IMPACT OF HEAVY METALS AND SALT STRESS ON *BRASSICA NAPUS* GROWTH AND DEVELOPMENT

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Abstract- Plant Stress refers to external conditions that negatively affecting growth, structure and yields of plants. Stress activate a wide range of plant responses like harmonal gene expression, cellular metabolism, altered in growth level, crop productivity, etc. The experiment was performed to determine the effect of heavy metals (Cadmium, Chromium and lead) and Salt (NaCl) stress on *Brassica Napus* growth and development. The seeds of *Brassica Napus* were treated with heavy metals and salts. Heavy metals and salt stress caused reductions in growth parameters (GP, SL, RL, FW, DW, RWC, VI, RSR, SWR, RWR). It has been concluded that heavy metals and salt stress negatively affected the growth of *Brassica Napus*

Index Terms- Plant stress, plant response, yield, hormonal gene expression, cellular metabolism, heavy metal stress, salt stress

I. INTRODUCTION

Plant stress mentioning to an outer environmental condition that harshely affecting plant physiological processes, growth, and plant productivity. Stress trigger a wide range of plant responses like altered gene expression, cellular metabolism, changes in growth rates, crop yields, etc. A plant stress usually reflects some sudden changes in environmental condition. However, in stress tolerant plant species, exposure to a particular stress leads to acclimation to specific stress in a time dependent manner **(Verma, Nizam *et al.*, 2013).** Salinity is one of the major abiotic stresses, badly affecting crop growth, particularly in arid and semi-arid regions of the world **(Ashrafi *et al.*, 2018)**.*Brassica Napus* is an annual to biennial herb, cultivated worldwide, and mostly adapted to temperate climates **(PROTA, 2018)**. *Brassica Napus* is cultivated and also has naturalized in 3disturbed habitats in temperate areas and tropical highlands **(PIER, 2018 & PROTA, 2018)**. It is reported as occurring in croplands, weedy fields, road sides, gravel areas along railroads, waste areas, pastures, open forests, orchards, gardens and riverbanks. *Brassica Napus* is listed as being a host of various phyto-plasmas that affect important crops and are transmitted by phloem tapping insects **(Casati *et al.*, 2016)**. *Brassica Napus* is reported as flowering between January to September, depending on climate and latitude **(Encyclopedia of Life, 2018)**.

Brassica Napus is mostly grown in temperate areas with mean annual temperatures of 5.6-25°C with a mean annual precipitation of 350 to 1600 mm **(PIER, 2018)**. The species survives early frosts, but temperatures below 3.8°C will kill most cultivars. *Brassica Napus* is one of the oldest Brassicaceae in cultivation **(PROTA, 2018)**. The products of various cultivars are sold in markets worldwide; for example, turnips, Chinese cabbage and pakchoi. The fruits are sold at markets in Mexico as bird food **(Vibrans, 2018)**.The oils from *Brassica Napus* seeds are used as cooking oil, industrial lubricant, lamp oil, for soap making and for biodiesel production. Brassica Napus is widely cultivated for its roots, leaves, seed oil and as a fodder **(PFAF, 2018)**. The flowers of some varieties are also consumed. The taproot (turnip), the leaves and flowers are used as a vegetable, boiled, fried, pickled or eaten as salads **(PROTA, 2018)**. Extracts of the plant are also used as insecticide against aphids, red spider mites and flies **(PFAF, 2018)**.

The effect of the aqueous extract of *Brassica Napus chinensis* was studied against bleomycin (BLM) induced pulmonary fibrosis. Aqueous extract of Brassica Napus chinensis 250 and 500 mg/kg showed significant protective effect against BLM induced pulmonary fibrosis in rats by normalizing the levels of glycoproteins (hexose, hexosamine and sialic acid) and improving the1ctiveity of Catalase (CAT) and Superoxide dismutase (SOD). The extract also improved pulmonary glutathione (GSH) content and depleted the lipid peroxidation levels in a dose dependent manner. The histo-pathological analysis also reveal the reversal of the lung architecture to near normal upon administration of plant extract. The pre-treatment of rats with *Brassica Napus* juice protected the rats against CCl4-induced hepatotoxicity. The treatment significantly reduced the serum GOT, GPT, alkaline phosphatase (ALP) and bilirubin level at a dose of 16 ml/ kg. The aim of this study was to ascertain the effects of salt and heavy metal stress on seed germination and seedling growth of *Brassica Napus*.

II. MATERIAL AND METHODS

Before conducting experiment petri plates along with filter paper were sterilized with the help of autoclave for one hour and then the seeds of *Brassica Napus* were surface sterilized, with a (1%) mercuric chloride solution for one minute and then washed three times with distilled water.

For conducting an experiment the petri dishes were arranged with 10 seeds (*Brassica Napus*) in each petri dish, treated with 3 mm concentration of different solutions.

The petri plates were kept at room temperature for seed germination and the reading of germinated seeds were recorded after every 24 hours. After 7 days, the experiment was terminated and Shoot length (SL), root length (RL) were measured while seedlings fresh weight (FW) and dry weight (DW) were taken. RWC, vigor index (VI), root shoot ratio (RSR), stem weight ratio

(SWR) and root weight ratio (RWR) were calculated as per given formula:

Germination percentage = (no. of germinated seed / total no. of seed) x 100

Vigor index = (mean of root length + mean of shoot length) x germination percentage

RWC = (fresh weight – dry weight) / fresh weight x 100

RSR = root dry weight / shoot dry weight

SWR = shoot dry weight / total dry weight

RWR = root dry weight / total dry weight

Collected data was analyzed statistically by using SPSS to analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($P < 0.05$).

III. RESULT

Present study showed that the (GP, SL, RL, FW, DW, RWC, VI, RSR, SWR, RWR) is negatively affected by salinity and heavy metal stress.

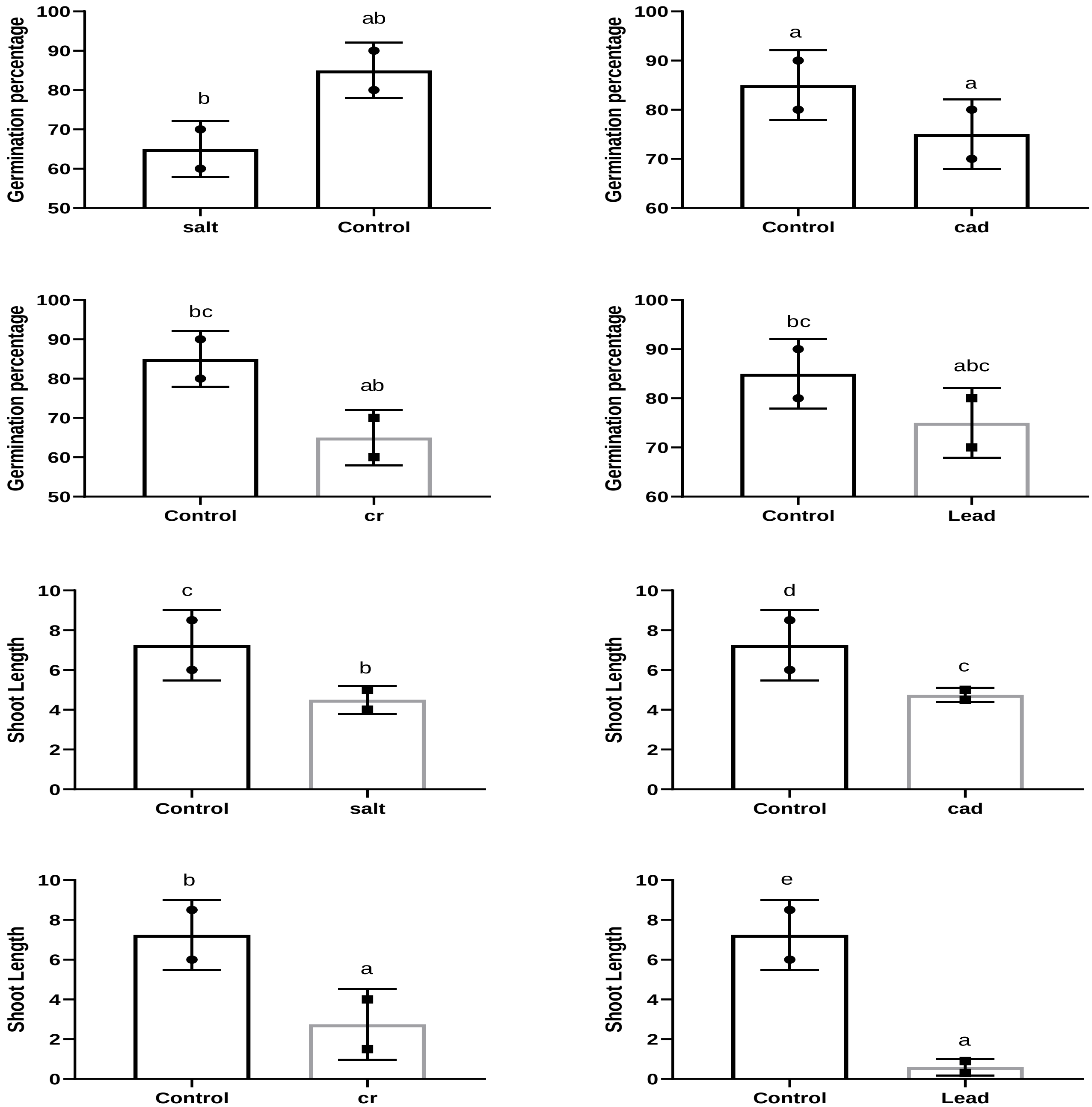

Fig. 1. Effect of heavy metals and salt stress on germination percentage (%), shoot length (cm) of *Brassica Napus*. Different letters on each bar show significance of mean at P<0.05

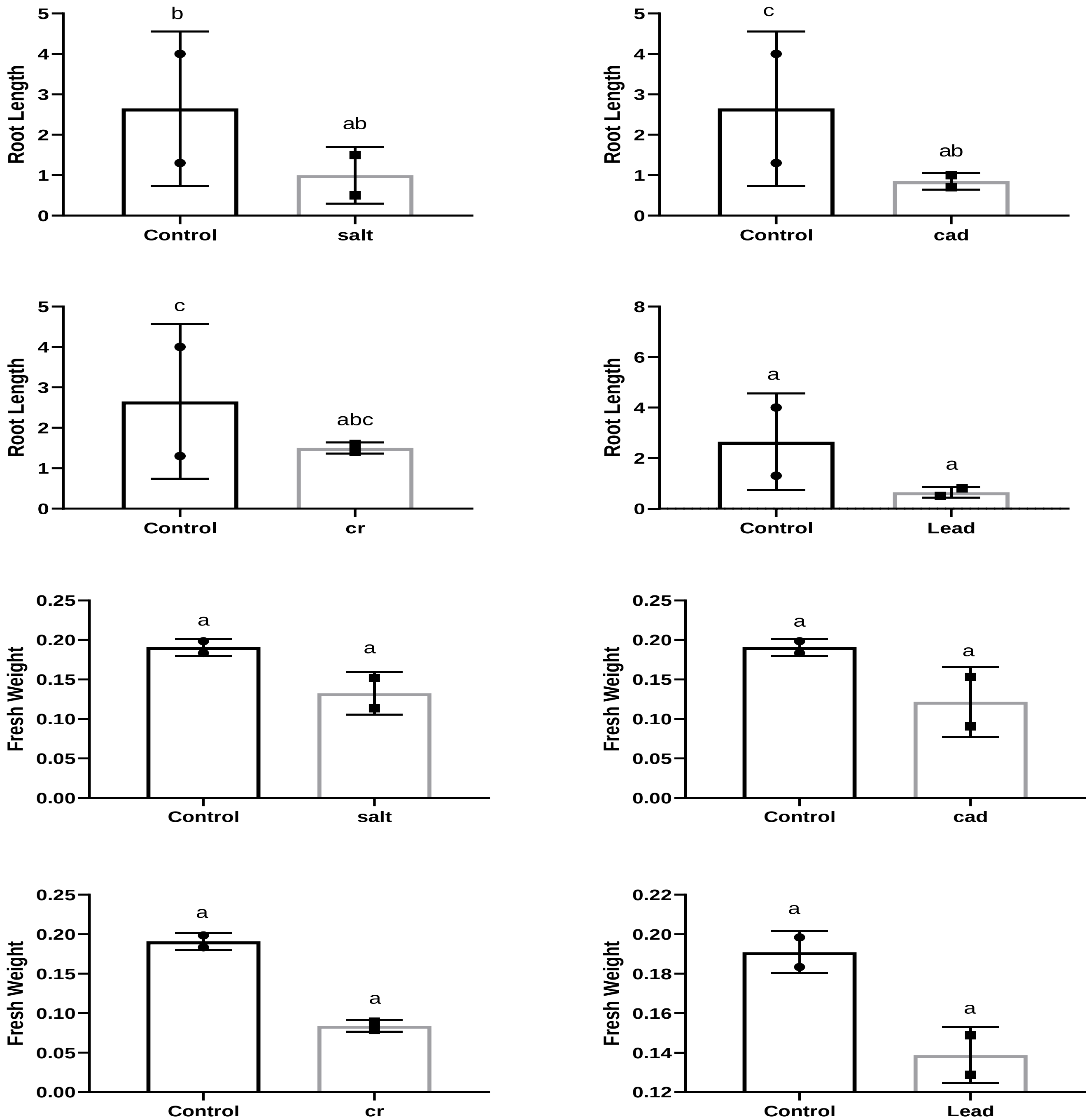

Fig. 2. Effect of heavy metals and salt stress on root length (cm), fresh weight (g) of *Brassica Napus*. Different letters on each bar show significance of mean at P<0.05

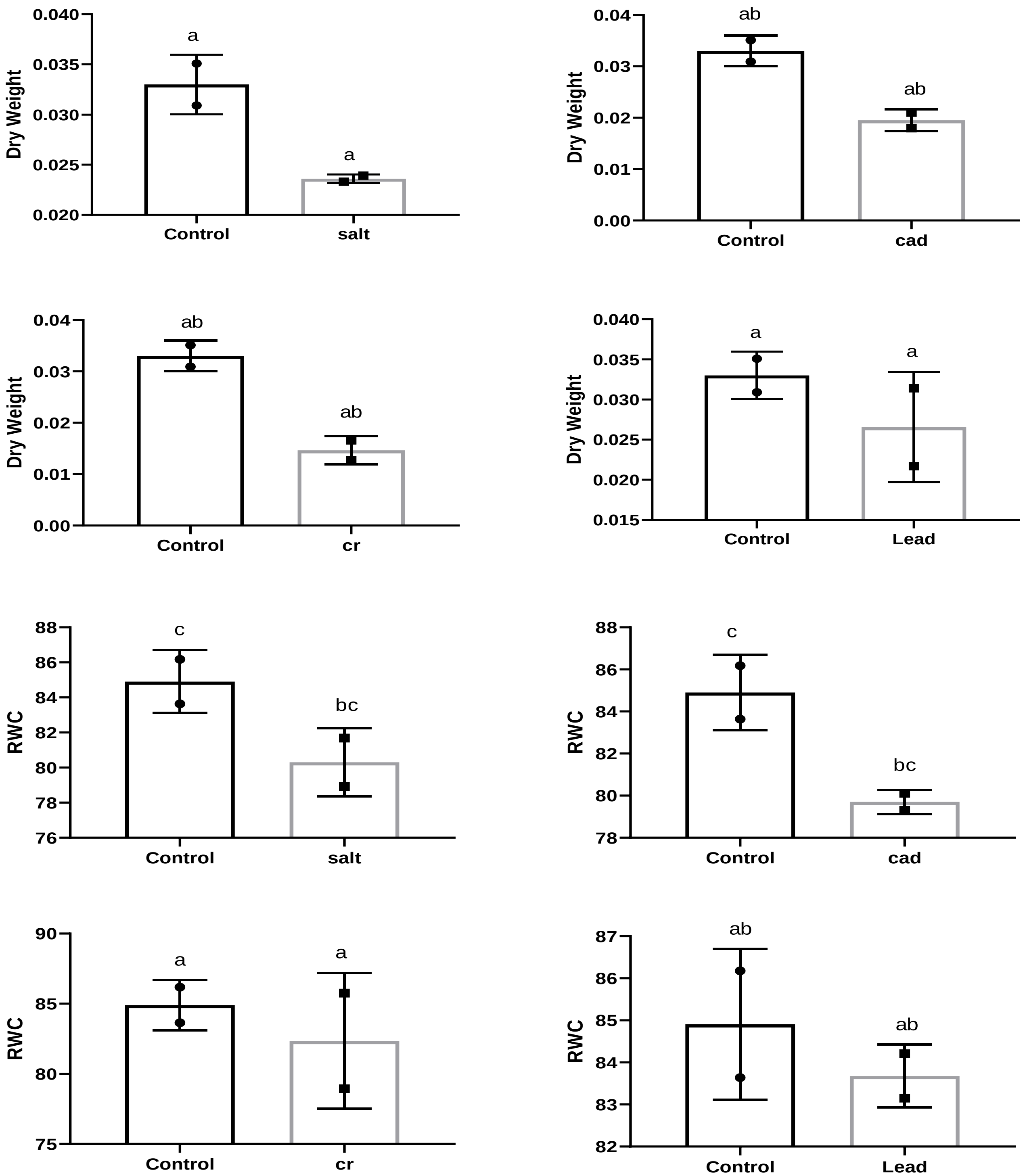

Fig. 3. Effect of heavy metals and salt stress on dry weight (g) and relative water content of *Brassica Napus*. Different letters on each bar show significance of mean at $P<0.05$

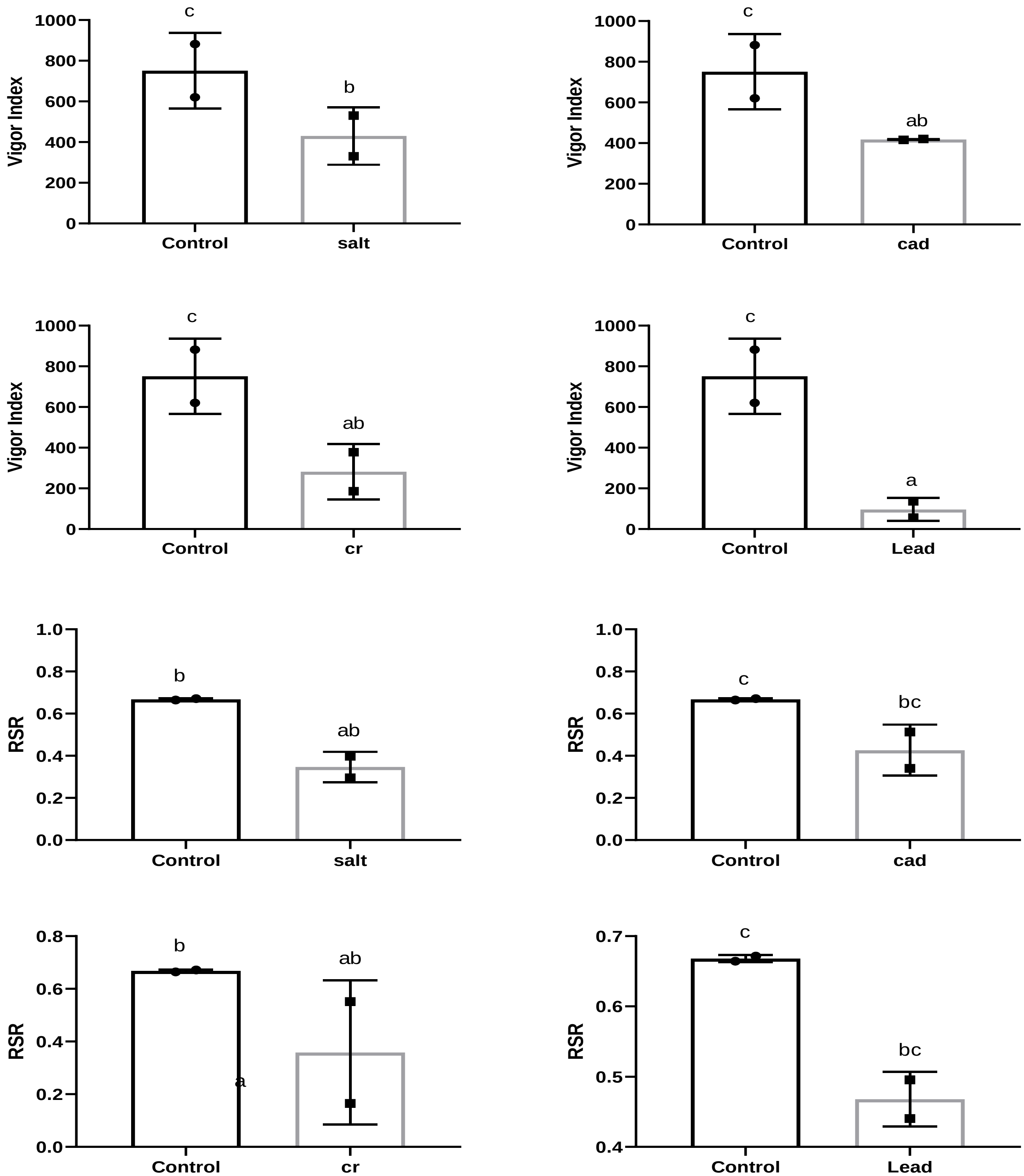

Fig. 4. Effect of heavy metals and salt stress on vigor index and root shoot ratio of *Brassica Napus*. Different letters on each bar show significance of mean at $P<0.05$

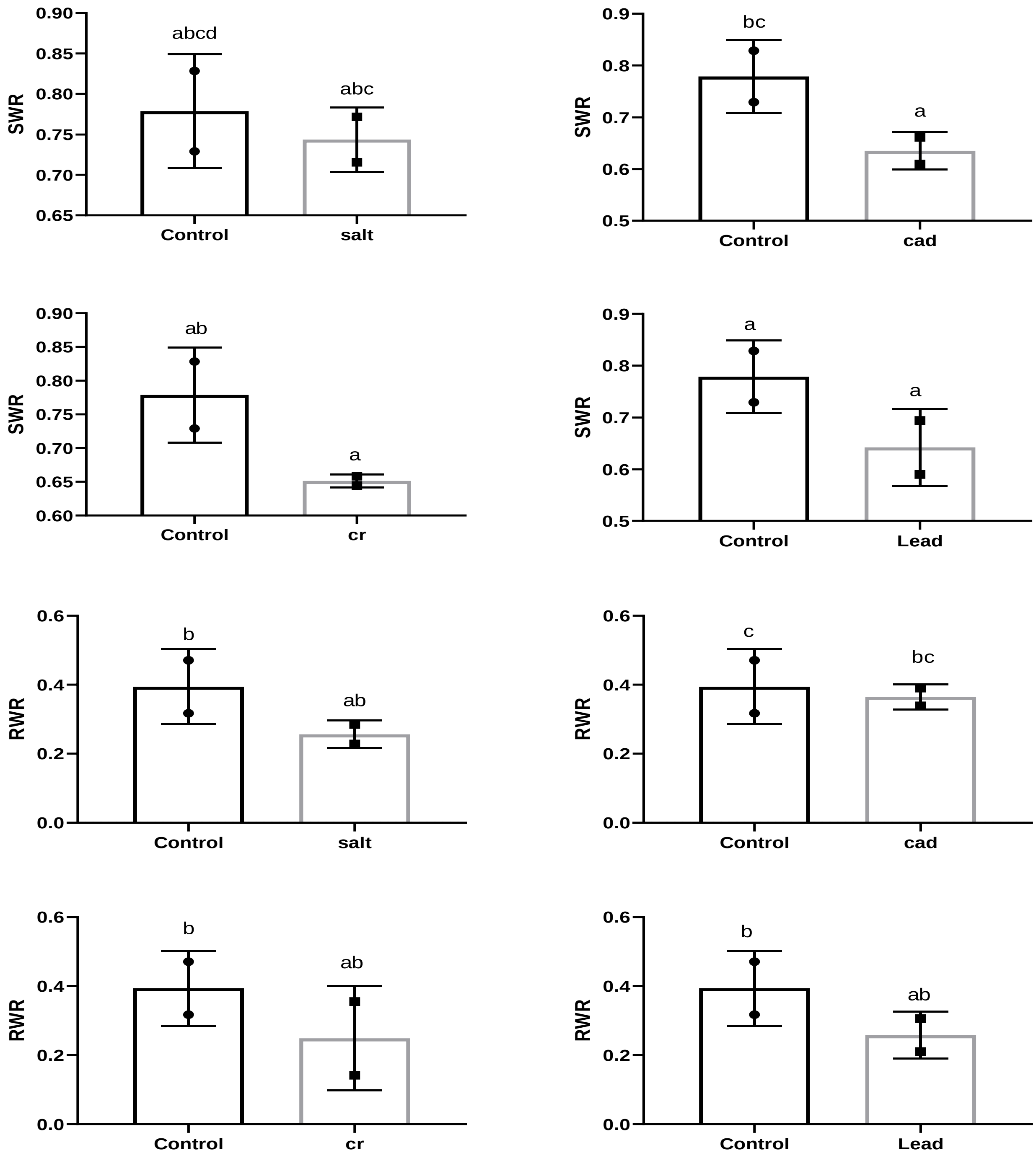

Fig. 1. Effect of heavy metals and salt stress on stem weight ratio and root weight ratio of *Brassica Napus*. Different letters on each bar show significance of mean at P<0.05

IV. DISCUSSIONS

Similar results were reported by **Theriappan *et al.* (2011)** stated that NaCl has inhibitory effect on the growth and germination of seeds of higher plants. **Begum *et al.* (1992)** stated that the deleterious effects of NaCl on seed germination and growth is due to osmotic stress, ion toxicities, ion imbalance or the combination of these factors. Our result showed similarity with the findings of **Shahid *et al.*, 2015; Chigbo & Batty, (2013)** described that increasing concentration of Cr reduced the germination rate of L. perenne, and this reduction was observed at more than 9.4 ppm Cr concentration. The reduced germination rate was related to the restricted oxygen uptake and physiological disorders in the supply of food reserves. Our result showed similarity with the findings of **Shahid *et al.*, 2015; Kranner & Colville, (2011)** stated that Cad reduced seed germination because Cd hamper water uptake and consequently seed germination does not take place. Our result showed similarity with the findings of **Chen et al. (2018)** analyzed that significant decreases in all germination parameters of cauliflower seeds were observed under 0.25 and 0.5 mM Pb stresses, whereas the inhibition was mitigated by the applications of NaHS, an H2S fast releaser. Similar results were also reported by **Asif et al. (2020)** described that Shoot length is negatively affected under saline conditions in comparison with control. **Munns, (1992)** revealed that old leaves senescence is encouraged by excessive salt accumulation which reduces carbohydrates supply along with PGR's to new growing regions thus affecting overall growth and minimizing growth. Our result showed similarity with the findings of **Dogan, (2019)** stated that increases in Cd concentrations in terms of the number of shoots per explant have more inhibitory effect than Cr and Pb. Cd is a non-redox metal and causes ROS directly via Haber–Weiss actions. Due to the toxic effect of Cd, excessive ROS production and oxidative stress occur in plants. Our results showed similarity with the findings of **Singh *et al.* (2015)** evaluated that significant ($P>0.05$) decline in growth parameters of the shoot length might be due to rise in Pb (at only 300 μM Pb) and Cr contents in cell wall components, consequently hindering cell division by inducing chromosomal aberrations and decreasing cell elongation, thus affecting the plant growth and development. Our result showed similarity with the findings of **Bah *et al.* (2011)** stated that Cr treatments caused significant reduction in root length. This reduction is related to high Cr concentration in plant tissues, so the plant utilize more energy to cope with the high Cr concentrations. Our result showed similarity with the findings of **Ahmad *et al.* (2005)** stated that Cadmium treatment of 50 Bg/g of soil had negative influence on root length than lead at 200 tig/g of soil. Root and dry weight of investigated species exhibited marked decline under highest cadmium treatment of 50 tg/g of soil in comparison to highest lead treatment of 200 Bg/g of soil. Result showed similarity with the findings of **Hao *et al.* (2015)** explained that lead stress caused a significant inhibition on wheat root growth, including the root length because high concentrations of lead resulted in the inhibitory effect, of root length. Our result showed similarity with the findings of **Farooq *et al.* (2016)** described that Fresh weight decreased under Cd stress. Decrease in plant growth and fresh biomass might be due to Cd-induced toxicity on photosynthetic apparatus **(Saidi *et al.*, 2013)**. Our result showed similarity with the findings of **Zaheer *et al.* (2020)** stated that Cr stress negatively affects the plant fresh weight of different plant species which depends upon a number of factors including plant species, dose, and duration of Cr application. Our result showed similarity with the findings of **Gupta *et al.* (2009)** analyzed that Pb treatment reduced the fresh weight and dry weight in comparison to control. Similar results were also reported by **Weisany *et al.* (2011)** stated that RWC significantly decreased with increasing salt stress. when plants were subjected to different salt treatments along with zinc, the relative water content significantly improved. Our result showed similarity with the findings of **Yuce *et al.* (2019)** determined that Cr stress cause damaging effects on relative water content. The high concentration of Cr in the Phaseolus vulgaris affected plant growth, reduced chlorophyll and sugar contents **Zeid, (2001)**. Our result showed similarity with the findings of **Alzahrani *et al.* (2020)** showed that Relative water content (RWC) in D. variabilis was found to decrease under Cd and Pb treatments, which confirmed our previous findings **(Qureshi *et al.*, 2005; Hakeem *et al.*, 2019)** in Pb and Cd stressed plants. This is due to stimulation of HM stress caused closure of stomata **(Brunet *et al.*, 2008)**. Similar results were also reported by **Shah *et al.* (2021)** described that vigor index was significantly reduced by saline stress due to osmotic and ion toxicity **(Parida & Das, 2005)**. Our result showed similarity with the findings of **Amin *et al.* (2014)** stated that chromium metal adversely affects the vigor index of G. Max. The toxic effect of chromium on the seeds increased with increasing the concentration of the metal. Our result showed similarity with the findings of **Espanany & Fallah, (2016)** stated that Increasing cadmium concentration causes a significant decrease ($P<0.01$) in dill vigor index. Our result showed similarity with the findings of **Tabatabaei & Ansari, (2019)** stated that increase in Pb stress, causes reduction in vigor index. Similar results were also reported by **Akram *et al.* 2007)** stated that Plant roots are the first organ to be exposed to salinity, and root growth is particularly sensitive to increase in salt concentration of medium, that's why roots are rapidly reduced or prevented by salinity **(Cramer *et al.*, 1988)**. Under saline conditions, depletion of O_2 deprives the plants of its primary energy source and accumulation of high levels of internal ethylene cause the inhibition of root elongation **(Koning & Jakson, 1979)** by reducing root growth. High salt concentration in nutrient medium cause stunted growth in plants **(Hernandez et al., 1995; Cherian et al., 1999; Takemuraet al., 2000)**. Salinity reduced plant growth either by increasing plant osmotic potential or specific ion toxicity **(Dionisio-Sese & Tobita, 2000)**. Our result also showed similarity with the findings of **Bah *et al.* (2011)** described that 1 mM Cd or Pb induced slight or even significant decrease in plant height and root/shoot dry weight especially in Pb treatment, demonstrating its hypertolerance to Cd and Pb stress. Cr treatment caused significant reduction in the plant height and dry weights of both the plant parts. Similar results were also reported by **Yousefi *et al.* (2017)** stated that Increase in salinity level from 10 to 50 dS/m led to a drastic decrease in measured traits of control samples, including root and stem length as well as their fresh and dry weights, whereas the seeds inoculated with A. lipoferum and Azotobacter + Azospirillum bacteria showed higher values of the mentioned traits even at 20 dS/m salinity level. Although hopbush seeds of the control treatment could germinate at 50 dS/m salinity level, root and stem growth were completely halted at salinity levels above 15 dS/m. This is while the treatments containing seeds inoculated with A. lipoferum and Azotobacter + Azospirillum bacteria could, in addition to germinating at up to 50 dS/m salinity levels, bring about root and stem growth at salinity levels of 20 and 50 dS/m. The reduction of hopbush species' root and stem growth under salinity stress can be attributed to reduced root colonization and weak nutrient absorption. Our result showed similarity with the findings of **Ehsan *et al.* (2014)** stated that cad stress reduced stem weight ratio. Cadmium supply reduced plant growth attributes and biomass. It is previously reported that excess Cd reduced plant growth and biomass by reducing mineral uptake and upsetting the biochemical and metabolic processes **(Gill *et al.*, 2011)**. Results showed that stem and root length reductions were detected more in Zheda 622 than in any other cultivar. The suppression of root organs might be due to cell division reduction in roots caused by metal toxicity **(Dey *et al.*, 2009; Ali *et al.*, 2013)**. Interestingly, we noticed that the percentage of root length deterioration was less than stem length deterioration in ZS 758. This is a clear indication that this cultivar has more tolerance/scavenging mechanisms against Cr-induced stress. Our result showed similarity with the findings of **Bharwana *et al.* (2014)** stated that Pb inhibited soluble protein contents in cotton plants in both roots and stems. This might be due to more oxidative damage occurring under Pb stress conditions that decreased the protein contents **(Gupta *et al.*, 2009)**. Similar results were also reported by **Akram *et al.* (2007)** described that Plant roots are the first organ to be exposed to salinity, and root growth is particularly sensitive to increase in salt concentration of medium, that's why roots are rapidly reduced or prevented by salinity **(Cramer *et al.*, 1988)**. The immediate response of salt stress is reduction in rate of leaf surface expansion **Wang & Nil, (2000)** and decrease in the fresh and dry weights of leaves, stem and roots **(Hernandez *et al.*, 1995; Ali Denar *et al.*, 1999; Chartzoulakis & Klapaki, 2000)**. Salinity reduced plant growth either by increasing plant osmotic potential or specific ion toxicity **(Dionisio-Sese & Tobita, 2000)**. In the present study, a significant decrease in shoot length, fresh and dry weights of shoot of all the hybrids was noted with the increase in salt concentration of medium. Our result showed similarity with the findings of **Gangwar & Singh (2011)** stated that Cr application cause reduction in root weight ratio. We reported that Cr and 100 M IAA alone as well as in combination decreased seed germination rate and growth of the pea seedlings which was accompanied by increased accumulation of Cr in roots and shoots. Exposure of the pea seedlings to Cr and IAA (250 M Cr + 100 M IAA) led to a 56% decrease in seed germination rate, 68 and 62% in root and shoot fresh mass and 60 and 54% in length of root and shoot, respectively, compared to control. Roots and shoots highly varied in their ability to accumulate Cr. Data of accumulation of Cr showed concentration dependent increase in Cr content in roots and shoots, however, Cr content was not detected in pea seedlings grown in control, 10 and 100 M IAA alone treatments. Our result showed similarity with the findings of **Nikolic *et al.* (2008)** described that Cd stress reduced root weight ratio. root growth apparently was less affected than leaf and stem growth. Root fresh mass decreased by ~44%, but shoot (stem + leaves) mass by more than 60%. The lower susceptibility of roots might be explained by accumulation of the metal in inactive form or else by mobility of Cd within the plant, facilitating its translocation to aerial parts **(Barceló *et al.*, 1988)**. **Titov *et al.* (1996)** reported higher sensitivity of roots in HM-treated wheat and barley seedlings. In maize cultivars, increased Cd in nutrient solution significantly reduced the dry weight of both leaves and roots **(Ekmekçi e*t al.*, 2008)**. Our result showed similarity with the findings of **Azmat *et al.* (2009)** stated that Pb stress cause reduction in root weight ratio. Results reported showed considerable reduction due to toxic metal Pb on relative growth rate, biomass production and water contents of Phaseolus mungo and Lens culinaris as reported earlier **(Godbold & Kettner, 1991)**. Fresh weight of the plant was affected by the Pb. The highest fresh weight production in leaves of Phaseolus mungo compared to Lens culinaris indicated that Phaseolus mungo was more tolerant as compared to Lens culinaris whereas fresh weight of both species showed acute toxicity of Pb on root and shoot. Relative growth rate of stress plants was considerably lower than that of none treated plant which may be attributed with the reduction in the root and shoot length. This results in growth inhibition, ratio of dry and fresh weight of total plant DW/FW.

V. CONCLUSION

The seeds of *Brassica Napus* were treated with different heavy metal and salt stress concentrations. All treatmentents showed reduction in *Brassica Napus* growth. It has been concluded that heavy metals and salt stress has negative impact on the growth of *Brassica Napus*

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