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Influence Of Seed Priming With $FeSO_4$ On Germination, Growth And Biochemical Aspects Of Mung bean (*Vigna Radiata* L.) Grown Under NaCl Stress

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Abstract

Salinity is the major abiotic factor that reduced the plant germination percentage, growth and productivity. However, micronutrients have an important role to reduce the salt stress effectively. This study was conducted to investigate the role of Fe via seed priming of *Vigna radiata* L. with two concentrations (100ppm & 200ppm) of $FeSO_4$ and grown under various levels (0-50-75-100mM) of NaCl. The results showed that NaCl stress reduced the germination percentage of *Vigna radiata* but seed priming with $FeSO_4$ improves the germination percentage. The seed priming with 100ppm showed maximum values of germination percentage (96.66±0.23), shoot length (37.84±0.08 cm), shoot fresh weight (26.87±0.067) and dry weight (9.05±0.08), root length (23.27±0.020), fresh weight of root (5.17±0.031), root dry weight (2.48±0.06), Proline (65.30±0.24) were observed. While 200ppm showed significantly maximum values of chlorophyll a& b contents, total soluble protein (0.372±0.18), Phenolic contents (95.57±0.12), and flavonoids (84.26±0.17). Seed priming with $FeSO_4$ has significant effects on the *Vigna radiata* L. under NaCl stress and improves the germination, growth, and biochemical parameters.

Key Words; *Vigna radiata*, Seed priming, $FeSO_4$, Proline, Phenolic, Flavonoids

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1. Introduction

The Mung bean (*Vigna radiata* L) commonly known as moong belongs to family Fabaceae and genus *Vigna*. The family is generally known as legume and pea family. The *Vigna radiata* is a summer season crop that is highly nutritive. (Islam *et al*., 2012). Mung bean in Pakistan is being cultivated on 146,000 hectares with annual 98,000 tons of production, marginal yield per unit area (Hanif *et al*., 2013). The uniqueness of the *Vigna radiata* is the root nodules that contain aerobic bacteria known as rhizobia. Which role in the fixation of atmospheric nitrogen in roots and increase soil fertility. (Ashraf *et al*., 2003). The mung bean also has a medicinal roles like in cancer prevention. It also exhibits insecticidal and antimicrobial activities. (Samreen *et al.,*2017). The *Vigna radiata* L. is a vital source of protein and has a significant part of the diet in many developing countries. The Vigna radiata contains 51% carbohydrates, 26% protein, and 3% vitamins. (Anjum *et al.,*2006).

The salinity is the major abiotic environmental factor that decreased crop growth and productivity. Salinity causes oxidative stress and changes the physiological and chemical behaviors of plants. Salt stress adversely affects the chlorophyll contents and membrane stability. (Parida *et al*., 2002). Due to an increase in salinity levels water and nutrient stress occur. The higher sodium concentrations prove toxic effects and cause physical damage to roots and decrease the water plus nutrient adsorption which causes significant reduction in photosynthesis, enzymatic process and protein synthesis (Tester and Davenport, 2003). Seed germination is negatively affected by salinity stresses (Zhu, 2002).

The supply of micronutrients is essential for efficient growth and yield of crops. However, micronutrient deficiencies in the soil are common and cause a problem in developing countries. (Singh, 2009). The influence of seed priming on crop establishment has been studied extensively. Generally, priming with the micronutrients improved seedling establishment. The iron is an important micronutrient that has a vital role in the structure of enzymes involved in the synthesis of amino acids. Iron is an important micronutrient for living organisms because iron has a vital role in DNA synthesis, photosynthesis, respiration. It activates many metabolic pathways. It is the component of many enzyme like cytochromes. (Rout *et al.,*2015).

Iron catalyzes the unique biochemical reactions and iron is a cofactor of almost 140 enzymes. Iron plays a vital role in plants like chlorophyll synthesis and the development of chloroplast. While the deficiencies of iron cause chlorosis and about 30% of crops are affected. (Sharma *et al.,*2003). The objectives of this study are to investigate the influence of seed priming with $FeSO_4$ on germination, physiological and biochemical attributes of *Vigna radiata* L. grown under various levels of NaCl stress.

2. Research Methodology

The seeds of *Vigna radiata* L. (cv. Chakwal) were collected from National Agriculture Research Center Islamabad.

2.1 Experimental Design

The seeds were surface sterilized with ethanol (70%) for 30 seconds then seeds were washed with distilled water. The seeds were primed with two concentrations (100ppm and 200ppm) of $FeSO_4$ for 1-hour and then seeds were dried. Later the seeds were grown under various levels (0mM, 50mM, 75mM and 100mM) of NaCl. The experiment was placed with 3 replicates and total of 36 pots were used. The germination percentage was observed after 24 hours and for a further 7 days to record a constant percentage of germination.

The germination percentage was calculated with the following formula;

Germination %= Number of germinated seeds in each Petri plate/ Total No. of seed sown in each petri×100

After harvesting the plant, length of shoot and root were measured separately with measuring tape, while a fresh and dry weight of shoot and root of *Vigna radiata* plants were measured separately with measuring balance.

2.2 Chlorophyll Contents determination

The chlorophyll contents were determined using the protocol of (Arnon, 1949). The following formula was used for final calculation;

Chl a = 12.7 x OD663 -2.69 x OD645

Chl b = 22.9 x OD645 - 4.68 x OD663

2.3 Biochemical Analysis of Plants

The proline content of leaves was determined by the protocol of (Bates *et al.*, 1973) and by using the method of (Singleton and Jones, 1999), Folin Ciocalteu with Gallic acid standard the Phenolics content was determined. The flavonoids content was determined by using the $AlCl_3$ protocol (Zhishen *et al.*, 1999).

The total soluble protein was determined by using the protocol of (Lowery *et al.*, 1951) and the final calculation was done by using the following formula;

Protein content (mg / g) = K value × Absorbance × Dilution Factor/ Weight of sample

K value = 19.6

3. Results and Discussion

Germination %

The results mentioned in the table-1 showed that salt stress strongly influenced the germination percentage of *Vigna radiata* L. It was observed that germination percentage strongly reduced under salt stress and germination percentage is inversely proportional to salt stress. When NaCl increased from 0mM to 100mM then germination percentage significantly ($P<0.05$) reduced. The reduction in germination under salt stress was also reported by (Bojovic *et al.*,2010; Bajehbaj *et al.*,2010). The comparison of both (100ppm and 200) concentrations showed that maximum significant ($P<0.05$) germination percentage (96.66±0.23) was recorded at 100ppm under 0mM NaCl and lowest germination percentage (65.66±0.36) were recorded in control plants. Salt stress reduced the germination either by osmotic pressure or by injurious effects of sodium and chloride ions. Salinity is the major abiotic factor reducing crop production throughout the world. (KhajehHosseini *et al.*, 2003). While the seed priming enhances many processes related to germination as the activities of germination enzymes which are crucial for breaking macromolecules essential for improving growth under salinity. Seed priming can regulate germination related genes (Ali *et al.*,2017). This study showed that the seed priming with $FeSO_4$ improved the germination percentage and priming with 100ppm $FeSO_4$ promote germination more than 200ppm. The positive effects of Fe on germination were also reported by (Nozoe *et al.*,2009).

Table 1. Effects of Seed priming with $FeSO_4$ on germination of *Vigna radiata* L. grown under various levels of NaCl (Mean values± S.E, n=3)

Treatments	0 mM	50 mM	75 mM	100 mM
Control	85.71±0.000	76.18 ±4.76	71.42± 0.00	65.66 ±4.76
$FeSO_4$ 100ppm	95.23± 4.76	90.47± 4.76	80.94± 4.76	76.18 ±4.76
$FeSO_4$ 200ppm	90.47 ±4.76	85.71± 0.000	80.94 ±4.76	71.42± 0.00

Shoot length (cm)

The fig.1-A showed that salts stress adversely affects the shoot length. It was observed that the shoot length of the *Vigna radiata* was inversely proportional to NaCl. As with the increased NaCl levels up to 100mM significantly ($P<0.05$) reduced the shoot length. Our results are inconsistent with Aymen *et al.*,2014**;** Ali *et al.*,2017. However, priming with $FeSO_4$ promoted this parameter more than control. The comparison was made between both (100ppm and 200ppm) and results revealed that 100ppm significantly ($P<0.05$) promotes this parameter more than 200ppm grown under various (0-50-75-100mM) levels of NaCl. The maximum shoot length (37.84±0.08 cm) was found at 50mM NaCl with 100ppm seed priming. The results are in accordance with the (Raju *et al.*,2017; Huda *et al.*,2009). The reduced photosynthesis is major cause of dwarf plant growth and elongation of the shoots in return cause lower fresh and dry weight. The role of primed seeds in improving crop growth more than control plants in Canola was also reported by (Basra *et al.*, 2003).

Fresh Weight of the shoot (g)

The results mentioned in (Fig.1-B) indicated that the fresh weight of shoots strongly reduced under NaCl stress. The increasing levels of NaCl (0-50-75-100mM) significantly ($P<0.05$) reduced the fresh weight of the shoot. The reduction in shoot fresh weight under various levels of NaCl was also described by (Ali *et al.*,2017; Shannon &Grieve,2000). While the comparison of both (100ppm & 200ppm) concentrations showed that the plants of primed seeds with 100ppm showed the fresh weight of shoots significantly ($P<0.05$) higher than 200ppm and control plants. The significantly ($P<0.05$) highest values (26.87±0.067) of shoot fresh weight were observed at 100ppm under 50mm NaCl and the lowest value (21.87±0.032) were found at 200ppm under 100mM NaCl. Hence seed priming with $FeSO_4$ improves biomass production. The Seed priming under various salinity levels has a strong effect on shoot fresh and dry weight of safflower. (Aymen *et al.*,2014).

The Dry weight of shoot (g)

The results mentioned in (fig.1-C) showed that the dry weight of shoots strongly reduced under NaCl. The increasing levels of salinity up to 100mM NaCl reduced the dry weight. The reduction in dry weight of shoot under NaCl was also reported by Ali *et al.*,2017; Shannon &Grieve,2000; Nabila begum *et al.*,2014. The comparison was made between 100ppm and 200ppm then results revealed that 100ppm promoted this parameter more than 200ppm and control group. However, the deficiencies of iron cause the chlorosis and stunted growth in return lower shoot length, fresh and dry weights.

Root length (cm)

The figure 1-D showed that root length is strongly reduced by various levels of NaCl. The reduction in root length under the various levels of NaCl was significant ($P<0.05$). The similar findings were also reported by Ali *et al.*,2017; Nabila begum *et al.*,2014; Demir & Arif, 2003. The comparison was made between both (100ppm and 200ppm) concentrations and results showed that priming with 100ppm $FeSO_4$ under NaCl (0-50-75-100mM) stress promotes root length more as compared to 200ppm. Romheld *et al.*,1982 reported that Fe induced the morphological changes in roots and the role of Fe in root elongation was also reported by (Nozoe *et al.*,2009; Greipsson *et al.*,1995).

Fresh weight of Root (g)

The figure 1-E showed that the fresh weight significantly ($P<0.05$) reduced under NaCl stress. The reduction in the fresh weight of roots under NaCl stress was also reported by Ali & Ashraf, 2011; Nabila begum *et al.*,2014. Salt stress is the major cause of the overproduction of ROS and damages the cellular structure and membrane stability that cause a reduction in biomass production (Ali and Ashraf,2011). When the comparison was made between both (100ppm and 200ppm) seed priming the results concluded that 100ppm $FeSO_4$ showed more promotion to this parameter than 200ppm under various levels of NaCl stress. The maximum fresh weight of root (5.17±0.031) was found at 100ppm under 50mM NaCl and the lowest value (3.23±0.026) was recorded at 200ppm under 100mM NaCl. Our results are supported by (Greipsson *et al.*,1995).

The Dry weight of Root (g)

Figure 1-F indicated that salinity adversely affects the root dry weight. It was observed that roots dry weight is inversely proportional to NaCl stress. As the NaCl increased from up to 100mM then root dry weight reduced. The similar effects of NaCl also reported by Begum *et al.*,2014; Zahed *et al.*,2016. The priming with 100ppm $FeSO_4$ sowed more significant ($P<0.05$) promotion to dry weight of root than 200ppm priming under various levels of NaCl

stress. The maximum value (2.48±0.06) were observed at 100ppm under 50mM NaCl, while lowest values (1.26±0.012) were found at 200ppm under 100mM NaCl. Our results are consistence with (Greipsson *et al.*,1995).

Chlorophyll a & b (mg/g)

The findings of this study described that salt stress strongly reduced photosynthetic pigments. It was observed that when NaCl increased up to 100mM then chlorophylls a & b decreased. Similar findings were also reported by Taibi *et al.*,2016; Moghadam *et al.*,2013. The reduction in the chlorophyll content is might have been degradation of chlorophyll by reactive oxygen species and chlorophyllase generated during photorespiration under salt stress. The salt stress-induced osmotic stress and sodium toxicity causes the formation of ROS, which can damage the chloroplast and mitochondria by damaging the cellular structure. (Singh *et al.*,1987). Salt stress interrupts the specific enzyme which is involved in the synthesis of green pigments (Souza *et al.*,2004). The results mentioned in (fig.2-A & B) showed that seed priming enhanced the chlorophyll a & b. The seed priming with 200ppm $FeSO_4$ promoted chlorophyll a & b more than 100ppm under NaCl stress. Iron is involved in the synthesis of chlorophyll and is responsible for dark green color to plants. Similar results were found by Babaein *et al.*, 2011 in sunflower crop and Galavi *et al.*, 2011 in safflower with application of an iron.

Proline Contents (mg/g)

The fig.2-C showed that proline contents are directly proportional to NaCl stress. The findings of this study showed that under increasing NaCl levels proline contents also increased (P<0.05). Our findings were in conformity with the findings of Batool *et al.*,2013. The comparison of both (100ppm and 200ppm) concentrations showed that seeds priming with 100ppm enhanced proline contents more than 200ppm. The Significantly higher values of proline (65.30±0.24) contents were observed at 100ppm under 100mM NaCl. Proline has an important role in decrease the harmful effect of salt stress and repairing processes under stress. Proline also acts as an osmoprotectant and is related to a component of salt resilience under salt stresses (Yu Lei and Shaozheng, 2000). Proline is accumulated in plants under salt stress and helps to tolerate saline environments. The proline may act as enzyme stabilizing. (Aymen *et al.*,2014).

Phenolic Contents (µg/mg)

The findings mentioned in (fig.2-D) showed that phenolic contents accumulate under NaCl stress. It was observed that phenolic contents are directly proportional to NaCl stress. As with the increase in NaCl levels, the phenolic contents also increased. Similar findings were reported by (Lim *et al.*,2012; Hanen *et al.*,2008). When the comparison was made between 100ppm and 200ppm then results showed that 200ppm promoted more than 100ppm under NaCl stress. The significantly (P<0.05) highest values (95.57±0.12) were found at 200ppm under 100mM while lowest phenolic contents (84.17±0.23) were found at 100ppm under 0mM NaCl showed. The higher salt concentrations disturb enzymatic activities that lead to decreased photosynthesis process in plants. The higher phenolic contents play an important role to overcome oxidative stress due to the salinity. The higher phenolic contents in plants under salt stress may be an adoptive mechanism (Minh *et al.*,2016).

Flavonoids (µg/mg)

The fig.2-E showed that flavonoids contents are directly proportional to NaCl stress. The findings showed that under increasing levels of NaCl the flavonoids contents also increased. (Vojodi Mehrabani *et al.*,2017). When the comparison was made between both (100ppm and 200ppm) concentrations then results showed that 200ppm promoted more this parameter more than 100ppm under various levels of NaCl stress. However, the maximum values of flavonoids (84.26±0.17) were found at 200ppm under 100mM NaCl, which showed that flavonoids contents accumulated under NaCl stress. The flavonoids are metabolites of plants that provide health benefits via cell signaling and antioxidant effects. Flavonoids are responsible for producing attractive colors to attract pollinating insects. The role of iron to promote flavonoids were reported by Shi *et al.*,2018.

Total Soluble Protein (mg/g)

The fig.2-F showed that total soluble protein is accumulated under salt stress. It was observed that when NaCl increased up to 100mM then total soluble protein also increased. The protein accumulation under salt stress act as a storage form of nitrogen that is utilized later. (Turan *et al.*,2007). In this study the protein under NaCl stress and seed priming with $FeSO_4$ increased. The effect of salt stress was more on control plants than the primed seeds plants. The comparison of both (100ppm & 200ppm) showed that plants grown from seeds primed with 200ppm showed more protein contents than 100ppm under various levels of NaCl. While the significant highest value of protein (0.411±0.18) was found at 200ppm under 75mM NaCl. The $FeSO_4$ improves the protein contents in *Vigna radiata* as reported by Ali *et al.*,2014; Shalu *et al.*,2014). Increment in protein may be because of iron which is significant components of the structure of compounds associated with amino acids synthesis and finally protein union, hence protein content increased with the use of these micronutrients. These results are agreed with the similar findings of Ravi *et al.*,2008 in safflower and Ebrahimian *et al.*,2010 in sunflower.

Conclusion

This study highlighted that NaCl stress affected the germination, physiological and biochemical aspects of *Vigna radiata* L. However, the use of micronutrients like Fe can reduce the injurious effect of salt stress. The seed priming with $FeSO_4$ promoted the germination percentage, Growth and biochemical (proline, protein, phenolics and flavonoids contents) under NaCl stress.

Figure 1. Effects of seed priming with $FeSO_4$ (100ppm and 200ppm) on (A) Shoot length, (B) Shoot Fresh weight, (C) Shoot dry weight, (D) Root length, (E) Root fresh weight, (F) Dry weight of root under various levels (0-50-75-100 mM) of NaCl

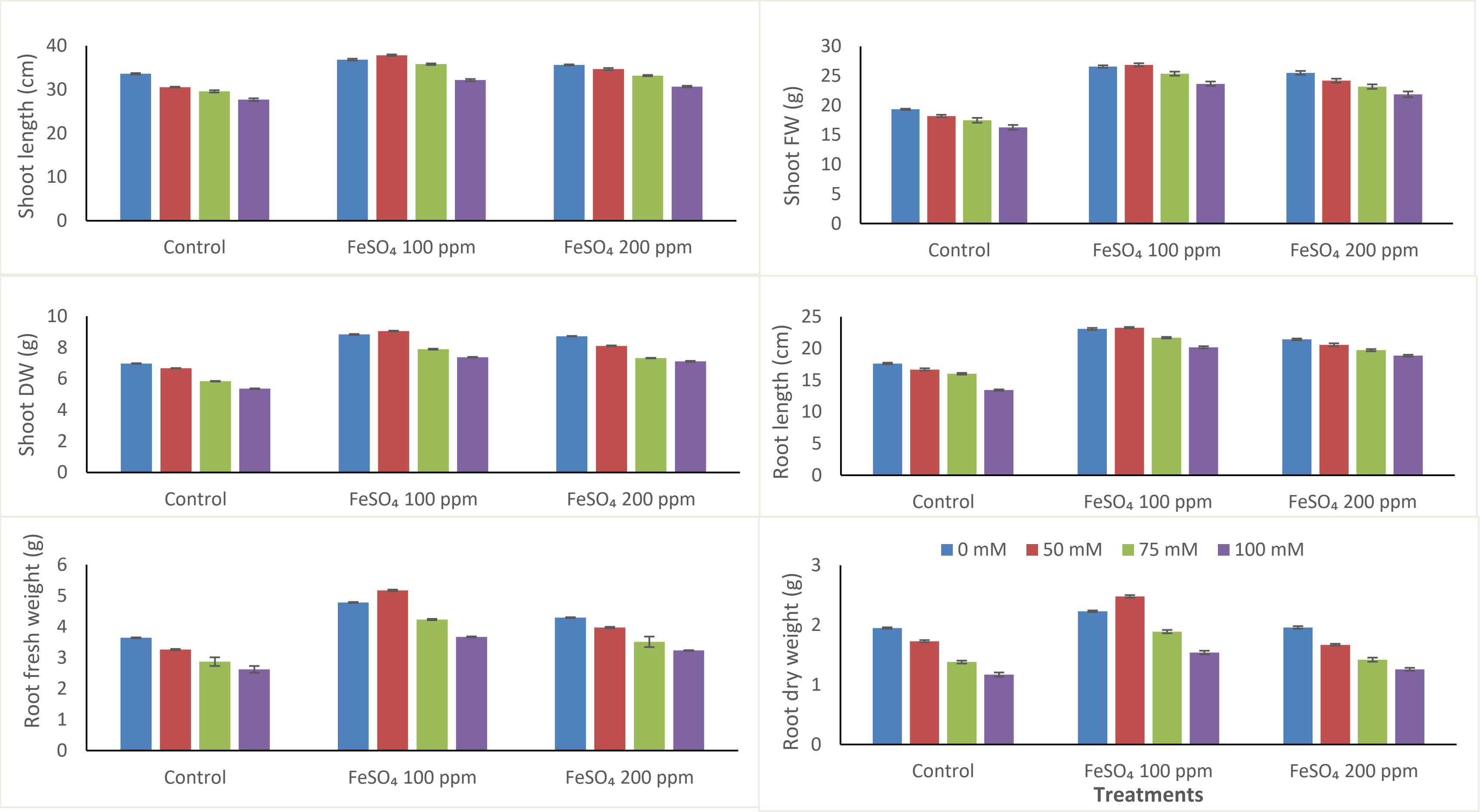
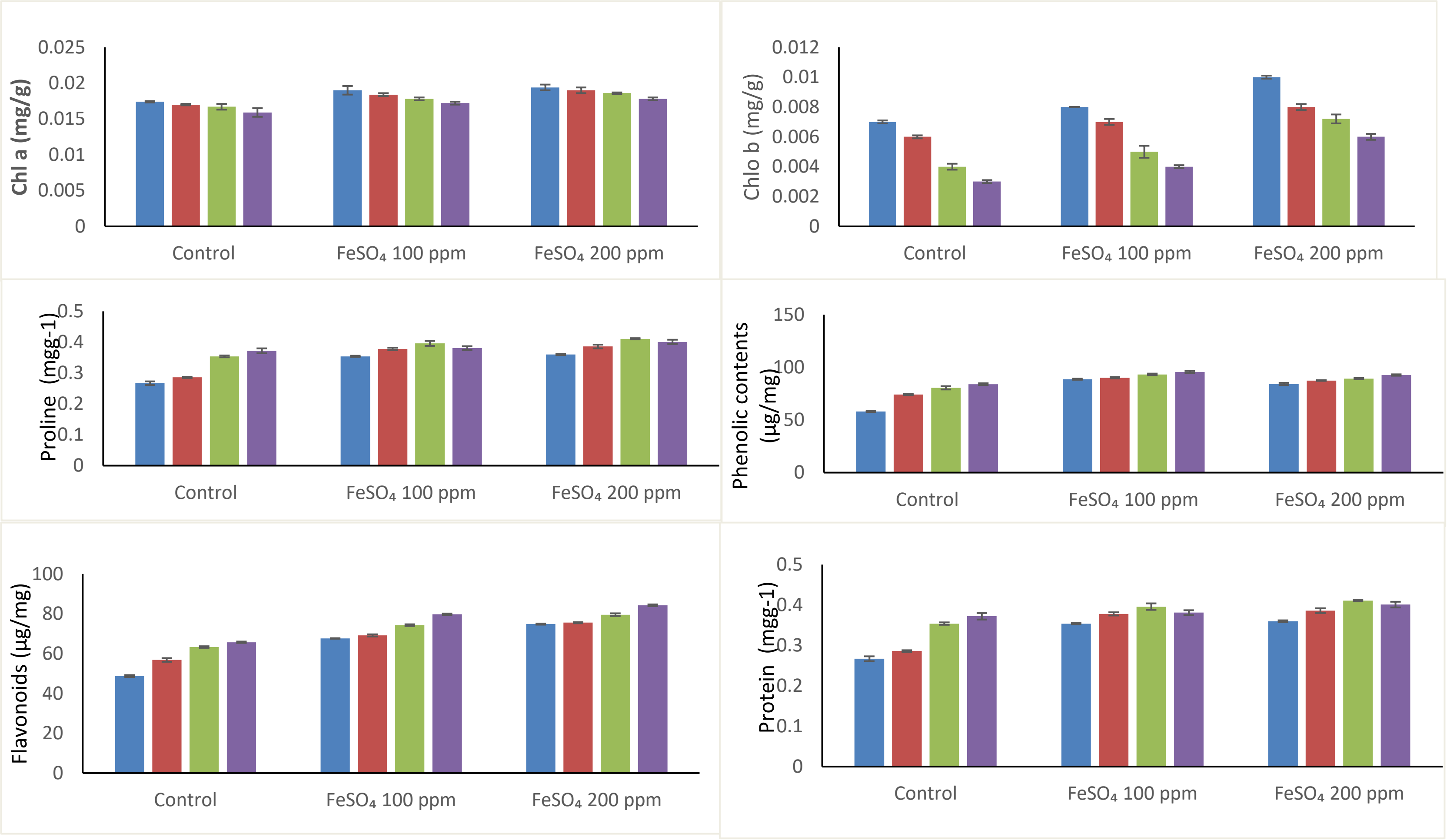


Figure 2. Effects of seed priming with $FeSO_4$ (100ppm and 200ppm) on (A)Chlorophyll a, (B) Chlorophyll b, (C) Proline, (D) Phenolic contents, (E) Flavonoids, (F) Total Soluble Protein under various levels (0-50-75-100 mM) of NaCl



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