MORPHO-PHYSIOLOGICAL FEEDBACK BY *CITRULLUS COLOCYNTHIS (* L.) UNDER SALINITY AND NICKEL STRESSES

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Abstract**:** Salinity and heavy metals in soil are dreadful hazards causing reduction in plant growth, development and yield values. Therefore a medicinal cucurbit weed *Citrullus colocynthis* L. was given exposure to a series of salt (NaCl), heavy metal ($NiCl_2$) and combined (NaCl and $NiCl_2$) levels prepared in Hoagland's solution. Germination and other growth parameters were promoted in low concentrations of NaCl (100mM), $NiCl_2$ (50 µM) and combined (100mM NaCl and 50 µM $NiCl_2$) while inhibited under separate and combined high level stresses 400mM NaCl, 200µM $NiCl_2$ and combined 400 mM NaCl and 200µM $NiCl_2$. Moderate levels (200mM of NaCl and 100µM of $NiCl_2$) slightly promoted growth parameters in all separate and combined treatments. In combined stresses, low salt concentration (100mM NaCl) mitigated the adverse effects of high level of heavy metal (200µM $NiCl_2$) and low heavy metal (50µM $NiCl_2$) also ameliorated the high salt complications (400mM NaCl). Variable results in morpho-physiological parameters were observed in the combined effect of salt and heavy metal. Growth parameters like length of shoot, root, fresh and dry weight, number of leaves, branches, flowers, leaf area index, total yield, rate of photosynthesis, chlorophylls, Ca^{2+}, K^+ and proteins were affected adversely with increasing stresses while some factors like free amino acids, free sugars, proline, antioxidants like superoxide dismutase and catalase, Na^+ and Cl^- increased with increasing stress levels. It is concluded that *Citrullus colocynth* produces adaptive chemicals when exposed to salt and nickel stresses and can tolerate low and moderate levels of salt and nickel.

Index-terms; Salinity stress, , Combined stress, *Citrullus*, NaCl Stress, Nickel ($NiCl_2$) Stress,

I. INTRODUCTION

Soil salinity is a rigorous dilemma to agricultural production (Cha-um *et al.*, 2009), hurting agricultural lands up to 50% and cultivated lands up to 20%, a frightening risk to the health as well as a severe ecological crisis of soil in the world (Conesa *et al.*, 2011). Soil salinity leads to a decline in plant growth, development and overall yield as it is a hazardous agent causing ionic, osmotic and oxidative stresses (Hussain & Dietz, 2016). About 0.8 billion hectares of land throughout the world are affected by salinity which is approximately 6% of the total area of the world (Shelke *et al.*, 2017). Among toxic salts, one of the most toxic salt is sodium chloride (NaCl) which comprises 50% of the entire soluble salts responsible for the addition of Na^+ and Cl^- which are dominating in saline soils (Li *et al.*, 2017). It has been documented that the stress trigger in most plants is Na^+, but, in several cases, Cl^- defeats Na^+ in toxicity (Li *et al.*, 2017). It was determined that the co-effects of heavy metal and salinity were definitely more severe in comparison to single stress (Ahmad *et al.*, 2016). Along with other heavy metals Nickel (Ni) is regarded as the most important environmental pollutant (Conesa 2011). Presently, the global level of Nickel has exceeded by about twenty to thirty folds more than the total range (Amari *et al.*, 2017). Duda (2008) announced Nickel (Ni) as "Allergen of the Year". A minor value of Nickel (Ni) is requisite for a standard plant as its deficiency is rarely reported whereas its toxicity is a concerning issue (Syu *et al.*, 2014). Nickel, a significant metal pollutant has crucial apprehension because of its rapidly escalating concentration in the soils of various areas of the world (Hussain *et al.*, 2013). The environment is being contaminated with Nickel by diverse nature as well as anthropogenic sources and accordingly going to become unusual in concentration. Natural sources of Ni may be weathering of rocks while anthropogenic sources are industries in which various Nickel compounds like Nickel (chloride, acetate, carbonate, oxide & hydroxide) are being used (Campel & Nikel, 2006). This variety of Nickel compounds accumulated in soil and is available for the plants easily taken up while absorption of water and other solutes, ultimately entering in the food chain and causing injurious effects on animal and human lives (Onaxpa 2018).

The toxic effects of accumulated sodium and chloride ions cause an increase in reactive oxygen species (ROS) resulting in decreased activity of stomata and electron transport system (Negrao et al., 2017). The excessive production of ROS (SOD & CAT etc.) can cause cell death due to the oxidation of proteins, lipids, carbohydrates, nucleic acids and chlorophyll (Hussain & Dietz, 2016). Adaptive defense mechanisms of plants to produce compatible solutes, ROS scavenging system and compartmentalization of toxic ions combat salinity (Abogadallah 2010). Regardless of toxication, Cl^- (chloride ions) have a dogmatic role in the turgor generation, pH, enzyme stability, balance of charge, modification in membrane potential, osmoregulation, volume control, and stomatal conductance leading to prevent water by minimizing its loss & use and photosynthetic competence of the plant (*Li et al.*, 2017). The presence of ions in a lower limit is beneficial but Surplus salt ions either in soil or in water cause considerable alterations in morpho-physiological characteristics of plants. During salinity stress, plants absorb excess Na^+ at the expense of K^+ & Ca^{++} consequential in extra Na+ contents of plant parts like leaves and stems, is increased, leading to nutritional disproportion resulting in decreased plant growth, inhibited physiological activities and reduced dry matter (Mahmoud *et al*., 2020).

Different plant organs accumulate Na+ and Cl^- under salt stress (Kurtar *et al*., 2016). Many researchers reported that long-term salt stress causes water deficiency and ion toxicity in older leaves while carbohydrate deficiency in young leaves (Kurtar *et al*., 2016). Therefore, the adaptive strategies of the plant under stress conditions determine the ability of salt resistance (Ors & Suarez 2017). Different plants could be different in physiological and biochemical tolerance mechanisms either at the complete plant level or at the cellular (Chang *et al*., 2014; Li *et al*. 2018). To select salt-tolerant cell lines, salinity tolerance expressed at the cellular level may be helpful to understand the mechanisms of salinity tolerance (Shelke *et al*., 2019). The degree of difference in responses to salinity and heavy metals in plants can be determined by measurement of altered germination percentage, growth parameters, and production of various compatible organic and inorganic solutes like antioxidants, etc (Kumar et al., 2017). All the life events of a plant depend upon the germination of the seed. Increasing salinity and heavy metal stress inhibits seed germination in glycophytes, while elevates in halophytes developing adaptations, for example, seed germination in *Kochia scoparia* is increased in high salinity stress (AlAhmadi & Kafi 2007).

Citrullus colocynth L. is a medicinal cucurbit weed, commonly known as 'Tumba' in India and Pakistan (Mahajan & Kumawat, 2013). There are multiple medicinal and biological uses of Citrullus including anticancer, antidiabetic, antioxidant, antilipidemic, anti-inflammatory, cytotoxic, insecticidal and antimicrobial (Hussain *et al*., 2014). The seeds of *C.colocynth* linoleic acid and oleic acid, fruit contain biochemical like Flavonoids, alkaloids, glycosides, terpenoids, cucurbitacins and colocynthosides (Hussain *et al.*, 2014).

It has been acknowledged that the harmful effects of salinity stress can be minimized or even ameliorated by the appliance of special micronutrients to plants, such as barley enhanced yield under salinity stress when provided Si micronutrient(Noreen *et al*., 2018). Recognition and understanding of plant abilities of salinity and heavy metal tolerance and resistance are of comprehensible attention in varietal improvement for reclamation of saline and heavy metal contaminated soils. Moreover, the use of heavy metals as micronutrients may prove helpful to the plants to ameliorate salinity stress and improve plant growth in a saline environment. Whereas soil contaminated with heavy metal may be turned into greenland if the plants are provided salts in lower concentrations. The study was conducted to determine firm morpho-physiological responses of *Citrullus colocynthis* L.towards single and combind stresses of salt (NaCl) and Nickel chloride ($NiCl_2$) .

II. MATERIAL AND METHOD

The experiment was carried out to study the adaptations against variable levels of salt and heavy metal stresses in the cucurbit weed *Citrullus colocynthis*. The practical conduct of the experiment was done in june 2018 in the Research area, Botanical Garden, Botany Department, The Islamia University of Bahawalpur.

Seeds of *Citrullus colocynthis* were collected from different sites of the thal desert of district Layyah (71.4774 E & 30.9057 N) for assessment of the response of species under salt and heavy metal stresses. The ripened fruits of the species were collected and seeds were obtained by removing the dried pulp. Healthy seeds were selected for further experiments.

To assess the tolerance potential and adaptive strategies of *Citrullus colocynthis* L. variable levels of NaCl and $NiCl_2$ were selected and added to the corresponding petri dishes to create 16 treatments. Control plants (T0) were grown without salt and metal while experimental groups include three individual salt and three individual heavy metal Ni treatments i.e. (least, moderate and severe salt and Ni stresses). For investigation of the combined effects of salt and heavy metal, nine treatments were maintained for combined levels of NaCl and $NiCl_2$ in the present experiment i.e.

Treatment #	**Single/combined stress**	**Salt level**	**Nickel level**
T0	control plants	Distilled water	Distilled water
T1	single	100mMol NaCl	
T2	single	200mMol NaCl	
T3	Single	400mMol NaCl	
T4	Single		50 µM $NiCl_2$
T5	Single		100 µM $NiCl_2$
T6	Single		200 µM $NiCl_2$
T7	Combined	100mMol NaCl	50 µM $NiCl_2$
T8	Combined	100mMol NaCl	100 µM $NiCl_2$
T9	Combined	100mMol NaCl	200 µM $NiCl_2$
T10	Combined	200mMol NaCl	50 µM $NiCl_2$
T11	Combined	200mMol NaCl	100 µM $NiCl_2$
T12	Combined	200mMol NaCl	200 µM $NiCl_2$
T13	Combined	400mMol NaCl	50 µM $NiCl_2$
T14	Combined	400mMol NaCl	100 µM $NiCl_2$
T15	Combined	400mMol NaCl	200 µM $NiCl_2$

The experiment was conducted in completely randomized design (CRD) with following factors (weed species, salinity, Heavy metal and salinity+ Heavy metal) with three replicates. Chemicals synthesized by Merrick laboratories were purchased from G.M Scientific store Multan cantt.

Laboratory experiment

To evaluate the seed germination percentage, a laboratory experiment was conducted in 9cm diameter Petri dishes. Each petri-plate was provided with whattman No.43 filter paper and twenty seeds were placed in each petri plate after soaking seeds for 24 hours in a 10% solution of sulphuric acid for breaking of the seed coat. All the petri plates were provided with Hoagland solution to maintain other nutrients. Petri plates were covered to protect from external irrelevant disturbances. Data for seed germination, radicle length, plumule length, fresh weight & dry weight of seedlings were collected from this experiment. The seed germination percentage was recorded according to the formula of Coolbear *et al*. (1984). Germination percentage $GP = \frac{\sum G}{N} \times 100$

Fresh weight and dry weight were calculated by electronic balance. The plant samples were preserved in a properly labeled vial containing preservatives (for anatomy), and paper bags (for taking dry weight).

Pot experiment

Soil analysis and sowing of seeds

The soil samples for the potted experiment were taken to be analyzed for physio-chemical characteristics which were EC1.97 ds/m, pH 8.1, soil texture sandy loam, organic matter 0.51%, available phosphorus 6ppm, and potassium 113ppm using EC meter, pH meter, hydrometer, loss on ignition, PFP-7 Jenway flame photometer (Jenway, PFP-7) using 200g dried soil for analysis. Seeds of *Catullus colocynthis* were soaked in 10% sulphuric acid solution in water for 24 hours to soften the seed coat. Twenty seeds of *Catullus colocynthis* were placed in each plastic pot (15cm diameter) containing soil with 40% sand, 30%silt and 30% clay to a depth of 1- 2 cm.

Morphological parameters Pots were treated with various levels of salt (NaCl, heavy metal ($NiCl_2$) and combined (salt NaCl+ heavy metal $NiCl_2$) according to the method described above. Pots were placed in the research area and left open. Adequate moisture was kept by providing water to the pots. After germination pots were given the treatment of salt, heavy metal, and combined salt+ heavy metal weekly. Morphological data were collected after four weeks at the vegetative stage and flowers at the reproductive stage while fruits were at maturation. Data was collected for fresh weight/mass (mg), dry weight/mass (mg), root length (cm), shoot length (cm), internodal length (cm), number of leaves, number of branches, number of tendrils, leaf area index (cm^2), number of flowers and gross weight of fruits.

Biochemical and physiological parameters

Jenway flame photometer was used for the determination of sodium (Na^+), Potassium (K^+) and Calcium (Ca^{++}). Jenway PCLM chloride meter was used for the determination of chloride (Cl^-). Ni^+ (aq) ion reacts with concentrated Cl^- ion in the presence of ethanol medium. Arnon method (1949) was used for the determination of chlorophyll a and bYemm & Willis method (1954) was used for the determination of total soluble sugars. Lowry *et al*. (1951) method was used for the determination of total soluble proteins. Bates *et al*. (1973) method was used for the determination of proline. Chance & Maehly (1955) method was used for the determination of Catalase. The activity of SOD was analyzed by method used by Giannopolitis &Ries (1977). Statistical analysis of the data was performed by using STATISTIX 8.1 software .

III. RESULTS

3.1 Laboratory experiment

Citrullus colocynth L. showed a significant increase in germination percentage, length of radicle and plumule, fresh weight and dry weight of seedling at lower salt (100mM NaCl), moderate level of salt (200mM NaCl), lower level of heavy metal (50 µM $NiCl_2$), combined lower salt and lower Heavy metal (100mM NaCl+ 50 µM $NiCl_2$), lower salt level + moderate heavy metal level (100mM NaCl + 100 µM $NiCl_2$) and moderate salt+ lower heavy metal level(200mM

NaCl+(50 µM NiCl$_2$), slight increase at moderate level of heavy metal (100 µM NiCl$_2$) while decrease at high level of salt (400mM NaCl), high level of heavy metal (200 µM NiCl$_2$), moderate salt level + high concentrations of heavy metal (200mM NaCl + 200 µM NiCl$_2$) and high level of salt + high level of heavy metal (400mM NaCl + 200 µM NiCl$_2$) (Fig.1).

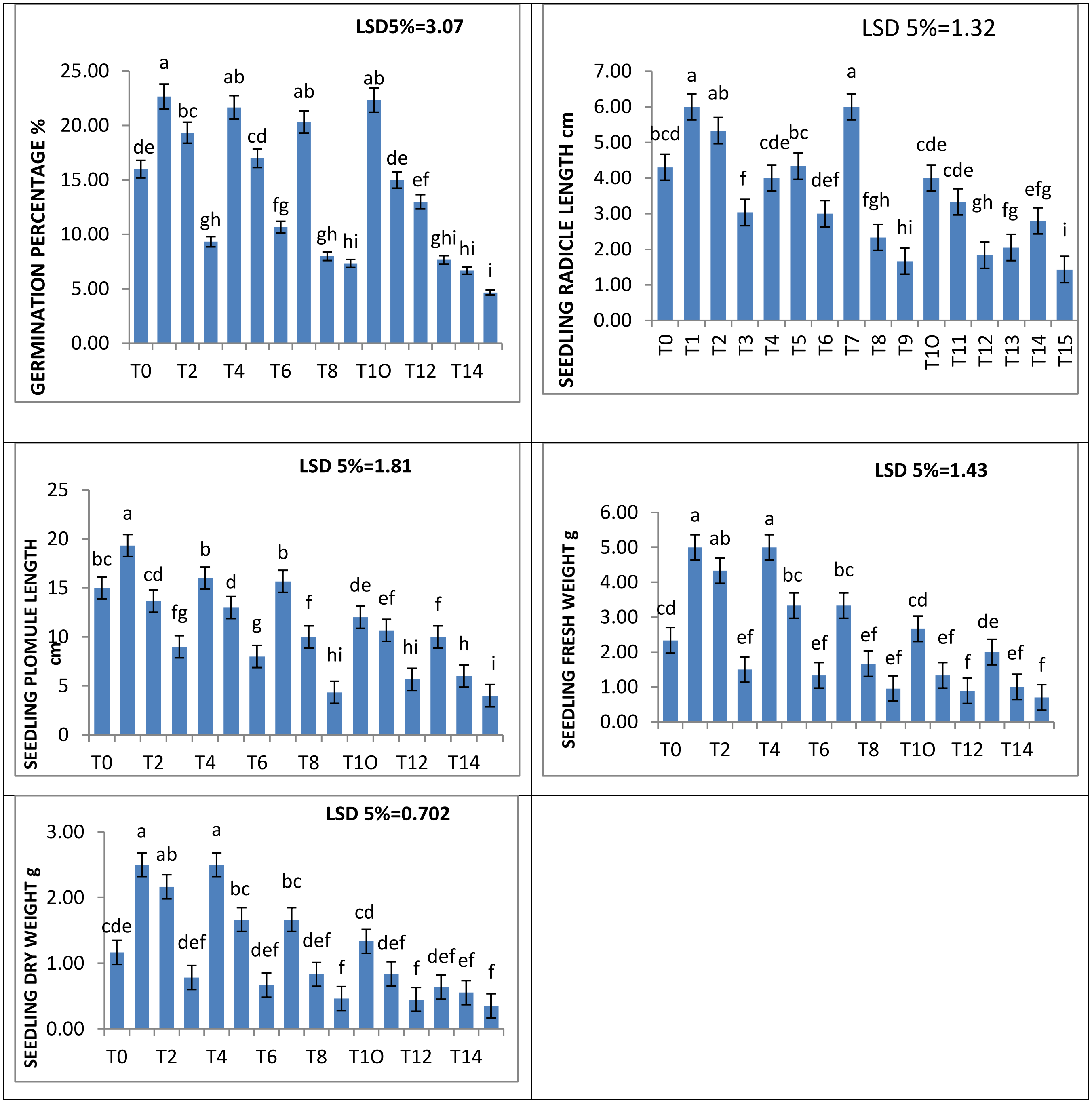


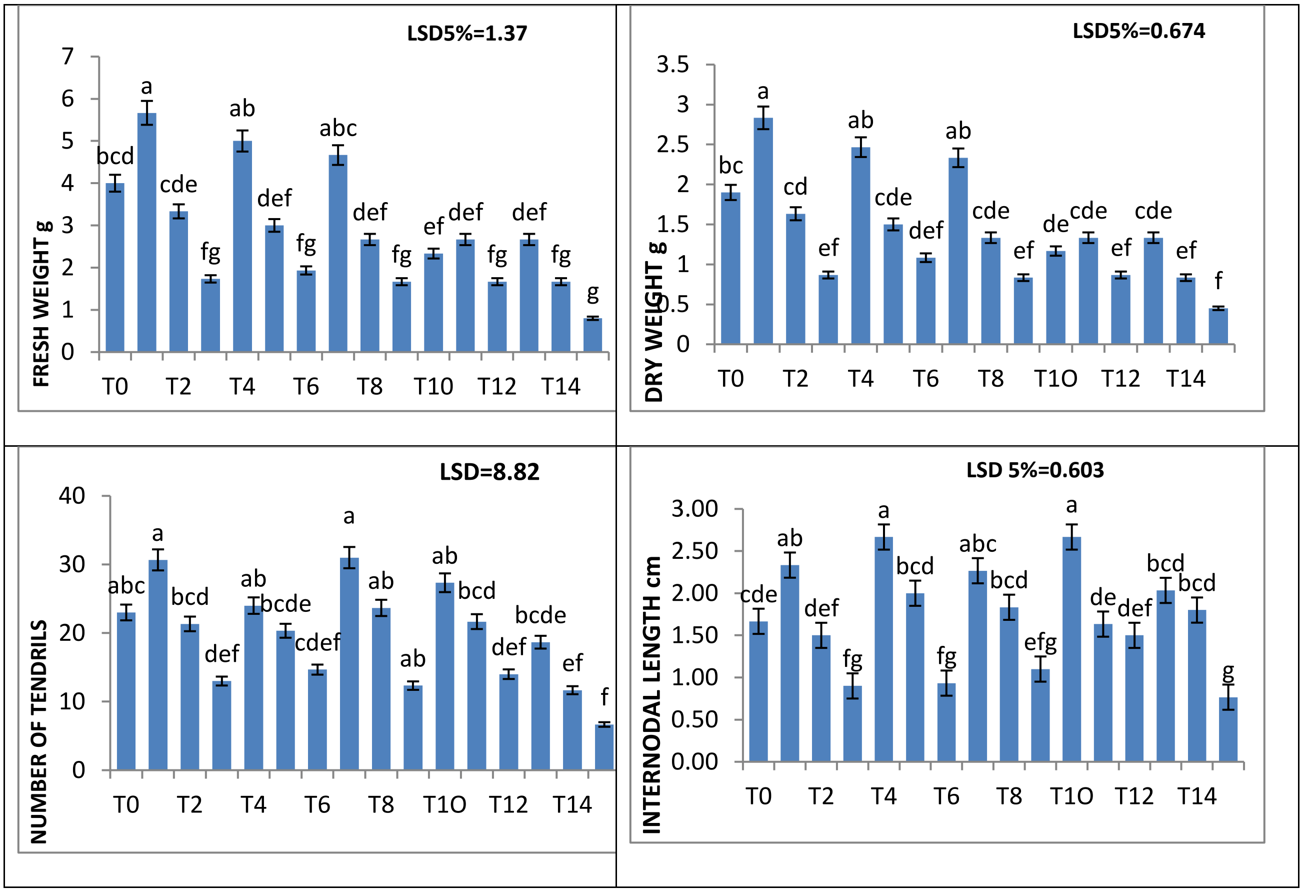
Fig.1. Effect of salt (NaCl), heavy metal (NiCl$_2$) and combined salt+ heavy metal (NaCl+NiCl$_2$) stresses on *Citrullus colocynth* in laboratory experiment.

3.2 Pot experiment

3.2.1 Morphological characters

Fresh weight, dry weight and number of branches of *Citrullus colocynthis* L. showed a significant increase under the stress of low salt (T1), low heavy metal (T4), and combined low salt and low heavy metal (T7). There was a gradual decrease in fresh and dry weight with increasing salt and heavy metal stress levels either separate or combined. A maximum decrease in these parameters was observed in combined high salt and high heavy metal (T15)(Fig.2).

The internodal length was significantly increased in low salt (T1), low heavy metal (T4), combined low salt and low heavy metal (T7), and combined low salt and moderate-heavy metal (T10). A slight increase in intermodal length was observed in moderate-heavy metal(T5), combined moderate salt and low heavy metal (T8), combined low salt and high heavy metal (T13), and combined moderate salt and high heavy metal (T14). Internodal length showed a neither positive nor negative effect in moderate salt (T2), combined moderate salt and moderate-heavy meal (T11), and combined high salt and moderate-heavy metal (T12). A considerable decrease in intermodal length was observed in high salt (T3), high heavy metal(T6), combined high salt and low heavy metal (T9), and combined high salt and high heavy metal (T15)(Fig.2).



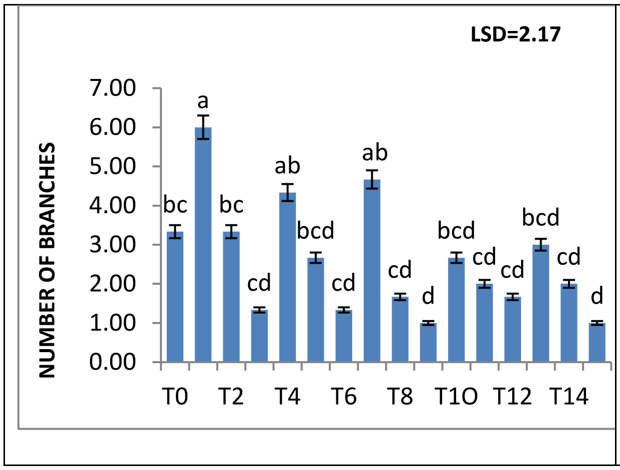


Fig.2. Effect of salt(NaCl), heavy metal ($NiCl_2$) and combined salt+ heavy metal ($NaCl+NiCl_2$) stresses on morphology of *Citrullus colocynth*.

The number of tendrils were significantly increased in low salt (T1), low heavy metal (T4), combined low salt and low heavy metal (T7), combined moderate salt and low heavy metal (T8), and combined low salt and moderate-heavy metal (T10). The number of tendrils were gradually decreased by increasing levels of salt and heavy metals concentrations. Maximum decrease was observed in combined high salt and high heavy metal(T15)(Fig.2).

Root length showed variable results. At the moderate levels of heavy metal(T5) and combined moderate salt and moderate-heavy metal(T11) root length was increased considerably, however moderate salt(T2) and combined low salt and moderate-heavy metal(T10) stresses does not affect root length while in all other levels of stresses, root length was decreased by increasing stress levels(Fig.3).

As compared to the control, shoot length was increased in low salt (T1), moderate salt (T2), low heavy metal (T4), moderate-heavy metal (T5), combined low salt and low heavy metal (T7), combined moderate salt and low heavy metal (T8), combined moderate salt and moderate-heavy metal (T11), and combined low salt and high heavy metal (T12). A little increase in shoot length was observed in combined low salt and moderate-heavy metal (T10) and combined low salt and high heavy metal (T13). Shoot length was decreased in high salt (T3), high heavy metal (T6), combined high salt and high heavy metal (T9), combined moderate salt and high heavy metal (14), and combined high salt and high heavy heavy metal (T15)(Fig.3).

The number of leaves showed a significant increase in moderate levels of salt (T2), moderate level of heavy metal (T5), combined low salt and low heavy metal (T7), a slight increase in low salt(T1), low heavy metal (T4), combined moderate salt and low metal (T8), combined low salt and moderate-heavy metal (T10) and combined moderate salt and moderate metal (T11) while a decrease in high salt(T3), high metal (T6), combined high salt and low metal (T9), combined moderate salt and high metal(T14) and combined high salt and high metal (T15)(Fig.3).

Leaf area index showed a gradual reduction with an increase in salt, heavy metal, or combined salt and heavy metal treatment as compared to the control. The least leaf area index was observed in combined high salt and high heavy metal level (T15)(Fig.3).

The number of flowers were considerably increased in low salt(T1) and low metal(T4), moderate salt (T2), moderate-heavy metal(T5), combined low salt and low metal (T7), combined moderate salt and low heavy metal (T8), combined low salt and moderate metal(T10) and combined low salt and high heavy metal (T13). A decrease in the number of flowers was observed in high salt(T3), high heavy metal (T6), combined high salt and low heavy metal(T9), combined high salt and moderate-heavy metal(T12), and combined high salt and high heavy metal(T15)(Fig.3).

The gross weight of fruit showed a considerable increase in low salt (T1), moderate salt (T2), low heavy metal (T4), moderate-heavy metal (T5), combined low salt and low heavy metal (T7), combined moderate salt and low heavy metal (T8), combined low salt and moderate-heavy metal(T10), combined moderate salt and moderate-heavy metal (T11). A slight decrease in the gross weight of fruit was observed in combined low salt and high heavy metal (T13) while there was a significant decrease in gross fruit weight in high salt (T3), high heavy metal (T6), combined high salt and low heavy metal (T9), combined high salt and moderate-heavy metal (T12), combined moderate salt and high heavy metal (T14), and combined high salt and high heavy metal (T15). There was an overall decrease in morphological parameters under the stress of high salt (T3), high heavy metal(T6), combined moderate salt and high heavy metal(T14), combined high salt and moderate-heavy metal(T12), and combined high salt and high heavy metal (T15)(Fig.3).

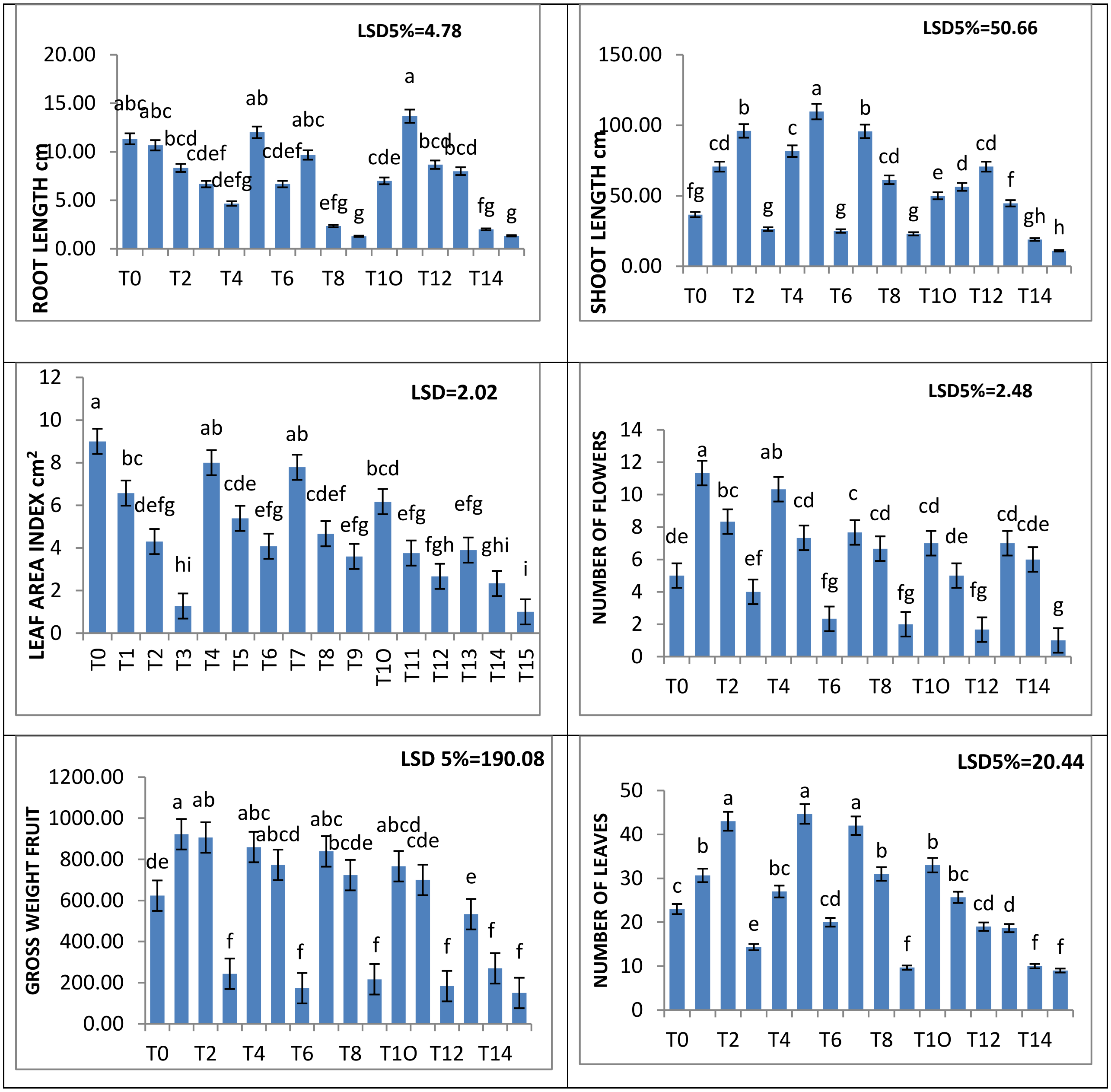
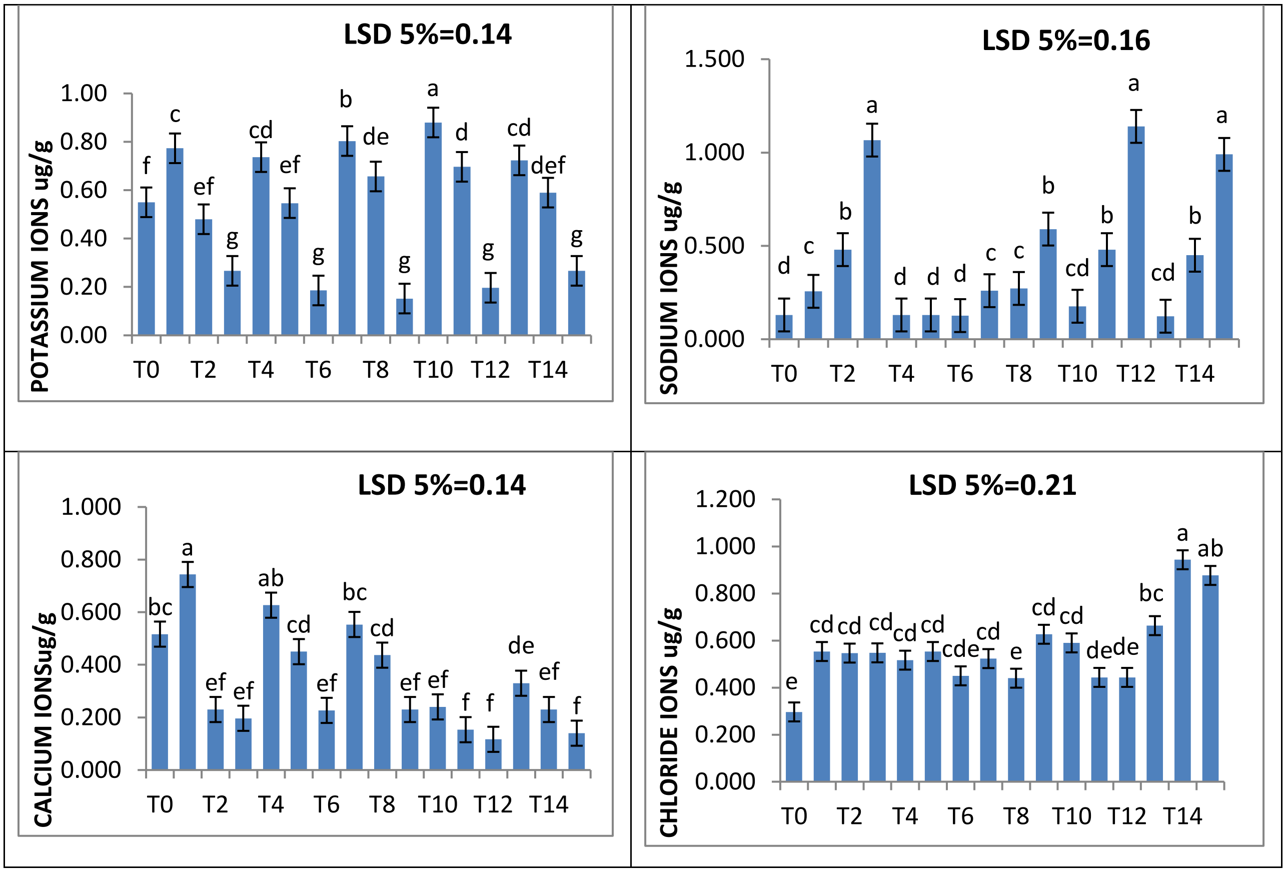


Fig.3. Effect of salt(NaCl), heavy metal ($NiCl_2$) and combined salt+ heavy metal ($NaCl+NiCl_2$) stresses on morphology of *Citrullus colocynth.*

3.3 Biochemical ions

Potassium ions were increased at low salt (T1), low heavy metal(T4), combined low salt and low heavy metal(T7), combined moderate salt and low heavy metal(T8), combined low salt and moderate-heavy metal (T10), combined moderate salt and moderate heavy metal (T11), combined low salt and high heavy metal (T13)and moderate salt and high heavy metal (T14) while decreased in a high level of salt(T3), high heavy metals(T6), combined high salt and low heavy metal(T9), combined high salt and moderate heavy metal (T12) and combined high salt and high heavy metal (T15)(Fig.4). Sodium ions increased with an increasing salt concentration in salt stress or combined salt and heavy metal stresses. Maximum sodium was recorded in high salt (T3), combined high salt and moderate heavy metal(T12), and combined high salt and high heavy metal(T15)(Fig.4). Calcium ions showed variation in various levels of stresses. At low stress of salt(T1), low heavy metal(T4), and combined low salt and low heavy metal(T7) calcium level was increased while gradual decrease by increasing salt(T2&T3), heavy metals(T5&T6), and combined salt and heavy metals(Fig.4). Chloride content increased in all trials whether treated with salt, heavy metal or combined salt & heavy metal stresses. Maximum chloride content was observed in combined moderate salt and high heavy metal (T14)(Fig.4). Nickel concentration increased with increasing heavy metal in heavy metal stress or combined salt and heavy metal. Maximum nickel concentration was observed in high salt and high heavy metal (T15) along with moderate salt and high heavy metal(T14), low salt and high heavy metal (T13), and high heavy metal(T6)(Fig.4).



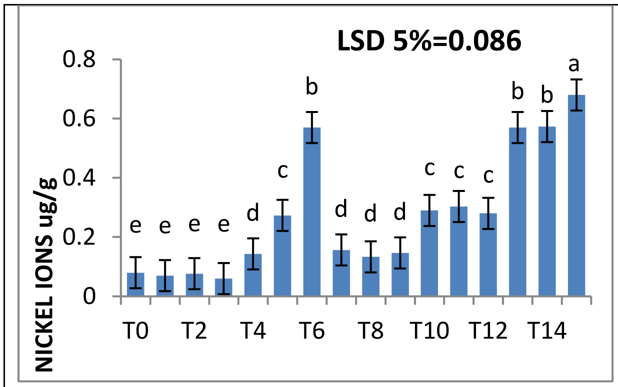


Fig.4. Effect of salt(NaCl), heavy metal ($NiCl_2$) and combined salt+ heavy metal ($NaCl+NiCl_2$) stresses on ions in shoot extract of *Citrullus colocynth*.

3.4 Physiological parameters

In comparison with control, the amount of chlorophyll a and b was significantly increased at low and moderate levels of salt, heavy metal, and combined low and moderate salt and heavy metals however a significant decrease in chlorophyll a and b was observed under the high level of salt (T3), heavy metal (T6) and combined high salt and heavy metal (T9) by increasing salt+ heavy metal stresses (T12, T14 & T15) (Fig-5).

It has been observed that the amount of free sugars showed variation in different concentrations of salt and heavy metals. A significant increase in sugars at a low stress levels of salt (T1), moderate level heavy metals(T5) and combined low salt and low heavy metal (T7), combined high salt and low heavy metal(T9), combined low salt and moderate heavy metal (T10), combined low salt and high heavy metal(T13), combined moderate salt and high heavy metal (T14) and combined high salt and high heavy metal (T15). Free sugars slightly decreased at high levels of salt (T3), high heavy metals (T6), and combined moderate metal and high salt (T12). A considerable decrease in sugars was recorded at moderate levels of salts (T2), low-level heavy metals (T4), combined moderate salt and low heavy metal levels (T8), and moderate salt and moderate heavy metal (T11). (Fig.5).

It has been observed that the amount of proteins significantly increased in moderate (T2)and high salt level (T3), combined low salt level and low heavy metal (T7), combined moderate salt and low heavy metal (T8), combined low salt and moderate heavy metal (T10) and combined moderate salt and moderate heavy metal(T11). There was a slight increase of proteins in a low level of heavy metal(T4), moderate levels of heavy metals (T5) combined low heavy metal and high salt(T9), combined high salt and moderate heavy metal (T12), combined low salt and high heavy metal (T13), combined moderate salt and high heavy metal(T14) and combined high salt and high heavy metal (T15). A decrease in proteins was recorded in low salt levels (T1)and high heavy metal (T6)(Fig.5).

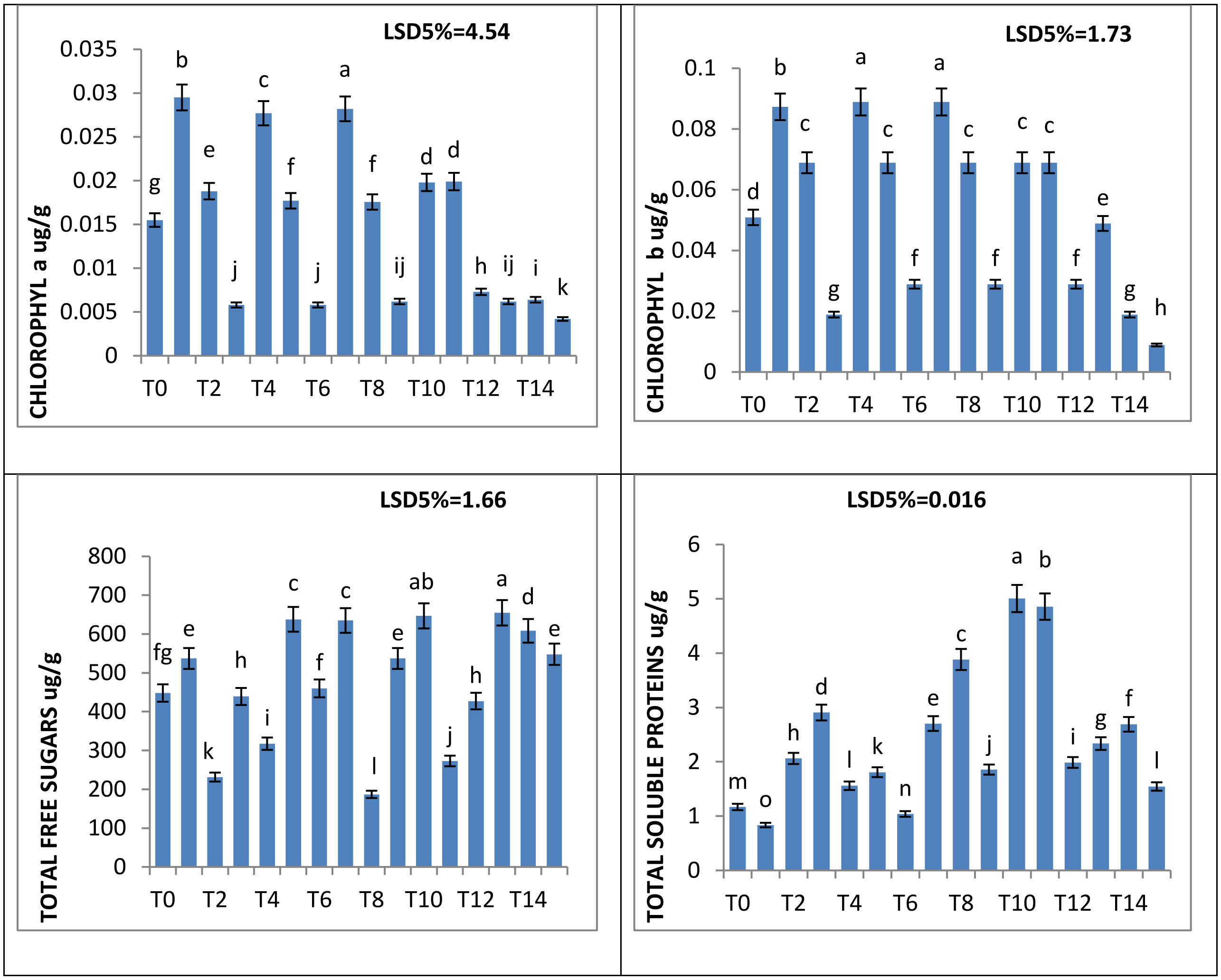


Fig.5. Effect of salt(NaCl), heavy metal ($NiCl_2$) and combined salt+ heavy metal ($NaCl+NiCl_2$) stresses on physiology in shoot extract of *Citrullus colocynth.*

Proline amplified employing the increasing salinity level as well as heavy metal. The maximum amount of proline was recorded in combined moderate salt and high heavy metal levels (T14) and the minimum level of proline was observed in a moderate level of heavy metal (T5), however the overall trend of proline was increased by increasing salt or heavy metal either single or combined(Fig.6).

Superoxide dismutase was increased in all levels of salt, heavy metal, and combined salt and heavy metals as compared to the control. A maximum increase in the amount of super oxidase dismutase was recorded in *Citrullus colocynth* grown under high heavy metal stress (T6). Catalase antioxidant enzyme activity increased with an increase in salinity level, heavy metal, and combined salt and heavy metal stress levels. A maximum increase in catalase was recorded in high heavy metal stress levels (T6)(Fig.6).

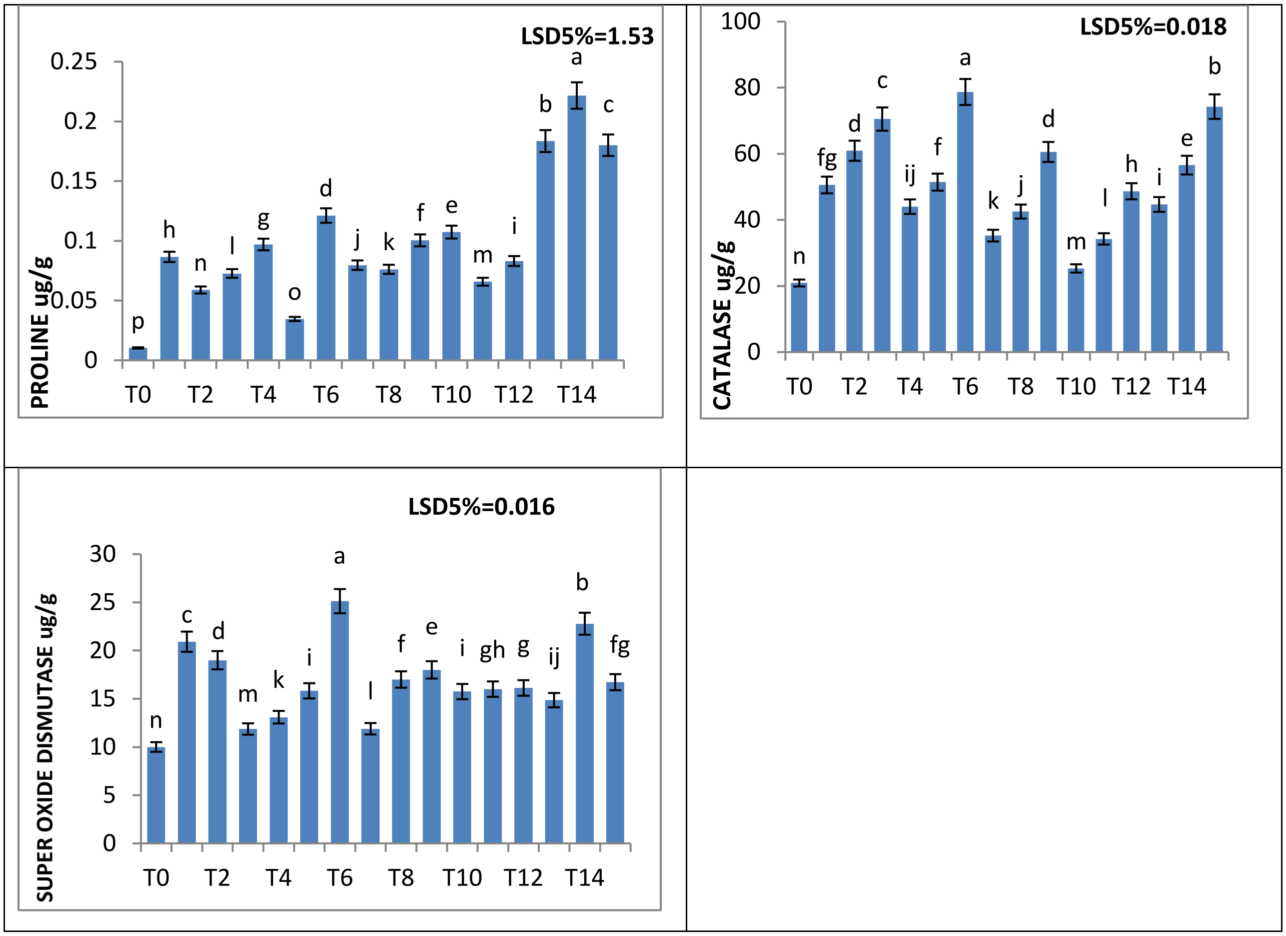


Fig.6. Effect of salt(NaCl), heavy metal ($NiCl_2$) and combined salt+ heavy metal ($NaCl+NiCl_2$) stresses on Proline, Catalase and Superoxide dismutase in shoot extract of *Citrullus colocynth*.

IV. DISCUSSION

Citrullus colocynth L. showed a positive response of seed germination, radicle and plumule length, fresh and dry weight of seedling at lower salt level(100mM NaCl), moderate salt level (200mM NaCl), lower heavy metal (50µM $NiCl_2$). Similar results were reported by Peralta *et al*., (2004) in *Medicago sativa* and this is due to absorption of more water and ionic balance for proper metabolism *(*Shultana, 2019). In present study, germination of *C.colocynth* was decreased in high level of salt (400mM NaCl), high level of heavy metal (200µM $NiCl_2$) and combined high salt level and high heavy metal level (400mM NaCl and 200µM $NiCl_2$) which is supported in mustard, Amaranthus and in *Vicia faba* because high levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity (Anaya *et al*., 2018; Emanuel *et al*., 2017; Jan *et al*., 2016).

Root length and shoot length of *C. colocynth* L. in the present study were highly retarded at high salt (400mM NaCl) and high heavy metal (200µM $NiCl_2$), also reported by many researchers (Alfaraas *et al*.,2016; Ain *et al*. (2016; Fatiha *et al*., 2019; Hussain *et al*., 2020). Excess salinity and heavy metals stresses minimize water uptake by the plant inducing negative impact on root length (Nikolic *et al*., 2008). Moreover salinity and heavy metal stress inhibit metabolic steps during cell division and elongation in high concentrations (Munns & Tester, 2008) main cause of which may be osmotic effect disturbed due to higher salts and heavy metals (Ahmad *et al*., 2020). Fresh and dry weight of *C.colocynth* in our studies was decreased at high salt (400mM NaCl), high heavy metal (20µM $NiCl_2$) and combined high salt and high heavy metal (400mM NaCl and 200μM $NiCl_2$). Our results are also supported by many reports like Chen *et al*. (2002), Pinera *et al*. (2016) and Yasmeen *et al*. (2018). Reduced root and shoot fresh weight was the result of decreased water intake, which as a result could cause decreased water content in plant tissue (Kahlon *et al*., 2018; Taghipour & Jalali, 2019). Sodium chloride absorbed in plants increased toxic ions concentrations resulting in disturbance of ionic balance of plant tissues (Rasheed *et al*., 2020).

Number and Leaf area of *C.colocynthis* L showed decrease in high salt (400mM NaCl), high heavy metal (200μM $NiCl_2$), combined high salt and low metal (400mM NaCl and 50μM $NiCl_2$). Similar findings have been reported by (Ain *et al*., 2016) and Kanwal *et al*. (2018). As the salt and heavy metal are accumulated in the root and shoot, leaf area cannot expand due to inhibited cell division (Ramezani *et al*., 2011). *C.colocynthis* L. revealed a considerable decrease in intermodal length, number of branches and number of flowers in high salt (400mM NaCl) and high heavy metal (200μM $NiCl_2$).This is similar to Kotagiri and Kolluru (2017) who noticed reduced plant growth under salinity stress. Reduction in leaves and flowers under salinity and heavy metals stresses also reported by Ain *et al*. (2016) and Khan *et al*. (2020).It may be due to accumulation of salt and heavy metal in shoot decreasing the cell division and elongation as studied in rice by Alizadeh *et al*. (2011). Branching of shoot depends upon mitotic activity in the meristematic cell which is disturbed by salinity and heavy metals (Gajewska *et al*., 2006).

C.colocynthis L. revealed a significant increase in Chloride contents at all levels of treatments as compared to control. High levels of chlorine are correlated with severe physiological dysfunction (Bazihizina *et al*., 2019; Ayub *et al*., 2020) positively in some plants and negatively in others (Kawtar *et al*., 2013; Bazihizina *et al*., 2019; Van zelm 2020). Potassium ions were increased at low salt level(100mM NaCl) and low heavy metal (50μM $NiCl_2$), while sodium ion was increased by increasing level of salt. Cytosolic homeostasis and ability of various plant tissues has been reported by retention of K^+ (Shabala & Pottosin, 2014).

C. colocynthis L. revealed significant decrease in chlorophyll a and b under high level of salt (400mM NaCl), high heavy metal (200μM $NiCl_2$), similar as photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of Nickel chloride studied by Islam *et al*. (2018). Molas (1997) also studied that effect of nickel in cabbage (*Brassica oleracea* L.) diminished the chlorophyll. There was gradual increase in proline by increasing levels of salinity and heavy metal stresses. Similar findings reported by Mehrian *et al*. (2015) that proline and free amino acids are increased in the plants under stress due to biosynthesis of amino acids and absence of translational factors. The accumulation of proline is also an adaptive response to salinity and heavy metal stress (Hayat *et al*., 2012). Salt tolerance is regulated by synchronized action of variable gene families involve in the initiation of variety of mechanisms such as water conservation strategies, the sequestration of toxic ions, adjustment of toxic metabolites and antioxidative defense (Gouveia *et al*., 2020). The elevated levels of salts cause reactive oxygen production (ROS) production including hydrogen peroxide, superoxide radicals and hydroxyl radicals (Luo *et al*., 2021).

It has been observed in our experiment on *C. colocynth*, antioxidant enzyme activities increased with the increasing level of salinity level as well as heavy metal. Superoxide dismutase was increased in all levels of salt, heavy metal and combined salt and heavy metals as compared to control. Maximum increase in amount of Superoxide dismutase was recorded in plants grown under in high heavy metal stress (200μM $NiCl_2$). Catalase antioxidant enzyme activities increased with increase in salinity level, heavy metal and combined salt and heavy metal stress levels. Maximum increase in catalase was recorded in high heavy metal stress level (200μM $NiCl_2$). Oxidative stress in salt tolerant plants was directly related with characters such as catalase and superoxide dismutase activity. Augmented commotion of the enzymes like CAT, SOD at higher salinities showed significant correlation between plant tolerance level and these antioxidant systems (Ashraf & Harris, 2004; Hussain *et al*., 2016). Metal toxicity is allied with oxidative stress indicated by the boost in quantity of hydroxyl radicals (OH^-), superoxide dismutase and catalase (Hao *et al*., 2006).

V. CONCLUSION

Citrullus colocynth L. can tolerate the stresses exerted by salinity and heavy metals either single stress or combined stress. Lower levels of stress positively affect on *Citrullus colocynth* L. by enhancing its morpho-physiological parameters. However higher stress levels adversely affect the germination, morphology, and physiology of plant *Citrullus colocynth* L. Lower levels of salts (100mM) and Nickel (50uM) were beneficial for the growth parameters. The plant also showed relatively better growth in moderate salt NaCl (200mM) and Nickel (100uM) as compared to the control . At higher concentrations of salt NaCl (400mM) and $NiCl_2$ (200uM) growth parameters were reduced. However, plant remained alive under high levels of stress which shows its tolerance. Production of ROS like superoxide dismutase (SOD) and catalase (CAT) indicates its strategy for the amelioration of negative effects of salts and heavy metals. Fewer proteins and a high level of amino acids also indicate its mechanism of salt and heavy metal tolerance. *Citrullus colocynth* L. is suggested for removal of salt and heavy metals from contaminated soils. It may be low-cost management for the rehabilitation of polluted soils with salts and heavy metals.

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Conflict of interest

Authors have no conflict of interest.

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