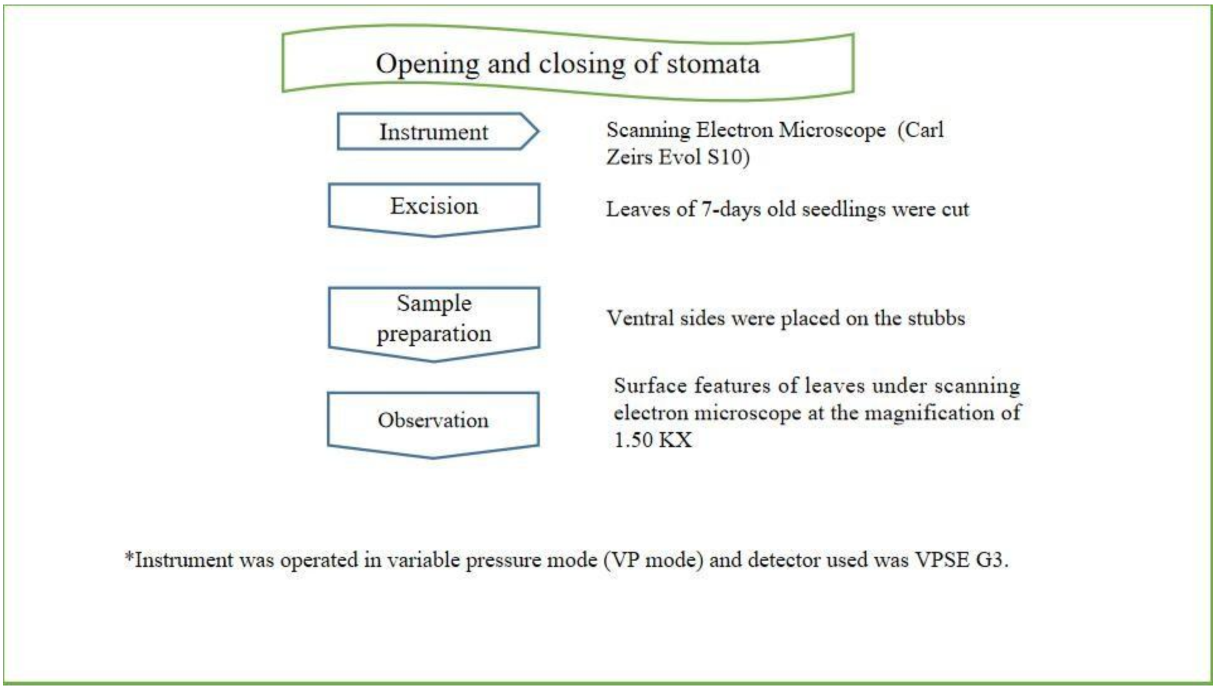
5.3.3 Raising of plants in *In-vivo*

Similar steps as shown in fig. 5.1 were followed for performing *in-vivo* study. Grow bags (16 × 30 cm) filled with a mixture of soil plus organic manure in 3:1 proportions, were used for sowing of *Trigonella* seeds. After 15, 30 and 45 days, harvesting of plants was done for further analytical study.

5.4 Growth analysis

5.4.1 **Germination percentage**	It was calculated by using the below mentioned formula $= \frac{total\ seeds\ germinated}{number\ of\ initial\ seeds\ used} \times 100$
5.4.2 **Measurement of morphological parameters**	In fenugreek seedlings, root and shoot length were measured in cm. Fresh weight was measured in grams using a weighing balance. Dry weight was taken by drying the samples at 80 ^{0}C for 24 h. Similarly, root length plus fresh and dry weights were measured in 15, 30 and 45 days old plants.
5.4.3 **Vigor index**	Following formula was used to calculate vigor index Vigor index = $(Root\ length + shoot\ length) \times germination\ percentage$
5.4.4 **Relative water content**	It was calculated both in seedlings and plants by using the following formula Relative water content$= \frac{Fresh\ weight - dry\ weight}{Turger\ weight - dry\ weight} \times 100$

5.4.5 Stomatal movements



Opening and closing of stomata

Instrument: Scanning Electron Microscope (Carl Zeirs Evol S10)

Excision: Leaves of 7-days old seedlings were cut

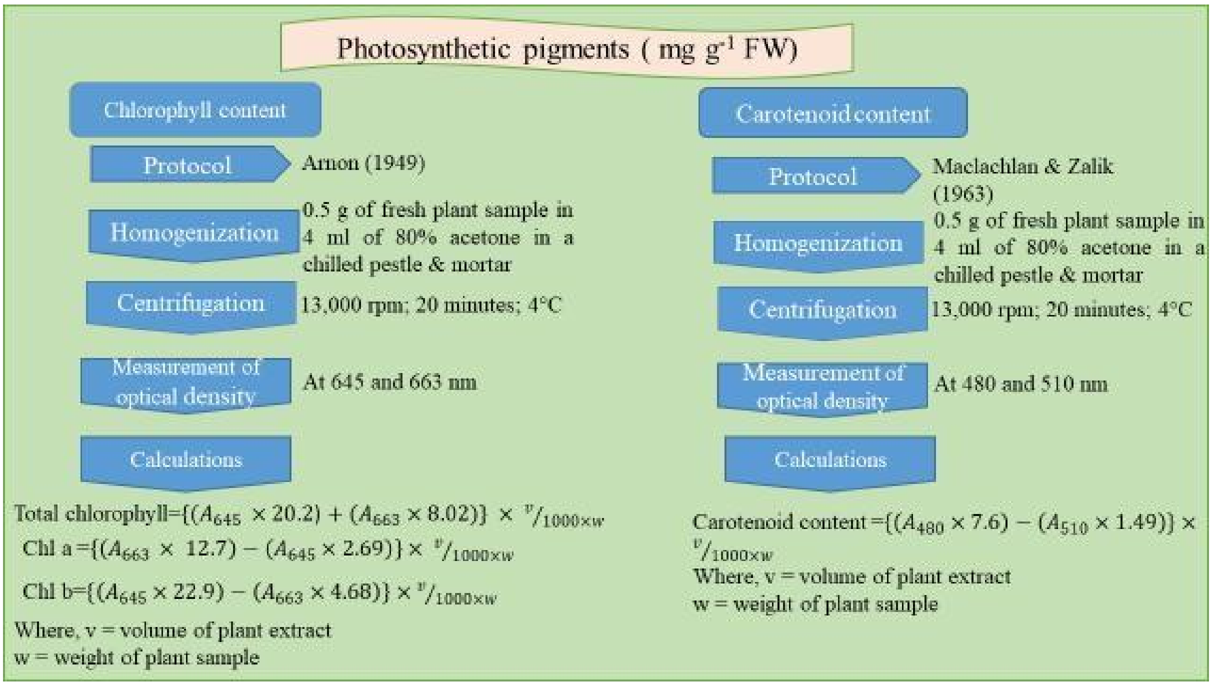
Sample preparation: Ventral sides were placed on the stubbs

Observation: Surface features of leaves under scanning electron microscope at the magnification of 1.50 KX

*Instrument was operated in variable pressure mode (VP mode) and detector used was VPSE G3.

5.5 Photosynthetic pigments

5.5.1 Chlorophylls and carotenoids



Photosynthetic pigments (mg g^{-1} FW)

Chlorophyll content

Protocol
Arnon (1949)

Homogenization
0.5 g of fresh plant sample in 4 ml of 80% acetone in a chilled pestle & mortar

Centrifugation
13,000 rpm; 20 minutes; 4°C

Measurement of optical density
At 645 and 663 nm

Calculations

Total chlorophyll=$\{(A_{645} \times 20.2) + (A_{663} \times 8.02)\} \times {}^{v}/_{1000 \times w}$

Chl a =$\{(A_{663} \times 12.7) - (A_{645} \times 2.69)\} \times {}^{v}/_{1000 \times w}$

Chl b=$\{(A_{645} \times 22.9) - (A_{663} \times 4.68)\} \times {}^{v}/_{1000 \times w}$

Where, v = volume of plant extract
w = weight of plant sample

Carotenoid content

Protocol
Maclachlan & Zalik (1963)

Homogenization
0.5 g of fresh plant sample in 4 ml of 80% acetone in a chilled pestle & mortar

Centrifugation
13,000 rpm; 20 minutes; 4°C

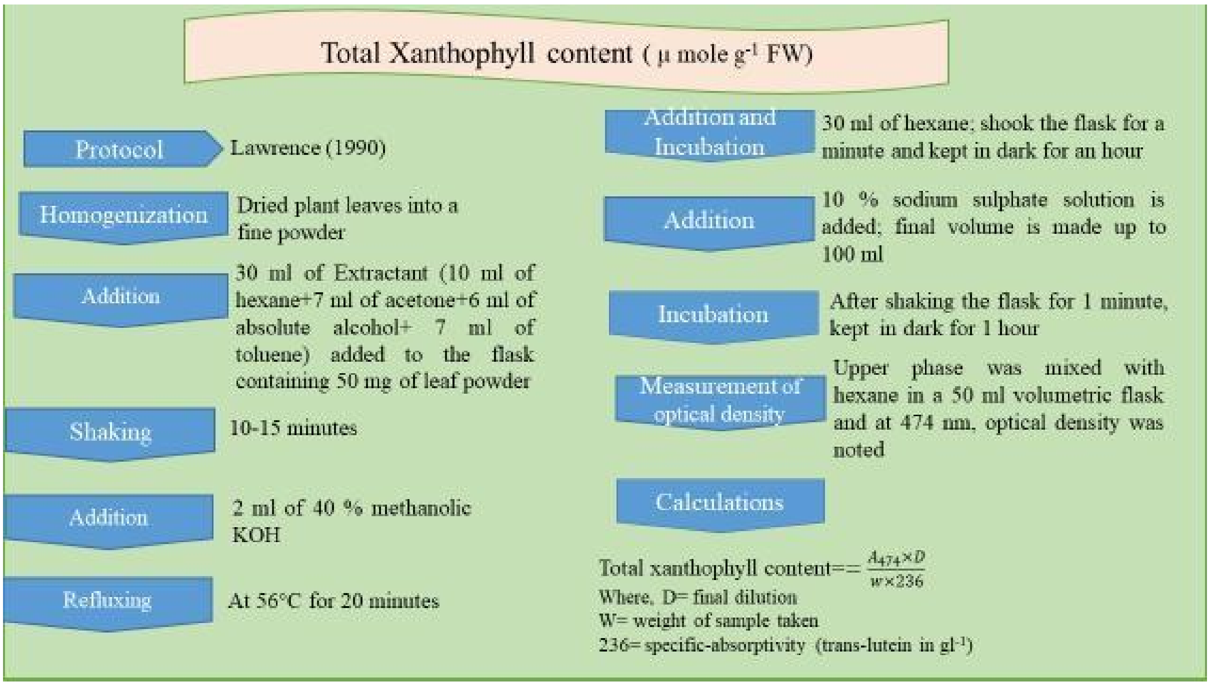
Measurement of optical density
At 480 and 510 nm

Calculations

Carotenoid content =$\{(A_{480} \times 7.6) - (A_{510} \times 1.49)\} \times {}^{v}/_{1000 \times w}$

Where, v = volume of plant extract
w = weight of plant sample

5.5.2 Xanthophylls



Total Xanthophyll content (μ mole g^{-1} FW)

Protocol
Lawrence (1990)

Homogenization
Dried plant leaves into a fine powder

Addition
30 ml of Extractant (10 ml of hexane+7 ml of acetone+6 ml of absolute alcohol+ 7 ml of toluene) added to the flask containing 50 mg of leaf powder

Shaking
10-15 minutes

Addition
2 ml of 40 % methanolic KOH

Refluxing
At 56°C for 20 minutes

Addition and Incubation
30 ml of hexane; shook the flask for a minute and kept in dark for an hour

Addition
10 % sodium sulphate solution is added; final volume is made up to 100 ml

Incubation
After shaking the flask for 1 minute, kept in dark for 1 hour

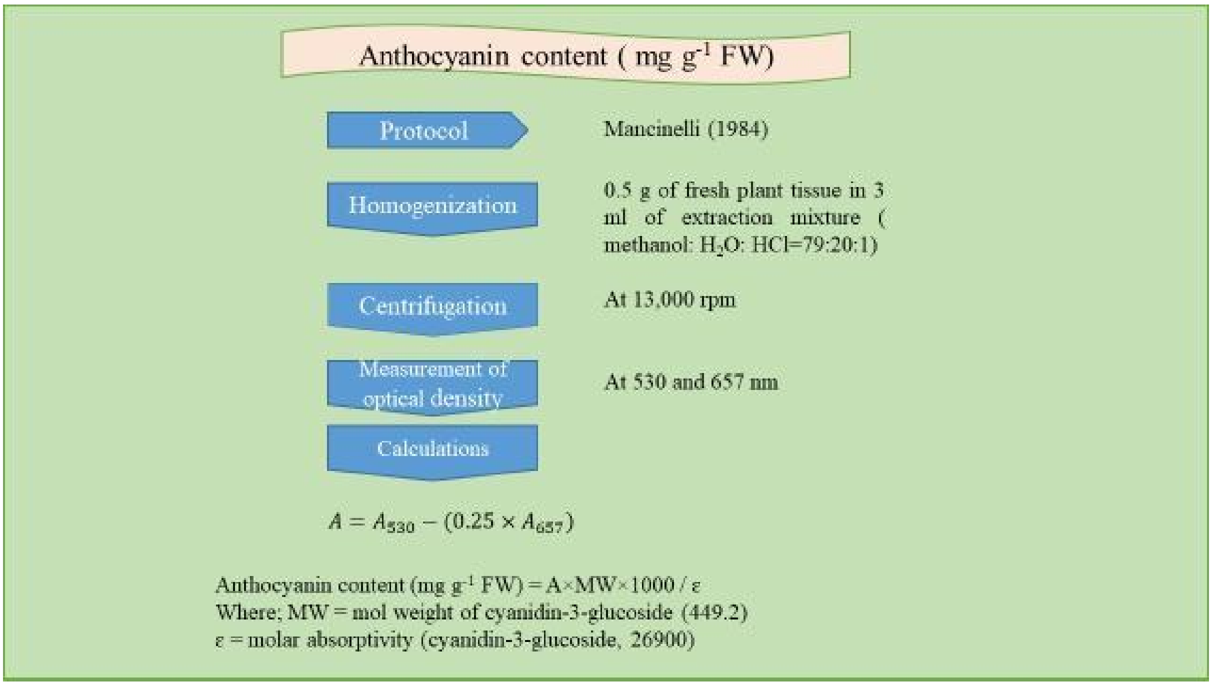
Measurement of optical density
Upper phase was mixed with hexane in a 50 ml volumetric flask and at 474 nm, optical density was noted

Calculations

Total xanthophyll content==$\frac{A_{474} \times D}{w \times 236}$
Where, D= final dilution
W= weight of sample taken
236= specific-absorptivity (trans-lutein in gl^{-1})

5.6 Metabolite content (mg g^{-1} FW)

5.6.1 Anthocyanins



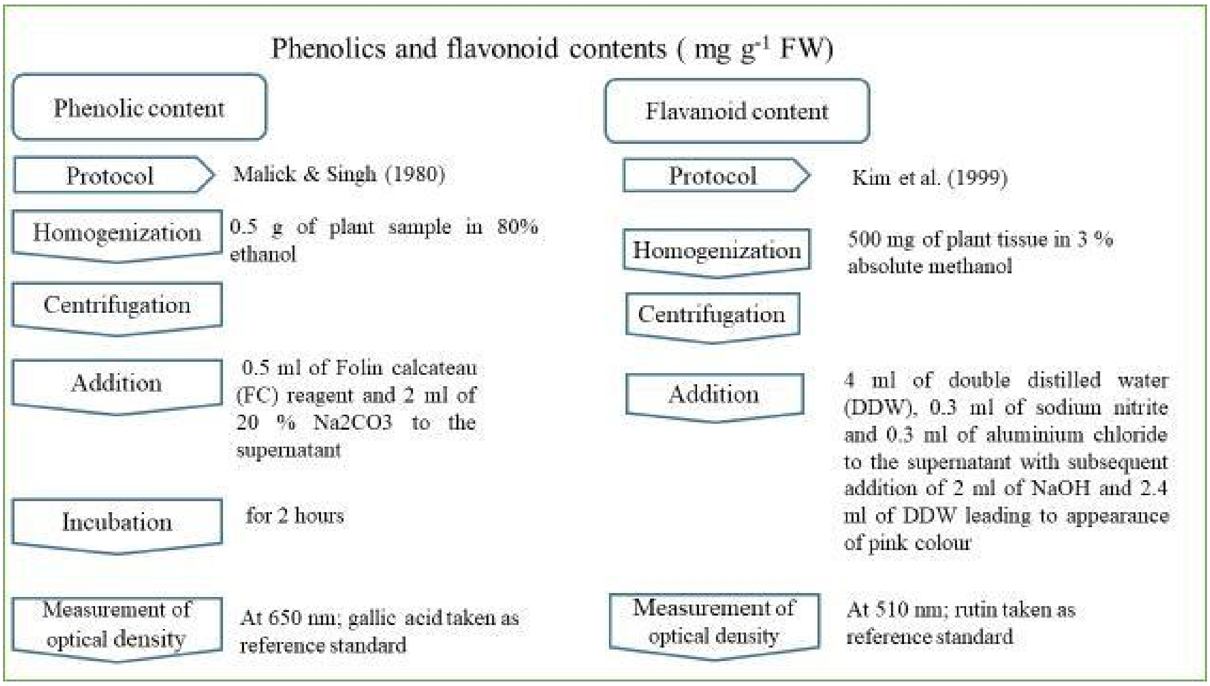
Anthocyanin content (mg g^{-1} FW)

Protocol	Mancinelli (1984)
Homogenization	0.5 g of fresh plant tissue in 3 ml of extraction mixture (methanol: H_2O: HCl=79:20:1)
Centrifugation	At 13,000 rpm
Measurement of optical density	At 530 and 657 nm
Calculations	

$$A = A_{530} - (0.25 \times A_{657})$$

Anthocyanin content (mg g^{-1} FW) = A×MW×1000 / ε
Where; MW = mol weight of cyanidin-3-glucoside (449.2)
ε = molar absorptivity (cyanidin-3-glucoside, 26900)

5.6.2 Phenolics and flavonoids



Phenolics and flavonoid contents (mg g^{-1} FW)

Phenolic content

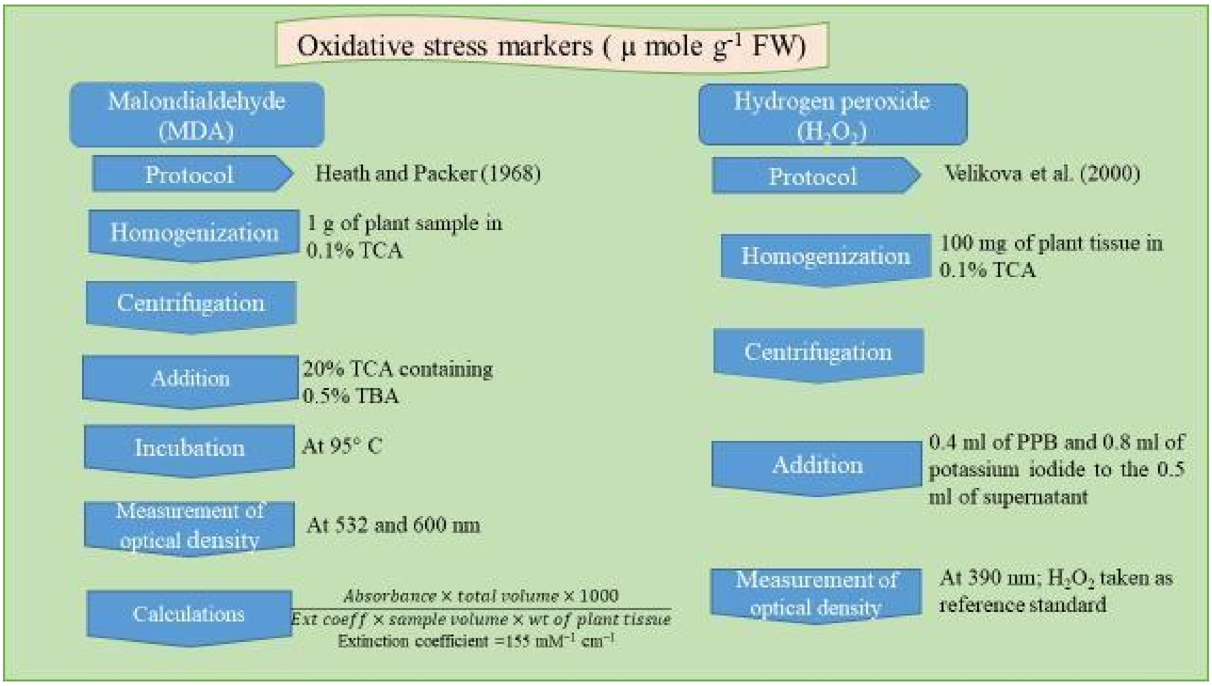
Protocol	Malick & Singh (1980)
Homogenization	0.5 g of plant sample in 80% ethanol
Centrifugation	
Addition	0.5 ml of Folin calcateau (FC) reagent and 2 ml of 20 % Na2CO3 to the supernatant
Incubation	for 2 hours
Measurement of optical density	At 650 nm; gallic acid taken as reference standard

Flavanoid content

Protocol	Kim et al. (1999)
Homogenization	500 mg of plant tissue in 3 % absolute methanol
Centrifugation	
Addition	4 ml of double distilled water (DDW), 0.3 ml of sodium nitrite and 0.3 ml of aluminium chloride to the supernatant with subsequent addition of 2 ml of NaOH and 2.4 ml of DDW leading to appearance of pink colour
Measurement of optical density	At 510 nm; rutin taken as reference standard

5.7 Oxidative damage

5.7.1 Oxidative stress markers [Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) content]



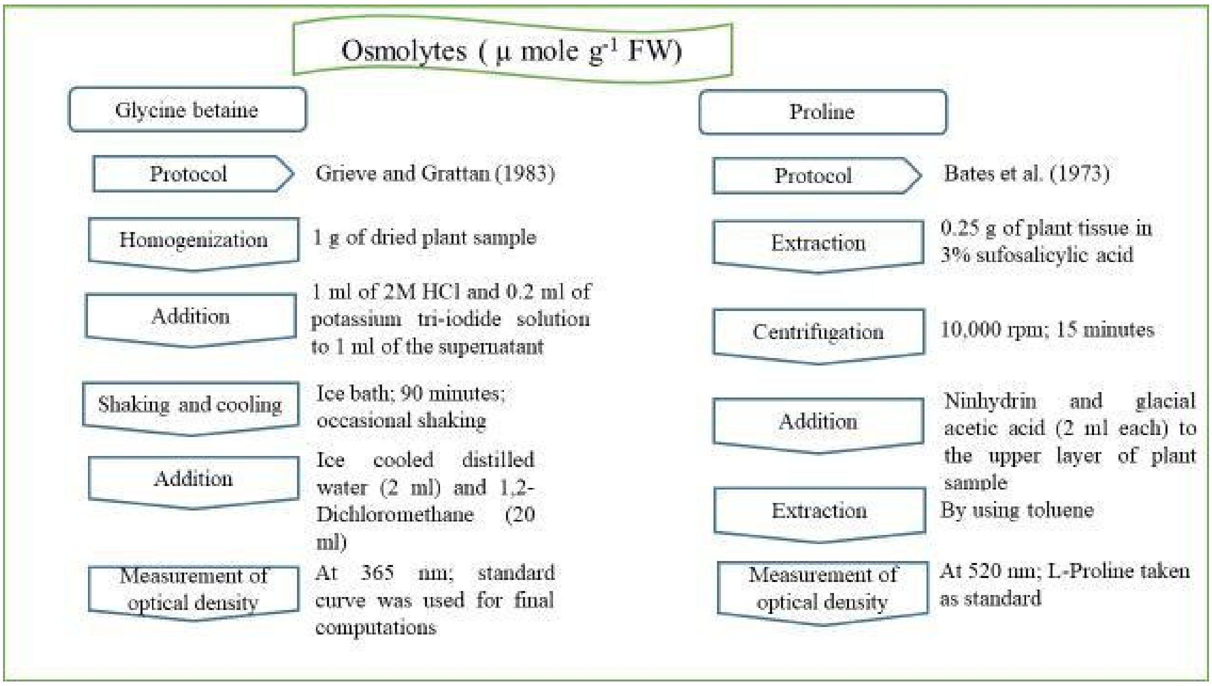
5.7.2 Histochemical studies by confocal microscopy

Roots of fenugreek seedlings were put to analysis to ascertain the membrane plus nuclear damage for which the procedures proposed by Callard et al. (1996) with that of Gutierrez-Alcala et al. (2000) were adopted by using a confocal microscope (Nikon AIR). Briefly, 1 cm long roots were excised from every sample with a subsequent thorough washing in water. To ascertain the membrane plus nuclear damage, two stains i.e. i) 10 μM of 2, 7-dichlorofluroscein dye and ii) 25 μM of propidium iodide were used consecutively to stain excised roots of *Trigonella foenum-graecum* seedlings. After incubating in the dark for half an hour, washing with PBS was performed. Stained slides were mounted in water to be observed with the help of confocal microscope.

5.8 Lead metal uptake (mg g^{-1} DW)

Extent of Pb uptake was estimated at highest Pb stress treatment of Pb III concentration using AAS-Atomic Absorption Spectrophotometer (Shimadzu model-6200). Briefly, plant samples were oven dried and then 1g of each sample was digested with 5 ml of HNO_3 + $HClO_4$ mixture having a proportion of 2:1. Afterwards, digested samples were cooled down and filtered. Double distilled water was added to each sample to make the final volume equal to 15 ml for the approximation of Pb contents.

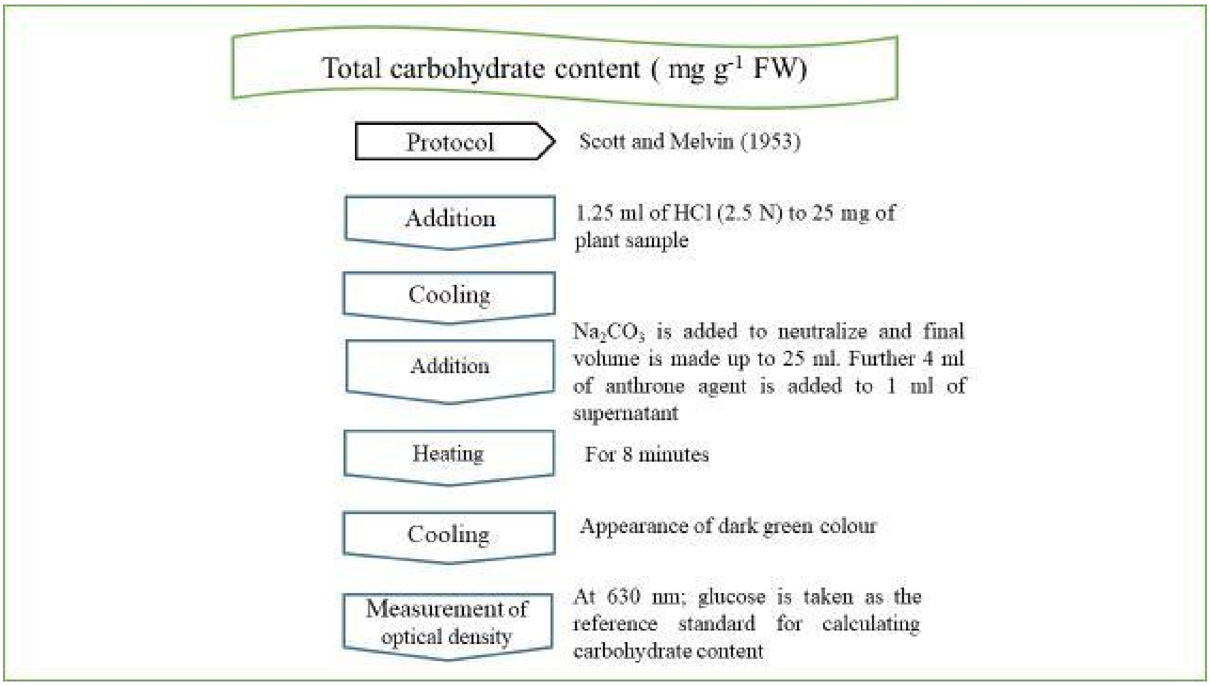
5.9 Estimation of Osmolytes (Glycine betaine and Proline content (μ mol g^{-1} FW)



Protocol	Grieve and Grattan (1983)
Homogenization	1 g of dried plant sample
Addition	1 ml of 2M HCl and 0.2 ml of potassium tri-iodide solution to 1 ml of the supernatant
Shaking and cooling	Ice bath; 90 minutes; occasional shaking
Addition	Ice cooled distilled water (2 ml) and 1,2-Dichloromethane (20 ml)
Measurement of optical density	At 365 nm; standard curve was used for final computations

Protocol	Bates et al. (1973)
Extraction	0.25 g of plant tissue in 3% sufosalicylic acid
Centrifugation	10,000 rpm; 15 minutes
Addition	Ninhydrin and glacial acetic acid (2 ml each) to the upper layer of plant sample
Extraction	By using toluene
Measurement of optical density	At 520 nm; L-Proline taken as standard

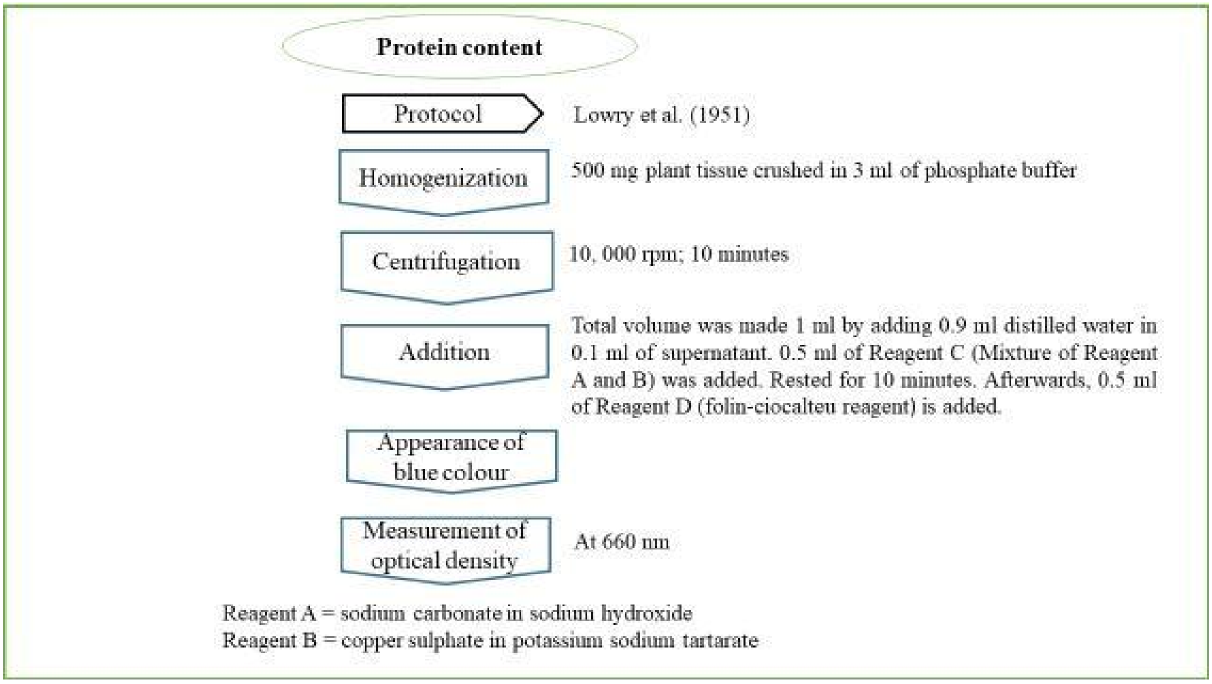
5.10 Total carbohydrates content (mg g^{-1} FW)



Protocol	Scott and Melvin (1953)
Addition	1.25 ml of HCl (2.5 N) to 25 mg of plant sample
Cooling	
Addition	Na_2CO_3 is added to neutralize and final volume is made up to 25 ml. Further 4 ml of anthrone agent is added to 1 ml of supernatant
Heating	For 8 minutes
Cooling	Appearance of dark green colour
Measurement of optical density	At 630 nm; glucose is taken as the reference standard for calculating carbohydrate content

5.11 Protein content and Antioxidant defense system

5.11.1 Protein content (mg g^{-1} FW)



Lowry et al. (1951)

500 mg plant tissue crushed in 3 ml of phosphate buffer

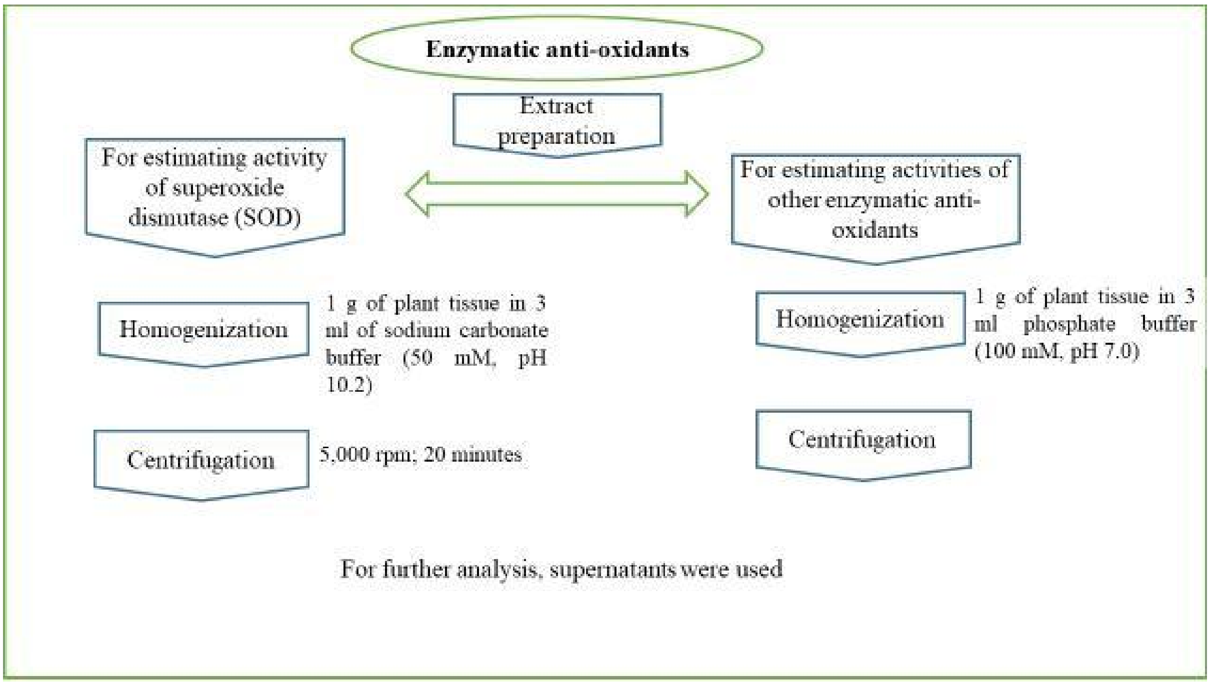
10, 000 rpm; 10 minutes

Total volume was made 1 ml by adding 0.9 ml distilled water in 0.1 ml of supernatant. 0.5 ml of Reagent C (Mixture of Reagent A and B) was added. Rested for 10 minutes. Afterwards, 0.5 ml of Reagent D (folin-ciocalteu reagent) is added.

At 660 nm

Reagent A = sodium carbonate in sodium hydroxide
Reagent B = copper sulphate in potassium sodium tartarate

5.11.2 Enzymatic antioxidants



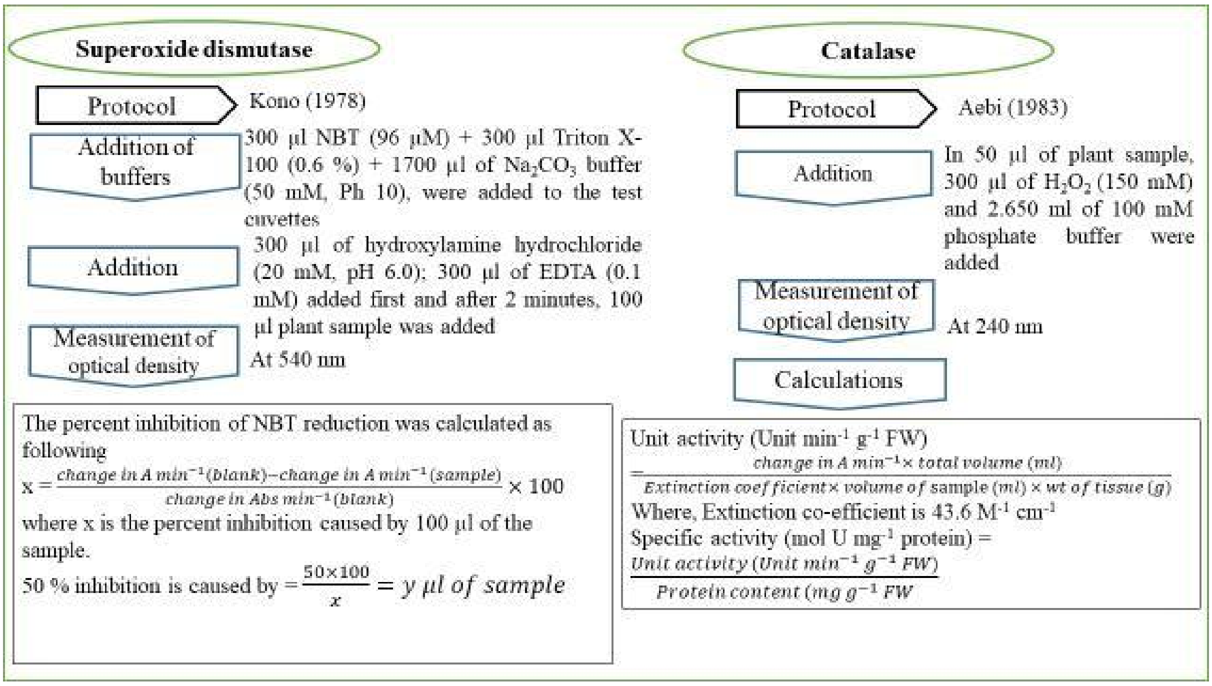
1 g of plant tissue in 3 ml of sodium carbonate buffer (50 mM, pH 10.2)

5,000 rpm; 20 minutes

1 g of plant tissue in 3 ml phosphate buffer (100 mM, pH 7.0)

For further analysis, supernatants were used

5.11.2.1 Superoxide dismutase(SOD) and Catalase (CAT); UA mg^{-1} protein



Superoxide dismutase

Protocol

Kono (1978)

Addition of buffers

300 µl NBT (96 µM) + 300 µl Triton X-100 (0.6 %) + 1700 µl of Na_2CO_3 buffer (50 mM, Ph 10), were added to the test cuvettes

Addition

300 µl of hydroxylamine hydrochloride (20 mM, pH 6.0); 300 µl of EDTA (0.1 mM) added first and after 2 minutes, 100 µl plant sample was added

Measurement of optical density

At 540 nm

The percent inhibition of NBT reduction was calculated as following

$$x = \frac{change\ in\ A\ min^{-1}(blank) - change\ in\ A\ min^{-1}(sample)}{change\ in\ Abs\ min^{-1}(blank)} \times 100$$

where x is the percent inhibition caused by 100 µl of the sample.

50 % inhibition is caused by $= \frac{50 \times 100}{x} = y\ \mu l\ of\ sample$

Catalase

Protocol

Aebi (1983)

Addition

In 50 µl of plant sample, 300 µl of H_2O_2 (150 mM) and 2.650 ml of 100 mM phosphate buffer were added

Measurement of optical density

At 240 nm

Calculations

Unit activity (Unit min^{-1} g^{-1} FW)

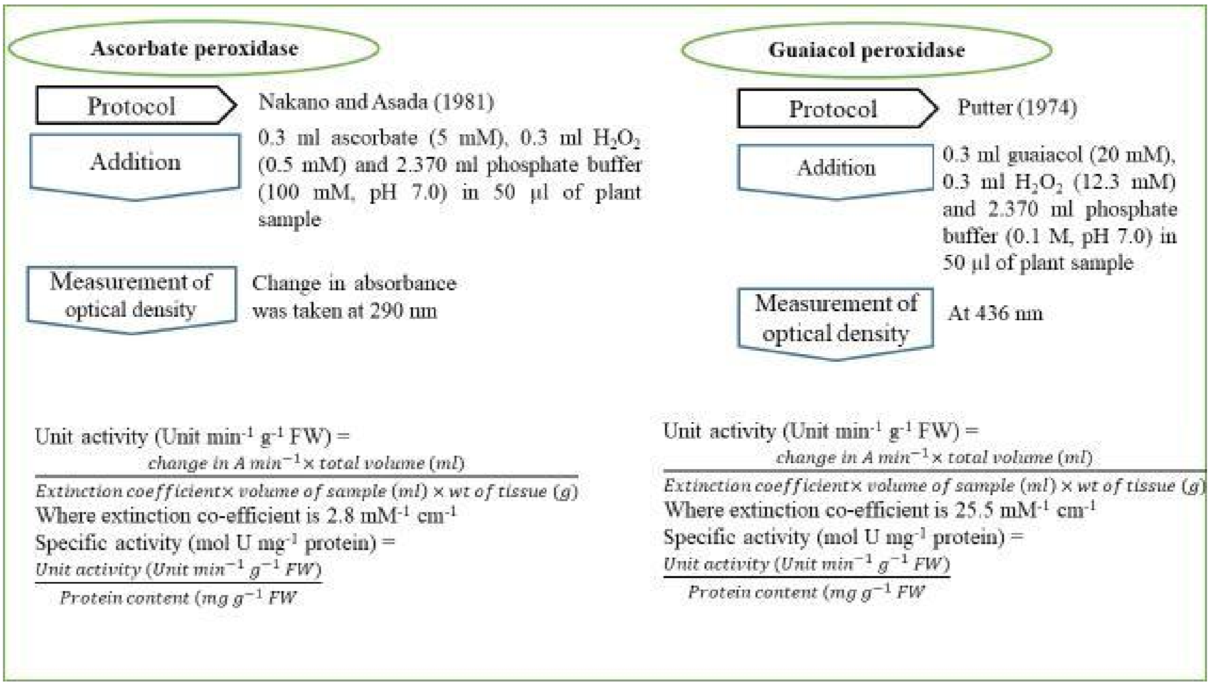
$$= \frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where, Extinction co-efficient is 43.6 M^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

5.11.2.2 Ascorbate peroxidase (APX; UA mg^{-1} protein); Guaiacol peroxidase (POD; UA mg^{-1} protein)



Ascorbate peroxidase

Protocol

Nakano and Asada (1981)

Addition

0.3 ml ascorbate (5 mM), 0.3 ml H_2O_2 (0.5 mM) and 2.370 ml phosphate buffer (100 mM, pH 7.0) in 50 µl of plant sample

Measurement of optical density

Change in absorbance was taken at 290 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 2.8 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

Guaiacol peroxidase

Protocol

Putter (1974)

Addition

0.3 ml guaiacol (20 mM), 0.3 ml H_2O_2 (12.3 mM) and 2.370 ml phosphate buffer (0.1 M, pH 7.0) in 50 µl of plant sample

Measurement of optical density

At 436 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

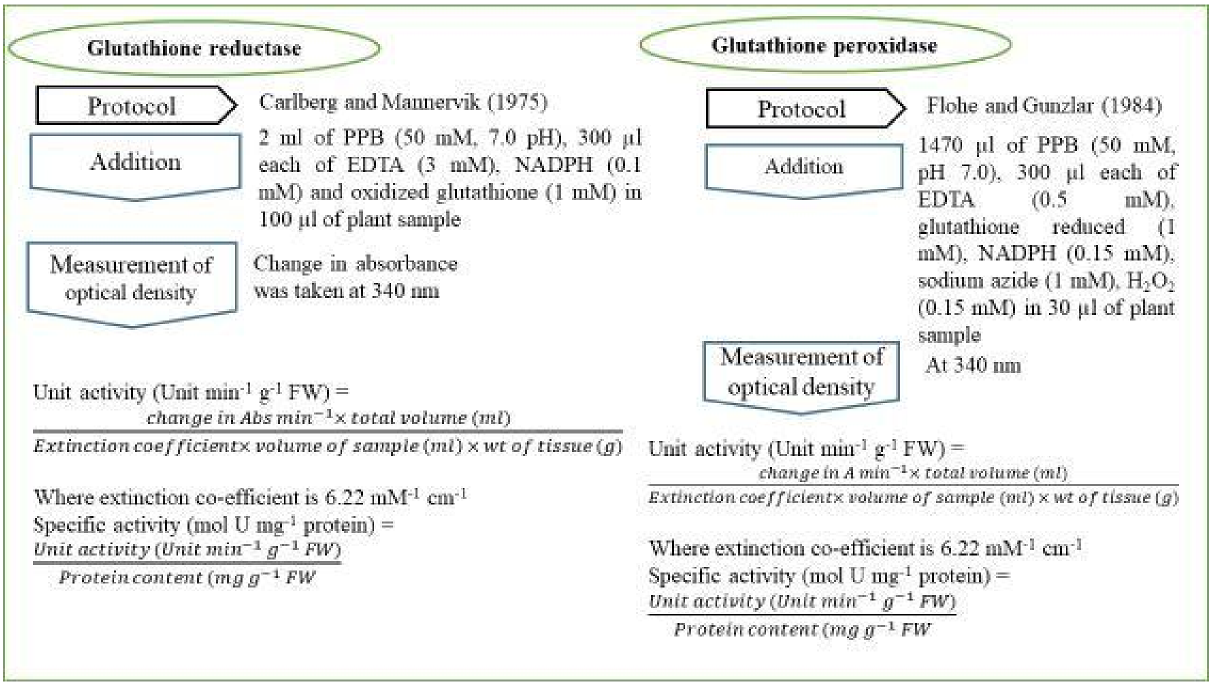
$$\frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 25.5 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

5.11.2.3 Glutathione reductase (GR; UA mg^{-1} protein); Glutathione peroxidase (GPOX; UA mg^{-1} protein)



Glutathione reductase

Protocol: Carlberg and Mannervik (1975)

Addition: 2 ml of PPB (50 mM, 7.0 pH), 300 µl each of EDTA (3 mM), NADPH (0.1 mM) and oxidized glutathione (1 mM) in 100 µl of plant sample

Measurement of optical density: Change in absorbance was taken at 340 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

Glutathione peroxidase

Protocol: Flohe and Gunzlar (1984)

Addition: 1470 µl of PPB (50 mM, pH 7.0), 300 µl each of EDTA (0.5 mM), glutathione reduced (1 mM), NADPH (0.15 mM), sodium azide (1 mM), H_2O_2 (0.15 mM) in 30 µl of plant sample

Measurement of optical density: At 340 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

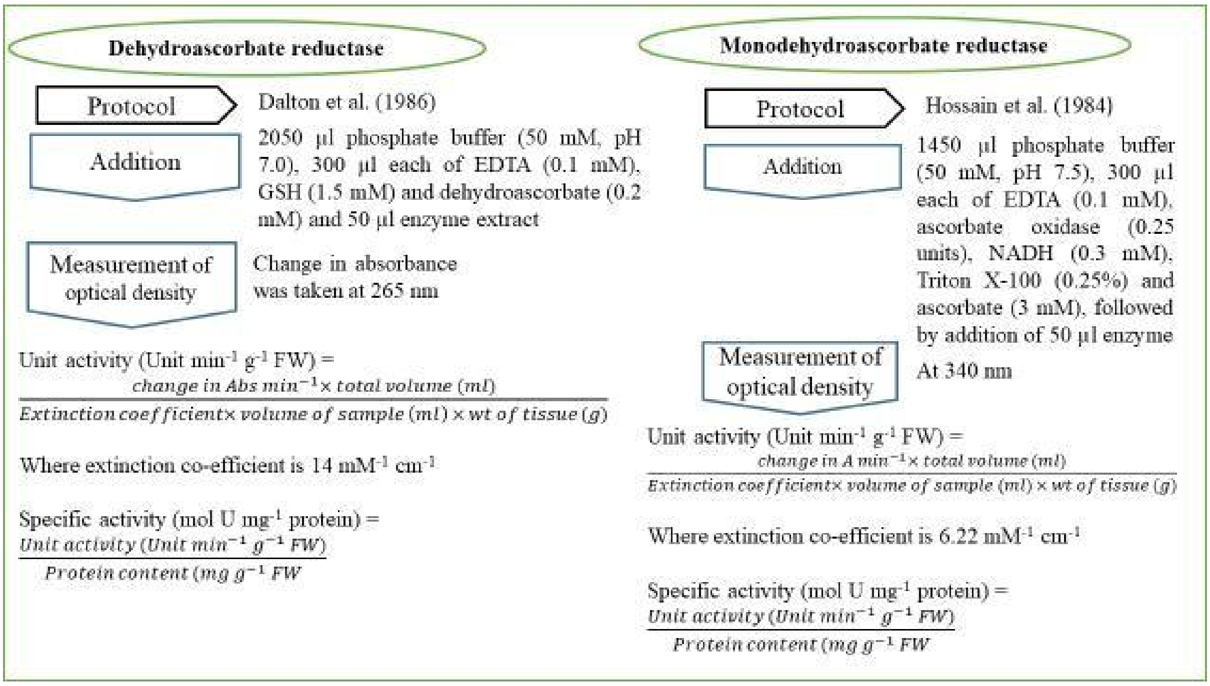
$$\frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

5.11.2.4 Dehydroascorbate reductase (DHAR; UA mg^{-1} protein); Monodehydroascorbate reductase (MDHAR; UA mg^{-1} protein)



Dehydroascorbate reductase

Protocol: Dalton et al. (1986)

Addition: 2050 µl phosphate buffer (50 mM, pH 7.0), 300 µl each of EDTA (0.1 mM), GSH (1.5 mM) and dehydroascorbate (0.2 mM) and 50 µl enzyme extract

Measurement of optical density: Change in absorbance was taken at 265 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

$$\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 14 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

Monodehydroascorbate reductase

Protocol: Hossain et al. (1984)

Addition: 1450 µl phosphate buffer (50 mM, pH 7.5), 300 µl each of EDTA (0.1 mM), ascorbate oxidase (0.25 units), NADH (0.3 mM), Triton X-100 (0.25%) and ascorbate (3 mM), followed by addition of 50 µl enzyme

Measurement of optical density: At 340 nm

Unit activity (Unit min^{-1} g^{-1} FW) =

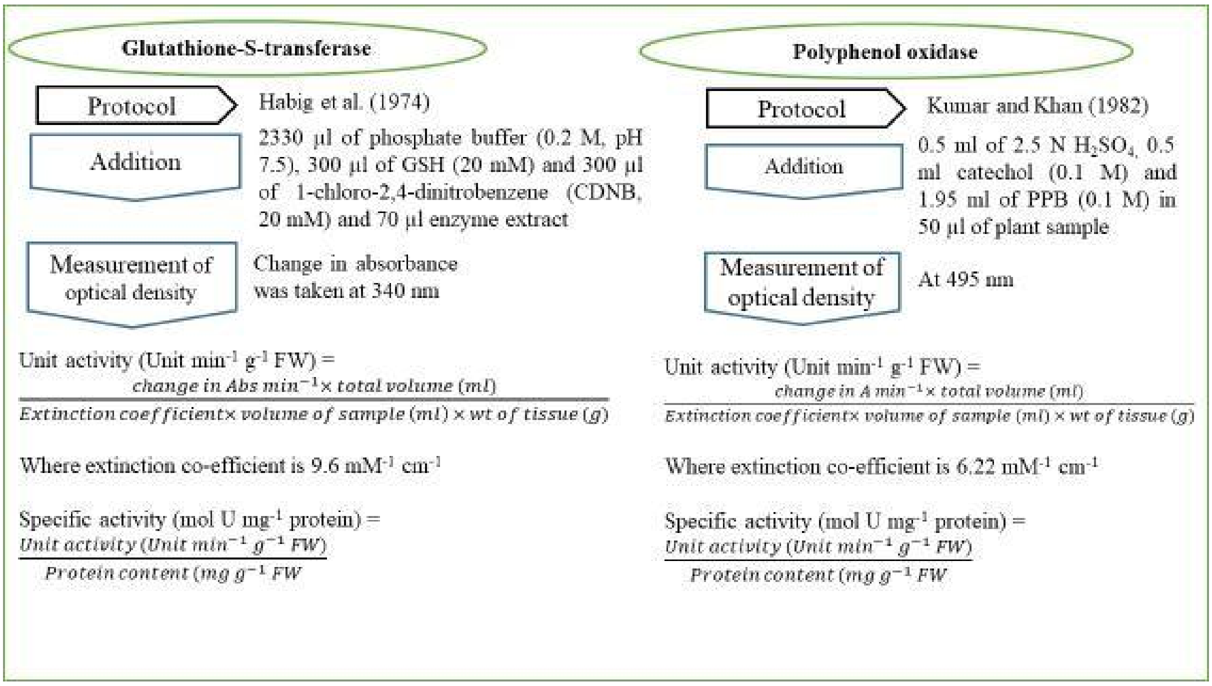
$$\frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$$

Where extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) =

$$\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$$

5.11.2.5 Glutathione-S-transferase (GST; UA mg^{-1} protein); Polyphenol oxidase (PPO; UA mg^{-1} protein)



Glutathione-S-transferase

Protocol: Habig et al. (1974)

Addition: 2330 µl of phosphate buffer (0.2 M, pH 7.5), 300 µl of GSH (20 mM) and 300 µl of 1-chloro-2,4-dinitrobenzene (CDNB, 20 mM) and 70 µl enzyme extract

Measurement of optical density: Change in absorbance was taken at 340 nm

Unit activity (Unit min^{-1} g^{-1} FW) = $\frac{change\ in\ Abs\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$

Where extinction co-efficient is 9.6 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

Polyphenol oxidase

Protocol: Kumar and Khan (1982)

Addition: 0.5 ml of 2.5 N H_2SO_4, 0.5 ml catechol (0.1 M) and 1.95 ml of PPB (0.1 M) in 50 µl of plant sample

Measurement of optical density: At 495 nm

Unit activity (Unit min^{-1} g^{-1} FW) = $\frac{change\ in\ A\ min^{-1} \times total\ volume\ (ml)}{Extinction\ coefficient \times volume\ of\ sample\ (ml) \times wt\ of\ tissue\ (g)}$

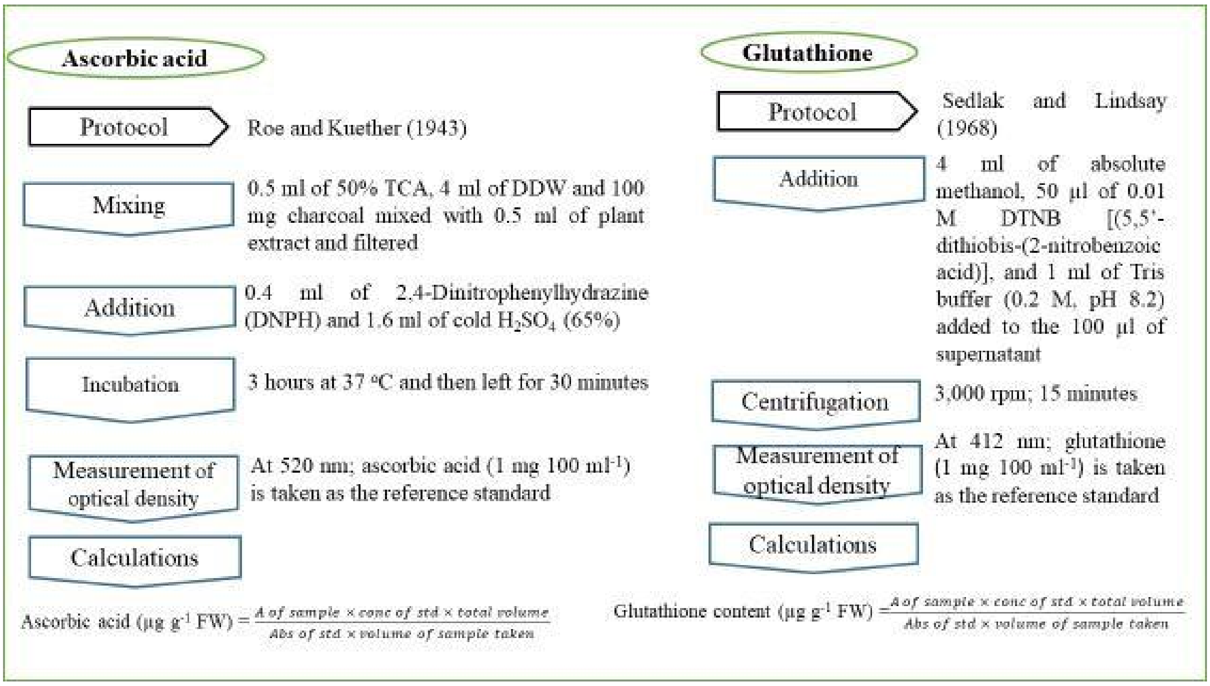
Where extinction co-efficient is 6.22 mM^{-1} cm^{-1}

Specific activity (mol U mg^{-1} protein) = $\frac{Unit\ activity\ (Unit\ min^{-1}\ g^{-1}\ FW)}{Protein\ content\ (mg\ g^{-1}\ FW}$

5.11.3 Non-enzymatic antioxidants

For the estimation of antioxidants of non-enzymatic nature in seedlings and plants, their extraction was performed in tris buffer (50 mM; 3 ml; pH 10.0) by taking about 1g of sample from each growth stage. It was subsequently centrifuged (13, 000 rpm; 20 minutes; 4 °C temperature). Afterwards, the supernatant was analysed for approximating the content of different non-enzymatic antioxidants.

5.11.3.1 Ascorbic acid (µg g^{-1} FW) and Glutathione content (µg g^{-1} FW)



Ascorbic acid

Protocol: Roe and Kuether (1943)

Mixing: 0.5 ml of 50% TCA, 4 ml of DDW and 100 mg charcoal mixed with 0.5 ml of plant extract and filtered

Addition: 0.4 ml of 2,4-Dinitrophenylhydrazine (DNPH) and 1.6 ml of cold H_2SO_4 (65%)

Incubation: 3 hours at 37 °C and then left for 30 minutes

Measurement of optical density: At 520 nm; ascorbic acid (1 mg 100 ml^{-1}) is taken as the reference standard

Calculations

Ascorbic acid (µg g^{-1} FW) = $\frac{A\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$

Glutathione

Protocol: Sedlak and Lindsay (1968)

Addition: 4 ml of absolute methanol, 50 µl of 0.01 M DTNB [(5,5'-dithiobis-(2-nitrobenzoic acid)], and 1 ml of Tris buffer (0.2 M, pH 8.2) added to the 100 µl of supernatant

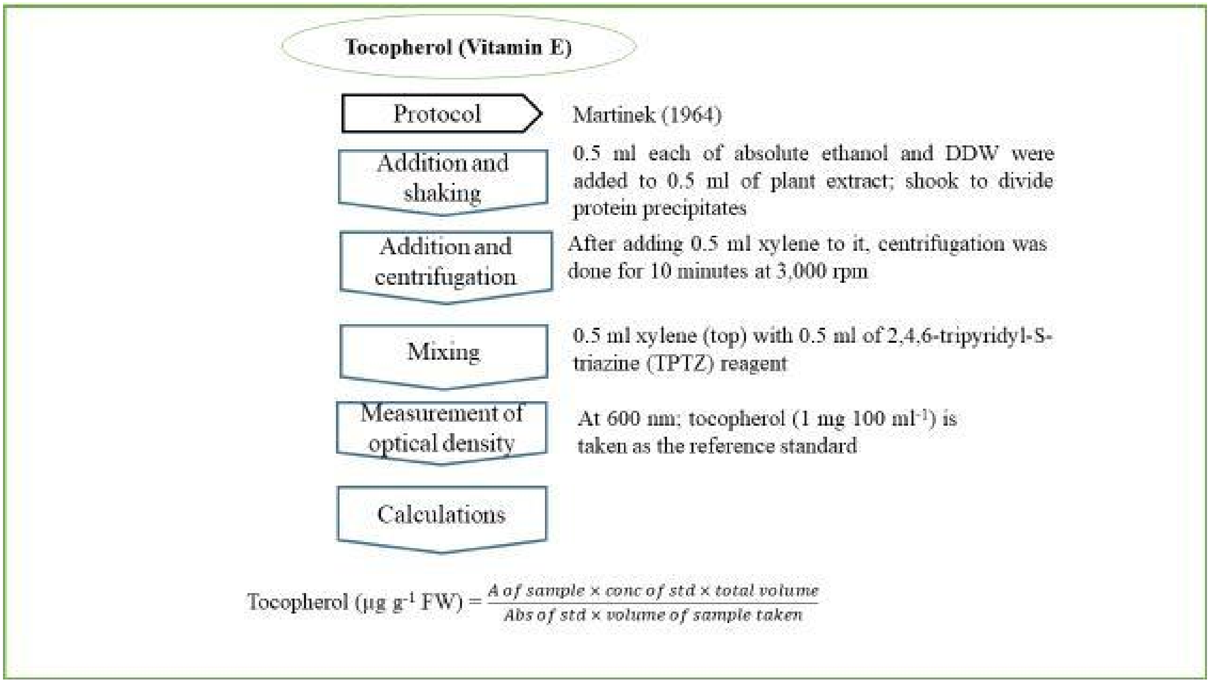
Centrifugation: 3,000 rpm; 15 minutes

Measurement of optical density: At 412 nm; glutathione (1 mg 100 ml^{-1}) is taken as the reference standard

Calculations

Glutathione content (µg g^{-1} FW) = $\frac{A\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$

5.11.3.2 Tocopherol (vitamin E;(μg g^{-1} FW)


Tocopherol (Vitamin E)

Protocol: Martinek (1964)

Addition and shaking: 0.5 ml each of absolute ethanol and DDW were added to 0.5 ml of plant extract; shook to divide protein precipitates

Addition and centrifugation: After adding 0.5 ml xylene to it, centrifugation was done for 10 minutes at 3,000 rpm

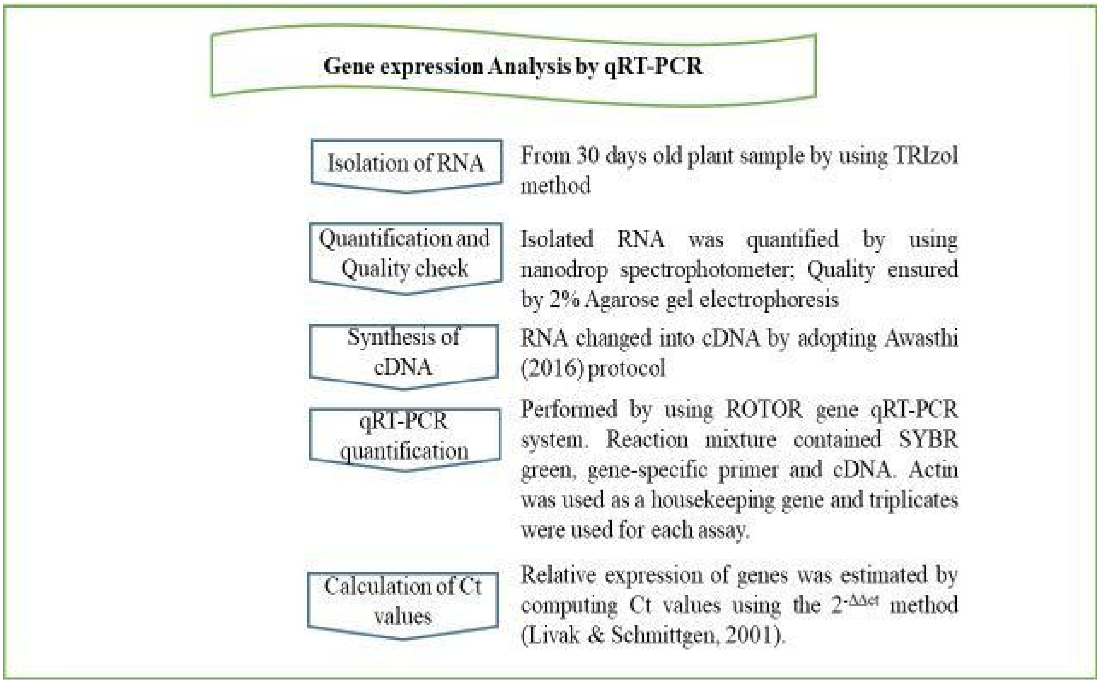
Mixing: 0.5 ml xylene (top) with 0.5 ml of 2,4,6-tripyridyl-S-triazine (TPTZ) reagent

Measurement of optical density: At 600 nm; tocopherol (1 mg 100 ml^{-1}) is taken as the reference standard

Calculations

$$\text{Tocopherol } (\mu g\ g^{-1}\ FW) = \frac{A\ of\ sample \times conc\ of\ std \times total\ volume}{Abs\ of\ std \times volume\ of\ sample\ taken}$$

5.13 Gene expression analysis


Gene expression Analysis by qRT-PCR

Isolation of RNA: From 30 days old plant sample by using TRIzol method

Quantification and Quality check: Isolated RNA was quantified by using nanodrop spectrophotometer; Quality ensured by 2% Agarose gel electrophoresis

Synthesis of cDNA: RNA changed into cDNA by adopting Awasthi (2016) protocol

qRT-PCR quantification: Performed by using ROTOR gene qRT-PCR system. Reaction mixture contained SYBR green, gene-specific primer and cDNA. Actin was used as a housekeeping gene and triplicates were used for each assay.

Calculation of Ct values: Relative expression of genes was estimated by computing Ct values using the $2^{-\Delta\Delta ct}$ method (Livak & Schmittgen, 2001).

Statistical analysis

One-way ANOVA i.e. analysis of variance was executed with the help of Tukey's test to evaluate the statistical difference of $P < 0.05$ significance level amongst the treatments by making use of SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Each experiment was performed in triplicates and data is exhibited in the form of means (± SEM) in the respective figures.

Chapter 6
Results
And
Discussion

List of Legends

Abbreviations	**Treatment**	**Legend**
CN	Control	⬜
T_1	Pb I	▨
T_2	Pb II	░
T_3	Pb III	▩
T_4	24-EBL	🟦
T_5	24-EBL + Pb I	▨
T_6	24-EBL+ Pb II	░
T_7	24-EBL+ Pb III	▩
T_8	Si	🟧
T_9	Si + Pb I	▨
T_{10}	Si+ Pb II	░
T_{11}	Si + Pb III	▩
T_{12}	24-EBL+Si	🟩
T_{13}	24-EBL+Si+Pb I	▨
T_{14}	24-EBL+Si+Pb II	░
T_{15}	24-EBL+Si+Pb III	▩

The perusal of lead (Pb) stress and its mitigation in *Trigonella* plants (pertaining to morphology, physio-chemistry and molecular nature) was recorded in the form of following significant observations:

6.1.1 Seedlings raised in *In vitro*

6.1.1.1 (a) Growth attributes

A notable decline in root length consequent to Pb stress treatments was recorded and summarized in fig. 6.1 and table 6.1 (a). The highest curtailment of 82.6% was marked at T_3 and it was around 61% in T_1 seedlings vis-a-vis CN plants. The results further divulged that 24-EBL and Si remarkably alleviated the Pb noxiousness by amplifying the root length. With regard to EBL pre-treated seedlings under Pb stress, maximum root length i.e. 2.93 cm was noted at T_5 concentration. Supplementation of Si also brought forth an improvement in the root length of Pb stressed seedlings with being its highest at T_9 treatment (2.2 cm). Root length results were comparatively better in case of EBL pre-treatment than Si augmentation while battling out the Pb stress from *Trigonella* seedlings. The cumulative treatment of 24-EBL + Si also amplified the root length in Pb exposed seedlings, exhibiting highest root length of 3.2 cm at T_{13}. An enhancement of 255.5% in root length was noticed at T_{13} in correlation to T_1 treatment seedlings. Seedlings at T_{14} treatment were recorded to have a 25.45% elevation than the seedlings of T_9 treatment. Additionally, a contrast made between T_{14} and T_{10} seedlings, 53.3% enhancement was shown by T_{14} seedlings. T_{14} treated seedlings had 20% increase in root length compared to control seedlings.

Shoot length exhibited Pb stress induced reduction, being at its lowest with a value of 1.37cm i.e. at T_3, 74.6% lower than CN seedlings. EBL and Si control seedlings displayed escalations of shoot length (Fig. 6.1 a; Table 6.1a). Under Pb toxicity, EBL treatment raised the shoot length to 5.83 cm at T_5 treatment as correlated to individual treatment of Pb I stressed seedlings, which stand at just 2.5 cm. On the other hand, Si treated stressed seedlings followed the same trend of enhancement in their shoot length. It made the shoot length to increase with its maximum value at T_9 (5cm). Collectively applied EBL and Si in Pb stress turned out to be more beneficial reaching its highest value of 6.53 cm at T_{14}. Shoot length recorded a reduction concomitant with every elevation in toxic concentrations of Pb in response to cumulative action of EBL + Si. T_9 seedlings exhibited 54% elevation of shoot length in correlation with T_2. Similarly, 203.65% augmentation was noted in T_7 than T_3. Furthermore, T_{14} had 22% more long shoots than CN seedlings.

A conspicuous negative impact on fresh weight of Pb stress exposed fenugreek seedlings was noted which was declined to its maximum at T_3 treatment, nearly 35% lesser than that of untreated seedlings kept as control (Fig. 6.1a; Table 6.1a). Further, pre-treatment of stressed seedlings with EBL i.e. T_5 treatment resulted in enhancing the fresh weight value to 1.35 g from being 1.06 at T_1 with a percentage increase of 33.96%. A similar improvement in fresh weight of stressed fenugreek seedlings was achieved with Si treatment, exhibiting a highest to lowest range of 1.35 ($T_{9)}$ to 1.06g (T_{11}) in treated seedlings. The highest augmentation in fresh weight took place at T_{13} (EBL + Si treatment in Pb I stressed seedlings) having a fresh weight value of 1.71g. Comparison of T_{13} with Pb III alone treated seedlings at T_3 exhibited a maximal enhancement of 106%. Similar correlation of T_{13} with CN, T_5 and T_9 treatments, exhibited a respective enhancement of 34.64%, 20.42% and 26.67%.

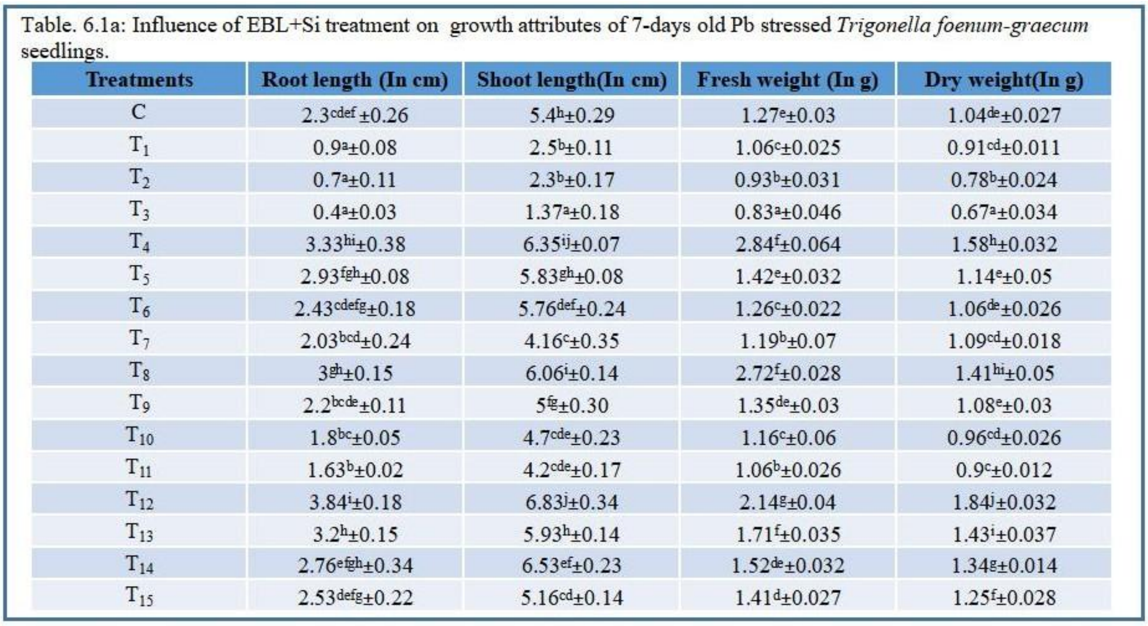


Table. 6.1a: Influence of EBL+Si treatment on growth attributes of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings.

Treatments	Root length (In cm)	Shoot length(In cm)	Fresh weight (In g)	Dry weight(In g)
C	$2.3^{cdef}\pm0.26$	$5.4^{h}\pm0.29$	$1.27^{e}\pm0.03$	$1.04^{de}\pm0.027$
T_1	$0.9^{a}\pm0.08$	$2.5^{b}\pm0.11$	$1.06^{c}\pm0.025$	$0.91^{cd}\pm0.011$
T_2	$0.7^{a}\pm0.11$	$2.3^{b}\pm0.17$	$0.93^{b}\pm0.031$	$0.78^{b}\pm0.024$
T_3	$0.4^{a}\pm0.03$	$1.37^{a}\pm0.18$	$0.83^{a}\pm0.046$	$0.67^{a}\pm0.034$
T_4	$3.33^{hi}\pm0.38$	$6.35^{ij}\pm0.07$	$2.84^{f}\pm0.064$	$1.58^{h}\pm0.032$
T_5	$2.93^{fgh}\pm0.08$	$5.83^{gh}\pm0.08$	$1.42^{e}\pm0.032$	$1.14^{e}\pm0.05$
T_6	$2.43^{cdefg}\pm0.18$	$5.76^{def}\pm0.24$	$1.26^{c}\pm0.022$	$1.06^{de}\pm0.026$
T_7	$2.03^{bcd}\pm0.24$	$4.16^{c}\pm0.35$	$1.19^{b}\pm0.07$	$1.09^{cd}\pm0.018$
T_8	$3^{gh}\pm0.15$	$6.06^{i}\pm0.14$	$2.72^{f}\pm0.028$	$1.41^{hi}\pm0.05$
T_9	$2.2^{bcde}\pm0.11$	$5^{fg}\pm0.30$	$1.35^{de}\pm0.03$	$1.08^{e}\pm0.03$
T_{10}	$1.8^{bc}\pm0.05$	$4.7^{cde}\pm0.23$	$1.16^{c}\pm0.06$	$0.96^{cd}\pm0.026$
T_{11}	$1.63^{b}\pm0.02$	$4.2^{cde}\pm0.17$	$1.06^{b}\pm0.026$	$0.9^{c}\pm0.012$
T_{12}	$3.84^{i}\pm0.18$	$6.83^{j}\pm0.34$	$2.14^{g}\pm0.04$	$1.84^{j}\pm0.032$
T_{13}	$3.2^{h}\pm0.15$	$5.93^{h}\pm0.14$	$1.71^{f}\pm0.035$	$1.43^{i}\pm0.037$
T_{14}	$2.76^{efgh}\pm0.34$	$6.53^{ef}\pm0.23$	$1.52^{de}\pm0.032$	$1.34^{g}\pm0.014$
T_{15}	$2.53^{defg}\pm0.22$	$5.16^{cd}\pm0.14$	$1.41^{d}\pm0.027$	$1.25^{f}\pm0.028$

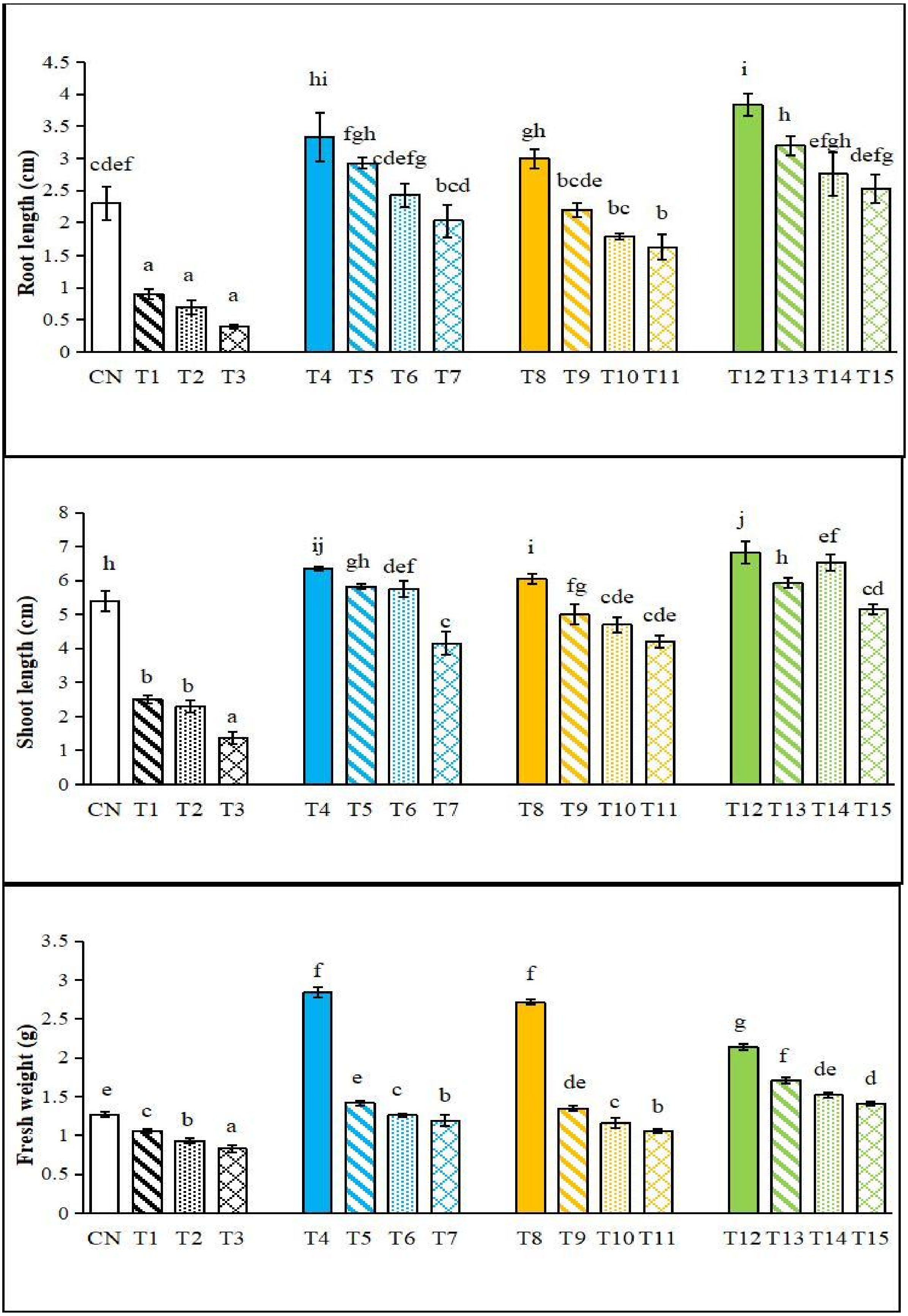


Fig. 6.1 (a): Influence of EBL+Si treatment on growth attributes in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

With regard to dry weight, a reduction of 12.5%, 25% and 35.57% was recorded respectively at T_1, T_2 and T_3 treatments of Pb alone stress vis-à-vis unstressed control seedlings. (Fig. 6.1 b ; Table 6.1 a).Treating Pb stress exposed seedlings individually with EBL and Si led to an improvement in dry weight with a highest percentage increase of 70.14% and 61.19% at T_5 and T_9 respectively in correlation to T_3 seedlings.

Cumulatively applied mitigants i.e. EBL and Si yielded much better outcomes in comparison to their individual applications with being at its highest around 1.43g of T_{13} treatment. A correlation drawn between T_7 and T_3 plus T_9 and T_3 revealed a respective augmentation of 62.68% and 61.19% in dry weights. T_{13} resulted in improving the dry weight by 37.5% in comparison to CN seedlings.

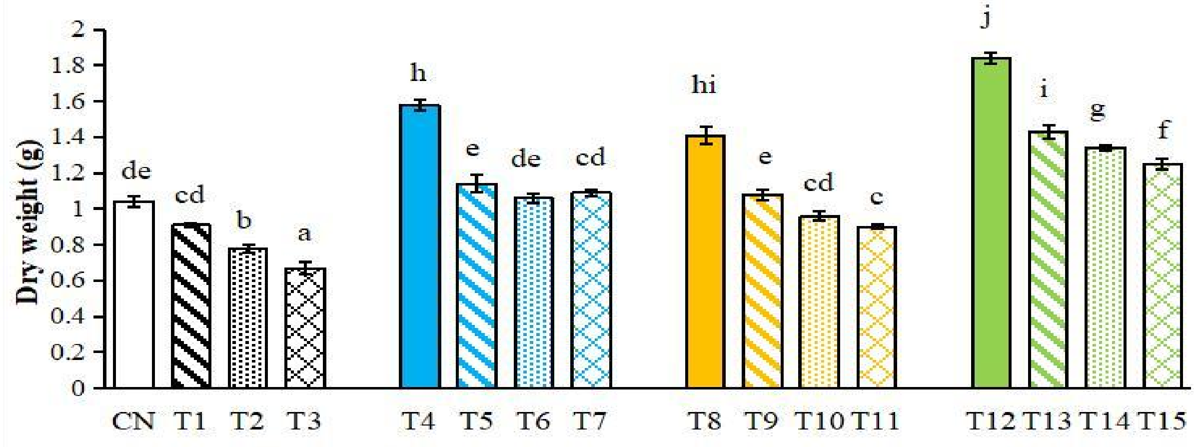


Fig. 6.1 (b): Influence of EBL+Si treatment on growth attributes (dry weight) in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Germination percentage of fenugreek seeds experienced a negative impact on account of Pb toxicity (Fig. 6.1c; Table 6.1b). Every elevation in toxic concentration of Pb lowered the germination percentage down i.e. 82.66% at T_1 got reduced to 72.88% of T_3 with being 80.3% around T_2. Control seeds exhibited 82.77% of germination percentage. Amongst all three discrete concentrations of Pb toxicity, pre-treatment of stressed seeds with EBL displayed the highest germination extent of 84.55% in T_6. Si augmentation to unstressed seedlings was observed to have higher value of germination percentage i.e. 92.11% in contrast to untreated CN plants. Furthermore, the Si treatment brought about an increase of 16.01% in germination in case of T_9 when contrasted against T_3, the treatment with highest concentration of Pb. Maximum percentage of germination was obtained when stressed seedlings were treated cumulatively with EBL plus Si at T_{14}, which was 22.04%, 5.93% and 5.19% greater than T_3, T_5 and T_9 respectively.

Curtailment of vigor index (VI) was also an outcome of stress induced by Pb metal in fenugreek as shown by T_1 seedlings (647%) with being reduced nearly half to that of untreated seedlings of control (1242%). Further, seedlings of T_2 treatment were noted to have VI value of 524% i.e. 19% lesser in contrast with T_1 treated seedlings (Fig. 6.1c; Table 6.1b). Individually supplemented EBL and Si under Pb metal stress elevated the VI values. Maximum value of VI (1429%) was observed around Pb I concentration in T_5 seedlings pre-treated with EBL. Exogenous augmentation of Si also escalated the VI values with being highest at T_9 treatment i.e. 1327% which revealed that 24-EBL was way more effective in enhancing the VI in response to Pb stress. However, when coupled, EBL and Si showed maximum elevation in the VI values amongst all treatments i.e. 1680% at T_{13} against 647% of Pb I alone augmented T_1 seedlings making the elevation as high as 159.66%. T_{13} seedlings correlated with T_5 and T_9 were recorded to exhibit respective increments of 17.56% and 26.60 % whereas in their comparison to untreated control seedlings, this enhancement stood at 35.26%.

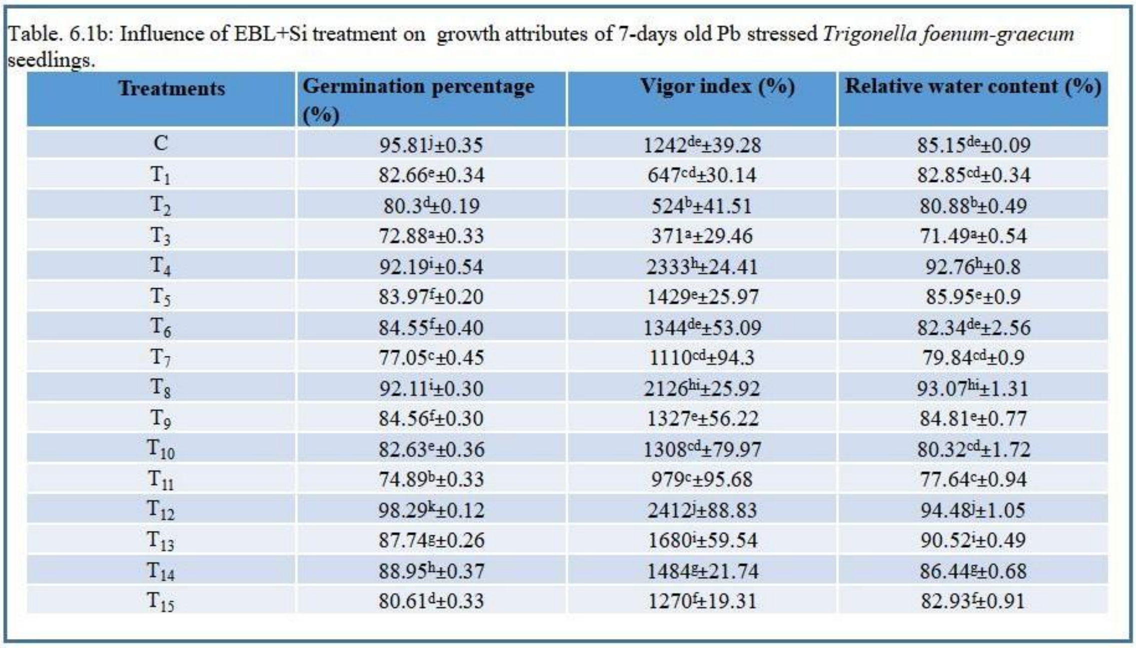


Table. 6.1b: Influence of EBL+Si treatment on growth attributes of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings.

Treatments	Germination percentage (%)	Vigor index (%)	Relative water content (%)
C	$95.81^{j}\pm0.35$	$1242^{de}\pm39.28$	$85.15^{de}\pm0.09$
T_1	$82.66^{e}\pm0.34$	$647^{cd}\pm30.14$	$82.85^{cd}\pm0.34$
T_2	$80.3^{d}\pm0.19$	$524^{b}\pm41.51$	$80.88^{b}\pm0.49$
T_3	$72.88^{a}\pm0.33$	$371^{a}\pm29.46$	$71.49^{a}\pm0.54$
T_4	$92.19^{i}\pm0.54$	$2333^{h}\pm24.41$	$92.76^{h}\pm0.8$
T_5	$83.97^{f}\pm0.20$	$1429^{e}\pm25.97$	$85.95^{e}\pm0.9$
T_6	$84.55^{f}\pm0.40$	$1344^{de}\pm53.09$	$82.34^{de}\pm2.56$
T_7	$77.05^{c}\pm0.45$	$1110^{cd}\pm94.3$	$79.84^{cd}\pm0.9$
T_8	$92.11^{i}\pm0.30$	$2126^{hi}\pm25.92$	$93.07^{hi}\pm1.31$
T_9	$84.56^{f}\pm0.30$	$1327^{e}\pm56.22$	$84.81^{e}\pm0.77$
T_{10}	$82.63^{e}\pm0.36$	$1308^{cd}\pm79.97$	$80.32^{cd}\pm1.72$
T_{11}	$74.89^{b}\pm0.33$	$979^{c}\pm95.68$	$77.64^{c}\pm0.94$
T_{12}	$98.29^{k}\pm0.12$	$2412^{j}\pm88.83$	$94.48^{j}\pm1.05$
T_{13}	$87.74^{g}\pm0.26$	$1680^{i}\pm59.54$	$90.52^{i}\pm0.49$
T_{14}	$88.95^{h}\pm0.37$	$1484^{g}\pm21.74$	$86.44^{g}\pm0.68$
T_{15}	$80.61^{d}\pm0.33$	$1270^{f}\pm19.31$	$82.93^{f}\pm0.91$

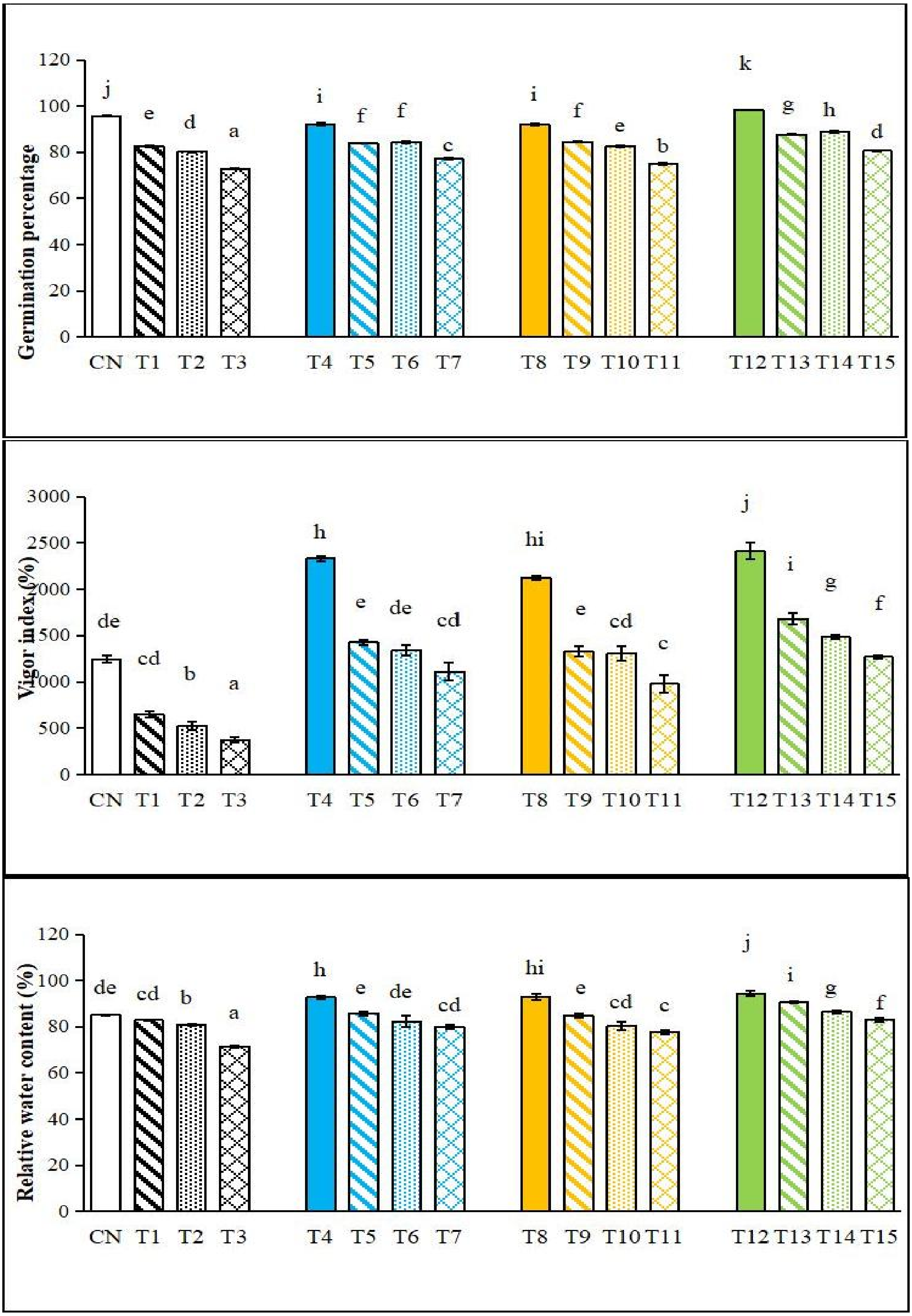
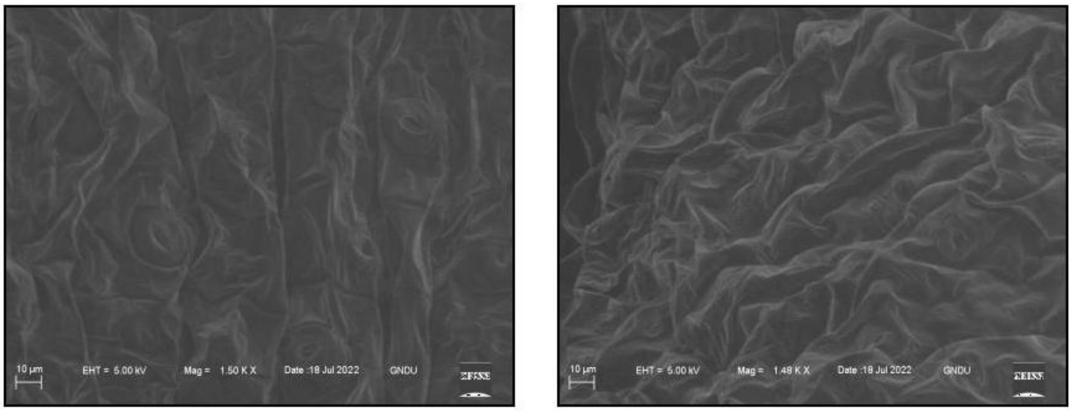


Fig. 6.1 (c): Influence of EBL+Si treatment on germination percentage, vigor index and RWC in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

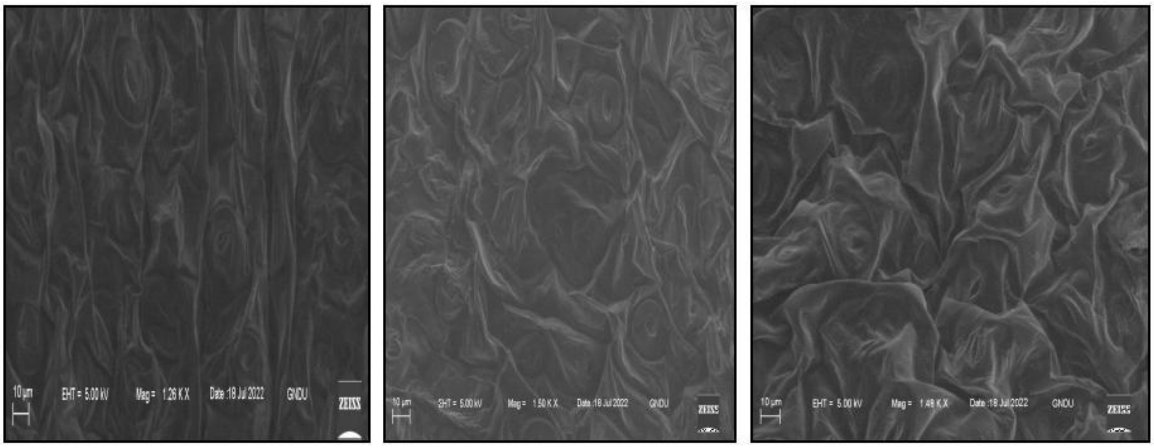
Ascertainment of RWC-“Relative water content” in Pb metal stressed fenugreek seedlings has been summed up in Fig. 6.1 (c) and Table 6.1 (b). A diminishing effect of Pb induced stress with increasing levels of Pb concentrations was evident from being 82.85% at T_1 treatment to 71.49% at T_3. Control seedlings were noted to have RWC value of 85.15%. Amongst the three discrete Pb applications in seedlings having EBL pre-treatment, highest value of RWC was observed at T_5 i.e. 85.95% (T_5). Si treatment without metal stress exhibited RWC value (93.06%) even higher than the CN seedlings Augmentation of exogenous Si under Pb toxicity had the least RWC value around T_{11} of 77.64%. EBL + Si treatments were noticed to have maximum RWC value at T_{13} i.e. 90.52%. T_{13} displayed a respective enhancement of 5.31%, 6.73% and 6.30% when correlated with T_5, T_9 and control seedlings.

6.1.1.1 (b) Stomatal physiology (opening and closing) by Scanning Electron Microscope

Ventral foliar surfaces in seedlings (7-days old) were examined under scanning electron microscope (Images 6.1 a-e). In the foliar part treated with the highest concentration of Pb i.e. Pb III (0.9 mM), stomatal closure along with shrinkage of guard cells was noted which was counteracted by the individual plus co-supplementation of 24-EBL and Si.



a) CN **b) Pb III (0.9 mM)**



c) 24-EBL (10^{-7}M) **d) Si (2mM)** **e) EBL + Si**

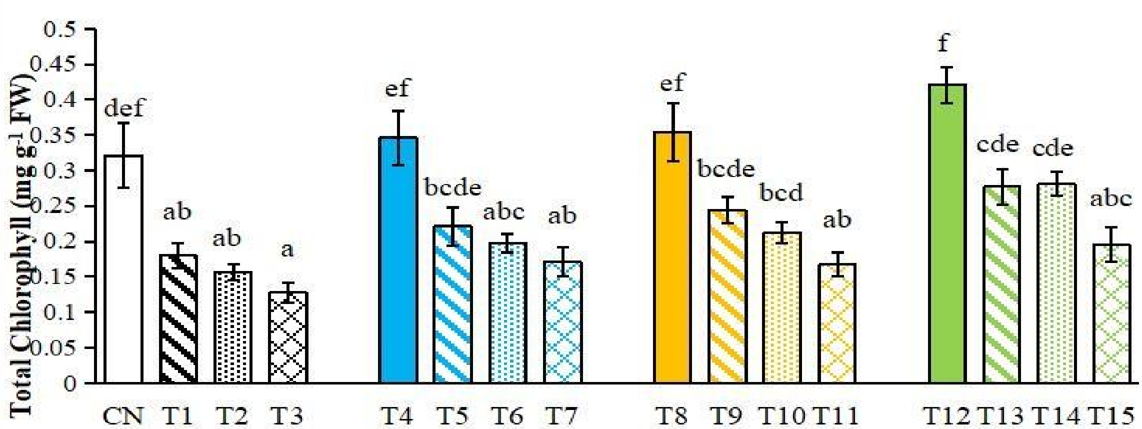
6.1.1.2 Photosynthetic pigments

Photosynthetic pigments - Chlorophyll a and Chlorophyll b along with total content of chlorophyll suffered negatively in their measures in Pb stress subjected seedlings (Fig. 6.2a And table 6.2a). Least amount of total chlorophyll i.e. 0.128 mg g^{-1} FW was noticed in T_3 seedlings which were augmented with maximum concentration of Pb. Treatment of stressed seedlings with EBL and Si alone concentrations, upregulated the amount of total chlorophyll in correlation to Pb alone treated T_3 seedlings, with being 0.221 mg g^{-1} FW at T_5 and 0.244 mg g^{-1} FW at T_9 which were respectively 72.65% and 90.62% in terms of percentage increase.

Synergistically treated seedlings with EBL and Si yielded better outcomes in improving the measures of total chlorophyll content in response to metal toxicity, when put to a contrast with individual treatments. Under stressed conditions, the highest level (0.277 mg g^{-1} FW) was noticed in T_{13} treated seedlings. An enhancement of 25.33% and 13.52% was shown by seedlings in T_{13} stage in correlation to T_5 and T_9 respectively. T_{13} results were 13.79% higher than the CN seedlings. Results were obtained in the similar fashion with respect to content of Chl a and b. The least amount of chl a (0.049 mg g^{-1} FW) and chl b (0.041 mg g^{-1} FW) were recorded at T_3 treatment. Furthermore, coupled supplementation with EBL and Si remarkably ameliorated the Pb induced diminishing impact by elevating the measures of these pigments and noted to be maximum around 0.211 and 0.166 mg g^{-1} FW for Chl a and b respectively at treatment T_{14}. An elevation of 30.24 and 25% in chl a and chl b respectively at T_5 together with 24.85% and 27.73% at T_9 was revealed when these treatments were contrasted against that of T_{14}.

Table. 6.2a:Influence of EBL+Si treatment on photosynthetic pigments of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	**Total Chl(mg g^{-1} FW)**	**Chl a(mg g^{-1} FW)**	**Chl b(mg g^{-1} FW)**
C	$0.321^{def}\pm0.046$	$0.174^{def}\pm0.032$	$0.096^{def}\pm0.013$
T_1	$0.18^{ab}\pm0.017$	$0.088^{ab}\pm0.011$	$0.077^{ab}\pm0.016$
T_2	$0.156^{ab}\pm0.011$	$0.074^{ab}\pm0.004$	$0.067^{ab}\pm0.006$
T_3	$0.128^{a}\pm0.014$	$0.049^{a}\pm0.007$	$0.041^{a}\pm0.013$
T_4	$0.346^{ef}\pm0.038$	$0.207^{fg}\pm0.037$	$0.173^{ef}\pm0.027$
T_5	$0.221^{bcde}\pm0.027$	$0.162^{def}\pm0.015$	$0.14^{def}\pm0.033$
T_6	$0.197^{abc}\pm0.013$	$0.153^{cd}\pm0.018$	$0.132^{cde}\pm0.02$
T_7	$0.171^{ab}\pm0.021$	$0.124^{bc}\pm0.026$	$0.12^{bc}\pm0.022$
T_8	$0.354^{ef}\pm0.041$	$0.197^{def}\pm0.03$	$0.162^{def}\pm0.025$
T_9	$0.244^{bcde}\pm0.018$	$0.169^{def}\pm0.03$	$0.137^{de}\pm0.021$
T_{10}	$0.212^{bcd}\pm0.015$	$0.137^{cd}\pm0.009$	$0.114^{cd}\pm0.015$
T_{11}	$0.167^{ab}\pm0.017$	$0.105^{bc}\pm0.019$	$0.095^{b}\pm0.011$
T_{12}	$0.421^{f}\pm0.025$	$0.255^{h}\pm0.048$	$0.225^{g}\pm0.017$
T_{13}	$0.277^{cde}\pm0.025$	$0.198^{efg}\pm0.049$	$0.166^{def}\pm0.035$
T_{14}	$0.281^{cde}\pm0.017$	$0.211^{fg}\pm0.055$	$0.175^{fg}\pm0.018$
T_{15}	$0.195^{abc}\pm0.024$	$0.204^{efg}\pm0.04$	$0.132^{cde}\pm0.025$



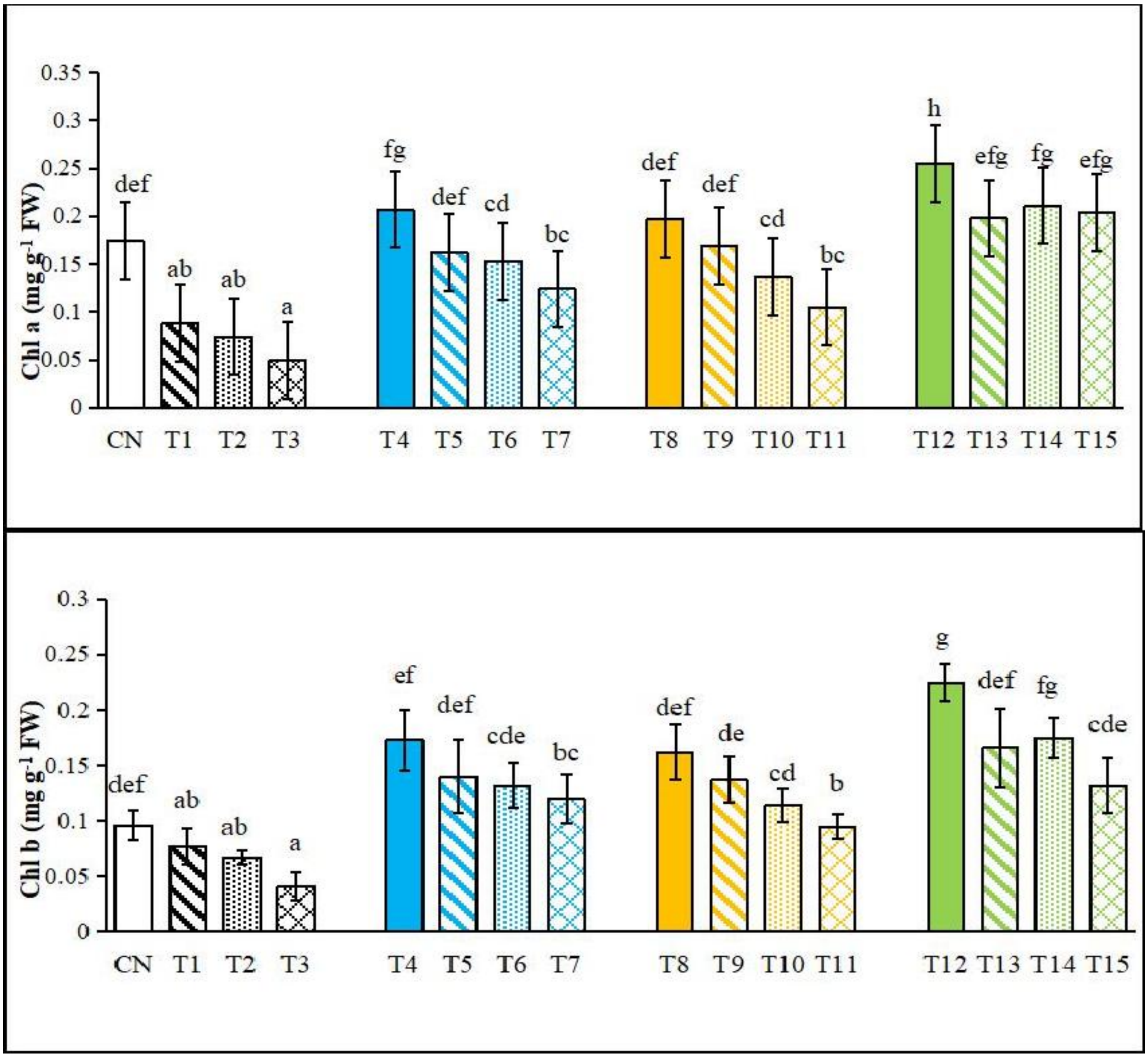


Fig. 6.2 (a): Influence of EBL+Si treatment on measures of photosynthetic pigments in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Pb stress was reported to diminish the measures of carotenoids (Fig.6.2b and Table 6.2b). Least carotenoid content was recorded at T_3 treatment with a value of 0.119 mg g^{-1} FW content. Pre-treatment of EBL against Pb exposure up-scaled the carotenoid amount with being its highest at 0.278 mg g^{-1} FW in case of T_5 concentration. Carotenoid measures got improved from 0.175 mg g^{-1} FW to 0.251 mg g^{-1} FW in Si treated T_9 seedlings, in correlation to Pb I exposed seedlings. Synergistically augmented EBL with Si further augmented the levels of carotenoids so that Pb induced toxic effects could be negated. The maximal level of 0.313 mg g^{-1} FW level was noted at T_{13} treatment while the minimal of 0.242 mg g^{-1} FW was reported at T_{15} treatment. Its amount recorded an upsurge of 19.01%, 12.6% and 24.7% at control, T_5 and T_9 treatments in correlation to T_{13} seedlings.

Lead toxicity lowered down the xanthophyll measures in 7 days old *In vitro* raised fenugreek seedlings (Fig. 6.2b; Table 6.2b). The maximal decline in the amount of xanthophyll content i.e., 1.19 mg g^{-1} FW was reported in case of T_3 metal stressed seedlings. A curtailment of 23.2% in xanthophyll levels was noted in T_2 augmented seedlings as opposed to T_1. In response to separate applications of EBL and Si, highest values of xanthophyll were reported at T_5, T_9 with values of 2.89 and 2.61 and the lowest being observed at T_7, T_{11} with values of 1.91 and 1.55 mg g^{-1} FW, respectively. Upsurge in the amount of xanthophyll was yielded when both EBL and Si were augmented synergistically in order to mitigate Pb stress. Xanthophyll measures were boosted from 1.55 to 3.41 mg g^{-1} FW at T_{13} concentration as opposed to T_2 treatment. Augmentation of xanthophyll measures by 28.67% occurred in T_{13} than T_9. An elevation of 32.25% was noted in T_{15} in comparison to T_{11}. T_{13} displayed 7.08% upsurge in measures of xanthophyll than control seedlings.

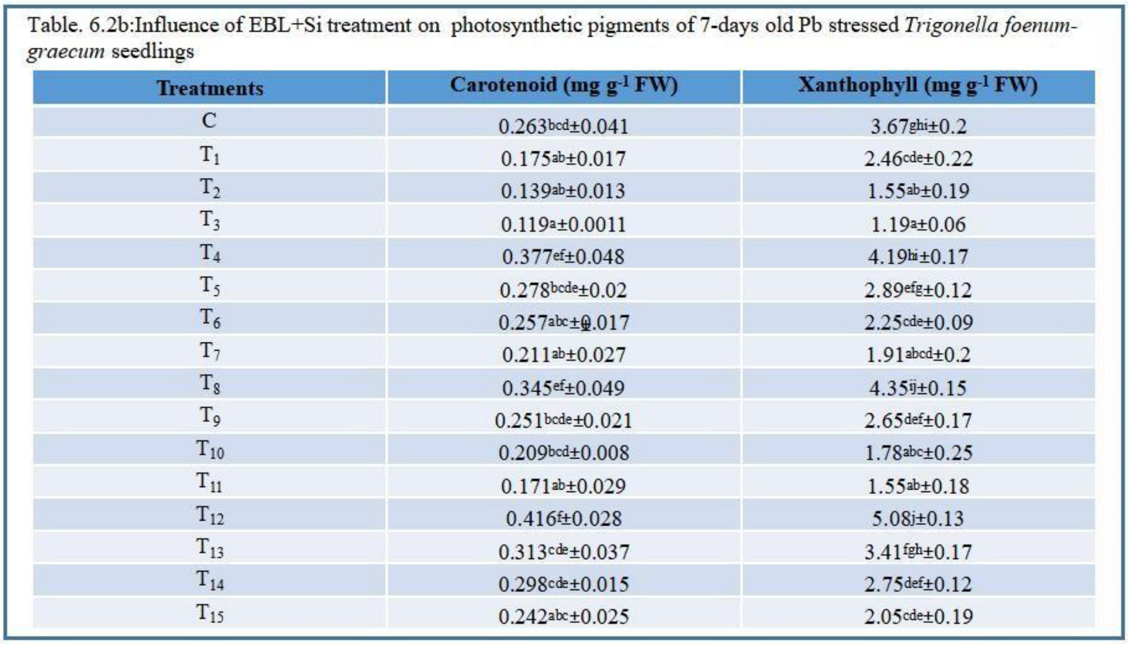


Table. 6.2b:Influence of EBL+Si treatment on photosynthetic pigments of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
C	$0.263^{bcd}\pm0.041$	$3.67^{ghi}\pm0.2$
T_1	$0.175^{ab}\pm0.017$	$2.46^{cde}\pm0.22$
T_2	$0.139^{ab}\pm0.013$	$1.55^{ab}\pm0.19$
T_3	$0.119^{a}\pm0.0011$	$1.19^{a}\pm0.06$
T_4	$0.377^{ef}\pm0.048$	$4.19^{hi}\pm0.17$
T_5	$0.278^{bcde}\pm0.02$	$2.89^{efg}\pm0.12$
T_6	$0.257^{abc}\pm0.017$	$2.25^{cde}\pm0.09$
T_7	$0.211^{ab}\pm0.027$	$1.91^{abcd}\pm0.2$
T_8	$0.345^{ef}\pm0.049$	$4.35^{ij}\pm0.15$
T_9	$0.251^{bcde}\pm0.021$	$2.65^{def}\pm0.17$
T_{10}	$0.209^{bcd}\pm0.008$	$1.78^{abc}\pm0.25$
T_{11}	$0.171^{ab}\pm0.029$	$1.55^{ab}\pm0.18$
T_{12}	$0.416^{f}\pm0.028$	$5.08^{j}\pm0.13$
T_{13}	$0.313^{cde}\pm0.037$	$3.41^{fgh}\pm0.17$
T_{14}	$0.298^{cde}\pm0.015$	$2.75^{def}\pm0.12$
T_{15}	$0.242^{abc}\pm0.025$	$2.05^{cde}\pm0.19$

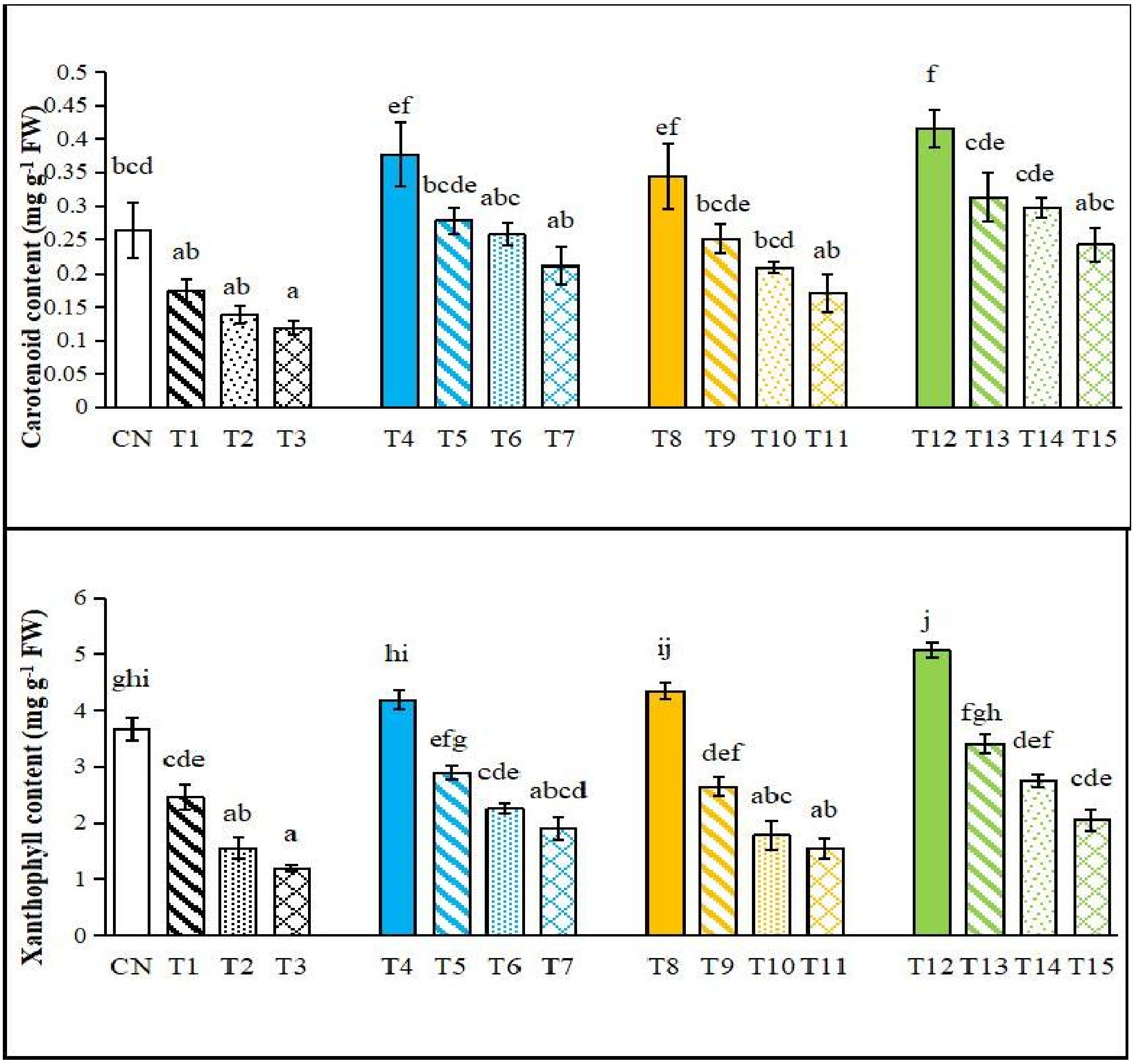


Fig. 6.2 (b): Influence of EBL+Si treatment on measures of photosynthetic pigments in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.1.3 Metabolites

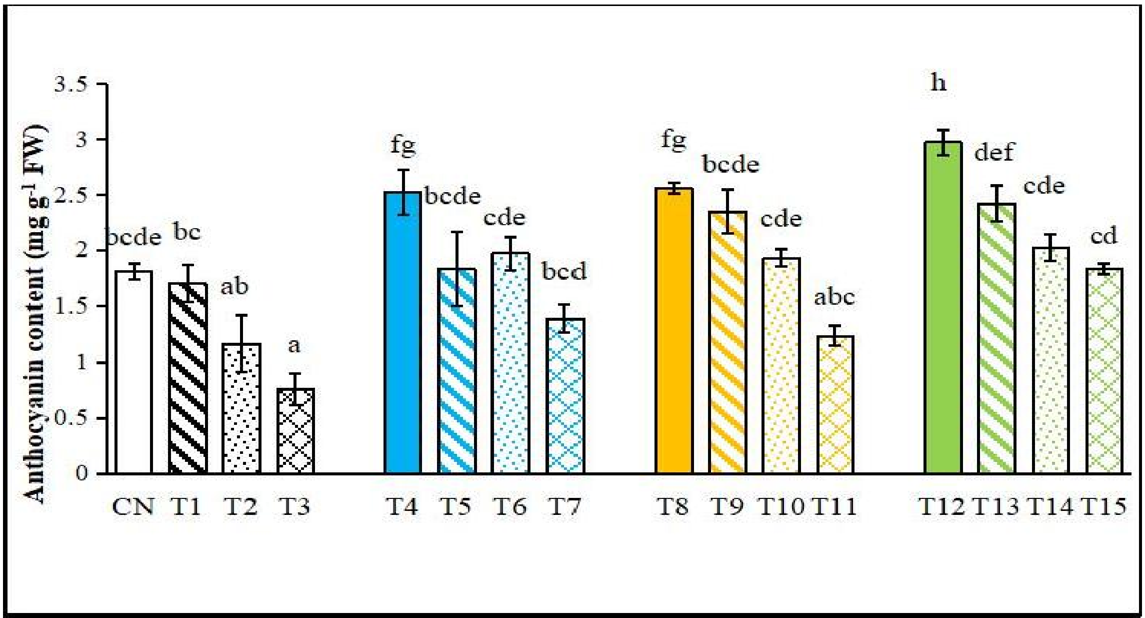
Anthocyanin was noted to have a declined content under Pb stress with being minimum (0.76 mg g^{-1} FW) at T_3 treatment which was 58.01% lower than the control (Fig. 6.3; Table 6.3). With respect to individually augmented mitigants i.e. EBL and Si in unstressed seedlings, T_8 seedlings (2.56 mg g^{-1} FW) were slightly better performers than T_4 seedlings (2.52 mg g^{-1} FW). Elevations in the anthocyanin levels were recorded at T_6 i.e. 1.96 mg g^{-1} FW. Anthocyanin content was enhanced from 1.7 to 1.83 mg g-1 FW as opposed to T_1 concentration. Feeding of Si against Pb toxicity led to the betterment of anthocyanin measures vis-à-vis seedlings stressed with Pb only. Anthocyanin content was noticed to be raised from 1.7 to 2.35 mg g^{-1} FW at T_9 concentration. Under Pb stress, Si augmentation was observed to be more efficient in elevating the anthocyanin measures in contrast to EBL treatment. Coupling of EBL with Si in combating Pb toxicity further elevated the measures of anthocyanin with a value of 2.42, 2.02 and 1.83 mg g^{-1} FW at T_{13}, T_{14} along with T_{15} augmented seedlings, respectively. T_7 showed a decrease of 10.79% when contrasted to T_{11} whereas T_{13} led to an enhancement of 33.70% than stress free seedlings under controlled conditions.

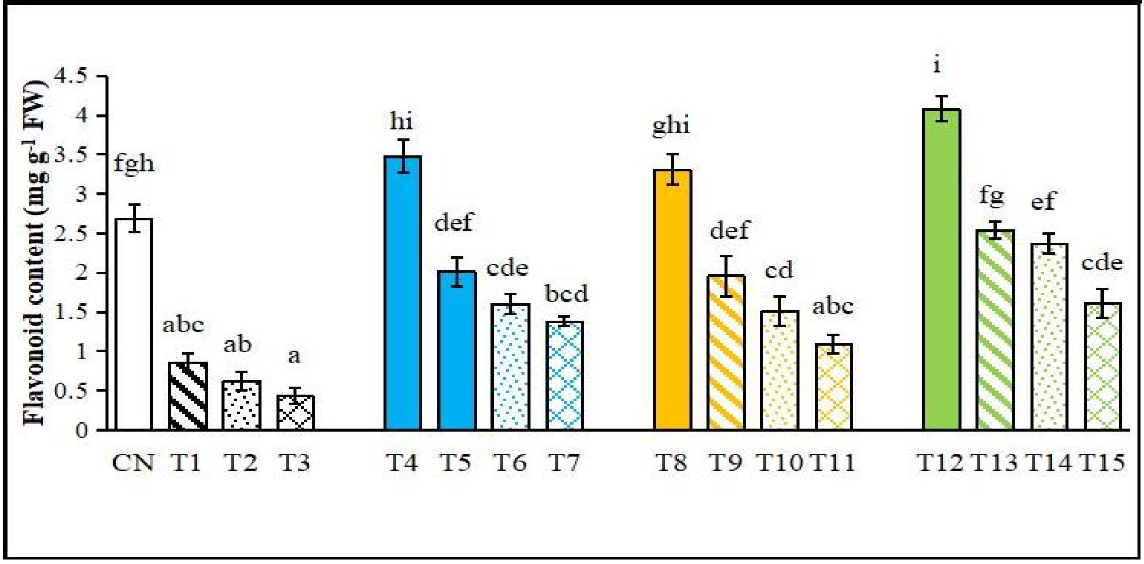
The least measures of flavonoid were recorded in T_3 seedlings augmented with maximum concentration of Pb only (0.43 mg g^{-1} FW; Fig. 6.3; Table 6.3). In all three treatments of Pb alone stress, a 68.02% at T_1, 76.95% at T_2 and 84.01% decrease at T_3 was observed when a correlation was made with control seedlings. Flavanoid content registered an upsurge of 133.72% from T_5 (EBL + Pb I) to Pb I alone treated T_1 seedlings. Silicon treatment was further recorded to escalate the measures of flavonoids vis-à-vis Pb alone treated seedlings. Levels of flavanoid were observed to be 1.96 at T_9, 1.51 at T_{10} and 1.09 mg g^{-1} FW at $T_{11,}$ respectively. Flavanoid content pertaining to cumulative application of EBL + Si, the maximum ($T_{13;}$ 2.54 mg g^{-1} FW) and minimum ($T_{15;}$ 1.61 mg g^{-1} FW) values were noted. Its measures registered an augmentation of 26.6% in T_{11} over T_7 seedlings. T_{13} further displayed a 5.57% decrement in relation to control seedlings.

With ascending levels of Pb stress, phenolics were noted to have a diminishing impact on their content (Fig. 6.3; Table 6.3). It was diminished to 0.564 mg g^{-1} FW (T_3) in correlation with control seedlings having 2.23 mg g^{-1} FW of phenolic content. Individually augmented EBL in stress free and stressed seedlings were recorded to have the highest measures of phenolics at T_4 (2.95 mg g^{-1} FW) and T_5 (2.14 mg g^{-1} FW) respectively. Exogenously applied Si, on the other hand, had the highest content in case of T_8 (2.72 mg g^{-1} FW) and T_9 (1.67 mg g^{-1} FW) for the same type of conditions. Combination of EBL and Si in Pb I stressed seedlings i.e. T_{13} showed greatest measures of phenolics at 2.67 mg g^{-1} FW while under control, EBL + Si made the phenolic content of T_{12} seedlings highest (3.35 mg g^{-1} FW) amongst all the treatments chosen for the present study. An increment of 51.77% was recorded in T_5 over T_1. Additionally, T_{13} seedlings exhibited 24.76% and 19.73% increment in correlation to T_5 and control seedlings respectively.

Table. 6.3: Influence of EBL+Si on measures of metabolites of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	Anthocyanin (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)
C	$1.81^{bcde}\pm0.07$	$2.69^{fgh}\pm0.18$	$2.23^{defg}\pm0.13$
T_1	$1.7^{bc}\pm0.17$	$0.86^{abc}\pm0.12$	$1.41^{abcd}\pm0.1$
T_2	$1.17^{ab}\pm0.26$	$0.62^{ab}\pm0.12$	$1.03^{ab}\pm0.09$
T_3	$0.76^{a}\pm0.14$	$0.43^{a}\pm0.1$	$0.564^{a}\pm0.07$
T_4	$2.52^{fg}\pm0.2$	$3.48^{hi}\pm0.21$	$2.95^{gh}\pm0.1$
T_5	$1.83^{bcde}\pm0.33$	$2.01^{def}\pm0.19$	$2.14^{defg}\pm0.22$
T_6	$1.97^{cde}\pm0.15$	$1.6^{cde}\pm0.13$	$1.81^{bcde}\pm0.18$
T_7	$1.39^{bcd}\pm0.12$	$1.38^{bcd}\pm0.06$	$1.16^{abc}\pm0.29$
T_8	$2.56^{fg}\pm0.05$	$3.31^{ghi}\pm0.19$	$2.72^{fgh}\pm0.11$
T_9	$2.35^{bcde}\pm0.2$	$1.96^{def}\pm0.26$	$1.67^{bcd}\pm0.17$
T_{10}	$1.93^{cde}\pm0.08$	$1.51^{cd}\pm0.18$	$1.58^{bcd}\pm0.15$
T_{11}	$1.24^{abc}\pm0.09$	$1.09^{abc}\pm0.11$	$0.945^{ab}\pm0.08$
T_{12}	$2.97^{h}\pm0.11$	$4.08^{i}\pm0.16$	$3.35^{h}\pm0.15$
T_{13}	$2.42^{def}\pm0.16$	$2.54^{fg}\pm0.11$	$2.67^{efgh}\pm0.11$
T_{14}	$2.02^{cde}\pm0.12$	$2.37^{ef}\pm0.13$	$2.26^{defg}\pm0.32$
T_{15}	$1.83^{cd}\pm0.05$	$1.61^{cde}\pm0.19$	$1.99^{cdef}\pm0.21$





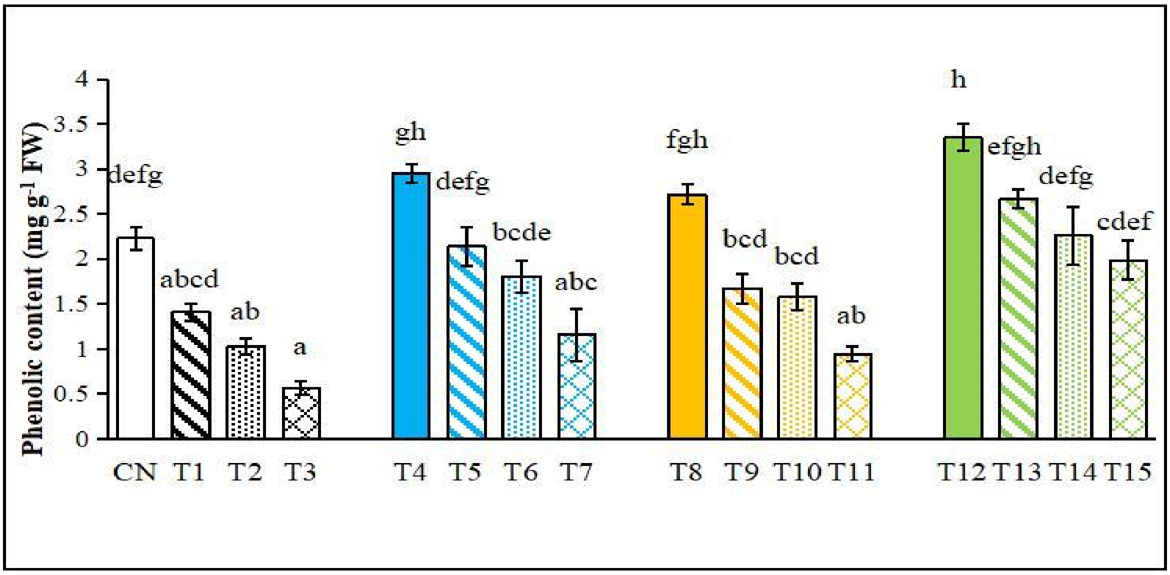


Fig. 6.3: Influence of EBL+Si treatment on measures of metabolites in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

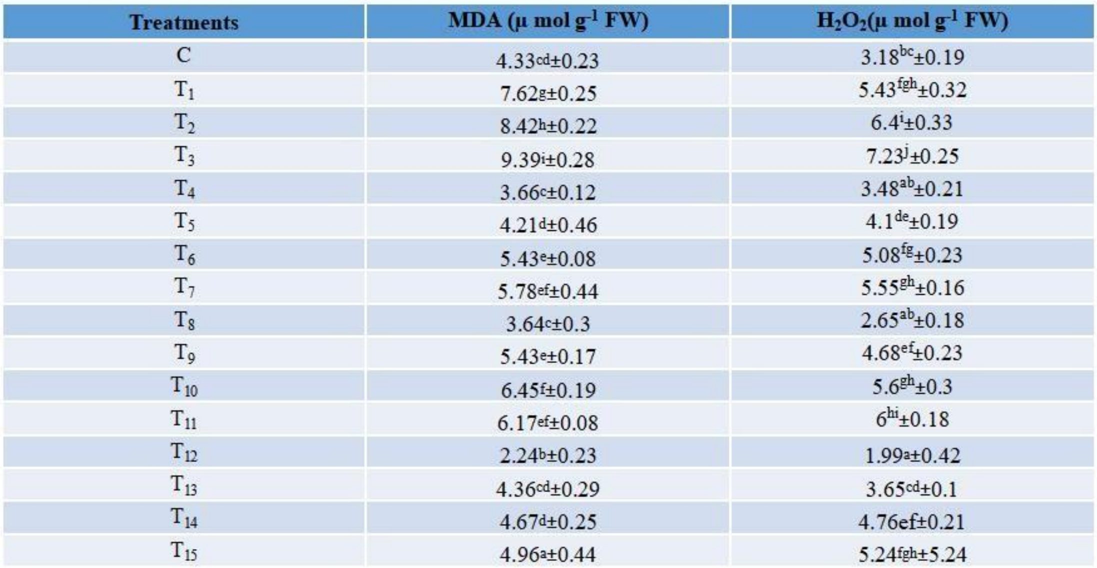
6.1.1.4 Oxidative damage

6.1.1.4.1 Oxidative stress markers

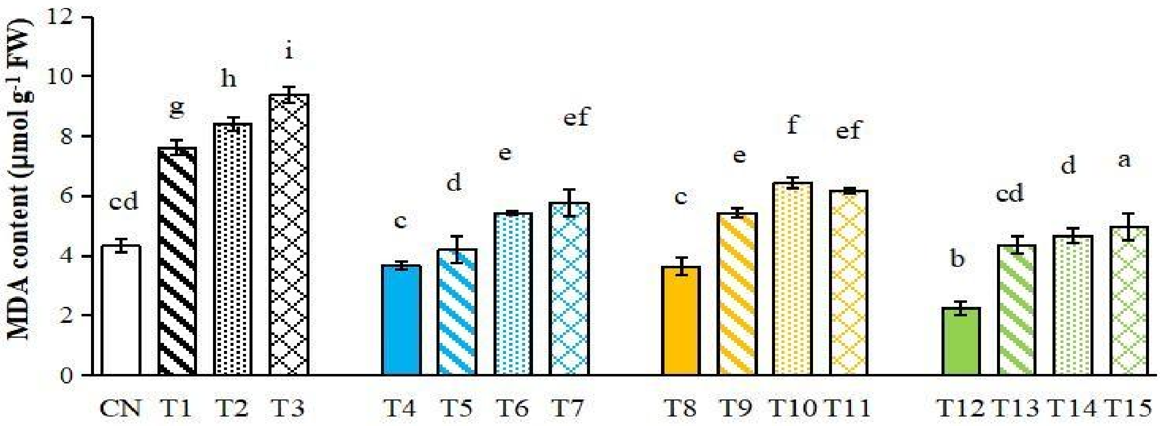
Extent of lipid peroxidation was noted to be enhanced because of Pb-mediated upsurge in the measures of MDA, when contrasted against control seedlings (Fig. 6.4; Table 6.4). An increment of 116.85% in the quantities of MDA was noted in T_3 seedlings, which were stressed with highest concentration of Pb, as opposed to the control seedlings. However, exogenously supplemented EBL and Si curtailed this Pb induced enhancement in MDA measures. With respect to individually applied EBL and Si to stressed seedlings, MDA contents were registered to be the most reduced at T_5 and T_9 with respective values of 4.21 and 5.43 µmol g^{-1} FW measures. Additionally, MDA values were diminished from 8.42 at T_2 to 4.67 µmol g^{-1} FW at T_{14} treated seedlings. A decrement of 44.75% was noted in T_5 over T_1. T_9 seedlings displayed 28.74% decline in MDA measures than T_1. T_{13} seedlings had 3.56% more measures of MDA in contrast to T_5. Besides this, T_{14} was recorded to have 7.85% higher MDA levels vis-a-vis control seedlings.

The measures of H_2O_2 were notably elevated in fenugreek seedlings upon getting subjected to Pb stress (Fig. 6.4; Table 6.4). An increase of 127.35% in the quantities of H_2O_2 got noticed in T_3 seedlings, which were stressed with highest concentration of Pb, as opposed to the control seedlings. Moreover, H_2O_2 measures were promoted to be 5.43 at T_1, 6.40 at T_2 and 7.23µmolg^{-1} FW at T_3 seedlings stressed with Pb metal alone. However, applying them with EBL, Si and EBL + Si against Pb toxicity diminished the amount of H_2O_2. The highest diminishing impact of mitigants was noticed in case of their coupled treatment at T_{13} with a value of 3.65 μmol g^{-1} FW. A decrement of 24% was noted in T_5 over T_1. T_9 seedlings registered a decline of 13.81% when correlated with T_1. Furthermore, T_{13} seedlings were recorded to have 10.9 % and 22% reduction when placed against T_5 and T_9, respectively.

Table. 6.4: Influence of EBL+Si on oxidative stress markers of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings



Treatments	MDA (μ mol g^{-1} FW)	H_2O_2(μ mol g^{-1} FW)
C	$4.33^{cd}\pm0.23$	$3.18^{bc}\pm0.19$
T_1	$7.62^{g}\pm0.25$	$5.43^{fgh}\pm0.32$
T_2	$8.42^{h}\pm0.22$	$6.4^{i}\pm0.33$
T_3	$9.39^{i}\pm0.28$	$7.23^{j}\pm0.25$
T_4	$3.66^{c}\pm0.12$	$3.48^{ab}\pm0.21$
T_5	$4.21^{d}\pm0.46$	$4.1^{de}\pm0.19$
T_6	$5.43^{e}\pm0.08$	$5.08^{fg}\pm0.23$
T_7	$5.78^{ef}\pm0.44$	$5.55^{gh}\pm0.16$
T_8	$3.64^{c}\pm0.3$	$2.65^{ab}\pm0.18$
T_9	$5.43^{e}\pm0.17$	$4.68^{ef}\pm0.23$
T_{10}	$6.45^{f}\pm0.19$	$5.6^{gh}\pm0.3$
T_{11}	$6.17^{ef}\pm0.08$	$6^{hi}\pm0.18$
T_{12}	$2.24^{b}\pm0.23$	$1.99^{a}\pm0.42$
T_{13}	$4.36^{cd}\pm0.29$	$3.65^{cd}\pm0.1$
T_{14}	$4.67^{d}\pm0.25$	4.76ef±0.21
T_{15}	$4.96^{a}\pm0.44$	$5.24^{fgh}\pm5.24$



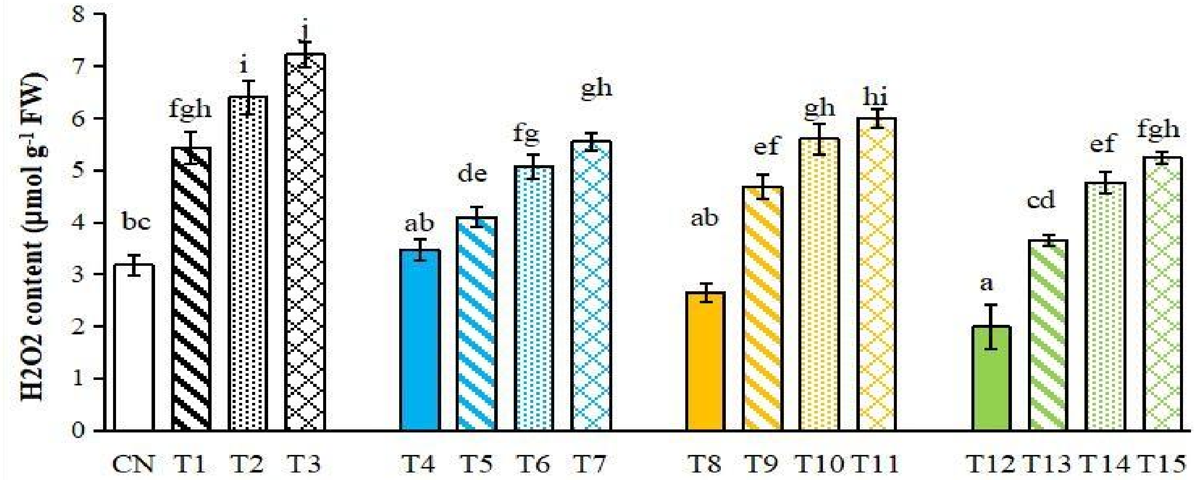
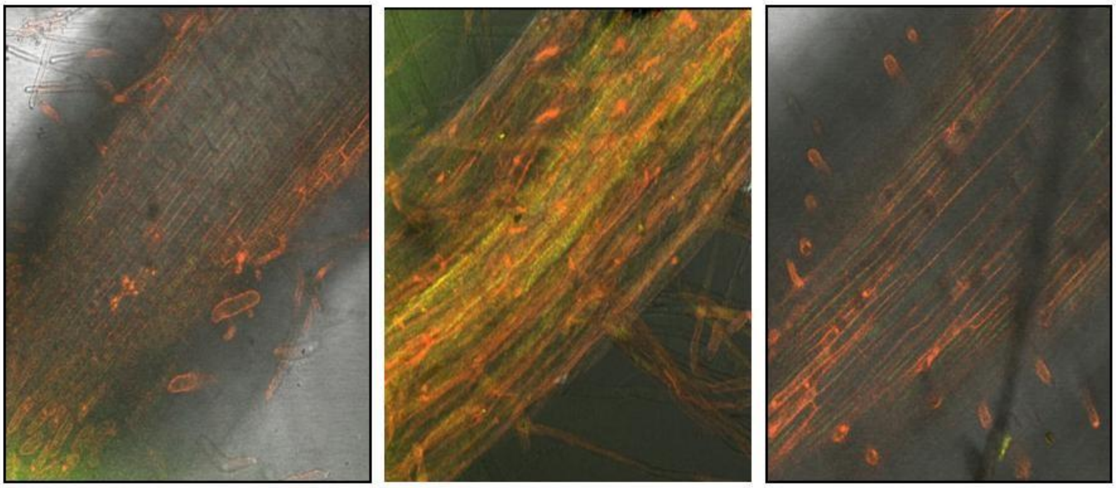


Fig. 6.4: Influence of EBL+Si treatment on measures of oxidative stress indicators in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

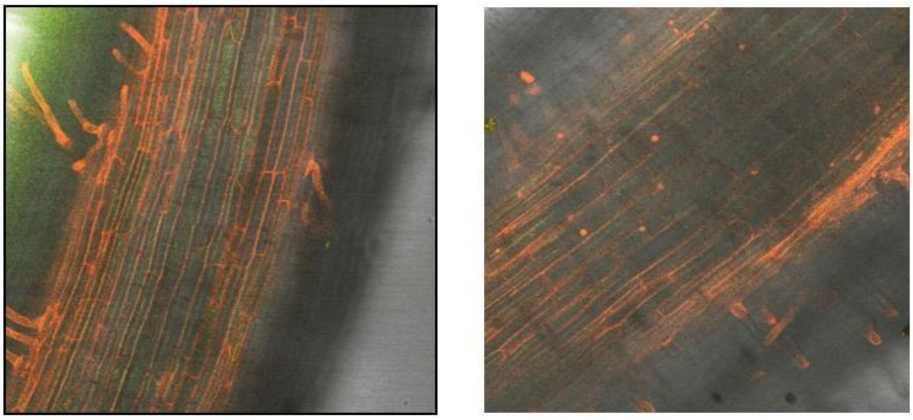
6.1.1.4.2 Histochemical studies (Confocal microscopy)

Oxidative damage in fenugreek root cells was discerned by using 2, 7-dichlorofluroscein dye under the confocal microscope (Image 6.2 a-e). Lead III had brought about the highest oxidative harm as depicted by greater intensity of green fluorescence, when contrasted against the control seedlings. However, membranal harm was mitigated lead stress exposed seedlings via supplementing them with EBL and Si, both individually plus cumulatively as noticeable by less brightness of green colour.

PI dye gives rise to a fluorescent composite by means of its interspersed distribution in nucleic acid. By crossing the membranal barrier in injured and dead cells, PI is able to stain the nuclear part. It was evident that Pb III augmented seedlings led to greater nuclear harm as shown by more strength of red colour. However, this nuclear harm was lessened in individual plus cumulative application of EBL and Si as noticeable by reduction in intensity and spread of PI induced red hue.



CN **Pb III (0.9 mM)** **24-EBL (10^{-7} M)**



Si (2mM) **EBL + Si**

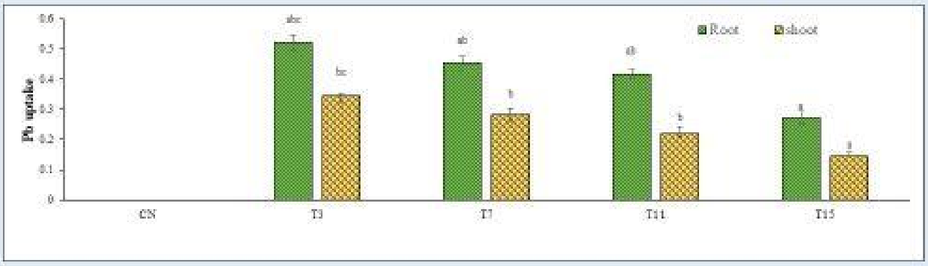
6.1.1.6 Lead metal uptake

No Pb content was detected in control samples i.e. *in vitro* grown 7-days old fenugreek seedlings (Fig 6.5; Table 6.5). Under individual stress of Pb, 0.520 and 0.344 mg g^{-1} DW Pb content was reported in the root plus shoot regions of fenugreek seedlings, respectively. EBL pre-treated seedlings of fenugreek diminished the Pb presence in root and shoot with 0.417 and 0.219 mg g^{-1} DW Pb content, respectively. Exogenous treatment of Si under metal stressed conditions lowered down the Pb content with 0.451 and 0.281 mg g^{-1} DW inside root and shoot, respectively. Co-supplementation of EBL and Si further reduced the Pb content in root plus shoot of fenugreek seedlings with 0.273 and 0.144 mg g^{-1} DW values of Pb.

Table 6.5

Treatments	Root (mg g^{-1} DW)	Shoot (mg g^{-1} DW)
C	ND	ND
T_3	$0.520^{abc}\pm0.0.26$	$0.344^{bc}\pm0.08$
T_7	$0.451^{ab}\pm0.023$	$0.281^{b}\pm0.023$
T_{11}	$0.417^{ab}\pm0.015$	$0.219^{b}\pm0.021$
T_{15}	$0.273^{a}\pm0.020$	$0.144^{a}\pm0.013$

Fig. 6.5



❑ **Influence of EBL + Si on Pb metal uptake by the 7-days old *Trigonella foenum-graecum* seedlings.**

6.1.1.7 Osmolytes

Significant inhibitions in the content of Proline took place on account of Pb toxicity in fenugreek seedlings (Fig. 6.6; Table 6.6). Every concentration of Pb i.e. T_1, T_2 plus T_3, registered a decline in the amount of proline with respective values of 0.85, 0.61, and 0.37 µ mol g^{-1} FW. Individually augmented EBL brought about a promotion of proline measures in stressed seedlings with being greatest at T_5 concentration (1.41 µ mol g^{-1} FW). Si too played a role in raising the levels of proline in response to metal stress. Proline levels were up-scaled from 0.37 to 1.41 µ mol g^{-1} FW in T_9 i.e. Si treated seedlings in contrast to T_3. Under Pb stress, maximum enhancement in proline measures (1.63 µ mol g^{-1} FW) was in T_{13} treatment. T13 seedlings exhibited 21.64% and 15.64% improvement over T_5 and T_9 treated seedlings.

Noteworthy reductions in the measures of glycine betaine were registered in Pb stress subjected seedlings (Fig. 6.6; Table 6.6). A decrement of 1.60 at T_1, 1.21 at T_2 and 0.70µ mol g^{-1} FW at T_3 in the content of glycine betaine was noted as opposed to control seedlings. Under the influence of EBL, the measures of glycine betaine were recorded to be 2.59 at T_5, 2.43 at T_6 and 1.42µ mol g^{-1} FW at T_7, respectively while Si treatment recorded glycine betaine content to be 2.34 at T_9, 2.58 at T_{10} and 1.55µ mol g^{-1} FW at T_{11}. Furthermore, combination of EBL with Si treatment in stress free conditions registered the highest quantities of glycine betaine (4.35 µ mol g^{-1} FW) amongst all the 16 different types of treatments chosen for the study. Glycine-betaine amount was further recorded to be curtailed as the intensity of Pb toxicity elevated as observed in coupled EBL + Si dosage with being highest at 3.46 µ mol g^{-1} FW in T_{13}. An escalation of 61.87% was registered in T_5 over T_1. T_9 seedlings displayed 46.25% increment than T_1. T_{13} seedlings exhibited nearly 34% improvement in glycine betaine as opposed to T_5. T_{13} showed 6.8% more measures of glycine betaine in contrast to control seedlings.

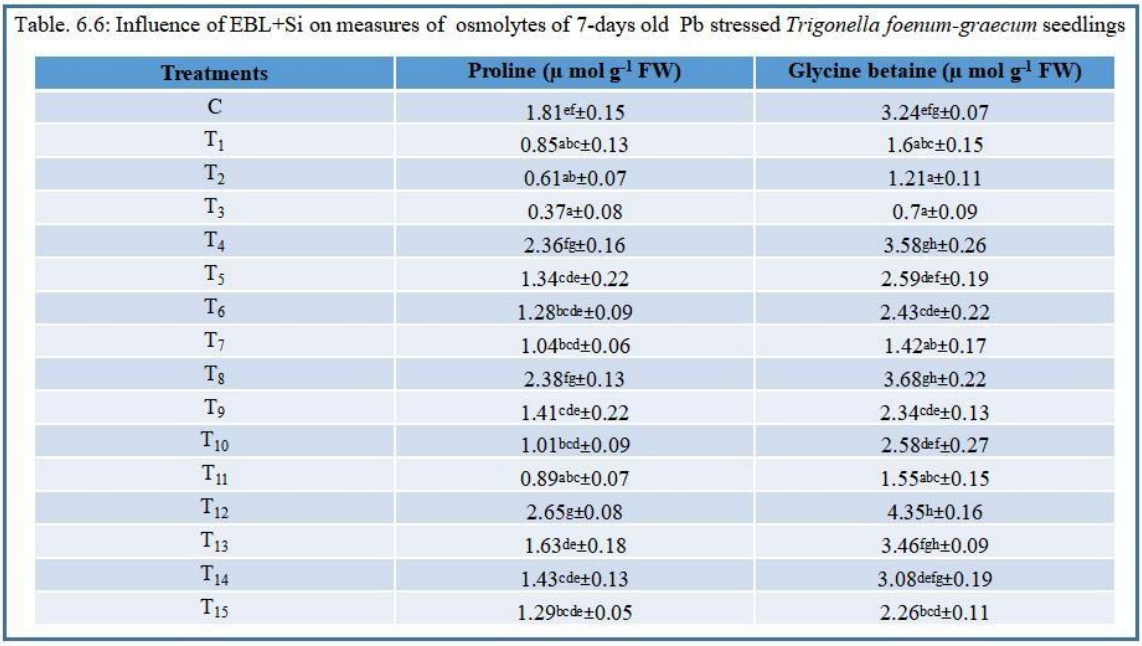


Table. 6.6: Influence of EBL+Si on measures of osmolytes of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	**Proline (µ mol g^{-1} FW)**	**Glycine betaine (µ mol g^{-1} FW)**
C	$1.81^{ef}\pm0.15$	$3.24^{efg}\pm0.07$
T_1	$0.85^{abc}\pm0.13$	$1.6^{abc}\pm0.15$
T_2	$0.61^{ab}\pm0.07$	$1.21^{a}\pm0.11$
T_3	$0.37^{a}\pm0.08$	$0.7^{a}\pm0.09$
T_4	$2.36^{fg}\pm0.16$	$3.58^{gh}\pm0.26$
T_5	$1.34^{cde}\pm0.22$	$2.59^{def}\pm0.19$
T_6	$1.28^{bcde}\pm0.09$	$2.43^{cde}\pm0.22$
T_7	$1.04^{bcd}\pm0.06$	$1.42^{ab}\pm0.17$
T_8	$2.38^{fg}\pm0.13$	$3.68^{gh}\pm0.22$
T_9	$1.41^{cde}\pm0.22$	$2.34^{cde}\pm0.13$
T_{10}	$1.01^{bcd}\pm0.09$	$2.58^{def}\pm0.27$
T_{11}	$0.89^{abc}\pm0.07$	$1.55^{abc}\pm0.15$
T_{12}	$2.65^{g}\pm0.08$	$4.35^{h}\pm0.16$
T_{13}	$1.63^{de}\pm0.18$	$3.46^{fgh}\pm0.09$
T_{14}	$1.43^{cde}\pm0.13$	$3.08^{defg}\pm0.19$
T_{15}	$1.29^{bcde}\pm0.05$	$2.26^{bcd}\pm0.11$

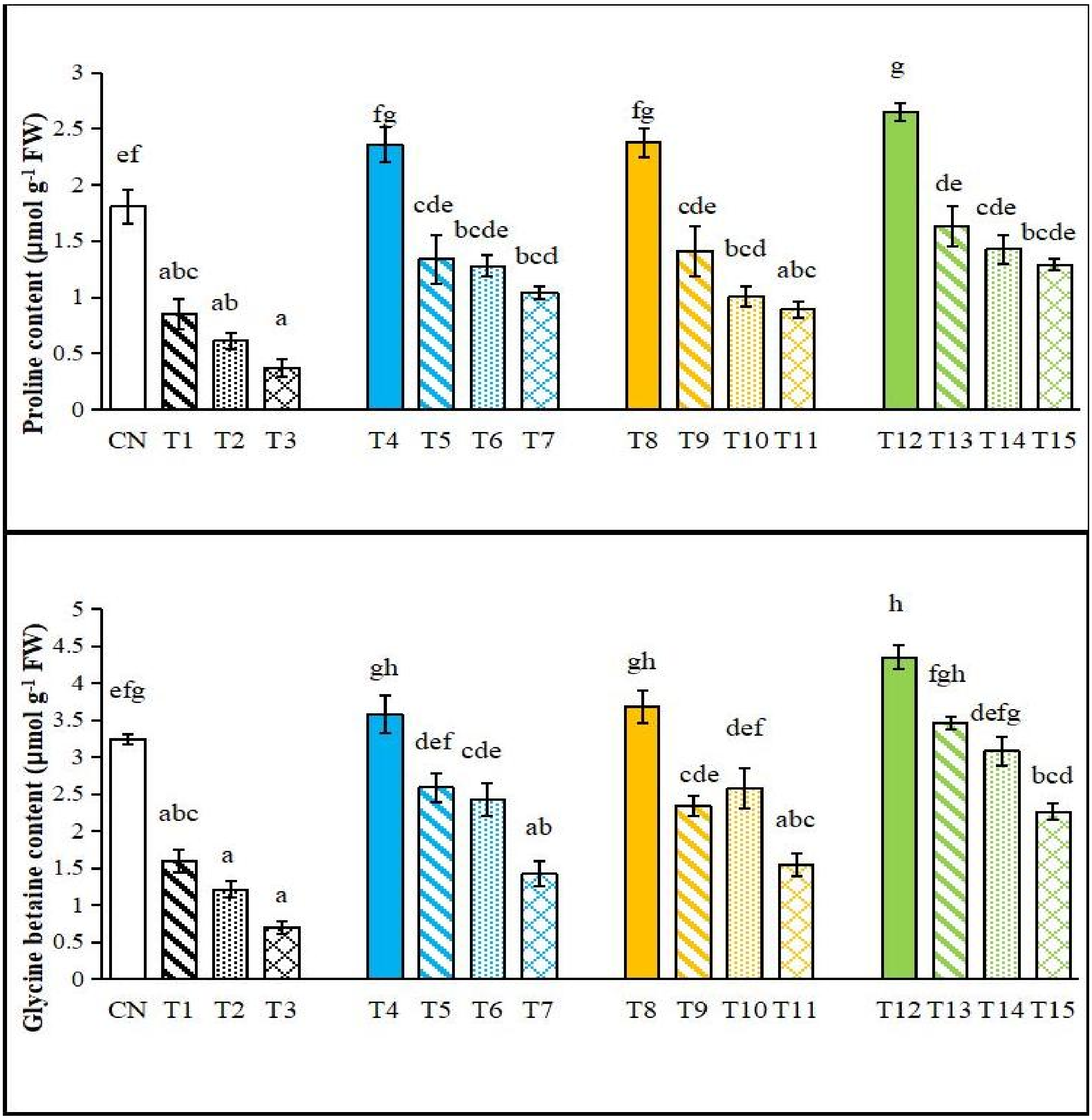


Fig. 6.6: Influence of EBL+Si treatment on measures of osmolytes in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.1.8 Total carbohydrates content

Total content of carbohydrates observed to have a diminishing effect on account of varying concentration of Pb stress in the study with being lowest around T_3 declined by Pb stress in fenugreek seedlings with the minimum of 0.48 mg g^{-1} FW at T_3 concentration (Fig. 6.7; Table 6.7). The most and least concentrations of carbohydrates in pre-treated EBL seedlings were recorded to be 4.55 at T_5 and 3.02 mg g^{-1} FW at T_7 respectively while the same values in case of Si were 4.26 at T_9 and 2.24 mg g^{-1} FW at T_{11}. When EBL and Si were co-applied together, promotion in the values of carbohydrate content was registered with being at its highest measure of 5.29 mg g^{-1} FW at T_{13}. T_{13} seedlings exhibited 16.26% improvement when correlated to T_5. T_{15} had 69.41% upsurge in carbohydrate measures against control seedlings.

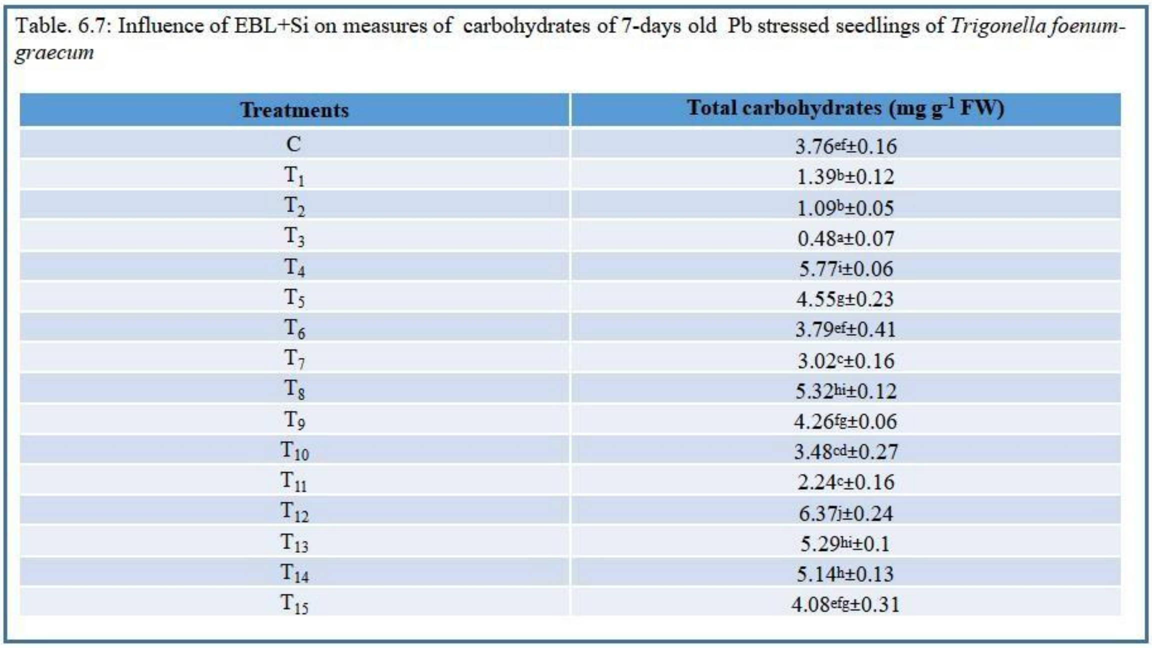


Table. 6.7: Influence of EBL+Si on measures of carbohydrates of 7-days old Pb stressed seedlings of *Trigonella foenum-graecum*

Treatments	Total carbohydrates (mg g^{-1} FW)
C	$3.76^{ef}\pm0.16$
T_1	$1.39^{b}\pm0.12$
T_2	$1.09^{b}\pm0.05$
T_3	$0.48^{a}\pm0.07$
T_4	$5.77^{i}\pm0.06$
T_5	$4.55^{g}\pm0.23$
T_6	$3.79^{ef}\pm0.41$
T_7	$3.02^{c}\pm0.16$
T_8	$5.32^{hi}\pm0.12$
T_9	$4.26^{fg}\pm0.06$
T_{10}	$3.48^{cd}\pm0.27$
T_{11}	$2.24^{c}\pm0.16$
T_{12}	$6.37^{j}\pm0.24$
T_{13}	$5.29^{hi}\pm0.1$
T_{14}	$5.14^{h}\pm0.13$
T_{15}	$4.08^{efg}\pm0.31$

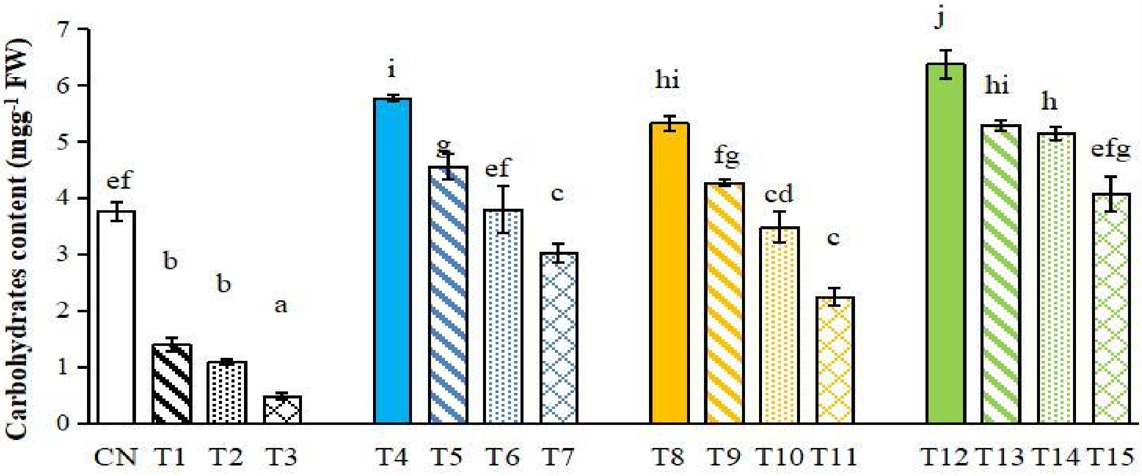


Fig. 6.7: Influence of EBL+Si treatment on measures of total carbohydrates in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.1.9 Protein content and Antioxidant defense system

6.1.1.9.1 Protein content and antioxidative enzymes

Lead metal stress caused a decline in the measures of protein content (Fig. 6.8a; Table 6.8a). In T_1 treated seedlings, amount of protein was 0.84 mg g^{-1} FW, which was 57% lower in correlation with control seedlings. The least amount of protein was recorded to be 0.35 mg g^{-1} FW at T_3 concentration. EBL pre-treatment of seedlings exhibited higher measures of protein in response to Pb stress, when contrasted against Si-augmented seedlings. The maximal (1.60

mg g^{-1} FW) and the minimal (1.16 mg g^{-1} FW) protein measures were noted at T_5 and T_7 concentrations, respectively when given pre-treatment of EBL. Supplementation with Si raised the measures of protein in response to stressed and stress free conditions. Seedlings in Si control showed 2.33 mg g^{-1} FW of protein content. Under Pb stress, the highest protein content of 1.52 mg g^{-1} FW was recorded in T_6. With respect to synergistic impact of EBL and Si, the maximum (2.23 mg g^{-1} FW) protein content was registered in T_{14} seedlings. Additionally, T_{13} seedlings exhibited 8.75% increase as compared to T_5 whereas T_{14} had 14.35% increment over control seedlings.

The extent of enzymatic activity was curtailed under Pb stress exposure in case of 7-days old fenugreek seedlings (Fig. 6.8a; Table 6.8a). Minimal enzymatic action of SOD was recorded at 1.74 UA mg^{-1} protein in T_3 metal stressed seedlings. Supplementing the stressed seedlings with 24-EBL improved the performance of SOD with a wide value range between the extremes of T_5 (4.34 UA mg^{-1} protein) and T_7 (2.49 UA mg^{-1} protein) respectively. Augmentation of Si to the seedlings under Pb toxicity, the greatest activity of SOD (3.42 UA mg^{-1} protein) was noted at T_5. In addition to this, EBL + Si + Pb I elevated the enzymatic action of SOD in T_{13} treatment (5.4 UA mg^{-1} protein). An increment of 71.54% was noted in T_5 over T_1. Seedlings of T_9 treatment displayed 35.17% increase than T_1. Further, seedlings at T_{13} treatment exhibited an elevation of 24.42% and 1.31% when correlated with T_5 and control.

Catalase action was adversely affected in response to Pb stress being minimum at 1.2 UA mg^{-1} protein in T_3 treatment (Fig. 6.8a; Table 6.8a). Applying the ameliorative EBL under Pb stress led to an improvement in CAT measures, which was maximum at 3.74 UA mg^{-1} protein in T_5 seedlings. Individually supplemented Si upgraded the CAT action than Pb alone augmented seedlings. Exogenous application of silicon with regard to Pb stress registered minimum CAT content at T_9 concentration (3.45 UA mg^{-1} protein). Its action was recorded to be curtailed with enhancement in Pb stress as is the case in EBL + Si, where the highest value of CAT activity was noted to be at 4.33 UA mg^{-1} protein in T_{13}. An increment of 34.05% was reported at T_5 when put against T_1. Further, T_9 seedlings had 23.65% enhancement over T_1 treatment. T_{13} seedlings displayed a respective increase of 15.77 and 22.31% in relation to T_5 and control.

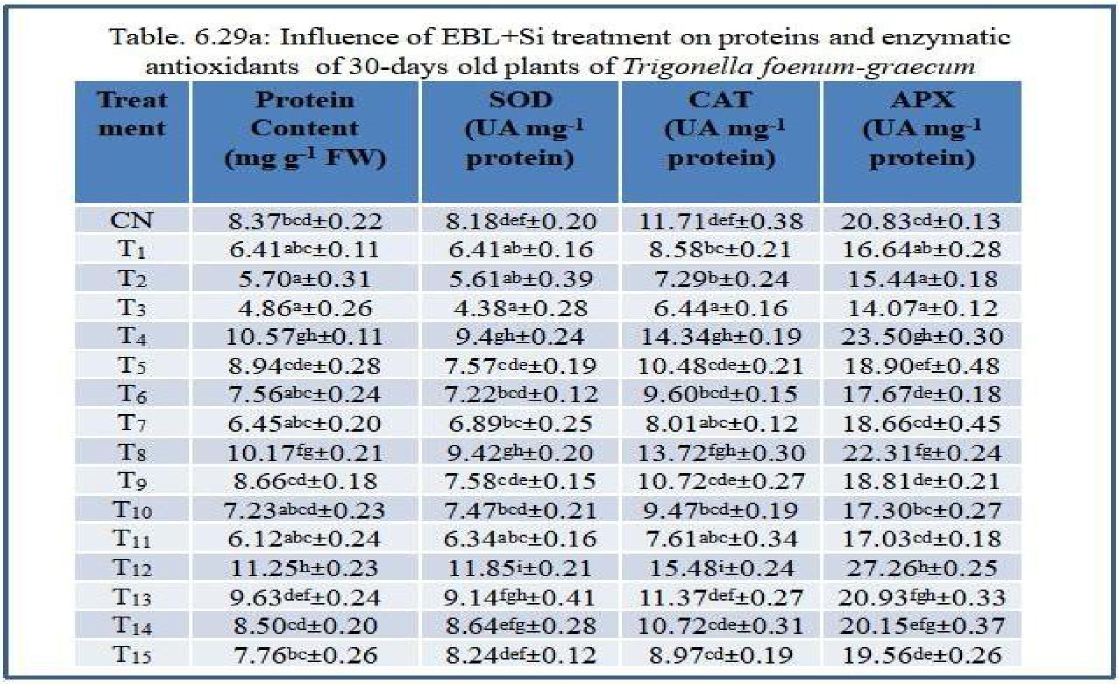
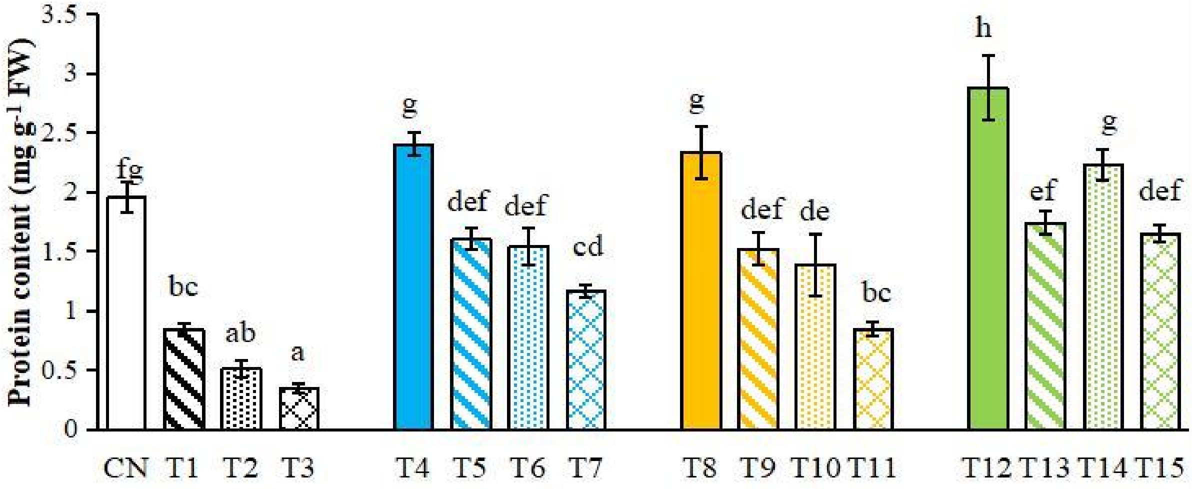


Table. 6.29a: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 30-days old plants of *Trigonella foenum-graecum*

Treatment	Protein Content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
CN	8.37^{bcd}±0.22	8.18^{def}±0.20	11.71^{def}±0.38	20.83^{cd}±0.13
T$_1$	6.41^{abc}±0.11	6.41^{ab}±0.16	8.58^{bc}±0.21	16.64^{ab}±0.28
T$_2$	5.70^{a}±0.31	5.61^{ab}±0.39	7.29^{b}±0.24	15.44^{a}±0.18
T$_3$	4.86^{a}±0.26	4.38^{a}±0.28	6.44^{a}±0.16	14.07^{a}±0.12
T$_4$	10.57^{gh}±0.11	9.4^{gh}±0.24	14.34^{gh}±0.19	23.50^{gh}±0.30
T$_5$	8.94^{cde}±0.28	7.57^{cde}±0.19	10.48^{cde}±0.21	18.90^{ef}±0.48
T$_6$	7.56^{abc}±0.24	7.22^{bcd}±0.12	9.60^{bcd}±0.15	17.67^{de}±0.18
T$_7$	6.45^{abc}±0.20	6.89^{bc}±0.25	8.01^{abc}±0.12	18.66^{cd}±0.45
T$_8$	10.17^{fg}±0.21	9.42^{gh}±0.20	13.72^{fgh}±0.30	22.31^{fg}±0.24
T$_9$	8.66^{cd}±0.18	7.58^{cde}±0.15	10.72^{cde}±0.27	18.81^{de}±0.21
T$_{10}$	7.23^{abcd}±0.23	7.47^{bcd}±0.21	9.47^{bcd}±0.19	17.30^{bc}±0.27
T$_{11}$	6.12^{abc}±0.24	6.34^{abc}±0.16	7.61^{abc}±0.34	17.03^{cd}±0.18
T$_{12}$	11.25^{h}±0.23	11.85^{i}±0.21	15.48^{i}±0.24	27.26^{h}±0.25
T$_{13}$	9.63^{def}±0.24	9.14^{fgh}±0.41	11.37^{def}±0.27	20.93^{fgh}±0.33
T$_{14}$	8.50^{cd}±0.20	8.64^{efg}±0.28	10.72^{cde}±0.31	20.15^{efg}±0.37
T$_{15}$	7.76^{bc}±0.26	8.24^{def}±0.12	8.97^{cd}±0.19	19.56^{de}±0.26



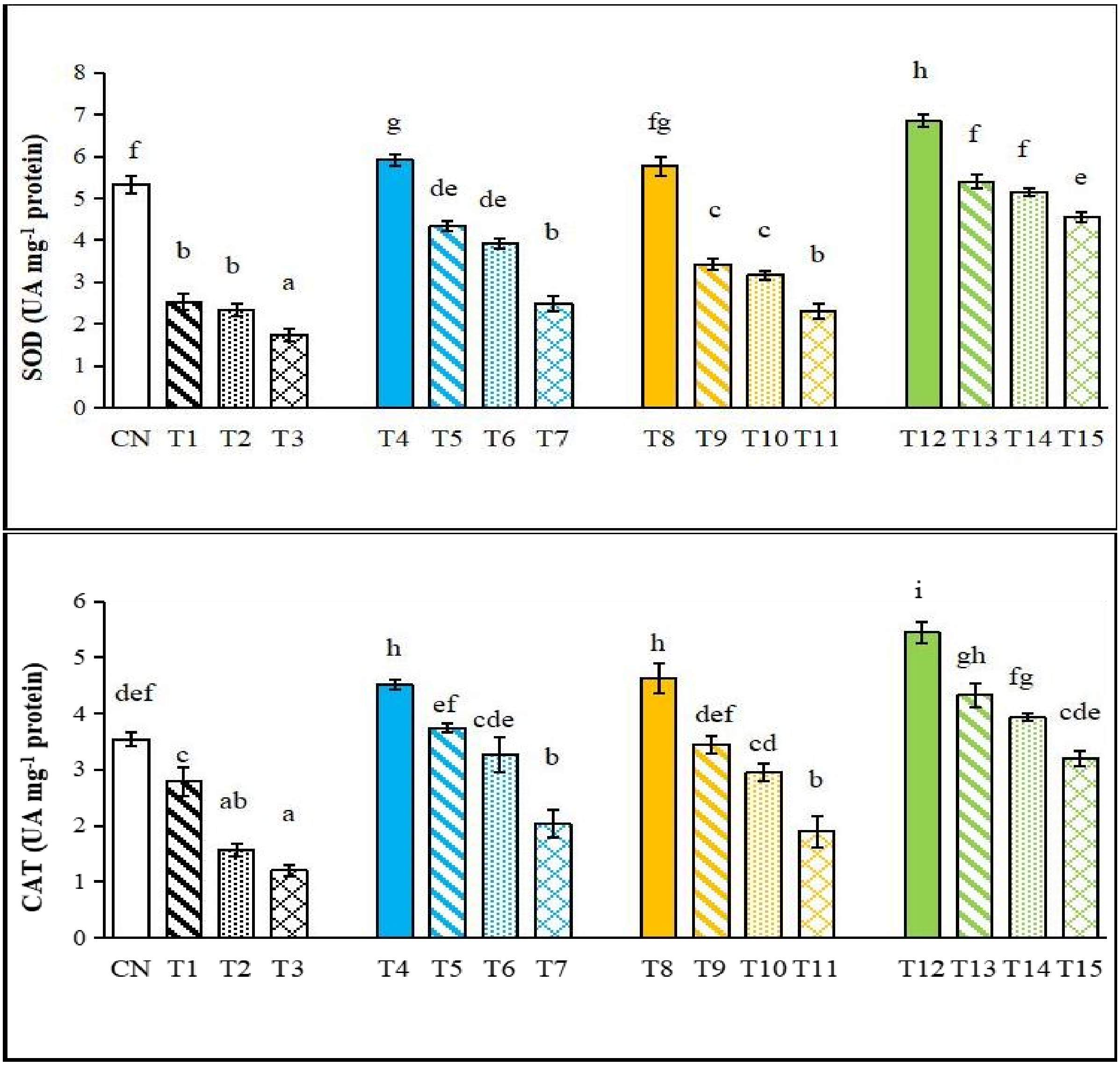


Fig. 6.8 (a): Influence of EBL+Si treatment on measures of proteins and enzymatic antioxidants in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

Lead toxicity caused the greatest decline in the enzymatic performance of APX as reported in case of T_3 (2.44 UA mg^{-1} protein) (Fig. 6.8b; Table 6.8a). Treating the fenugreek seedlings with EBL and Si in an individual manner reportedly upgraded the APX measures in contrast noted at T_6 with a value of 5.38 UA mg^{-1} protein whereas in Si treated seedlings, such an elevated activity was found at T_9 metal stressed seedlings (5.44 UA mg^{-1} protein). In response to Pb stress, the maximum elevation in enzymatic activity was at T_{13} (6.24 UA mg^{-1} protein) amongst all the selected treatments where EBL and Si are co-applied. An increment of 49.01% was noted in T_5 over T_1. T_9 seedlings displayed 53.23% escalation in correlation to T_1. Further, T_{13} seedlings recorded 17.95 % and 39.59% increase as compared to T_5 and control, respectively.

Lead toxicity notably declined the activity of POD, in relation to control (Fig. 6.8b; Table 6.8b). In respect to Pb stress conditions, enzymatic performance of POD was recorded to be at its lowest at T_3 concentration with a content value of 2.44 UA mg^{-1} protein. Maximum (5.38 UA mg^{-1} protein) and minimum (4.51 UA mg^{-1} protein) levels of POD were noticed in T_6 and T_7 stressed seedlings when given a 24-EBL treatment. Supplementing the fenugreek seedlings with Si resulted in maximum elevation in the enzymatic levels at T_9 (5.44 UA mg^{-1} protein). In co-supplementation, EBL + Si were more effective in up-scaling the action of POD enzyme vis-à-vis their individual treatments. It can be corroborated by the highest values of this enzyme i.e. 6.24 UA mg^{-1} protein at T_{13} treatment (EBL + Si + Pb I). An increment of 16.44% was noted in T_5 over T_1. Seedlings at T_9 stage recorded 18.79 % escalations over T_1. Furthermore, T_{13} seedlings exhibited 18.79% and 16.48% upsurge when correlated with T_5 and control.

Highest decline in the measures of GR on account of Pb stress took place at T_3 concentration (3.01 UA mg^{-1} protein) (Fig. 6.8b; Table 6.8b). Seedlings of control recorded an activity level of 5.78 UA mg^{-1} protein with respect to GR. A notable diminishing impact of Pb stress was mitigated by EBL application at T_6 (4.57 UA mg^{-1} protein) while the same was accomplished by Si treatment in T_9 cultivars (4.88 UA mg^{-1} protein). The highest elevation in GR's performance with content of 5.51 UA mg^{-1} protein was recorded at T_{13} treatment. An upsurge of 9.17% was seen in T_5 over T_1. Whereas T_{13} seedlings exhibited a 23.09% increment at T5 with a 5.01% decline in relation to control.

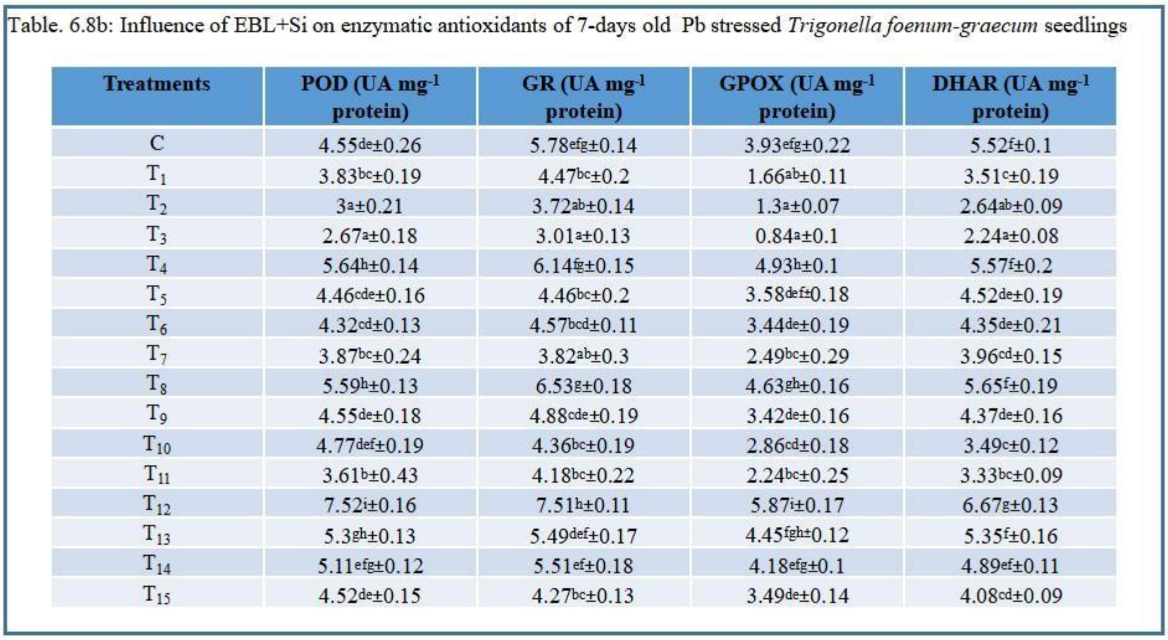
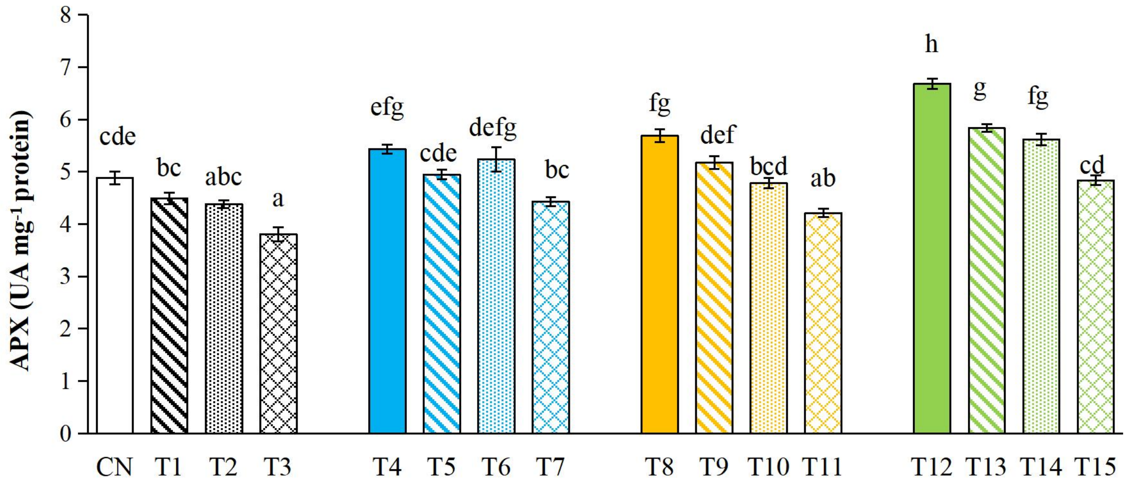


Table. 6.8b: Influence of EBL+Si on enzymatic antioxidants of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
C	$4.55^{de}\pm0.26$	$5.78^{efg}\pm0.14$	$3.93^{efg}\pm0.22$	$5.52^{f}\pm0.1$
T_1	$3.83^{bc}\pm0.19$	$4.47^{bc}\pm0.2$	$1.66^{ab}\pm0.11$	$3.51^{c}\pm0.19$
T_2	$3^{a}\pm0.21$	$3.72^{ab}\pm0.14$	$1.3^{a}\pm0.07$	$2.64^{ab}\pm0.09$
T_3	$2.67^{a}\pm0.18$	$3.01^{a}\pm0.13$	$0.84^{a}\pm0.1$	$2.24^{a}\pm0.08$
T_4	$5.64^{h}\pm0.14$	$6.14^{fg}\pm0.15$	$4.93^{h}\pm0.1$	$5.57^{f}\pm0.2$
T_5	$4.46^{cde}\pm0.16$	$4.46^{bc}\pm0.2$	$3.58^{def}\pm0.18$	$4.52^{de}\pm0.19$
T_6	$4.32^{cd}\pm0.13$	$4.57^{bcd}\pm0.11$	$3.44^{de}\pm0.19$	$4.35^{de}\pm0.21$
T_7	$3.87^{bc}\pm0.24$	$3.82^{ab}\pm0.3$	$2.49^{bc}\pm0.29$	$3.96^{cd}\pm0.15$
T_8	$5.59^{h}\pm0.13$	$6.53^{g}\pm0.18$	$4.63^{gh}\pm0.16$	$5.65^{f}\pm0.19$
T_9	$4.55^{de}\pm0.18$	$4.88^{cde}\pm0.19$	$3.42^{de}\pm0.16$	$4.37^{de}\pm0.16$
T_{10}	$4.77^{def}\pm0.19$	$4.36^{bc}\pm0.19$	$2.86^{cd}\pm0.18$	$3.49^{c}\pm0.12$
T_{11}	$3.61^{b}\pm0.43$	$4.18^{bc}\pm0.22$	$2.24^{bc}\pm0.25$	$3.33^{bc}\pm0.09$
T_{12}	$7.52^{i}\pm0.16$	$7.51^{h}\pm0.11$	$5.87^{i}\pm0.17$	$6.67^{g}\pm0.13$
T_{13}	$5.3^{gh}\pm0.13$	$5.49^{def}\pm0.17$	$4.45^{fgh}\pm0.12$	$5.35^{f}\pm0.16$
T_{14}	$5.11^{efg}\pm0.12$	$5.51^{ef}\pm0.18$	$4.18^{efg}\pm0.1$	$4.89^{ef}\pm0.11$
T_{15}	$4.52^{de}\pm0.15$	$4.27^{bc}\pm0.13$	$3.49^{de}\pm0.14$	$4.08^{cd}\pm0.09$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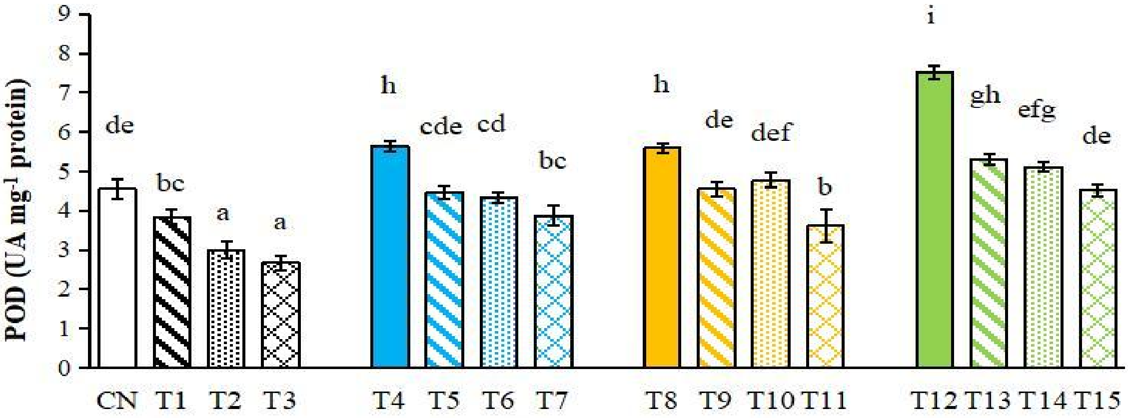
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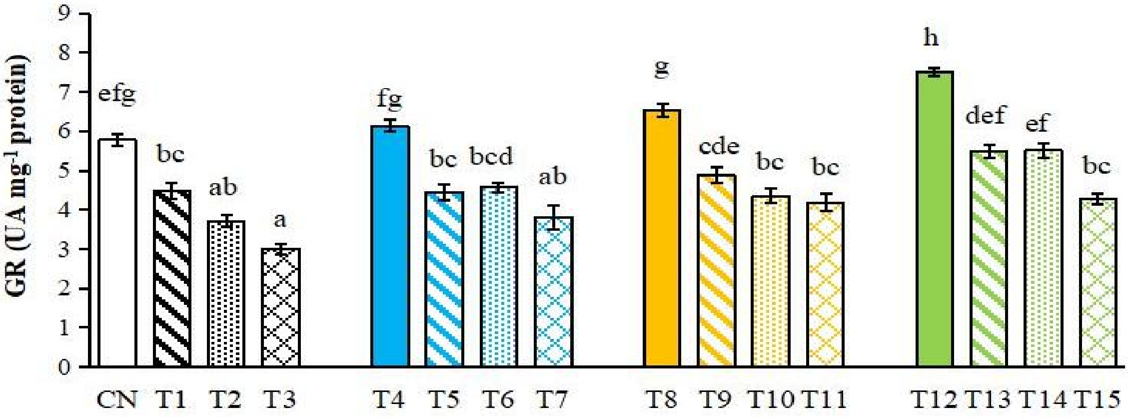
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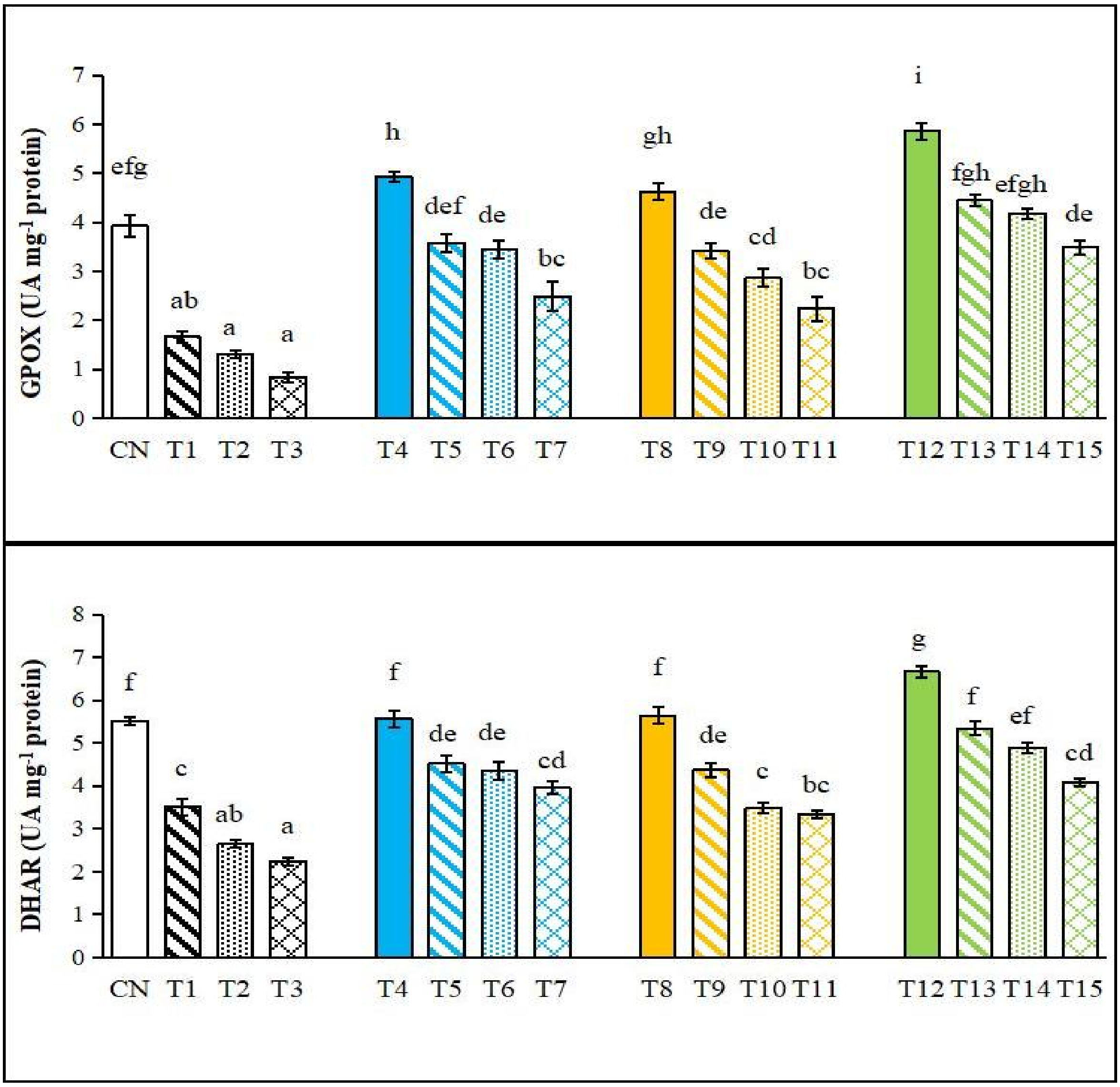


Fig. 6.8 (b): Influence of EBL + Si treatment on measures of enzymatic antioxidants in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

The enzymatic performance of GPOX got declined in Pb exposed fenugreek seedlings with 1.66, 1.3, and 0.84 UA mg^{-1} protein at Pb treatment concentrations of T_1, T_2 and T_3, respectively (Fig. 6.8c; Table 6.8b). Individually augmented EBL and Si with respect to Pb alone toxicity resulted in boosting the levels of GPOX with respective values of 3.58 and 3.42 UA mg^{-1} protein at T_5 and T_9. Further, curtailment of enzymatic action was reported with every escalation in Pb concentrations as was evident in cumulative application (EBL + Si) having highest levels of activity at T_{13} (4.45 UA mg^{-1} protein). An improvement of 115.66% was noted in T_5 over T_1. Further, T_{13} seedlings exhibited 24.30% and 13.23% increase in correlation to T_5 and control.

DHAR's enzymatic action suffered from Pb induced diminishing effect in in vitro raised seedlings (Fig. 6.8c; Table 6.8b). Maximum enzymatic levels were observed in T_1 seedlings

(3.51 UA mg-1 protein) with respect to control. In individually treated seedlings with EBL and Si, the greatest measures of enzyme were noted at T_5 and T_7 with 4.52 and 3.96 UA mg^{-1} protein values respectively. On the other hand, under the influence of same mitigants, minimal content values were at T_9 (4.37 UA mg-1 protein) and T_{11} (3.33 UA mg-1 protein). Further augmentation of enzymatic performance of DHAR in response to metal exposure took place at T_{13} (5.35 UA mg^{-1} protein). An elevation of 28.71% was reported in T_5 over T_1. T_9 seedlings displayed 24.14% escalation than T_1. Further, T_{13} seedlings exhibited 18.36% upsurge as opposed to T_5 and a 3.07 % decline in correlation with control.

MDHAR enzyme's performance level was adversely affected as a consequence of lead stress (Fig. 6.8c; Table 6.8c). The least amount of activity was visible at 1.9 UA mg^{-1} protein in T_2 treatment. MDHAR levels were escalated from 3.28 to 5.05 UA mg^{-1} protein in EBL pre-augmented seedlings of T_5 treatment. Supplementing the fenugreek seedlings with Si augmented the enzymatic levels with being highest in T_9 treatment (4.96 UA mg^{-1} protein. The maximum MDHAR activity level (5.37 UA mg^{-1} protein) got recorded in coupled supplementation of EBL and Si at T_{14} (5.45 UA mg^{-1} protein). An upsurge of 53.96% was recorded in T_5 over T_1 whereas T_9 seedlings showed 51.21% escalation in relation to T_1. Seedlings at T_{13} treatment recorded 6.36% and 19.06% increment in correlation with T_5 and control.

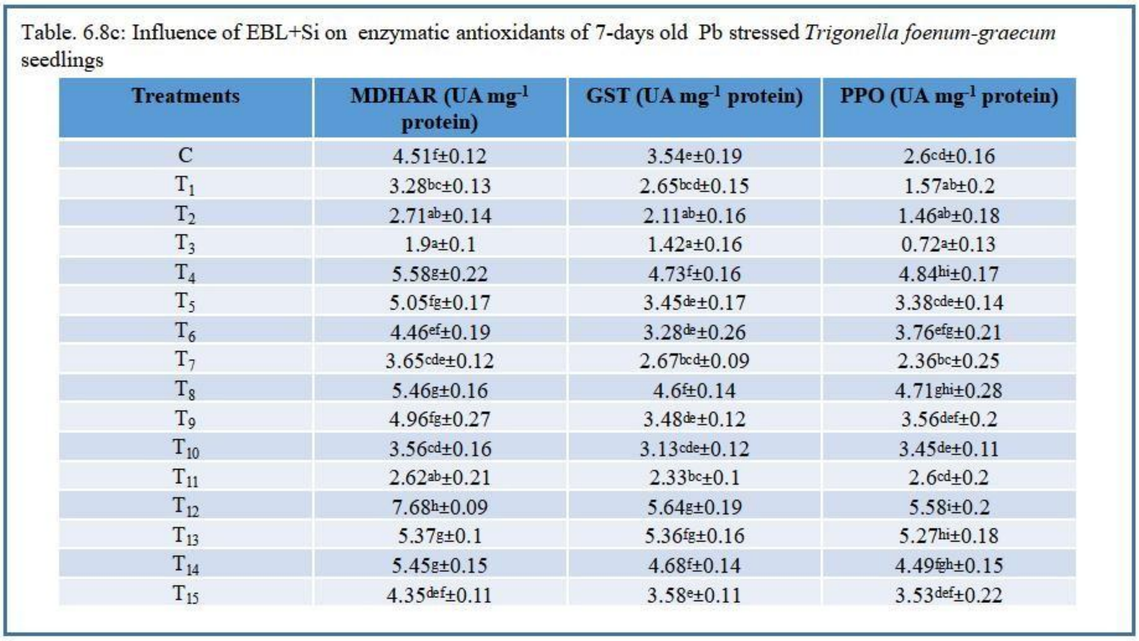


Table. 6.8c: Influence of EBL+Si on enzymatic antioxidants of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings

Treatments	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
C	$4.51^{f}\pm0.12$	$3.54^{e}\pm0.19$	$2.6^{cd}\pm0.16$
T_1	$3.28^{bc}\pm0.13$	$2.65^{bcd}\pm0.15$	$1.57^{ab}\pm0.2$
T_2	$2.71^{ab}\pm0.14$	$2.11^{ab}\pm0.16$	$1.46^{ab}\pm0.18$
T_3	$1.9^{a}\pm0.1$	$1.42^{a}\pm0.16$	$0.72^{a}\pm0.13$
T_4	$5.58^{g}\pm0.22$	$4.73^{f}\pm0.16$	$4.84^{hi}\pm0.17$
T_5	$5.05^{fg}\pm0.17$	$3.45^{de}\pm0.17$	$3.38^{cde}\pm0.14$
T_6	$4.46^{ef}\pm0.19$	$3.28^{de}\pm0.26$	$3.76^{efg}\pm0.21$
T_7	$3.65^{cde}\pm0.12$	$2.67^{bcd}\pm0.09$	$2.36^{bc}\pm0.25$
T_8	$5.46^{g}\pm0.16$	$4.6^{f}\pm0.14$	$4.71^{ghi}\pm0.28$
T_9	$4.96^{fg}\pm0.27$	$3.48^{de}\pm0.12$	$3.56^{def}\pm0.2$
T_{10}	$3.56^{cd}\pm0.16$	$3.13^{cde}\pm0.12$	$3.45^{de}\pm0.11$
T_{11}	$2.62^{ab}\pm0.21$	$2.33^{bc}\pm0.1$	$2.6^{cd}\pm0.2$
T_{12}	$7.68^{h}\pm0.09$	$5.64^{g}\pm0.19$	$5.58^{i}\pm0.2$
T_{13}	$5.37^{g}\pm0.1$	$5.36^{fg}\pm0.16$	$5.27^{hi}\pm0.18$
T_{14}	$5.45^{g}\pm0.15$	$4.68^{f}\pm0.14$	$4.49^{fgh}\pm0.15$
T_{15}	$4.35^{def}\pm0.11$	$3.58^{e}\pm0.11$	$3.53^{def}\pm0.22$

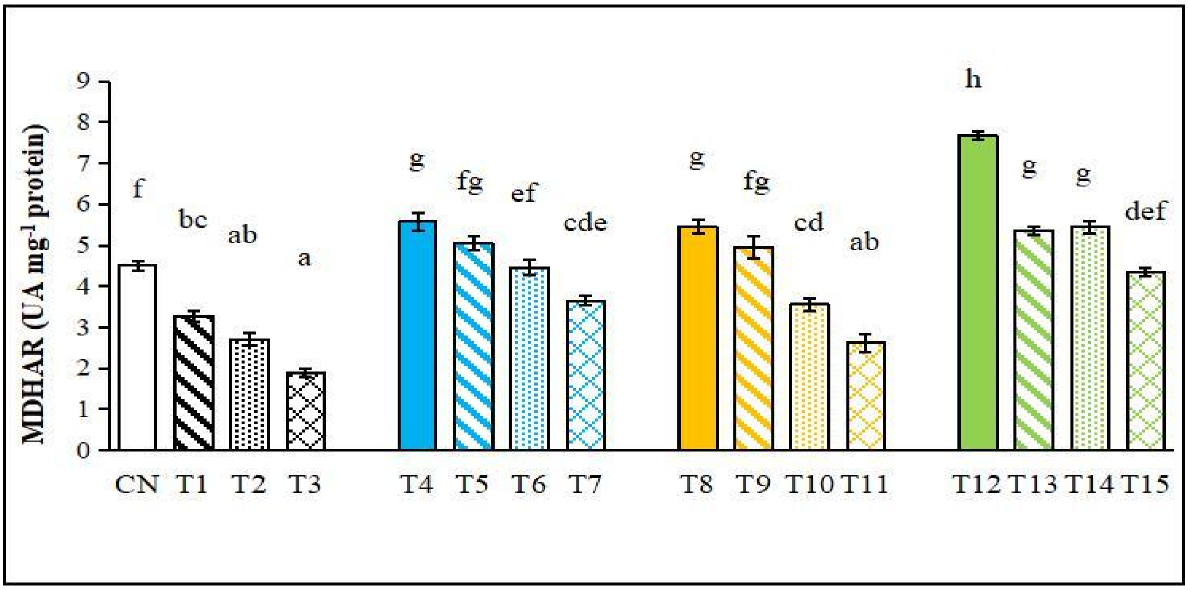


Fig. 6.8 (c): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

GST enzyme had activity level of 2.65, 2.11 and 1.42 UA mg^{-1} protein at different concentrations of lead i.e. T_1, T_2 and T_3, respectively (Fig. 6.8d; Table 6.8c). Maximal and minimal values of GST content were recorded at T_5 (3.45 UA mg^{-1} protein) and T_7 (2.67 UA mg^{-1} protein) in EBL augmented seedlings. In Si augmented seedlings under stressful conditions, highest GST content of 3.48 UA mg^{-1} protein was noted in T_9 seedlings. EBL + Si upscaled the activity levels of GST in response to T_{13} concentration (5.36 UA mg^{-1} protein) An increment of 30.18% was noted in T_5 over T_1. Further, T_9 seedlings showed 31.32% upsurge opposed to T_1. T_{13} seedlings showed increments of 55.36% and 51.41% at T_5 and control.

Measures of PPO enzyme were at their minimum at the highest treatment value of Pb stress i.e. T_3 (0.72 UA mg^{-1} protein) in contrast to control plants (Fig. 6.8d; Table 6.8c). However, in EBL augmented seedlings under no stress conditions, highest activity was measured at T_4 of 4.71 UA mg^{-1} protein value. Applying EBL to Pb stressed fenugreek seedlings registered minimal activity of this enzyme at 2.36 UA mg^{-1} protein at T_7. Further, the Si treatment had the highest measures of PPO activity in case of T_9 cultivars (3.56 UA mg^{-1} protein) in metal stressed conditions. Whereas cumulatively applied EBL and Si made the activity level of PPO as high as 5.27 UA mg^{-1} protein at T_{13} treatment. An increase of 60.97% was noticed in T_5 than T_1. T_{13} seedlings showed 39.01% and 33.45% increase as compared to T_5 and control seedlings, respectively.

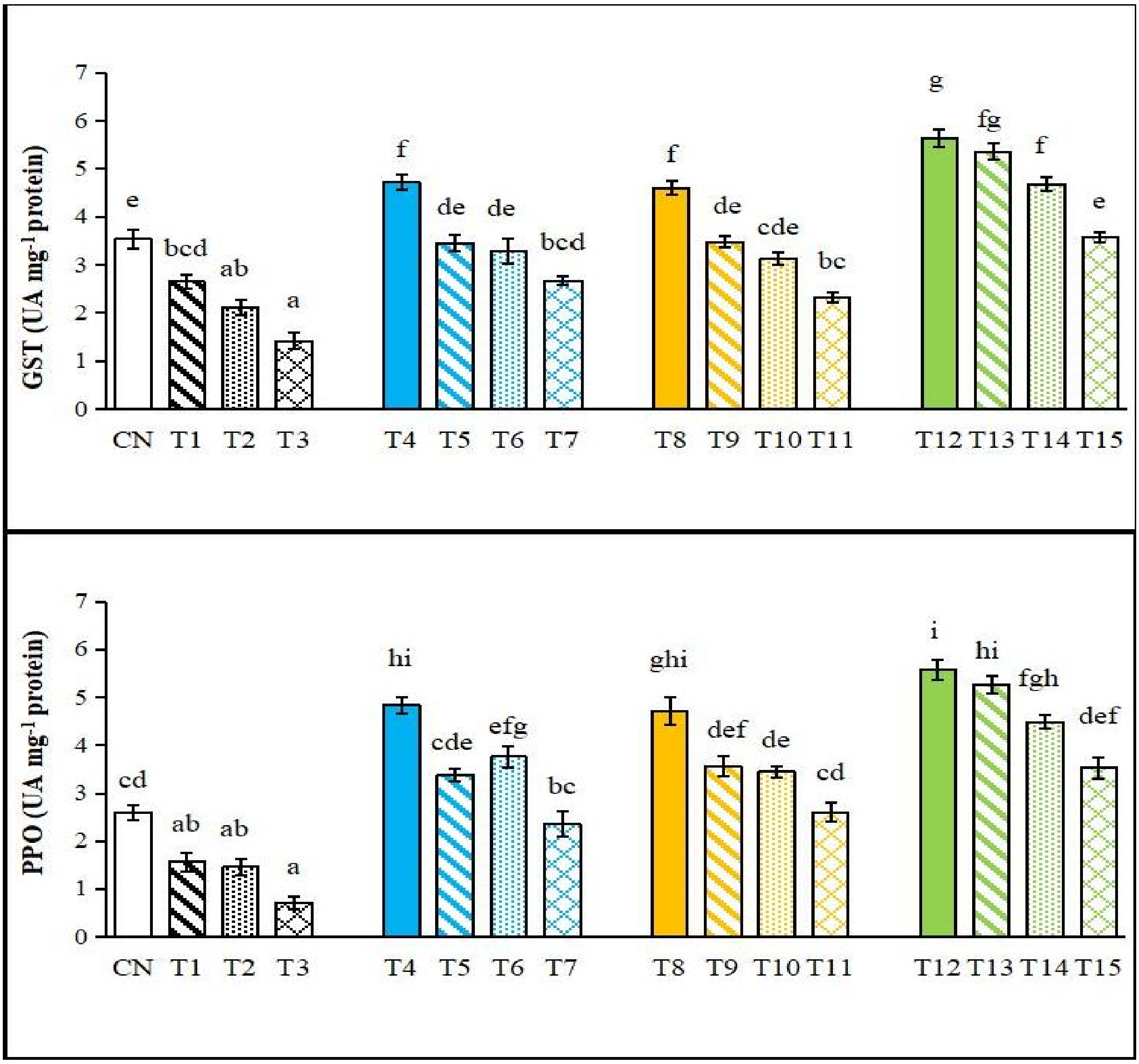


Fig. 6.8 (d): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

6.1.1.8.2 Non-enzymatic antioxidants

Amount of ascorbic acid was registered to be diminished at each of the Pb concentration selected for carrying out this study (Fig. 6.9; Table 6.9). The topmost vale of ascorbic acid was noted at 1.71 µg g^{-1} FW in response to T_1 concentration amongst every discrete level of Pb stress. Minimum values of Ascorbic acid in EBL and Si alone applications with regard to stressed seedlings, were 1.80 µg g^{-1} FW at T_7 and 1.52 at T_{11} seedlings. However, synergistic action of EBL +Si was observed to have highest value of this antioxidant at T_{13} i.e. 3.33 µg g^{-1} FW at T_{14}. An elevation of 57.89% and 43.27% was reported in T_5 and T_9 over T_1 metal stressed seedlings. T_{13} seedlings showed 28.43% and 7.11 % increase as compared to T_5 and control, respectively.

Lead toxicity recorded a decline in the measures of glutathione with being minimum at T_1 (0.365 µg g^{-1} FW) in contrast to control seedlings (Fig. 6.9; Table 6.9). However, pre-treated EBL seedlings displayed highest values of 2.85 µg g^{-1} FW and 1.92 µg g^{-1} FW of glutathione in response to stress free and stressful environments respectively. With respect to Si treatment, these values were found to be 2.41 µg g^{-1} FW and 1.87 µg g^{-1} FW respectively. Coupling of EBL with Si for mitigating the harms of Pb toxicity, exhibited glutathione content 2.55 µg g^{-1} FW in T_{13} stress exposed seedlings. Moreover, T_{13} seedlings exhibited an increment of 32.81% and 33.50% in glutathione when correlated with T_5 and control, respectively.

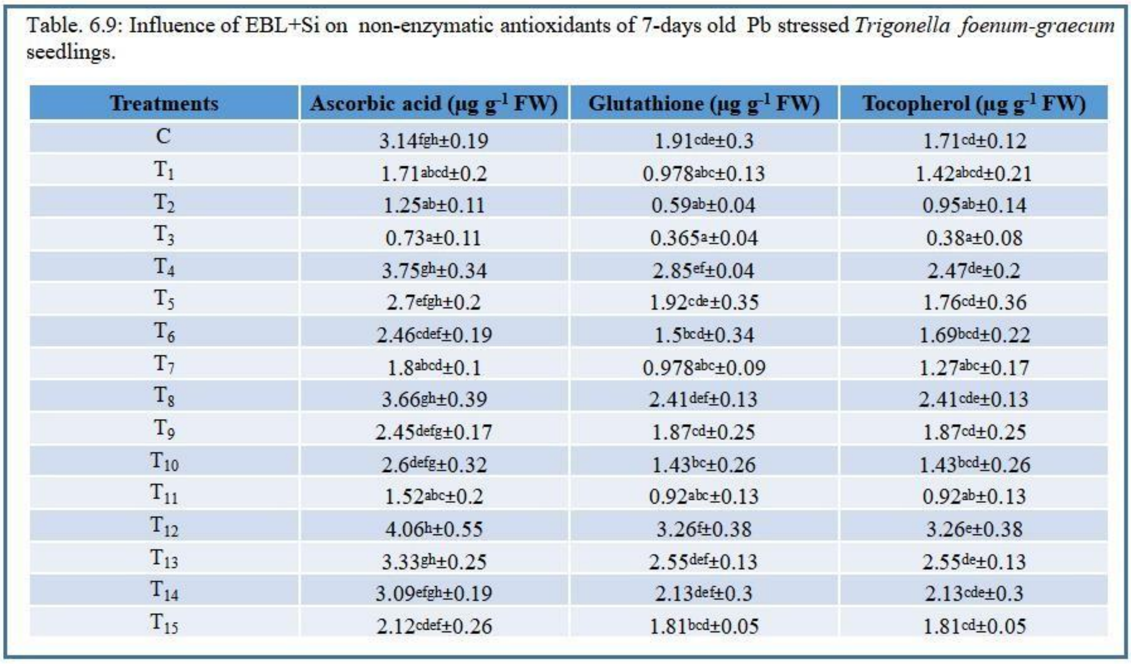
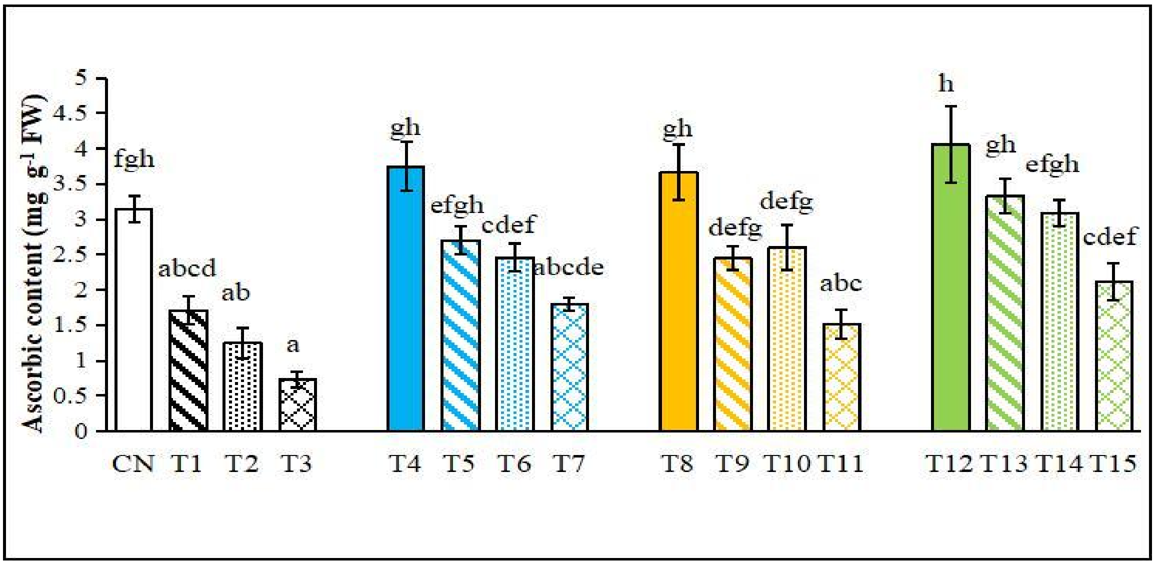


Table. 6.9: Influence of EBL+Si on non-enzymatic antioxidants of 7-days old Pb stressed *Trigonella foenum-graecum* seedlings.

Treatments	Ascorbic acid (µg g^{-1} FW)	Glutathione (µg g^{-1} FW)	Tocopherol (µg g^{-1} FW)
C	3.14^{fgh}±0.19	1.91^{cde}±0.3	1.71^{cd}±0.12
T_1	1.71^{abcd}±0.2	0.978^{abc}±0.13	1.42^{abcd}±0.21
T_2	1.25^{ab}±0.11	0.59^{ab}±0.04	0.95^{ab}±0.14
T_3	0.73^{a}±0.11	0.365^{a}±0.04	0.38^{a}±0.08
T_4	3.75^{gh}±0.34	2.85^{ef}±0.04	2.47^{de}±0.2
T_5	2.7^{efgh}±0.2	1.92^{cde}±0.35	1.76^{cd}±0.36
T_6	2.46^{cdef}±0.19	1.5^{bcd}±0.34	1.69^{bcd}±0.22
T_7	1.8^{abcd}±0.1	0.978^{abc}±0.09	1.27^{abc}±0.17
T_8	3.66^{gh}±0.39	2.41^{def}±0.13	2.41^{cde}±0.13
T_9	2.45^{defg}±0.17	1.87^{cd}±0.25	1.87^{cd}±0.25
T_{10}	2.6^{defg}±0.32	1.43^{bc}±0.26	1.43^{bcd}±0.26
T_{11}	1.52^{abc}±0.2	0.92^{abc}±0.13	0.92^{ab}±0.13
T_{12}	4.06^{h}±0.55	3.26^{f}±0.38	3.26^{e}±0.38
T_{13}	3.33^{gh}±0.25	2.55^{def}±0.13	2.55^{de}±0.13
T_{14}	3.09^{efgh}±0.19	2.13^{def}±0.3	2.13^{cde}±0.3
T_{15}	2.12^{cdef}±0.26	1.81^{bcd}±0.05	1.81^{cd}±0.05



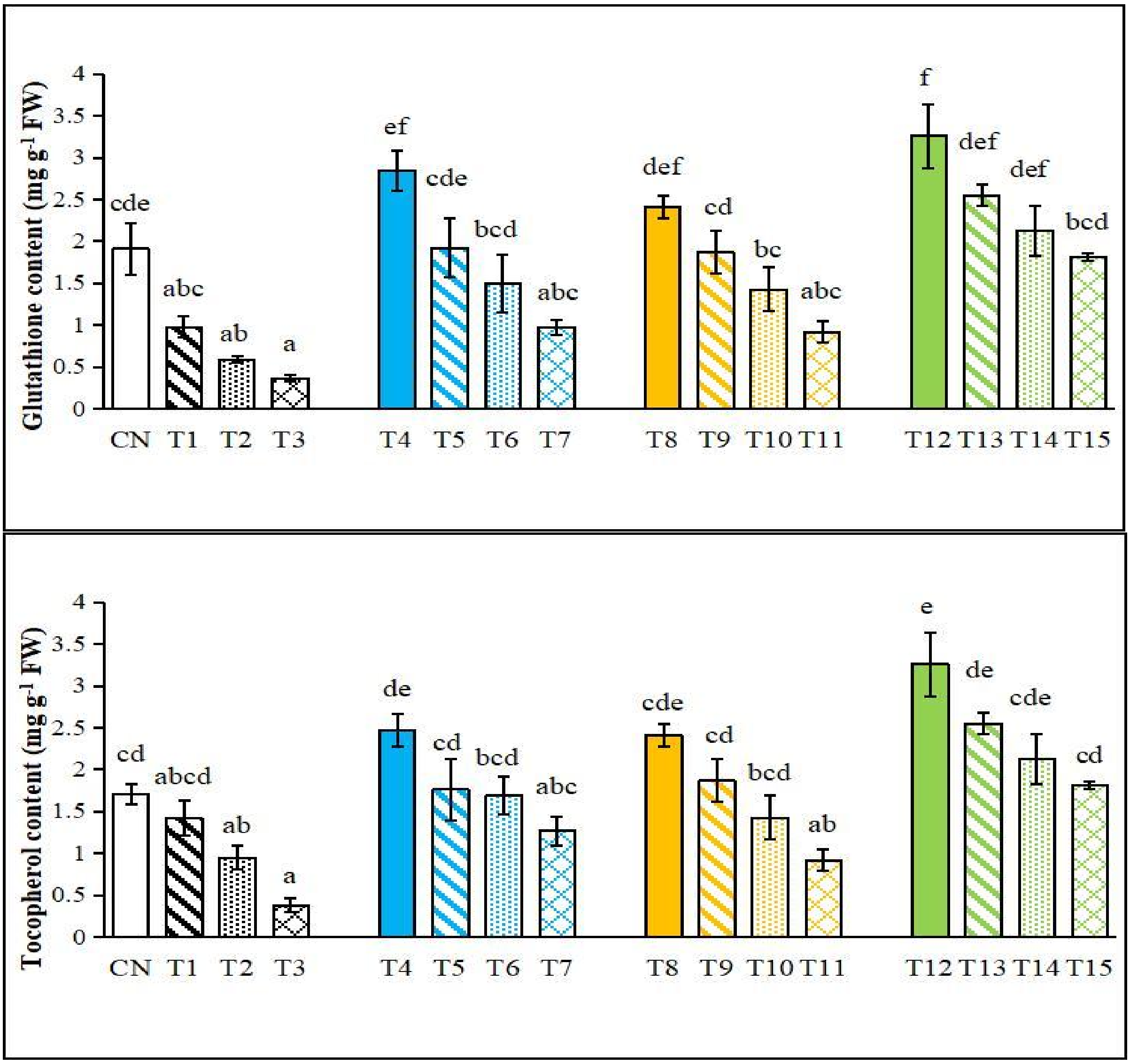
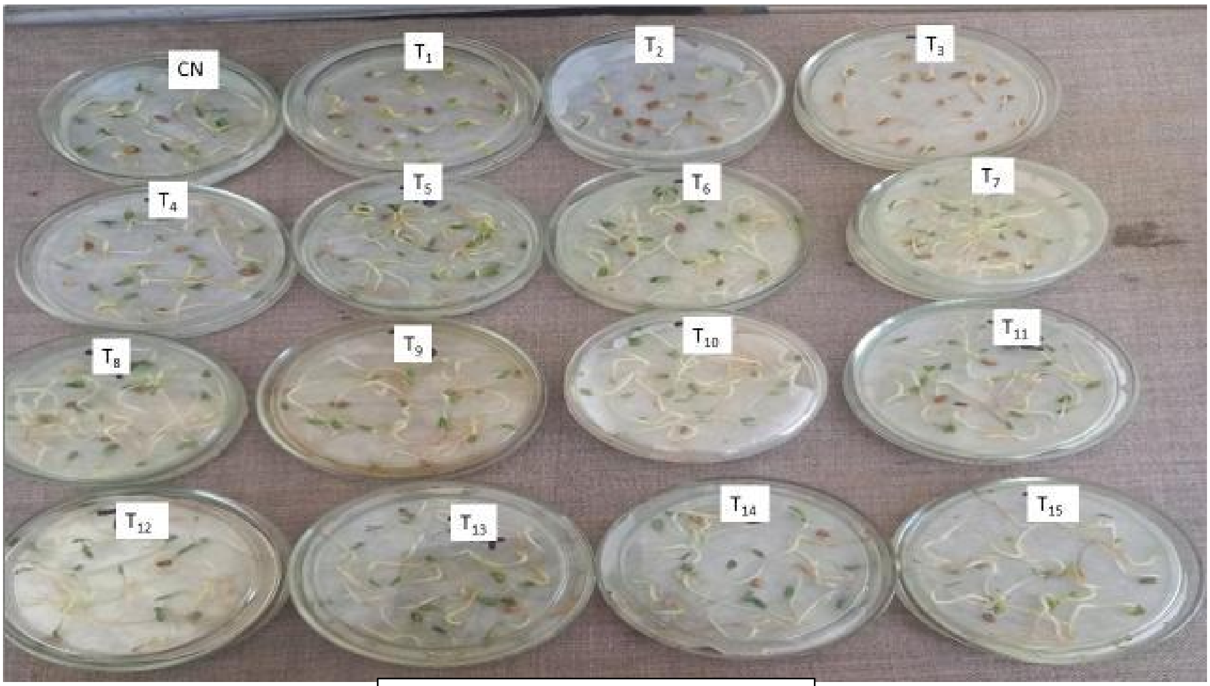
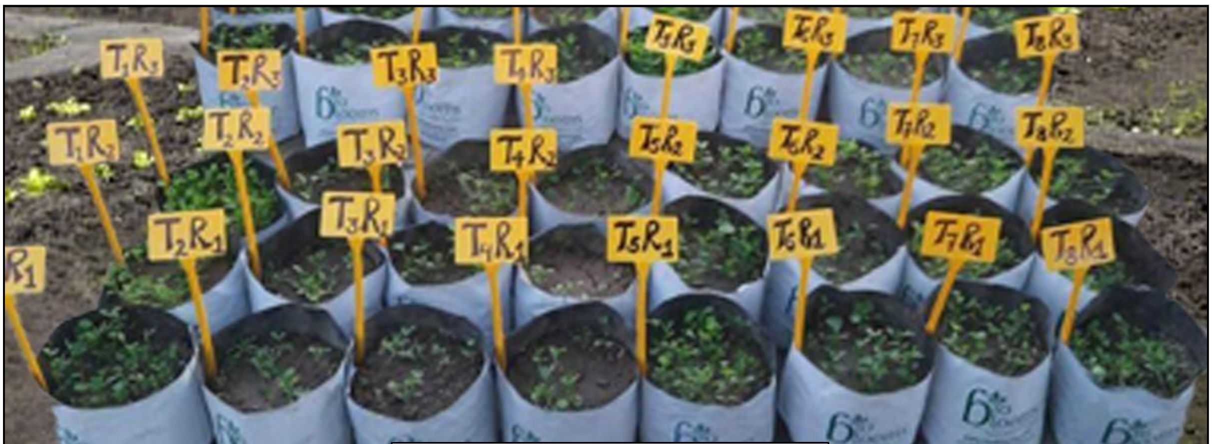


Fig. 6.9: Influence of EBL+Si treatment on measures of non-enzymatic antioxidants in 7 days old Pb stressed *Trigonella foenum-graecum* seedlings. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Tocopherol content exhibited a drastic decline at every chosen level of Pb toxicity during the present work (Fig. 6.9; Table 6.9). Minimum measures of tocopherol were recorded to be of 0.38 µg g^{-1} FW at T_3 amongst all the three distinct concentrations of Pb. And in case of EBL and Si alone augmented seedlings under Pb induced stress, the highest values of this antioxidant were recorded to be 1.76 at T_5 and 1.87 µg g^{-1} FW at T_9, respectively. However, EBL + Si together displayed a highest value of tocopherol content of 2.55 µg g^{-1} FW in T_{13}. An upsurge of 23.94% was recorded in T_5 over T_1. Similarly, T_{13} seedlings displayed a promotion of 45% as opposed to T_5. Escalation with an increment of 49.12% at T_{13} was observed when correlated against control seedlings.



a) *In vitro* growth of plants



b) *In vivo* growth of plants

Image 6.3 (a-b): Individual and cumulative treatment of EBL and Si in Pb stressed *Trigonella foenum-graecum* species raised in both *In vitro* and *In vivo*.

6.1.2 Plants raised in *In vivo* conditions*:* 15 days old stage

6.1.2.1 Growth attributes

A notable decline in root length consequent to Pb stress treatments was recorded and summarized in (Fig. 6.10a; Table 6.10a). The highest curtailment of 58.66% was marked at T_3 and it was above 34.44% in T_1 plants vis-a-vis CN plants. The results further divulged that 24-EBL and Si remarkably alleviated the Pb noxiousness by amplifying the root length. With regard to EBL pre-treated plants under Pb stress, maximum root length i.e. 3.56 cm was noted at T_5 concentration. Supplementation of Si also brought forth an improvement in the root length of Pb stressed plants with being its highest at T_9 treatment (3.76 cm). Root length results were comparatively better in case of Si pre-treatment than EBL augmentation while battling out the Pb stress from *Trigonella* plants. The cumulative treatment of 24-EBL + Si also amplified the root length in Pb exposed plants, exhibiting highest root length of 8.10 cm at T_{13}. An enhancement of 193.478% in root length got noticed at T_{13} in correlation to T_1 treatment plants. Plants at T_{13} treatment were recorded to have a 115.42% elevation than the plants of T_9 treatment. Additionally, a contrast made between T_{13} and T_{10} plants, 150.77% enhancement was shown by T_{13} plants. T_{14} treated plants had 29.45% increase in root length compared to control plants.

Shoot length exhibited Pb stress induced reduction, being at its lowest with a value of 4.24 cm i.e. at T_3, 55.41% lower than CN plants. EBL and Si control plants displayed escalations of shoot length (Fig. 6.10a; Table 6.10a). Under Pb toxicity, EBL treatment raised the shoot length to 9.44 cm at T_5 treatment as correlated to individual treatment of Pb I concentration which stands at 8.39 cm. On the other hand, Si treated stressed plants followed the same trend of enhancement in their shoot length. It made the shoot length to increase with its maximum value at T_9 (9.8 cm). Collectively applied EBL and Si in Pb stress turned out to be more beneficial reaching its highest value of 10.13 cm at T_{13}. Shoot length recorded a reduction concomitant with every elevation in toxic concentrations of Pb in response to cumulative action of EBL + Si. T_9 plants exhibited 55.30% elevation of shoot length in correlation with T_2. Similarly, 48.34% augmentation was noted in T_7 than T_3. Furthermore, T_{13} had 6.51% more long shoots than CN plants.

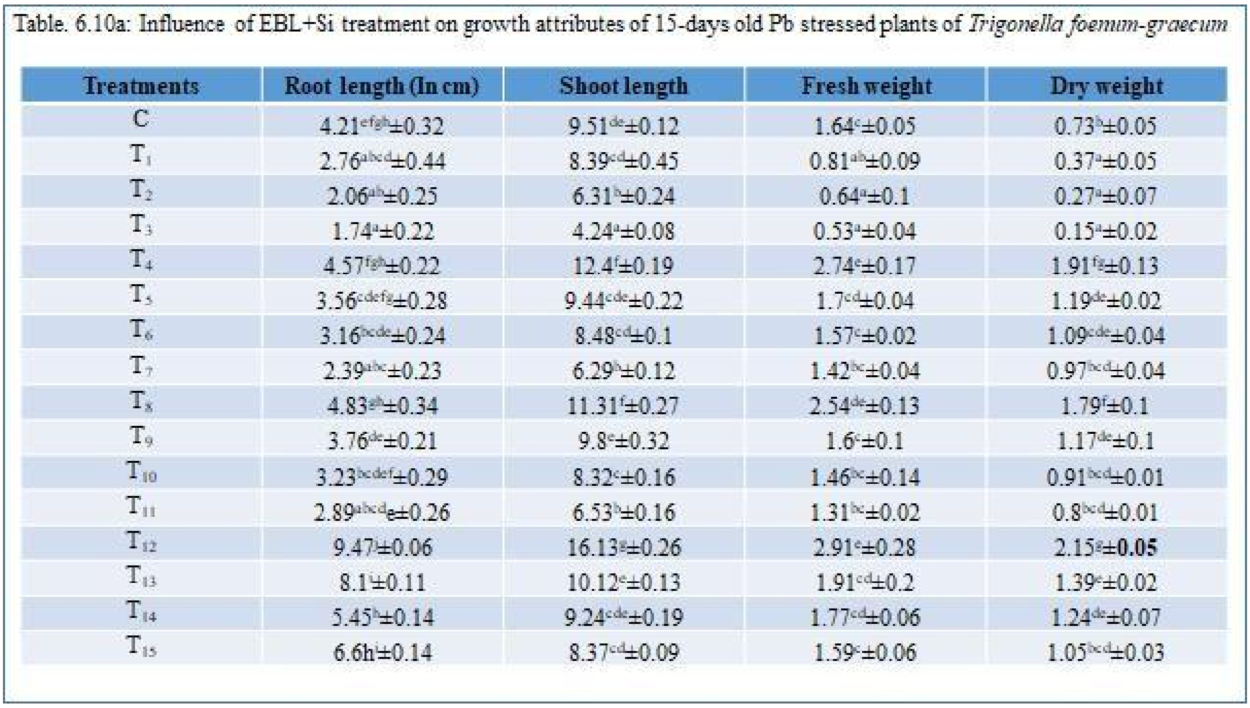
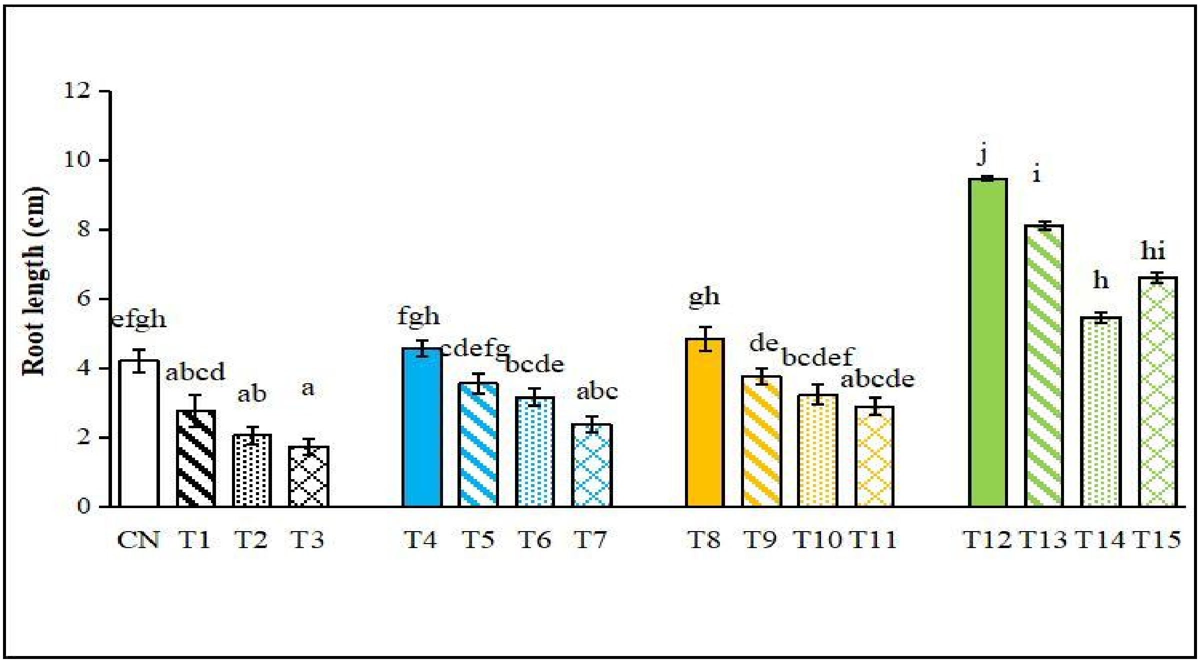


Table. 6.10a: Influence of EBL+Si treatment on growth attributes of 15-days old Pb stressed plants of *Trigonella foemum-graecum*

Treatments	Root length (In cm)	Shoot length	Fresh weight	Dry weight
C	$4.21^{efgh}\pm0.32$	$9.51^{de}\pm0.12$	$1.64^{c}\pm0.05$	$0.73^{b}\pm0.05$
T_1	$2.76^{abcd}\pm0.44$	$8.39^{cd}\pm0.45$	$0.81^{ab}\pm0.09$	$0.37^{a}\pm0.05$
T_2	$2.06^{ab}\pm0.25$	$6.31^{b}\pm0.24$	$0.64^{a}\pm0.1$	$0.27^{a}\pm0.07$
T_3	$1.74^{a}\pm0.22$	$4.24^{a}\pm0.08$	$0.53^{a}\pm0.04$	$0.15^{a}\pm0.02$
T_4	$4.57^{fgh}\pm0.22$	$12.4^{f}\pm0.19$	$2.74^{e}\pm0.17$	$1.91^{fg}\pm0.13$
T_5	$3.56^{cdefg}\pm0.28$	$9.44^{cde}\pm0.22$	$1.7^{cd}\pm0.04$	$1.19^{de}\pm0.02$
T_6	$3.16^{bcde}\pm0.24$	$8.48^{cd}\pm0.1$	$1.57^{c}\pm0.02$	$1.09^{cde}\pm0.04$
T_7	$2.39^{abc}\pm0.23$	$6.29^{b}\pm0.12$	$1.42^{bc}\pm0.04$	$0.97^{bcd}\pm0.04$
T_8	$4.83^{gh}\pm0.34$	$11.31^{f}\pm0.27$	$2.54^{de}\pm0.13$	$1.79^{f}\pm0.1$
T_9	$3.76^{de}\pm0.21$	$9.8^{e}\pm0.32$	$1.6^{c}\pm0.1$	$1.17^{de}\pm0.1$
T_{10}	$3.23^{bcdef}\pm0.29$	$8.32^{c}\pm0.16$	$1.46^{bc}\pm0.14$	$0.91^{bcd}\pm0.01$
T_{11}	$2.89^{abcd}e\pm0.26$	$6.53^{b}\pm0.16$	$1.31^{bc}\pm0.02$	$0.8^{bcd}\pm0.01$
T_{12}	$9.47^{j}\pm0.06$	$16.13^{g}\pm0.26$	$2.91^{e}\pm0.28$	$2.15^{g}\pm$**0.05**
T_{13}	$8.1^{i}\pm0.11$	$10.12^{e}\pm0.13$	$1.91^{cd}\pm0.2$	$1.39^{e}\pm0.02$
T_{14}	$5.45^{h}\pm0.14$	$9.24^{cde}\pm0.19$	$1.77^{cd}\pm0.06$	$1.24^{de}\pm0.07$
T_{15}	$6.6h^{i}\pm0.14$	$8.37^{cd}\pm0.09$	$1.59^{c}\pm0.06$	$1.05^{bcd}\pm0.03$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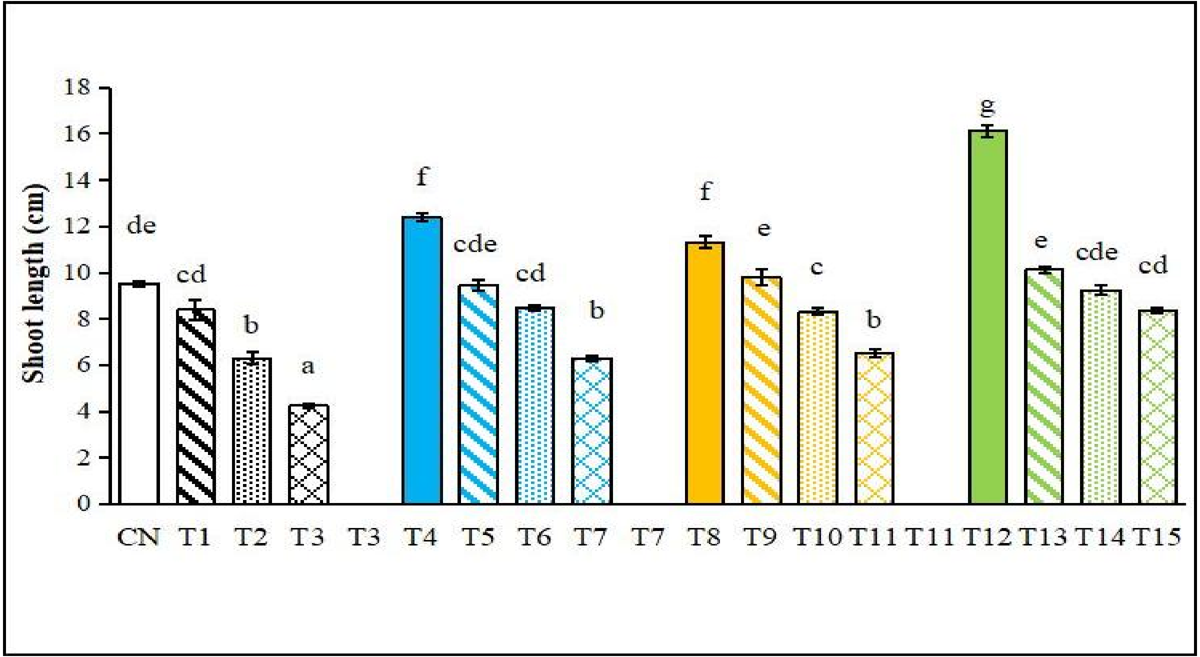
T15


Shoot length (cm)
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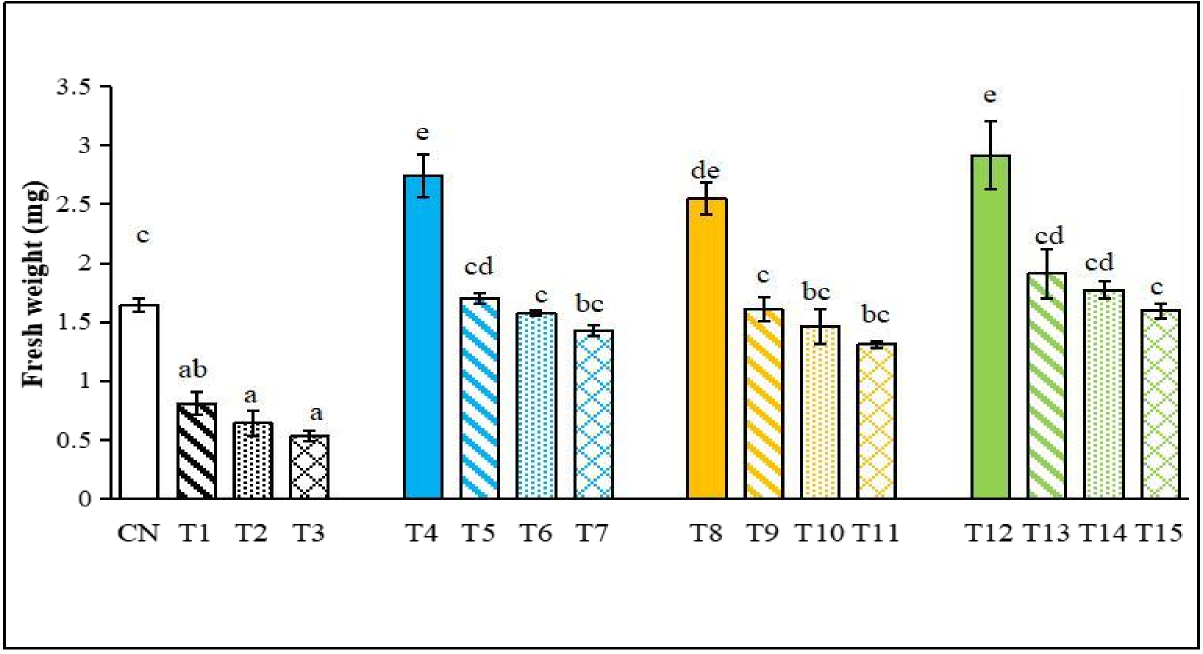
2
4
6
8
10
12
14
16
18
de
cd
b
a
f
cde
cd
b
f
e
c
b
g
e
cde
cd
CN
T1
T2
T3
T3
T4
T5
T6
T7
T7
T8
T9
T10
T11
T11
T12
T13
T14
T15


Fresh weight (mg)
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0.5
1
1.5
2
2.5
3
3.5
c
ab
a
a
e
cd
c
bc
de
c
bc
bc
e
cd
cd
c
CN
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
T13
T14
T15

Fig. 6.10 (a): Influence of EBL+Si treatment on growth attributes in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

A conspicuous negative impact on fresh weight of Pb stress exposed fenugreek plants was noted which was declined to its maximum at T_3 treatment i.e. 67.68% lesser than that of untreated plants kept as control (Fig. 6.10a; Table 6.10a). Further, pre-treatment of stressed plants with EBL i.e. T_5 treatment resulted in enhancing the fresh weight value to 1.70 g from being 0.81 g at T_1 with a percentage increase of 109.8%. A similar improvement in fresh weight of stressed fenugreek plants was achieved with Si treatment, exhibiting a highest to lowest range of 1.60g (T_9) to 1.31g (T_{11}) in treated plants. The highest augmentation in fresh weight took place at T_{13} (EBL + Si treatment in Pb I stressed plants) having a fresh weight value of 1.91g. Comparison of T_{13} with Pb III alone treated plants at T_3 exhibited a maximal enhancement of 260.3%. Similar correlation of T_{13} with CN, T_5 and T_9 treatments, exhibited a respective enhancement of 16.46%, 12.35% and 19.37%.

With regard to dry weight, a reduction of 48.50%, 63.31% and 78.53% was recorded respectively at T_1, T_2 and T_3 treatments of Pb alone stress vis-à-vis unstressed control plants. (Fig. 6.10b; Table 6.10a).Treating Pb stress exposed plants individually with EBL and Si led to an improvement in dry weight with a highest percentage increase of 653.6 and 642.8% at T_5 and T_9 respectively in correlation to T_3 plants.

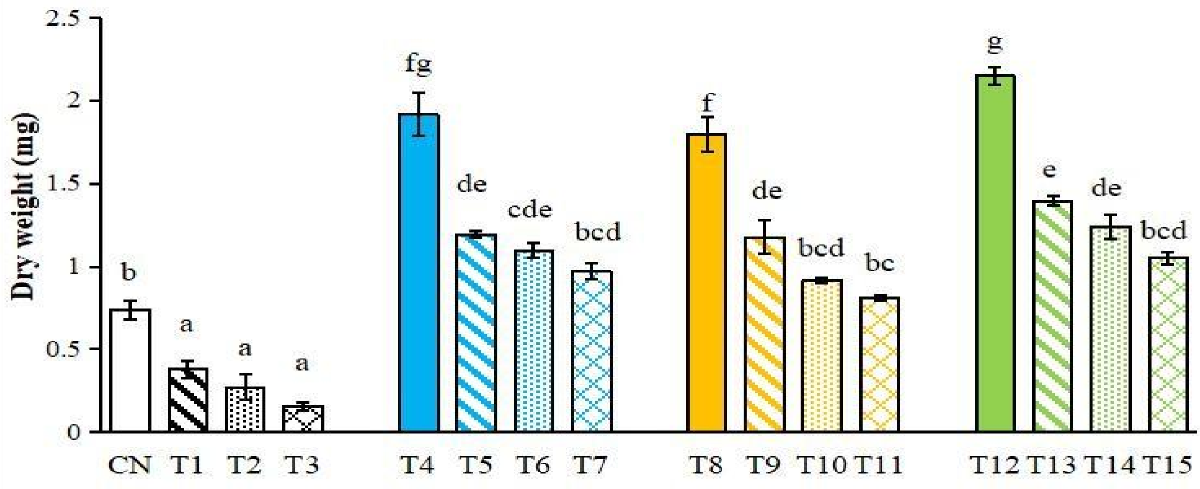


Fig. 6.10 (b): Influence of EBL+Si treatment on growth attributes in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Cumulatively applied mitigants i.e. EBL and Si yielded much better outcomes in comparison to their individual applications with being at its highest around 1.43g of T_{13} treatment. A correlation drawn between T_7 and T_3 plus T_9 and T_3 revealed a respective augmentation of 62.68% and 34.32% in dry weights. T_{13} resulted in improving the dry weight by 37.5% in comparison to CN plants.

Germination percentage of fenugreek seeds experienced a negative impact on account of Pb toxicity (Fig. 6.10c; Table 6.10b). Every elevation in toxic concentration of Pb lowered the germination percentage down i.e. 67.47% at T_1 got reduced to 52.37% of T_3 with being 56.08% around T_2. Control seeds exhibited 84.19% of germination percentage. Amongst all three discrete concentrations of Pb toxicity, pre-treatment of stressed seeds with EBL displayed the highest germination extent of 78.08% in T_5. Si augmentation to unstressed plants was observed to have higher value of germination percentage i.e. 88.44% in contrast to untreated CN plants. Furthermore, the Si treatment brought about an increase of 42.04% in germination in case of T_9 when contrasted against T_3, the treatment with highest concentration of Pb. Maximum percentage of germination was obtained when stressed plants were treated cumulatively with EBL plus Si at T_{15}, which was 64.96 %, 10.64% and 16.13% greater than T_3, T_5 and T_9 respectively.

Reduction in vigor index (VI) was also an outcome of stress induced by Pb metal in fenugreek as shown by T_1 plants (864%) with being reduced nearly half to that of untreated plants of control (1213.81%). Further, plants of T_2 treatment were noted to have VI value of 560% i.e. 35.18% lesser in contrast with T_1 treated plants (Fig. 6.10c; Table 6.10b). Individually supplemented EBL and Si under Pb metal stress elevated the VI values. Maximum value of VI (1357%) was observed around Pb I concentration in T_5 plants pre-treated with EBL. Exogenous augmentation of Si also escalated the VI values with being highest at T_9 treatment i.e. 1300%, which revealed that 24-EBL was way more effective in enhancing the VI in response to Pb stress. However, when coupled, EBL and Si showed maximum elevation in the VI values amongst all treatments i.e. 1548% at T_{13} against 864% of Pb I alone augmented T_1 plants making the elevation as high as 79.16%. T_{13} plants correlated with T_5 and T_9 were recorded to exhibit a respective increase of 14.07% and 19.07 % whereas in their comparison to untreated control plants, this enhancement stood at 27.61%.

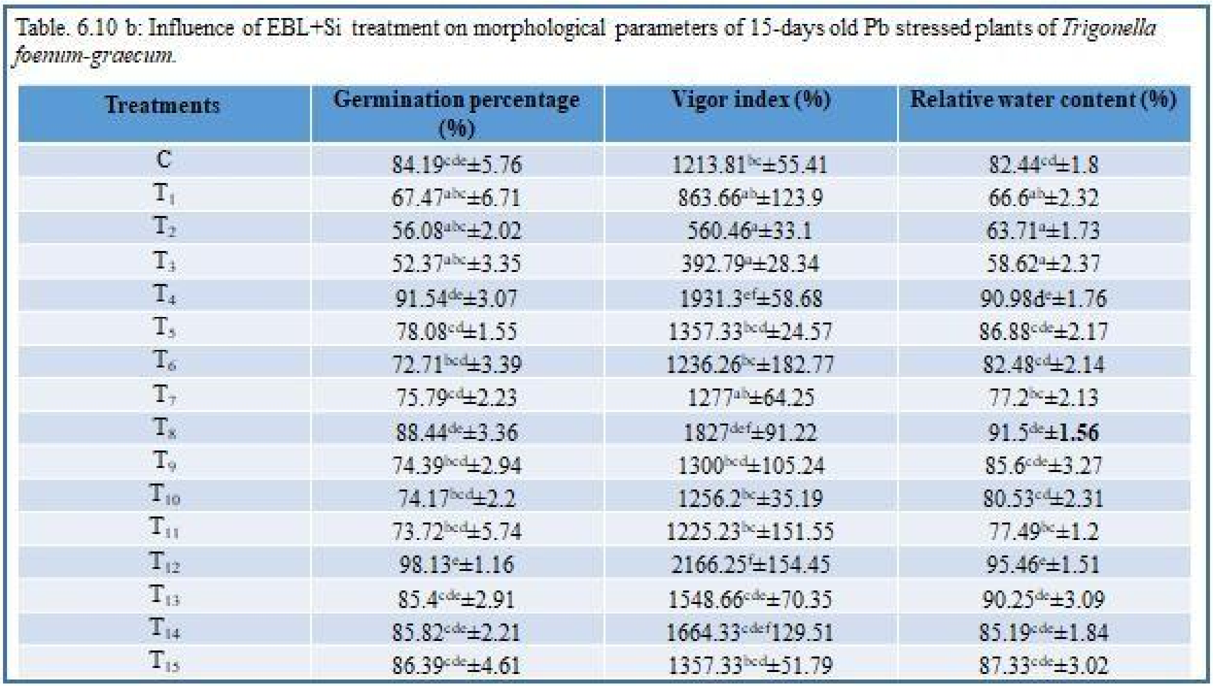
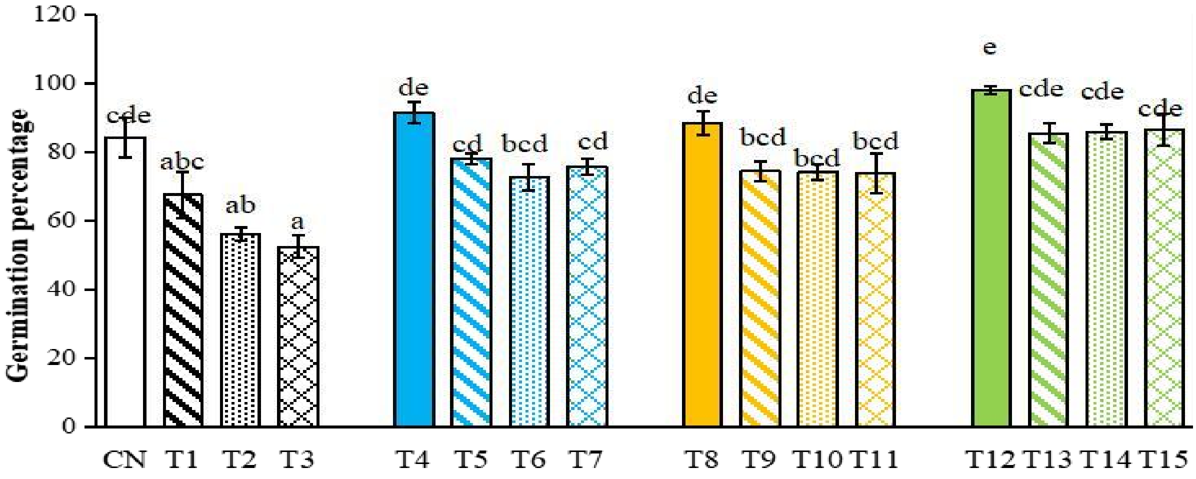


Table. 6.10 b: Influence of EBL+Si treatment on morphological parameters of 15-days old Pb stressed plants of *Trigonella foenum-graecum.*

Treatments	Germination percentage (%)	Vigor index (%)	Relative water content (%)
C	$84.19^{cde}\pm5.76$	$1213.81^{bc}\pm55.41$	$82.44^{cd}\pm1.8$
T_1	$67.47^{abc}\pm6.71$	$863.66^{ab}\pm123.9$	$66.6^{ab}\pm2.32$
T_2	$56.08^{abc}\pm2.02$	$560.46^{a}\pm33.1$	$63.71^{a}\pm1.73$
T_3	$52.37^{abc}\pm3.35$	$392.79^{a}\pm28.34$	$58.62^{a}\pm2.37$
T_4	$91.54^{de}\pm3.07$	$1931.3^{ef}\pm58.68$	$90.98d^{e}\pm1.76$
T_5	$78.08^{cd}\pm1.55$	$1357.33^{bcd}\pm24.57$	$86.88^{cde}\pm2.17$
T_6	$72.71^{bcd}\pm3.39$	$1236.26^{bc}\pm182.77$	$82.48^{cd}\pm2.14$
T_7	$75.79^{cd}\pm2.23$	$1277^{ab}\pm64.25$	$77.2^{bc}\pm2.13$
T_8	$88.44^{de}\pm3.36$	$1827^{def}\pm91.22$	$91.5^{de}\pm$**1.56**
T_9	$74.39^{bcd}\pm2.94$	$1300^{bcd}\pm105.24$	$85.6^{cde}\pm3.27$
T_{10}	$74.17^{bcd}\pm2.2$	$1256.2^{bc}\pm35.19$	$80.53^{cd}\pm2.31$
T_{11}	$73.72^{bcd}\pm5.74$	$1225.23^{bc}\pm151.55$	$77.49^{bc}\pm1.2$
T_{12}	$98.13^{e}\pm1.16$	$2166.25^{f}\pm154.45$	$95.46^{e}\pm1.51$
T_{13}	$85.4^{cde}\pm2.91$	$1548.66^{cde}\pm70.35$	$90.25^{de}\pm3.09$
T_{14}	$85.82^{cde}\pm2.21$	$1664.33^{cdef}129.51$	$85.19^{cde}\pm1.84$
T_{15}	$86.39^{cde}\pm4.61$	$1357.33^{bcd}\pm51.79$	$87.33^{cde}\pm3.02$



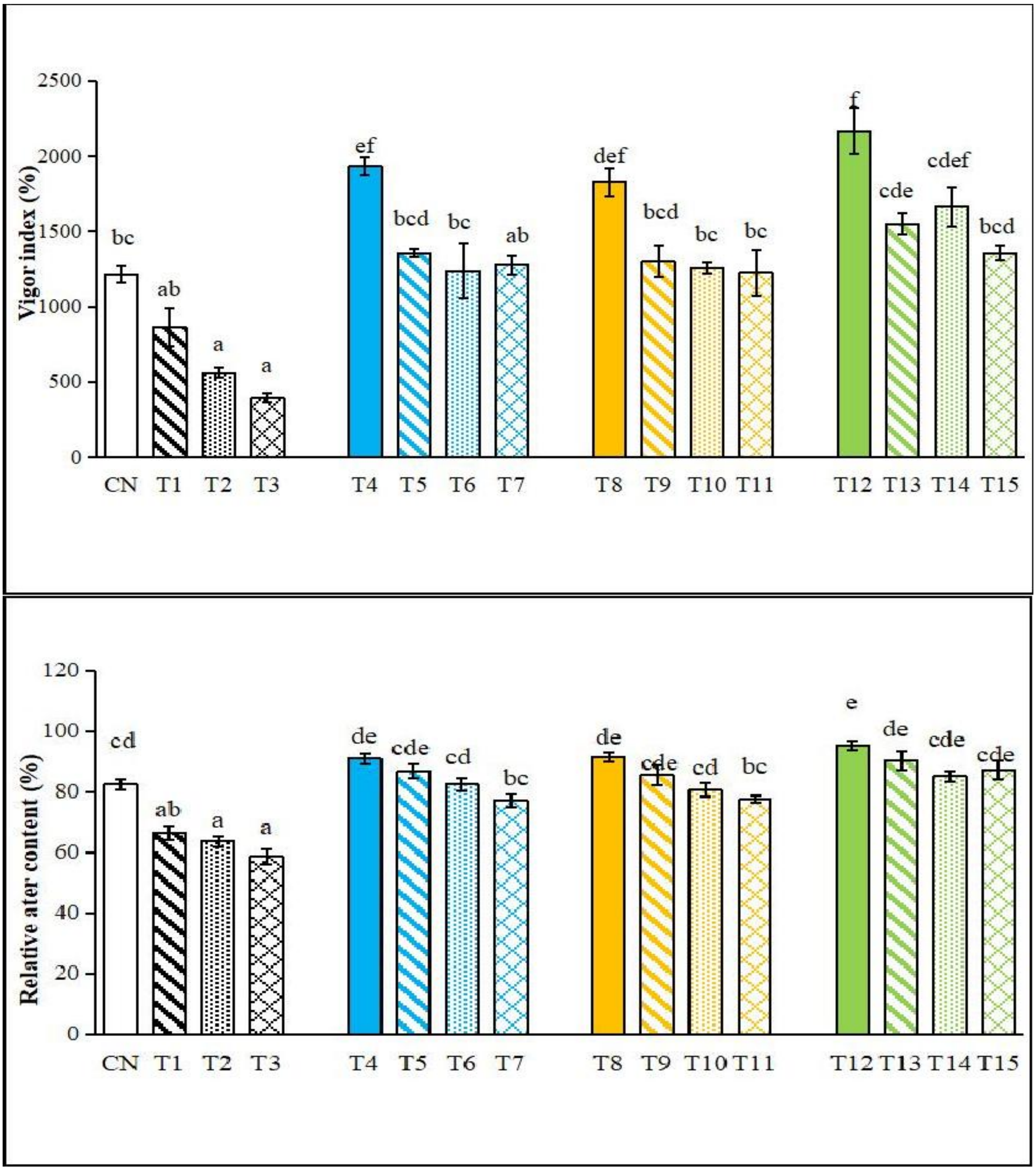


Fig. 6.10 (c): Influence of EBL+Si treatment on germination percentage, vigor index and RWC in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05

Ascertainment of RWC-"Relative water content" in Pb metal stressed fenugreek plants has been summed up in Fig. 6.10c and Table 6.10b. A diminishing effect of Pb induced stress with increasing levels of Pb concentrations was evident from being 66.6% at T_1 treatment to 58.62% at T_3. Control plants were noted to have RWC value of 82.44%. Amongst the three discrete Pb applications in plants having EBL pre-treatment, highest value of RWC was observed at T_5 i.e. 86.88% (T_5). Si treatment without metal stress exhibited RWC value (91.5%) even higher than the CN plants Augmentation of exogenous Si under Pb toxicity had the least RWC value around T_{11} of 77.49%. EBL + Si treatments were noticed to have maximum RWC value at T_{13} i.e. 90.25%. T_{13} displayed a respective enhancement of 4.12%, 5.67 and 9.72%.

6.1.2.2 Photosynthetic activity

6.1.2.2.1 Photosynthetic pigments

Pigments - Chlorophyll a and Chlorophyll b along with total content of chlorophyll suffered negatively in their measures in Pb stress subjected plants (Fig. 6.11a; Table 6.11a). Least amount of total chlorophyll i.e. 1.6 mg g^{-1} FW was noticed in T_3 plants which were augmented with maximum concentration of Pb. Treatment of stressed plants with EBL and Si alone concentrations, upregulated the amount of total chlorophyll in correlation to Pb alone treated T_3 plants, with being 3.72 mg g^{-1} FW at T_5 and 3.46 mg g^{-1} FW at T_9 which were respectively 132.5% and 116.25% in terms of percentage increase. Synergistically treated plants with EBL and Si yielded better outcomes in improving the measures of total chlorophyll content in response to metal toxicity, when put to a contrast with individual treatments. Under stressed conditions, the highest level (4.01 mg g^{-1} FW) was noticed in T_{13} treated plants. An enhancement of 7.79% and 15.89% was shown by plants in T_{13} stage in correlation to T_5 and T_9 respectively. T_{13} results were 20.05% higher than the CN plants. Results were obtained in the similar fashion with respect to content of Chl a and b. The least amount of chl a (1.62 mg g^{-1} FW) and chl b (1.31 mg g^{-1} FW) were recorded at T_3 treatment. Furthermore, coupled supplementation with EBL and Si remarkably ameliorated the Pb induced diminishing impact by elevating the measures of these pigments and noted to be maximum around 3.66 and 2.97 mg g^{-1} FW for Chl a and b respectively at treatment T_{14} and T_{13} . An elevation of 18.44 and 15.56% in chl a and chl b respectively at T_5 together with 32.13% and 30.83% at T_9 was T_{14} and T_{13} respectively.

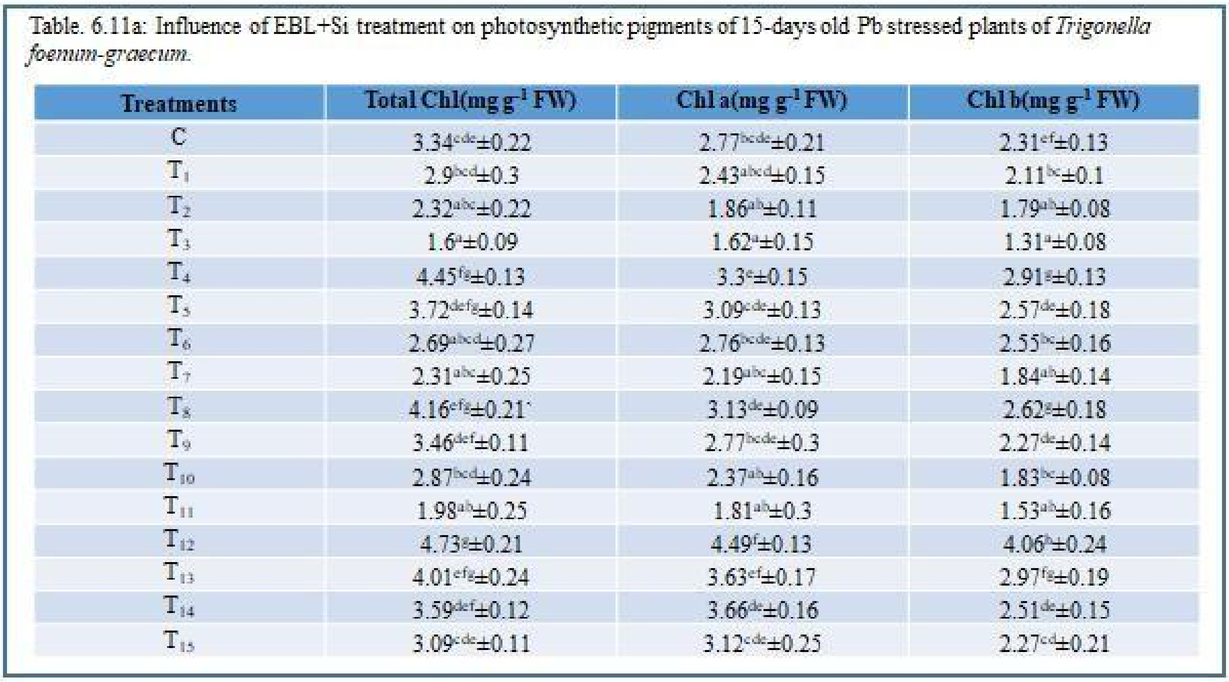
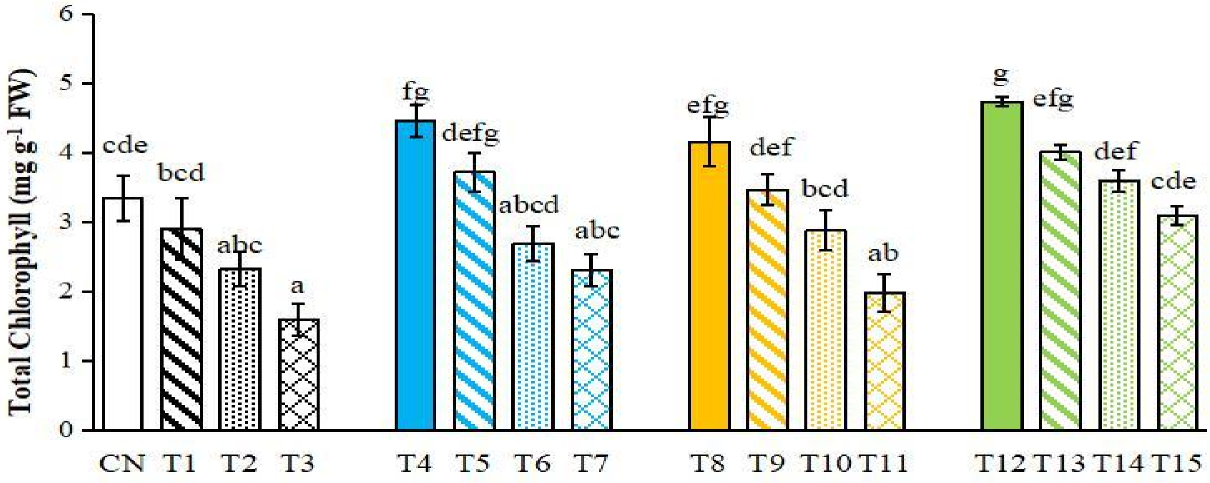


Table. 6.11a: Influence of EBL+Si treatment on photosynthetic pigments of 15-days old Pb stressed plants of *Trigonella foemum-graecum.*

Treatments	Total Chl(mg g^{-1} FW)	Chl a(mg g^{-1} FW)	Chl b(mg g^{-1} FW)
C	$3.34^{cde}\pm0.22$	$2.77^{bcde}\pm0.21$	$2.31^{ef}\pm0.13$
T_1	$2.9^{bcd}\pm0.3$	$2.43^{abcd}\pm0.15$	$2.11^{bc}\pm0.1$
T_2	$2.32^{abc}\pm0.22$	$1.86^{ab}\pm0.11$	$1.79^{ab}\pm0.08$
T_3	$1.6^{a}\pm0.09$	$1.62^{a}\pm0.15$	$1.31^{a}\pm0.08$
T_4	$4.45^{fg}\pm0.13$	$3.3^{e}\pm0.15$	$2.91^{g}\pm0.13$
T_5	$3.72^{defg}\pm0.14$	$3.09^{cde}\pm0.13$	$2.57^{de}\pm0.18$
T_6	$2.69^{abcd}\pm0.27$	$2.76^{bcde}\pm0.13$	$2.55^{bc}\pm0.16$
T_7	$2.31^{abc}\pm0.25$	$2.19^{abc}\pm0.15$	$1.84^{ab}\pm0.14$
T_8	$4.16^{efg}\pm0.21$	$3.13^{de}\pm0.09$	$2.62^{g}\pm0.18$
T_9	$3.46^{def}\pm0.11$	$2.77^{bcde}\pm0.3$	$2.27^{de}\pm0.14$
T_{10}	$2.87^{bcd}\pm0.24$	$2.37^{ab}\pm0.16$	$1.83^{bc}\pm0.08$
T_{11}	$1.98^{ab}\pm0.25$	$1.81^{ab}\pm0.3$	$1.53^{ab}\pm0.16$
T_{12}	$4.73^{g}\pm0.21$	$4.49^{f}\pm0.13$	$4.06^{h}\pm0.24$
T_{13}	$4.01^{efg}\pm0.24$	$3.63^{ef}\pm0.17$	$2.97^{fg}\pm0.19$
T_{14}	$3.59^{def}\pm0.12$	$3.66^{de}\pm0.16$	$2.51^{de}\pm0.15$
T_{15}	$3.09^{cde}\pm0.11$	$3.12^{cde}\pm0.25$	$2.27^{cd}\pm0.21$



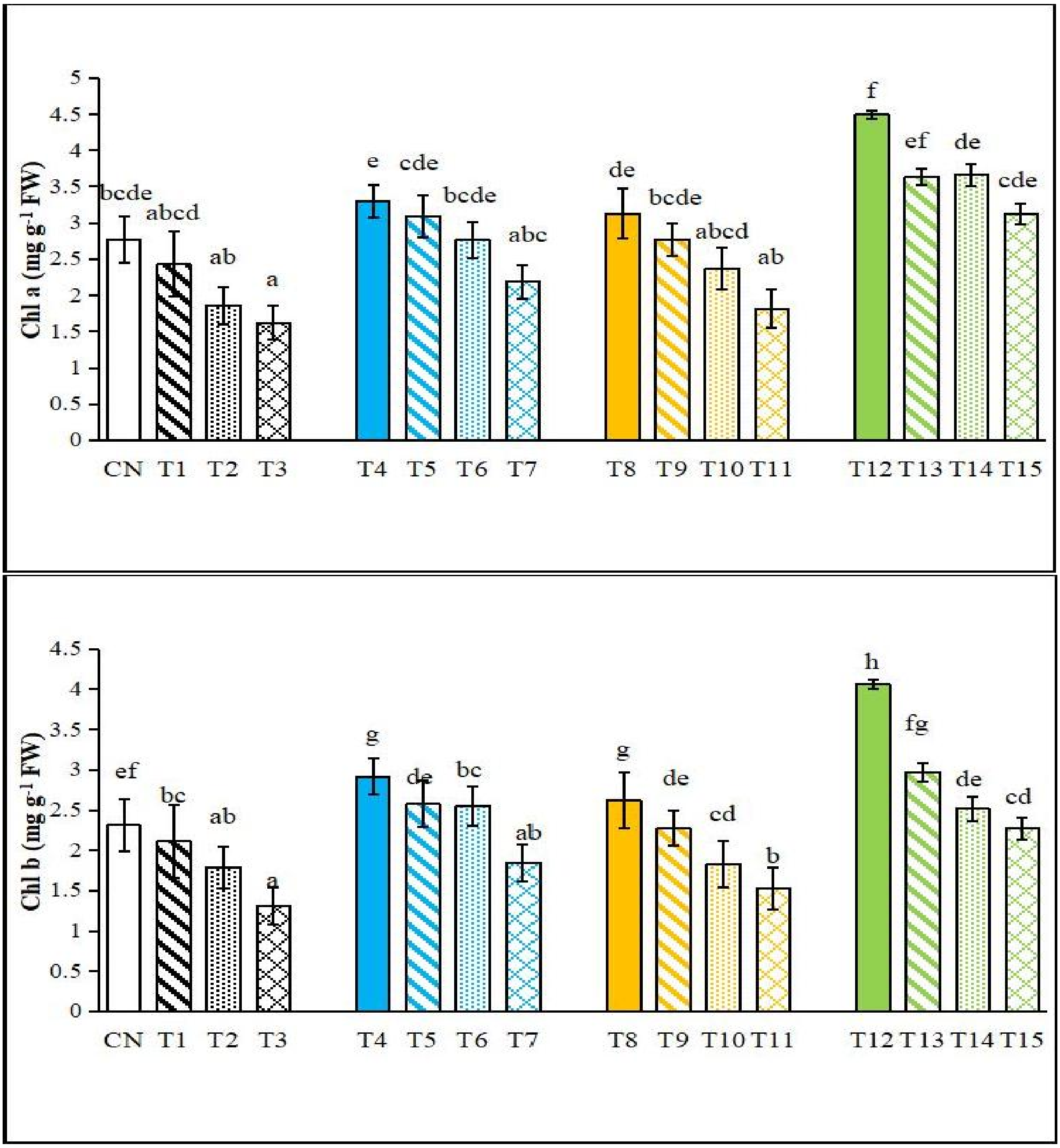


Fig. 6.11 (a): Influence of EBL + Si treatment on measures of photosynthetic pigments in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Pb stress was reported to diminish the measures of carotenoids (Fig. 6.11b; Table 6.11b). Least carotenoid content was recorded at T_1 treatment with a value of 4.65 mg g^{-1} FW content. Pre-treatment of EBL against Pb exposure up-scaled the carotenoid amount with being its highest at 5.49 mg g^{-1} FW in case of T_5 concentration. Carotenoid measures got improved from 4.65 mg g^{-1} FW to 5.69 mg g^{-1} FW in Si treated T_{10} plants, in correlation to Pb I exposed plants. Synergistically augmented EBL with Si further augmented the levels of carotenoids so that Pb induced toxic effects could be negated. The maximal level of 6.15 mg g^{-1} FW level was noted at T_{13} treatment while the minimal of 5.41 mg g^{-1} FW was reported at

T_{15} treatment. Its amount recorded an upsurge of 12.43%, 12.02% and 13.67% at control, T_5 and T_9 treatments in correlation to T_{13} plants.

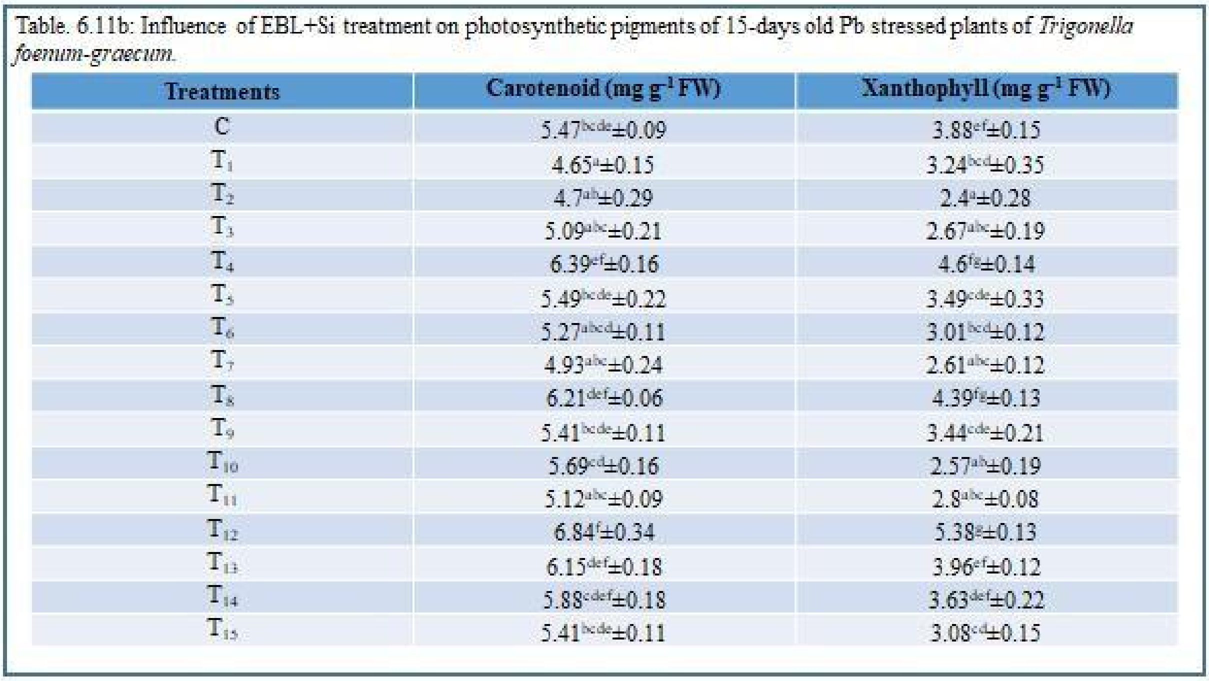


Table. 6.11b: Influence of EBL+Si treatment on photosynthetic pigments of 15-days old Pb stressed plants of *Trigonella foenum-graecum*.

Treatments	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
C	$5.47^{bcde}\pm0.09$	$3.88^{ef}\pm0.15$
T_1	$4.65^{a}\pm0.15$	$3.24^{bcd}\pm0.35$
T_2	$4.7^{ab}\pm0.29$	$2.4^{a}\pm0.28$
T_3	$5.09^{abc}\pm0.21$	$2.67^{abc}\pm0.19$
T_4	$6.39^{ef}\pm0.16$	$4.6^{fg}\pm0.14$
T_5	$5.49^{bcde}\pm0.22$	$3.49^{cde}\pm0.33$
T_6	$5.27^{abcd}\pm0.11$	$3.01^{bcd}\pm0.12$
T_7	$4.93^{abc}\pm0.24$	$2.61^{abc}\pm0.12$
T_8	$6.21^{def}\pm0.06$	$4.39^{fg}\pm0.13$
T_9	$5.41^{bcde}\pm0.11$	$3.44^{cde}\pm0.21$
T_{10}	$5.69^{cd}\pm0.16$	$2.57^{ab}\pm0.19$
T_{11}	$5.12^{abc}\pm0.09$	$2.8^{abc}\pm0.08$
T_{12}	$6.84^{f}\pm0.34$	$5.38^{g}\pm0.13$
T_{13}	$6.15^{def}\pm0.18$	$3.96^{ef}\pm0.12$
T_{14}	$5.88^{cdef}\pm0.18$	$3.63^{def}\pm0.22$
T_{15}	$5.41^{bcde}\pm0.11$	$3.08^{cd}\pm0.15$

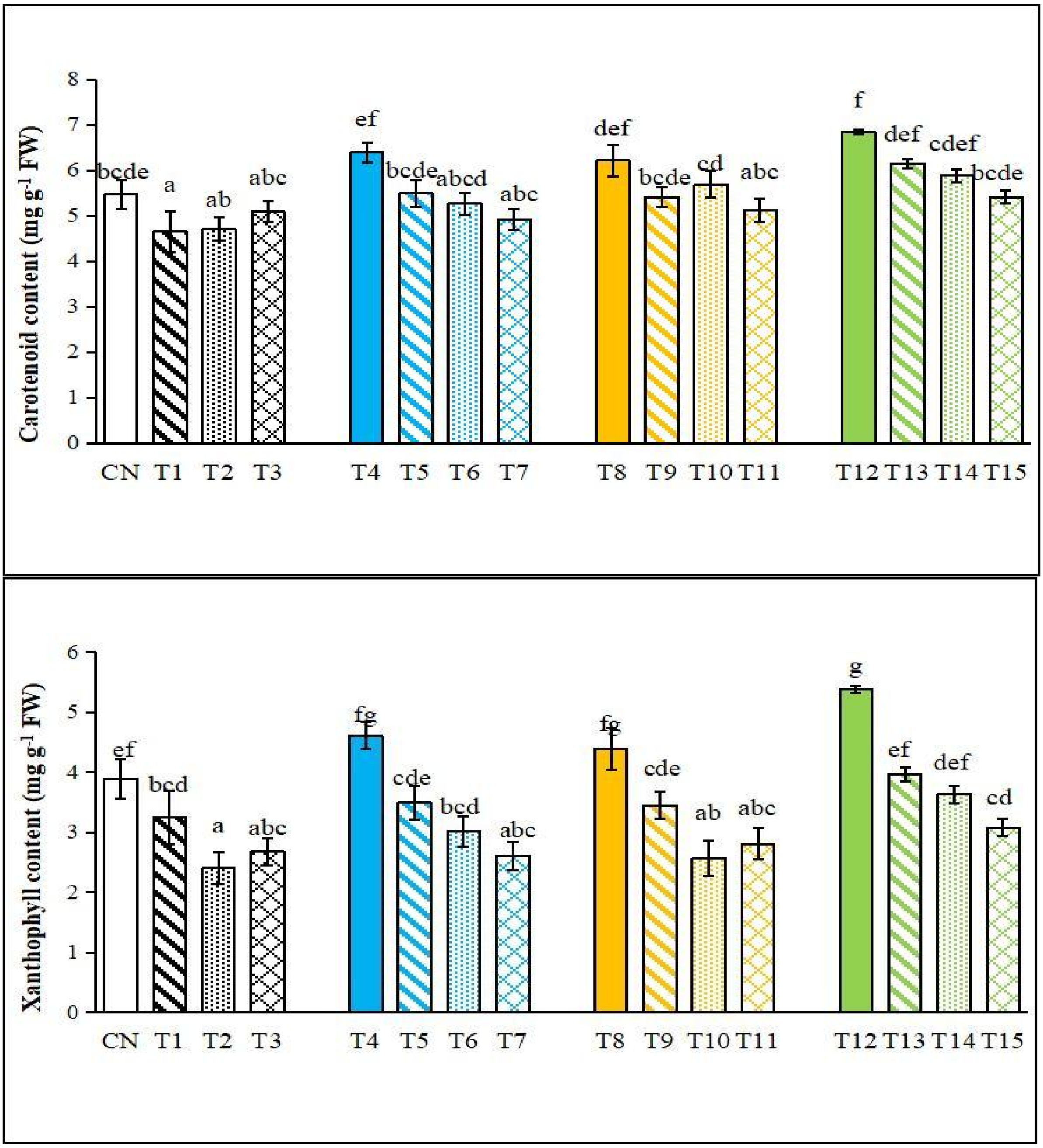


Fig. 6.11 (b): Influence of EBL+Si treatment on measures of photosynthetic pigments in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

Lead toxicity lowered down the xanthophyll measures in 15 days old *In vivo* raised fenugreek plants (Fig. 6.11b; Table 6.11b). The maximal decline in the amount of xanthophyll content i.e., 2.4 mg g^{-1} FW was reported in case of T_2 metal stressed plants. A curtailment of 25.92% in xanthophyll levels was noted in T_2 augmented plants as opposed to T_1. In response to separate applications of EBL and Si, highest values of xanthophyll were reported at T_5, T_9 with values of 3.49 and 3.44 and the lowest being observed at T_7, T_{10} with values of 2.61 and 2.57 mg g^{-1} FW, respectively. Upsurge in the amount of xanthophyll was yielded when both EBL and Si were augmented synergistically in order to mitigate Pb stress. Xanthophyll measures were boosted from 2.4 to 3.96 mg g^{-1} FW at T_{13} concentration as opposed to T_2 treatment. Augmentation of xanthophyll measures by 15.11% occurred in T_{13} than T_9. An elevation of 10% was noted in T_{15} in comparison to T_{11}. T_{13} displayed 2.06% upsurge in measures of xanthophyll than control plants.

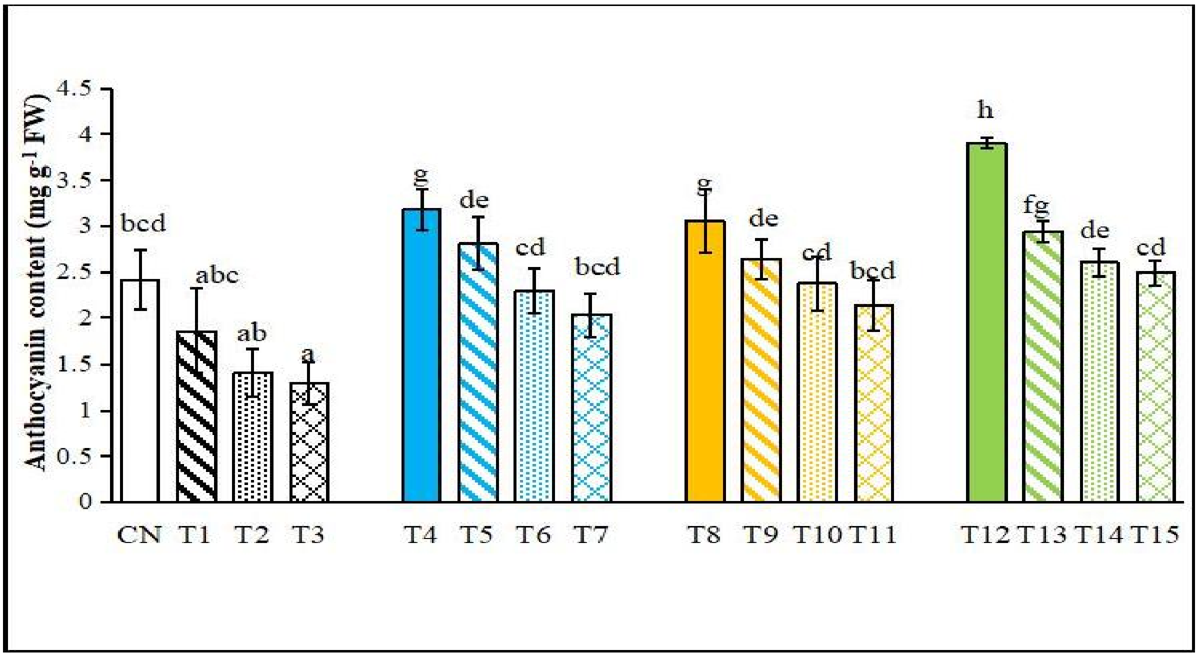
6.1.2.3 Metabolites

Anthocyanin was noted to have a declined content under Pb stress with being minimum (1.3 mg g^{-1} FW) at T_3 treatment which was 46.05% lower than the control (Fig. 6.12; Table 6.12). With respect to individually augmented mitigants i.e. EBL and Si in unstressed plants, T_4 plants (3.18 mg g^{-1} FW) were slightly better performers than T_8 plants (3.05 mg g^{-1} FW). Elevations in the anthocyanin levels were recorded at T_6 i.e. Anthocyanin content was enhanced from 1.86 to 2.29 mg g-1 FW as opposed to T_1 concentration. Feeding of Si against Pb toxicity led to the betterment of anthocyanin measures vis-à-vis plants stressed with Pb only. Anthocyanin content was noticed to be raised from 1.86 to 2.64 mg g^{-1} FW at T_9 concentration. Under Pb stress, Si augmentation was observed to be more efficient in elevating the anthocyanin measures in contrast to EBL treatment. Coupling of EBL with Si in combating Pb toxicity further elevated the measures of anthocyanin with a value of 2.94, 2.60 and 2.49 mg g^{-1} FW at T_{13}, T_{14} along with T_{15} augmented plants, respectively. T_7 showed a decrease of 5.41% when contrasted to T_{11} whereas T_{13} led to an enhancement of 22% than stress free plants under controlled conditions.

The least measures of flavonoid were recorded in T_3 plants augmented with maximum concentration of Pb only (1.24 mg g^{-1} FW; Fig. 6.12; Table 6.12). In all three treatments of Pb alone stress, a 15.74% at T_1, 28.7% at T_2 and 42.59% decrease at T_3 was observed when a correlation was made with control plants. Flavanoid content registered an upsurge of 43.4% from T_5 (EBL + Pb I) to Pb I alone treated T_1 plants. Silicon treatment was further recorded to escalate the measures of flavonoids vis-à-vis Pb alone treated plants. Levels of flavanoid were observed to be 2.73 at T_9, 2.58 at T_{10} and 2.39 mg g^{-1} FW at T_{11}, respectively. Flavanoid content pertaining to cumulative application of EBL + Si, the maximum ($T_{13;}$ 3.34 mg g^{-1} FW) and minimum ($T_{15;}$ 3.04 mg g^{-1} FW) values were noted. Its measures registered an augmentation of 10.13% in T_{11} over T_7 plants. T_{13} further displayed a 54.62% increment in relation to control plants.

Table. 6.12: Influence of EBL+Si treatment on metabolites of 15-days old Pb stressed plants of *Trigonella foenum-graecum*.

Treatments	Anthocyanin (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)
CN	$2.41^{bcd}\pm0.21$	$2.16^{defg}\pm0.17$	$3.43^{ef}\pm0.10$
T_1	$1.86^{abc}\pm0.36$	$1.82^{abc}\pm0.09$	$2.93^{bcd}\pm0.11$
T_2	$1.41^{ab}\pm0.21$	$1.54^{ab}\pm0.13$	$2.47^{a}\pm0.15$
T_3	$1.3^{a}\pm0.17$	$1.24^{a}\pm0.08$	$2.30^{abc}\pm0.17$
T_4	$3.18^{g}\pm0.37$	$3.39^{gh}\pm0.08$	$4.43^{fg}\pm0.13$
T_5	$2.80^{de}\pm0.18$	$2.61^{cdefg}\pm0.18$	$3.58^{cde}\pm0.12$
T_6	$2.29^{cd}\pm0.19$	$2.78^{defg}\pm0.24$	$3.21^{bcd}\pm0.17$
T_7	$2.03^{bcd}\pm0.37$	$2.17^{bcde}\pm0.16$	$2.72^{abc}\pm0.10$
T_8	$3.05^{g}\pm0.32$	$2.89^{efg}\pm0.12$	$3.90^{fg}\pm0.06$
T_9	$2.64^{de}\pm0.11$	$2.73^{defg}\pm0.13$	$3.43^{cde}\pm0.13$
T_{10}	$2.37^{cd}\pm0.06$	$2.58^{cdefg}\pm0.20$	$2.76^{ab}\pm0.10$
T_{11}	$2.14^{bcd}\pm0.19$	$2.39^{bcdef}\pm0.17$	$2.62^{abc}\pm0.12$
T_{12}	$3.91^{h}\pm0.10$	$4.03^{h}\pm0.17$	$4.87^{g}\pm0.21$
T_{13}	$2.94^{fg}\pm0.12$	$3.34^{gh}\pm0.26$	$3.94^{ef}\pm0.22$
T_{14}	$2.60^{de}\pm0.11$	$3.15^{fgh}\pm0.13$	$3.43^{def}\pm0.20$
T_{15}	$2.49^{cd}\pm0.08$	$3.04^{efg}\pm0.18$	$2.83^{cd}\pm0.60$


Anthocyanin content (mg g^{-1} FW)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
bcd
abc
ab
a
g
de
cd
bcd
g
de
cd
bcd
h
fg
de
cd
CN
T1
T2
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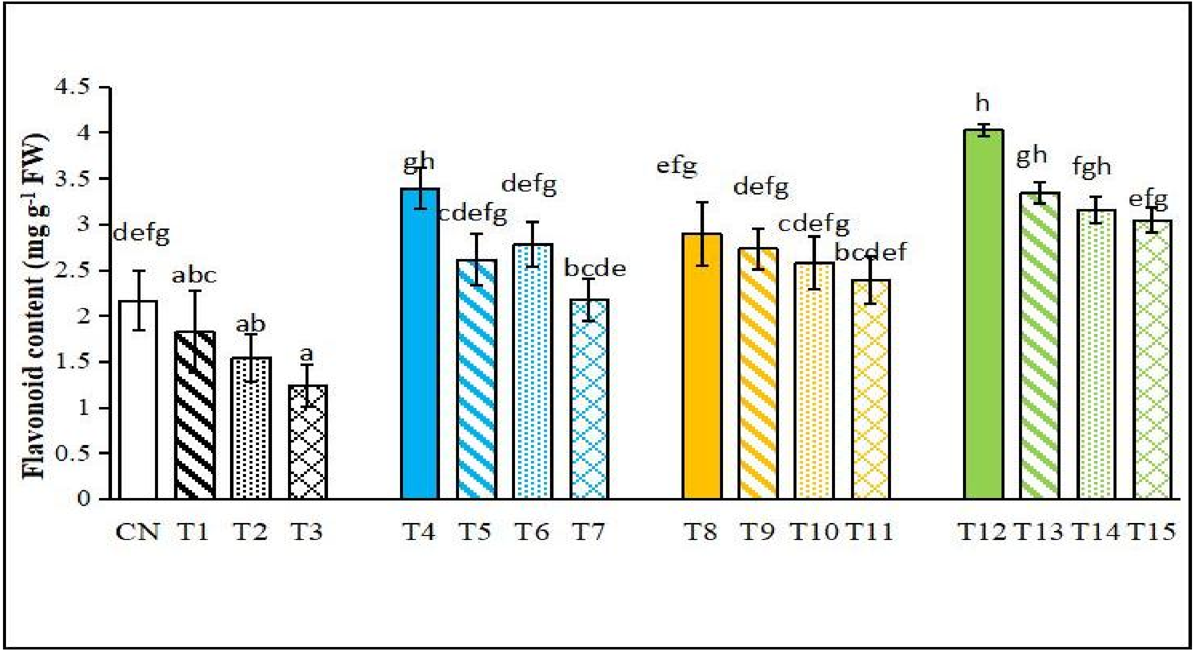
Flavonoid content (mg g^{-1} FW)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
defg
abc
ab
a
gh
cdefg
defg
bcde
efg
defg
cdefg
bcdef
h
gh
fgh
efg
CN
T1
T2
T3
T4
T5
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T8
T9
T10
T11
T12
T13
T14
T15

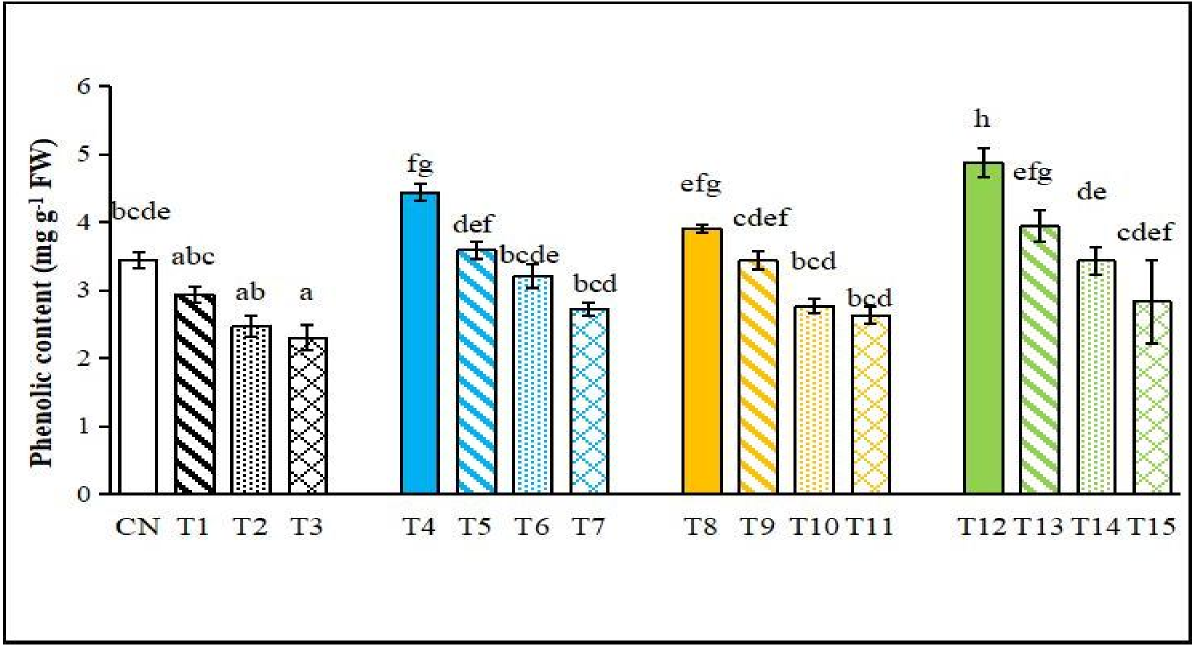

Phenolic content (mg g^{-1} FW)
6
5
4
3
2
1
0
bcde
abc
ab
a
fg
def
bcde
bcd
efg
cdef
bcd
bcd
h
efg
de
cdef
CN
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
T13
T14
T15

Fig. 6.12: Influence of EBL+Si treatment on measures of metabolites in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

With ascending levels of Pb stress, phenolics were noted to have a diminishing impact on their content (Fig. 6.12; Table 6.12). It was diminished to 2.30 mg g^{-1} FW (T_3) in correlation with control plants having 3.43 mg g^{-1} FW of phenolic content. Individually augmented EBL in stress free and stressed plants were recorded to have the highest measures of phenolics at T_4 (4.43 mg g^{-1} FW) and T_5 (3.58 mg g^{-1} FW) respectively. Exogenously applied Si, on the other hand, had the same values to be highest in case of T_8 (3.90 mg g^{-1} FW) and T_9 (3.43 mg g^{-1} FW). Combination of EBL and Si in Pb I stressed plants i.e. T_{13} showed greatest measures of phenolics at 3.94 mg g^{-1} FW while under control, EBL + Si made the phenolic content of T_{12} plants (highest 4.87 mg g^{-1} FW) amongst all the treatments chosen for the present study. An increment of 22.18% was recorded in T_5 over T_1. Additionally, T_{13} plants exhibited 10.05% and 14.86% increment in correlation to T_5 and control plants respectively.

6.1.2.4 Oxidative damage

6.1.2.4.1 Oxidative stress markers

Extent of lipid peroxidation was noted to be enhanced because of Pb-mediated upsurge in the measures of MDA, when contrasted against control plants (Fig. 6.13; Table 6.13). An increment of 41.71% in the quantities of MDA was noted in T_3 plants, which were stressed with highest concentration of Pb, as opposed to the control plants. However, exogenously supplemented EBL and Si curtailed this Pb induced enhancement in MDA measures. With respect to individually applied EBL and Si to stressed plants, MDA contents were registered to be the most reduced at T_5 and T_9 with respective values of 3.00 and 3.44 µmol g^{-1} FW measures. Additionally, MDA values were diminished from 4.16 at T_2 to 2.55 µmol g^{-1} FW at T_{14} treated plants. A decrement of 25.92% was noted in T_5 over T_1. T_9 plants displayed 15.06% decline in MDA measures than T_1. T_{14} plants had 15% depletion in contrast to T_5. Besides this, T_{14} recorded 24.55% decline vis-a-vis controlplants.

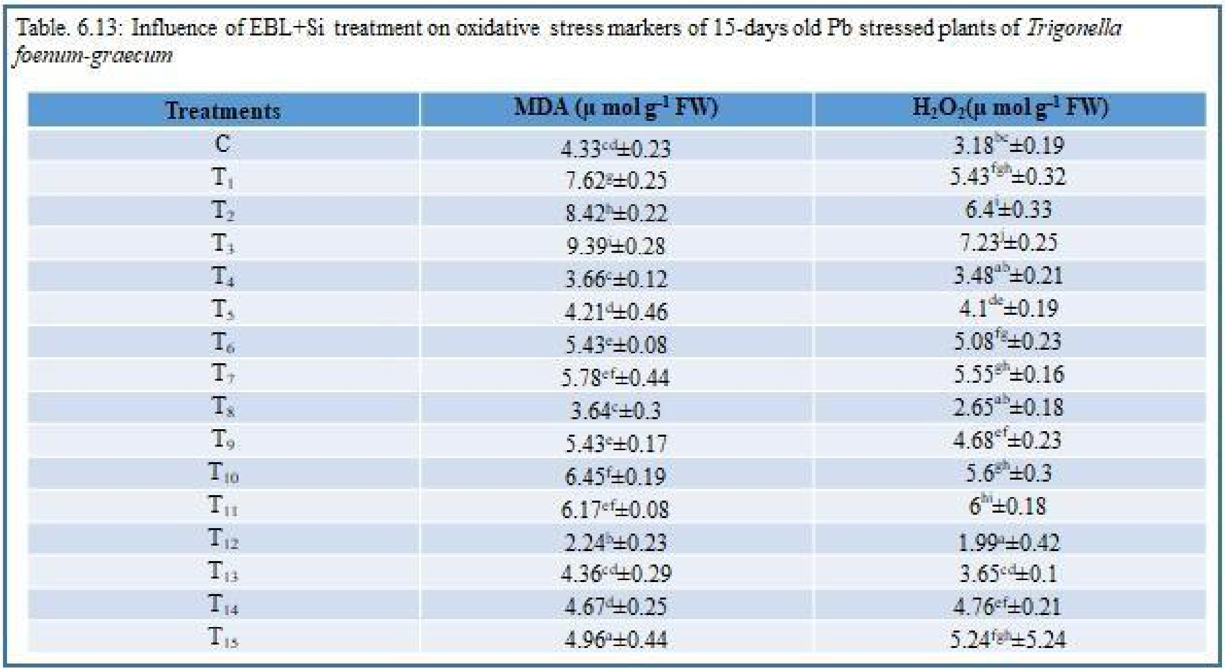


Table. 6.13: Influence of EBL+Si treatment on oxidative stress markers of 15-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatments	**MDA (μ mol g^{-1} FW)**	**H_2O_2(μ mol g^{-1} FW)**
C	$4.33^{cd}\pm0.23$	$3.18^{bc}\pm0.19$
T_1	$7.62^{g}\pm0.25$	$5.43^{fgh}\pm0.32$
T_2	$8.42^{h}\pm0.22$	$6.4^{i}\pm0.33$
T_3	$9.39^{i}\pm0.28$	$7.23^{j}\pm0.25$
T_4	$3.66^{c}\pm0.12$	$3.48^{ab}\pm0.21$
T_5	$4.21^{d}\pm0.46$	$4.1^{de}\pm0.19$
T_6	$5.43^{e}\pm0.08$	$5.08^{fg}\pm0.23$
T_7	$5.78^{ef}\pm0.44$	$5.55^{gh}\pm0.16$
T_8	$3.64^{c}\pm0.3$	$2.65^{ab}\pm0.18$
T_9	$5.43^{e}\pm0.17$	$4.68^{ef}\pm0.23$
T_{10}	$6.45^{f}\pm0.19$	$5.6^{gh}\pm0.3$
T_{11}	$6.17^{ef}\pm0.08$	$6^{hi}\pm0.18$
T_{12}	$2.24^{b}\pm0.23$	$1.99^{a}\pm0.42$
T_{13}	$4.36^{cd}\pm0.29$	$3.65^{cd}\pm0.1$
T_{14}	$4.67^{d}\pm0.25$	$4.76^{ef}\pm0.21$
T_{15}	$4.96^{a}\pm0.44$	$5.24^{fgh}\pm5.24$

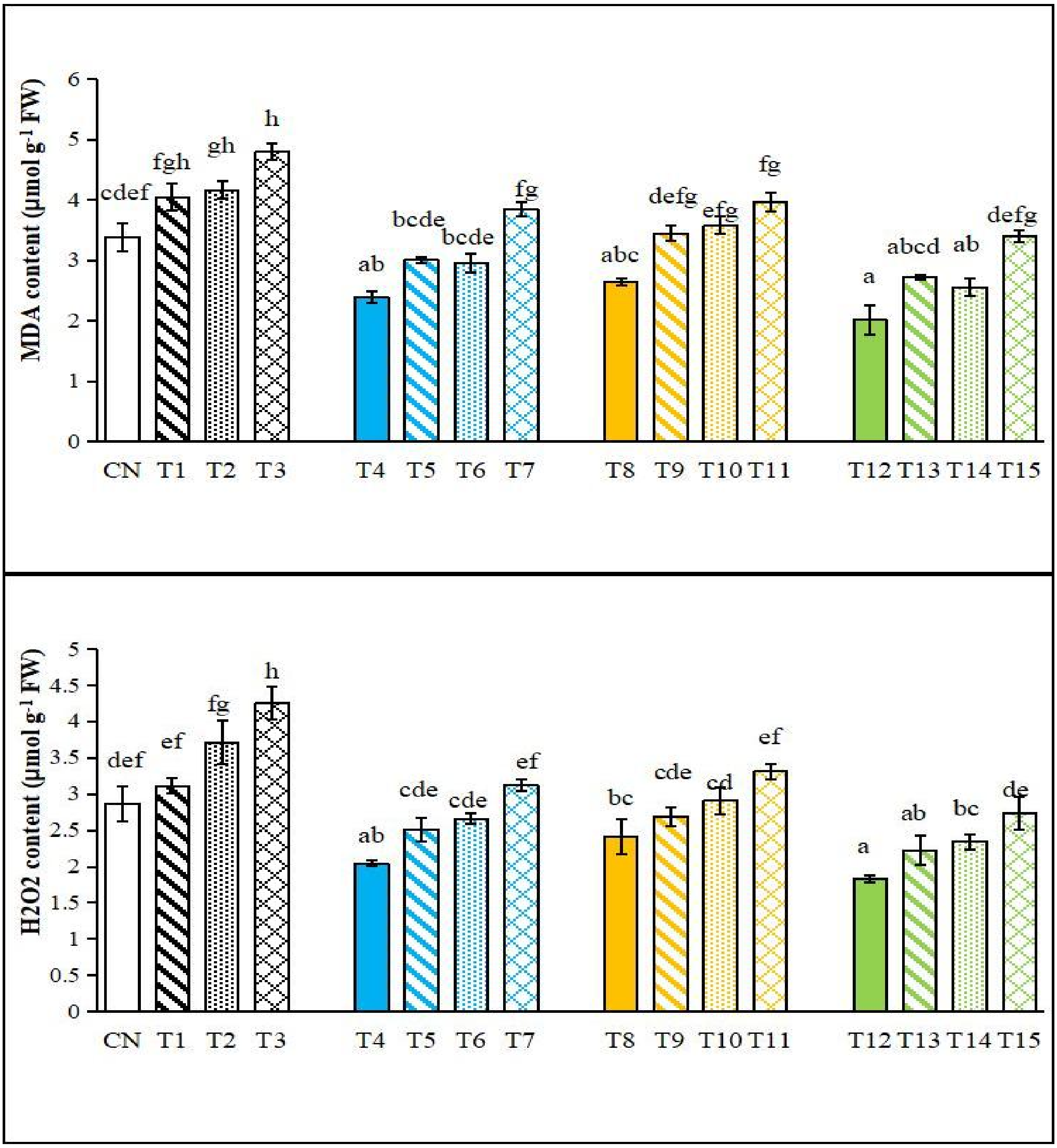


Fig. 6.13: Influence of EBL + Si treatment on measures of oxidative stress indicators in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

The measures of H_2O_2 were notably elevated in fenugreek plants upon getting subjected to Pb stress (Fig. 6.13; Table 6.13). An increase of 48% in the quantities of H_2O_2 got noticed in T_3 plants, which were stressed with highest concentration of Pb, as opposed to the control plants. Moreover, H_2O_2 measures were promoted to be 3.11 at T_1, 3.71 at T_2 and 4.25 µmolg^{-1} FW at T_3 treatments stressed with Pb metal alone. However, applying them with EBL, Si and EBL + Si against Pb toxicity diminished the amount of H_2O_2. The highest diminishing impact of mitigants was noticed in case of their coupled treatment at T_{13} with a value of 2.22 µmol g^{-1} FW. A decrement of 19.29% was noted in T_5 over T_1. T_9 plants registered a decline of 13.5%

when correlated with T_1. Furthermore, T_{13} plants were recorded to have 11.55 % and 7.17% reduction when placed against T_5 and $T_{9,}$ respectively.

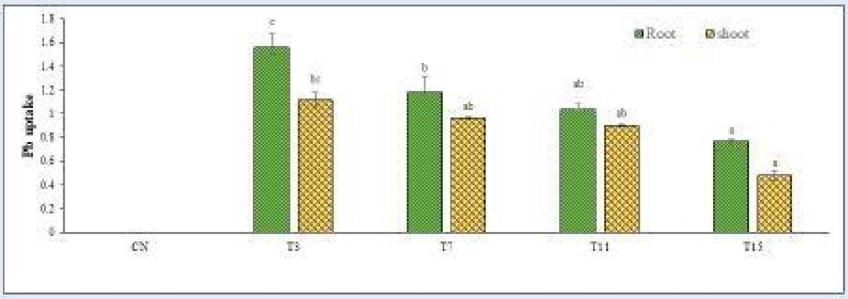
6.1.2.5 Lead metal uptake

No Pb content was detected in control samples i.e. *in vivo* grown 15 days old fenugreek plants (Fig 6.14; Table 6.14). Under individual stress of Pb, 1.56 and 1.116 mg g^{-1} DW Pb content was reported in the root plus shoot regions of fenugreek plants, respectively. EBL pre-treated plants of fenugreek diminished the Pb presence in root and shoot with 1.17 and 0.965 mg g^{-1} DW Pb content, respectively.Exogenous treatment of Si under metal stressed conditions lowered down the Pb content with 1.03 and 0.899 mg g^{-1} DW inside root and shoot, respectively. Co-supplementation of EBL and Si further reduced the Pb content in root plus shoot of fenugreek plants with 0.766 and 0.483 mg g^{-1} DW values of Pb.

Table 6.14

Treatments	Root (mg g^{-1} DW)	Shoot (mg g^{-1} DW)
C	ND	ND
T_3	$1.56^{c}\pm0.0.26$	$1.116^{bc}\pm0.06$
T_7	$1.17^{b}\pm0.023$	$0.965^{ab}\pm0.006$
T_{11}	$1.03^{ab}\pm0.015$	$0.899^{ab}\pm0.007$
T_{15}	$0.766^{a}\pm0.012$	$0.483^{a}\pm0.04$

Fig. 6.14

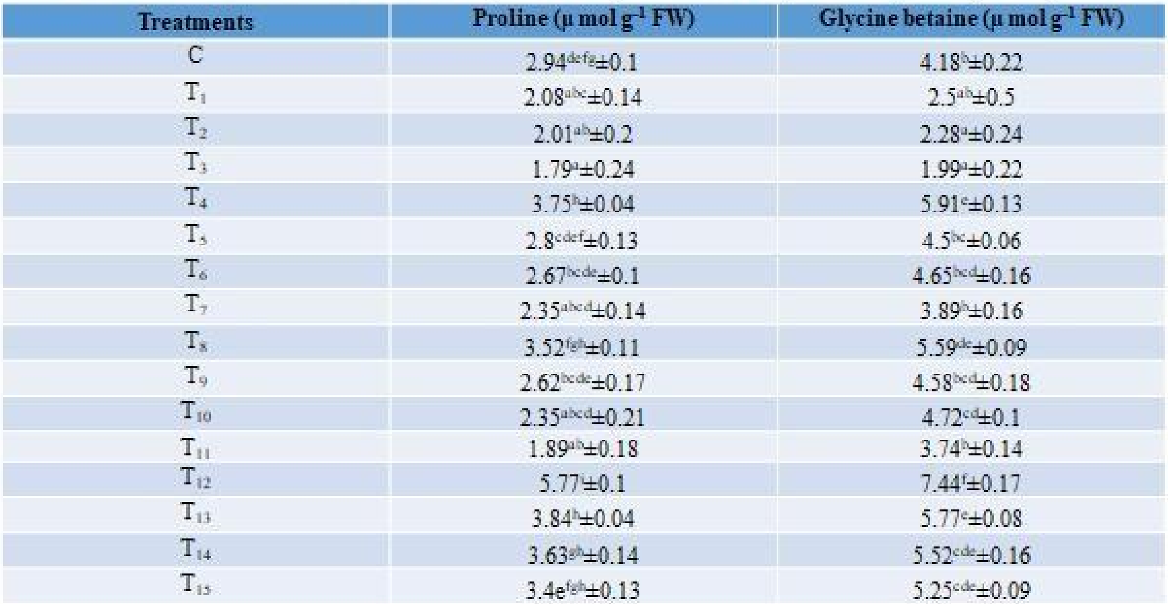


☐ **Influence of EBL + Si on Pb metal uptake by the 15-days old *Trigonella foenum-graecum plants.***

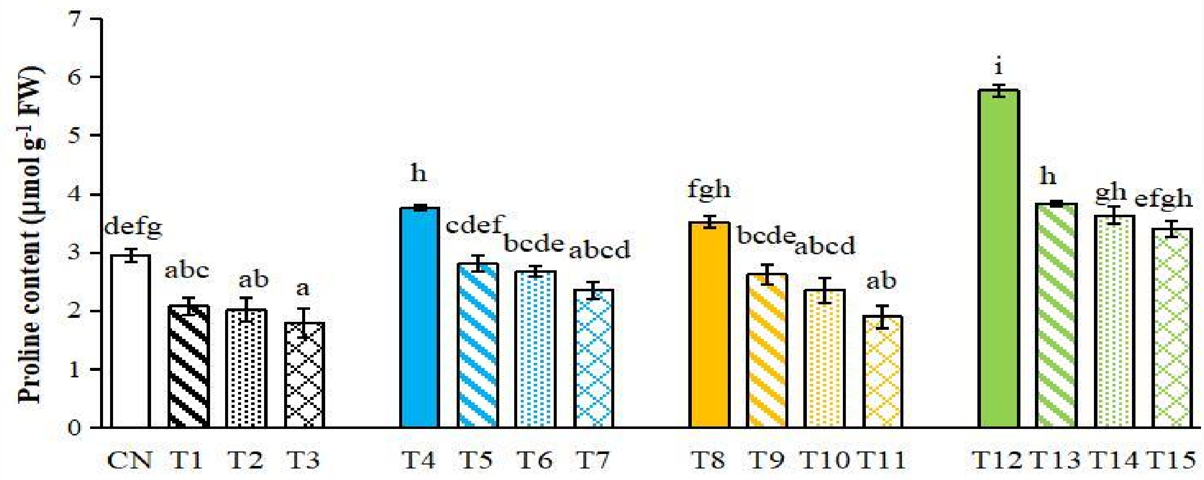
6.1.2.6 Osmolytes

Significant inhibitions in the content of proline took place on account of Pb toxicity in fenugreek plants (Fig 6.15; Table 6.15). Every concentration of Pb i.e. T_1, T_2 plus T_3, registered a decline in the amount of proline with respective values of 2.08, 2.01, and 1.79 µ mol g^{-1} FW. Individually augmented EBL brought about a promotion of proline measures in stressed plants with being greatest at T_5 concentration (2.80 µ mol g^{-1} FW). Si too played a role in raising the levels of proline in response to metal stress. Proline levels were up-scaled from 1.79 to 2.62 µ mol g^{-1} FW in T_9 i.e. Si treated plants in contrast to $T_{3.}$ Under Pb stress, maximum enhancement in proline measures (3.84 µ mol g^{-1} FW) was in T_{13} treatment. T_{13} plants exhibited 37.14% and 46.56% improvement over T_5 and T_9 treated plants.

Table. 6.15: Influence of EBL+Si treatment on osmolytes of 15-days old Pb stressed plants of *Trigonella foenum-graecum*.



Treatments	Proline (μ mol g^{-1} FW)	Glycine betaine (μ mol g^{-1} FW)
C	2.94^{defg}±0.1	4.18^{b}±0.22
T_1	2.08^{abc}±0.14	2.5^{ab}±0.5
T_2	2.01^{ab}±0.2	2.28^{a}±0.24
T_3	1.79^{a}±0.24	1.99^{a}±0.22
T_4	3.75^{h}±0.04	5.91^{e}±0.13
T_5	2.8^{cdef}±0.13	4.5^{bc}±0.06
T_6	2.67^{bcde}±0.1	4.65^{bcd}±0.16
T_7	2.35^{abcd}±0.14	3.89^{b}±0.16
T_8	3.52^{fgh}±0.11	5.59^{de}±0.09
T_9	2.62^{bcde}±0.17	4.58^{bcd}±0.18
T_{10}	2.35^{abcd}±0.21	4.72^{cd}±0.1
T_{11}	1.89^{ab}±0.18	3.74^{b}±0.14
T_{12}	5.77^{i}±0.1	7.44^{f}±0.17
T_{13}	3.84^{h}±0.04	5.77^{e}±0.08
T_{14}	3.63^{gh}±0.14	5.52^{cde}±0.16
T_{15}	3.4e^{fgh}±0.13	5.25^{cde}±0.09



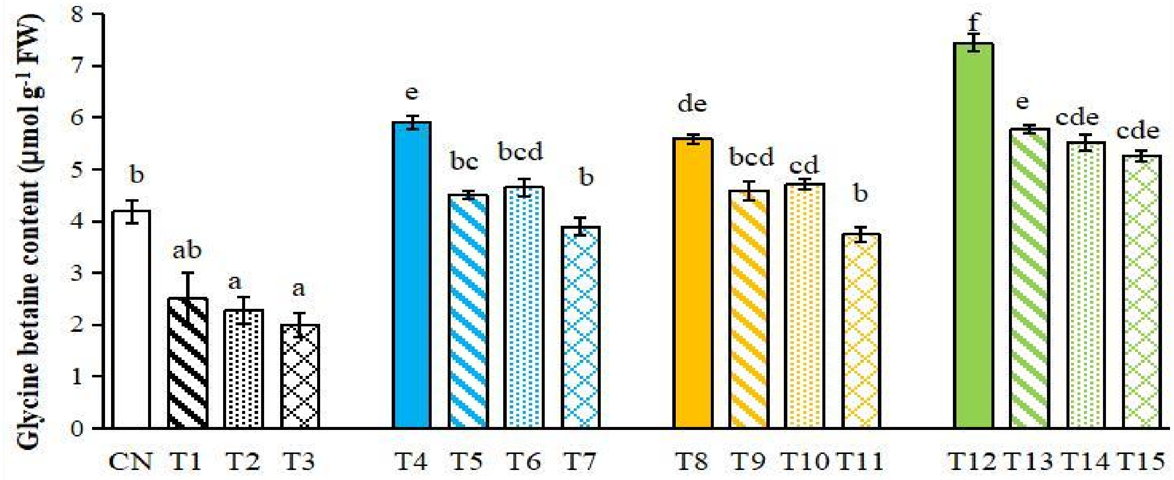


Fig. 6.15: Influence of EBL+Si treatment on measures of osmolytes in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Noteworthy reductions in the measures of glycine-betaine were registered in Pb stress subjected plants (Fig 6.15; Table 6.15). A decrement of 2.50 at T_1, 2.28 at T_2 and 1.99 µ mol g^{-1} FW at T_3 in the content of glycine-betaine was noted as opposed to control plants.. Under the influence of EBL, the measures of glycine betaine were recorded to be 4.50 at T_5, 4.65 at T_6 and 3.89 µ mol g^{-1} FW at T_7 respectively while Si treatment recorded glycine betaine content to be 4.58 at T_9, 4.72 at T_{10} and 3.74 µ mol g^{-1} FW at T_{11}. Furthermore, combination of EBL with Si treatment in stress free conditions registered the highest quantities of glycine betaine (7.44 µ mol g^{-1} FW) amongst all the 16 different types of treatments chosen for the study. Glycine-betaine amount was further recorded to be curtailed as the intensity of Pb toxicity elevated as observed in coupled EBL + Si dosage with being highest at 5.77 µ mol g^{-1} FW in T_{13}. An escalation of 80% was registered in T_5 over T_1. T_9 plants displayed 83.2% increment than T_1. T_{13} plants exhibited nearly 28.22% improvement in glycine betaine as opposed to T_5. T_{13} showed 38.03% more measures of glycine betaine in contrast to control plants.

6.1.2.7 Total carbohydrates content

Total content of carbohydrates was observed to have a diminishing effect on account of varying concentration of Pb stress in the study with being lowest around T_3 (4.14 mg g^{-1} FW) (Fig. 6.16; Table 6.16). The most and least concentrations of carbohydrates in pre-treated EBL plants were recorded to be 5.73 at T_5 and 4.85 mg g^{-1} FW at T_7 respectively while the same values in case of Si were 5.69 at T_9 and 4.91 mg g^{-1} FW at T_{11}. When EBL and Si were co-applied together, promotion in the values of carbohydrate content was registered with being at its highest measure of 6.80 mg g^{-1} FW at T_{13}. T_{13} plants exhibited 18.67% improvement when correlated to T_5. T_{15} had 26.81% upsurge in carbohydrate measures against control plants.

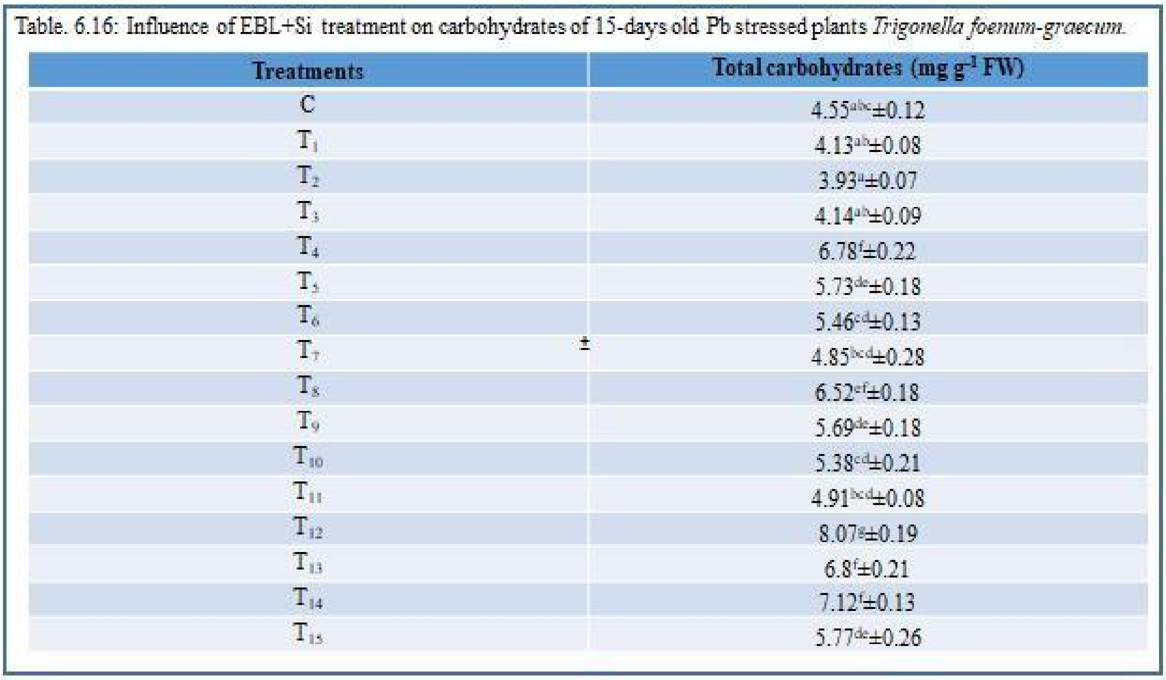


Table. 6.16: Influence of EBL+Si treatment on carbohydrates of 15-days old Pb stressed plants *Trigonella foenum-graecum.*

Treatments	Total carbohydrates (mg g^{-1} FW)
C	$4.55^{abc}\pm0.12$
T_1	$4.13^{ab}\pm0.08$
T_2	$3.93^{a}\pm0.07$
T_3	$4.14^{ab}\pm0.09$
T_4	$6.78^{f}\pm0.22$
T_5	$5.73^{de}\pm0.18$
T_6	$5.46^{cd}\pm0.13$
T_7 ±	$4.85^{bcd}\pm0.28$
T_8	$6.52^{ef}\pm0.18$
T_9	$5.69^{de}\pm0.18$
T_{10}	$5.38^{cd}\pm0.21$
T_{11}	$4.91^{bcd}\pm0.08$
T_{12}	$8.07^{g}\pm0.19$
T_{13}	$6.8^{f}\pm0.21$
T_{14}	$7.12^{f}\pm0.13$
T_{15}	$5.77^{de}\pm0.26$

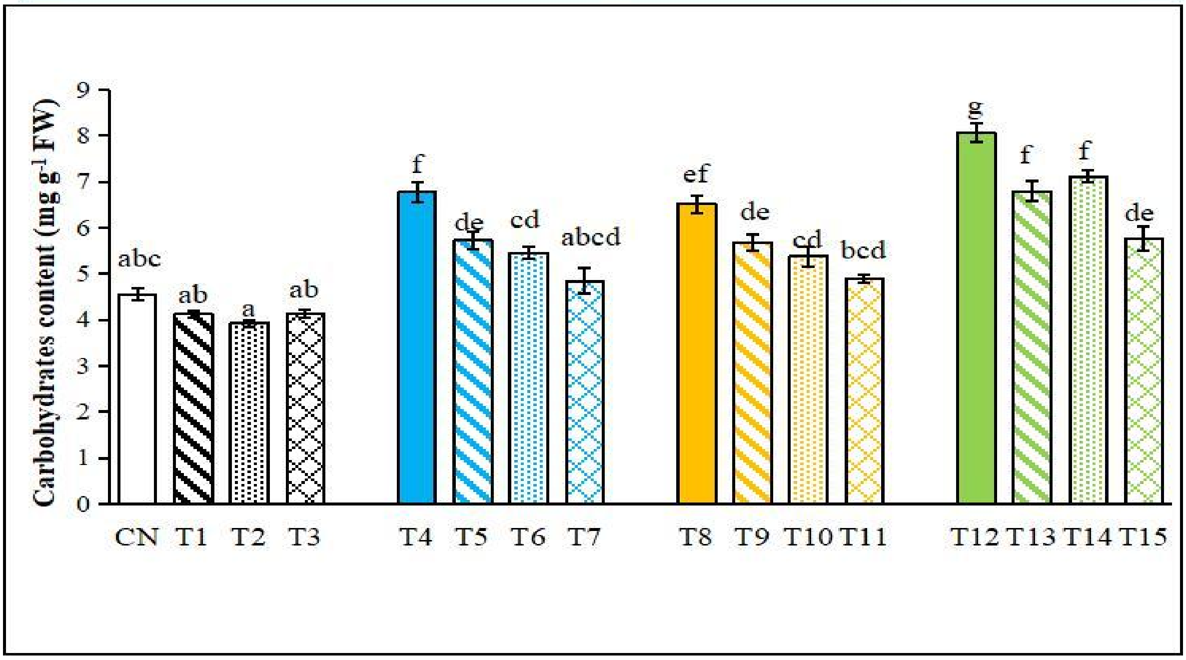


Fig. 6.16: Influence of EBL+Si treatment on measures of total carbohydrates in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

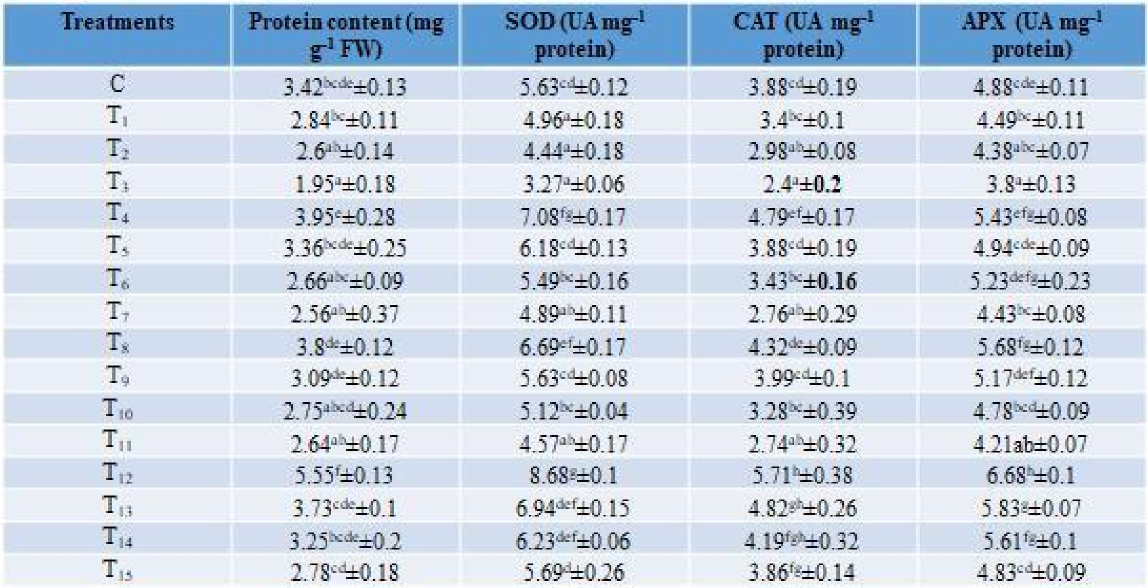
6.1.2.8 Protein content and Antioxidant defense system

6.1.2.8.1 Protein content and antioxidative enzymes

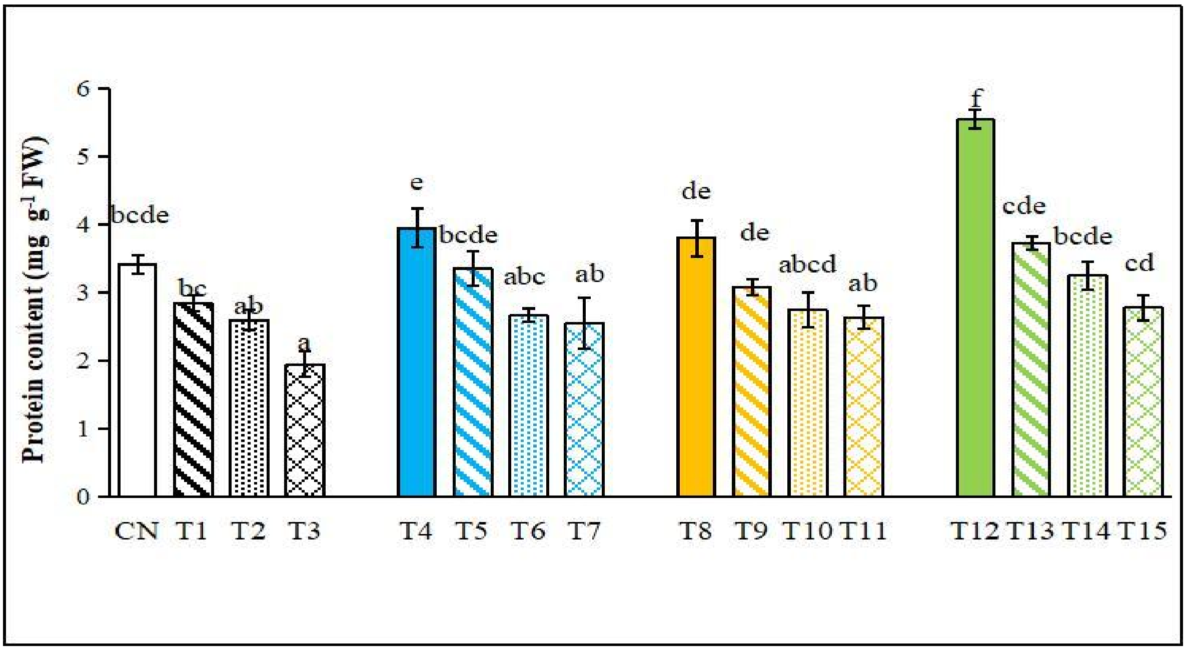
Lead metal stress caused a decline in the measures of protein content (Fig 6.17a; Table 6.17a). In T_1 treated plants, amount of protein was 2.84 mg g^{-1} FW, which was 16.95% lower in correlation with control plants. The least amount of protein was recorded to be 1.95 mg g^{-1} FW at T_3 concentration. EBL pre-treatment of plants exhibited higher measures of protein in response to Pb stress, when contrasted against Si-augmented plants. The maximal (3.36 mg g^{-1} FW) and the minimal (2.56 mg g^{-1} FW) protein measures were noted at T_5 and T_7 concentrations, respectively when given pre-treatment of EBL. Supplementation with Si raised the measures of protein in response to stressed and stress free conditions. Plants in Si control showed 3.80 mg g^{-1} FW of protein content. Under Pb stress, the highest protein content of 3.09 mg g^{-1} FW was recorded in T_9. With respect to synergistic impact of EBL and Si, the maximum (3.73 mg g^{-1} FW) protein content was registered in T_{13} plants. Additionally, T_{13} plants exhibited 11.01% and 9.06% increment as compared to T_5 and control plants, respectively.

The extent of enzymatic activity was curtailed under Pb stress exposure in case of 15-days old fenugreek plants (Fig 6.17a; Table 6.17a). Minimal enzymatic action of SOD activity was recorded at 3.27 UA mg^{-1} protein in T_3 metal stressed plants. Supplementing the stressed plants with 24-EBL improved the performance of SOD with a wide value range between the extremes of T_5 (6.18 UA mg^{-1} protein) and T_7 (4.89 UA mg^{-1} protein) respectively. Augmentation of Si to the plants under Pb toxicity, the greatest activity of SOD (5.63 UA mg^{-1} protein) was noted at T_9. In addition to this, EBL + Si + Pb I elevated the enzymatic action of SOD in T_{13} treatment (6.94 UA mg^{-1} protein). An increment of 24.59% was noted in T_5 over T_1. Plants of T_9 treatment displayed 13.5% increase than T_1. Further, plants at T_{13} treatment exhibited an elevation of 12.29% and 23.26% when respectively correlated with T_5 and control.

Table. 6.17 a: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 15-days old Pb stressed plants of *Trigonella foenum-graecum.*



Treatments	Protein content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
C	$3.42^{bcde} \pm 0.13$	$5.63^{cd} \pm 0.12$	$3.88^{cd} \pm 0.19$	$4.88^{cde} \pm 0.11$
T_1	$2.84^{bc} \pm 0.11$	$4.96^{a} \pm 0.18$	$3.4^{bc} \pm 0.1$	$4.49^{bc} \pm 0.11$
T_2	$2.6^{ab} \pm 0.14$	$4.44^{a} \pm 0.18$	$2.98^{ab} \pm 0.08$	$4.38^{abc} \pm 0.07$
T_3	$1.95^{a} \pm 0.18$	$3.27^{a} \pm 0.06$	2.4^{a} **±0.2**	$3.8^{a} \pm 0.13$
T_4	$3.95^{e} \pm 0.28$	$7.08^{fg} \pm 0.17$	$4.79^{ef} \pm 0.17$	$5.43^{efg} \pm 0.08$
T_5	$3.36^{bcde} \pm 0.25$	$6.18^{cd} \pm 0.13$	$3.88^{cd} \pm 0.19$	$4.94^{cde} \pm 0.09$
T_6	$2.66^{abc} \pm 0.09$	$5.49^{bc} \pm 0.16$	3.43^{bc} **±0.16**	$5.23^{defg} \pm 0.23$
T_7	$2.56^{ab} \pm 0.37$	$4.89^{ab} \pm 0.11$	$2.76^{ab} \pm 0.29$	$4.43^{bc} \pm 0.08$
T_8	$3.8^{de} \pm 0.12$	$6.69^{ef} \pm 0.17$	$4.32^{de} \pm 0.09$	$5.68^{fg} \pm 0.12$
T_9	$3.09^{de} \pm 0.12$	$5.63^{cd} \pm 0.08$	$3.99^{cd} \pm 0.1$	$5.17^{def} \pm 0.12$
T_{10}	$2.75^{abcd} \pm 0.24$	$5.12^{bc} \pm 0.04$	$3.28^{bc} \pm 0.39$	$4.78^{bcd} \pm 0.09$
T_{11}	$2.64^{ab} \pm 0.17$	$4.57^{ab} \pm 0.17$	$2.74^{ab} \pm 0.32$	4.21ab±0.07
T_{12}	$5.55^{f} \pm 0.13$	$8.68^{g} \pm 0.1$	$5.71^{h} \pm 0.38$	$6.68^{h} \pm 0.1$
T_{13}	$3.73^{cde} \pm 0.1$	$6.94^{def} \pm 0.15$	$4.82^{gh} \pm 0.26$	$5.83^{g} \pm 0.07$
T_{14}	$3.25^{bcde} \pm 0.2$	$6.23^{def} \pm 0.06$	$4.19^{fgh} \pm 0.32$	$5.61^{fg} \pm 0.1$
T_{15}	$2.78^{cd} \pm 0.18$	$5.69^{d} \pm 0.26$	$3.86^{fg} \pm 0.14$	$4.83^{cd} \pm 0.09$


Protein content (mg g^{-1} FW)
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3
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5
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bcde
bc
ab
a
e
bcde
abc
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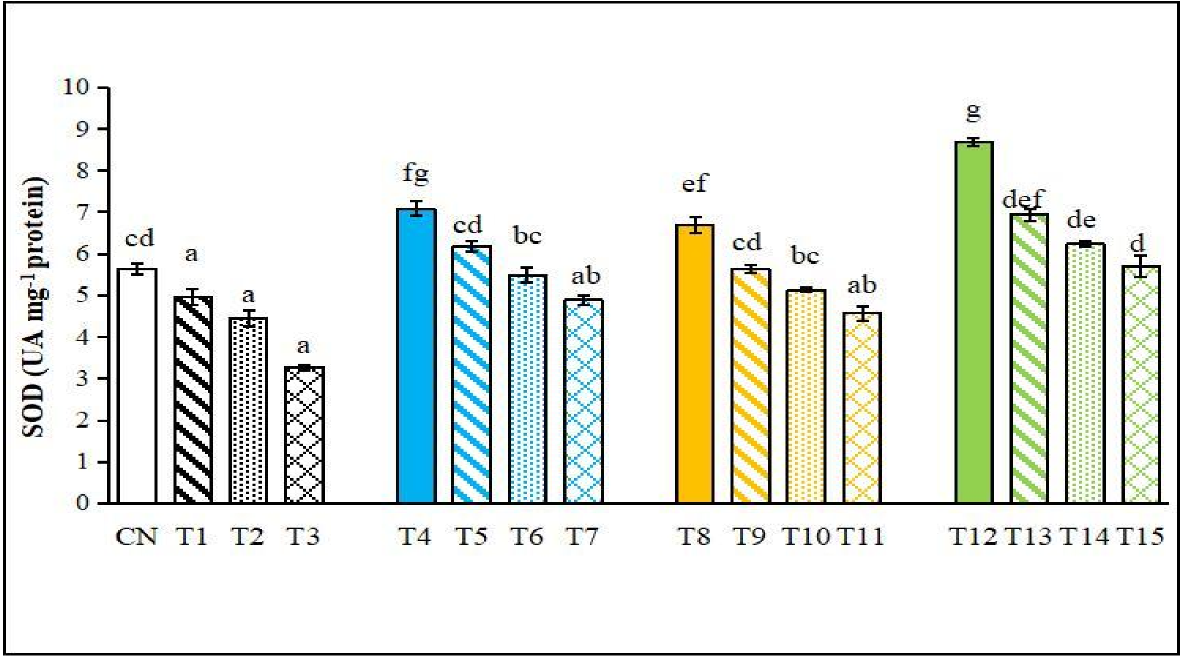
SOD (UA mg^{-1} protein)
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3
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5
6
7
8
9
10
cd
a
a
a
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ab
g
def
de
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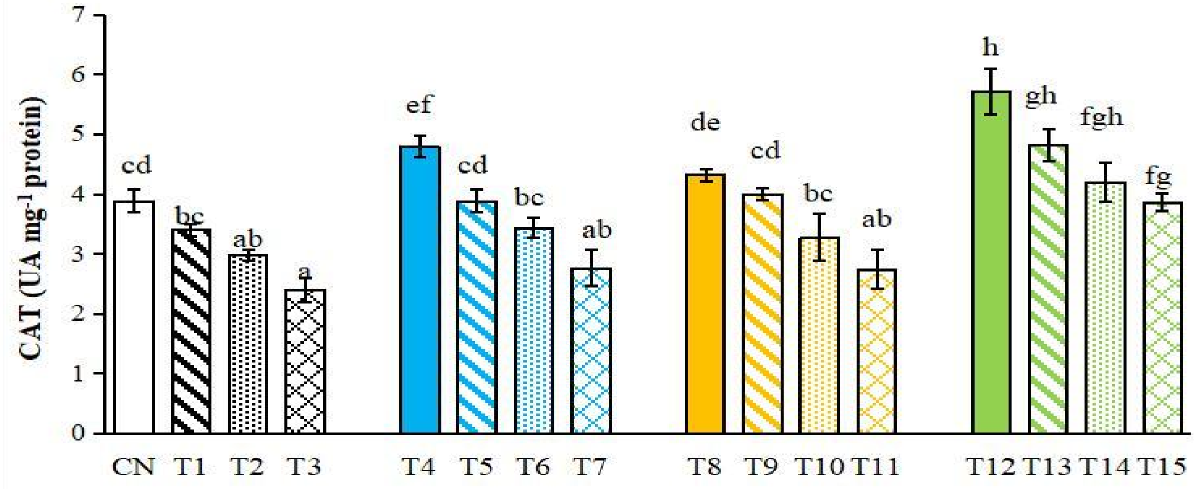


Fig. 6.17 (a): Influence of EBL+Si treatment on measures of proteins and enzymatic antioxidants in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

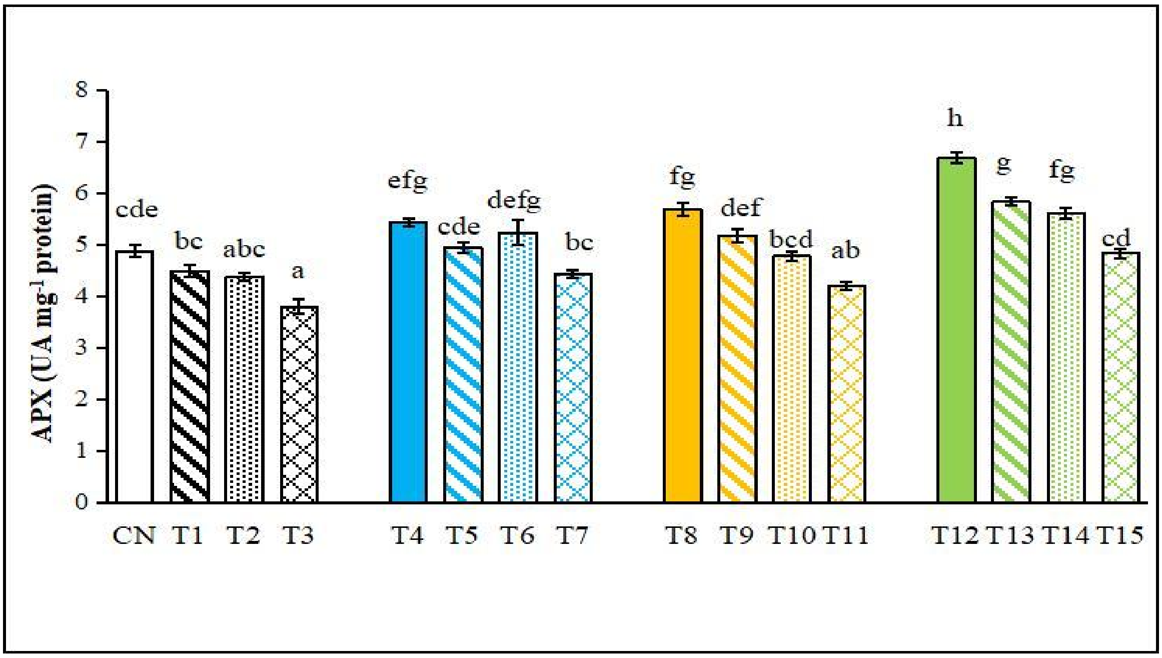
Catalase action was adversely affected in response to Pb stress being minimum at 2.40 UA mg^{-1} protein in T_3 treatment (Fig. 6.17a; Table 6.17a). Applying the ameliorative EBL under Pb stress led to an improvement in CAT measures, which was maximum at 3.88 UA mg^{-1} protein in T_5 plants. Individually supplemented Si upgraded the CAT action than Pb alone augmented plants. Exogenous application of silicon with regard to Pb stress registered minimum CAT content at T_9 concentration (3.99 UA mg^{-1} protein). Its action was recorded to be curtailed with enhancement in Pb stress, as is the case in EBL + Si, where the highest value of CAT activity was noted to be at 4.82 UA mg^{-1} protein in T_{13}. An increment of 14.11% was reported at T_5 when put against T_1. Further, T_9 plants had 17.35% enhancement over T_1 treatment. T_{13} plants displayed a respective increment of 24.22 % each in relation to T_5 and control.

Lead toxicity caused a greatest decline in the enzymatic performance of APX as reported in case of T_3 (3.80 UA mg^{-1} protein) (Fig. 6.17b; Table 6.17a). Treating the fenugreek plants with EBL and Si in an individual manner reportedly upgraded the APX measures in contrast with Pb alone treatments. The maximum activity level in EBL augmented plants was noted at T_6 with a value of 5.23 UA mg^{-1} protein whereas in Si treated plants, such an elevated activity was found at T_9 metal stressed plants (5.17 UA mg^{-1} protein). In response to Pb stress, the maximum elevation in enzymatic activity was at T_{13} (5.83 UA mg^{-1} protein) amongst all the selected treatments where EBL and Si are co-applied. An increment of 10.02% was noted in

T_5 over T_1. T_9 plants displayed 15.14% escalation in correlation to T_1. Further, T_{13} plants recorded 18.01 % and 19.46% increase as compared to T_5 and control, respectively.

Lead toxicity notably declined the activity of POD, in relation to control (Fig 6.17b; Table 6.17b). In respect to Pb stress conditions, enzymatic performance of POD was recorded to be at its lowest at T_3 concentration with a content value of 4.21 UA mg^{-1} protein. Maximum (5.28 UA mg^{-1} protein) and minimum (4.69 UA mg^{-1} protein) levels of POD were noticed in T_5 and T_7 stressed plants when given a 24-EBL treatment. Supplementing the fenugreek plants with Si resulted in maximum elevation in the enzymatic levels at T_9 (5.52 UA mg^{-1} protein). In co-supplementation, EBL + Si were more effective in up-scaling the action of POD enzyme vis-à-vis their individual treatments. It can be corroborated by the highest values of this enzyme i.e. 6.10 UA mg^{-1} protein at T_{13} treatment (EBL + Si + Pb I). An increment of 0.18% was noted in T_5 over T_1. Plants at T_9 stage recorded 4.74 % escalations over T_1. Furthermore, T_{13} plants exhibited an upsurge of 15.53% when correlated with both T_5 and control conditions.

Highest decline in the measures of GR on account of Pb stress took place at T_3 concentration (2.75 UA mg^{-1} protein) (Fig. 6.17b; Table 6.17b). Plants of control recorded an activity level of 4.48 UA mg^{-1} protein with respect to GR. A notable diminishing impact of Pb stress was mitigated by EBL application at T_5 (4.48 UA mg^{-1} protein) while the same was accomplished by Si treatment in T_9 cultivars (4.72 UA mg^{-1} protein). The highest elevation in GR's performance with content of 5.74 UA mg^{-1} was recorded at T_{13} treatment. An upsurge of 10.61% was seen in T_5 over T_1. Whereas T_{13} plants exhibited a 28.82% increment at both treatments of T_5 and control.



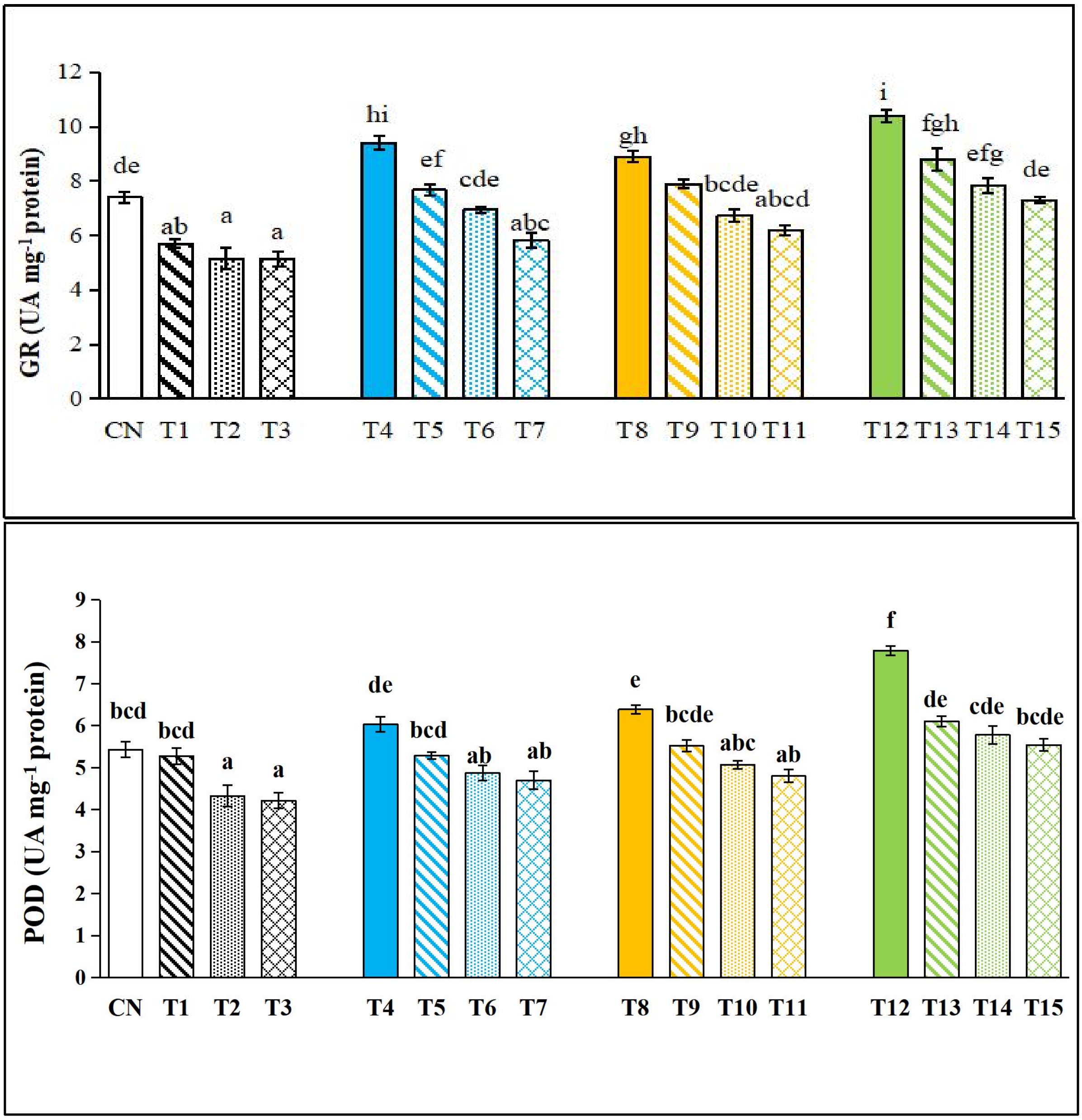


Fig. 6.17 (b): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

The enzymatic performance of GPOX got declined in Pb exposed fenugreek plants with 3.49, 3.35, and 2.78 UA mg^{-1} protein at Pb treatment concentrations of T$_1$, T$_2$ and T$_3$, respectively (Fig. 6.17c; Table 6.17b). Individually augmented EBL and Si with respect to Pb alone toxicity resulted in boosting the levels of GPOX with respective values of 4.84 and 4.93 UA mg^{-1} protein at T$_5$ and T$_9$. Further, curtailment of enzymatic action was reported with every escalation in Pb concentrations as was evident in cumulative application (EBL + Si) having highest levels of activity at T$_{13}$ (6.33 UA mg^{-1} protein). An improvement of 38.68% was noted in T_5 over T_1. Further, T_{13} plants exhibited 30.78% and 69.70% increase in correlation to T_5 and control.

DHAR's enzymatic action suffered from Pb induced diminishing effect in in vitro raised plants (Fig. 6.17c; Table 6.17b). Maximum enzymatic levels were observed in T_1 plants (5.59 UA mg^{-1} protein) with respect to control. In individually treated plants with EBL and Si, the greatest measures of enzyme were noted at T_5 and T_7 with 6.68 and 5.17 UA mg^{-1} protein values respectively. On the other hand, under the influence of same mitigants, minimal content values were at T_9 (6.42 UA mg-1 protein) and T_{11} (4.90 UA mg-1 protein). Further augmentation of enzymatic performance of DHAR in response to metal exposure took place at T_{13} (7.32 UA mg^{-1} protein). An elevation of 19.49% was reported in T_5 over T_1. T_9 plants displayed 14.84% escalation than T_1. Further, T_{13} plants exhibited 9.58% upsurge as opposed to T_5 and a 17.12 % decline in correlation with control.

MDHAR enzyme's performance level was adversely affected as a consequence of lead stress (Fig. 6.20c; Table 6.17c). The least amount of activity was visible at 3.71 UA mg^{-1} protein in T_3 treatment. MDHAR levels were escalated from 5.03 to 5.31 UA mg^{-1} protein in EBL pre-augmented plants of both the T_5 and T_6 treatment. Supplementing the fenugreek plants with Si augmented the enzymatic levels with being highest in T_9 treatment (5.61 UA mg^{-1} protein. The maximum MDHAR activity level (7.93 UA mg^{-1} protein) got recorded in coupled supplementation of EBL and Si at $T_{13.}$ A decrement of 3.80% was recorded in T_5 over T_1 whereas T_9 plants showed 1.63% escalation in relation to T_1. Plants at T_{13} treatment recorded 49.34% and 35.09% increment in correlation with T_5 and control.

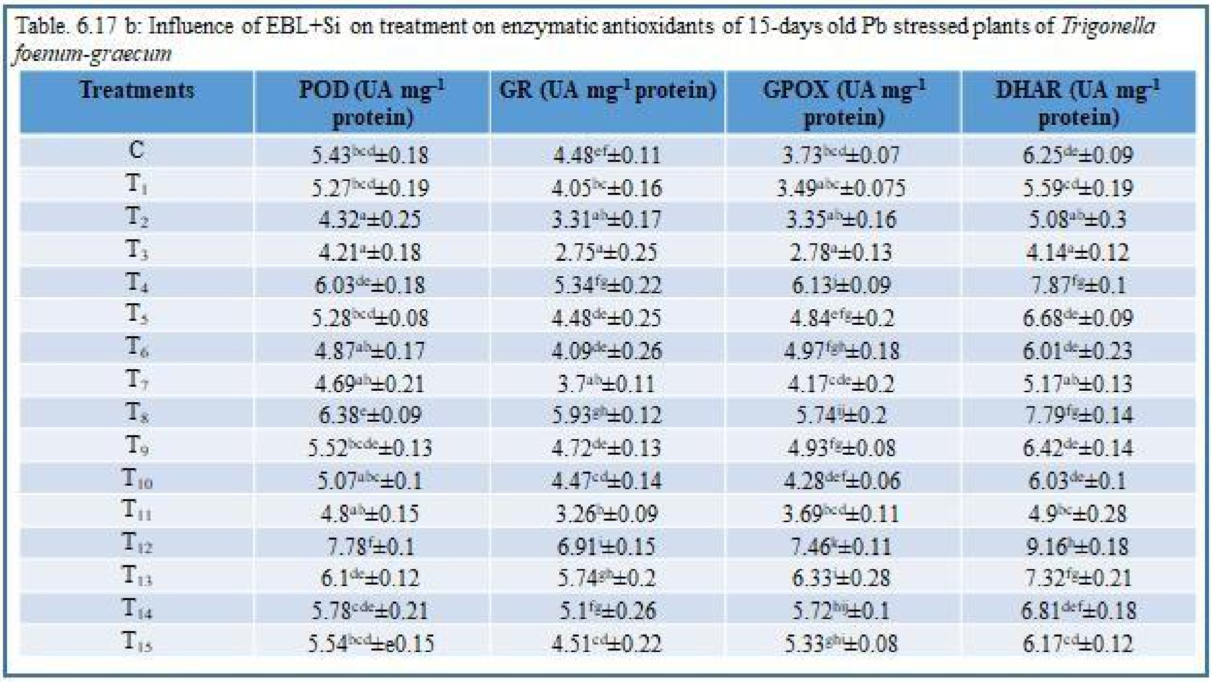


Table. 6.17 b: Influence of EBL+Si on treatment on enzymatic antioxidants of 15-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatments	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
C	5.43^{bcd}±0.18	4.48^{ef}±0.11	3.73^{bcd}±0.07	6.25^{de}±0.09
T_1	5.27^{bcd}±0.19	4.05^{bc}±0.16	3.49^{abc}±0.075	5.59^{cd}±0.19
T_2	4.32^{a}±0.25	3.31^{ab}±0.17	3.35^{ab}±0.16	5.08^{ab}±0.3
T_3	4.21^{a}±0.18	2.75^{a}±0.25	2.78^{a}±0.13	4.14^{a}±0.12
T_4	6.03^{de}±0.18	5.34^{fg}±0.22	6.13^{i}±0.09	7.87^{fg}±0.1
T_5	5.28^{bcd}±0.08	4.48^{de}±0.25	4.84^{efg}±0.2	6.68^{de}±0.09
T_6	4.87^{ab}±0.17	4.09^{de}±0.26	4.97^{fgh}±0.18	6.01^{de}±0.23
T_7	4.69^{ab}±0.21	3.7^{ab}±0.11	4.17^{cde}±0.2	5.17^{ab}±0.13
T_8	6.38^{e}±0.09	5.93^{gh}±0.12	5.74^{ij}±0.2	7.79^{fg}±0.14
T_9	5.52^{bcde}±0.13	4.72^{de}±0.13	4.93^{fg}±0.08	6.42^{de}±0.14
T_{10}	5.07^{abc}±0.1	4.47^{cd}±0.14	4.28^{def}±0.06	6.03^{de}±0.1
T_{11}	4.8^{ab}±0.15	3.26^{b}±0.09	3.69^{bcd}±0.11	4.9^{bc}±0.28
T_{12}	7.78^{f}±0.1	6.91^{i}±0.15	7.46^{k}±0.11	9.16^{h}±0.18
T_{13}	6.1^{de}±0.12	5.74^{gh}±0.2	6.33^{i}±0.28	7.32^{fg}±0.21
T_{14}	5.78^{cde}±0.21	5.1^{fg}±0.26	5.72^{hij}±0.1	6.81^{def}±0.18
T_{15}	5.54^{bcd}±e0.15	4.51^{cd}±0.22	5.33^{ghi}±0.08	6.17^{cd}±0.12

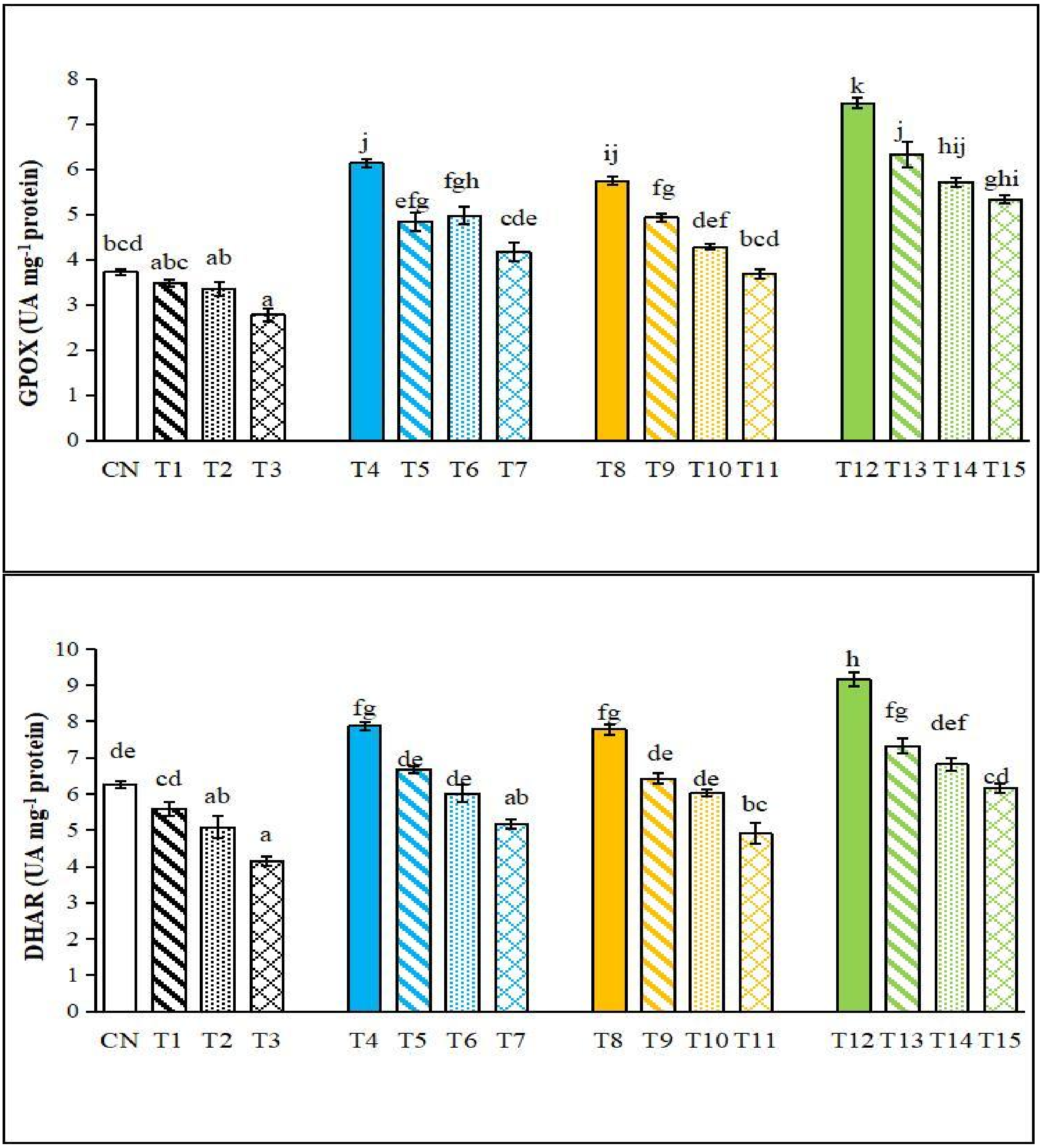


Fig. 6.17 (c): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

GST enzyme had activity level of 4.30, 3.68 and 3.26 UA mg^{-1} protein at different concentrations of lead i.e. at T_1, T_2 and T_3 (Fig. 6.17d; Table 6.17c). Maximal and minimal values of GST content were recorded at T_5 (4.98 UA mg^{-1} protein) and T_7 (3.86 UA mg^{-1} protein) in EBL augmented plants. In Si augmented plants under stressful conditions, highest GST content of 4.73 UA mg^{-1} protein was noted in T_9 plants. EBL + Si upscaled the activity levels of GST in response to T_{13} concentration (6.68 UA mg^{-1} protein). An increment of 15.81% was noted in T_5 over T_1. Further, T_9 plants showed 10% upsurge opposed to T_1. T_{13} plants showed increment of 34.13% at both T_5 and control.

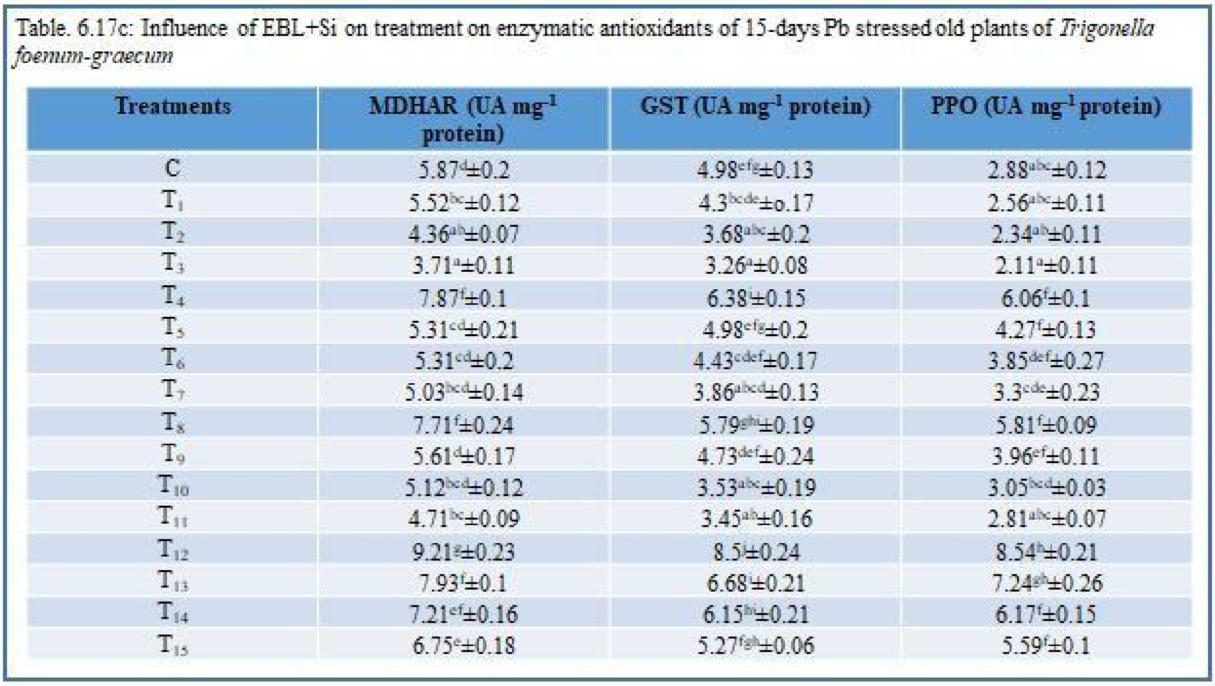
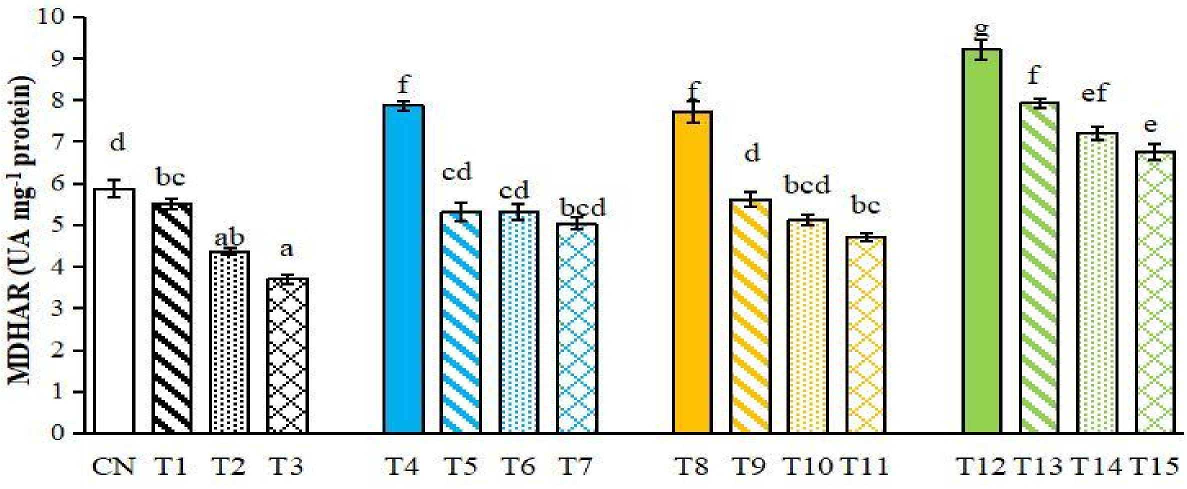


Table. 6.17c: Influence of EBL+Si on treatment on enzymatic antioxidants of 15-days Pb stressed old plants of *Trigonella foenum-graecum*

Treatments	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
C	5.87^{d}±0.2	4.98^{efg}±0.13	2.88^{abc}±0.12
T_1	5.52^{bc}±0.12	4.3^{bcde}±o.17	2.56^{abc}±0.11
T_2	4.36^{ab}±0.07	3.68^{abc}±0.2	2.34^{ab}±0.11
T_3	3.71^{a}±0.11	3.26^{a}±0.08	2.11^{a}±0.11
T_4	7.87^{f}±0.1	6.38^{i}±0.15	6.06^{f}±0.1
T_5	5.31^{cd}±0.21	4.98^{efg}±0.2	4.27^{f}±0.13
T_6	5.31^{cd}±0.2	4.43^{cdef}±0.17	3.85^{def}±0.27
T_7	5.03^{bcd}±0.14	3.86^{abcd}±0.13	3.3^{cde}±0.23
T_8	7.71^{f}±0.24	5.79^{ghi}±0.19	5.81^{f}±0.09
T_9	5.61^{d}±0.17	4.73^{def}±0.24	3.96^{ef}±0.11
T_{10}	5.12^{bcd}±0.12	3.53^{abc}±0.19	3.05^{bcd}±0.03
T_{11}	4.71^{bc}±0.09	3.45^{ab}±0.16	2.81^{abc}±0.07
T_{12}	9.21^{g}±0.23	8.5^{j}±0.24	8.54^{h}±0.21
T_{13}	7.93^{f}±0.1	6.68^{i}±0.21	7.24^{gh}±0.26
T_{14}	7.21^{ef}±0.16	6.15^{hi}±0.21	6.17^{f}±0.15
T_{15}	6.75^{e}±0.18	5.27^{fgh}±0.06	5.59^{f}±0.1



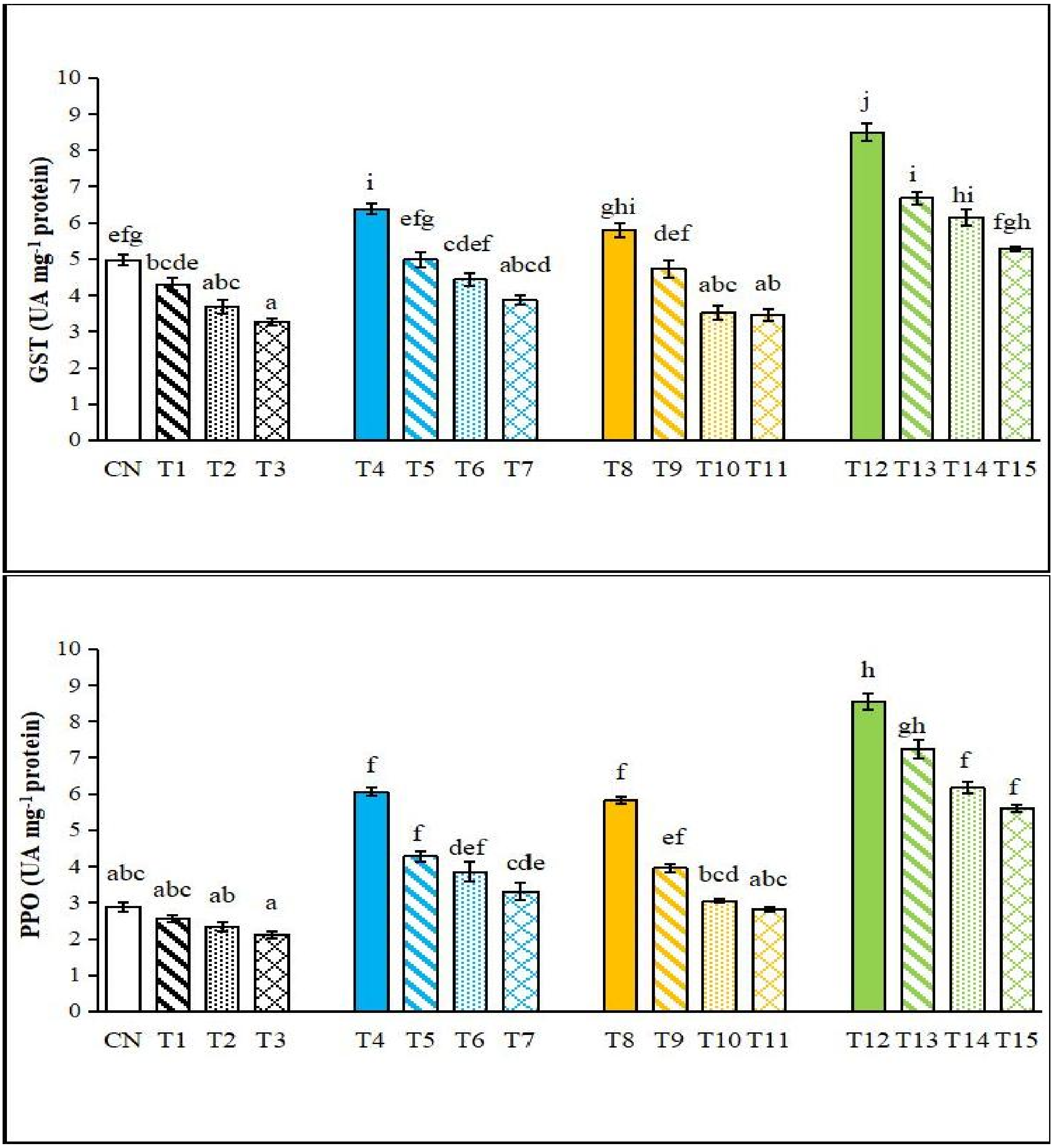


Fig. 6.17 (d): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Measures of PPO enzyme were at their minimum at the highest treatment value of Pb stress i.e. T_3 (2.11 UA mg^{-1} protein) in contrast to control plants (Fig. 6.17d; Table 6.17c). However, in EBL augmented plants under no stress conditions, highest activity was measured at T_4 of 6.06 UA mg^{-1} protein value. Applying EBL to Pb stressed fenugreek plants registered minimal activity of this enzyme at 3.30 UA mg^{-1} protein at T_7. Further, the Si treatment had the highest measures of PPO activity in case of T_9 cultivars (3.96 UA mg^{-1} protein) in metal stressed conditions. Whereas cumulatively applied EBL and Si made the activity level of PPO as high as 7.24 UA mg^{-1} protein at T_{13} treatment. An increase of 54.68% was noticed in

T_5 than T_1. T_{13} plants showed 69.55% and 151.38% increase as compared to T_5 and control plants, respectively.

6.1.2.8.2 Non-enzymatic antioxidants

Amount of ascorbic acid was registered to be diminished at each of the Pb concentration selected for carrying out this study (Fig. 6.18; Table 6.18). The topmost value of ascorbic acid was noted at 2.87 µg g^{-1} FW in response to T_1 concentration amongst every discrete level of Pb stress. Minimum values of Ascorbic acid in EBL and Si alone applications with regard to stressed plants, were 2.42 µg g^{-1} FW at T_7 and 2.20 at T_{11} plants. However, synergistic action of EBL +Si was observed to have highest value of this antioxidant at T_{13} i.e. 4.48 µg g^{-1} FW at T_{14}. An elevation of 21.95% and 13.58% was reported in T_5 and T_9 over T_1 metal stressed plants. T_{13} plants showed 28% and 25.13 % increase as compared to T_5 and control, respectively.

Lead toxicity recorded a decline in the measures of glutathione with being minimum at T_1 (2.93 µg g^{-1} FW) in contrast to control plants (Fig. 6.18; Table 6.18). However, pre-treated EBL plants displayed highest values of 4.58 µg g^{-1} FW and 3.80 µg g^{-1} FW of glutathione in response to stress free and stressful environments respectively. With respect to Si treatment, these values were found to be 4.45 µg g^{-1} FW and 3.61 µg g^{-1} FW respectively. Coupling of EBL with Si for mitigating the harms of Pb toxicity, exhibited glutathione content 4.64 µg g^{-1} FW in T_{13} stress exposed plants. Moreover, T_{13} plants exhibited an increment of 22.10% and 19.89% in glutathione when correlated with T_5 and control, respectively.

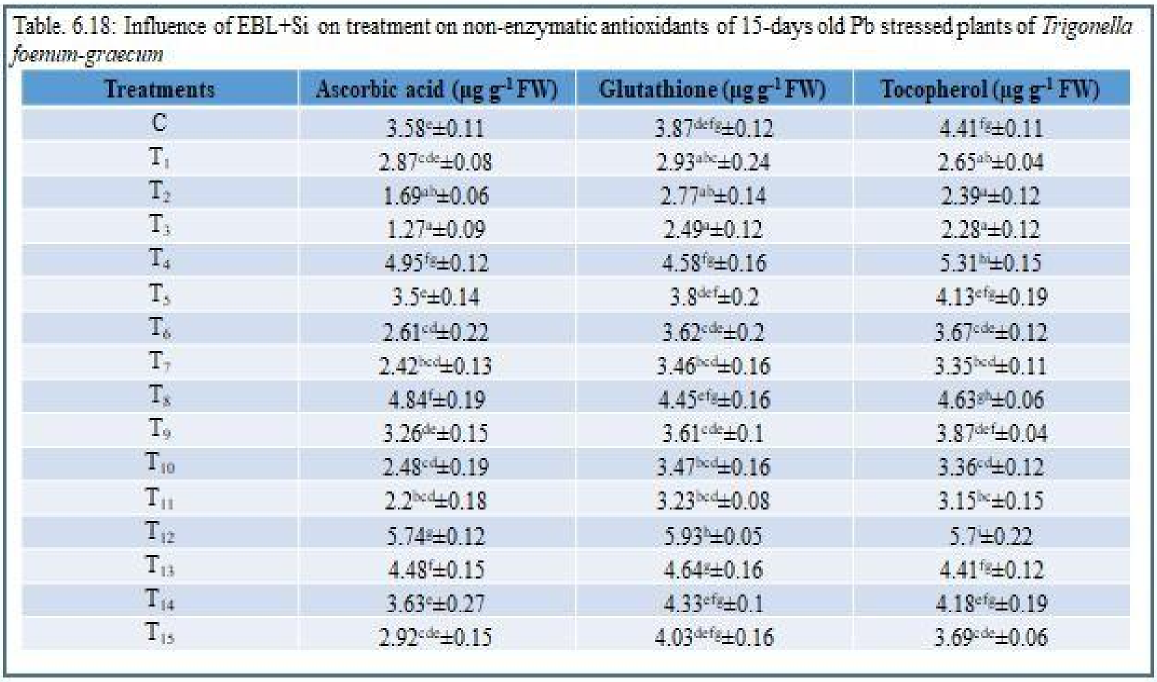
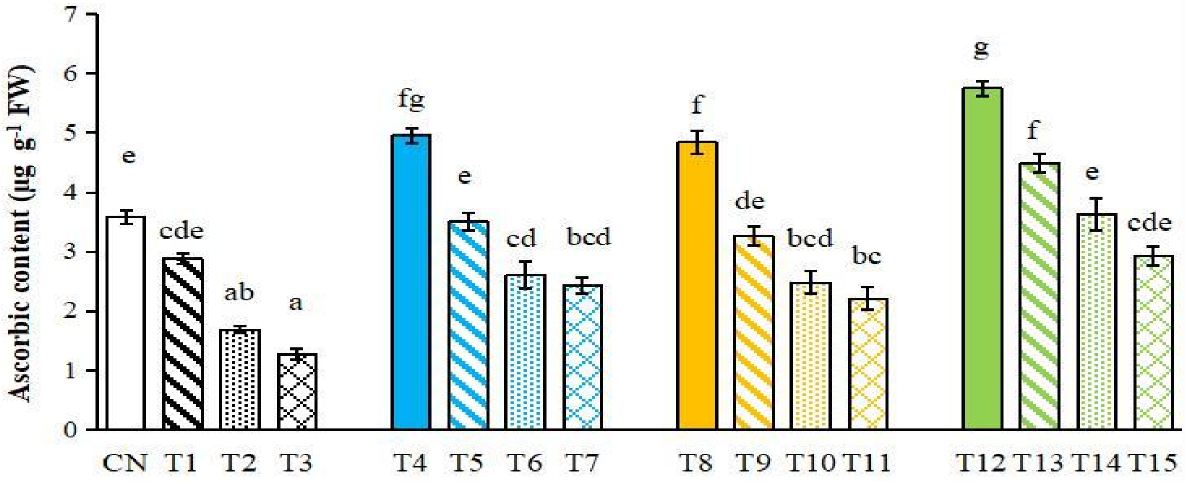
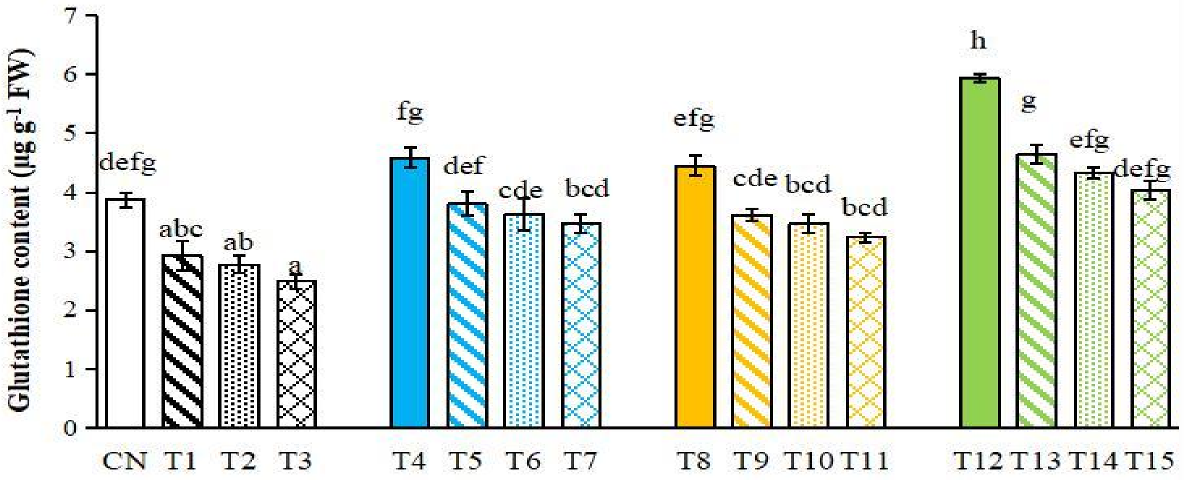


Table. 6.18: Influence of EBL+Si on treatment on non-enzymatic antioxidants of 15-days old Pb stressed plants of *Trigonella foemum-graecum*

Treatments	Ascorbic acid (µg g^{-1} FW)	Glutathione (µg g^{-1} FW)	Tocopherol (µg g^{-1} FW)
C	$3.58^{e}\pm0.11$	$3.87^{defg}\pm0.12$	$4.41^{fg}\pm0.11$
T_1	$2.87^{cde}\pm0.08$	$2.93^{abc}\pm0.24$	$2.65^{ab}\pm0.04$
T_2	$1.69^{ab}\pm0.06$	$2.77^{ab}\pm0.14$	$2.39^{a}\pm0.12$
T_3	$1.27^{a}\pm0.09$	$2.49^{a}\pm0.12$	$2.28^{a}\pm0.12$
T_4	$4.95^{fg}\pm0.12$	$4.58^{fg}\pm0.16$	$5.31^{hi}\pm0.15$
T_5	$3.5^{e}\pm0.14$	$3.8^{def}\pm0.2$	$4.13^{efg}\pm0.19$
T_6	$2.61^{cd}\pm0.22$	$3.62^{cde}\pm0.2$	$3.67^{cde}\pm0.12$
T_7	$2.42^{bcd}\pm0.13$	$3.46^{bcd}\pm0.16$	$3.35^{bcd}\pm0.11$
T_8	$4.84^{f}\pm0.19$	$4.45^{efg}\pm0.16$	$4.63^{gh}\pm0.06$
T_9	$3.26^{de}\pm0.15$	$3.61^{cde}\pm0.1$	$3.87^{def}\pm0.04$
T_{10}	$2.48^{cd}\pm0.19$	$3.47^{bcd}\pm0.16$	$3.36^{cd}\pm0.12$
T_{11}	$2.2^{bcd}\pm0.18$	$3.23^{bcd}\pm0.08$	$3.15^{bc}\pm0.15$
T_{12}	$5.74^{g}\pm0.12$	$5.93^{h}\pm0.05$	$5.7^{i}\pm0.22$
T_{13}	$4.48^{f}\pm0.15$	$4.64^{g}\pm0.16$	$4.41^{fg}\pm0.12$
T_{14}	$3.63^{e}\pm0.27$	$4.33^{efg}\pm0.1$	$4.18^{efg}\pm0.19$
T_{15}	$2.92^{cde}\pm0.15$	$4.03^{defg}\pm0.16$	$3.69^{cde}\pm0.06$


Ascorbic content (μg g-1 FW)
7
6
5
4
3
2
1
0
e
cde
ab
a
fg
e
cd
bcd
f
de
bcd
bc
g
f
e
cde
CN T1 T2 T3
T4 T5 T6 T7
T8 T9 T10 T11
T12 T13 T14 T15


Glutathione content (μg g-1 FW)
7
6
5
4
3
2
1
0
defg
abc
ab
a
fg
def
cde
bcd
efg
cde
bcd
bcd
h
g
efg
defg
CN T1 T2 T3
T4 T5 T6 T7
T8 T9 T10 T11
T12 T13 T14 T15

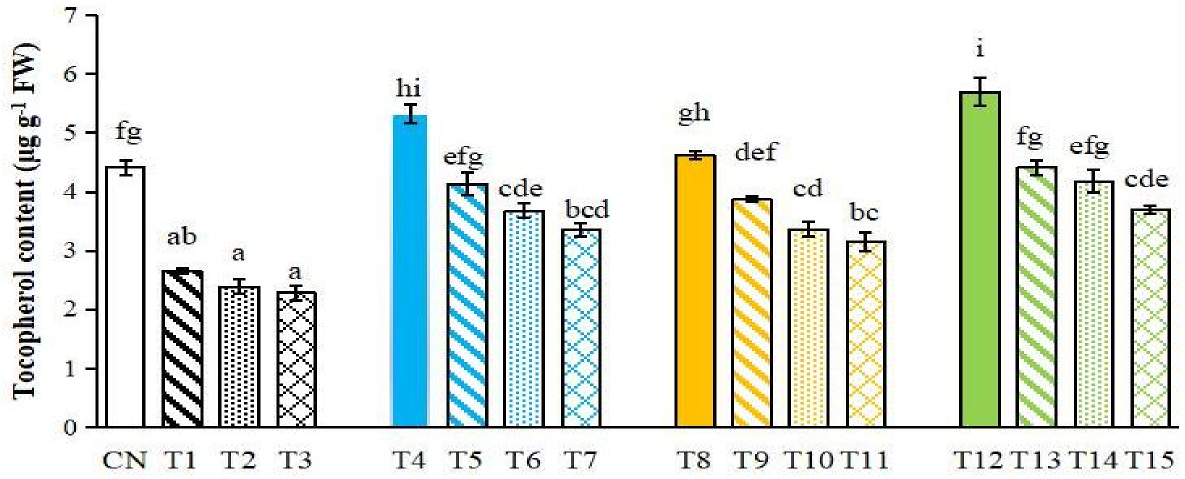

Tocopherol content (μg g-1 FW)
7
6
5
4
3
2
1
0
fg
ab
a
a
hi
efg
cde
bcd
gh
def
cd
bc
i
fg
efg
cde
CN T1 T2 T3
T4 T5 T6 T7
T8 T9 T10 T11
T12 T13 T14 T15

Fig. 6.18: Influence of EBL+Si treatment on measures of non - enzymatic antioxidants in 15 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Tocopherol content exhibited a drastic decline at every chosen level of Pb toxicity during the present work (Fig. 6.18; Table 6.18). Minimum measures of tocopherol were recorded to be of 2.28 µg g^{-1} FW at T_3 amongst all the three distinct concentrations of Pb. In addition, in case of EBL and Si alone augmented plants under Pb induced stress, the highest values of this antioxidant were recorded to be 4.13 at T_5 and 3.87 µg g^{-1} FW at T_9, respectively. However, EBL + Si together displayed a highest value of tocopherol content of 4.41 µg g^{-1} FW in T_{13}. An upsurge of 55.84% was recorded in T_5 over T_1. Similarly, T_{13} plants displayed a promotion of 6.77% as opposed to T_5. T_{13} was observed to be having the same content of tocopherol as that of control plants.

6.1.3 Plants raised in *In vivo* conditions*:* 30 days old stage

6.1.3.1 Growth attributes

In response to Pb stress, the root length was recorded to be at its lowest at T_3 (2.62 cm) (Fig. 6.19a; Table 6.19a). EBL pre-treatment promoted the root length of 30 days old stressed plants with being maximum and minimum at T_5 and T_7 with the longest and shortest roots at values of 6.62 and 5.11, respectively. EBL application displayed superior results in enhancing root lengths under stress than Si application. EBL and Si further intensified the growth in fenugreek roots with the longest roots being seen at 8.2 cm in T_{13}. T_3 exhibited 60.42% drop in root length over T_5 plants. An increment of 30.57% was noticed in T_{13} versus T_9 plants. T_{13} recorded 43.85% elevation contrary to control plants.

Shoot length exhibited Pb stress induced reduction, being at its lowest with a value of 6.88 cm i.e. at T_3, 70.43% lower than CN plants. EBL and Si control plants displayed escalations of shoot length (Fig. 6.19a; Table 6.19a). Under Pb toxicity, EBL treatment raised the shoot length to 23.17 cm at T_5 treatment as correlated to individual treatment of Pb I stressed plants, which stand at just 17.31 cm. On the other hand, Si treated stressed plants followed the same trend of enhancement in their shoot length. It made the shoot length to increase with its maximum value at T_9 (20.13cm). Collectively applied EBL and Si in Pb stress turned out to be more beneficial, attaining its highest value of 29.22 cm at T_{14}. Shoot length recorded a reduction concomitant with every elevation in toxic concentrations of Pb in response to cumulative action of EBL + Si. T_9 plants exhibited 58.50% elevation of shoot length in correlation with T_2. Similarly, 177.76% augmentation was noted in T_7 than T_3. Furthermore, T_{14} had 25.56% more long shoots than CN plants.

Fresh weight was reportedly marred in response to Pb exposure with greatest reduction being noticed at T_3 treated plants (1.17 g) in (Fig. 6.19a; Table 6.19a). In EBL pre-treated plants, the maximal and minimal enhancement values stood at i.e., 2.24 g and 1.79 g fresh weights in T_5 and T_6 treated plants, respectively.T_{10} elevated the fresh weight from 1.63 g to 2.17 g, as opposed to T_2 treated plants. The maximum improvement in fresh weight was noted in case of integrated EBL +Si treated plants with highest being at T_{13} with 3.49 g fresh weight. T_5 recorded 91.45% increase in contrast to T_3. An amplification of 55.80% and 17.50% was registered in T_{13} over T_5 and control plants.

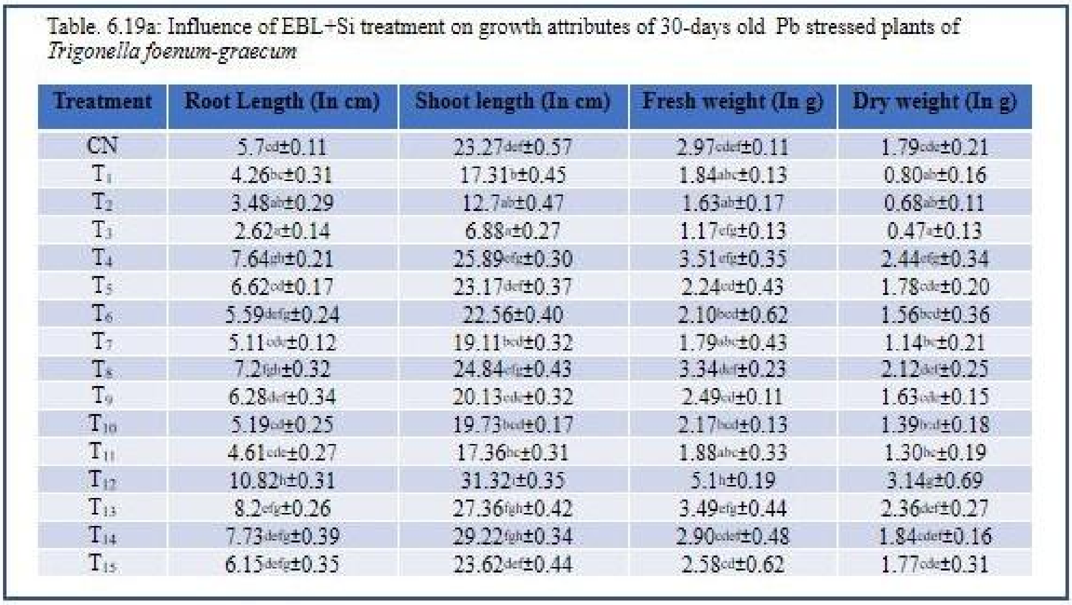
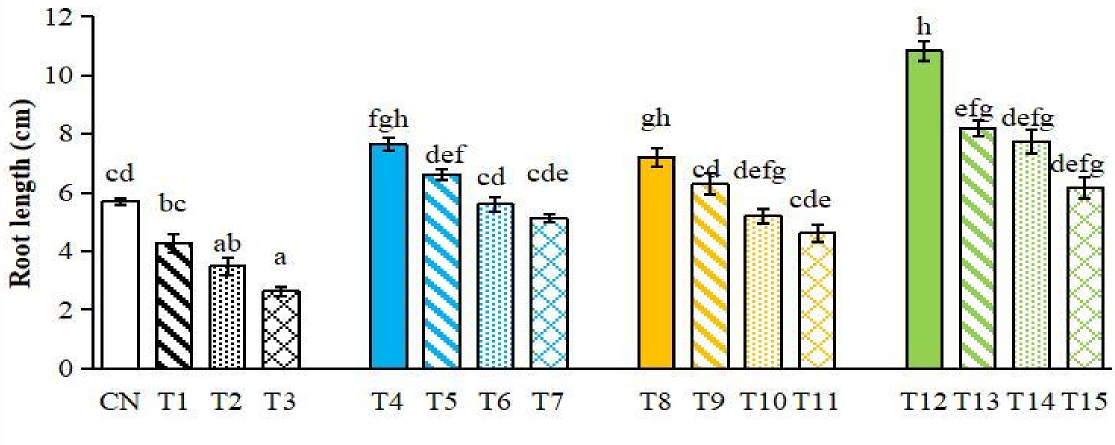


Table. 6.19a: Influence of EBL+Si treatment on growth attributes of 30-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Root Length (In cm)	Shoot length (In cm)	Fresh weight (In g)	Dry weight (In g)
CN	$5.7^{cd}\pm0.11$	$23.27^{def}\pm0.57$	$2.97^{cdef}\pm0.11$	$1.79^{cde}\pm0.21$
T_1	$4.26^{bc}\pm0.31$	$17.31^{b}\pm0.45$	$1.84^{abc}\pm0.13$	$0.80^{ab}\pm0.16$
T_2	$3.48^{ab}\pm0.29$	$12.7^{ab}\pm0.47$	$1.63^{ab}\pm0.17$	$0.68^{ab}\pm0.11$
T_3	$2.62^{a}\pm0.14$	$6.88^{a}\pm0.27$	$1.17^{efg}\pm0.13$	$0.47^{a}\pm0.13$
T_4	$7.64^{gh}\pm0.21$	$25.89^{efg}\pm0.30$	$3.51^{efg}\pm0.35$	$2.44^{efg}\pm0.34$
T_5	$6.62^{cd}\pm0.17$	$23.17^{def}\pm0.37$	$2.24^{cd}\pm0.43$	$1.78^{cde}\pm0.20$
T_6	$5.59^{defg}\pm0.24$	22.56 ± 0.40	$2.10^{bcd}\pm0.62$	$1.56^{bcd}\pm0.36$
T_7	$5.11^{cde}\pm0.12$	$19.11^{bcd}\pm0.32$	$1.79^{abc}\pm0.43$	$1.14^{bc}\pm0.21$
T_8	$7.2^{fgh}\pm0.32$	$24.84^{efg}\pm0.43$	$3.34^{def}\pm0.23$	$2.12^{def}\pm0.25$
T_9	$6.28^{def}\pm0.34$	$20.13^{cde}\pm0.32$	$2.49^{cd}\pm0.11$	$1.63^{cde}\pm0.15$
T_{10}	$5.19^{cd}\pm0.25$	$19.73^{bcd}\pm0.17$	$2.17^{bcd}\pm0.13$	$1.39^{bcd}\pm0.18$
T_{11}	$4.61^{cde}\pm0.27$	$17.36^{bc}\pm0.31$	$1.88^{abc}\pm0.33$	$1.30^{bc}\pm0.19$
T_{12}	$10.82^{h}\pm0.31$	$31.32^{i}\pm0.35$	$5.1^{h}\pm0.19$	$3.14^{g}\pm0.69$
T_{13}	$8.2^{efg}\pm0.26$	$27.36^{fgh}\pm0.42$	$3.49^{efg}\pm0.44$	$2.36^{def}\pm0.27$
T_{14}	$7.73^{defg}\pm0.39$	$29.22^{fgh}\pm0.34$	$2.90^{cdef}\pm0.48$	$1.84^{cdef}\pm0.16$
T_{15}	$6.15^{defg}\pm0.35$	$23.62^{def}\pm0.44$	$2.58^{cd}\pm0.62$	$1.77^{cde}\pm0.31$



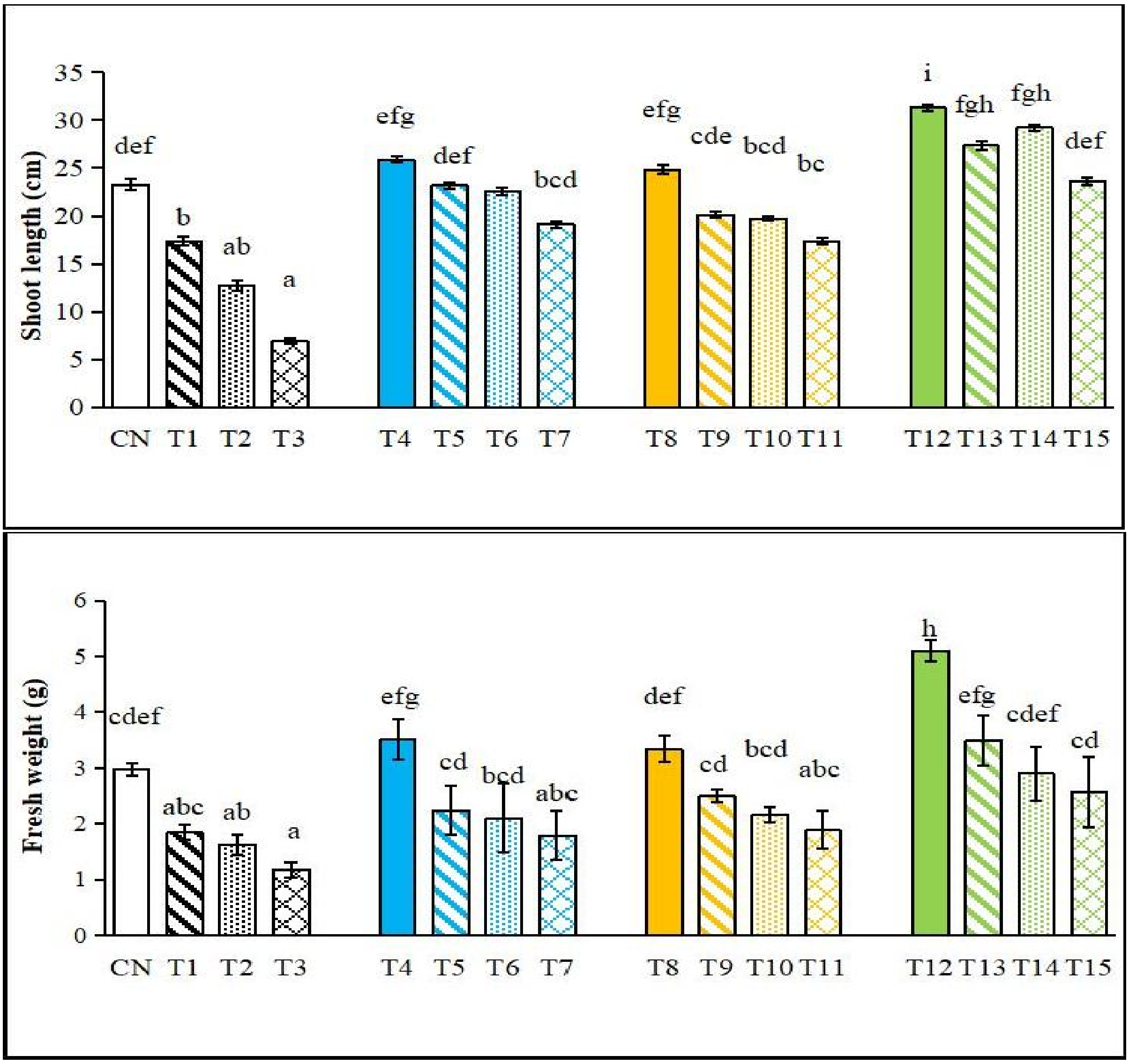


Fig. 6.19 (a): Influence of EBL+Si treatment on growth attributes in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Notable difference was exhibited among T_1, T_2 and T_3 plants with respect to reduction in their dry weights (Fig. 6.19b; Table 6.19a) with 0.80, 0.68 and 0.47 g dry weights values, respectively. Individually added EBL and Si in mitigating the Pb induced stress registered an upsurge in the value of dry weights with maximum being around 1.78 and 1.63 g at T_5 and T_9 concentrations, respectively. EBL and Si together yielded better results in enhancing dry weight vis-à-vis their individual applications. EBL alone addition exhibited greater dry weight (1.56 g) at T_6 versus T_{10} treatment which had only 1.39 g dry weight. T_5 recorded an upsurge of 9.20% in contrast to T_9. An amplification of 32.58% was recorded in T_{13} over T_5.

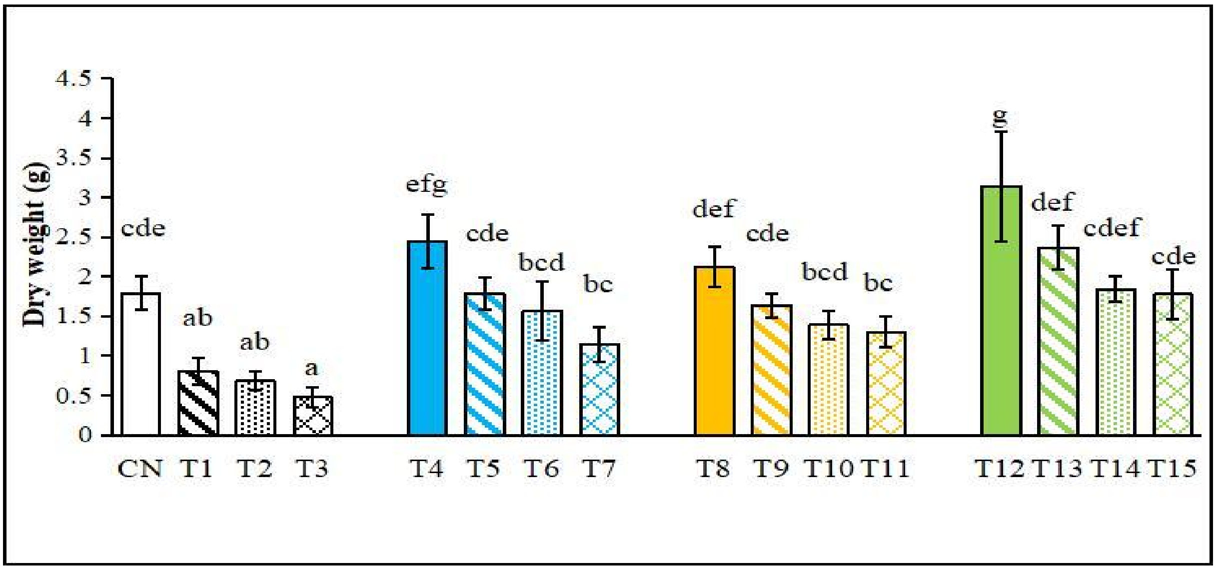


Fig. 6.19 (b): Influence of EBL+Si treatment on dry weight in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Abridgement of vigor index (VI) was also noticed in Pb metal stressed fenugreek as shown by T_1 plants (1204.7%) with being reduced 44.17% to that of untreated plants of control (2157.9%). Further, plants of T_2 treatment were noted to have VI value of 861.5% i.e. 28.48% lesser in contrast with T_1 treated plants (Fig. 6.19c; Table 6.19b). Individually supplemented EBL and Si under Pb metal stress elevated the VI values. Maximum value of VI (2376.7%) was observed around Pb I concentration in T_5 plants pre-treated with EBL. Exogenous augmentation of Si also escalated the VI values with being highest at T_9 treatment i.e. 1967.4% which revealed that 24-EBL was way more effective in enhancing the VI in response to Pb stress. However, when coupled, EBL and Si showed maximum elevation in the VI values amongst all treatments i.e. 3161.8% at T_{13} against 1204.7% of Pb I alone augmented T_1 plants making the elevation as high as 162.4%. T_{13} plants correlated with T_5 and T_9 were recorded to exhibit a respective increase of 33.03% and 60.7 % whereas in their comparison to untreated control plants, this enhancement stood at 46.52%.

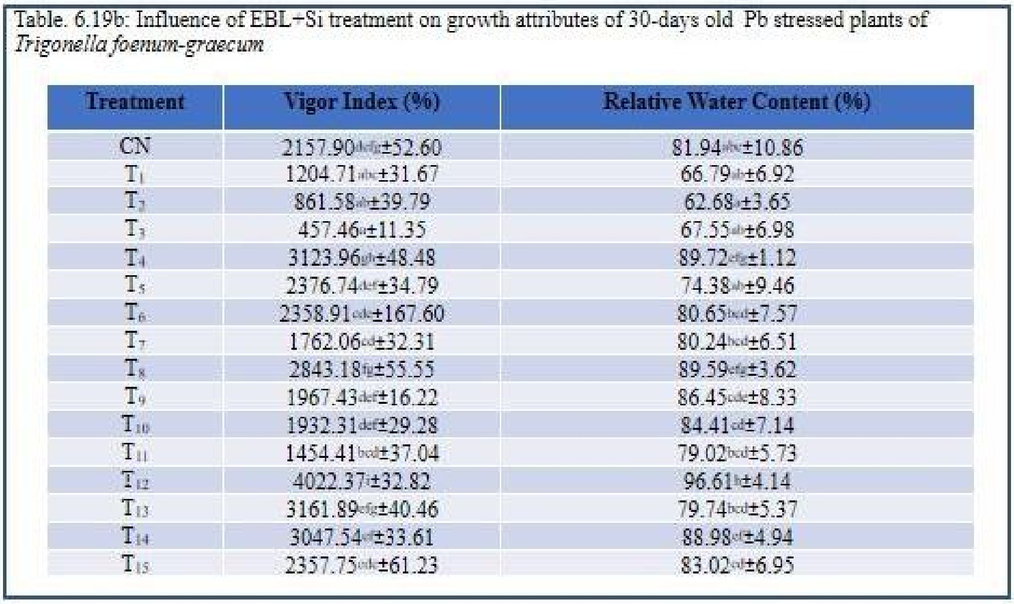


Table. 6.19b: Influence of EBL+Si treatment on growth attributes of 30-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Vigor Index (%)	Relative Water Content (%)
CN	2157.90^{defg}±52.60	81.94^{abc}±10.86
T_1	1204.71^{abc}±31.67	66.79^{ab}±6.92
T_2	861.58^{ab}±39.79	62.68^{a}±3.65
T_3	457.46^{a}±11.35	67.55^{ab}±6.98
T_4	3123.96^{gh}±48.48	89.72^{efg}±1.12
T_5	2376.74^{def}±34.79	74.38^{ab}±9.46
T_6	2358.91^{cde}±167.60	80.65^{bcd}±7.57
T_7	1762.06^{cd}±32.31	80.24^{bcd}±6.51
T_8	2843.18^{fg}±55.55	89.59^{efg}±3.62
T_9	1967.43^{def}±16.22	86.45^{cde}±8.33
T_{10}	1932.31^{def}±29.28	84.41^{cd}±7.14
T_{11}	1454.41^{bcd}±37.04	79.02^{bcd}±5.73
T_{12}	4022.37^{i}±32.82	96.61^{h}±4.14
T_{13}	3161.89^{efg}±40.46	79.74^{bcd}±5.37
T_{14}	3047.54^{ef}±33.61	88.98^{ef}±4.94
T_{15}	2357.75^{cde}±61.23	83.02^{cd}±6.95

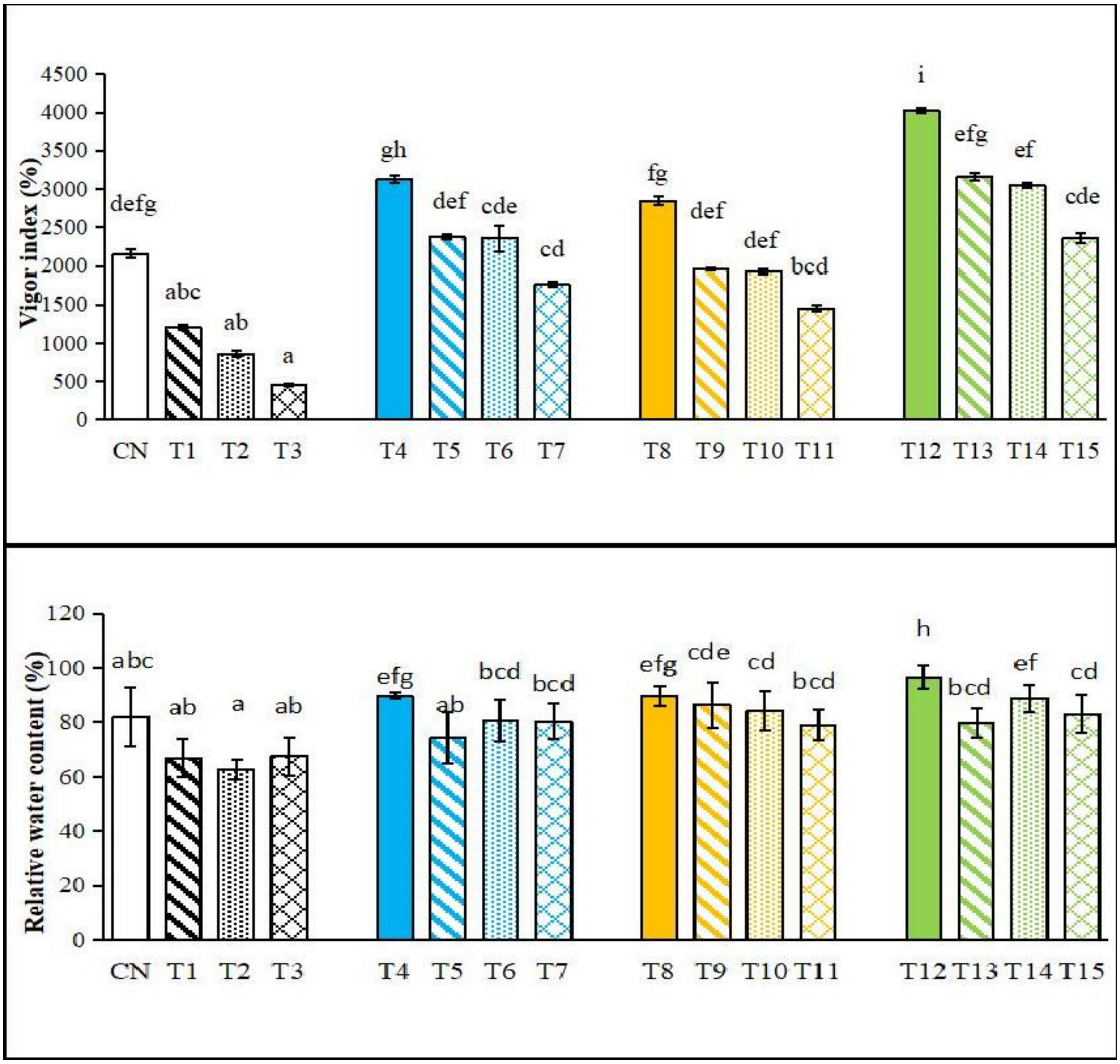


Fig. 6.19 (c): Influence of EBL+Si treatment on vigor index and RWC in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment

level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

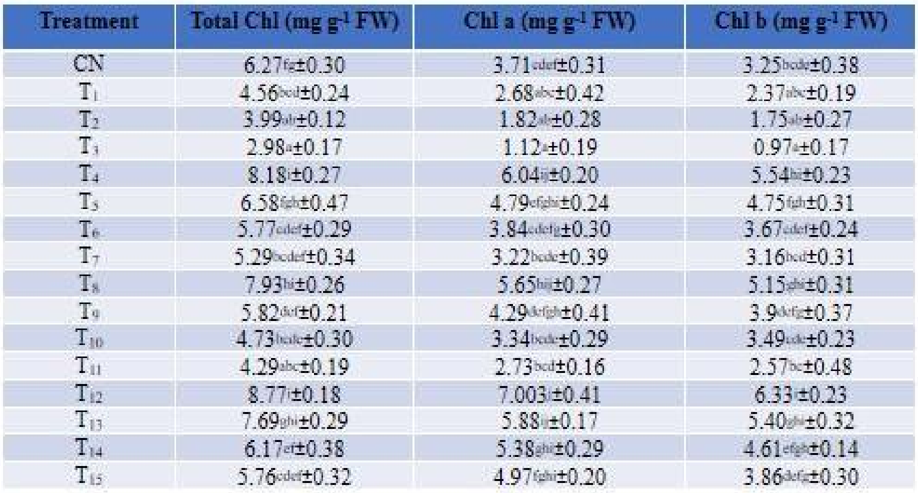
Relative water content was diminished on account of Pb stress with lowest relative water content (62.68%) recorded in T_2 plants (Fig. 6.19c; Table 6.19b). EBL treatment with reference to unstressed conditions noted to have relative water content of 89.72% (T_4) which was higher in contrast to control plants. Amongst all the three Pb treatments in EBL-pre-treated seedlings, the optimum relative water content of 80.65% was noted in T_6. Silicon addition against Pb stress recorded the lowest relative water content of 79.02% in T_{11} plants. T_{14} exhibited greater relative water content with being highest at 88.98%.

6.1.3.2 Photosynthetic activity

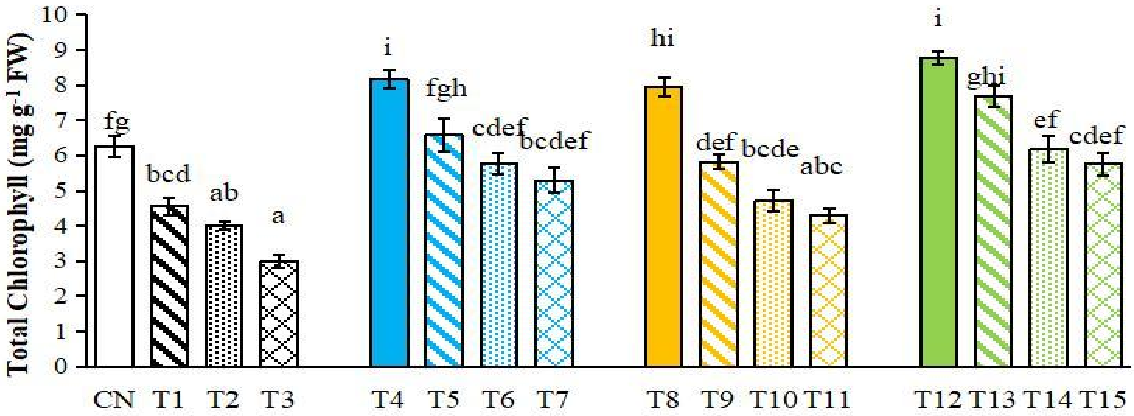
6.1.3.2.1 Photosynthetic pigments

Photosynthetic pigments had documented a drastic decline as an aftermath of Pb stress (Fig. 6.20a; Table 6.20a). Almost 52.47% drop in the measures of total chlorophyll was recorded at T_3 versus control conditions i.e., from 6.27 to 2.98 mg g^{-1} FW. EBL and Si applied in an individual manner, promoted its quantities in reference to Pb stress where the highest values among them were reported of 6.58 and 5.82 mg g^{-1} FW at T_5 and T_9. Synergistically supplemented EBL and Si under stress registered far superior outcomes in amplifying the measures of total chlorophyll vis-à-vis their individual treatments. The largest quantities were reported to be in case of 7.69 mg g^{-1} FW in T_{13} plants. Further, T_5 displayed 44.29% increment as opposed to T_1 whereas T_9 exhibited 27.63% upsurge in contrast to T_1. An upgradation of 16.86% was recorded in T_{13} over T_5. Similar outcomes were obtained with respect to content values of chl a and chl b in fenugreek plants. The lowest measure of chl a (1.12 mg g^{-1} FW) and chl b (0.97 mg g^{-1} FW) were registered at T_3 concentration. EBL and Si notably ameliorated the Pb-induced noxiousness by boosting their levels. The integrated treatment of EBL and Si further augmented their contents in response to stressful conditions with being the highest around 5.88 and 5.40 mg g^{-1} FW in T_{13} plants. Furthermore, T_5 recorded 78.73% and 100.42% increment versus T_1 whereas T_9 exhibited 60.07% and 64.55% upsurge contrary to T_1 in reference to chl a and chl b, respectively.

Table. 6.20a: Influence of EBL+Si treatment on photosynthetic pigments of 30-days old plants of *Trigonella foenum-graecum*



Treatment	Total Chl (mg g^{-1} FW)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)
CN	6.27^{fg}±0.30	3.71^{cdef}±0.31	3.25^{bcde}±0.38
T_1	4.56^{bcd}±0.24	2.68^{abc}±0.42	2.37^{abc}±0.19
T_2	3.99^{ab}±0.12	1.82^{ab}±0.28	1.75^{ab}±0.27
T_3	2.98^{a}±0.17	1.12^{a}±0.19	0.97^{a}±0.17
T_4	8.18^{i}±0.27	6.04^{ij}±0.20	5.54^{hi}±0.23
T_5	6.58^{fgh}±0.47	4.79^{efghi}±0.24	4.75^{fgh}±0.31
T_6	5.77^{cdef}±0.29	3.84^{cdefg}±0.30	3.67^{cdef}±0.24
T_7	5.29^{bcdef}±0.34	3.22^{bcde}±0.39	3.16^{bcd}±0.31
T_8	7.93^{hi}±0.26	5.65^{hij}±0.27	5.15^{ghi}±0.31
T_9	5.82^{def}±0.21	4.29^{defgh}±0.41	3.9^{defg}±0.37
T_{10}	4.73^{bcde}±0.30	3.34^{bcde}±0.29	3.49^{cde}±0.23
T_{11}	4.29^{abc}±0.19	2.73^{bcd}±0.16	2.57^{bc}±0.48
T_{12}	8.77^{i}±0.18	7.003^{j}±0.41	6.33^{i}±0.23
T_{13}	7.69^{ghi}±0.29	5.88^{ij}±0.17	5.40^{ghi}±0.32
T_{14}	6.17^{ef}±0.38	5.38^{ghi}±0.29	4.61^{efgh}±0.14
T_{15}	5.76^{cdef}±0.32	4.97^{fghi}±0.20	3.86^{defg}±0.30



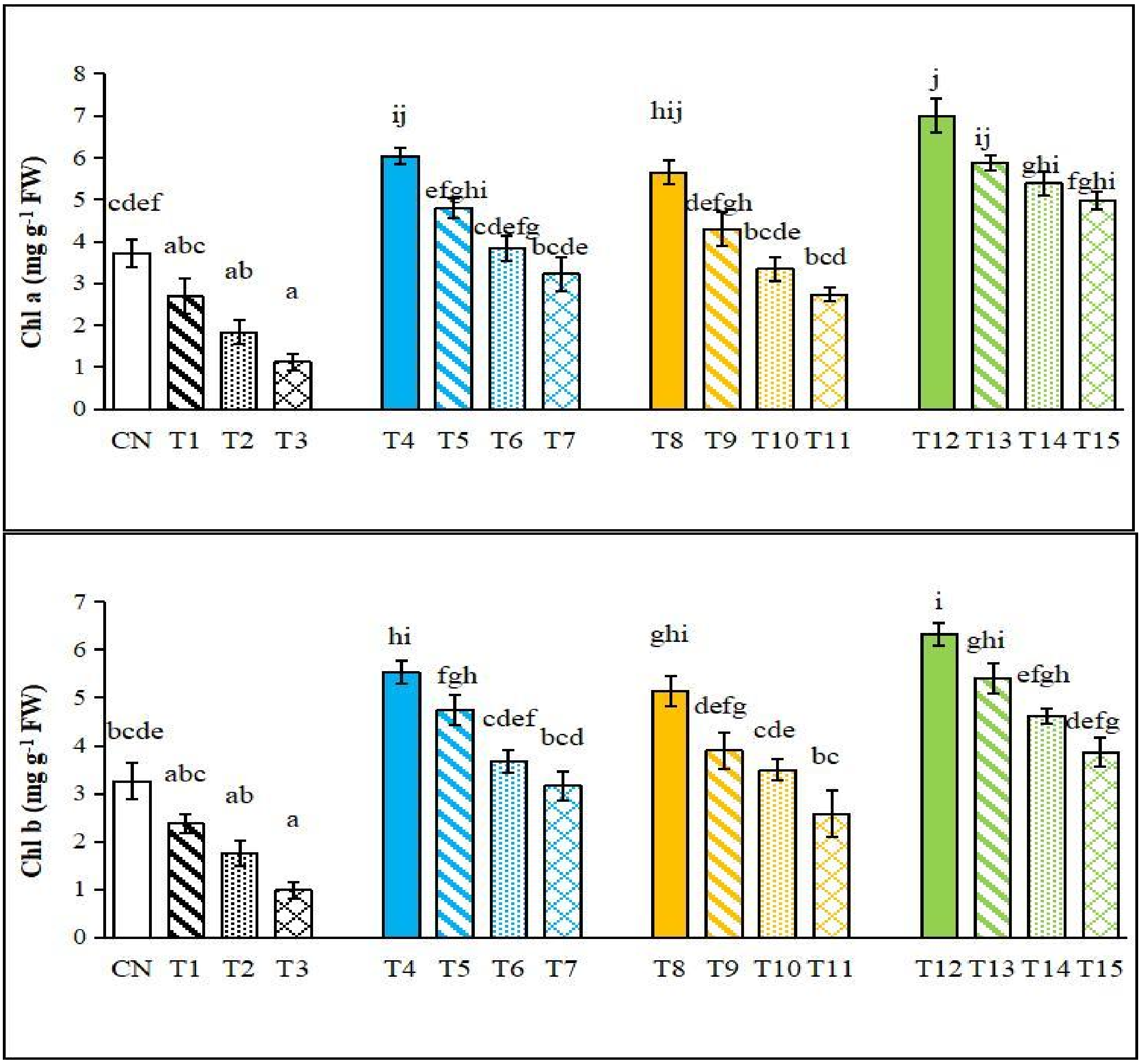


Fig. 6.20 (a): Influence of EBL+Si treatment on measures of photosynthetic pigments in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

It was documented that Pb stress diminished the carotenoid measures (Fig. 6.20b; Table 6.20b). Carotenoid quantities depleted with elevated levels of Pb toxicity with the greatest decrement at T_3 (4.09 mg g^{-1} FW). Carotenoid measures were scaled up from 6.11 to 7.31 mg g^{-1} FW in T_5 plants contrary to T_1 stressed plants. Si addition brought forth maximum escalation in the carotenoid content of 7.12 mg g^{-1} FW at T_9 genotypes. The largest amount of carotenoid i.e. 8.75 mg g^{-1} FW was reported at T_{13} while the most diminished content stood at T_{15} (7.14 mg g^{-1} FW). A respective augmentation of 19.69% and 13.34% was noted in T_{13} versus T_5 and control plants.

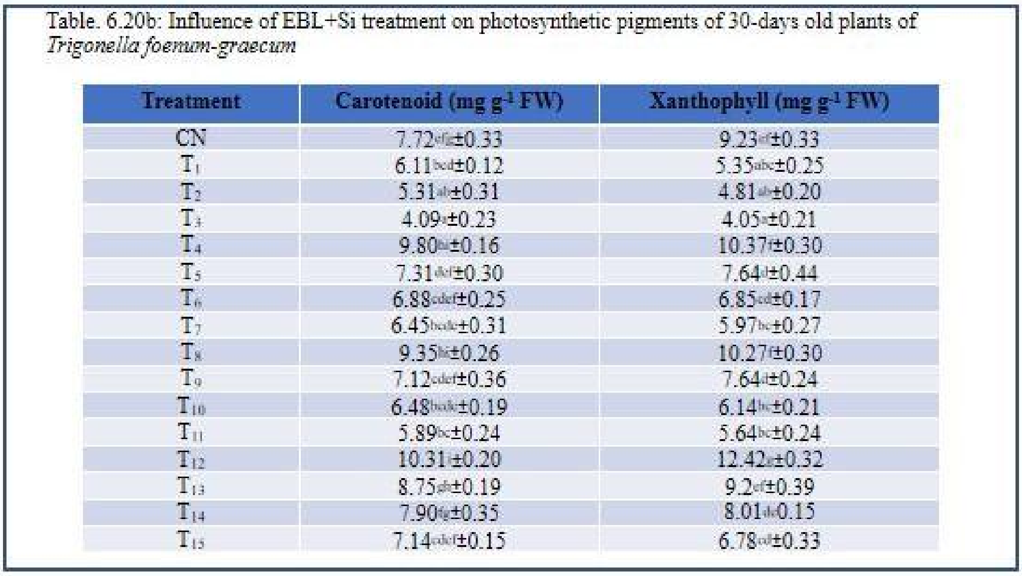
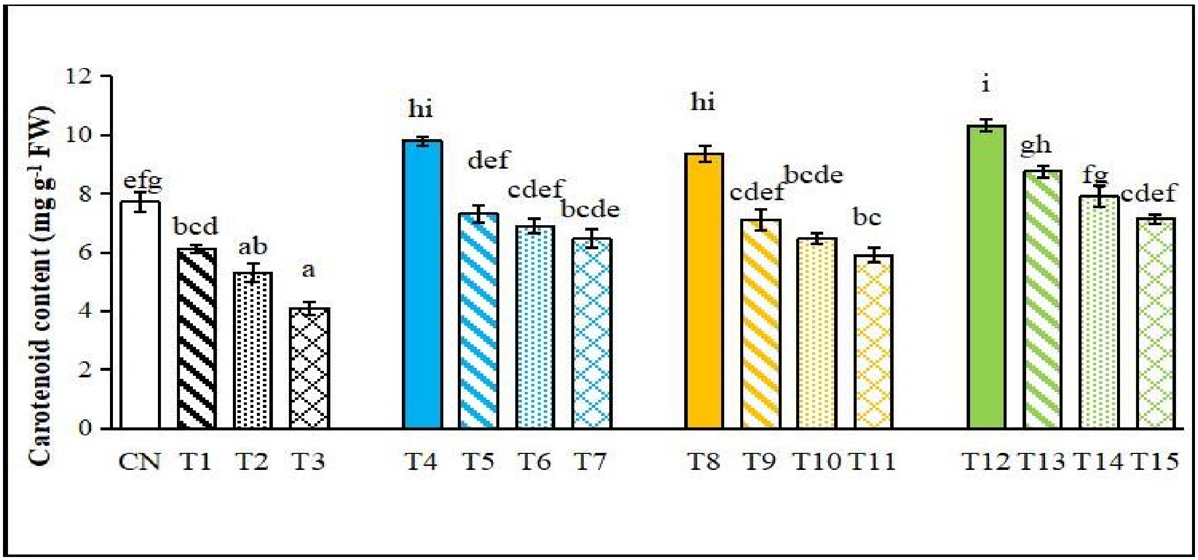


Table. 6.20b: Influence of EBL+Si treatment on photosynthetic pigments of 30-days old plants of *Trigonella foemum-graecum*

Treatment	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
CN	$7.72^{efg}\pm0.33$	$9.23^{ef}\pm0.33$
T_1	$6.11^{bcd}\pm0.12$	$5.35^{abc}\pm0.25$
T_2	$5.31^{ab}\pm0.31$	$4.81^{ab}\pm0.20$
T_3	$4.09^{a}\pm0.23$	$4.05^{a}\pm0.21$
T_4	$9.80^{hi}\pm0.16$	$10.37^{f}\pm0.30$
T_5	$7.31^{def}\pm0.30$	$7.64^{d}\pm0.44$
T_6	$6.88^{cdef}\pm0.25$	$6.85^{cd}\pm0.17$
T_7	$6.45^{bcde}\pm0.31$	$5.97^{bc}\pm0.27$
T_8	$9.35^{hi}\pm0.26$	$10.27^{f}\pm0.30$
T_9	$7.12^{cdef}\pm0.36$	$7.64^{d}\pm0.24$
T_{10}	$6.48^{bcde}\pm0.19$	$6.14^{bc}\pm0.21$
T_{11}	$5.89^{bc}\pm0.24$	$5.64^{bc}\pm0.24$
T_{12}	$10.31^{i}\pm0.20$	$12.42^{g}\pm0.32$
T_{13}	$8.75^{gh}\pm0.19$	$9.2^{ef}\pm0.39$
T_{14}	$7.90^{fg}\pm0.35$	$8.01^{de}0.15$
T_{15}	$7.14^{cdef}\pm0.15$	$6.78^{cd}\pm0.33$



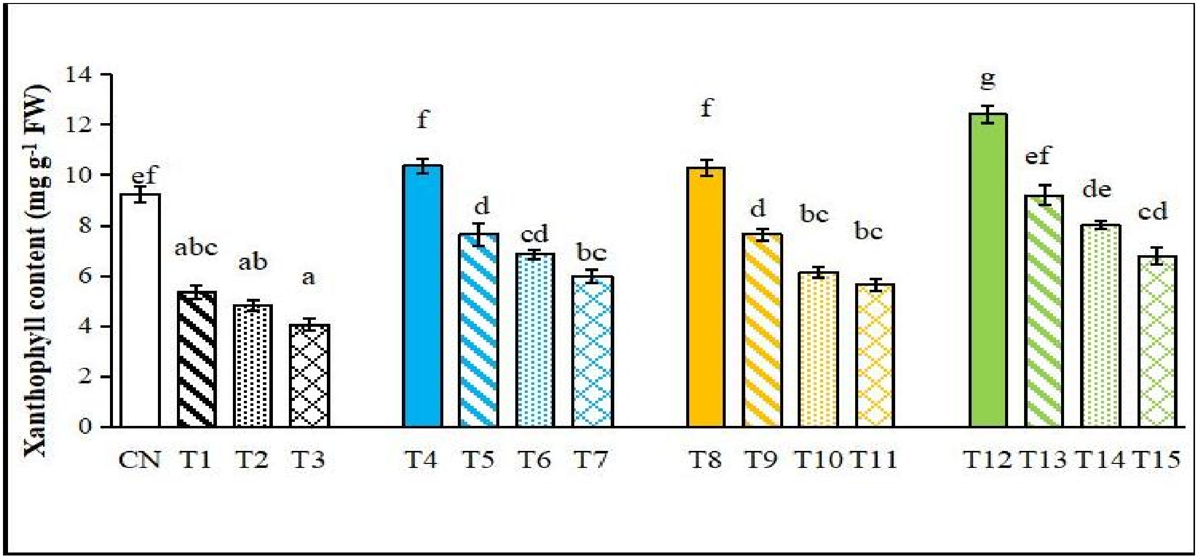


Fig. 6.20 (b): Influence of EBL+Si treatment on measures of photosynthetic pigments in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Lead stress significantly depleted the content of xanthophyll with a maximal reduction of 4.05 mg g^{-1} FW in T_3 plants (Fig. 6.20b; Table 6.20b). EBL and Si treatments scaled up the measures of xanthophyll when fenugreek was subjected to Pb stress. Individually added EBL and Si mitigated the Pb stress in terms of promoted levels of xanthophyll having highest content at T_5 and T_9 treatments i.e 7.64 mg g^{-1} FW in each one of them. Diminution of xanthophyll content was directly influenced by increasing levels of Pb stress in EBL and Si alone applications. The highest xanthophyll was registered to be 9.2 mg g^{-1} FW at T_{13} concentration. In comparison to T_1, T_5 and T_9, both recorded an increment of 42.80%. A respective upsurge of 20.41% was noticed in T_{13} over T_5 plants.

6.1.3.3 Metabolites

Lead stress brought forth noteworthy reductions in the amount of anthocyanin with the greatest reduction of 2.76 mg g^{-1} FW at T_3 (Fig. 6.21; Table 6.21). Individually augmented EBL and Si under Pb stress reportedly elevated the levels of anthocyanin with being largest around 6.79 mg g^{-1} FW in T_9. EBL was far superior versus Si in boosting the anthocyanin content in response to Pb toxicity. The optimal value of anthocyanin content (8.31 mg g^{-1} FW) was recorded in the case of coupling of stress plus its mitigants i.e. T_{13}, among all the treatments used in the study. T_5 exhibited an increment of 80.19% contrary to T_1 whereas T_9 registered an elevation of 64% versusT_1. An upsurge of 22.38% was recorded in T_{13} over T_9.

Both EBL and Si in absence of Pb stress brought about an improvement in flavonoids in *Trigonella* plants among all the other treatments (Fig. 6.21; Table 6.21). A maximum decrement in the flavonoids i.e. 3.04 mg g^{-1} FW was noticed at T_3. Depletion in the quantities of flavonoids was noticed as the level of Pb stress was scaled up in Pb alone augmented plants. In EBL pre-applied plants against Pb stress, minimal content of flavanoid seas registered at T_7 (4.93 mg g^{-1} FW). Individually treated stressed fenugreek plants with Si led to a promotion in the values from 3.04 of T_3 to 6.59 mg g^{-1} FW in T_5 plants. In addition to this, the combination of EBL and Si largely attenuated Pb toxicity in fenugreek plants having optimum content of 7.33 mg g^{-1} FW at T_{14} treatment. An upsurge of 37.57% at T_5 was reported in correlation to T_1 whereas T_9 exhibited 21.92% enhancement over T_1. An amplification of 25.51% and 18.8% was registered in T_{13} vis-à-vis T_9 and control plants.

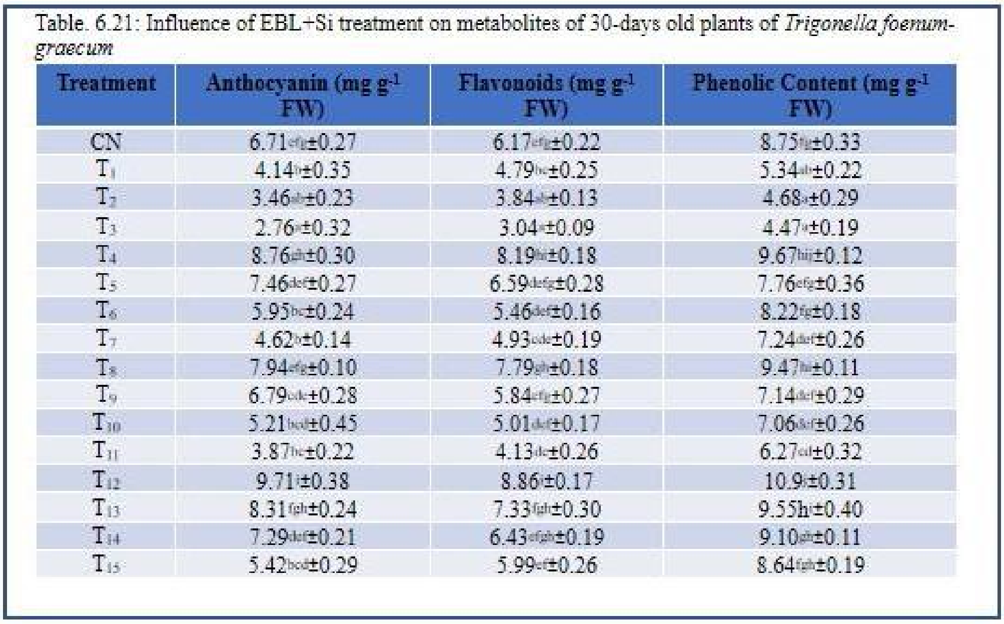
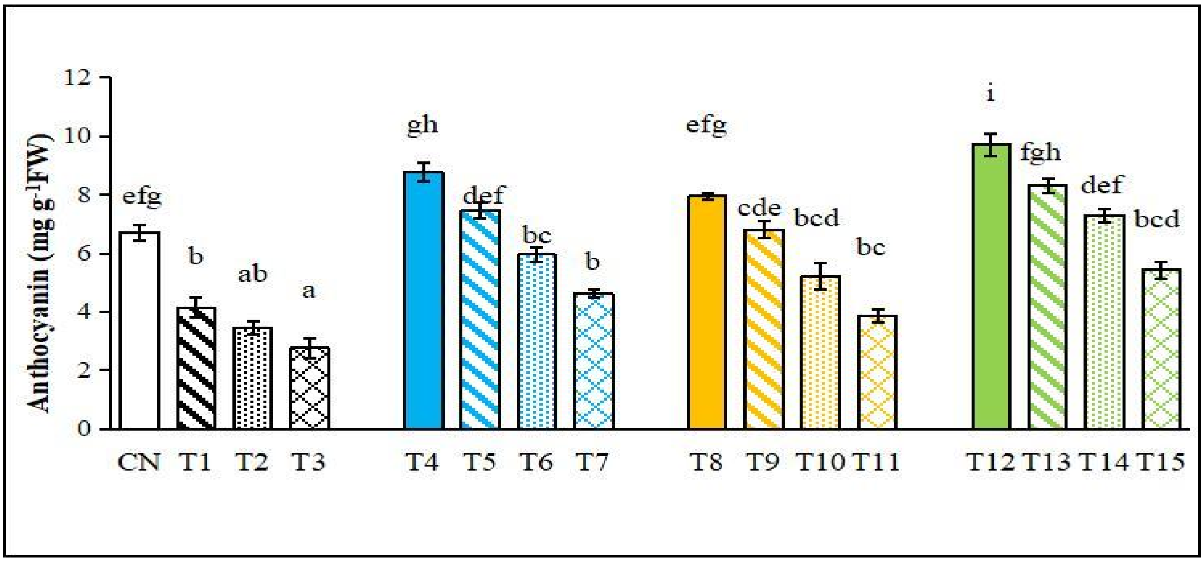


Table. 6.21: Influence of EBL+Si treatment on metabolites of 30-days old plants of *Trigonella foenum-graecum*

Treatment	Anthocyanin (mg g^{-1} FW)	Flavonoids (mg g^{-1} FW)	Phenolic Content (mg g^{-1} FW)
CN	6.71^{efg}±0.27	6.17^{efg}±0.22	8.75^{fg}±0.33
T_1	4.14^{b}±0.35	4.79^{bc}±0.25	5.34^{ab}±0.22
T_2	3.46^{ab}±0.23	3.84^{ab}±0.13	4.68^{a}±0.29
T_3	2.76^{a}±0.32	3.04^{a}±0.09	4.47^{a}±0.19
T_4	8.76^{gh}±0.30	8.19^{hi}±0.18	9.67^{hij}±0.12
T_5	7.46^{def}±0.27	6.59^{defg}±0.28	7.76^{efg}±0.36
T_6	5.95^{bc}±0.24	5.46^{def}±0.16	8.22^{fg}±0.18
T_7	4.62^{b}±0.14	4.93^{cde}±0.19	7.24^{def}±0.26
T_8	7.94^{efg}±0.10	7.79^{gh}±0.18	9.47^{hi}±0.11
T_9	6.79^{cde}±0.28	5.84^{efg}±0.27	7.14^{def}±0.29
T_{10}	5.21^{bcd}±0.45	5.01^{def}±0.17	7.06^{def}±0.26
T_{11}	3.87^{bc}±0.22	4.13^{de}±0.26	6.27^{cd}±0.32
T_{12}	9.71^{i}±0.38	8.86^{i}±0.17	10.9^{i}±0.31
T_{13}	8.31^{fgh}±0.24	7.33^{fgh}±0.30	9.55h^{i}±0.40
T_{14}	7.29^{def}±0.21	6.43^{efgh}±0.19	9.10^{gh}±0.11
T_{15}	5.42^{bcd}±0.29	5.99^{ef}±0.26	8.64^{fgh}±0.19



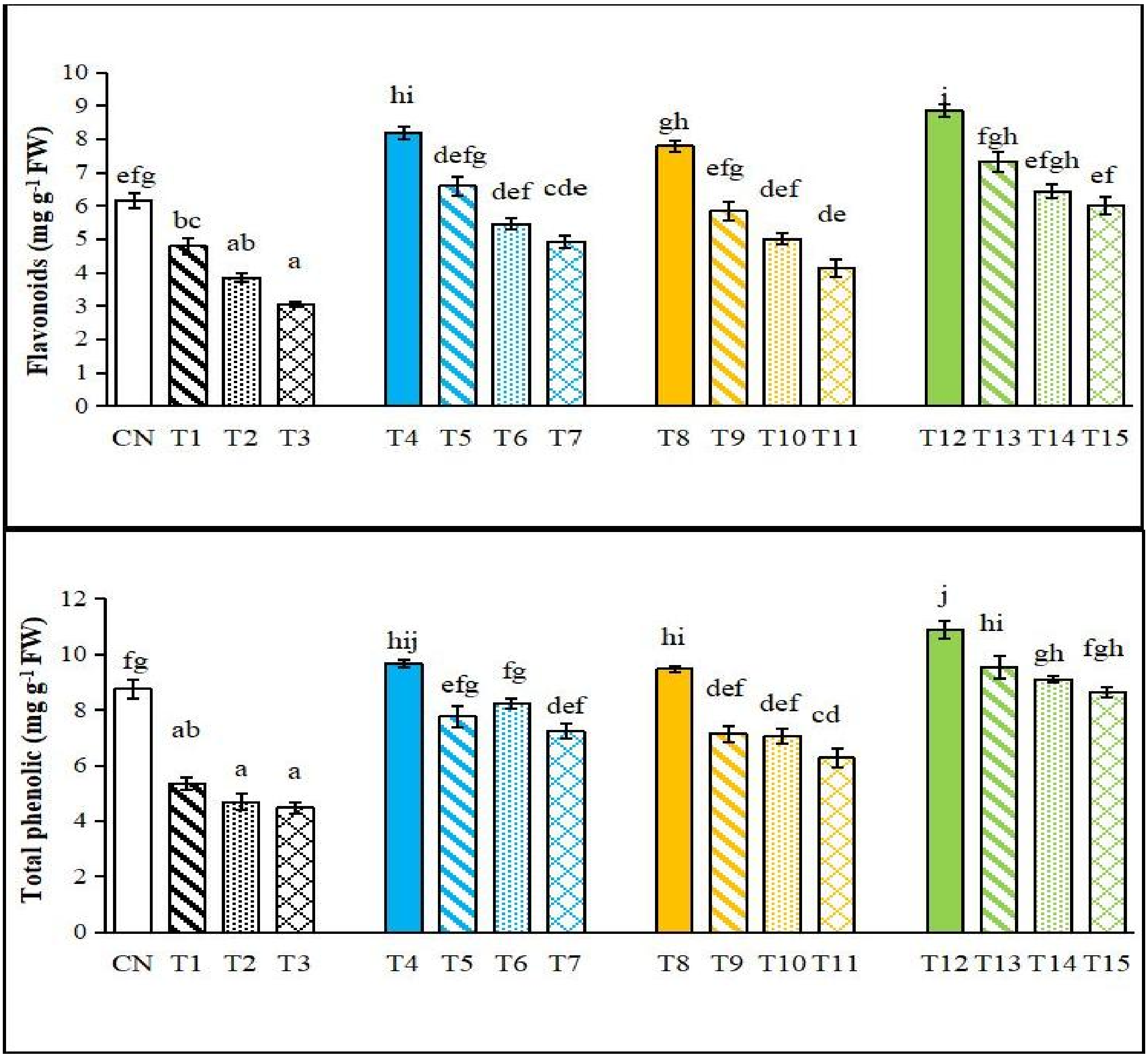


Fig. 6.21: Influence of EBL+Si treatment on measures of metabolites in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Severe reductions in phenolic content were the outcome of Pb exposure in fenugreek plants with greatest decline recorded at T_3 treatment (Fig. 6.21; Table 6.21). T_7 was found to have 7.24 mg g^{-1} FW phenolic measures, whereas T_{11} had 6.27 mg g^{-1} FW of phenolic content. The largest measures of phenolics were documented at 9.55 mg g^{-1} FW in T_{13} treatment, as a correlation was drawn amongst all other treatments selected for carrying out the current study with reference to Pb stress. T_6 recorded 75.64% enhancement as opposed to T_2 whereas T_{10} had 50.85% elevation over T_2. An enhancement percentage of 23.06% was registered in T_{13} over T_5. Furthermore, T_{14} treatment exhibited an escalation of 4% versus control plants.

6.1.3.4 Oxidative stress markers

MDA levels were boosted in reference to Pb toxicity in fenugreek plants with being at lowest and at zenith at T_2 and T_3 having 10.50 and 10.71 µmol g^{-1} FW concentrations, respectively) (Fig. 6.22; Table 6.22). Addition of EBL, Si and EBL +Si at Pb III concentration i.e., T_7, T_{11} and T_{15} recorded MDA contents of 5.34, 4.75 and 4.24 µmol g^{-1} FW, respectively. T_5 had a reduction of 48.64% in correlation to T_1 whereas T_9 registered a decline of 43.82% over $T_{1.}$ About 21.17% drop was noted in T_{14} versus T_{10}. T_{15} treatment exhibited 22.20% depletion as compared to control plants.

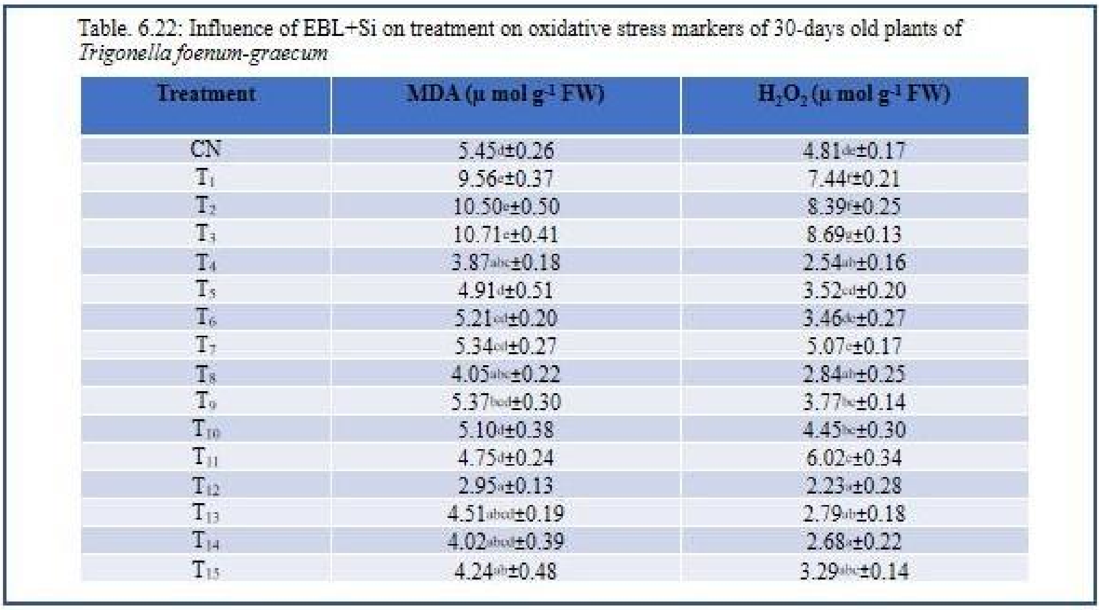


Table. 6.22: Influence of EBL+Si on treatment on oxidative stress markers of 30-days old plants of *Trigonella foenum-graecum*

Treatment	MDA (μ mol g^{-1} FW)	H_2O_2 (μ mol g^{-1} FW)
CN	5.45^{d}±0.26	4.81^{de}±0.17
T_1	9.56^{e}±0.37	7.44^{f}±0.21
T_2	10.50^{e}±0.50	8.39^{f}±0.25
T_3	10.71^{e}±0.41	8.69^{g}±0.13
T_4	3.87^{abc}±0.18	2.54^{ab}±0.16
T_5	4.91^{d}±0.51	3.52^{cd}±0.20
T_6	5.21^{cd}±0.20	3.46^{de}±0.27
T_7	5.34^{cd}±0.27	5.07^{e}±0.17
T_8	4.05^{abc}±0.22	2.84^{ab}±0.25
T_9	5.37^{bcd}±0.30	3.77^{bc}±0.14
T_{10}	5.10^{d}±0.38	4.45^{bc}±0.30
T_{11}	4.75^{d}±0.24	6.02^{e}±0.34
T_{12}	2.95^{a}±0.13	2.23^{a}±0.28
T_{13}	4.51^{abcd}±0.19	2.79^{ab}±0.18
T_{14}	4.02^{abcd}±0.39	2.68^{a}±0.22
T_{15}	4.24^{ab}±0.48	3.29^{abc}±0.14

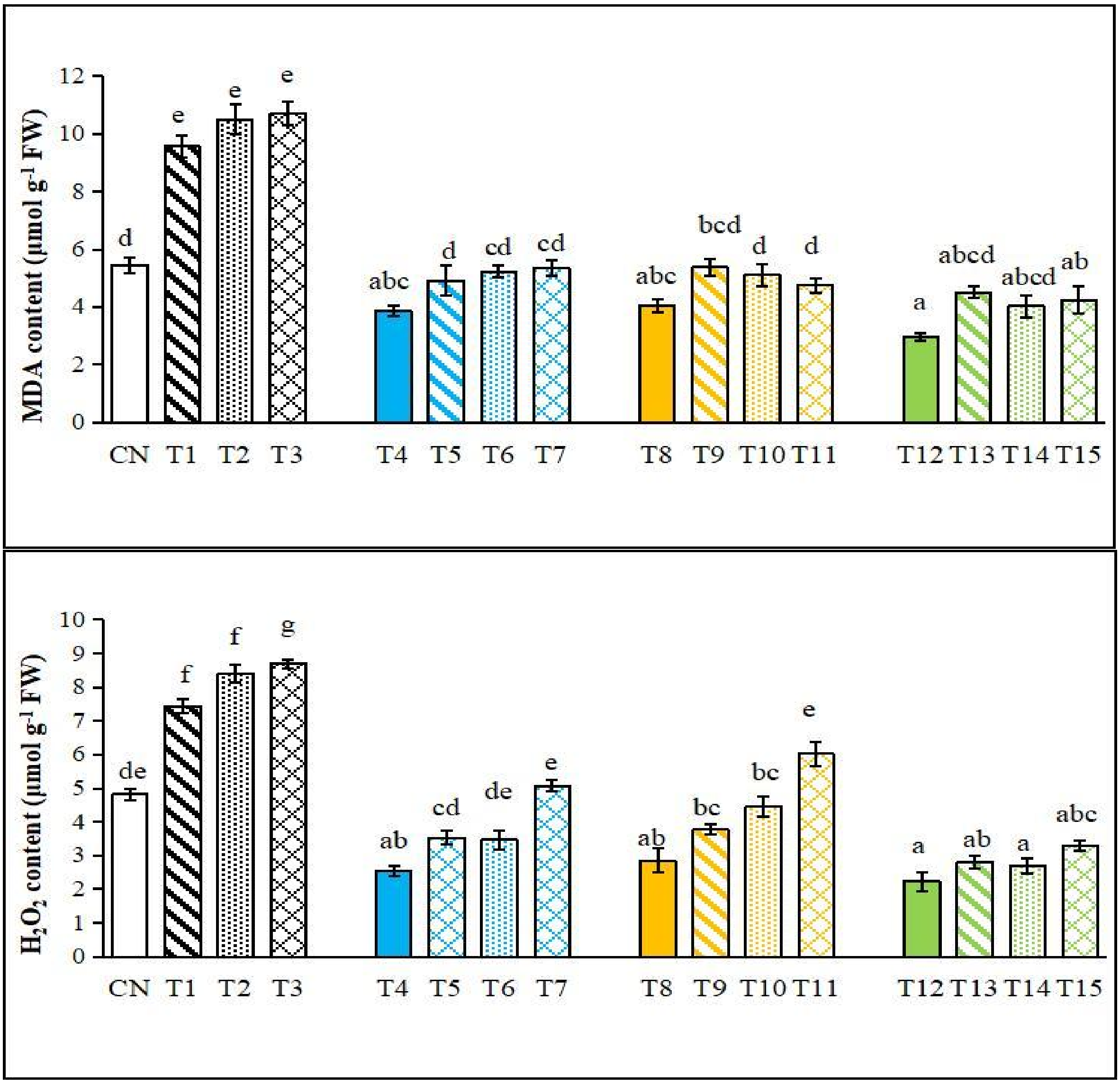


Fig. 6.22: Influence of EBL+Si treatment on measures of oxidative stress indicators in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every

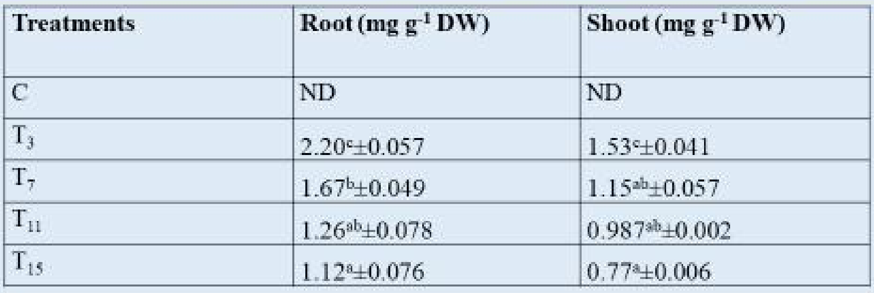
treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Results followed the same trend for H_2O_2 level, which was noticed to be elevated with the increments in the Pb concentration (Fig. 6.22; Table 6.22). The greatest measures of H_2O_2 (8.69 µmol g^{-1} FW) was recorded at T_3 concentration. Moreover, H_2O_2 measures were diminished as the level of Pb elevated with respect to individual plus coupled treatments of EBL and Si. Minimum quantities of H_2O_2 content (2.68 µmol g^{-1} FW) were documented in the T_{15} plants amongst all other treatments. T_5 had a reduction of 52.68% as opposed to T_1 whereas T_9 was noted to have a decline of 49.32% versus T_1. A depletion of 45.34% was noted in T_{15} versus T_{11}. T_{15} exhibited 31.60% decline than control plants.

6.1.3.5 Lead metal uptake

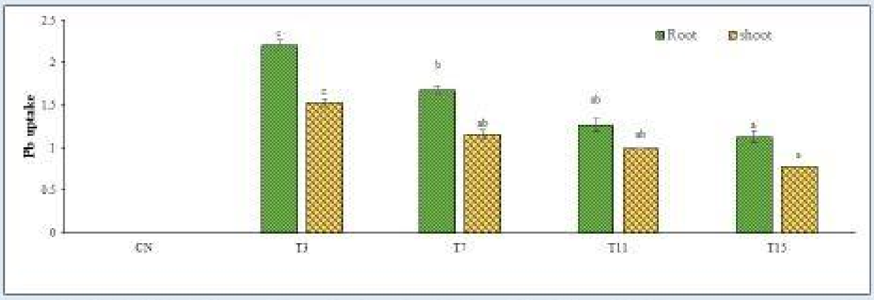
Roots of fenugreek were noticed to have more extent of Pb uptake than the foliar regions of in 30-days old growth stage (Fig. 6.23; Table 6.23). Metal content of 2.20 and 1.53 mg g^{-1} DW was detected in root and leaves, respectively in plants supplied solely with Pb. Pre-treatment of EBL showed 1.67 and 1.15 mg g^{-1} DW inside root and leaves, respectively in response to Pb while foliar augmentation of Si exhibited 1.26 and 0.987 mg g^{-1} DW metal content. EBL and Si co-application was registered to have 1.12 and 0.77 mg g^{-1} DW Pb measures inside root and leaves, respectively.

Table 6.23



Treatments	Root (mg g^{-1} DW)	Shoot (mg g^{-1} DW)
C	ND	ND
T_3	2.20^c±0.057	1.53^c±0.041
T_7	1.67^b±0.049	1.15^{ab}±0.057
T_{11}	1.26^{ab}±0.078	0.987^{ab}±0.002
T_{15}	1.12^a±0.076	0.77^a±0.006

Fig. 6.23



❑ **Influence of EBL + Si on Pb metal uptake by the 30-days old *Trigonella foenum-graecum* seedlings.**

6.1.3.6 Osmolytes

Proline content was scaled down in fenugreek plants due to their subjection to Pb stress (Fig. 6.24; Table 6.24). Proline measures of 2.9, 2.31 and 1.9 µ mol g^{-1} FW were noticed at T_1, T_2 and T_3 treatments, respectively. The untreated control plants showed 3.87 µ mol g^{-1} FW amount of proline. EBL pre-treated control plants (T_4) were reported to have 5.81 μ mol g^{-1} FW proline quantities being higher by 50.12% in contrast to control genotypes. Only a 19.87% decrement was documented with regard to proline content in Si treated fenugreek plants under Pb I stress (T_9) when contrasted with Si control plants (T_8). Proline content was improved from 1.90 of T_3 to 3.09 μ mol g^{-1} FW in T_7 plants. Remarkable enhancements in proline measures were found in stressed plants where EBL and Si were applied in coupled manner. The proline measures were recorded to be prime at T_{14} (5.39 μ mol g^{-1} FW) owing to the synergistic impact of both the mitigants. T_5 plants were noted to have an increment of 33.10% contrary to T_1 whereas T_9 had an escalation of 30.68% over T_1. An upsurge of 17.09% was noted in T_{13} versus T_5.

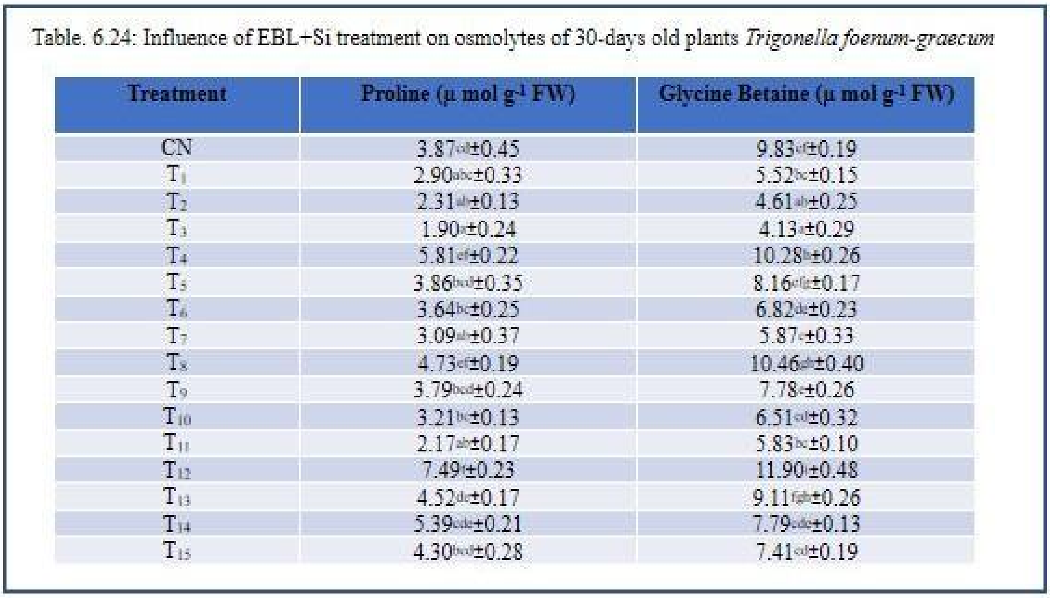


Table. 6.24: Influence of EBL+Si treatment on osmolytes of 30-days old plants *Trigonella foenum-graecum*

Treatment	Proline (µ mol g^{-1} FW)	Glycine Betaine (µ mol g^{-1} FW)
CN	3.87^{cd}±0.45	9.83^{ef}±0.19
T$_1$	2.90^{abc}±0.33	5.52^{bc}±0.15
T$_2$	2.31^{ab}±0.13	4.61^{ab}±0.25
T$_3$	1.90^{a}±0.24	4.13^{a}±0.29
T$_4$	5.81^{ef}±0.22	10.28^{h}±0.26
T$_5$	3.86^{bcd}±0.35	8.16^{efg}±0.17
T$_6$	3.64^{bc}±0.25	6.82^{de}±0.23
T$_7$	3.09^{ab}±0.37	5.87^{c}±0.33
T$_8$	4.73^{ef}±0.19	10.46^{gh}±0.40
T$_9$	3.79^{bcd}±0.24	7.78^{e}±0.26
T$_{10}$	3.21^{bc}±0.13	6.51^{cd}±0.32
T$_{11}$	2.17^{ab}±0.17	5.83^{bc}±0.10
T$_{12}$	7.49^{f}±0.23	11.90^{i}±0.48
T$_{13}$	4.52^{de}±0.17	9.11^{fgh}±0.26
T$_{14}$	5.39^{cde}±0.21	7.79^{cde}±0.13
T$_{15}$	4.30^{bcd}±0.28	7.41^{cd}±0.19

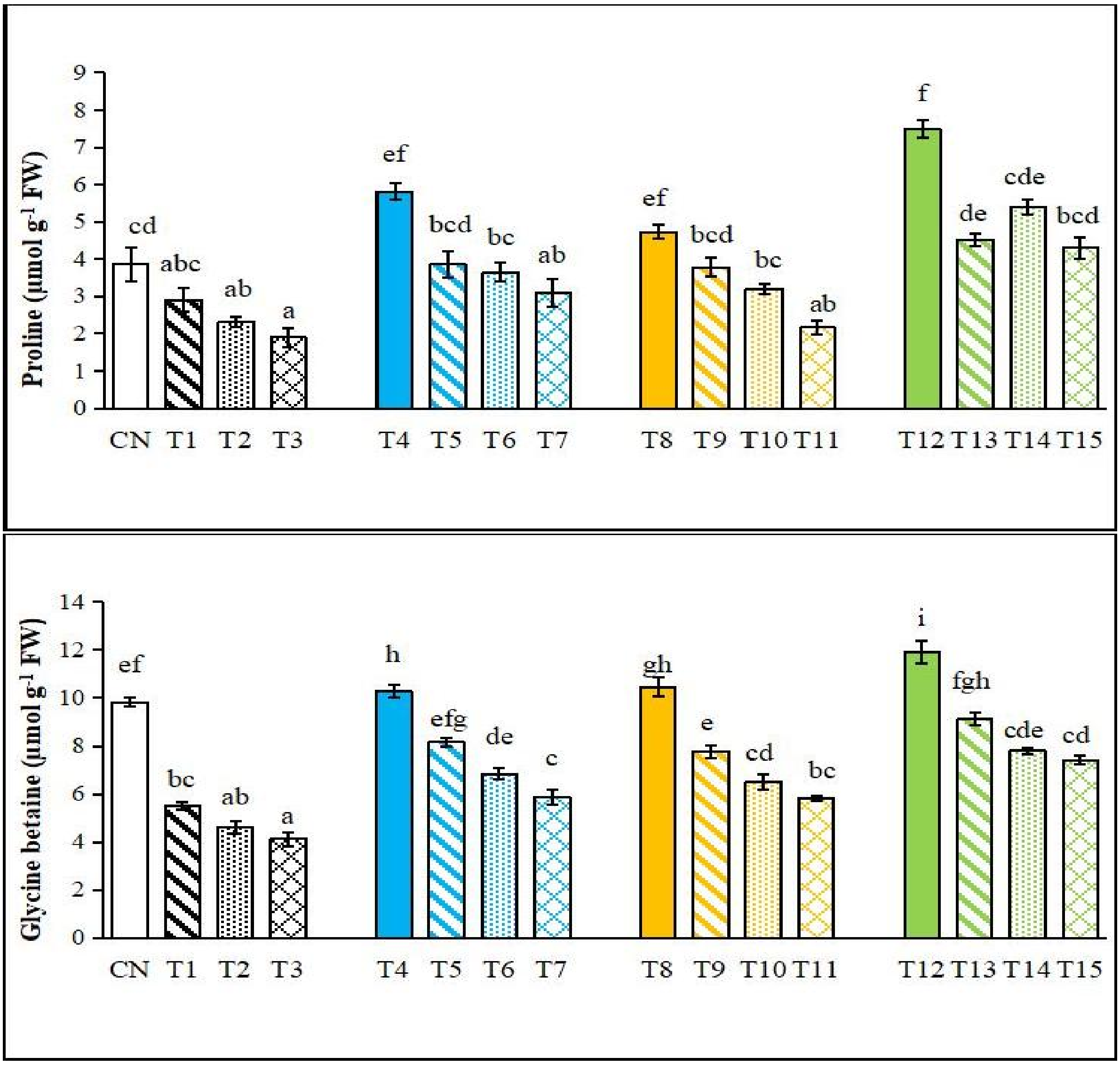


Fig. 6.24: Influence of EBL+Si treatment on measures of osmolytes in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together

with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Notable reductions in the quantities of glycine betaine were documented as a consequence of Pb stress in fenugreek plants (Fig. 6.24; Table 6.24). T_3 plants (4.13 µ mol g^{-1} FW) recorded a depletion of 57.98% vis-à-vis control plants (4.17 µ mol g^{-1} FW). EBL plus Si augmentation remarkably attenuated the Pb-induced stress in fenugreek plants promoting the content of glycine betaine. The highest levels of glycine betaine noted in individual treatment of EBL and Si were 8.16 and 7.78 µ mol g^{-1} FW at T_5 and T_9, respectively. The coupling of EBL and Si control plants (T_{12}) reported to register 11.90 µ mol g^{-1} FW glycine betaine content. Under conditions of stress, the maximal increment in glycine betaine content was of 9.11 µ mol g^{-1} FW at T_{13} fenugreek plants when given a combination of EBL with Si. T_5 recorded 47.82% increment in contrast to T_1 whereas T_9 had 40.94% elevation over T_1. An augmentation of 17.09% was noticed in T_{13} than T_9. T_{13} plants registered 7.32% elevation than control plants.

6.1.3.7 Total carbohydrates

Lead stress resulted in depletion of the measures of carbohydrates with being lowest at T_3 (2.47 mg g^{-1} FW) (Fig. 6.25; Table 6.25). Plants subjected to Pb I (T_1) underwent a 43.30% reduction when correlated against control plants. Pre-treatment of EBL in fenugreek under stressful conditions reported to have the highest measures of carbohydrates of 6.16 mg g^{-1} FW in T_6 plants. Foliar spraying of Si in order to mitigate stressful conditions recorded maximum and minimum quantities of carbohydrates at T_9 and T_{11} with 5.43 and 4.51 mg g^{-1} FW contents. Coupled addition of EBL and Si registered the minimum content value of 6.94 mg g^{-1} FW at T_{14} concentration. T_6 had an increment of 95.55% as opposed to T_2 whereas T_{10} exhibited 65.07% upsurge over T_2. An upscaling of 26.10% was noted in T_{13} over T_5. T_{13} plants were noticed to have 11.12% elevation versus control plants.

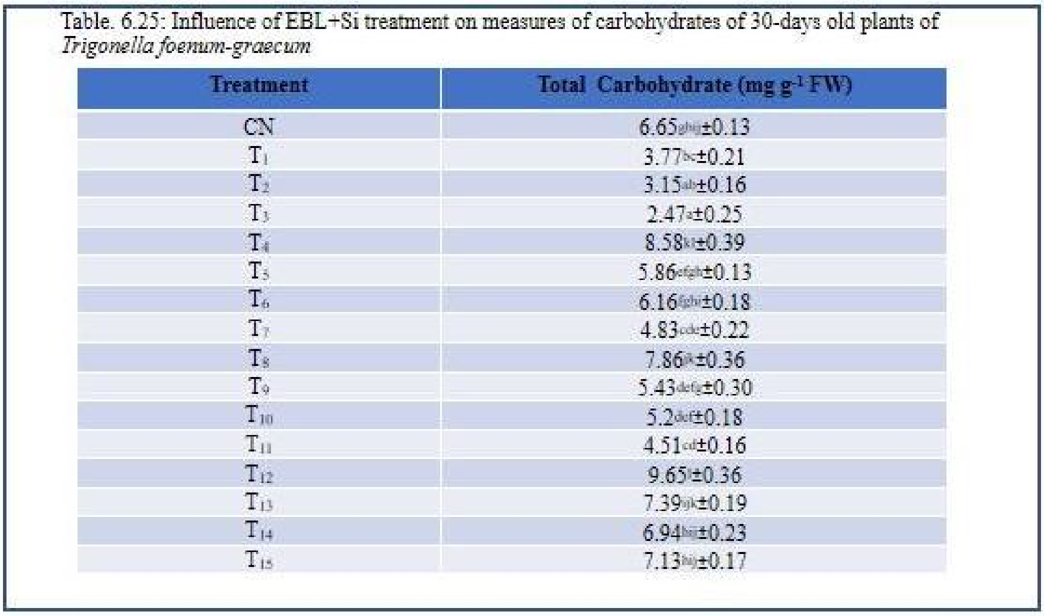


Table. 6.25: Influence of EBL+Si treatment on measures of carbohydrates of 30-days old plants of *Trigonella foenum-graecum*

Treatment	Total Carbohydrate (mg g^{-1} FW)
CN	6.65^{ghij}±0.13
T_1	3.77^{bc}±0.21
T_2	3.15^{ab}±0.16
T_3	2.47^{a}±0.25
T_4	8.58^{kl}±0.39
T_5	5.86^{efgh}±0.13
T_6	6.16^{fghi}±0.18
T_7	4.83^{cde}±0.22
T_8	7.86^{jk}±0.36
T_9	5.43^{defg}±0.30
T_{10}	5.2^{def}±0.18
T_{11}	4.51^{cd}±0.16
T_{12}	9.65^{l}±0.36
T_{13}	7.39^{ijk}±0.19
T_{14}	6.94^{hij}±0.23
T_{15}	7.13^{hij}±0.17

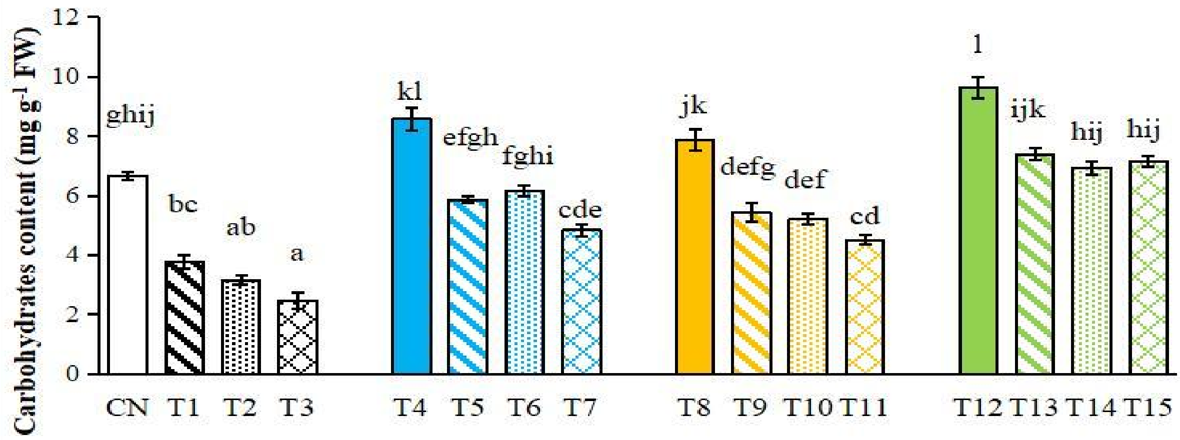


Fig. 6.25: Influence of EBL+Si treatment on measures of total carbohydrates in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.3.8 Protein content and antioxidant defense system

6.1.3.8.1 Protein content and antioxidative enzymes

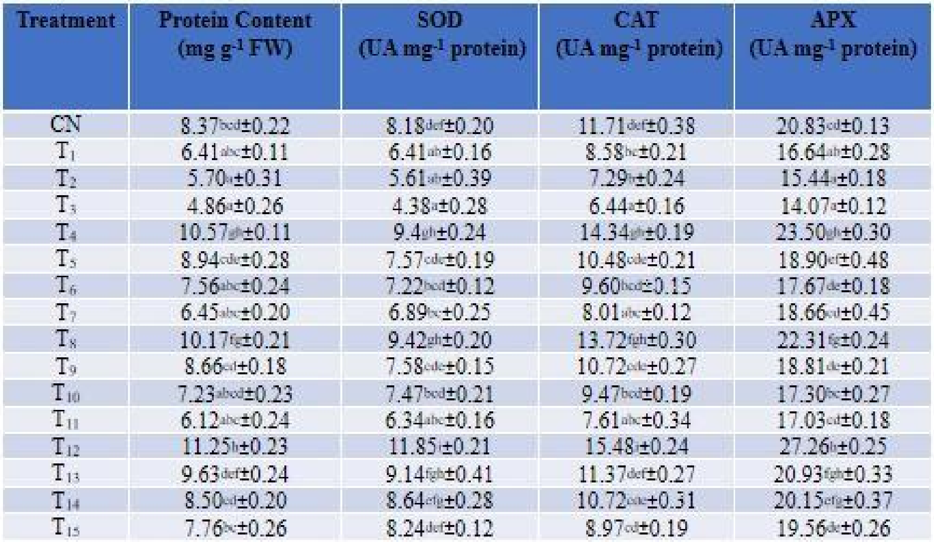
Protein content recorded a drastic drop on account of Pb induced stress in 30 days old fenugreek plants (Fig. 6.26a; Table 6.26a). Measures of protein were noted to be 6.41, 5.70 and 4.86 mg g^{-1} FW at T_1, T_2 and T_3 concentrations, respectively. Pre-treatment of EBL in fenugreek exhibited highest value of 8.94 mg g^{-1} FW at T_5 in response to Pb stress. Si control fenugreek (T_8) plants registered a content of 10.17 mg g^{-1} FW which was observed to be 8.66 mg g^{-1} FW at T_9 under stressed conditions. Coupled addition of EBL and Si showed 9.63, 8.5

and 7.76 mg g^{-1} FW protein quantities at T_{13}, T_{14} and T_{15} concentrations, respectively. T_6 exhibited 32.63% increment in protein level in correlation to T_2 whereas T_{10} exhibited 26.84% improvement over T_2. An upsurge of 7.71% was noted in T_{13} versus T_5. T_{13} plants had 15.05% higher protein content than control plants.

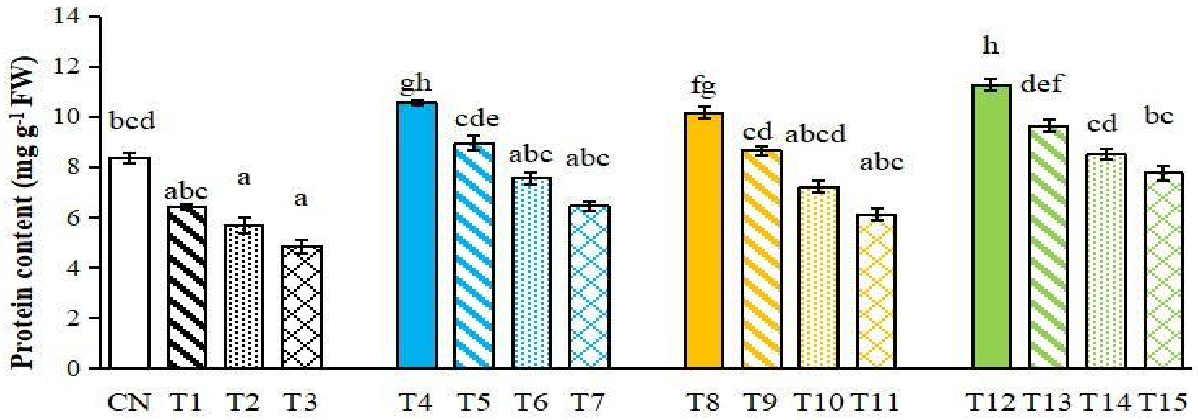
Lead exposure scaled down the activity of SOD enzyme with 6.41 (T_1), 5.61 (T_2) and 4.38 UA mg^{-1} protein (T_3) content values (Fig. 6.26a; Table 6.26a). EBL pre-treatment under stressed conditions upgraded the functioning of the SOD enzyme with the optimum activity of 7.57 UA mg^{-1} protein in T_5 plants. Silicon control fenugreek plants (T_8) exhibited 9.42 UA mg^{-1} protein. SOD activity was maximum (9.14 UA mg^{-1} protein) under Pb toxicity in T_{13} treated plants. T_5 recorded 18.09% improvement in contrast to T_1 whereas T_9 had an elevation of 18.25% over T_1. An upsurge of 20.73% was noted in T_{13} than T_5. T_{13} plants had an increase of 11.73% compared with control plants.

Estimated CAT activity in control plants turned out to be 11.71 UA mg^{-1} protein, whereas T_1, T_2 and T_3 treated plants had its values to stand at 8.58, 7.29 and 6.44 UA mg^{-1} protein (Fig. 6.26a; Table 6.26a). Optimal value of CAT activity in response of Pb stress was detected in EBL pre-treated plants of T_5 with 10.48 UA mg^{-1} protein. Applying the second mitigant i.e. Si under stressful conditions exhibited 10.72, 9.47 and 7.61 UA mg^{-1} protein at T_9, T_{10} and T_{11} concentrations. Combination of EBL and Si registered the highest CAT activity of 11.37 UA mg^{-1} protein at T_{13} level. T_5 recorded 22.14% increase contrary to T_1 whereas T_9 had an elevation of 24.94% over T_1. An upsurge of 18.43% was noticed in T_{13} than T_6. A decrease of 2.9 % was reported in T_{13} plants versus control plants.

Table. 6.26a: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 30-days old plants of *Trigonella foenum-graecum*



Treatment	Protein Content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
CN	8.37^{bcd}±0.22	8.18^{def}±0.20	11.71^{def}±0.38	20.83^{cd}±0.13
T_1	6.41^{abc}±0.11	6.41^{ab}±0.16	8.58^{bc}±0.21	16.64^{ab}±0.28
T_2	5.70^{a}±0.31	5.61^{ab}±0.39	7.29^{b}±0.24	15.44^{a}±0.18
T_3	4.86^{a}±0.26	4.38^{a}±0.28	6.44^{a}±0.16	14.07^{a}±0.12
T_4	10.57^{gh}±0.11	9.4^{gh}±0.24	14.34^{gh}±0.19	23.50^{gh}±0.30
T_5	8.94^{cde}±0.28	7.57^{cde}±0.19	10.48^{cde}±0.21	18.90^{ef}±0.48
T_6	7.56^{abc}±0.24	7.22^{bcd}±0.12	9.60^{bcd}±0.15	17.67^{de}±0.18
T_7	6.45^{abc}±0.20	6.89^{bc}±0.25	8.01^{abc}±0.12	18.66^{cd}±0.45
T_8	10.17^{fg}±0.21	9.42^{gh}±0.20	13.72^{fgh}±0.30	22.31^{fg}±0.24
T_9	8.66^{cd}±0.18	7.58^{cde}±0.15	10.72^{cde}±0.27	18.81^{de}±0.21
T_{10}	7.23^{abcd}±0.23	7.47^{bcd}±0.21	9.47^{bcd}±0.19	17.30^{bc}±0.27
T_{11}	6.12^{abc}±0.24	6.34^{abc}±0.16	7.61^{abc}±0.34	17.03^{cd}±0.18
T_{12}	11.25^{h}±0.23	11.85^{i}±0.21	15.48^{i}±0.24	27.26^{h}±0.25
T_{13}	9.63^{def}±0.24	9.14^{fgh}±0.41	11.37^{def}±0.27	20.93^{fgh}±0.33
T_{14}	8.50^{cd}±0.20	8.64^{efg}±0.28	10.72^{cde}±0.31	20.15^{efg}±0.37
T_{15}	7.76^{bc}±0.26	8.24^{def}±0.12	8.97^{cd}±0.19	19.56^{de}±0.26



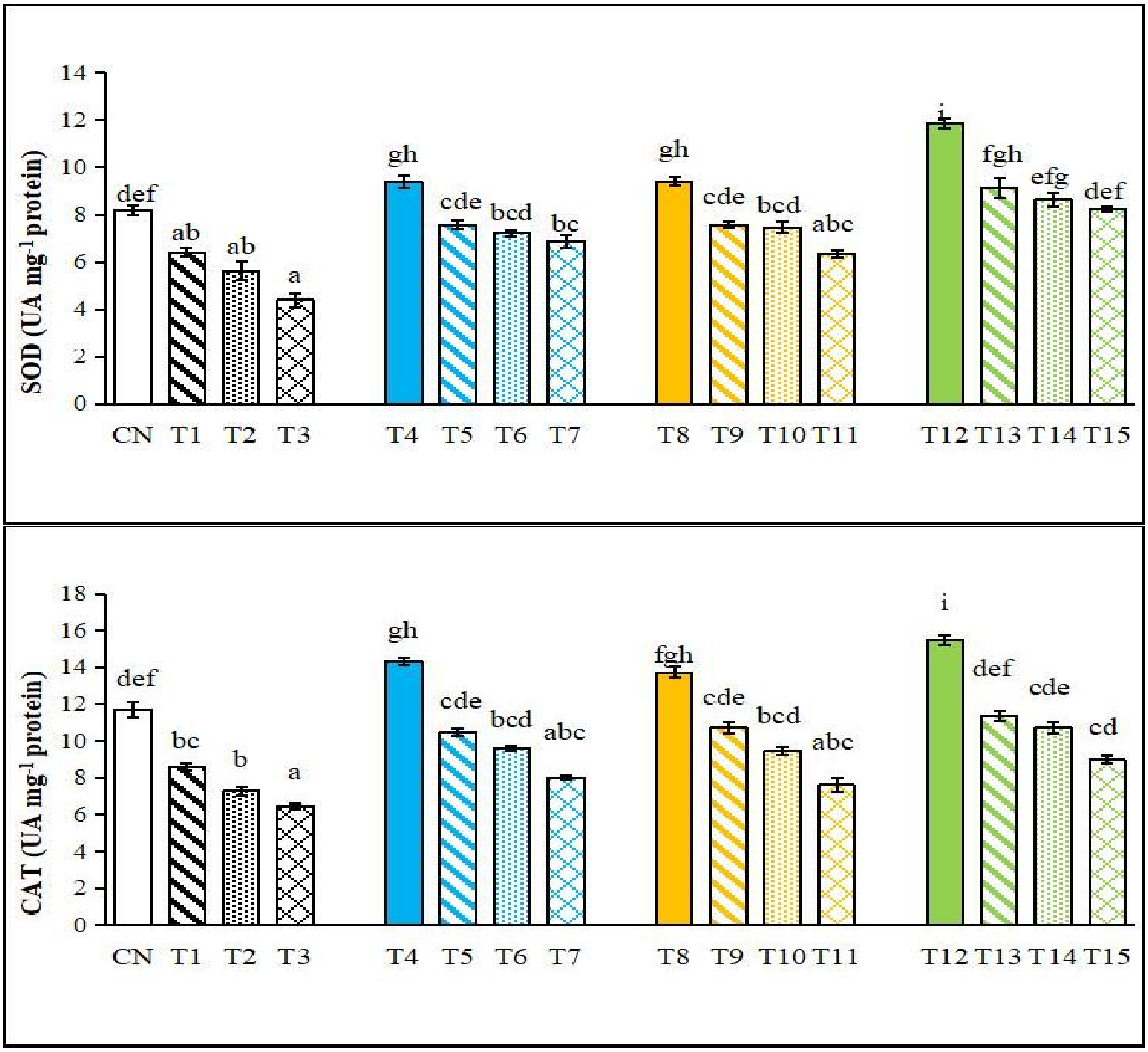


Fig. 6.26 (a): Influence of EBL+Si treatment on measures of protein and enzymatic antioxidants in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Lead exposure scaled down the functional aspect of APX enzyme with the highest depletion of 14.07 UA mg^{-1} protein at T_3 treatment (Fig. 6.26b; Table 6.26a). Application of EBL and Si alone reportedly enhanced the APX measures. The optimum content of 18.9 UA mg^{-1} protein resultant of EBL treatment was noted at T_5 while in Si treated plants, greater APX activity (18.81 UA mg^{-1} protein) was in T_9 plants. Under Pb stress, the optimum level of APX activity was recorded at combined treatments of EBL + Si ($T_{13;}$ 20.93 UA mg^{-1} protein). T_5 was noted to have an increase of 13.58% contrary to T_1 while in case of T_9, an elevation of 13.04% over T_1 was reported. An upsurge of 20.98% was reported in T_{14} when contrasted with T_{10}. T_{14} plants had a 0.43% increment in comparison to control plants.

Functioning of POD enzyme got negatively affected in T_1 stressed plants with 7.32 UA mg^{-1} protein activity in correlation to control plants (Fig. 6.26b; Table 6.26b). Control plants in

EBL and Si (T_4 and T_8) registered POD levels of 12.57 and 11.88 UA mg^{-1} protein, which were far higher in contrast to the control plants. Under stressful conditions, the optimal values of enzymatic activities with reference to individually added EBL and Si, were exhibited at T_5 and T_9 treatments with 9.66 and 8.78 UA mg^{-1} protein, respectively. T_{13} topped amongst all the treatments in terms of having highest level of POD activity (11.99 UA mg^{-1} protein) under Pb stress. T_7 had an increment of 47.81% contrary to T_3 whereas T_{11} exhibited 17.33% improvement over T_3. An increment of 36.56% was noted in T_{13} than T_9.

The functional aspect of the GR enzyme suffered adversely with regard to Pb stress having minimal levels of GR activity of 5.14 UA mg^{-1} protein at T_2 concentration (Fig. 6.26b; Table 6.26b). Enzymatic action of GR was noticed to be depleted with the elevations in Pb stress. Foliar spray of Si under Pb stress upgraded the GR activity. Performance of GR enzyme was escalated from 5.82 to 7.67 UA mg^{-1} protein in T_7 plants. Addition of Si to stressed plants recorded the highest plus lowest levels of GR activity at T_9 and T_{11} with 7.89 and 6.19 UA mg^{-1} protein, respectively. Coupled addition of EBL and Si in case of Pb exposure registered the highest measures of GR activity (8.79 UA mg^{-1} protein) at T_{13}. T_5 exhibited a 34.56% increment contrary to T_1 whereas T_9 exhibited 38.42% elevation over T_1. A boost of 11.4% was recorded in T_{13} over T_9. T_{13} had 18.78% amplification over control plants.

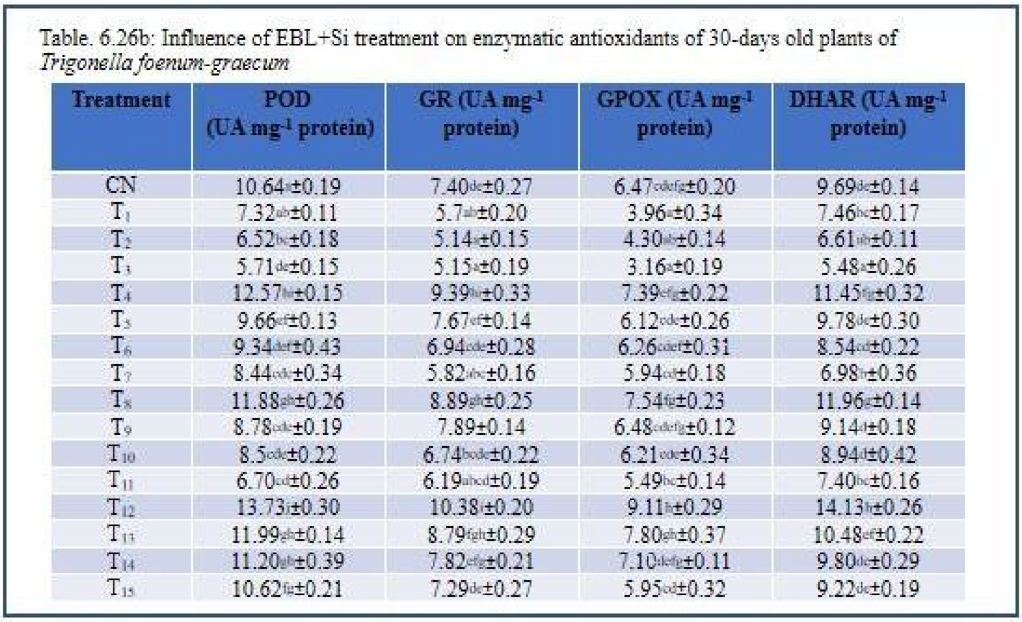
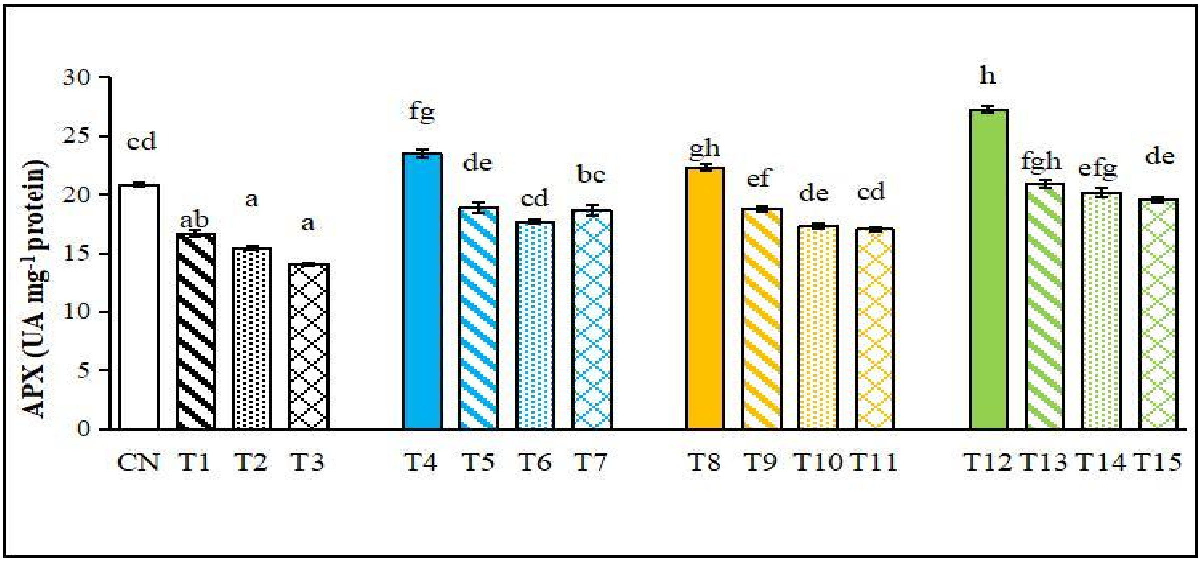


Table. 6.26b: Influence of EBL+Si treatment on enzymatic antioxidants of 30-days old plants of *Trigonella foenum-graecum*

Treatment	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
CN	10.64^{a}±0.19	7.40^{de}±0.27	6.47^{cdefg}±0.20	9.69^{de}±0.14
T_1	7.32^{ab}±0.11	5.7^{ab}±0.20	3.96^{a}±0.34	7.46^{bc}±0.17
T_2	6.52^{bc}±0.18	5.14^{a}±0.15	4.30^{ab}±0.14	6.61^{ab}±0.11
T_3	5.71^{de}±0.15	5.15^{a}±0.19	3.16^{a}±0.19	5.48^{a}±0.26
T_4	12.57^{hi}±0.15	9.39^{hi}±0.33	7.39^{efg}±0.22	11.45^{fg}±0.32
T_5	9.66^{ef}±0.13	7.67^{ef}±0.14	6.12^{cde}±0.26	9.78^{de}±0.30
T_6	9.34^{def}±0.43	6.94^{cde}±0.28	6.26^{cdef}±0.31	8.54^{cd}±0.22
T_7	8.44^{cde}±0.34	5.82^{abc}±0.16	5.94^{cd}±0.18	6.98^{b}±0.36
T_8	11.88^{gh}±0.26	8.89^{gh}±0.25	7.54^{fg}±0.23	11.96^{g}±0.14
T_9	8.78^{cde}±0.19	7.89±0.14	6.48^{cdefg}±0.12	9.14^{d}±0.18
T_{10}	8.5^{cde}±0.22	6.74^{bcde}±0.22	6.21^{cde}±0.34	8.94^{d}±0.42
T_{11}	6.70^{cd}±0.26	6.19^{abcd}±0.19	5.49^{bc}±0.14	7.40^{bc}±0.16
T_{12}	13.73^{i}±0.30	10.38^{i}±0.20	9.11^{h}±0.29	14.13^{h}±0.26
T_{13}	11.99^{gh}±0.14	8.79^{fgh}±0.29	7.80^{gh}±0.37	10.48^{ef}±0.22
T_{14}	11.20^{gh}±0.39	7.82^{efg}±0.21	7.10^{defg}±0.11	9.80^{de}±0.29
T_{15}	10.62^{fg}±0.21	7.29^{de}±0.27	5.95^{cd}±0.32	9.22^{de}±0.19



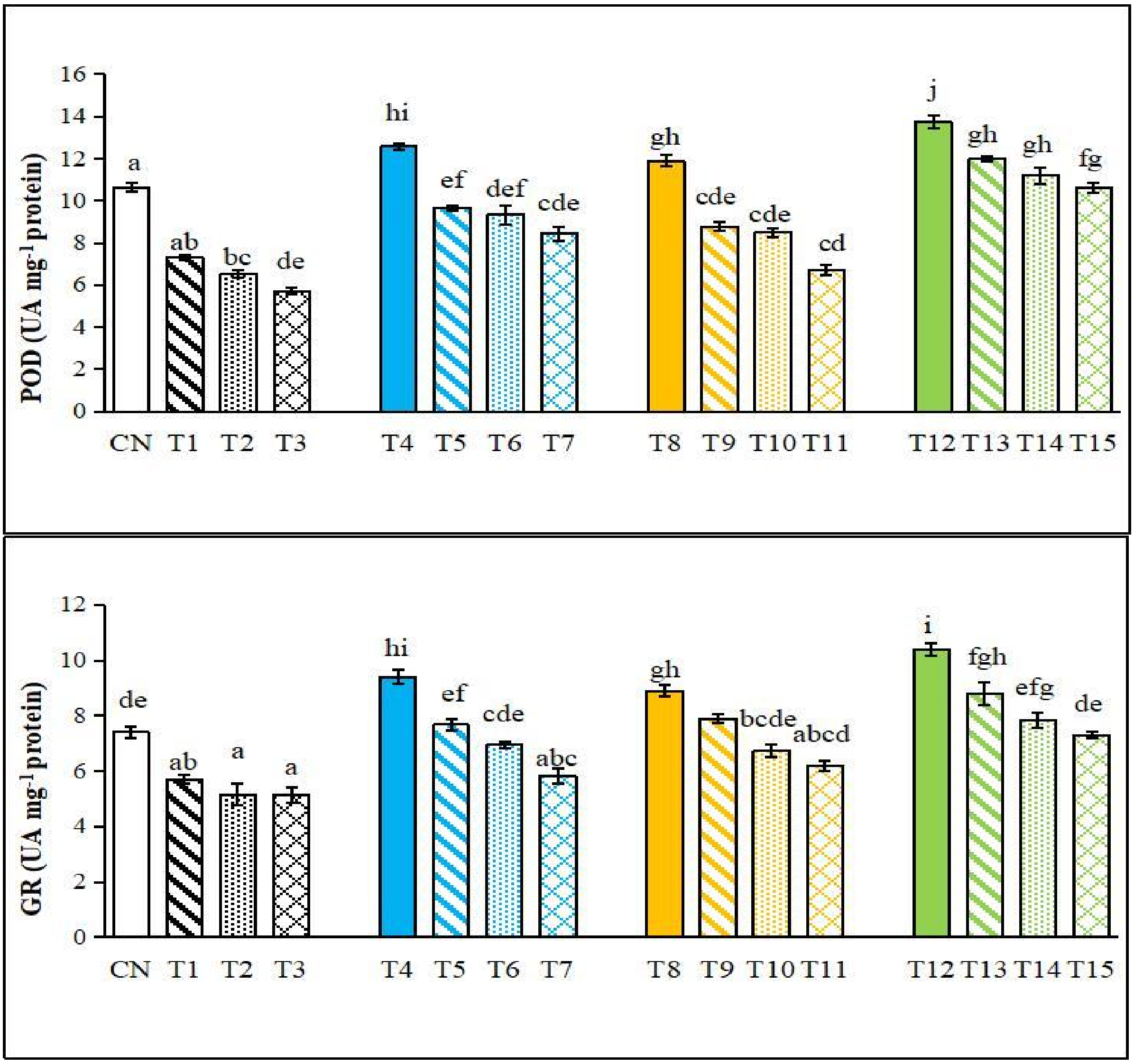
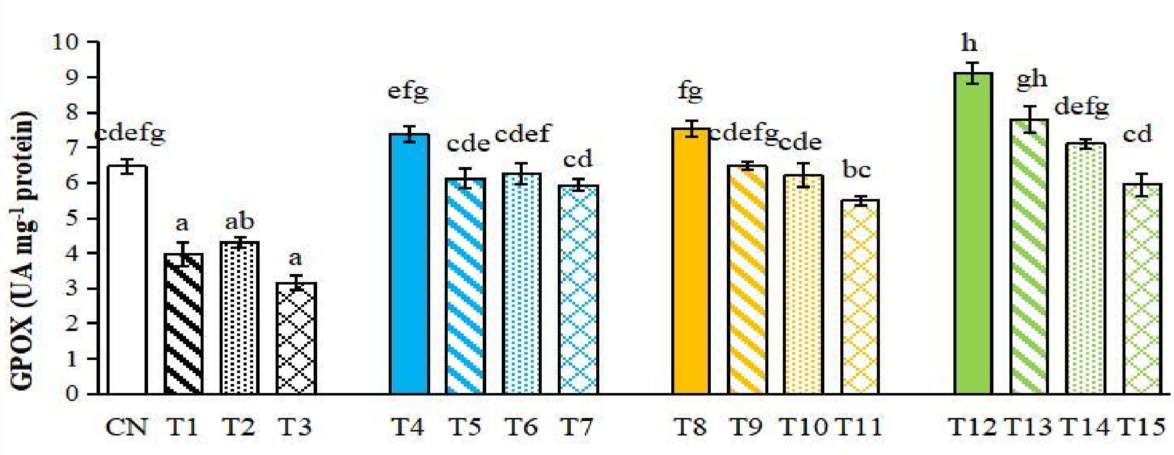


Fig. 6.26 (b): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Performance of GPOX enzyme was scaled down in Pb stressed cultivars having 3.96, 4.3 and 3.16 UA mg^{-1} protein at T_1, T_2 and T_3 treatments, respectively (Fig. 6.26b; Table 6.26b). Individually, both EBL and Si upregulated the levels of GPOX activity than Pb alone stressed cultivars. Application of EBL under Pb stress up-scaled the activity level of GPOX with the maximum measure at T_6 (6.26 UA mg^{-1} protein). Augmentation of Si under Pb showed optimum level of GPOX activity of 6.48 UA mg^{-1} protein at T_9. Further, the maximal and minimal measures of GPOX, with respect to coupled application of EBL and Si, were noted to be 7.8 and 5.95 UA mg^{-1} protein at T_{13} and T_{15} concentrations, respectively. T_5 had 54.54% increase contrary to T_1 whereas T_9 exhibited 63.63% upsurge over T_1. An improvement of 20.37% was reported in T_{13} over T_9. T_{13} recorded 20.55% increment as opposed to control plants.

Enzymatic performance of DHAR was downgraded in fenugreek plants upon being subjected to Pb stress (Fig. 6.26c; Table 6.26b). Among Pb alone exposed plants, minimum level of DHAR activity was noticed in T_3 cultivars (5.48 UA mg^{-1} protein). EBL feeding elevated the DHAR activity under stressful conditions, among which the minimum activity level of 6.98 UA mg^{-1} protein was noted at T_7. With reference to Si addition under Pb exposure, the highest measure of its activity was noted at T_9 (9.14 UA mg^{-1} protein). EBL + Si further upgraded the DHAR levels under stressful conditions with lowest DHAR activity of 9.22 UA mg^{-1} protein at T_{15}. T_5 recorded 31.09% increment in contrast to T_1 whereas T_9 showed 22.52% elevation than T_1. An augmentation of 7.15% was noted in T_{13} over T_5.

The lowest level of MDHAR activity was noted in T_3 plants (6.67 UA mg^{-1} protein) (Fig. 6.26c; Table 6.26c). Pre-treatment of EBL in unstressed plants (T_4) exhibited 14.16 UA mg^{-1} protein whereas its supplementation under stressful conditions resulted in 10.96, 10.32 and 8.82 UA mg^{-1} protein activity at T_5, T_6 and T_7 concentrations, respectively. A decline of 45.74% in the quantities of this enzyme was recorded in T_{11} stressed fenugreek plants versus their respective control cultivars (T_8). Only 19.76, 22.4 and 28.08% curtailments were recorded in T_{13}, T_{14} and T_{15} stressed plants when augmented with a combined application of EBL and Si over T_{12} plants. T_5 had an increment of 23.28% contrary to T_1 whereas T_9 recorded 21.37% elevation over T_1. An upsurge of 10.76% was noted in T_{13} versus T_5. T_{13} had a decline of 4.03% over control plants.



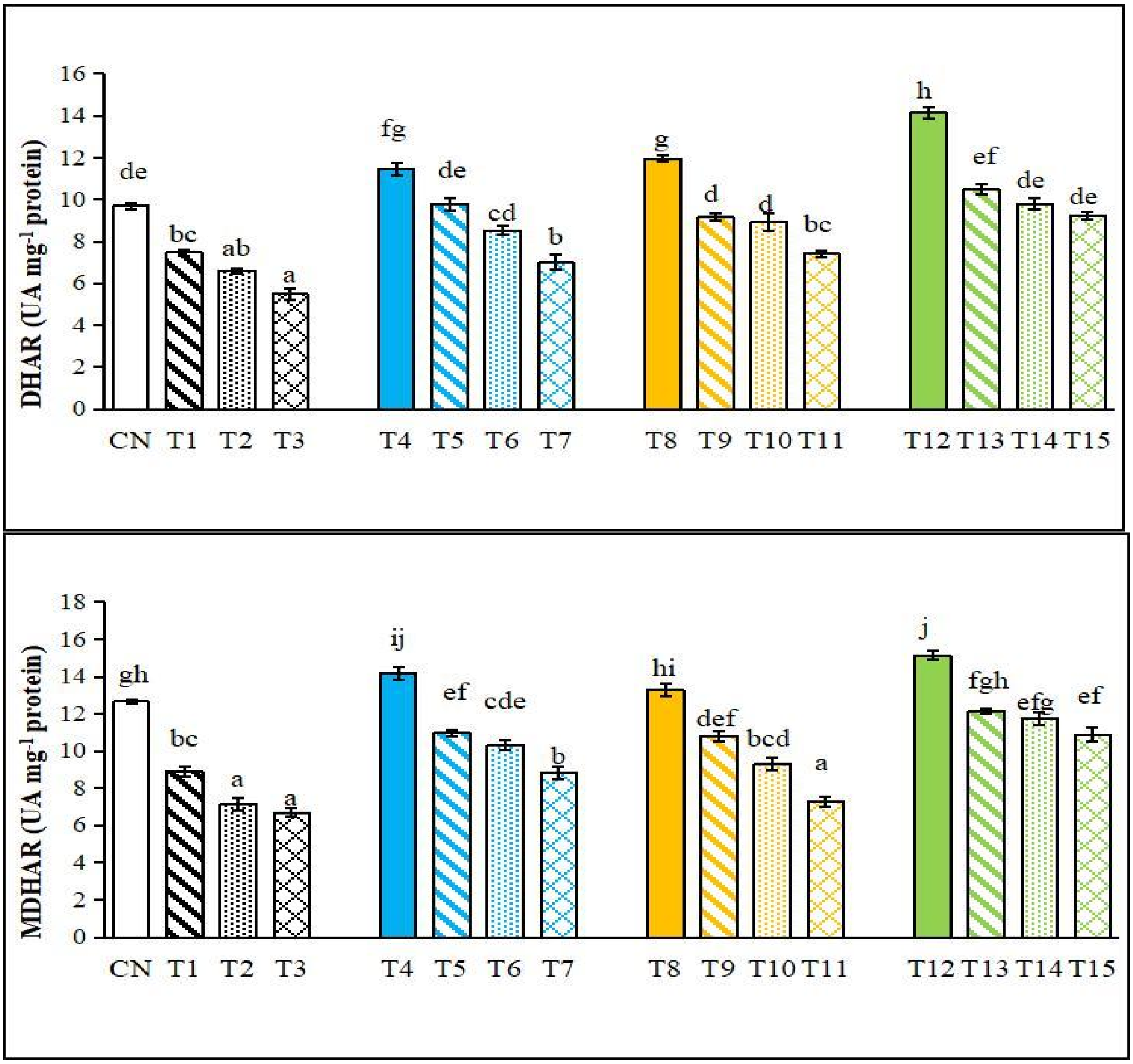


Fig. 6.26 (c): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

Lead stress curtailed the functioning of GST in 30-days old fenugreek plants with the lowest amount being recorded at 3.42 UA mg^{-1} protein in T_3 metal stressed plants (Fig. 6.26d; Table 6.26c). Individual augmentation of EBL and Si under stressful conditions registered the maximum levels of GST activity under Pb I stress with 7.70 and 7.58 UA mg^{-1} protein at T_5 and T_{10}, respectively. Combined EBL and Si control plants (T_{12}) exhibited 12.77 UA mg^{-1} protein activity while in T_{13}, T_{14} and T_{15}, combination of EBL and Si showed 8.84, 8.26 and 7.96 UA mg^{-1} protein activities, respectively. T_5 exhibited 38.98% increase contrary to T_1 whereas T_9 showed 24.36% upgradation than T_1. An augmentation of 28.3% was noted in T_{13} over T_9. T_{13} had an elevation of 9% as opposed to control plants.

Minimum level of PPO activity was noted in Pb III treated plants with 4.65 UA mg^{-1} protein (Fig. 6.26d; Table 6.26c). However, augmentation of EBL under Pb stress scaled up the performance of PPO with 10.01 UA mg^{-1} protein at T_7 concentration. Whereas with concern to Si-treated plants subjected to stressed conditions, maximum enzymatic action of PPO of 9.43 UA mg^{-1} protein was documented at T_{11}. Highest plus lowest PPO levels in the case of EBL and Si combination were noticed at T_{13} and T_{15} with 11.39 and 10.34 UA mg^{-1} protein activities, respectively. T_7 had an increment of 115.2% in contrast to T_3. An intensification of 10.18% was found in T_{15} than T_{11}. T_{13} had 34% increment than control plants.

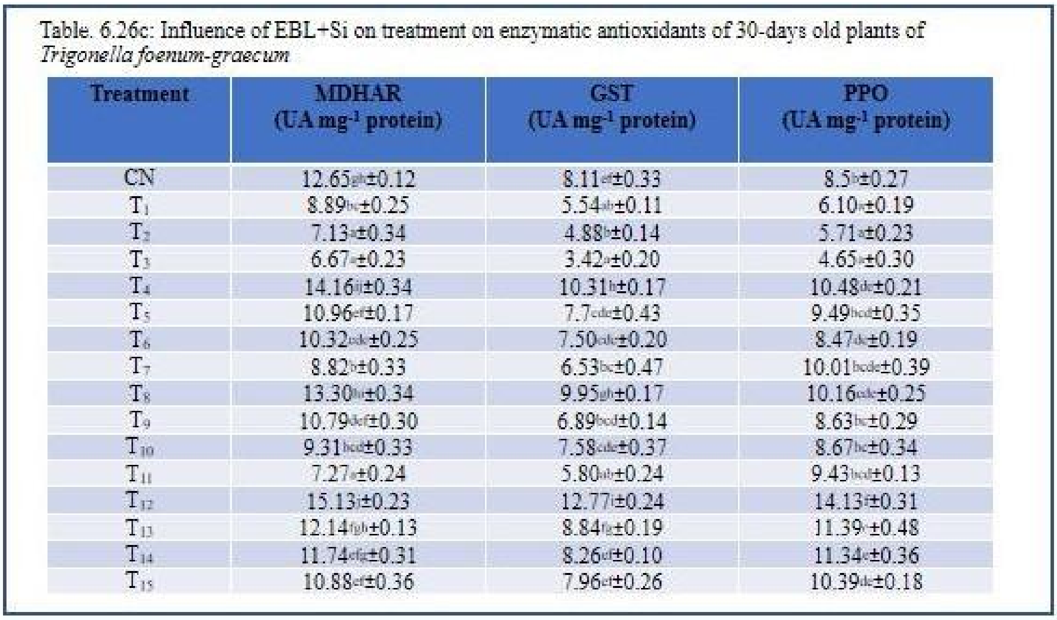


Table. 6.26c: Influence of EBL+Si on treatment on enzymatic antioxidants of 30-days old plants of *Trigonella foenum-graecum*

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
CN	$12.65^{gh}\pm0.12$	$8.11^{ef}\pm0.33$	$8.5^{b}\pm0.27$
T_1	$8.89^{bc}\pm0.25$	$5.54^{ab}\pm0.11$	$6.10^{a}\pm0.19$
T_2	$7.13^{a}\pm0.34$	$4.88^{b}\pm0.14$	$5.71^{a}\pm0.23$
T_3	$6.67^{a}\pm0.23$	$3.42^{a}\pm0.20$	$4.65^{a}\pm0.30$
T_4	$14.16^{ij}\pm0.34$	$10.31^{h}\pm0.17$	$10.48^{de}\pm0.21$
T_5	$10.96^{ef}\pm0.17$	$7.7^{cde}\pm0.43$	$9.49^{bcd}\pm0.35$
T_6	$10.32^{cde}\pm0.25$	$7.50^{cde}\pm0.20$	$8.47^{de}\pm0.19$
T_7	$8.82^{b}\pm0.33$	$6.53^{bc}\pm0.47$	$10.01^{bcde}\pm0.39$
T_8	$13.30^{hi}\pm0.34$	$9.95^{gh}\pm0.17$	$10.16^{cde}\pm0.25$
T_9	$10.79^{def}\pm0.30$	$6.89^{bcd}\pm0.14$	$8.63^{bc}\pm0.29$
T_{10}	$9.31^{bcd}\pm0.33$	$7.58^{cde}\pm0.37$	$8.67^{bc}\pm0.34$
T_{11}	$7.27^{a}\pm0.24$	$5.80^{ab}\pm0.24$	$9.43^{bcd}\pm0.13$
T_{12}	$15.13^{j}\pm0.23$	$12.77^{i}\pm0.24$	$14.13^{f}\pm0.31$
T_{13}	$12.14^{fgh}\pm0.13$	$8.84^{fg}\pm0.19$	$11.39^{e}\pm0.48$
T_{14}	$11.74^{efg}\pm0.31$	$8.26^{ef}\pm0.10$	$11.34^{e}\pm0.36$
T_{15}	$10.88^{ef}\pm0.36$	$7.96^{ef}\pm0.26$	$10.39^{de}\pm0.18$

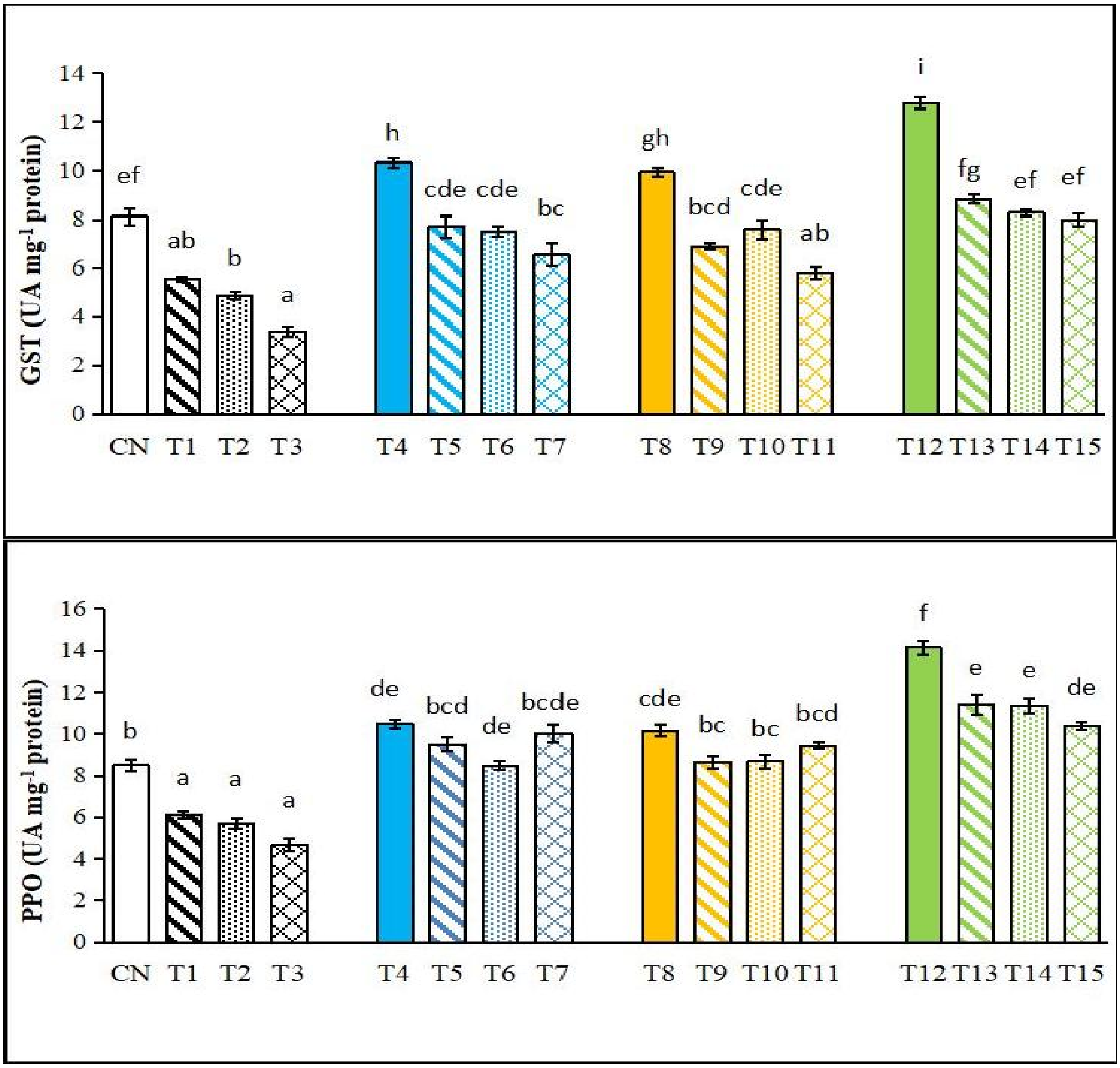


Fig. 6.26 (d): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every

treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.3.8.2 Non-enzymatic antioxidants

The measures of ascorbic acid recorded a diminishing impact in case of Pb treated plants (Fig. 6.27; Table 6.27). The lowest value of ascorbic acid content (3.71 µg g^{-1} FW) was estimated at T_2 concentration. Maximum ascorbic quantity i.e., 10.86 µg g^{-1} FW was at EBL + Si treated plants i.e., T_{12}. At T_{13}, T_{14} and T_{15} in reference to EBL and Si co-augmented plants, ascorbic acid values of 8.64, 8.22 and 7.27 µg g^{-1} FW, respectively were registered. T_6 documented 109.7% increment in contrast to T_2 whereas T_{10} showed 88.94% elevation over T_2. An amplification of 23.25% was found in T_{13} than T_{10}. T_{13} made 29.53% elevation in contrast to control plants.

Glutathione registered a depletive effect with regard to its content as the level of Pb elevated (Fig. 6.27; Table 6.27). Minimum measures of glutathione content i.e., 4.53 µg g^{-1} FW were at T_3 concentration with a 41.54% decline than control cultivars. EBL and Si (control) genotypes (T_4 and T_8) had a respective improvement of 13.29% and 10.06%, over control plants. Alone addition of EBL resulted in improving its amount by being 7.41, 6.99 and 6.53 µg g^{-1} FW at T_5, T_6 and T_7 concentrations, respectively. Co-supplementation of EBL with Si under Pb stress elevated the glutathione levels to 8.9 at T_{13}, 9.07 at T_{14} and 8.24 µg g^{-1} FW at T_{15}, respectively. T_6 brought about an increment of 38.69% over T_2 treatment whereas T_{10} recorded 25% elevation over T_2. An improvement of 43.96% was noted in T_{14} than T_{10}. T_{14} registered an elevation of 17.03% versus control.

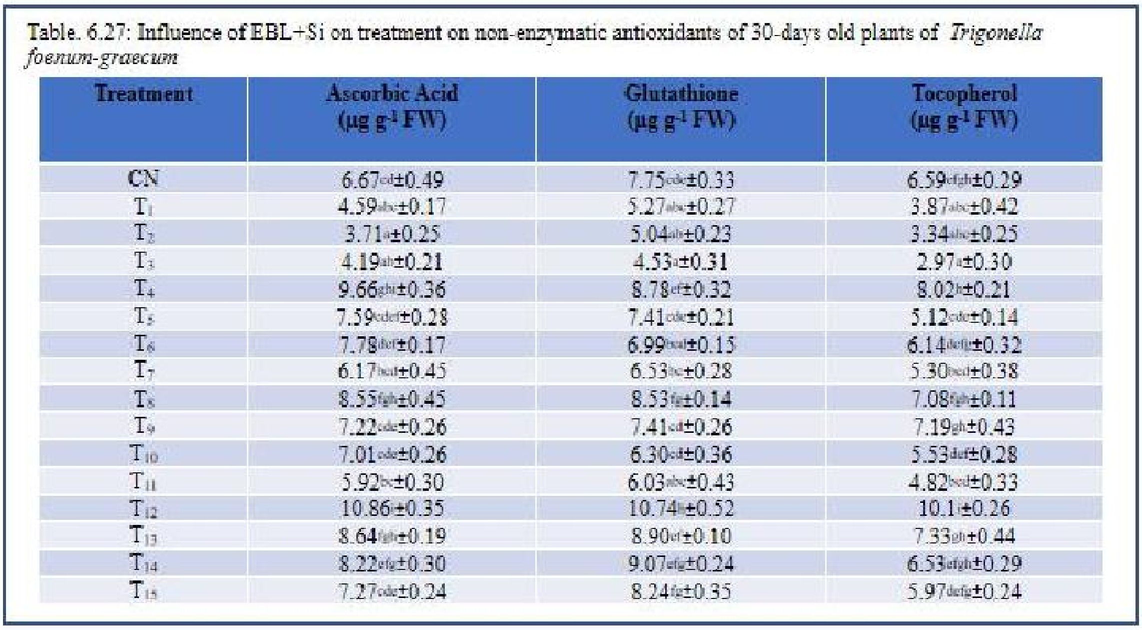
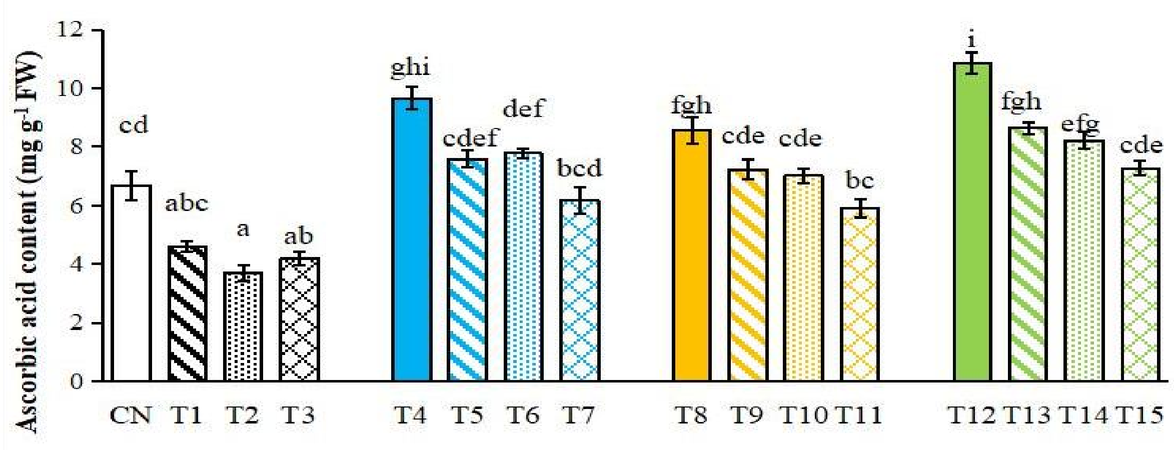


Table. 6.27: Influence of EBL+Si on treatment on non-enzymatic antioxidants of 30-days old plants of *Trigonella foenum-graecum*

Treatment	Ascorbic Acid ($\mu g\ g^{-1}$ FW)	Glutathione ($\mu g\ g^{-1}$ FW)	Tocopherol ($\mu g\ g^{-1}$ FW)
CN	$6.67^{cd}\pm0.49$	$7.75^{cde}\pm0.33$	$6.59^{efgh}\pm0.29$
T_1	$4.59^{abc}\pm0.17$	$5.27^{abc}\pm0.27$	$3.87^{abc}\pm0.42$
T_2	$3.71^{a}\pm0.25$	$5.04^{ab}\pm0.23$	$3.34^{abc}\pm0.25$
T_3	$4.19^{ab}\pm0.21$	$4.53^{a}\pm0.31$	$2.97^{a}\pm0.30$
T_4	$9.66^{ghi}\pm0.36$	$8.78^{ef}\pm0.32$	$8.02^{h}\pm0.21$
T_5	$7.59^{cdef}\pm0.28$	$7.41^{cde}\pm0.21$	$5.12^{cde}\pm0.14$
T_6	$7.78^{def}\pm0.17$	$6.99^{bcd}\pm0.15$	$6.14^{defg}\pm0.32$
T_7	$6.17^{bcd}\pm0.45$	$6.53^{bc}\pm0.28$	$5.30^{bcd}\pm0.38$
T_8	$8.55^{fgh}\pm0.45$	$8.53^{fg}\pm0.14$	$7.08^{fgh}\pm0.11$
T_9	$7.22^{cde}\pm0.26$	$7.41^{cd}\pm0.26$	$7.19^{gh}\pm0.43$
T_{10}	$7.01^{cde}\pm0.26$	$6.30^{cd}\pm0.36$	$5.53^{def}\pm0.28$
T_{11}	$5.92^{bc}\pm0.30$	$6.03^{abc}\pm0.43$	$4.82^{bcd}\pm0.33$
T_{12}	$10.86^{i}\pm0.35$	$10.74^{h}\pm0.52$	$10.1^{i}\pm0.26$
T_{13}	$8.64^{fgh}\pm0.19$	$8.90^{ef}\pm0.10$	$7.33^{gh}\pm0.44$
T_{14}	$8.22^{efg}\pm0.30$	$9.07^{efg}\pm0.24$	$6.53^{efgh}\pm0.29$
T_{15}	$7.27^{cde}\pm0.24$	$8.24^{fg}\pm0.35$	$5.97^{defg}\pm0.24$



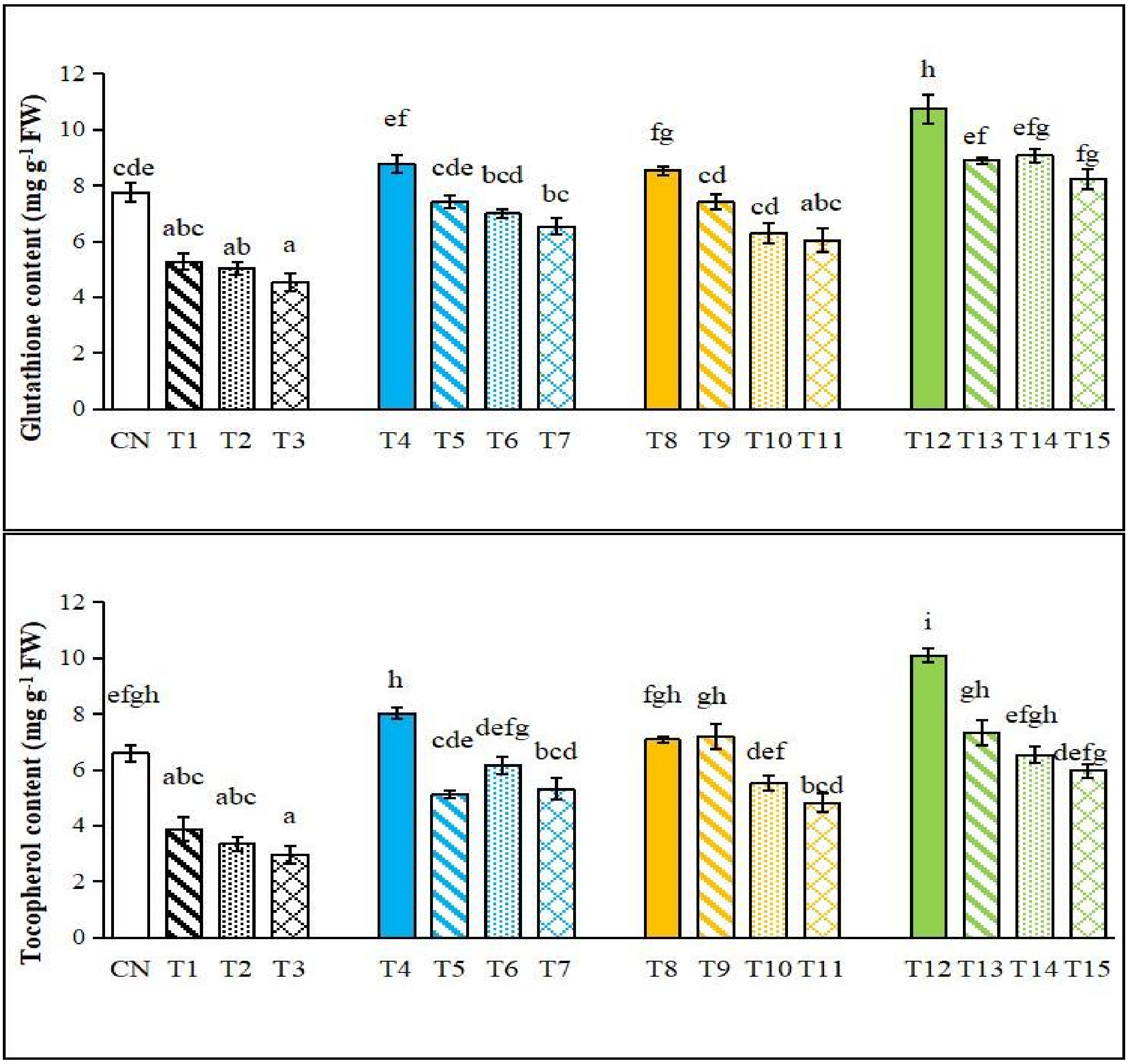


Fig. 6.27: Influence of EBL+Si treatment on measures of non-enzymatic antioxidants in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

Tocopherol share was negatively linked with Pb treatment as the metal depleted its content (Fig. 6.27; Table 6.27). The amount of tocopherol which was reported at T_3 treatment (2.97 µg g^{-1} FW) was the least amongst all three Pb concentrations chosen in the study. In case of individually applied of EBL and Si under Pb stress, highest content value of tocopherol was recorded at T_6 and T_9 with 6.14 and 7.19 µg g^{-1} FW, respectively. The tocopherol levels of 7.33 µg g^{-1} FW was observed in T_{15} plants. T_6 recorded 83.8% increment in comparison to T_2 whereas T_{10} showed 65.56% elevation than T_2. An amplification of 18.08% was noted in T_{14} than T_{10}.

6.1.3.9 Gene expression

Under control, 2.61 fold change in gene expression was noticed (Fig. 6.28; Table 6.28). Lowest expression of SOD gene was observed in Pb III stressed plants with 1.01 fold change. Individual treatment with EBL and Si under Pb stress showed 3.5 and 5.3 fold change. However, co-application of EBL+Si further induced the gene expression of SOD gene with 6.52 fold change under stressed conditions.

Treatment	**Relative gene expression**	
	SOD	**CAT**
CN	$2.61^{b}\pm0.46$	$3.66^{b}\pm0.23$
T_3	$1.01^{a}\pm0.1$	$1.73^{a}\pm0.48$
T_7	$3.48^{b}\pm0.1$	$5.84^{c}\pm0.19$
T_{11}	$5.29^{c}\pm0.28$	$5.36^{bc}\pm0.54$
T_{15}	$6.52^{c}\pm0.39$	$6.46^{c}\pm0.36$

Table 6.28: Influence of EBL+Si treatment on comparative gene expression in 30 days old Pb stressed *Trigonella foenum-graecum* plants.

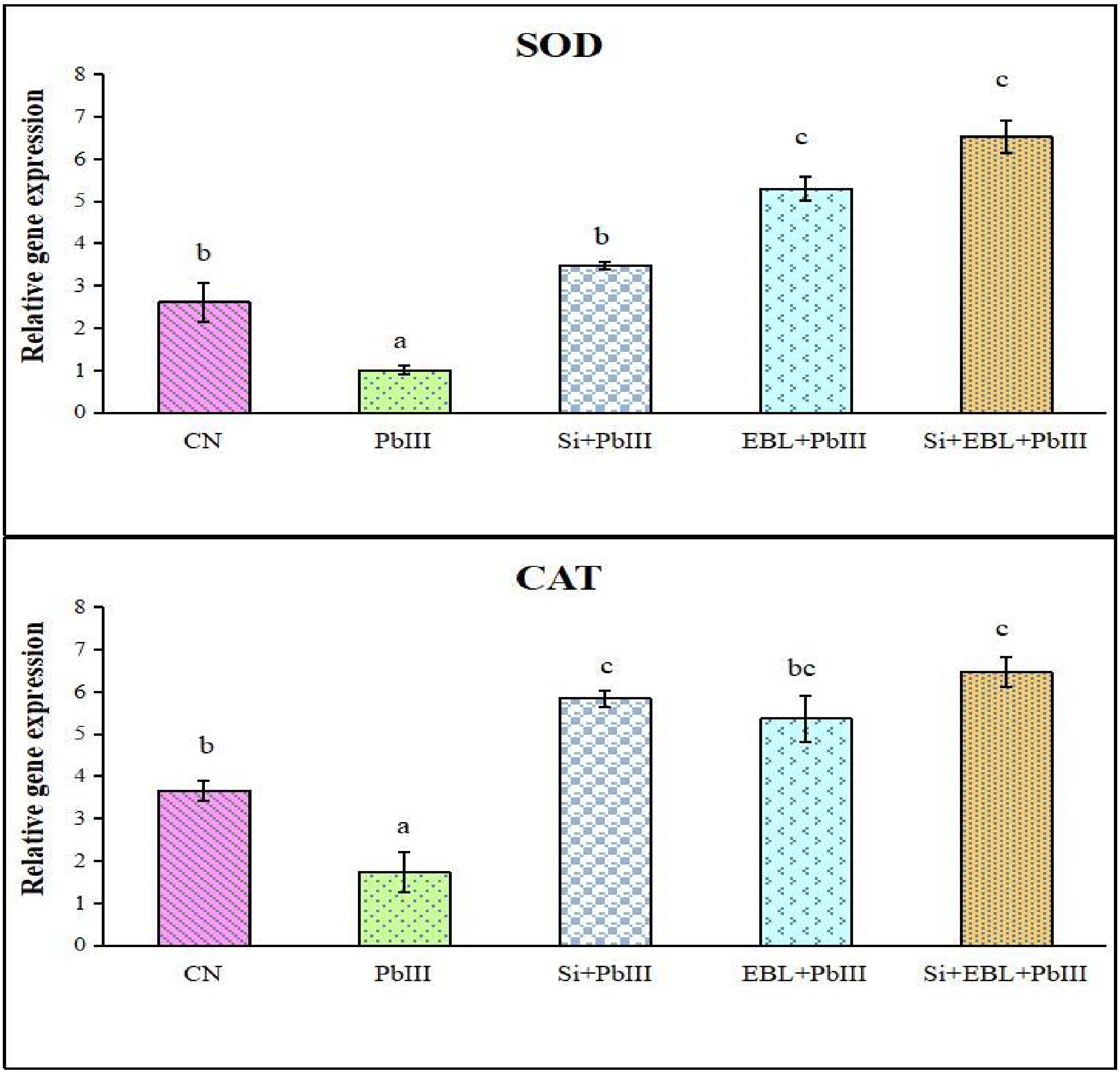


Fig. 6.28: Influence of EBL+Si treatment on stress responsive SOD and CAT genes in 30 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

Similarly, expression of CAT gene was also influenced with minimum expression in Pb III stressed plants with 1.22 fold change (Fig. 6.28; Table 6.28). A fold change of 3.66 was found in control plants. Application of EBL and Si alone under stressed conditions increased the expression of CAT gene with 5.84 and 5.36 fold change, respectively. Application of EBL and Si in coupled manner resulted in 6.46 fold change in CAT expression under Pb III which was highest among all the samples.

6.1.4. Plants raised in *In vivo* conditions*:* 45 days old plants

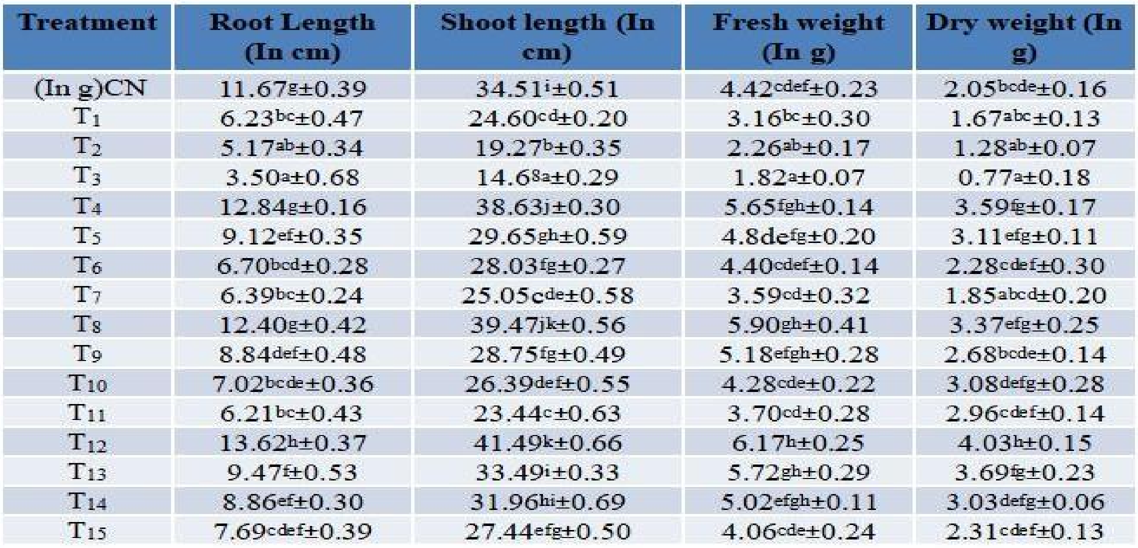
6.1.4.1 Growth attributes

A notable inhibition in root growth consequent to Pb stress treatments was recorded and summarized in fig. and table 6.29a. The highest curtailment of 70% was marked at T_3 and it was around 46.61% in T_1 plants vis-a-vis CN plants. The results further divulged that 24-EBL and Si remarkably alleviated the Pb noxiousness by amplifying the root length. With regard to EBL pre-treated plants under Pb stress, maximum root length i.e. 9.12 cm was noted at T_5 concentration. Supplementation of Si also brought forth an improvement in the root length of Pb stressed plants with being its highest at T_9 treatment (8.84 cm). Root length results were comparatively better in case of Si pre-treatment than EBL augmentation while battling out the Pb stress from *Trigonella* plants. The cumulative treatment of 24-EBL + Si also amplified the root length in Pb exposed plants, exhibiting highest root length of 9.47 cm at T_{13}. An enhancement of 52% in root length got noticed at T_{13} in correlation to T_1 treatment plants. Plants at T_{13} treatment were recorded to have a 7.12% elevation than the plants of T_9treatment. Additionally, in a contrast made between T_{13} and T_{10} plants, 34.9% enhancement was shown by T_{13} plants. T_{14} treated plants had 24.07% increase in root length compared to control plants.

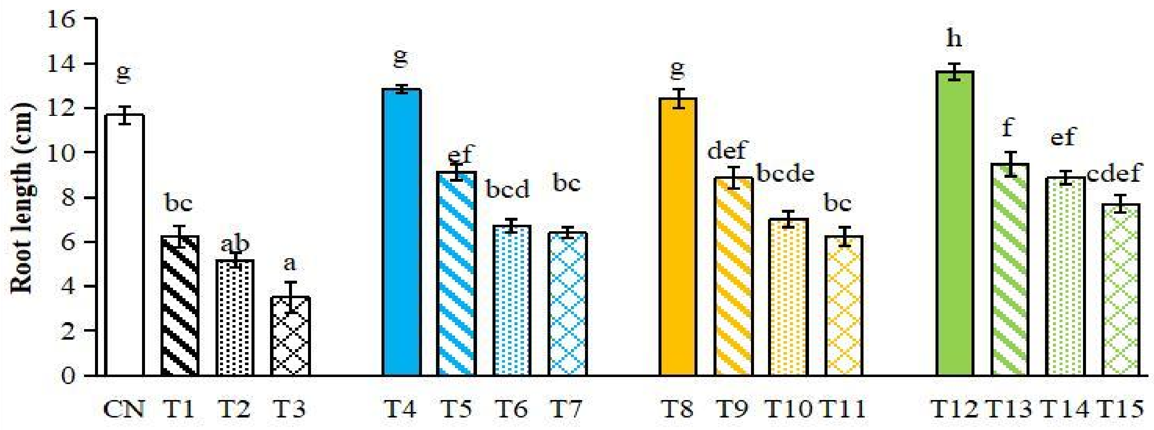
Shoot length was recorded to be reduced under Pb stress with the shortest shoot length of 14.68 cm at T_3 concentration. EBL and Si control plants showed boosted the elongation of shoots (Fig. 6.29a; Table 6.29a). With elevation in stress levels, shoot length further declined. Shoot length was markedly enhanced in EBL pre-treated stressed plants with highest value being reported in T_5 treated plants (29.65 cm) as opposed to Pb I alone stressed plants having a length of only 24.60 cm. Under Pb stress, Si spraying promoted shoot length with the greatest being around 28.75 cm in T_9 treated fenugreek plants. Collectively applied EBL and Si proved more beneficial with the optimum length being recorded at 33.49 cm in T_{13} concentration. T_5 plants showed 20.52% increment in shoot length over T_1. Similarly, 59.67% increase was noticed in T_{11} than T_3. Shoot length was respectively enhanced by 13% and 16.48% in T_{13} than T_5 and T_9. T_{13} showed 2.95% increment versus control plants.

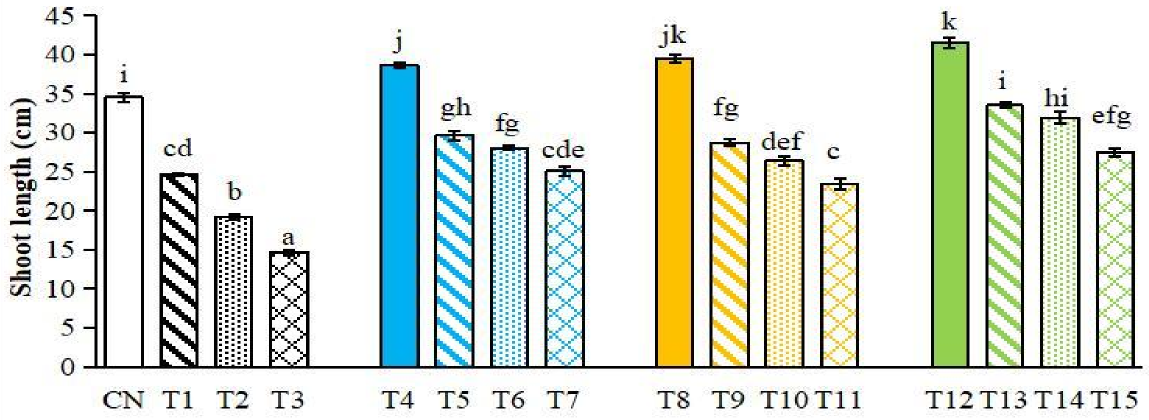
Fresh weight was substantially affected under metal stress with maximum reduction in fresh weight (1.82 g) in T_3 plants (Fig. 6.29a; Table 6.29a). EBL pre-treated plants had an improvement in the fresh weight with the highest and lowest fresh weights i.e., 4.8 and 3.59 g being noticed at T_5 and T_7, respectively. Combination of EBL and Si was determined to be more efficient in scaling up the fresh weights as compared to their alone treatments in response to Pb stress. The greatest increment in fresh weight with regard to combined application was reported at T_{13} with 5.72 g fresh weight. It was escalated by 7.91% in T_5 than T_9. T_{13} treatment recorded 10.23% increment over T_9. T_{13} showed 29.18% augmentation over control plants.

Table. 6.29a: Influence of EBL+Si treatment on growth attributes of 45-days old Pb stressed plants of *Trigonella foenum-graecum*



Treatment	Root Length (In cm)	Shoot length (In cm)	Fresh weight (In g)	Dry weight (In g)
(In g)CN	11.67^{g}±0.39	34.51^{i}±0.51	4.42^{cdef}±0.23	2.05^{bcde}±0.16
T$_1$	6.23^{bc}±0.47	24.60^{cd}±0.20	3.16^{bc}±0.30	1.67^{abc}±0.13
T$_2$	5.17^{ab}±0.34	19.27^{b}±0.35	2.26^{ab}±0.17	1.28^{ab}±0.07
T$_3$	3.50^{a}±0.68	14.6^{8a}±0.29	1.82^{a}±0.07	0.77^{a}±0.18
T$_4$	12.84^{g}±0.16	38.63^{j}±0.30	5.65^{fgh}±0.14	3.59^{fg}±0.17
T$_5$	9.12^{ef}±0.35	29.65^{gh}±0.59	4.8de^{fg}±0.20	3.11^{efg}±0.11
T$_6$	6.70^{bcd}±0.28	28.03^{fg}±0.27	4.40^{cdef}±0.14	2.28^{cdef}±0.30
T$_7$	6.39^{bc}±0.24	25.05c^{de}±0.58	3.59^{cd}±0.32	1.85^{abcd}±0.20
T$_8$	12.40^{g}±0.42	39.47^{jk}±0.56	5.90^{gh}±0.41	3.37^{efg}±0.25
T$_9$	8.84^{def}±0.48	28.75^{fg}±0.49	5.18^{efgh}±0.28	2.68^{bcde}±0.14
T$_{10}$	7.02^{bcde}±0.36	26.39^{def}±0.55	4.28^{cde}±0.22	3.08^{defg}±0.28
T$_{11}$	6.21^{bc}±0.43	23.44^{c}±0.63	3.70^{cd}±0.28	2.96^{cdef}±0.14
T$_{12}$	13.62^{h}±0.37	41.49^{k}±0.66	6.17^{h}±0.25	4.03^{h}±0.15
T$_{13}$	9.47^{f}±0.53	33.49^{i}±0.33	5.72^{gh}±0.29	3.69^{fg}±0.23
T$_{14}$	8.86^{ef}±0.30	31.96^{hi}±0.69	5.02^{efgh}±0.11	3.03^{defg}±0.06
T$_{15}$	7.69^{cdef}±0.39	27.44^{efg}±0.50	4.06^{cde}±0.24	2.31^{cdef}±0.13





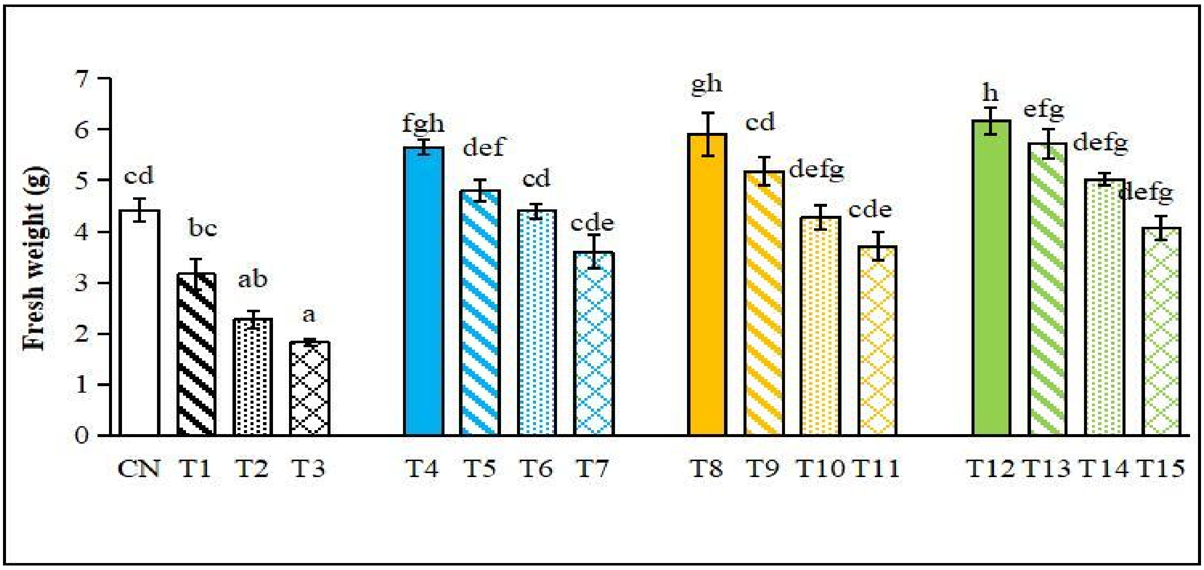


Fig. 6.29 (a): Influence of EBL+Si treatment on growth attributes in Pb stressed 45 days old *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

T_1, T_2 plus T_3 plants were noted to have 1.67, 1.28 and 0.77 g dry weights (Fig. 6.29b; Table 6.29a). An improvement in dry weights was noticed in Pb-treated plants when treated with EBL. Application of EBL and Si in individual format under Pb stress recorded maximum 3.11 and 2.68 g dry weights at T_5 and T_9, respectively. Better results were displayed by EBL + Si with reference to dry weights when contrasted against their individual treatments. The greatest dry weight of 3.69 g was noted at T_{13}. It was scaled up by 13.82% in T_5 over T_9. A 18.64% enhancement was detected in T_{13} over T_5. T_{15} treatment had 13.8% increase with respect to T_9.

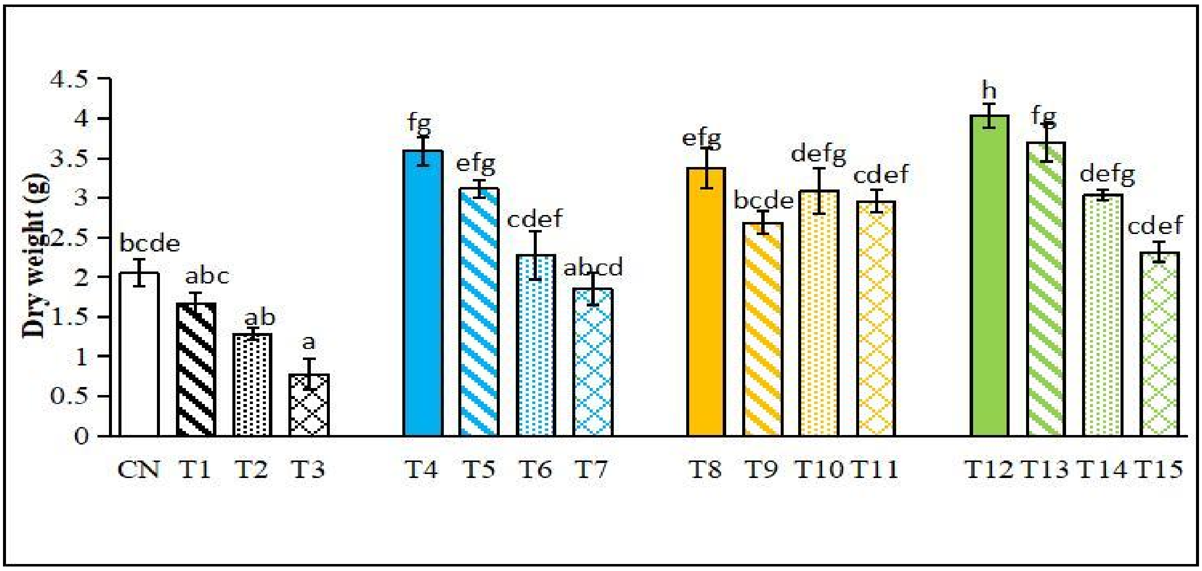


Fig. 6.29 (b): Influence of EBL+Si treatment on growth attributes in Pb stressed 45 days old *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together

with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Decrement of vigor index (VI) was also a consequence of stress induced by Pb metal in fenugreek as shown by T_1 plants (1712%) with being reduced nearly half to that of untreated plants of control (3203%). Further, plants of T_2 treatment were noted to have VI value of 1031% i.e. 39.77% lesser in contrast with T_1 treated plants (Fig. 6.29c; Table 6.29b). Individually supplemented EBL and Si under Pb metal stress elevated the VI values. Maximum value of VI (2688%) was observed around Pb I concentration in T_5 plants pre-treated with EBL. Exogenous augmentation of Si also escalated the VI values with being highest at T_9 treatment i.e. 2113% which revealed that 24-EBL was way more effective in enhancing the VI in response to Pb stress. However, when coupled, EBL and Si showed maximum elevation in the VI values amongst all treatments i.e. 3206% at T_{13} against 1712% of Pb I alone augmented T_1 plants making the elevation as high as 87.26%. T_{13} plants correlated with T_5 and T_9 were recorded to exhibit a respective increase of 19.26% and 51.72%.

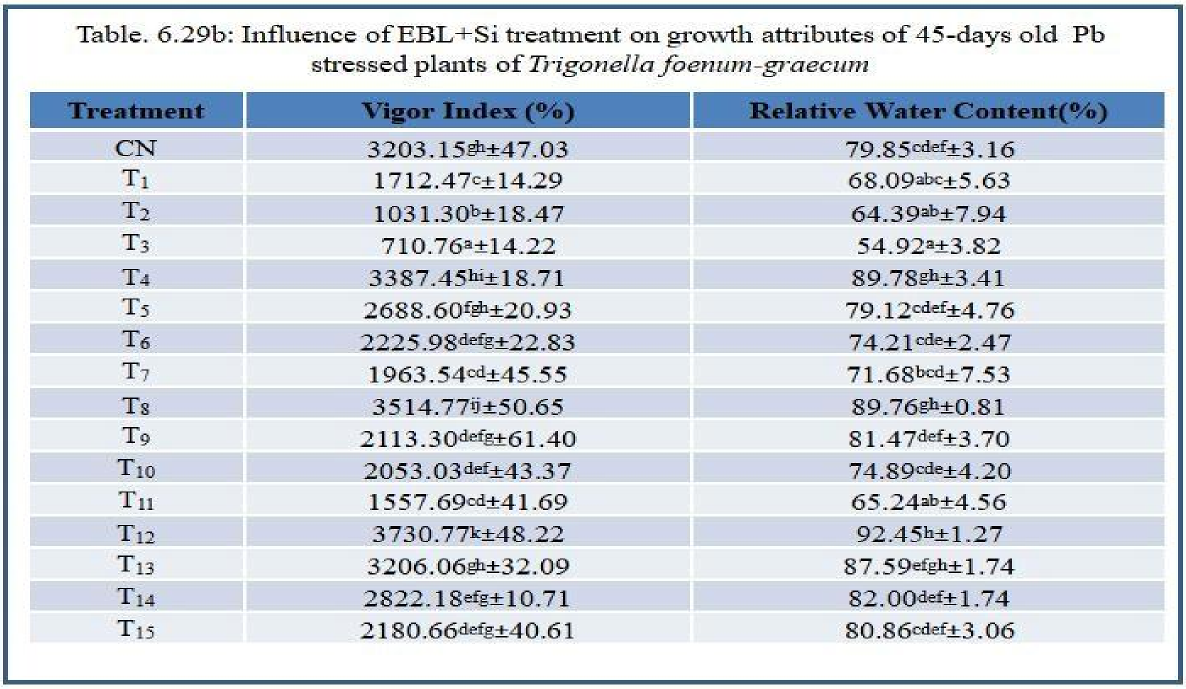
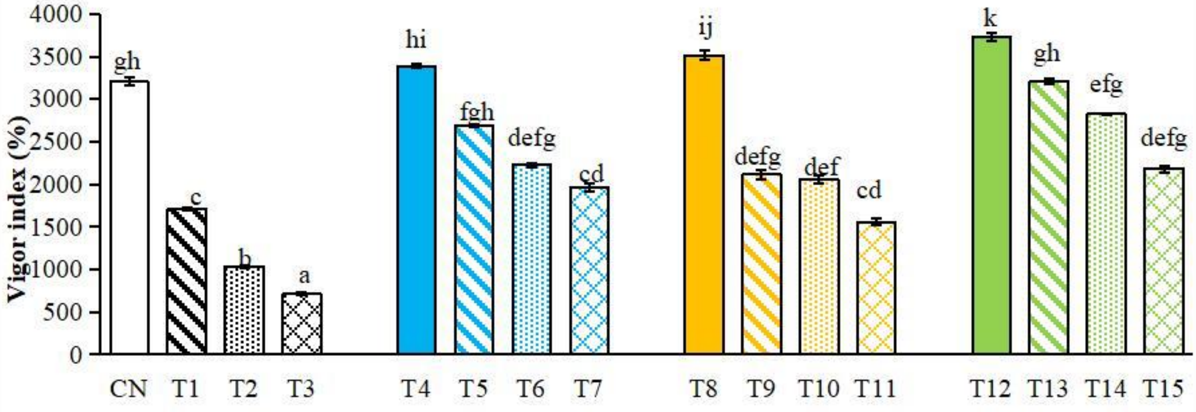


Table. 6.29b: Influence of EBL+Si treatment on growth attributes of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Vigor Index (%)	Relative Water Content(%)
CN	3203.15^{gh}±47.03	79.85^{cdef}±3.16
T_1	1712.47^{c}±14.29	68.09^{abc}±5.63
T_2	1031.30^{b}±18.47	64.39^{ab}±7.94
T_3	710.76^{a}±14.22	54.92^{a}±3.82
T_4	3387.45^{hi}±18.71	89.78^{gh}±3.41
T_5	2688.60^{fgh}±20.93	79.12^{cdef}±4.76
T_6	2225.98^{defg}±22.83	74.21^{cde}±2.47
T_7	1963.54^{cd}±45.55	71.68^{bcd}±7.53
T_8	3514.77^{ij}±50.65	89.76^{gh}±0.81
T_9	2113.30^{defg}±61.40	81.47^{def}±3.70
T_{10}	2053.03^{def}±43.37	74.89^{cde}±4.20
T_{11}	1557.69^{cd}±41.69	65.24^{ab}±4.56
T_{12}	3730.77^{k}±48.22	92.45^{h}±1.27
T_{13}	3206.06^{gh}±32.09	87.59^{efgh}±1.74
T_{14}	2822.18^{efg}±10.71	82.00^{def}±1.74
T_{15}	2180.66^{defg}±40.61	80.86^{cdef}±3.06



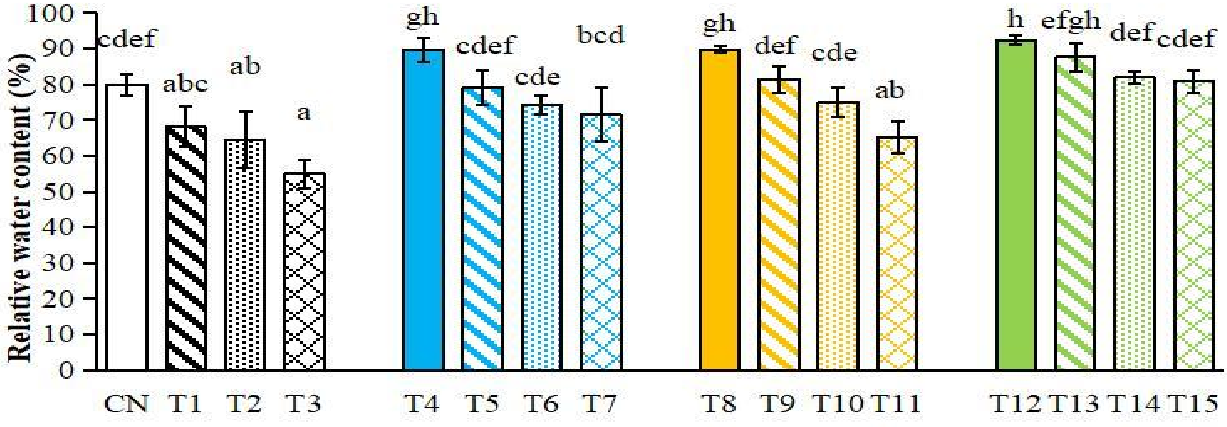


Fig. 6.29 (c): Influence of EBL+Si treatment on Vigor index and RWC in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment

level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

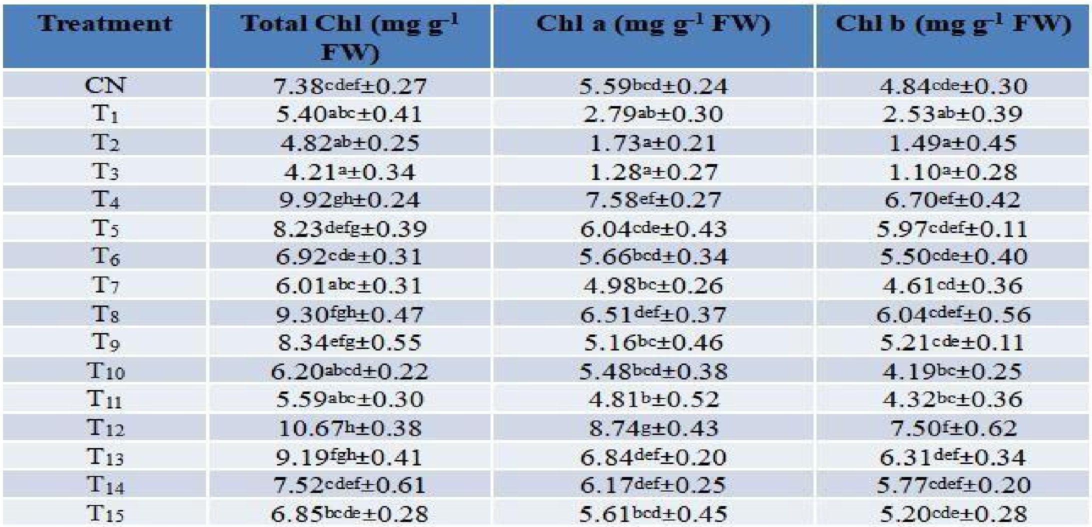
Lead exposure in fenugreek plants consequently gave rise to reduction in the value of relative water content with the least relative water content at 54.92% in T_3 (Fig. 6.29c; Table 6.29b). Pre-treatment with EBL under stressful circumstances resulted in maximum relative water content of 79.12% at T_5 followed by 71.68% at T_7. Foliar application of the second mitigant i.e. Si under unstressed conditions (T_8) showed 89.76% relative water content. T_9, T_{10} and T_{11} recorded 81.47, 74.89 and 65.24% relative water content. The relative water content value of 87.59% was detected at T_{13} when applied with EBL and Si together. It was elevated by 16.19% in T_5 contrary to T_1. An upsurge of 19.66% was noticed in T_9 than T_1. T_{13} treatment had 7.52% more content value than T_9.

6.1.4.2 Photosynthetic activity

6.1.4.2.1 Photosynthetic pigments

Photosynthetic pigments were documented to have a significant decline as an aftermath of Pb stress (Fig. 6.30a; Table 6.30a). Almost 43% drop in the measures of total chlorophyll was recorded at T_3 versus control conditions i.e., from 7.38 to 4.21 mg g^{-1} FW. EBL and Si applied in an individual manner, promoted its quantities in reference to Pb stress where the highest values among them were reported of 8.23 and 8.34 mg g^{-1} FW at T_5 and T_9. Synergistically supplemented EBL and Si under stress registered far superior outcomes in amplifying the measures of total chlorophyll vis-à-vis their individual treatments. The largest quantities were reported to be in case of 9.19 mg g^{-1} FW in T_{13} plants. Further, T_5 displayed 52.4% increment as opposed to T_1 whereas T_9 exhibited 54.4% upsurge in contrast to T_1. An upgradation of 11.67% was recorded in T_{13} over T_5. Similar outcomes were obtained with respect to content values of chl a and chl b in fenugreek plants. The lowest measure of chl a (1.28 mg g^{-1} FW) and chl b (1.10 mg g^{-1} FW) were registered at T_3 concentration. EBL and Si notably ameliorated the Pb-induced noxiousness by boosting their levels. The integrated treatment of EBL and Si further augmented their contents in response to stressful conditions with being the highest around 6.84 and 6.31 mg g^{-1} FW in T_{13} plants. Furthermore, T_5 recorded 116.48% and 135.96% increment versus T_1 whereas T_9 exhibited 85% and 105.92% upsurge contrary to T_1 in reference to chl a and chl b, respectively.

Table. 6.30a : Influence of EBL+Si treatment on photosynthetic pigments of 45-days old Pb stressed plants of *Trigonella foenum-graecum*



Treatment	Total Chl (mg g^{-1} FW)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)
CN	$7.38^{cdef}\pm0.27$	$5.59^{bcd}\pm0.24$	$4.84^{cde}\pm0.30$
T_1	$5.40^{abc}\pm0.41$	$2.79^{ab}\pm0.30$	$2.53^{ab}\pm0.39$
T_2	$4.82^{ab}\pm0.25$	$1.73^{a}\pm0.21$	$1.49^{a}\pm0.45$
T_3	$4.21^{a}\pm0.34$	$1.28^{a}\pm0.27$	$1.10^{a}\pm0.28$
T_4	$9.92^{gh}\pm0.24$	$7.58^{ef}\pm0.27$	$6.70^{ef}\pm0.42$
T_5	$8.23^{defg}\pm0.39$	$6.04^{cde}\pm0.43$	$5.97^{cdef}\pm0.11$
T_6	$6.92^{cde}\pm0.31$	$5.66^{bcd}\pm0.34$	$5.50^{cde}\pm0.40$
T_7	$6.01^{abc}\pm0.31$	$4.98^{bc}\pm0.26$	$4.61^{cd}\pm0.36$
T_8	$9.30^{fgh}\pm0.47$	$6.51^{def}\pm0.37$	$6.04^{cdef}\pm0.56$
T_9	$8.34^{efg}\pm0.55$	$5.16^{bc}\pm0.46$	$5.21^{cde}\pm0.11$
T_{10}	$6.20^{abcd}\pm0.22$	$5.48^{bcd}\pm0.38$	$4.19^{bc}\pm0.25$
T_{11}	$5.59^{abc}\pm0.30$	$4.81^{b}\pm0.52$	$4.32^{bc}\pm0.36$
T_{12}	$10.67^{h}\pm0.38$	$8.74^{g}\pm0.43$	$7.50^{f}\pm0.62$
T_{13}	$9.19^{fgh}\pm0.41$	$6.84^{def}\pm0.20$	$6.31^{def}\pm0.34$
T_{14}	$7.52^{cdef}\pm0.61$	$6.17^{def}\pm0.25$	$5.77^{cdef}\pm0.20$
T_{15}	$6.85^{bcde}\pm0.28$	$5.61^{bcd}\pm0.45$	$5.20^{cde}\pm0.28$

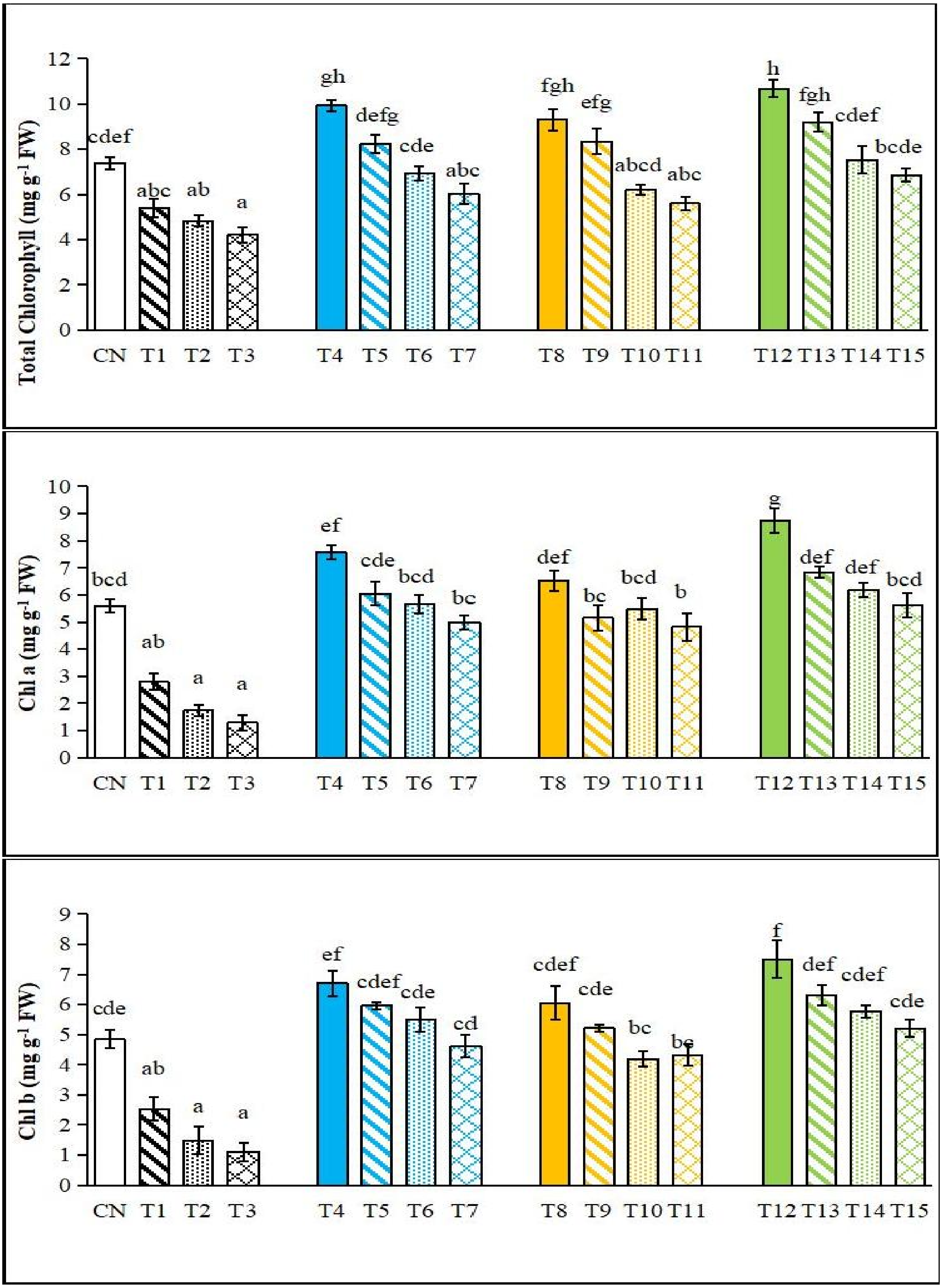


Fig. 6.30 (a): Influence of EBL+Si treatment on photosynthetic pigments in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

It was documented that Pb stress diminished the carotenoid measures (Fig. 6.30b; Table 6.30b). Carotenoid quantities depleted with elevated levels of Pb toxicity with the greatest decrement at T_3 (5.38 mg g^{-1} FW). Carotenoid measures were scaled up from 7.37 to 9.92 mg g^{-1} FW in T_5 plants contrary to T_1 stressed plants. Si addition brought forth maximum escalation in the carotenoid content of 10.76 mg g^{-1} FW at T_9 genotypes. The largest amount of carotenoid i.e. 11.16 mg g^{-1} FW was reported at T_{13} while the most diminished content stood at T_{15} (7.14 mg g^{-1} FW). A respective augmentation of 12.5% and 11.42% was noted in T_{13} versus T_5 and control plants.

Lead stress significantly depleted the content of xanthophyll with a maximal reduction of 7.02 mg g^{-1} FW in T_3 plants (Fig. 6.30b; Table 6.30b). EBL and Si treatments scaled up the measures of xanthophyll when fenugreek was subjected to Pb stress. Individually added EBL and Si mitigated the Pb stress in terms of promoted levels of xanthophyll having highest content at T_5 and T_9 treatments i.e 12.91 mg g^{-1} FW and 12.63 mg g^{-1} FW, respectively. Diminution of xanthophyll content was directly influenced by increasing levels of Pb stress in EBL and Si alone applications. The highest xanthophyll was registered to be 14.5 mg g^{-1} FW at T_{13} concentration. In comparison to T_1, T_5 and T_9, recorded an increment of 42.96% and 39.86%, respectively. A respective upsurge of 12.31% was noticed in T_{13} over T_5 plants.

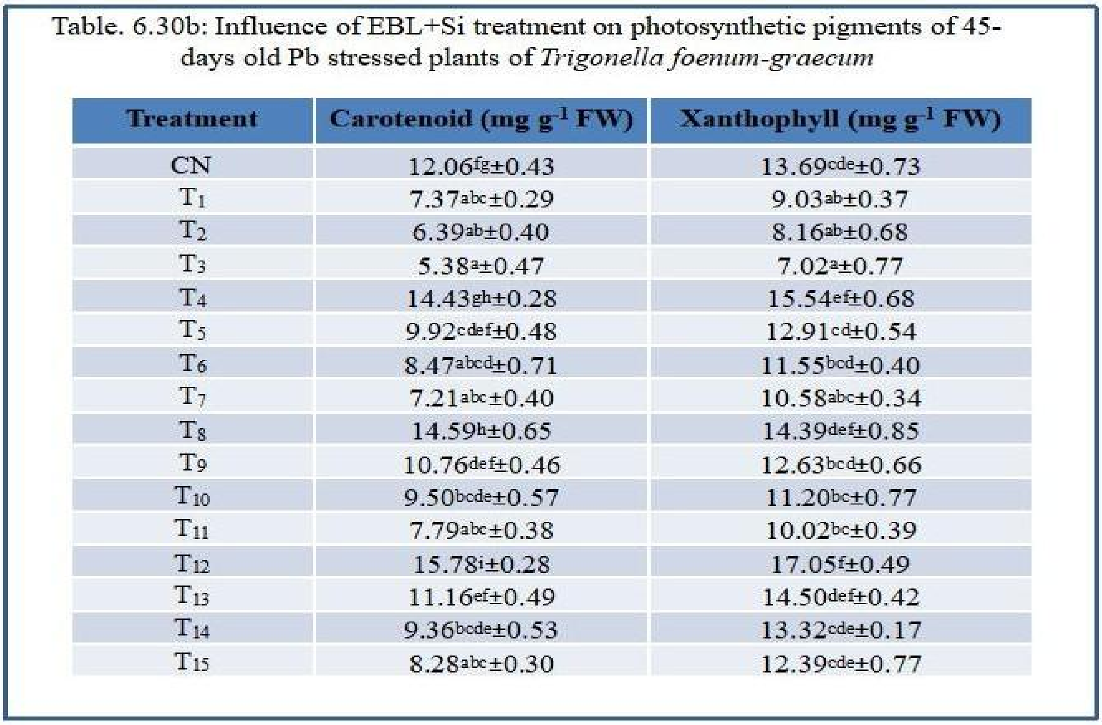


Table. 6.30b: Influence of EBL+Si treatment on photosynthetic pigments of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Carotenoid (mg g^{-1} FW)	Xanthophyll (mg g^{-1} FW)
CN	$12.06^{fg}\pm0.43$	$13.69^{cde}\pm0.73$
T_1	$7.37^{abc}\pm0.29$	$9.03^{ab}\pm0.37$
T_2	$6.39^{ab}\pm0.40$	$8.16^{ab}\pm0.68$
T_3	$5.38^{a}\pm0.47$	$7.02^{a}\pm0.77$
T_4	$14.43^{gh}\pm0.28$	$15.54^{ef}\pm0.68$
T_5	$9.92^{cdef}\pm0.48$	$12.91^{cd}\pm0.54$
T_6	$8.47^{abcd}\pm0.71$	$11.55^{bcd}\pm0.40$
T_7	$7.21^{abc}\pm0.40$	$10.58^{abc}\pm0.34$
T_8	$14.59^{h}\pm0.65$	$14.39^{def}\pm0.85$
T_9	$10.76^{def}\pm0.46$	$12.63^{bcd}\pm0.66$
T_{10}	$9.50^{bcde}\pm0.57$	$11.20^{bc}\pm0.77$
T_{11}	$7.79^{abc}\pm0.38$	$10.02^{bc}\pm0.39$
T_{12}	$15.78^{i}\pm0.28$	$17.05^{f}\pm0.49$
T_{13}	$11.16^{ef}\pm0.49$	$14.50^{def}\pm0.42$
T_{14}	$9.36^{bcde}\pm0.53$	$13.32^{cde}\pm0.17$
T_{15}	$8.28^{abc}\pm0.30$	$12.39^{cde}\pm0.77$

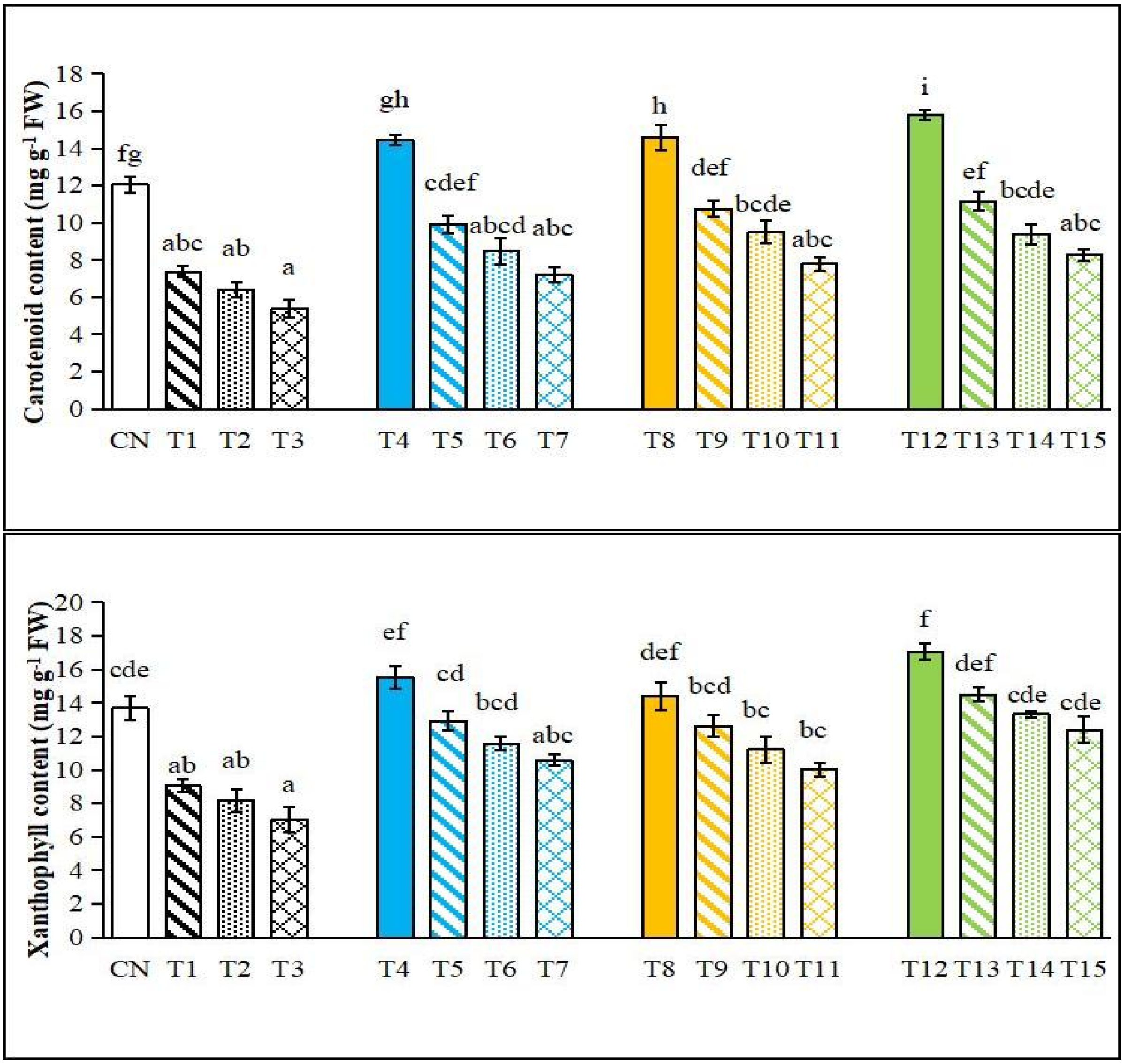


Fig. 6.30 (b): Influence of EBL+Si treatment on photosynthetic pigments in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.4.3 Metabolites

Anthocyanin was noted to have a declined content under Pb stress with being minimum (6.87 mg g^{-1} FW) at T_3 treatment which was 35.5% lower than the control (Fig. 6.31; Table 6.31). With respect to individually augmented mitigants i.e. EBL and Si in unstressed plants, T_4 plants (12.60 mg g^{-1} FW) were slightly better performers than T_8 plants (11.87 mg g^{-1} FW). Elevations in the anthocyanin levels were recorded at T_5 i.e. anthocyanin content was enhanced from 8.71 to 11.20 mg g-1 FW as opposed to T_1 concentration. Feeding of Si against Pb toxicity led to the betterment of anthocyanin measures vis-à-vis plants stressed with Pb only. Anthocyanin content was noticed to be raised to highest value of 10.34 mg g^{-1} FW at T_9 concentration. Under Pb stress, Si augmentation was observed to be more efficient in elevating the anthocyanin measures in contrast to EBL treatment. Integration of EBL with

Si in combating Pb toxicity further elevated the measures of anthocyanin with a value of 12.45, 11.19 and 10.30 mg g^{-1} FW at T_{13}, T_{14} along with T_{15} augmented plants, respectively. T_7 showed a decline of 8.71% when contrasted to T_{11} whereas T_{13} led to an enhancement of 17.12% than stress free plants under controlled conditions.

The minimal quantities of flavonoid were recorded in T_3 plants augmented with maximum concentration of Pb only (5.95 mg g^{-1} FW; Fig. 6.31; Table 6.31). In all three treatments of Pb alone stress, a 33.64% at T_1, 46.38% at T_2 and 53.22% decrease at T_3 was observed when a correlation was made with control plants. Flavanoid content registered an upsurge of 35.9% from T_5 (EBL + Pb I) to Pb I alone treated T_1 plants. Silicon treatment was further recorded to escalate the measures of flavonoids vis-à-vis Pb alone treated plants. Levels of flavanoid were observed to be 10.97 at T_9, 8.61 at T_{10} and 8.24 mg g^{-1} FW at T_{11}, respectively. Flavanoid content pertaining to cumulative application of EBL + Si, the maximum (T_{13}; 14.61 mg g^{-1} FW) and minimum (T_{15}; 11.37 mg g^{-1} FW) values were noted. Its measures registered a decrement of 12.24% in T_{11} over T_7 plants. Furthermore, T_{13} displayed a 14.85% augmentation in relation to control plants.

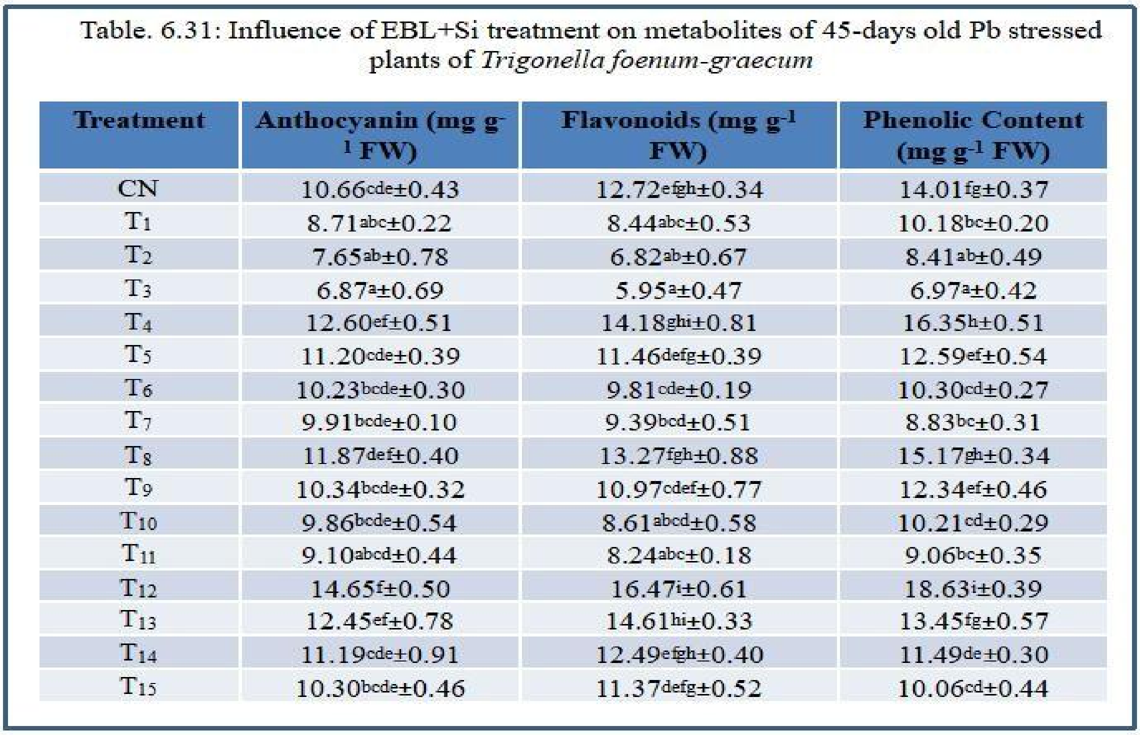


Table. 6.31: Influence of EBL+Si treatment on metabolites of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Anthocyanin (mg g^{-1} FW)	Flavonoids (mg g^{-1} FW)	Phenolic Content (mg g^{-1} FW)
CN	10.66^{cde}±0.43	12.72^{efgh}±0.34	14.01^{fg}±0.37
T_1	8.71^{abc}±0.22	8.44^{abc}±0.53	10.18^{bc}±0.20
T_2	7.65^{ab}±0.78	6.82^{ab}±0.67	8.41^{ab}±0.49
T_3	6.87^{a}±0.69	5.95^{a}±0.47	6.97^{a}±0.42
T_4	12.60^{ef}±0.51	14.18^{ghi}±0.81	16.35^{h}±0.51
T_5	11.20^{cde}±0.39	11.46^{defg}±0.39	12.59^{ef}±0.54
T_6	10.23^{bcde}±0.30	9.81^{cde}±0.19	10.30^{cd}±0.27
T_7	9.91^{bcde}±0.10	9.39^{bcd}±0.51	8.83^{bc}±0.31
T_8	11.87^{def}±0.40	13.27^{fgh}±0.88	15.17^{gh}±0.34
T_9	10.34^{bcde}±0.32	10.97^{cdef}±0.77	12.34^{ef}±0.46
T_{10}	9.86^{bcde}±0.54	8.61^{abcd}±0.58	10.21^{cd}±0.29
T_{11}	9.10^{abcd}±0.44	8.24^{abc}±0.18	9.06^{bc}±0.35
T_{12}	14.65^{f}±0.50	16.47^{i}±0.61	18.63^{i}±0.39
T_{13}	12.45^{ef}±0.78	14.61^{hi}±0.33	13.45^{fg}±0.57
T_{14}	11.19^{cde}±0.91	12.49^{efgh}±0.40	11.49^{de}±0.30
T_{15}	10.30^{bcde}±0.46	11.37^{defg}±0.52	10.06^{cd}±0.44

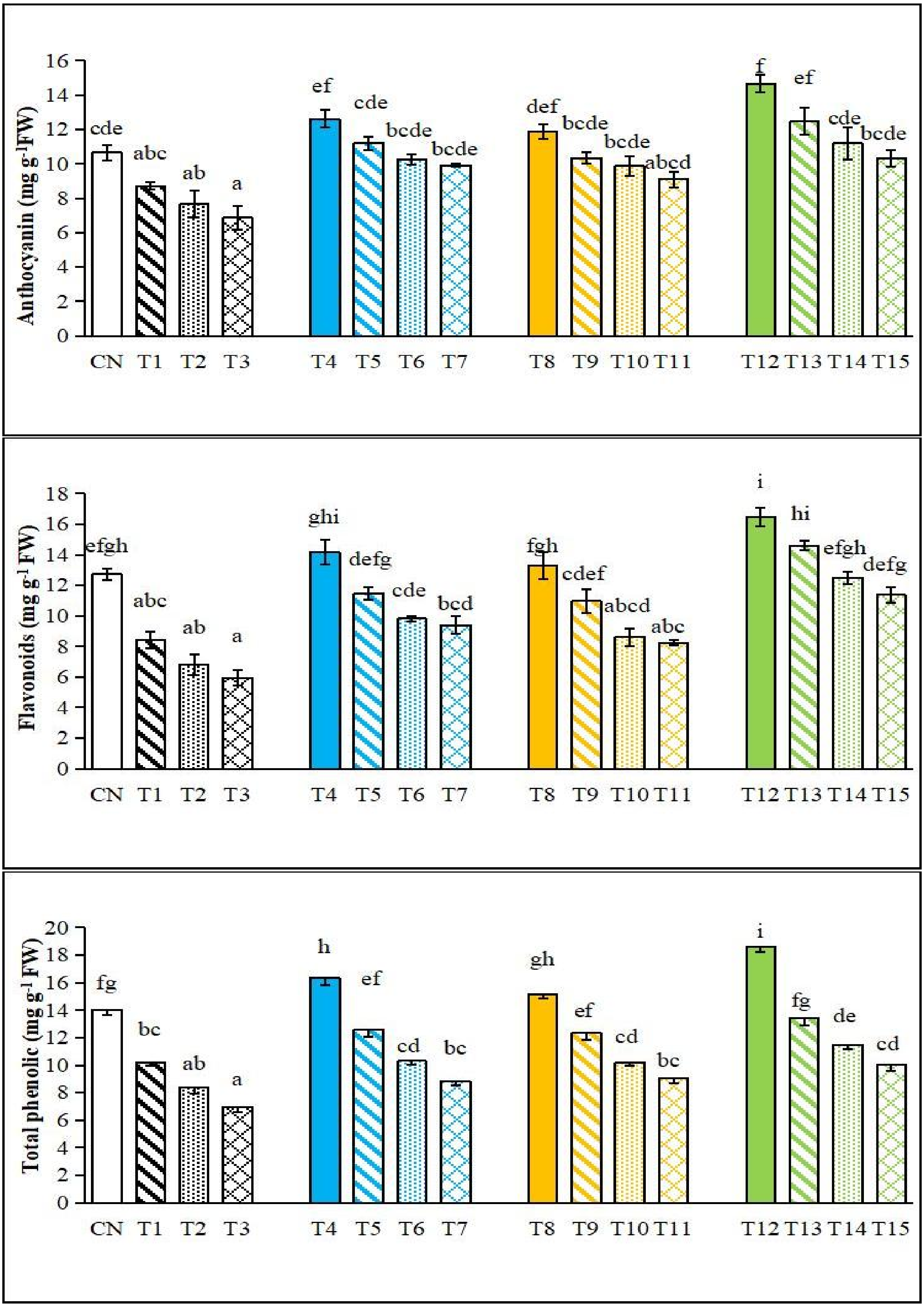


Fig. 6.31: Influence of EBL+Si treatment on measures of metabolites in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

With ascending levels of Pb stress, phenolics were noted to have a diminishing impact on their content (Fig. 6.31; Table 6.31). It was diminished to 6.97 mg g^{-1} FW (T_3) in correlation with control plants having 14.01 mg g^{-1} FW of phenolic content. Individually augmented EBL in stress free and stressed plants were recorded to have the highest measures of phenolics at T_4 (16.35 mg g^{-1} FW) and T_5 (12.59 mg g^{-1} FW) respectively. Exogenously applied Si, on the other hand, had the highest values in case of T_8 (15.17 mg g^{-1} FW) and T_9 (12.34 mg g^{-1} FW) for the same conditions. Combination of EBL and Si in Pb I stressed plants i.e. T_{13} showed greatest measures of phenolics at 13.45 mg g^{-1} FW while under control, EBL + Si made the phenolic content of T_{12} plants (highest 18.63 mg g^{-1} FW) amongst all the treatments chosen for the present study. An increment of 23.67% was recorded in T_5 over T_1. Additionally, T_{13} plants exhibited decrements of 6.83% and 3.99%, in correlation to T_5 and control plants respectively.

6.1.4.4 Oxidative damage

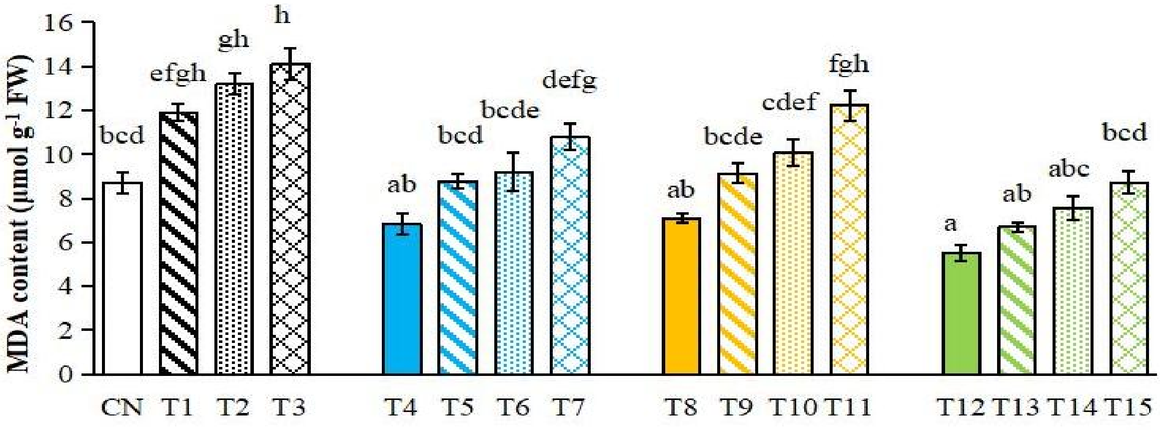
6.1.2.4.1 Oxidative stress markers

Extent of lipid peroxidation was noted to be enhanced because of Pb-mediated upsurge in the measures of MDA, when contrasted against control plants (Fig. 6.32; Table 6.32). An increment of 62% in the quantities of MDA was noted in T_3 plants, which were stressed with highest concentration of Pb, as opposed to the control plants. However, exogenously supplemented EBL and Si curtailed this Pb induced enhancement in MDA measures. With respect to individually applied EBL and Si to stressed plants, MDA contents were registered to be the most reduced at T_5 and T_9 with respective values of 8.77 and 9.14 µmol g^{-1} FW measures. Additionally, MDA values were diminished from 13.17 at T_2 to 7.56 µmol g^{-1} FW at T_{14} treated plants. A decrement of 26.24% was noted in T_5 over T_1. T_9 plants displayed 23.12% decline in MDA measures than T_1. T_{13} plants had 23.83% depletion in contrast to T_5. Besides this, T_{13} recorded 23.21% decline vis-a-vis control plants.

The measures of H_2O_2 were notably elevated in fenugreek plants upon getting subjected to Pb stress (Fig. 6.32; Table 6.32). An increase of 37.2% in the quantities of H_2O_2 got noticed in T_3 plants, which were stressed with highest concentration of Pb, as opposed to the control plants. Moreover, H_2O_2 measures were promoted to be 14.36 at T_1, 15.24 at T_2 and 16.79µmolg^{-1}FW at T_3 treatments stressed with Pb metal alone. However, applying them with EBL, Si and EBL + Si against Pb toxicity diminished the amount of H_2O_2. The highest diminishing impact of mitigants was noticed in case of their coupled treatment at T_{13} with a value of 6.90 µmol g^{-1} FW. A decrement of 20.75% was noted in T_5 over T_1. T_9 plants registered a decline of 13.37% when correlated with T_1. Furthermore, T_{13} plants were recorded to have 39.36 % and 44.53% reduction when placed against T_5 and T_9, respectively.

Table. 6.32: Influence of EBL+Si on treatment on oxidative stress markers of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	MDA (μ mol g^{-1} FW)	H_2O_2 (μ mol g^{-1} FW)
CN	8.7^{bcd}±0.50	10.68^{cd}±0.41
T$_1$	11.89^{efgh}±0.39	14.36^{fgh}±0.45
T$_2$	13.17^{gh}±0.47	15.24^{gh}±0.29
T$_3$	14.09^{h}±0.74	16.79^{h}±0.48
T$_4$	6.83^{ab}±0.48	7.08^{ab}±0.24
T$_5$	8.77^{bcd}±0.31	11.38^{cde}±0.44
T$_6$	9.19^{bcde}±0.85	12.46^{def}±0.63
T$_7$	10.77^{defg}±0.59	14.01^{fg}±0.35
T$_8$	7.09^{ab}±0.20	7.65^{ab}±0.65
T$_9$	9.14^{bcde}±0.44	12.44^{def}±0.50
T$_{10}$	10.08^{cdef}±0.58	13.73^{efg}±0.46
T$_{11}$	12.22^{fgh}±0.70	14.82^{fgh}±0.39
T$_{12}$	5.49^{a}±0.36	5.16^{a}±0.39
T$_{13}$	6.68^{ab}±0.22	6.90^{a}±0.57
T$_{14}$	7.56^{abc}±0.54	9.54^{bc}±0.45
T$_{15}$	8.71^{bcd}±0.50	11.09^{cd}±0.70



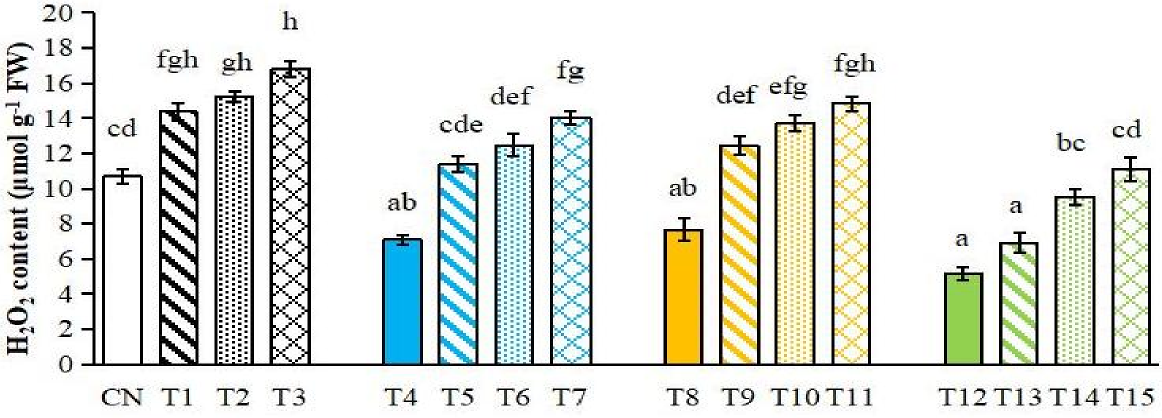
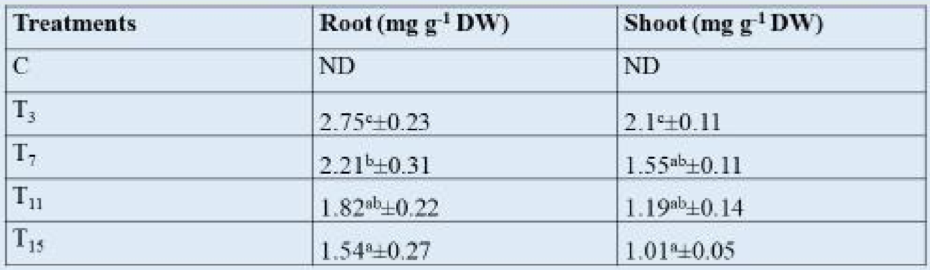


Fig. 6.32: Influence of EBL+Si treatment on oxidative stress indicators in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.4.5 Lead metal uptake

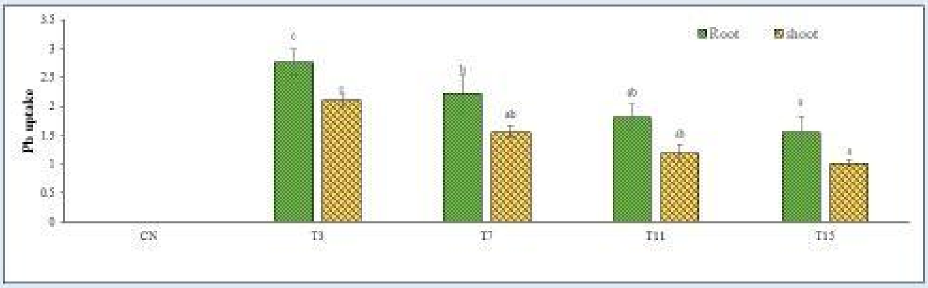
No Pb content was detected in control samples i.e. *in vivo* grown 45 days old fenugreek plants (Fig. 6.33; Table 6.33). Under individual stress of Pb, 2.75 and 2.1 mg g^{-1} DW Pb content was reported in the root plus shoot regions of fenugreek plants, respectively. EBL pre-treated plants of fenugreek diminished the Pb presence in root and shoot with 2.21 and 1.55 mg g^{-1} DW Pb content, respectively. Exogenous treatment of Si under metal stressed conditions lowered down the Pb content with 1.82 and 1.19 mg g^{-1} DW inside root and shoot, respectively. Co-supplementation of EBL and Si further reduced the Pb content in root plus shoot of fenugreek plants with 1.54 and 1.01 mg g^{-1} DW values of Pb.

Table 6.33



Treatments	Root (mg g^{-1} DW)	Shoot (mg g^{-1} DW)
C	ND	ND
T_3	$2.75^{c}\pm0.23$	$2.1^{c}\pm0.11$
T_7	$2.21^{b}\pm0.31$	$1.55^{ab}\pm0.11$
T_{11}	$1.82^{ab}\pm0.22$	$1.19^{ab}\pm0.14$
T_{15}	$1.54^{a}\pm0.27$	$1.01^{a}\pm0.05$

Fig. 6.33



❑ **Influence of EBL + Si on Pb metal uptake by the 45-days old *Trigonella foenum-graecum* seedlings.**

6.1.4.6 Osmolytes

Consequential decline in the content of proline took place on account of Pb toxicity in fenugreek plants (Fig. 6.34; Table 6.34). Every concentration of Pb i.e. T_1, T_2 plus T_3, registered a reduction in the amount of proline with respective values of 4.59, 3.9, and 3.64 µ mol g^{-1} FW. Individually augmented EBL brought about a promotion of proline measures in stressed plants with being greatest at T_5 concentration (7.96 µ mol g^{-1} FW). Si too played a role in raising the levels of proline in response to metal stress. Proline levels were up-scaled from 3.64 to 5.73 µ mol g^{-1} FW in T_9 i.e. Si treated plants in contrast to T_3. Under Pb stress, maximum enhancement in proline measures (8.4 µ mol g^{-1} FW) was in T_{13} treatment. T_{13} plants exhibited 5.52% and 46.59% improvement over T_5 and T_9 treated plants.

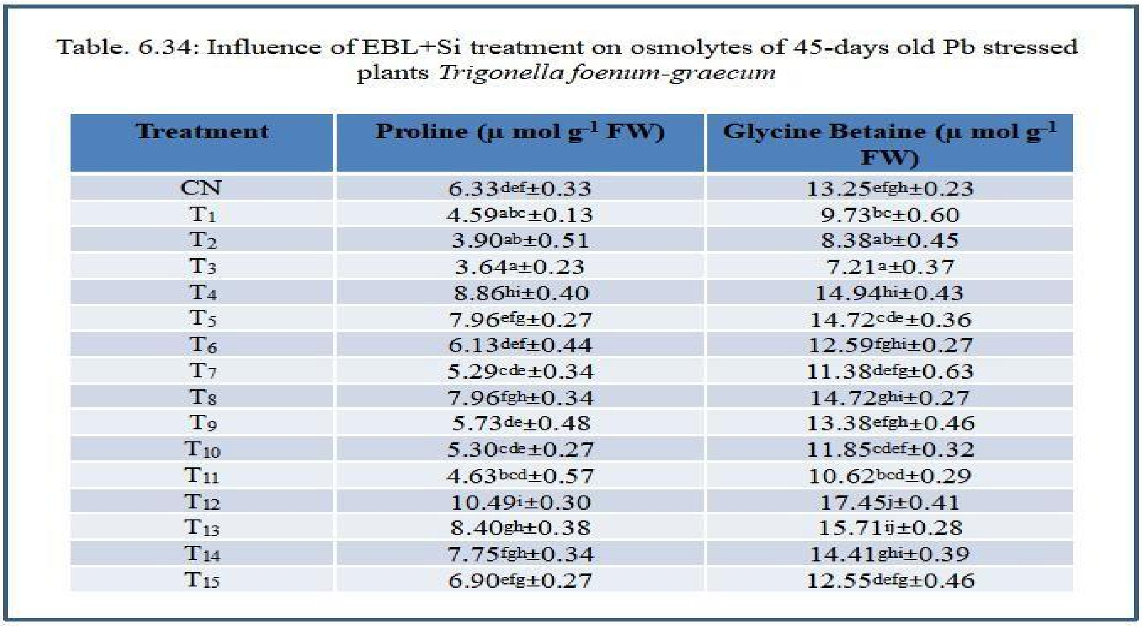


Table. 6.34: Influence of EBL+Si treatment on osmolytes of 45-days old Pb stressed plants *Trigonella foenum-graecum*

Treatment	Proline (µ mol g^{-1} FW)	Glycine Betaine (µ mol g^{-1} FW)
CN	6.33^{def}±0.33	13.25^{efgh}±0.23
T_1	4.59^{abc}±0.13	9.73^{bc}±0.60
T_2	3.90^{ab}±0.51	8.38^{ab}±0.45
T_3	3.64^{a}±0.23	7.21^{a}±0.37
T_4	8.86^{hi}±0.40	14.94^{hi}±0.43
T_5	7.96^{efg}±0.27	14.72^{cde}±0.36
T_6	6.13^{def}±0.44	12.59^{fghi}±0.27
T_7	5.29^{cde}±0.34	11.38^{defg}±0.63
T_8	7.96^{fgh}±0.34	14.72^{ghi}±0.27
T_9	5.73^{de}±0.48	13.38^{efgh}±0.46
T_{10}	5.30^{cde}±0.27	11.85^{cdef}±0.32
T_{11}	4.63^{bcd}±0.57	10.62^{bcd}±0.29
T_{12}	10.49^{i}±0.30	17.45^{j}±0.41
T_{13}	8.40^{gh}±0.38	15.71^{ij}±0.28
T_{14}	7.75^{fgh}±0.34	14.41^{ghi}±0.39
T_{15}	6.90^{efg}±0.27	12.55^{defg}±0.46

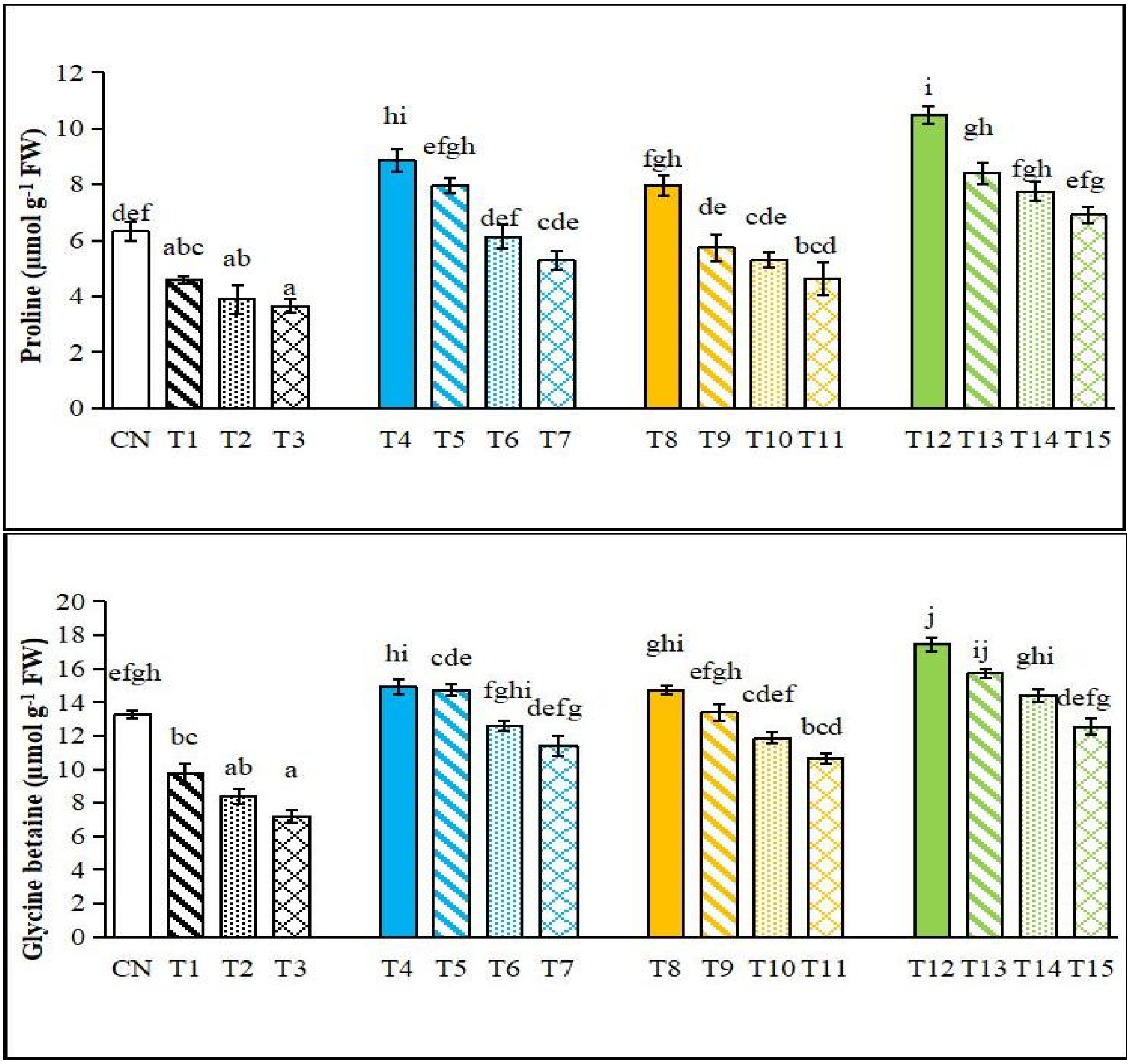


Fig. 6.34: Influence of EBL+Si treatment on measures of osmolytes in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Remarkable reductions in the measures of glycine-betaine were registered in Pb stress subjected plants (Fig. 6.34; Table 6.34). A decrement of 9.73 at T_1, 8.38 at T_2 and 7.21 µ mol g^{-1} FW at T_3 in the content of glycine-betaine was noted as opposed to control plants.. Under the influence of EBL, the measures of glycine betaine were recorded to be 14.72 at T_5, 12.59 at T_6 and 11.38 µ mol g^{-1} FW at T_7 respectively while Si treatment recorded glycine betaine content to be 13.38 at T_9, 11.85 at T_{10} and 10.62 µ mol g^{-1}FW at T_{11}. Furthermore, combination of EBL with Si treatment in stress free conditions registered the highest quantities of glycine betaine (17.45 µ mol g^{-1} FW) amongst all the 16 different types of treatments chosen for the study. Glycine-betaine amount was further recorded to be curtailed as the intensity of Pb toxicity elevated as observed in coupled EBL + Si dosage with being highest at 15.71 µ mol g^{-1} FW in T_{13}. An escalation of 51.28% was registered in T_5 over T_1.

T_9 plants displayed 37.51% increment than T_1. T_{13} plants exhibited nearly 6.72% improvement in glycine betaine as opposed to T_5. T_{13} showed 18.56% more measures of glycine betaine in contrast to control plants.

6.1.4.7 Total carbohydrates content

Total content of carbohydrates observed to have a diminishing effect on account of varying concentration of Pb stress in the study with being lowest around T_3 declined by Pb stress in fenugreek plants with the minimum of 5.25 mg g^{-1} FW at T_3 concentration (Fig. 6.35; Table 6.35). The most and least concentrations of carbohydrates in pre-treated EBL plants were recorded to be 10.8 at T_5 and 7.74 mg g^{-1} FW at T_7 respectively while the same values in case of Si were 11.33 at T_9 and 8.01 mg g^{-1} FW at $T_{11.}$ When EBL and Si were co-applied, promotion in the values of carbohydrate content was registered with being at its highest measure of 14.25 mg g^{-1} FW at T_{13}. T_{13} plants exhibited 16.26% improvement when correlated to T_5. T_{13} had 30.58% upsurge in carbohydrate measures against control plants.

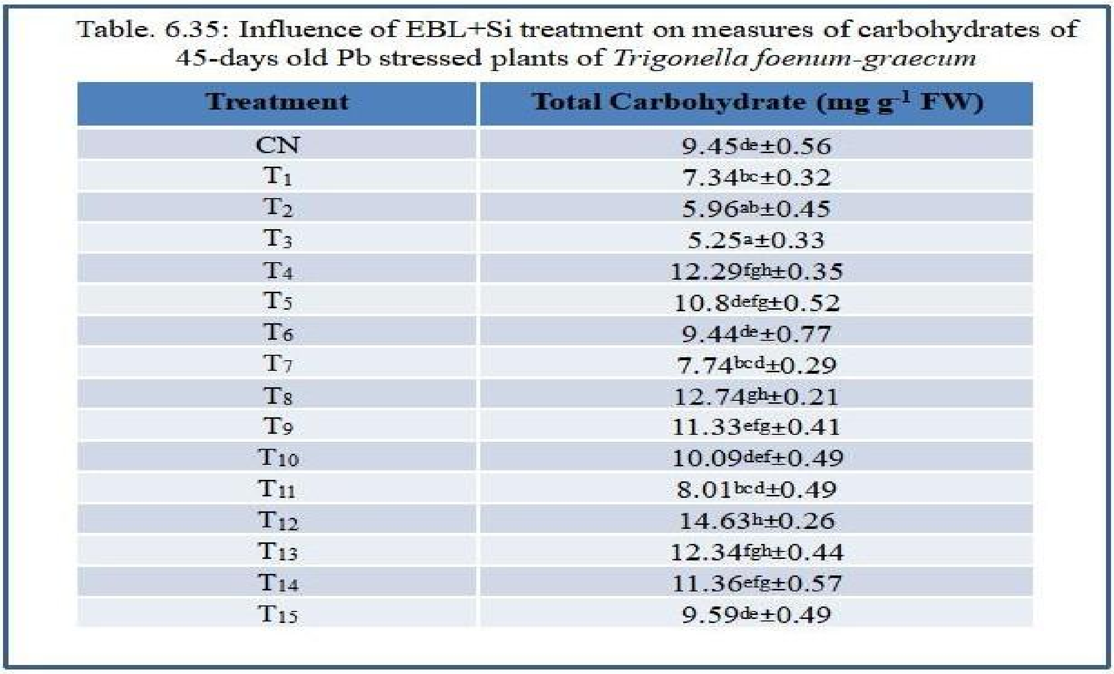


Table. 6.35: Influence of EBL+Si treatment on measures of carbohydrates of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Total Carbohydrate (mg g^{-1} FW)
CN	9.45^{de}±0.56
T_1	7.34^{bc}±0.32
T_2	5.96^{ab}±0.45
T_3	5.25^{a}±0.33
T_4	12.29^{fgh}±0.35
T_5	10.8^{defg}±0.52
T_6	9.44^{de}±0.77
T_7	7.74^{bcd}±0.29
T_8	12.74^{gh}±0.21
T_9	11.33^{efg}±0.41
T_{10}	10.09^{def}±0.49
T_{11}	8.01^{bcd}±0.49
T_{12}	14.63^{h}±0.26
T_{13}	12.34^{fgh}±0.44
T_{14}	11.36^{efg}±0.57
T_{15}	9.59^{de}±0.49

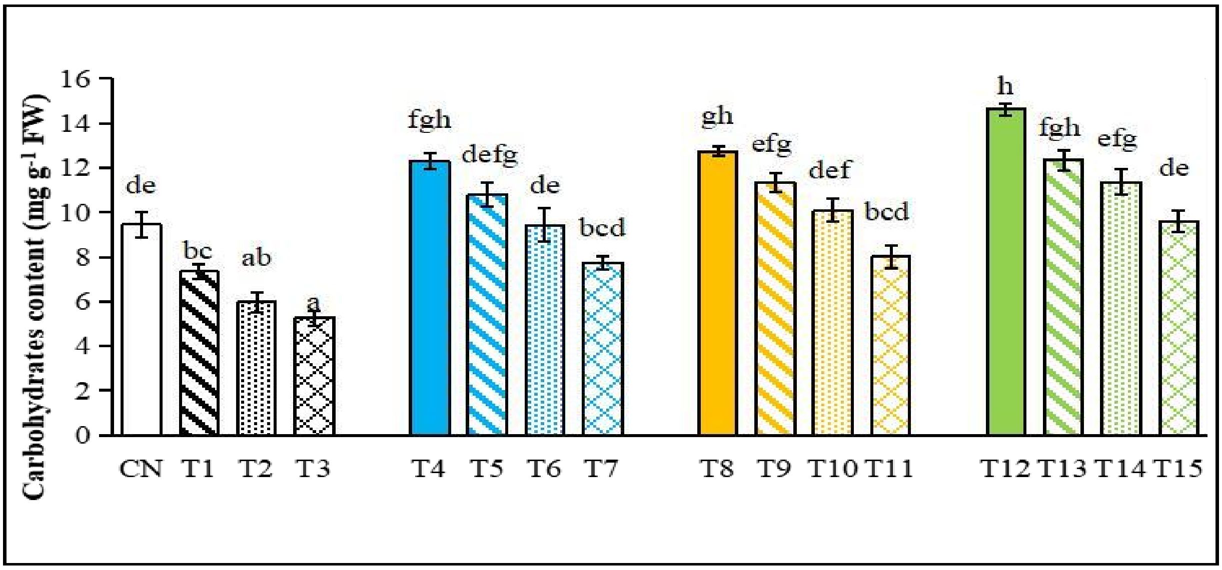


Fig. 6.35: Influence of EBL+Si treatment on measures of total carbohydrates in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at $P<0.05$.

6.1.4.8 Protein content and Antioxidant defense system

6.1.4.8.1 Protein content and antioxidative enzymes

Lead metal stress caused a decline in the measures of protein content (Fig. 6.36a; Table 6.36a). In T_1 treated plants, amount of protein was 8.10 mg g^{-1} FW, which was 28.7% lower in correlation with control plants. The least amount of protein was recorded to be 6.26 mg g^{-1} FW at T_3 concentration. EBL pre-treatment of plants exhibited higher measures of protein in response to Pb stress, when contrasted against Si-augmented plants, with the highest protein of 9.5 mg g^{-1} FW at T_5. Supplementation with Si raised the measures of protein in response to stressed and stress free conditions. Plants in Si control showed 12.72 mg g^{-1} FW of protein content. Under Si + Pb stress, the highest protein content of 9.68 mg g^{-1} FW was recorded in T_9. With respect to synergistic impact of EBL and Si, the maximum (10.24 mg g^{-1} FW) protein content was registered in T_{13} plants. Additionally, T_{13} plants exhibited 7.78% increase as compared to T_5 whereas T_{14} had 16.28 % increment over control plants.

The extent of enzymatic activity was curtailed under Pb stress exposure in case of 45-days old fenugreek plants (Fig. 6.36a; Table 6.36a). Minimal enzymatic action of SOD activity was recorded at 7.4 UA mg^{-1} protein in T_1 metal stressed plants. Supplementing the stressed plants with 24-EBL improved the performance of SOD with a wide value range between the extremes of T_5 (9.27 UA mg^{-1} protein) and T_7 (7.14 UA mg^{-1} protein) respectively. Augmentation of Si to the plants under Pb toxicity, the greatest activity of SOD (8.68 UA mg^{-1} protein) was noted at T_9. In addition to this, EBL + Si + Pb I elevated the enzymatic action of SOD in T_{13} treatment (11.51 UA mg^{-1} protein). An increment of 25.27% was noted in T_5 over T_1. Plants of T_9 treatment displayed 17.3% increase than T_1. Further, plants at T_{13} treatment exhibited an elevation of 24.2% and 0.7% when correlated with T_5 and control.

Catalase action was adversely affected in response to Pb stress being minimum at 12.95 UA mg^{-1} protein in T_3 treatment (Fig. 6.36a; Table 6.36a). Applying the ameliorative EBL under Pb stress led to an improvement in CAT measures which was maximum at 18.97 UA mg^{-1} protein in T_5 plants. Individually supplemented Si upgraded the CAT action than Pb alone augmented plants. Exogenous application of silicon with regard to Pb stress registered minimum CAT content at T_9 concentration (19.77 UA mg^{-1} protein). Its action was recorded to be curtailed with enhancement in Pb stress as is the case in EBL + Si, where the highest value of CAT activity was noted to be at 21.04 UA mg^{-1} protein in T_{13}. An increment of 15.7% was reported at T_5 when put against T_1. Further, T_9 plants had 20.54% enhancement over T_1 treatment. T_{13} plants displayed a respective decrease of 27.37 and 8.5% in relation to T_5 and control.

Lead toxicity caused a greatest decline in the enzymatic performance of APX as reported in case of T_3 (15.97 UA mg^{-1} protein) (Fig. 6.36b; Table 6.36a). Treating the fenugreek plants with EBL and Si in an individual manner reportedly upgraded the APX measures in contrast with Pb alone treatments. The maximum activity level in EBL augmented plants was noted at T_5 with a value of 24.2 UA mg^{-1} protein whereas in Si treated plants, such an elevated activity was found at T_9 metal stressed plants (24.63 UA mg^{-1} protein). In response to Pb stress, the maximum elevation in enzymatic activity was at T_{13} (26.82 UA mg^{-1} protein) amongst all the selected treatments where EBL and Si are co-applied. An increment of 25.25% was noted in T_5 over T_1. T_9 plants displayed 27.5% escalation in correlation to T_1. Further, T_{13} plants recorded 10.82 % and 10.9% increase as compared to T_5 and control, respectively.

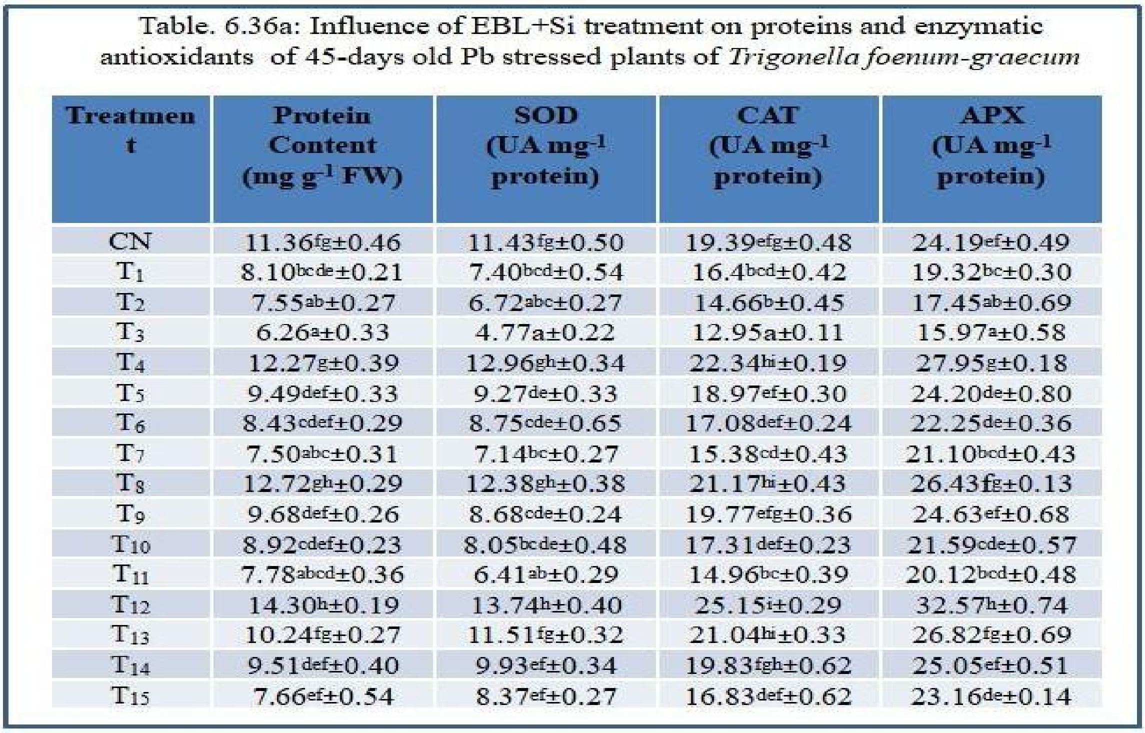
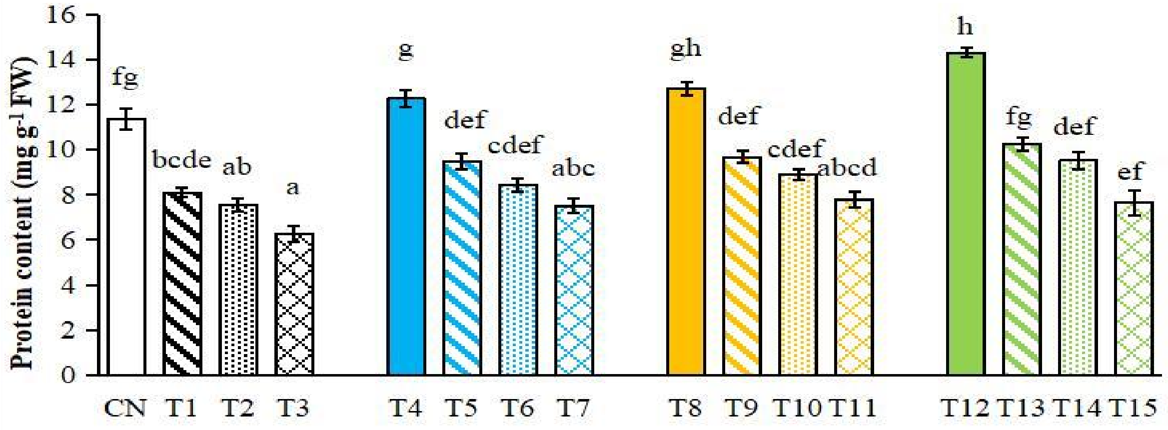


Table. 6.36a: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	Protein Content (mg g^{-1} FW)	SOD (UA mg^{-1} protein)	CAT (UA mg^{-1} protein)	APX (UA mg^{-1} protein)
CN	$11.36^{fg}\pm0.46$	$11.43^{fg}\pm0.50$	$19.39^{efg}\pm0.48$	$24.19^{ef}\pm0.49$
T_1	$8.10^{bcde}\pm0.21$	$7.40^{bcd}\pm0.54$	$16.4^{bcd}\pm0.42$	$19.32^{bc}\pm0.30$
T_2	$7.55^{ab}\pm0.27$	$6.72^{abc}\pm0.27$	$14.66^{b}\pm0.45$	$17.45^{ab}\pm0.69$
T_3	$6.26^{a}\pm0.33$	$4.77a\pm0.22$	$12.95a\pm0.11$	$15.97^{a}\pm0.58$
T_4	$12.27^{g}\pm0.39$	$12.96^{gh}\pm0.34$	$22.34^{hi}\pm0.19$	$27.95^{g}\pm0.18$
T_5	$9.49^{def}\pm0.33$	$9.27^{de}\pm0.33$	$18.97^{ef}\pm0.30$	$24.20^{de}\pm0.80$
T_6	$8.43^{cdef}\pm0.29$	$8.75^{cde}\pm0.65$	$17.08^{def}\pm0.24$	$22.25^{de}\pm0.36$
T_7	$7.50^{abc}\pm0.31$	$7.14^{bc}\pm0.27$	$15.38^{cd}\pm0.43$	$21.10^{bcd}\pm0.43$
T_8	$12.72^{gh}\pm0.29$	$12.38^{gh}\pm0.38$	$21.17^{hi}\pm0.43$	$26.43^{fg}\pm0.13$
T_9	$9.68^{def}\pm0.26$	$8.68^{cde}\pm0.24$	$19.77^{efg}\pm0.36$	$24.63^{ef}\pm0.68$
T_{10}	$8.92^{cdef}\pm0.23$	$8.05^{bcde}\pm0.48$	$17.31^{def}\pm0.23$	$21.59^{cde}\pm0.57$
T_{11}	$7.78^{abcd}\pm0.36$	$6.41^{ab}\pm0.29$	$14.96^{bc}\pm0.39$	$20.12^{bcd}\pm0.48$
T_{12}	$14.30^{h}\pm0.19$	$13.74^{h}\pm0.40$	$25.15^{i}\pm0.29$	$32.57^{h}\pm0.74$
T_{13}	$10.24^{fg}\pm0.27$	$11.51^{fg}\pm0.32$	$21.04^{hi}\pm0.33$	$26.82^{fg}\pm0.69$
T_{14}	$9.51^{def}\pm0.40$	$9.93^{ef}\pm0.34$	$19.83^{fgh}\pm0.62$	$25.05^{ef}\pm0.51$
T_{15}	$7.66^{ef}\pm0.54$	$8.37^{ef}\pm0.27$	$16.83^{def}\pm0.62$	$23.16^{de}\pm0.14$



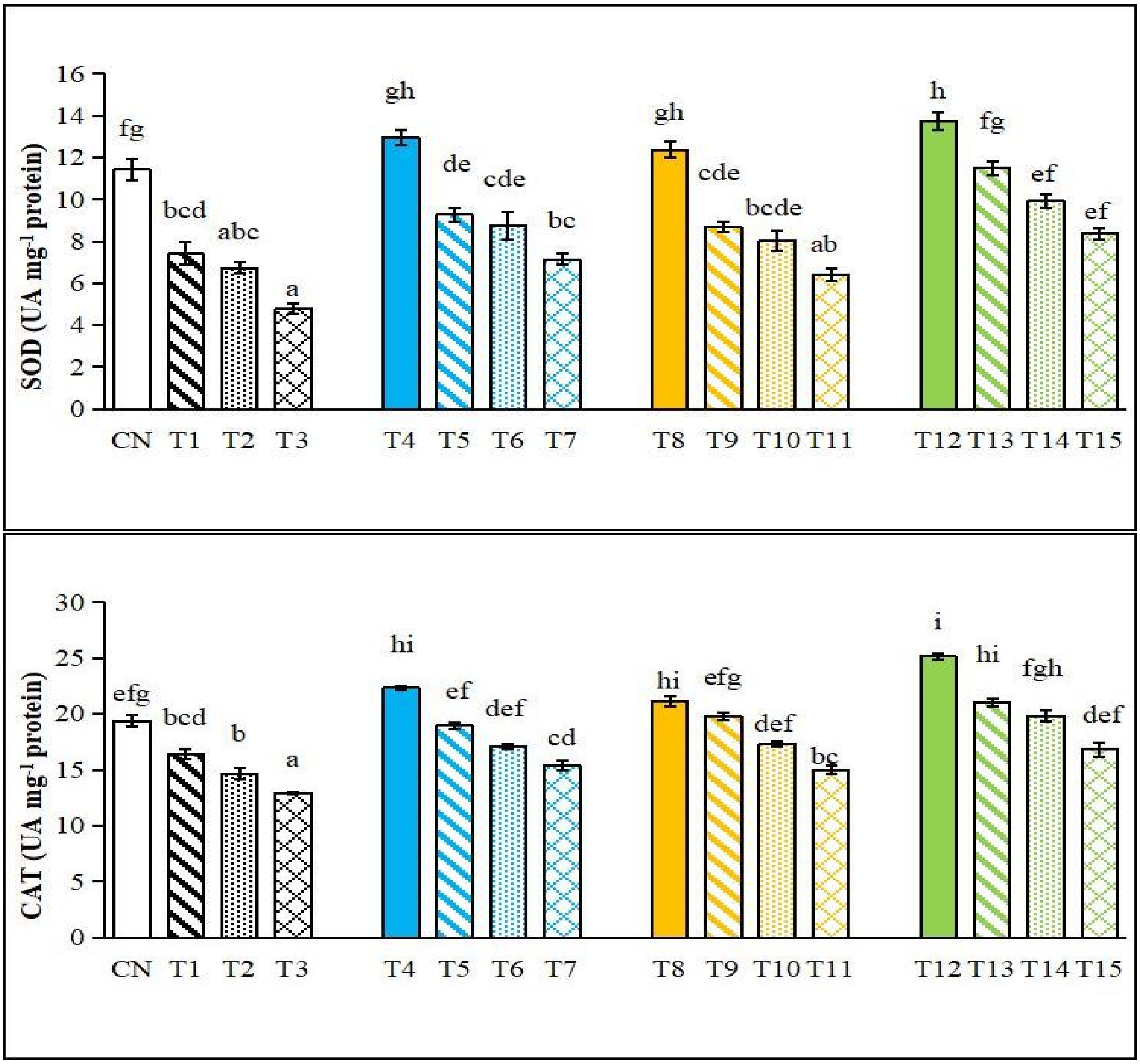


Fig. 6.36 (a): Influence of EBL+Si treatment on measures of proteins and enzymatic antioxidants in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Lead toxicity notably declined the activity of POD, in relation to control (Fig. 6.36b; Table 6.36b). In respect to Pb stress conditions, enzymatic performance of POD was recorded to be at its lowest at T_3 concentration with a content value of 10.66 UA mg^{-1} protein. Maximum (18.66 UA mg^{-1} protein) and minimum (15.25 UA mg^{-1} protein) levels of POD were noticed in T_5 and T_7 stressed plants when given a 24-EBL treatment. Supplementing the fenugreek plants with Si resulted in maximum elevation in the enzymatic levels at T_9 (18.03 UA mg^{-1} protein). In co-supplementation, EBL + Si were more effective in up-scaling the action of POD enzyme vis-à-vis their individual treatments. It can be corroborated by the highest values of this enzyme i.e. 21.97 UA mg^{-1} protein at T_{13} treatment (EBL + Si + Pb I). An increment of 30% was noted in T_5 over T_1. Plants at T_9 stage recorded 25.64% escalations over T_1. Furthermore, T_{13} plants exhibited a respective upsurge of 17.73% and 13.77% when correlated with T_5 and control conditions.

Highest decline in the measures of GR on account of Pb stress took place at T_3 concentration (6.64 UA mg^{-1} protein) (Fig. 6.36b; Table 6.36b). Plants of control recorded an activity level of 11.31 UA mg^{-1} protein with respect to GR. A notable diminishing impact of Pb stress was mitigated by EBL application at T_5 (11.15 UA mg^{-1} protein) while the same was accomplished by Si treatment in T_9 cultivars (10.88 UA mg^{-1} protein). The highest elevation in GR's performance with content of 13.98 UA mg^{-1} protein was recorded at T_{13} treatment. An upsurge of 15.8% was seen in T_5 over T_1. T_{13} plants exhibited a respective increment of 25.38% and 23.6% at treatments of T_5 and control.

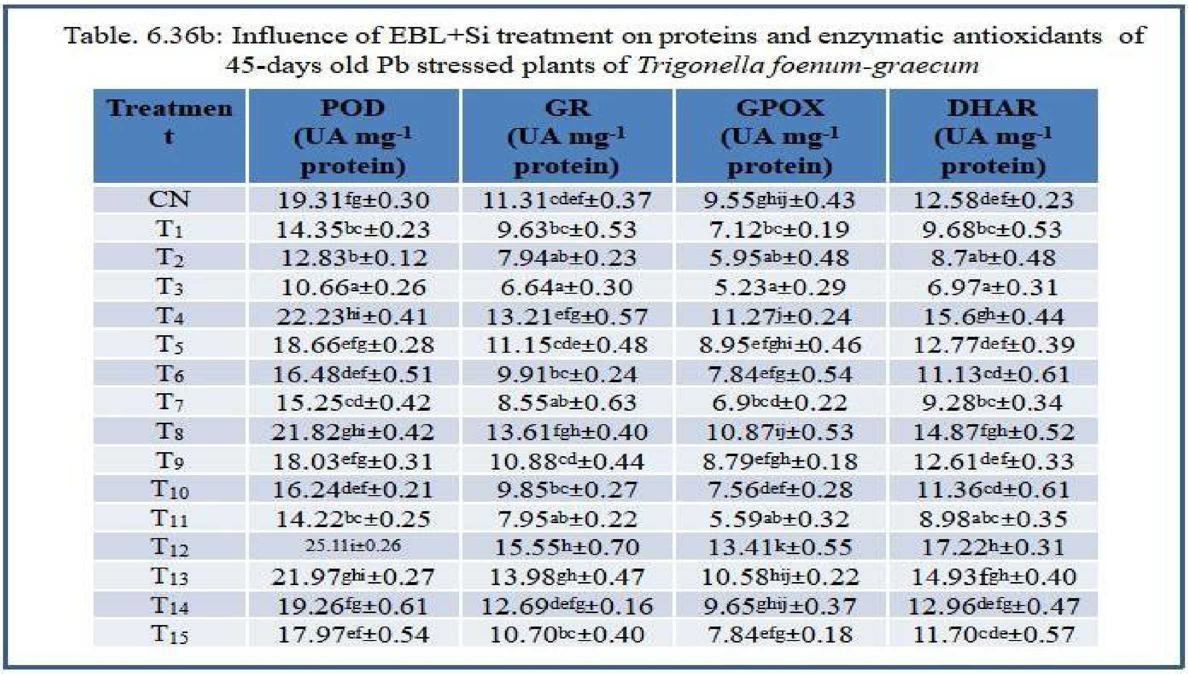
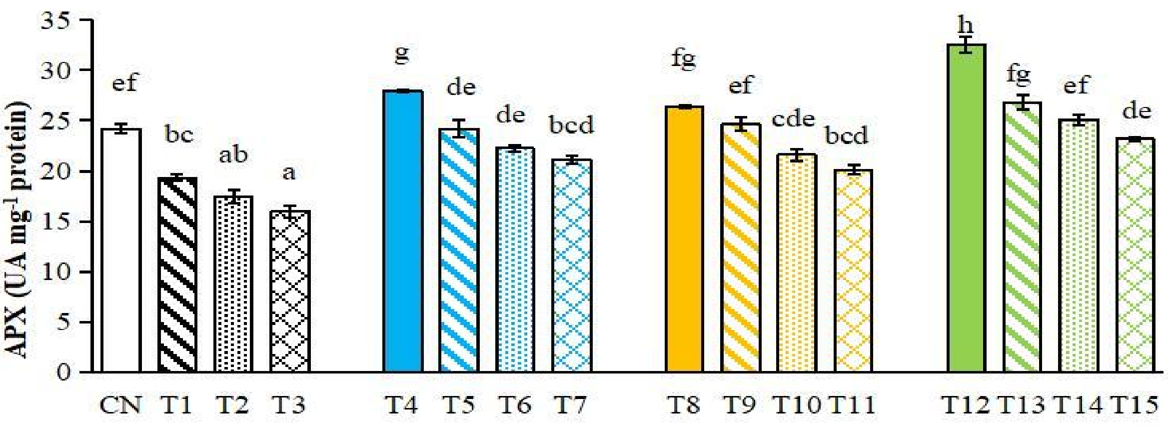


Table. 6.36b: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	POD (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	GPOX (UA mg^{-1} protein)	DHAR (UA mg^{-1} protein)
CN	$19.31^{fg}\pm0.30$	$11.31^{cdef}\pm0.37$	$9.55^{ghij}\pm0.43$	$12.58^{def}\pm0.23$
T_1	$14.35^{bc}\pm0.23$	$9.63^{bc}\pm0.53$	$7.12^{bc}\pm0.19$	$9.68^{bc}\pm0.53$
T_2	$12.83^{b}\pm0.12$	$7.94^{ab}\pm0.23$	$5.95^{ab}\pm0.48$	$8.7^{ab}\pm0.48$
T_3	$10.66^{a}\pm0.26$	$6.64^{a}\pm0.30$	$5.23^{a}\pm0.29$	$6.97^{a}\pm0.31$
T_4	$22.23^{hi}\pm0.41$	$13.21^{efg}\pm0.57$	$11.27^{j}\pm0.24$	$15.6^{gh}\pm0.44$
T_5	$18.66^{efg}\pm0.28$	$11.15^{cde}\pm0.48$	$8.95^{efghi}\pm0.46$	$12.77^{def}\pm0.39$
T_6	$16.48^{def}\pm0.51$	$9.91^{bc}\pm0.24$	$7.84^{efg}\pm0.54$	$11.13^{cd}\pm0.61$
T_7	$15.25^{cd}\pm0.42$	$8.55^{ab}\pm0.63$	$6.9^{bcd}\pm0.22$	$9.28^{bc}\pm0.34$
T_8	$21.82^{ghi}\pm0.42$	$13.61^{fgh}\pm0.40$	$10.87^{ij}\pm0.53$	$14.87^{fgh}\pm0.52$
T_9	$18.03^{efg}\pm0.31$	$10.88^{cd}\pm0.44$	$8.79^{efgh}\pm0.18$	$12.61^{def}\pm0.33$
T_{10}	$16.24^{def}\pm0.21$	$9.85^{bc}\pm0.27$	$7.56^{def}\pm0.28$	$11.36^{cd}\pm0.61$
T_{11}	$14.22^{bc}\pm0.25$	$7.95^{ab}\pm0.22$	$5.59^{ab}\pm0.32$	$8.98^{abc}\pm0.35$
T_{12}	$25.11^{i}\pm0.26$	$15.55^{h}\pm0.70$	$13.41^{k}\pm0.55$	$17.22^{h}\pm0.31$
T_{13}	$21.97^{ghi}\pm0.27$	$13.98^{gh}\pm0.47$	$10.58^{hij}\pm0.22$	$14.93^{fgh}\pm0.40$
T_{14}	$19.26^{fg}\pm0.61$	$12.69^{defg}\pm0.16$	$9.65^{ghij}\pm0.37$	$12.96^{defg}\pm0.47$
T_{15}	$17.97^{ef}\pm0.54$	$10.70^{bc}\pm0.40$	$7.84^{efg}\pm0.18$	$11.70^{cde}\pm0.57$



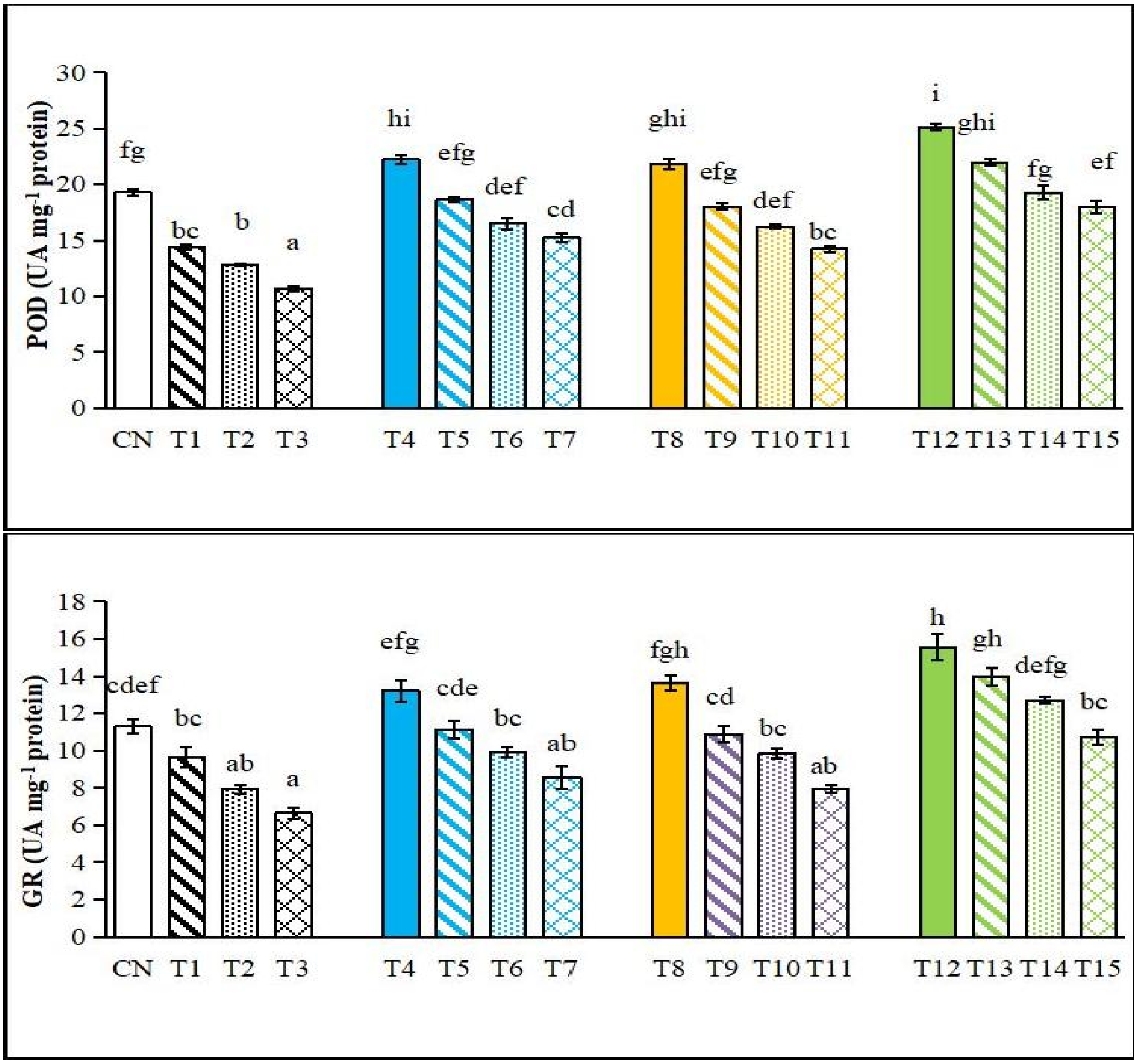


Fig. 6.36 (b): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

The enzymatic performance of GPOX got declined in Pb exposed fenugreek plants with 7.12, 5.95, and 5.23 UA mg^{-1} protein at Pb treatment concentrations of T_1, T_2 and T_3, respectively (Fig. 6.36c; Table 6.36b). Individually augmented EBL and Si with respect to Pb alone toxicity resulted in boosting the levels of GPOX with respective values of 8.95 and 8.79 UA mg^{-1} protein at T_5 and T_9. Further, curtailment of enzymatic action was reported with every escalation in Pb concentrations as was evident in cumulative application (EBL + Si) having highest levels of activity at T_{13} (10.58 UA mg^{-1} protein). An improvement of 25.7% was noted in T_5 over T_1. Further, T_{13} plants exhibited 18.21% and 10.8% increase in correlation to T_5 and control.

DHAR's enzymatic action suffered from Pb induced diminishing effect in *In vivo* raised plants (Fig. 6.36c; Table 6.36b). Maximum enzymatic levels were observed in T_1 plants (9.68

UA mg^{-1} protein) with respect to control. In individually treated plants with EBL and Si, the greatest measures of enzyme were noted at T_5 and T_7 with 12.77 and 9.28 UA mg^{-1} protein values respectively. On the other hand, under the influence of same mitigants, minimal content values were at T_9 (12.61 UA mg-1 protein) and T_{11} (8.98 UA mg-1 protein). Further augmentation of enzymatic performance of DHAR in response to metal exposure took place at T_{13} (14.93 UA mg^{-1} protein). An elevation of 31.92% was reported in T_5 over T_1. T_9 plants displayed 16.91% escalation than T_1. Further, T_{13} plants exhibited 30.26% upsurge as opposed to T_5 and a 18.68 % decline in correlation with control.

MDHAR enzyme's performance level was adversely affected as a consequence of lead stress (Fig. 6.36c; Table 6.36c). The least amount of activity was visible at 7.23 UA mg^{-1} protein in T_3 treatment. MDHAR levels were escalated 14.5 and 12.15 UA mg^{-1} protein in EBL pre-augmented plants of T_5 and T_6 treatment. Supplementing the fenugreek plants with Si augmented the enzymatic levels with being highest in T_9 treatment (14.14 UA mg^{-1} protein. The maximum MDHAR activity level (16.13 UA mg^{-1} protein) got recorded in coupled supplementation of EBL and Si at T_{13}. A decrement of 26.41% was recorded in T_5 over T_1 whereas T_9 plants showed 23.27% escalation in relation to T_1. Plants at T_{13} treatment recorded 11.24% and 1.31% increment in correlation with T_5 and control.

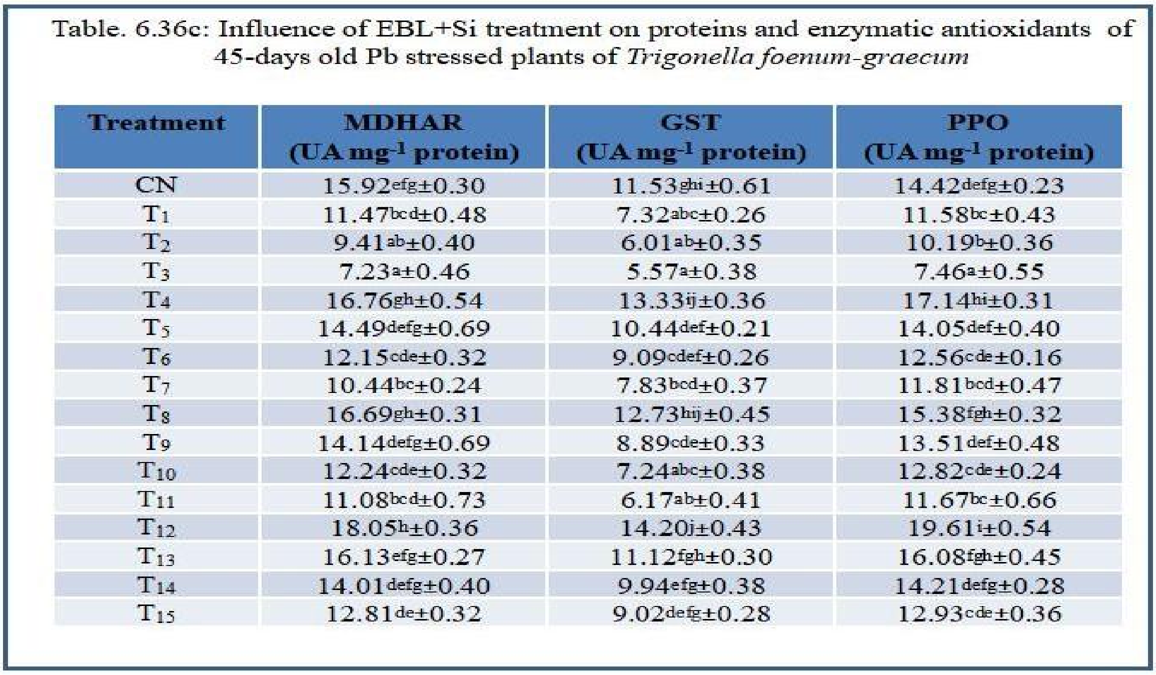


Table. 6.36c: Influence of EBL+Si treatment on proteins and enzymatic antioxidants of 45-days old Pb stressed plants of *Trigonella foenum-graecum*

Treatment	MDHAR (UA mg^{-1} protein)	GST (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
CN	$15.92^{efg}\pm0.30$	$11.53^{ghi}\pm0.61$	$14.42^{defg}\pm0.23$
T_1	$11.47^{bcd}\pm0.48$	$7.32^{abc}\pm0.26$	$11.58^{bc}\pm0.43$
T_2	$9.41^{ab}\pm0.40$	$6.01^{ab}\pm0.35$	$10.19^{b}\pm0.36$
T_3	$7.23^{a}\pm0.46$	$5.57^{a}\pm0.38$	$7.46^{a}\pm0.55$
T_4	$16.76^{gh}\pm0.54$	$13.33^{ij}\pm0.36$	$17.14^{hi}\pm0.31$
T_5	$14.49^{defg}\pm0.69$	$10.44^{def}\pm0.21$	$14.05^{def}\pm0.40$
T_6	$12.15^{cde}\pm0.32$	$9.09^{cdef}\pm0.26$	$12.56^{cde}\pm0.16$
T_7	$10.44^{bc}\pm0.24$	$7.83^{bcd}\pm0.37$	$11.81^{bcd}\pm0.47$
T_8	$16.69^{gh}\pm0.31$	$12.73^{hij}\pm0.45$	$15.38^{fgh}\pm0.32$
T_9	$14.14^{defg}\pm0.69$	$8.89^{cde}\pm0.33$	$13.51^{def}\pm0.48$
T_{10}	$12.24^{cde}\pm0.32$	$7.24^{abc}\pm0.38$	$12.82^{cde}\pm0.24$
T_{11}	$11.08^{bcd}\pm0.73$	$6.17^{ab}\pm0.41$	$11.67^{bc}\pm0.66$
T_{12}	$18.05^{h}\pm0.36$	$14.20^{j}\pm0.43$	$19.61^{i}\pm0.54$
T_{13}	$16.13^{efg}\pm0.27$	$11.12^{fgh}\pm0.30$	$16.08^{fgh}\pm0.45$
T_{14}	$14.01^{defg}\pm0.40$	$9.94^{efg}\pm0.38$	$14.21^{defg}\pm0.28$
T_{15}	$12.81^{de}\pm0.32$	$9.02^{defg}\pm0.28$	$12.93^{cde}\pm0.36$

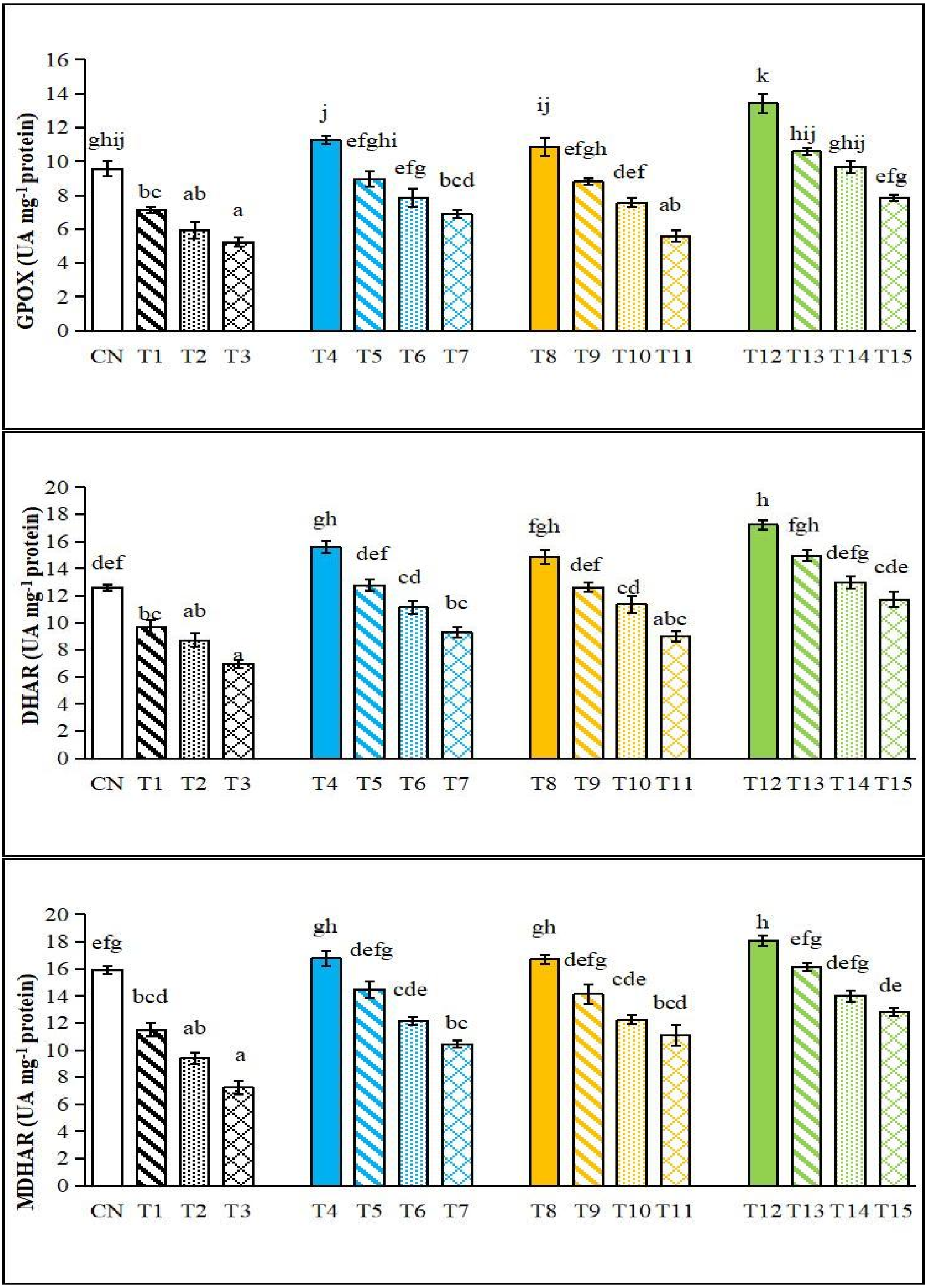
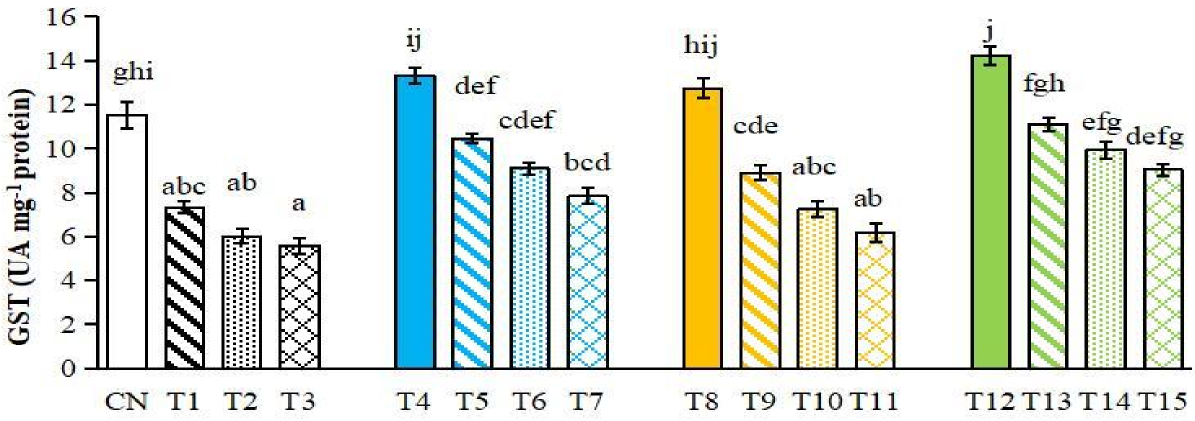


Fig. 6.36 (c): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

GST enzyme had activity level of 7.32, 6.01 and 5.57 UA mg^{-1} protein at different concentrations of lead i.e. at T_1, T_2 and T_3 (Fig. 6.36d; Table 6.36c). Maximal and minimal values of GST content were recorded at T_5 (10.44 UA mg^{-1} protein) and T_7 (7.83 UA mg^{-1} protein) in EBL augmented plants. In Si augmented plants under stressful conditions, highest GST content of 8.89 UA mg^{-1} protein was noted in T_9 plants. EBL + Si up-scaled the activity levels of GST in response to T_{13} concentration (11.12 UA mg^{-1} protein). An increment of 42.62% was noted in T_5 over T_1. Further, T_9 plants showed 21.44% upsurge opposed to T_1. T_{13} plants showed increment of 6.51% over T_5 and decline of 3.55% over control.

Measures of PPO enzyme were at their minimum at the highest treatment value of Pb stress i.e. T_3 (7.46 UA mg^{-1} protein) in contrast to control plants (Fig. 6.36d; Table 6.36c). However, in EBL augmented plants under no stress conditions, highest activity was measured at T_4 of 17.14 UA mg^{-1} protein value. Applying EBL to Pb stressed fenugreek plants registered minimal activity of this enzyme at 11.81 UA mg^{-1} protein at T_7. Further, the Si treatment had the highest measures of PPO activity in case of T_9 cultivars (13.51 UA mg^{-1} protein) in metal stressed conditions. Whereas cumulatively applied EBL and Si made the activity level of PPO as high as 16.08 UA mg^{-1} protein at T_{13} treatment. An increase of 21.32% was noticed in T_5 than T_1. T_{13} plants showed 14.44% and 11.51% increase as compared to T_5 and control plants, respectively.



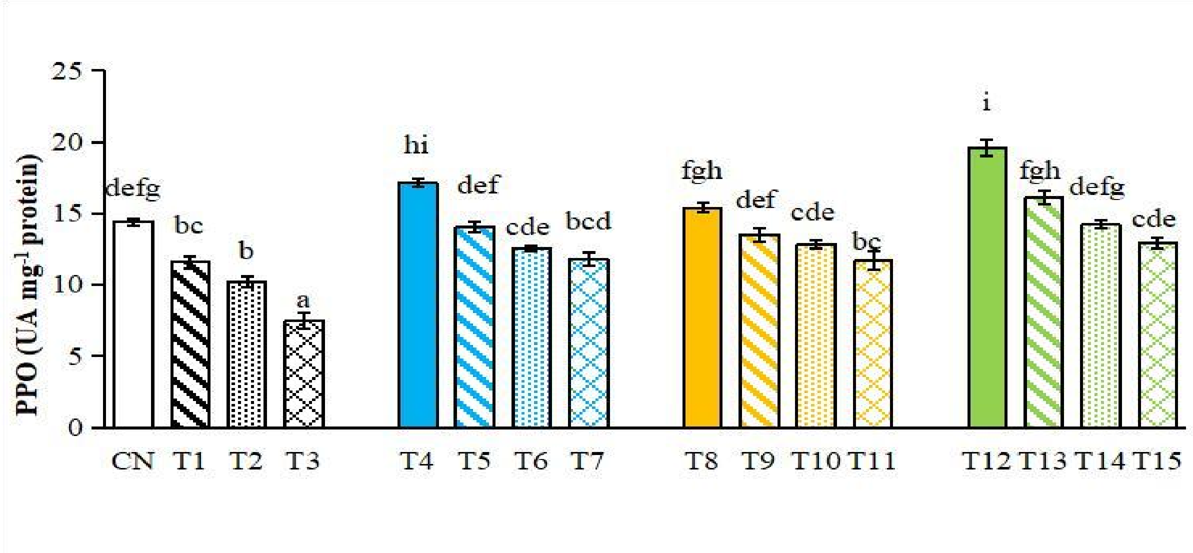


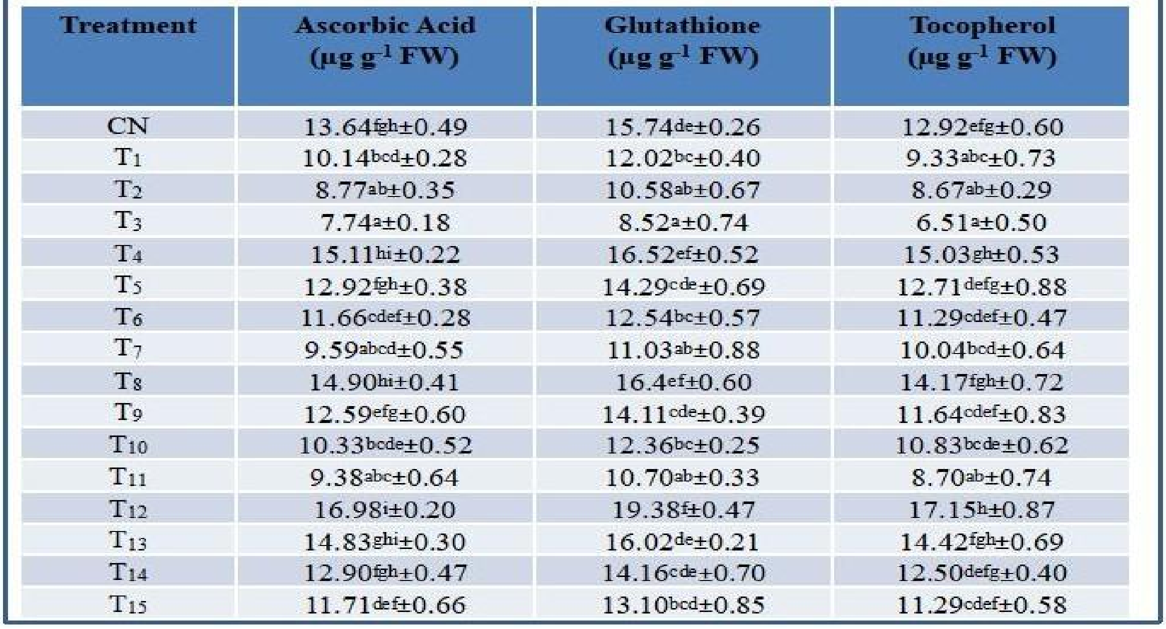
Fig. 6.36 (d): Influence of EBL+Si treatment on measures of enzymatic antioxidants in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

6.1.4.8.2 Non-enzymatic antioxidants

Amount of ascorbic acid was registered to be diminished at each of the Pb concentration selected for carrying out this study (Fig. 6.37; Table 6.37). The topmost value of ascorbic acid was noted at 10.14 µg g^{-1} FW in response to T_1 concentration amongst every discrete level of Pb stress. Minimum values of Ascorbic acid in EBL and Si alone applications with regard to stressed plants, were 9.6 µg g^{-1} FW at T_7 and 9.38 at T_{11} plants. However, synergistic action of EBL + Si was observed to have highest value of this antioxidant at T_{13} i.e. 14.83 µg g^{-1} FW at T_{14}. An elevation of 27.41% and 24.16% was reported in T_5 and T_9 over T_1 metal stressed plants. T_{13} plants showed 14.78% and 8.72% increase as compared to T_5 and control, respectively.

Lead toxicity recorded a decline in the measures of glutathione with being minimum at T_3 (8.52 µg g^{-1} FW) in contrast to control plants (Fig. 6.37; Table 6.37). However, pre-treated EBL plants displayed highest values of 16.52 µg g^{-1} FW and 14.29 µg g^{-1} FW of glutathione in response to stress free and stressful environments respectively. With respect to Si treatment, these values were found to be 16.4 µg g^{-1} FW and 14.11 µg g^{-1} FW respectively. Coupling of EBL with Si for mitigating the harms of Pb toxicity, exhibited glutathione content 16.02 µg g^{-1} FW in T_{13} stress exposed plants. Moreover, T_{13} plants exhibited an increment of 12.1% and 1.77% in glutathione when correlated with T_5 and control, respectively.

Table. 6.37: Influence of EBL+Si on treatment on non-enzymatic antioxidants of 45-days old Pb stressed plants of *Trigonella foenum-graecum*



Treatment	Ascorbic Acid (µg g^{-1} FW)	Glutathione (µg g^{-1} FW)	Tocopherol (µg g^{-1} FW)
CN	$13.64^{fgh}\pm0.49$	$15.74^{de}\pm0.26$	$12.92^{efg}\pm0.60$
T$_1$	$10.14^{bcd}\pm0.28$	$12.02^{bc}\pm0.40$	$9.33^{abc}\pm0.73$
T$_2$	$8.77^{ab}\pm0.35$	$10.58^{ab}\pm0.67$	$8.67^{ab}\pm0.29$
T$_3$	$7.74^{a}\pm0.18$	$8.52^{a}\pm0.74$	$6.51^{a}\pm0.50$
T$_4$	$15.11^{hi}\pm0.22$	$16.52^{ef}\pm0.52$	$15.03^{gh}\pm0.53$
T$_5$	$12.92^{fgh}\pm0.38$	$14.29^{cde}\pm0.69$	$12.71^{defg}\pm0.88$
T$_6$	$11.66^{cdef}\pm0.28$	$12.54^{bc}\pm0.57$	$11.29^{cdef}\pm0.47$
T$_7$	$9.59^{abcd}\pm0.55$	$11.03^{ab}\pm0.88$	$10.04^{bcd}\pm0.64$
T$_8$	$14.90^{hi}\pm0.41$	$16.4^{ef}\pm0.60$	$14.17^{fgh}\pm0.72$
T$_9$	$12.59^{efg}\pm0.60$	$14.11^{cde}\pm0.39$	$11.64^{cdef}\pm0.83$
T$_{10}$	$10.33^{bcde}\pm0.52$	$12.36^{bc}\pm0.25$	$10.83^{bcde}\pm0.62$
T$_{11}$	$9.38^{abc}\pm0.64$	$10.70^{ab}\pm0.33$	$8.70^{ab}\pm0.74$
T$_{12}$	$16.98^{i}\pm0.20$	$19.38^{f}\pm0.47$	$17.15^{h}\pm0.87$
T$_{13}$	$14.83^{ghi}\pm0.30$	$16.02^{de}\pm0.21$	$14.42^{fgh}\pm0.69$
T$_{14}$	$12.90^{fgh}\pm0.47$	$14.16^{cde}\pm0.70$	$12.50^{defg}\pm0.40$
T$_{15}$	$11.71^{def}\pm0.66$	$13.10^{bcd}\pm0.85$	$11.29^{cdef}\pm0.58$

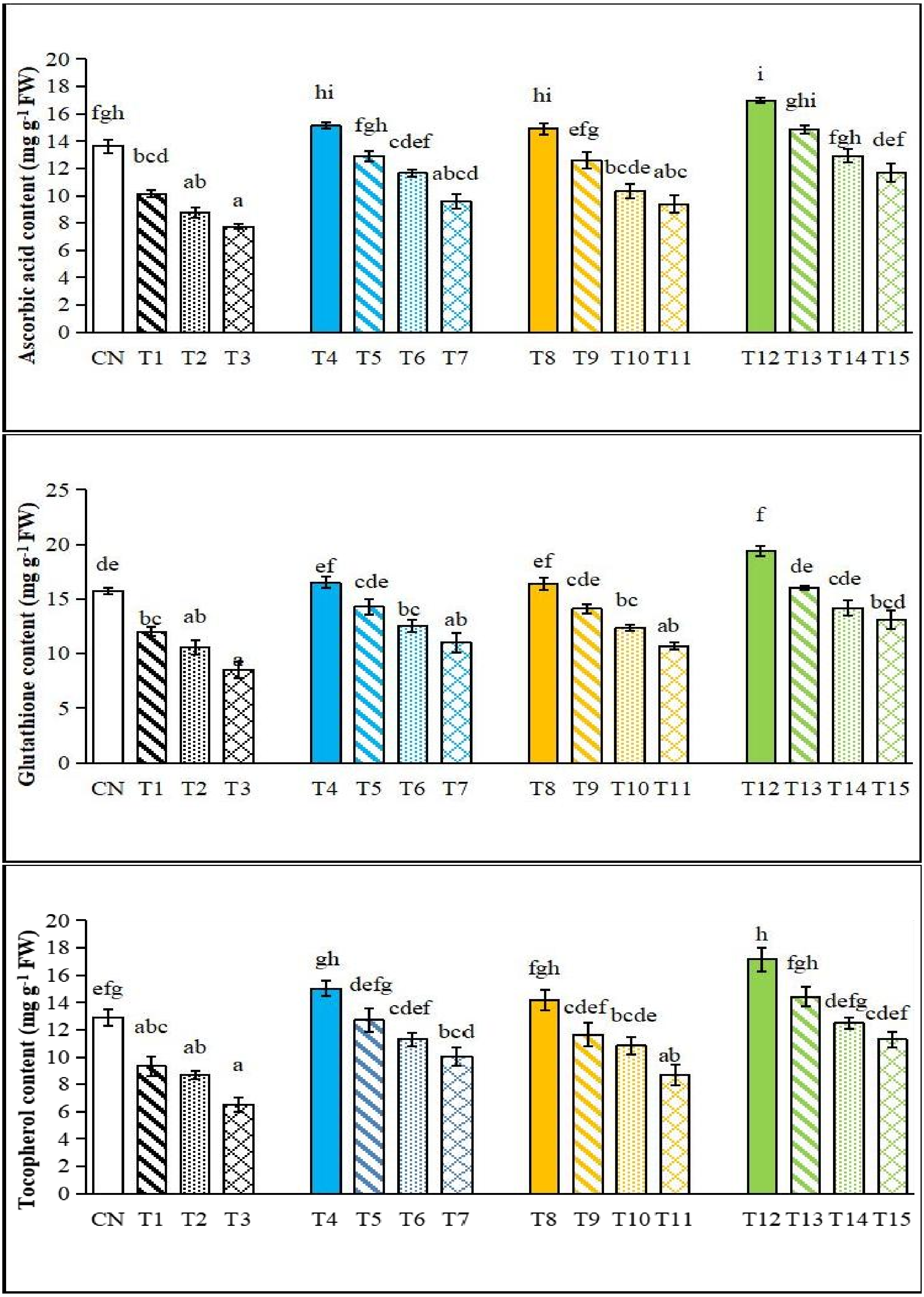


Fig. 6.37: Influence of EBL+Si treatment on measures of non-enzymatic antioxidants in 45 days old Pb stressed *Trigonella foenum-graecum* plants. Each value depicts the mean of three replicates for every treatment level together with standard error of mean. Means inside a column followed by dissimilar letter/s differ significantly at P<0.05.

Tocopherol content exhibited a drastic decline at every chosen level of Pb toxicity during the present work (Fig. 6.37: Table 6.37). Minimum measures of tocopherol were recorded to be of 6.51 µg g^{-1} FW at T_3 amongst all the three distinct concentrations of Pb. And in case of EBL and Si alone augmented plants under Pb induced stress, the highest values of this antioxidant were recorded to be 12.71 at T_5 and 11.64 µg g^{-1} FW at T_9, respectively. However, EBL + Si together displayed a highest value of tocopherol content of 14.42 µg g^{-1} FW in T_{13}. An upsurge of 36.22% was recorded in T_5 over T_1. Similarly, T_{13} plants displayed a promotion of 26.75% as opposed to T_5. T_{13} was observed to be having the 11.6% elevation in tocopherol as that of control plants.

6.2 Discussion

Heavy metals have a ubiquitous distribution and become pernicious for both plants and human beings in exceeding concentrations. Various ecological and biological processes especially the decomposition and mineralization of organic matter get adversely affected on account of their toxicity (Keely et al. 2003). Plants, in particular, when chance upon to grow in soils defiled by the toxic levels of heavy metals, suffers from numerous aftermaths. Retardation in optimal pace of growth and other metabolic processes hampering the plant produce and its quality, more specifically in the case of economically significant plants, have been documented as phytotoxic outcomes of heavy metals (Shiowatana *et al.*, 2001). To curb the problem of metal induced phytotoxicity, effective mitigation strategies are needed to be looked for. The ambit of heavy metal toxicity and its amelioration in commercially important crops is delimited in the present study to evaluate the after-effects of Pb toxicity in *Trigonella foenum-graecum* plants where two mitigants-24-EBL and Si were utilized in individual plus cumulative manner. The outcomes of this endeavour have been discussed as follows:

6.2.1 Plant growth

In present work, germination percentage of fenugreek plants recorded a drastic decline in response to discrete levels of Pb stress. However, when these plants were exogenously supplemented with 24-EBL and Si, a notable induction in seed germination took place. These outcomes are concomitant with the results of Sharma & Bhardwaj (2006) where 24-EBL enhanced the extent of seed germination in metal stressed seeds of *Brassica juncea*. Similarly, Si treatment was reported to mitigate negative impacts of As and Al on seed germination in *Oryza sativa* and *Zea mays* respectively (Khan & Gupta, 2018; Delavar et al., 2017) and Si NPs improved different parameters of seed germination in *Phyllostachys edulis* subjected to Cd toxicity (Emamverdian et al., 2021).

The possible reasons for these findings include the role of Si in resource mobilization via enhancing the action of hydrolytic enzyme amylase inside the seeds or through modulating levels of phytohormones in the form of higher gibberellic acid content than ABA as noticed in *Zea mays* subjected to Al stress (Delavar et al., 2017). Various Si transporter genes (LSi1, LSi2 and LSi6) in As stress affected *Oryza sativa* seeds were up/downregulated by Si treatment (Khan & Gupta, 2018). Furthermore, upregulation of enzymatic action of antioxidants, CAT in particular, together with enhancement in gene expression levels regulating hydrolytic enzymes like esterases plus acid phosphatases in Cd stress exposed seeds of lettuce have been observed to take place under the influence of Si priming (Pereira et al., 2021).

In the present research, revelations regarding noxious impact of Pb stress upon various growth related attributes have been recorded in fenugreek plants. Oxidation of key phytohormones like auxin by lead metal could be held accountable for these growth-impeding outcomes (Agami & Mohamed, 2013). Lead stress mediated inhibition of growth is more evidently observed in case of roots which is possibly on account of higher accumulation of lead inside them (Liu et al., 2009).

However, upgradation of growth characteristics could be achieved by supplementing 24-EBL and Si to stressed plants as reported in the present work. Our results are similar to Jan et al., 2018 where Cd stress induced reduction in growth plus biomass of *Pisum sativum* was made better by cumulative treatment with EBL and Si. 24-EBL with that of Si cast a diminutive impact on metal uptake while improving absorption rates of other essential nutrients plus photosynthetic pigments. All these changes contribute to enhancement in plant growth as well as its biomass as suggested by Ren et al. (2023) and Maia et al. (2022) in EBL treated genotypes plus An et al. (2022) and El-Beltagi et al. (2020) in case of Si treatment. Improvement in growth attributes by EBL supplementation might be linked with its role in regulating the process of cell division, elongation and maturation or in stimulating the activity of xyloglucan endo-transglycosylase, the enzyme responsible for expanding the cell walls (Saeidnejad et al., 2012; Li et al., 2008).

Accumulation of Si in foliar bundle cells modulates the structure of photosynthetic apparatus, subsequently leading to betterment of light use efficiency of PSII and photosynthesis thereof. This is the major reason behind silicon-facilitated increase of the plant biomass (Adrees et al., 2015; Torabi et al., 2013). Silicon not only provides mechanical support but also improves growth parameters in plants subjected to stress by diminishing the translocation of heavy metals inside the plants (Shi et al., 2005). Furthermore, the cumulative effect of 24-EBL with that of Si on growth attributes of Pb stress subjected fenugreek plants could be taking place on account of their synergistic mechanism of action.

6.2.2 Photosynthetic system

Anabolic reactions of photosynthesis are facilitated by chlorophylls as they participate in transformation of radiant energy into biochemical energy stored as ATP molecules (Zhou et al., 2018). Rate of photosynthetic process is directly regulated by every type of modulation in the quantities of pigments (Zhang et al., 2011). This study showed that Pb exposure of fenugreek plants resulted in denigration of pigment measures. This decline is either associated with lowered production of biosynthetic intermediate-porphobilinogen (PBG) needed for chlorophyll formation (Cenkci et al., 2010) or with the substitution of central Mg ion by Pb itself (Harpaz-Saad et al. 2007). Our outcomes resonate with the findings in Pb exposed maize and rice plants where notable reduction in photosynthetic pigments took place (Zanganeh et al., 2021; Ashraf et al., 2017). Curtailment of functional efficiency of photosynthesis assisting enzymes namely Fd-NADP$^+$ reductase and δ-aminolevulinic acid dehydratase might be responsible for the decline in photosynthetic attributes under stress (Islam et al., 2013; Gupta et al. 2009). In plants such as rice, rose geranium and salvinia, a significant drop in carotenoid levels was observed on account of Pb contamination (Ashraf et al., 2017; Leal-Alvarado *et al.*, 2016; Rao and Raghu, 2016). Similar reduction in carotenoid measures in *Acorus calamus* subjected to Sb toxicity was noticed and was reasoned to be linked with stress mediated impairment of electron transport (Zhou et al., 2018).

In the present study, 24-EBL and Si individually and cumulatively brought about improvements in different photosynthetic parameters in response to Pb toxicity. Similar findings were reported in grapes and cabbage plants subjected to Cd toxicity where photosynthetic pigments were enhanced by the respective supplementation with 24-EBL and Si (Li et al. 2022; Wu et al., 2018). Carotenoids prevent the photo-oxidation of chlorophylls via modulating the ROS measures inside the chloroplast as reported by Amooaghaie et al. (2018). Besides this, EBL and Si supplementation in tomato and maize plants when subjected to Pb and Cd stress respectively not only enhanced the measures of photosynthetic pigments but also the rate of electron transfer, efficiency of photosystem II along with net photosynthetic rate and protection of chloroplast (Maia et al., 2022; Vaculik et al., 2015). The possible reasons behind the EBL and Si facilitated enhancement in the measures of pigments might be a) upgradation of enzymatic action of RuBP carboxylase-oxygenase with that of b) reduction in metal uptake, producing a stimulating effect on the functional efficiency of both the photosystems (Siddiqui et al., 2018; Adrees et al., 2015)

Akin to our outcomes, the coupled application of EBL and Si remarkably escalated the measures of pigments, chlorophyll fluorescence, quantum yield in Cd stress exposed pea seedlings vis-à-vis their individual treatments by boosting mineral absorption (especially Mg to be utilized for synthesizing chlorophyll) due to Si and mitigating photo-oxidative disruptions due to EBL (Jan et al., 2018).

6.2.3 Metabolites

Phenolic compounds formed by either phenylpropanoid or shikimic acid pathway, possess antioxidative attributes (Ren & Sun, 2014). Current experimental work divulged the information about decline in the measures of metabolites (anthocyanin, flavanoid plus phenolics) in response to all the discrete concentrations of heavy metal lead. However, EBL as well as Si brought about an upsurge in their quantities in stress exposed fenugreek plants. Results were obtained on the similar lines in As stressed tomato plants where SiNPs escalated the levels of phenolics along with flavanoids (González-Moscoso et al. 2019) and in Al stressed maize plants where Si treatment enhanced the production of phenolics (Wang et al., 2004). Flavonoids have a say over ROS homeostasis under stress exposure on account of their antioxidant competence. An escalation in the measures of phenols was recorded in Al exposed barley plants (both roots and shoots) when given Si treatment (Vega et al., 2019). EBL facilitated enhancement was recorded in phenolic plus anthocyanin levels in the As exposed seedling stage of coriander when supplemented with triacontanol (Asadi Karam et al., 2017). This coupled treatment of EBL+Si might be operative to hinder the formation of $O_2^{\cdot-}$ and thereby ROS production, in order to alleviate the Pb induced stress in fenugreek.

6.2.4 Oxidative stress

Higher measure of Pb causes excessive production and accumulation of ROS, which oxidatively damages the cellular components and upsets the redox equilibrium (Yadav et al. 2010, Mobeen et al., 2021). A burst of ROS is responsible for disrupting the membranal constitution, especially the lipid part resulting in electrolyte leakage. In the current study, such a negative influence is hinted at by the enhanced levels of stress markers i.e. MDA and H_2O_2 in fenugreek plants subjected to Pb stress. Supplementation of these plants with 24-EBL and Si remarkably reduced the Pb mediated generation of both MDA plus H_2O_2. Similar outcomes have been reached at with 24-EBL supplementation by Liu et al. (2023) in Zn stressed *Citrullus lanatus*, Zhong et al. (2020) in Pb stressed *Festuca arundinacea*, and Nazir et al. (2019) in Ni stressed *Cucumis sativus* and with Si treatment by Saleem et al. (2022) and Thind et al., (2021) in Cd stressed *Zea mays* and *Triticum aestivum* respectively.

24-EBL facilitated decline in the genesis of ROS and lipid peroxidation in the membranes thereof, might be taking place by escalation in the endogenous measures of growth regulators like salicylic acid and ethylene which combat metal toxicity via their intricate cross talks (Fariduddin et al., 2013). On the other hand, Si treatment lowers down the formation of free radicals through the maintenance of water status and osmolytes, as reported in Cd stress exposed peas (Rahman et al., 2017). Cumulative treatment of 24-EBL with Si, regulated lipid peroxidation, diminished MDA content with stabilization of membranes more efficiently when correlated with their individual application in Pb stressed fenugreek plants through enhanced scavenging of ROS.

6.2.5 Metal uptake

Lead levels were more in Pb treated *Trigonella* plants and exhibited further increase with every elevation in concentrations during the current experimental work whereas EBL and Si lowered down its measures, especially in the roots. The outcomes further revealed that greater proportions of lead metal are accumulated inside the roots in contrast to aerial plant parts, which is concomitant with the findings of Kumar et al. (2017) and Yilmaz et al., (2019). Silicon application led to lowering down of metal quantities in shoots vis-à-vis roots in case of field grown cereal crops (Sohail et al., 2020). Endodermal casparian strips grow more in thickness because of Si supplementation, thereby impairing the normal process of metal translocation in plants. This subsequently enhances the deposition of Si plus lignin in dermal parts. Silicon-facilitated decline in Pb content could be possibly due to formation of Pb silicate complexes resulting in decrease in the free ionic form of Pb together with restricted transport of heavy metals through symplastic regions (Cao et al., 2017; Ji et al., 2017).

Moreover, Si treatment encounters the enhancement in inflow of sulphur rich compounds, which participate in synthesis of phytochelatins contributing towards obstructing the metal transport while elevating the passage of essential ions in different tissues of plants (Saleem et al., 2022; Rahman et al., 2017). Besides, Si lowers the absorption of metals by roots through escalating the genesis of diverse root exudates, which carry out metal chelation (Etesami & Jeong, 2018; Kidd et al., 2001). Current study also revealed that individually applied 24-EBL to the Pb stressed *Trigonella* plants declined the uptake of Pb which resonates with the research outcomes of Kohli et al. (2018) and Li et al. (2009). For reducing metal uptake, 24-EBL also synthesizes phytochelatins, sequesters the heavy metals, promotes the uptake of inorganic ions of essential nature and maintains ion homeostasis, especially among sodium, potassium, calcium, magnesium ions in the upper foliar and petiolar regions (Ahmad et al., 2018; Waisi et al., 2017). Therefore, when both EBL and Si are supplemented in combination, better osmotic attunement and ion homeostasis lead to reduction in Pb uptake.

6.2.6 Osmolytes

The solute potential of plants is declined by a varied range of environmental stresses, which not only intensifies the electrolyte leakage but also negatively harms the cellular turgidity and thereby the cell growth. For putting up with these stresses, plants tend to generate osmolytes like proline and glycine betaine (Arif et al., 2021). Metabolic functions are protected by the enhanced measures of these osmolytes. Osmolytes were further observed to deter the harmful impact of metal toxicity through amendments in metal ion chelation and maintenance of optimal hydration levels (Ahmad et al., 2015; Ahanger et al., 2015). Osmolytes were reportedly reduced during the current experimental work that were normalised and even enhanced further by 24-EBL and Si feeding in Pb stress affected fenugreek plants. Metal stressed plants such as coriander (Pb), wheat (Cd) and licorice (Al) when exogenously supplemented with Si, brought about an significant elevation in the measures of osmolytes (Yazdani et al., 2021; Fatemi et al., 2020; Howladar et al., 2018). Over-accumulation of osmolytes in Si treated plants upscale the water uptake efficiency via stimulation of expression of genes and TFs (transcription factors) regulating aquaporins as observed in *Sorghum* (Khattab et al., 2014; Liu et al., 2013).

The osmolytes upon getting stocked up in higher quantities assist in maintaining redox equilibrium while enhancing the enzymatic efficiency so that ROS could be scavenged and cellular processes be protected thereof (Ahanger & Ahmad, 2019). EBL supplementation brings forth the same amendments to mitigate the metal stress in different plant varieties namely mung bean, mustard and peach trees suffering Al, Cu and chilling stress respectively (Siddiqui et al., 2018; Gao et al., 2016; Ali et al., 2008). Gao et al. in 2016 hinted at the role of alterations in P5CS enzyme so that it contributes in promoting the synthesis of proline while suppressing the action of proline dehydrogenase, thus reducing its consumption. Osmolytes tend to regulate both the glyoxalase and antioxidant systems for upgrading the stress forbearance (Hasanuzzaman et al., 2014). Carboxylase activity of Rubisco was protected from getting photoinhibited by proline levels (Sivakumar et al., 2000). Cumulatively applied EBL and Si during the current study escalated the formation and accumulation of osmolytes in Pb stress exposed fenugreek plants, most probably because of their synergistic influence in upregulating the genes of proline biosynthesis (Gao et al., 2016) with that of TFs managing water relations (Liu et al., 2013).

6.2.7 Carbohydrates

The starch is synthesized through fixation of carbon dioxide inside chloroplasts- the photosynthetic apparatus which also stores and distributes it to different sink organs where energy supply is needed. Remarkable alterations on the carbon metabolism have been recorded in response to metal stress, which ultimately degrades the nutritional composition of such plants (Wahid et al., 2007). The carbohydrates produced during photosynthetic process not only propel the cellular metabolism but also provide protection to various cellular regions (Muller et al., 2011). In the present experimental work, a considerable reduction in the content of carbohydrates was noticed in Pb stressed fenugreek plants, which was improved by the synergistic treatment with 24-EBL and Si. Piling up of carbohydrates in different plant parts plays a key role in regulating oxidative "PPP"-i.e. Pentose phosphate pathway via acting as ROS scavenger, especially of free radicals (Ende and Peshev, 2013; Hu et al., 2012; Van den Ende & Valluru, 2009). Carbohydrates measures were significantly upscaled by supplying 24-EBL to Pb stress subjected *Brassica juncea* plants (Kohli et al., 2019) and Si in Cd stressed *Triticum aestivum* plants (Heile et al., 2021; Howladar et al., 2018). Epibrassinolide/EBL and Si facilitated upsurge in the measures of carbohydrates in fenugreek plants might be linked to the enhancement in photosynthetic rates. Organic molecules required to battle out stressful conditions are made available by carbohydrates and these also function as the principal component of energy inception (Siddiqui et al., 2019, 2020).

6.2.8 Antioxidant defense system

6.2.8.1 Proteins + Antioxidative enzymes

Current research outcomes divulged the details about reduced measures of proteins in response to Pb induced toxicity in fenugreek plants, which were renormalized and then enhanced further by supplementing these plants with 24-EBL and Si. These findings are in the agreement with earlier works where 24-EBL enhanced protein levels in *Pleioblastus pygmaeus* (Emamverdian et al., 2020) and *Gossypium* sp. (Bharwana et al., 2013) exposed to Pb stress and Si performed the same action in Pb stressed *Festuca arundinacea* (Zhong et al., 2020) and *Brassica juncea* (Dalyan et al., 2018; Kohli et al., 2018).

Oxidative stress induced by ROS generation is chiefly mitigated by innate defense system of the plants operated by antioxidants (Schieber et al., 2014). Enzymes which are integral part of this antioxidative defense mechanism include APX, CAT, DHAR, GPOX, GR, GST, MDHAR, POD and SOD and they act as ROS scavengers (Das & Roychoudhury, 2014). Every enzyme listed here exhibits a peculiar action to regulate ROS levels, for instance, a) scavenging of H_2O_2 to H_2O occurs by APX and CAT b) upkeep of ascrorbte measures by DHAR plus MDHAR c) conjugating the glutathione with hydrophobic or electrophilic compounds by GST d) extracellular scavenging of H_2O_2 by POD and e) removing the superoxide radicals, are some major functions performed by them. Our outcomes recorded diminutive impact of Pb toxicity on the measures of all these enzymes in fenugreek plants as noticed in case of SOD in *Vicia faba* (Abusalima et al., 2020) and *Lymantria dispar* (Jiang et al., 2020); CAT in *Brassica campestris* (Zhong et al., 2020); DHAR and MDHAR in *Triticum aestivum* (Hasanuzzaman et al., 2018) and APX in *Brassica napus* (Kanwal et al., 2014).

In the present study, cumulative treatment of 24-EBL and Si elevated the enzymatic action of different antioxidants. Similar findings have been reported in Cd stress exposed *Cicer arietinum* (Ahmad et al., 2016); As stressed *Triticum aestivum* and *B. juncea* (Pandey et al., 2016; Geng et al., 2018), Ni stressed *B. campestris* and *Oryza sativa* (Abd_Allah et al., 2019; Hasanuzzaman et al., 2019) and Cd stressed *Triticum aestivum* plants (Xuebin et al., 2020) when supplemented with Si. Additionally, 24-EBL supplementation in Pb stress exposed *Oryza sativa* (Guedes et al., 2021), *Acutodesmus obliquus* (Talarek-Karwel et al., 2019) and *Brasssica campestris* (Nandikonda & Sadhu, 2019) brought about a notable enhancement in the enzymatic efficiency of almost every key antioxidant.

This functional role of 24-EBL in upgradation of activity of antioxidative enzymes for the amelioration of stressful conditions could be come across in various research works (Arora et al., 2012; Sharma et al., 2011). Further, EBL comes off as playing a major part in upscaling the measures of antioxidant enzymes under heavy metal toxicity via enhancing the expression of genes coding for them (Kohli et al., 2018). Moreover, ameliorative potential of EBL against heavy metals could also be attributed to the action of BSK 1 i.e BR signaling kinase which facilitates an upsurge in the measures of Salicylic acid to negate the oxidative harm (Deng et al., 2016). Besides this, stimulated functioning of GR plus GST by EBL feeding could be associated with elevation in pool of GSH with that of curtailed activity of NADPH oxidase for the mitigation of toxicity induced by heavy metals (Kanwar et al., 2012). Antioxidants as the likes of Ascorbic acid, DHAR plus MDHAR are also upscaled by EBL as reported by Wang et al. (2018) in pakchoi and Soares et al. (2016) in *Solanum nigrum*.

Conclusively, treatment with 24-EBL plus Si in a coupled manner could be possibly engaged in upregulating the expression levels of genes regulating biosynthesis of various antioxidant enzymes as well as non-enzymes.

6.2.8.2 Non-enzymatic antioxidants

Antioxidants of non-enzymatic nature, for instance, glutathione cast a remarkable influence in making plants more tolerant and able to combat heavy metal toxicity. Owing to their role to act as reductants, both ascorbic acid and glutathione facilitate the stabilization of cellular membranes. Free radicals like OH· gets directly scavenged by these non-enzymatic antioxidants (Foyer & Noctor, 2011). Lead metal exposure in the present study lowered down the content of the sulphur containing antioxidant glutathione. This outcome is substantiated by the similar findings in *Gongauloux polyedea* under Pb induced stress (Okamoto, *et al*., 2001). This reduction is possibly associated with less de-novo generation of glutathione coupled with its over usage in producing phytochelatins which would chelate toxic metallic ions (Nareshkumar et al., 2015). During the current experimental work, 24-EBL, Si and 24-EBL+ Si when applied individually and cumulatively, showed an upsurge in the content of all the antioxidants in Pb stressed *Trigonella* plants.

Corresponding to these findings, Si application led to an increase in the amounts of AsA and GSH in response to As stress (Kaya & Ashraf, 2022) in tomato, Cd stress (Thind et al., 2020) in wheat, Ni stress (Abd_Allah et al., 2019) in *Brassica juncea* and Al stress (De-sousa et al., 2019) in maize. Akin to this, Liu et al. (2023) in Zn stressed *Citrullus lanatus,* Zhou et al. (2018) in Cu stressed *Vitis vinifera* and Osman & Rady (2012) in Cd and Pb stress exposed *Solanum lycopersicum*, also recorded in EBL facilitated escalation in the levels of non-enzymatic antioxidants.

6.2.9 Gene expression

Levels of expression in genes regulating SOD and CAT enzymes were recorded to be enhanced during the present work in response to stressful environments. Along the same lines, Si treatment was noticed to elevate the extent of SOD gene expression in Cu stress exposed *Arabidopsis thaliana* plants (Khandekar & Leisner, 2011). Moreover, different genes coding for a varied range of antioxidative enzymes were recorded to be upregulated by 24-EBL in *Citrullus lanatus* and *Brassica juncea* subjected respectively to Zn and Pb stress (Liu et al., 2023; Kohli et al., 2018). The extent of expression was upregulated further upon 24-EBL and Si supplementation. The coupled application of EBL with that of Si could have a possible role in upregulating the expression of biosynthetic genes linked with the stimulation of antioxidants (enzymatic plus non- enzymatic) which belong to AsA–GSH cycle. Alleviation of metal induced toxicity by 24-EBL supplementation might be operational on account of promoted action of enzymatic antioxidants which in turn upregulate the corresponding gene expression levels. The outcomes of the current research hint at the possible modulation of Si induced upscaling of antioxidative enzymes via 24-EBL so that the performance and expression levels of antioxidative genes exhibit an upsurge.

Major effects of Pb toxicity in *Trigonella foenum-graecum* and their alleviation by treating these plants with 24-EBL and Si have been summarized in the fig. 6.2.1.

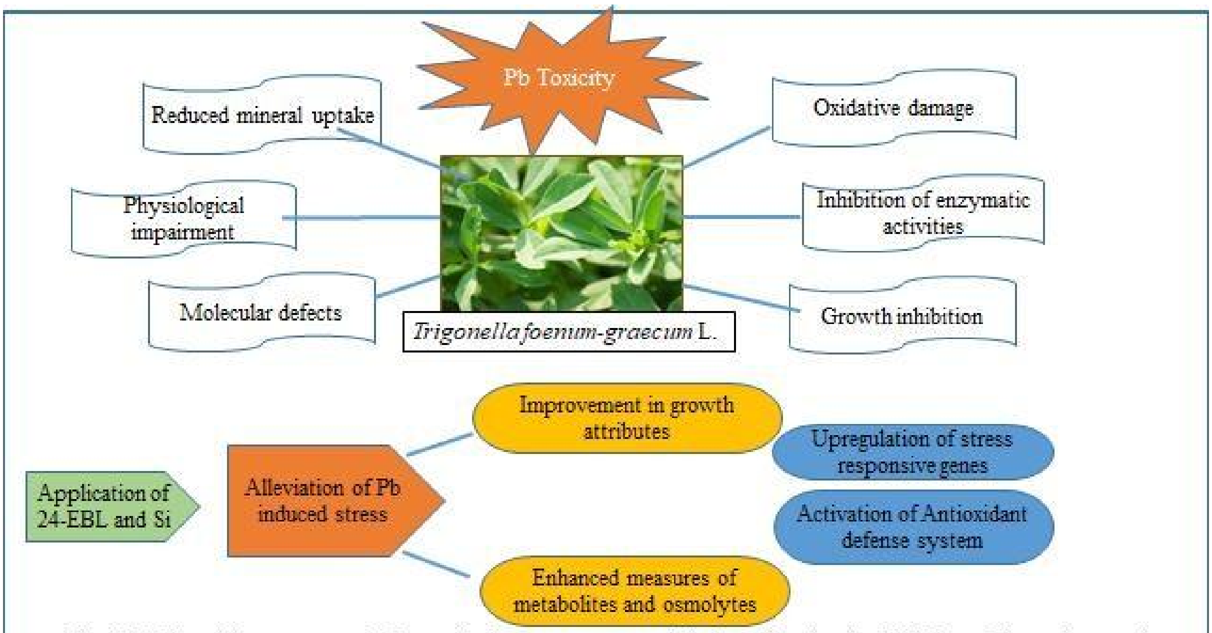


Fig. 6.2.1 Pb toxicity symptoms in *Trigonella foenum-graecum* and their amelioration by 24-EBL and Si supplementation.

Chapter 7
Summary and Conclusions

Heavy metal Pb, a recognized ecologically toxic pollutant of non-biodegradable nature, has become a serious threat to food security. Considering this, mitigation of Pb generated stress in fenugreek by using efficient and environmentally safe measures becomes more pertinent. Therefore, the present experimental work was executed for evaluating the impact of two such amelioratives i.e. EBL and Si in Pb metal stressed fenugreek plants. Following objectives were intended to be achieved:

- *In vitro* study of *Trigonella foenum-graecum* L. seedlings exposed to Pb stress along with the supplementation of BRs and Si.
- Monitoring the effects of Pb induced stress in *Trigonella foenum-graecum* L. plants.
- Analysis of potency of BRs and Si in mitigating Pb toxicity stress in *Trigonella foenum-graecum* L. plants.
- Comparative study of gene expression of Pb stress associated genes in *Trigonella foenum-graecum* L. in response to the BRs and Si.

To realize these objectives, seeds of fenugreek (*Trigonella foenum-graecum* var. Palam somya) were subjected to surface sterilization. One lot of the seeds was immersed in 24-EBL (10^{-7} M) for 8 hours, while simultaneously soaking the other lot in distilled water. Lead nitrate and silicic acid (2 mM) were used to supply Pb and Si to these plants. Harvesting of *In vitro* grown 7 days old seedlings and *in vivo* grown 15, 30, 45 days old plants was performed followed by their subjection to analytical study. Various attributes of these stages which were evaluated have been mentioned below:

- Morphological: a) length (root + shoot) b) biomass (dry + fresh) c) vigor index d) germination percentage
- Photosynthetic Pigments: a) Chlorophylls, b) carotenes and c) xanthophylls
- Secondary metabolites: a) anthocyanins b) phenolics and c) flavanoids
- Oxidative damage indicators: a) Malondialdehyde (MDA) and b) Hydrogen peroxide (H_2O_2)
- Membranal and nuclear impairment (Confocal microscopy)
- Metal content inside seedlings as well as plants (by usage of AAS- Atomic Absorption Spectrophotometry)
- Osmolytes: a) glycine betaine b) proline and c) total sugars
- Protein content and Antioxidative enzymes (APX, CAT, DHAR, GR, GPOX, GST, MDHAR, PPO and SOD)

- Non-enzymatic antioxidants: a) Ascorbic acid (AsA) b) Glutathione (GdISH) and c) **α-** tocopherol (Vit. E)
- Gene expression: stress responsive genes (qRT-PCR)

Next, statistical assessment of these parameters was executed with SPSS 16.0.

Discrete measures of Pb metal resulted in an adverse influence on not only the length but also on the biomass and vigor index of fenugreek plants. However, supplementing these stressed plants with EBL and Si brought about an improvement in these parameters. Water status expressed as RWC- Relative water content exhibited a reduction in response to Pb metal toxicity. On the similar lines, results were obtained for the photosynthetic pigments having the maximum curtailment at 0.9 mM of Pb. Water content along with measures of different photosynthetic contents displayed an enhancement when these Pb stressed plants were subjected to EBL and Si treatment.

Measures of secondarily formed metabolites namely Phenolics, anthocyanins and flavonoids were noted down to be diminished in Pb exposed seedlings plus plants of *T. foenum-graecum.* However, EBL and Si supplementation brought forth an augmentation in the content of these metabolites, particularly in case of their coupled treatment as opposed to individual ones.

Elevation in the extent of MDA along with H_2O_2 was recorded with being highest at 0.9 mM concentration. However, exogenous supplementation of EBL and Si lowered down their measures in order to alleviate the oxidative damage. Impairments in membranal and nuclear structures were noticed in Pb stressed seedlings but this damage was mitigated by EBL and Si treatment.

Lead metal content was recorded to be higher in both stressed seedlings and *in vivo* grown plants, particularly inside their root regions. The levels of metal displayed a decline when the different stages (seedling + plants) of fenugreek plants were supplemented with EBL and Si.

Additionally, osmolytes inclusive of total soluble sugars, glycine betaine and proline exhibited a drastic reduction in their measures with maximum decline at 0.9 mM concentration of Pb. This metal stress induced reduction was ameliorated and improved further when fenugreek plants were subjected to EBL and Si treatments.

Seedlings together with plants of *T. foenum-graecum* were recorded to have reduced measures of protein in response to Pb stress, which was made better by supplicating the stressed plants with EBL and Si.

Enzymatic action of antioxidants namely SOD, APX, CAT etc. was diminished in both the metal stressed stages i.e. seedlings plus plants. However, treating these stages with EBL and

Si enhanced their activity level even under stressful environments. It was noticed that different antioxidants of non-enzymatic nature were ebbed in their contents inside Pb stressed plants while being up-scaled upon getting supplemented with EBLand Si.

Lead induced stress brought about upregulation in the expression levels of genes namely SOD and CAT. However, supplementation of metal stressed plants with EBL and Si in individual plus cumulative format enhanced the extent of their expression.

Overall, a conclusion could be drawn from this experimental work that individual plus coupled usage of EBL and Si is a favourable strategy to mitigate the negative influence of Pb metal generated stress in fenugreek plants which makes the various aspects (morpho-anatomical, biochemical and molecular) of metal stressed plants better. Treatment T_{13} having the combination of 24-EBL and Si with that of lowest concentration of Pb i.e. Pb I has shown maximum efficiency over all the other treatments in mitigation Pb metal stress.

Chapter 8
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Publications

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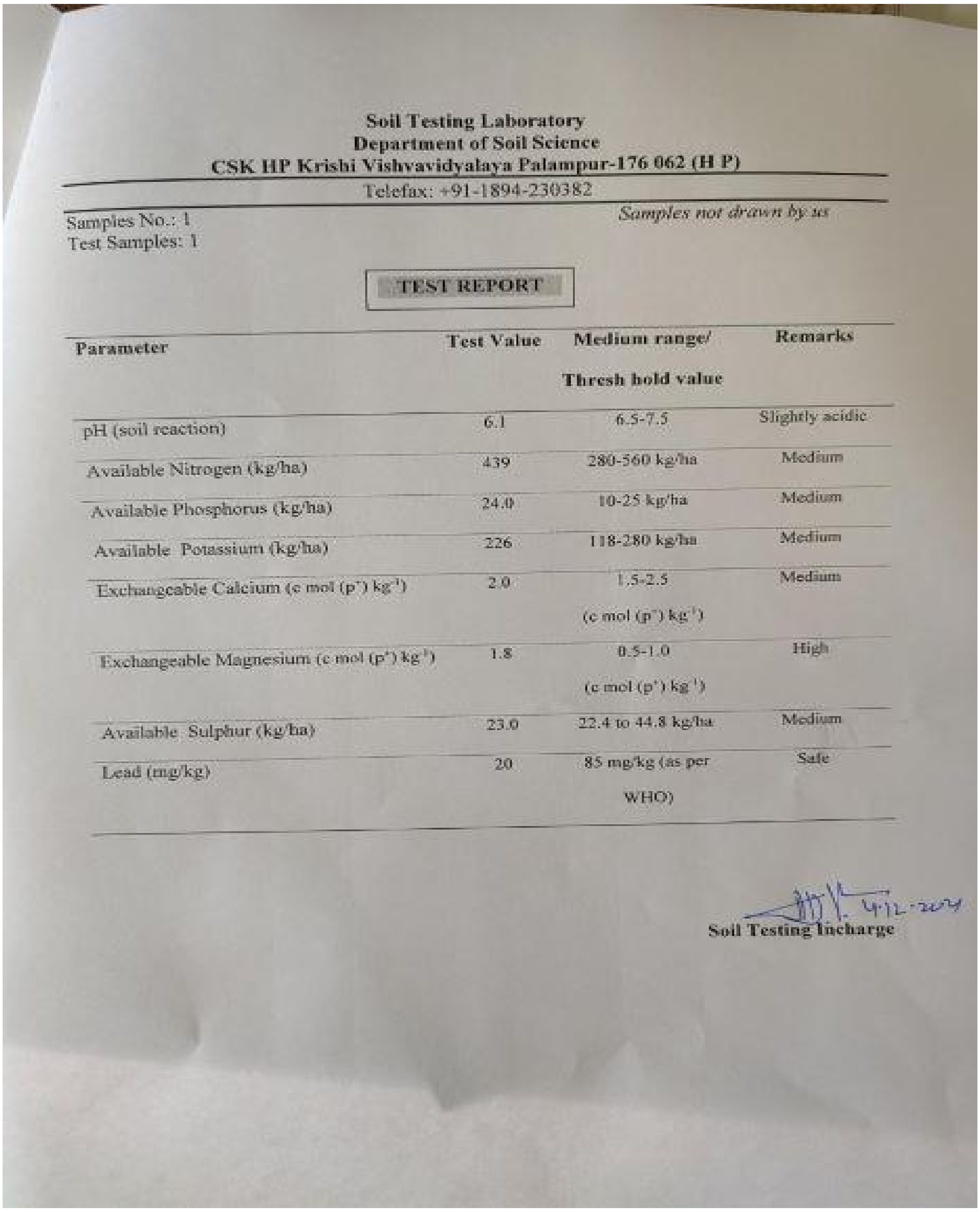
Conferences/ Workshops Attended

1. Oral presentation on the title “Himalayan Ecosystem: A Perusal of Govt.schemes for its conservation in Himachal Pradesh” “ 1st International Conference on Recent Trends in science and technology for environment conservation and sustainable development”, 29-30 Sep., 2023, organized by Dept. of botany and Zoology, SGC, Nadaun.
2. Participated in 3 Days International Virtual workshop on “Molecular Dynamic Simulation in Bioscience Research held from 4-6th August, 2023, organized by Quaxon Bio & IT solutions, India in collaboration with, Ministry of MSME, Govt. of India.
3. Oral presentation on the title “Role of Silicon in amelioration of Pb metal city in fenugreek plants ” “ 5th International Conference on Advances in Agriculture, technology and allied sciences”, 4-5 June, 2022, organized by Centurion University, Orissa.
4. Oral presentation on the title “Cumulative impact of EBL and Si in mitigation of Pb stress in 30 days old fenugreek plants” “ 7th International Conference on Global Research Initiatives for Sustainable Agriculture and Allied Sciences”, 21-23 Nov., 2022, organized by Aastha Foundation, Meerut (U.P.).
5. Oral presentation on the title “Analysis of Himachal Pradesh policy of solid waste management in urban areas” at National Conference on Hazardous waste: Education, Research and Management Strategies held at Govt. College, Una (H.P.) (20-21 Dec., 2019).
6. Oral presentation entitled “Coronavirus pandemic and our mental health” held on 11th Dec.2021, organized by IQAC, Sidharth Govt. Utkrisht College, Nadaun, Hamirpur (H.P.).

Appendix-2



Soil Testing Laboratory
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CSK HP Krishi Vishvavidyalaya Palampur-176 062 (H P)
Telefax: +91-1894-230382

Samples No.: 1
Test Samples: 1

Samples not drawn by us

TEST REPORT

Parameter	**Test Value**	**Medium range/ Thresh hold value**	**Remarks**
pH (soil reaction)	6.1	6.5-7.5	Slightly acidic
Available Nitrogen (kg/ha)	439	280-560 kg/ha	Medium
Available Phosphorus (kg/ha)	24.0	10-25 kg/ha	Medium
Available Potassium (kg/ha)	226	118-280 kg/ha	Medium
Exchangeable Calcium (c mol (p$^+$) kg^{-1})	2.0	1.5-2.5 (c mol (p$^+$) kg^{-1})	Medium
Exchangeable Magnesium (c mol (p$^+$) kg^{-1})	1.8	0.5-1.0 (c mol (p$^+$) kg^{-1})	High
Available Sulphur (kg/ha)	23.0	22.4 to 44.8 kg/ha	Medium
Lead (mg/kg)	20	85 mg/kg (as per WHO)	Safe

4·12-2024
Soil Testing Incharge