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Research Article

ENHANCING SPINACH (*SPINACIA OLERACEA* L.) SEED GERMINATION, LEAF BIOMASS PRODUCTION UNDER SALINE WATER STRESS: THE IMPLICATIONS OF ZINC AND COW MANURE

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Abstract

Plant growth and productivity is restricted due to significant influence of salt stress on the essential physiological processes. Zinc supplementation holds promise as a technique to improve the resilience under salt stress. An open field experiment was conducted to assess the leaf growth parameters of spinach (*Spinacia oleracea*) including seed germination, plant area, leaf area, length, width, leaf fresh weight as well as dry weight, and Number of leaves per plant. Spinach plants were irrigated with a defined electrical conductivity (EC) level of 4.16 dSm^{-1} in clay loam, non-saline soil. Cow manure, a form of organic waste rich in macronutrients and micronutrients was also investigated. This study focuses on examining the response of zinc and cow manure on spinach growth under salinity stress. Salinity stress resulted in reduction in seed germination, leaf area, width, length, leaf fresh and dry weight, with no significant effect on the number of leaves. High doses of zinc and cow manure (0.20g Zinc+ 150g Cow Manure + Salinity) meaningfully enhanced performance by increasing seed germination by 15-20%, leaf area 130 cm^2, length 8.41 cm, width 4.8 cm , leaf fresh 0.67g and leaf dry weight 0.51g but didn't affect the number of leaves. Overall, our results demonstrate that the application of zinc and cow manure induced resistance to salinity stress and impacts positively on Spinach growth. Future research can focus on how zinc affects enzymatic activities and various mechanisms under salt stress.

Keywords: *Leafy vegetables, abiotic stress, organic fertilizer, micronutrients, Sodium Chloride, Organic Agriculture*
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1. INTRODUCTION

Green leafy vegetables such as spinach, are known for their rich nutrient contents, including bioactive compounds and antioxidants that play a part in averting age-related diseases and aging (Gupta and Prakash, 2009; Punchay et al., 2020). Spinach is cultivated globally on approximately 921,000 hectares of land, with an annual production exceeding 26 million tons, particularly in Asia where Annual Production is 25 million tons (Chapagain and James, 2013; FAO, 2018).
However, spinach production faces several challenges resulting in a yield gap, including poor seed germination, inadequate irrigation water quality and quantity, saline soils, suboptimal

cultivation practices, and improper use of chemicals and fertilizers (Debnath et al., 2021). Salinity, brought on by climate change, natural processes and anthropogenic activities poses a significant threat to sustainable crop production worldwide. Approximately 20% (45 million hectares) of irrigated land, which produces one-third of the food in the world, is affected by salinity. Furthermore, it is estimated that the part of agricultural land destroyed by salinization increases by 10 million hectares annually, with 50% of all of arable land worldwide projected to be impacted by salinity by 2050 (Pimentel et al., 2004; Shrivastava and Kumar, 2015; Qureshi, 2020). This salt induced land degradation under the impact of climate change has emerged a daunting environmental issue which continues to threaten agricultural productivity (Saqib et al. 2023).

Salinity stress adversely affects ion homeostasis, nutrient disproportion, along with various physio-chemical, and molecular processes crucial for plant development, therefore, decreasing plant growth (Munns, 2002; Shahid et al., 2020). In leafy vegetables, salinity stress can enhance the accumulation of flavonoids, polyphenols, phenolics, bioactive leaf pigments, and antioxidant activity (Sarker and Oba, 2019). Particularly spinach is regarded as a vegetable that is sensitive to salt (Bergman et al., 2001; Zhao et al., 2021), with a salinity threshold (ECt) typically below 2.5 dS/m for most vegetable crops, including leafy vegetables (Snapp et al., 1991; Machado and Serralheiro, 2017).

Salinity influences yield and seed germination, the first and most important stage of a plant's growth cycle. According to research, salt has a negative impact on the germination process in various plants for example *Posidonia*, *Oryza sativa*, *Triticum aestivum*, *Brassica sp*. and *Zea mays* (Gul et al., 2013) and *Ipomoea aquatic* (Ibrahim et al., 2019).

The administration of specific nutrients (silicon, zinc, boron, potassium) and organic substances (salicylic acid, humic acid, aspartic acid) has demonstrated promising impact in improving salt tolerance in vegetable crops. Through the enhancement of physiological, catabolic/anabolic, and molecular responses, bio stimulant chemicals can help in abiotic stress reduction and improve plant development (Cristofano et al., 2021). Zinc is a micronutrient that is necessary for plant growth. It is a component of many enzymes, proteins, membranes, and proteins that bind DNA stability. Moreover, it contributes to the formation of indole acetic acid (IAA), protein synthesis, gene regulation, protein metabolism, and defense against oxidative damage (Brown et al., 1993; Aravind and Prasad, 2004) (Marschner, 1995; Alloway, 2008).

The stability of the earth's systems and environmental processes, as well as agriculture and food production, depend on maintaining the health of the soil (Arshad et al. 2022). In developing countries, the continuous use of inorganic fertilizers and limited technical knowledge among farmers leads to nutrient imbalances and environmental and soil pollution (Azza, 2007). Farmers are looking for organic fertilizers because of the growing cost of inorganic fertilizers. These fertilizers can increase soil fertility by boosting certain physicochemical qualities. These characteristics include pH, electrical conductivity, microbial population, soil organic matter, water-holding capacity, cation exchange capacity, soil texture, and nutrient availability and uptake (Agbede et al., 2008; Muhammad and Khattak, 2009) and soil texture (Agbede et al., 2008; Muhammad and Khattak, 2009). Furthermore, the incorrect handling and the elimination of organic waste from various sources has been made worse by urbanization, which has led to environmental deterioration and possible bio-magnification of food chain contamination (Mrabet et al., 2012;

Benjawan et al., 2015). Similar to this, growing urbanization is making it more difficult to properly manage and dispose of organic waste from various sources, which is also lowering environmental quality and eventually food chain contaminating through bio-magnification (Mrabet et al., 2012; Benjawan et al., 2015). Cow dung is an organic waste product that has natural qualities and doesn't harm soil. It supplies macronutrients like calcium, phosphorus, potassium, and sulphur as well as micronutrients like iron, zinc, boron, cobalt, and molybdenum. (Preliminary survey, 2019). In addition, manure improves soil microbial activity, water retention, cation exchange capacity, and soil structure (Magdalena and Sudiarso, 2013). Cow dung takes the form of solid manure, or feces mixed with food scraps and urine, it has long-term benefits and provides plants with sustenance, which contains micro and macro elements like N, P, K, Mg, Ca, and Mn that plants need.

(Anwar et al., 2017) investigated spinach shoots' dry biomass and found that P and K contents were increased by applying manure that had been co-composted at various ratios with leaf litter. On the other hand, when more leaf litter was added together with a manure amendment, the amounts of N, Zn, Fe, Cu, and Cd in spinach decreased. (Hashimi et al., 2019) indicated that cow manure application without applied N showed adverse impact on spinach yields. (Kaho et al., 2020) results showed that the height of the plants, number of leaves, the fresh weight, and the dry weight of spinach (*Amaranthus spp*.) were all significantly impacted by the application of cow along with atonic manure on dry soil. (Fajeriana et al., 2022) stated that the development and yield of red spinach were significantly impacted by the application of bokashi fertilizer (organic fertilizer created from the fermentation of organic materials such as compost and manure by using decomposing microorganisms such as fermenting microbes or fungi) on oxisol top soil planting media. Similar to other parts of the world, Pakistan uses a lot of chemical fertilizers to boost vegetable yields. In areas where the use of chemical fertilizers is too much, expanding the use of organic fertilizers instead of chemical fertilizers is crucial for establishing a sustainable agriculture system that, in the long run, protects human health, the environment, and natural resources in vegetable production.

It is important to examine how cow dung affects plant production if you want to boost output and quality, as well as prevent environmental pollution in spinach cultivation. Simultaneously, it is also important to examine the ability of zinc to increase salt tolerance in spinach to get maximum yield.

This study set out to investigate the effects of zinc and cow dung on the germination of seeds, the growth of plants, and the yield of spinach produced in Rahim Yar Khan's open fields under salt stress.

2. MATERIALS AND METHODS

2.1. Growing Conditions

The study was conducted from November 5th,2021 to January 25th,2021. A pot experiment was conducted in the field conditions at KFUEIT, RYK (28°23'30''N 70°22'27''E). The soil used as growing medium was collected from Khwaja Fareed University’s nursery area (28°23'30''N 70°22'27''E) air-dried, and mixed. The soil type was determined to be clay loam with a slightly acidic pH of 6. Local spinach seeds were sowed in 36 pots with 20g seed per pot (7 inch pot size). The average temperature during this period ranged from 15 °C (58.9°F) to 22 °C (71.5°F) and the average humidity during this period varied between 49% to 56%.

2.2. Experimental Treatments

Pots were randomized with 6 treatments along with 6 replications and different doses of cow manure and zinc were used i.e. T1 Control, T2 Salinity stress (4.1 dsm-1), T3 0.10g Zinc, 75g (30 t/ha) cow manure and Salinity stress (4.1 dsm-1), T4 0.10g Zinc, 150g (60 t/ha) Cow manure and

Salinity stress (4.1 dsm-1), T5 0.20g Zinc, 75g (30 t/ha) cow manure and salinity stress (4.1 dsm-1) , T6 0.20g zinc, 150g (60 t/ha) cow manure and Salinity (4.1 dsm-1) in total 36 pots of 20cm each. salinity stress was given by applying underground NaCl salt-water (E.C; 4.16 dsm-1) in clay loam, non-saline soil in respective pots excluding control.

Cow manure was applied (top dressed on soil) after 7 days of Sowing to T3, T4, T5 & T6 as per treatment plan. Different doses of zinc were given with every irrigation as per treatments. Irrigation was done twice a week when needed.

2.3. Data collection

Number of germinated seeds were examined daily from day 7-12. As the roots poked over the pericarp by 2 mm, the seeds were considered to be germinated as done by (Gairola et al., 2011). It took 12 days for seeds to germinate. After 12 days, the percentage of seeds that germinated was identified using the following formula (Arias et al., 2018).

$$\text{GP} = (\text{Number of seeds germinated} / \text{Total number of seeds sown}) \times 100$$

After 82 days of sowing, the plants were harvested, and the leaves were separated to measure the growth metrics. The quantity of leaves per plant were counted. For the purpose of data analysis, the leaf growth parameters were measured on every leaf on every plant, with the average value being obtained. Using a 30 cm ruler, the leaf width of each tagged plant in the pots was measured. This was measured in the midpoint of the leaf length, from one end of the leaf to the other. From the leaf stalk to the leaf apex, the leaf length was calculated using the meter ruler.

Following a distilled water wash to get rid of any remaining soil particles, the plants were allowed to air dry. An analytical balance was then used to determine the fresh weight (g) of leaves. For 24 hours leaves were placed in oven at 24°C for estimation of dry weight as performed by (Kaho et al., 2020).

2.4. Statistical analysis

The Difference in germination percentage, leaf area, leaf length, leaf width, fresh weight, dry weight and number of leaves per plant were calculated using one-way ANOVA via LSD test at $P \leq 0.05$

3. RESULTS

3.1. Germination Percentage

Spinach seeds germinated more readily were under T6 (0.20g Zinc + 150g (60 t/ha) cow manure + salinity) whereas the least germinated seeds were under T2 (salinity stressed). The percentage of germination of spinach seeds with increasing salinity level was slightly decreased. Germination percentage was increase 20 percent in T6 (0.20g Zinc + 150g (60 t/ha) cow manure + salinity) comparing with T2 salinity stress, while increased 15 percent comparing with the control as shown in Figure 1.

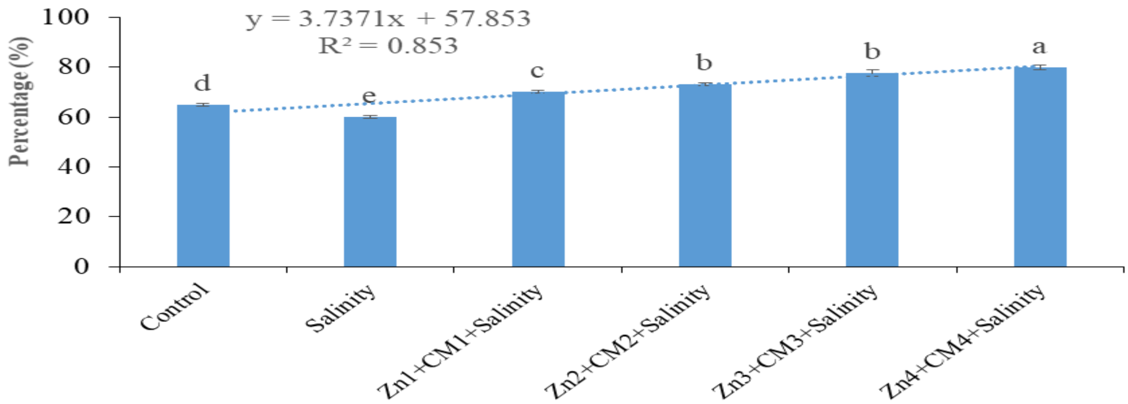

Figure 1. Effect of different treatments on the germination (%) of Spanish. Different letter on error bars represents the difference at significance level (*P* 0.005).

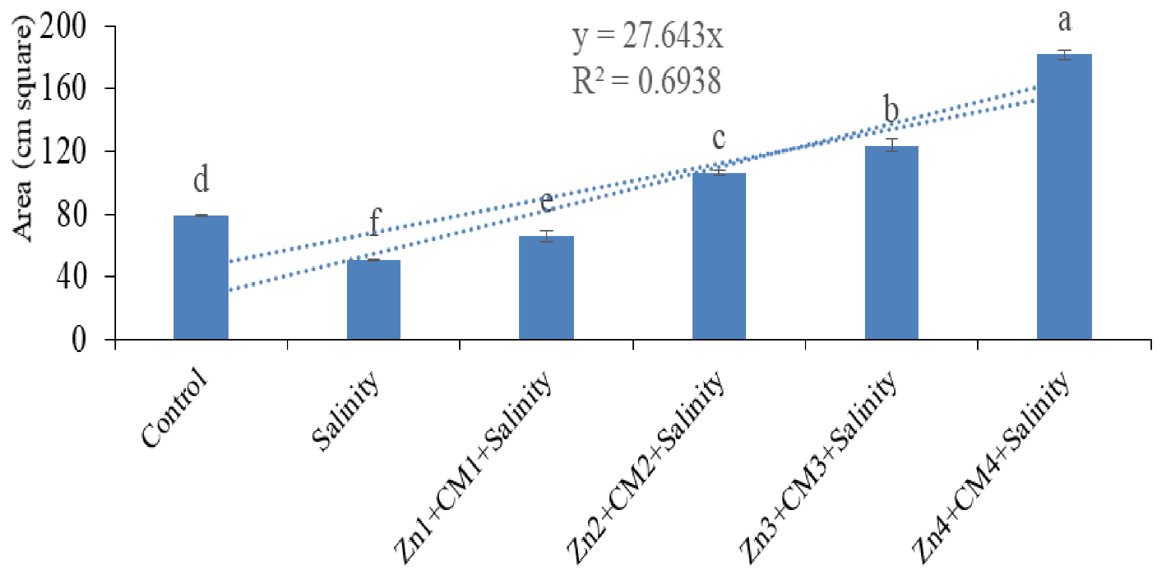

Figure 2. Effect of different treatments on the area (cm^2) of Spanish leaves. Different letter on error bars represents the difference at significance level (*P* <0.005)

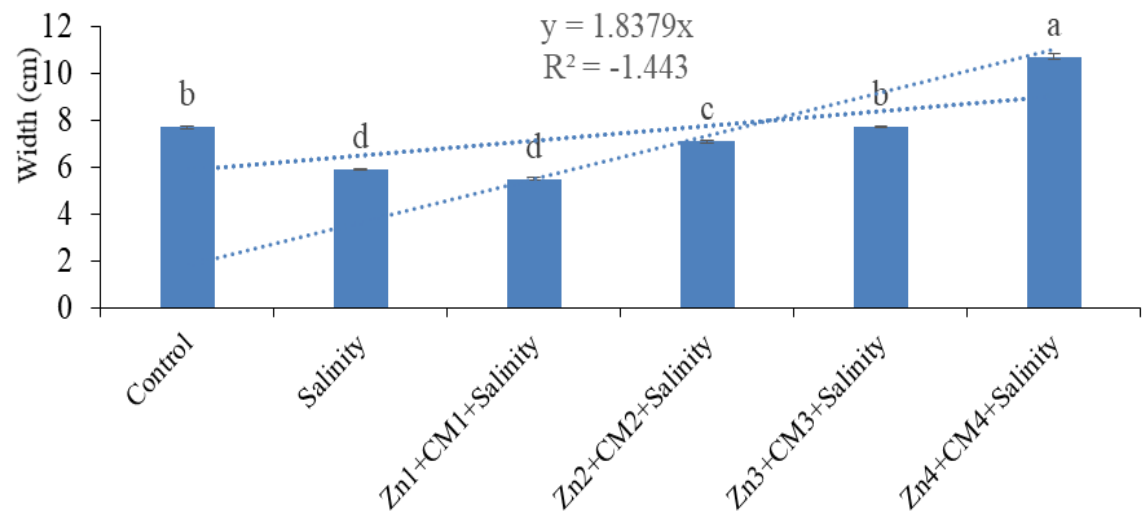

Figure 3. Effect of different treatments on the width (cm) of Spanish leaves. Different letter on error bars represents the difference at significance level (*P* <0.005).

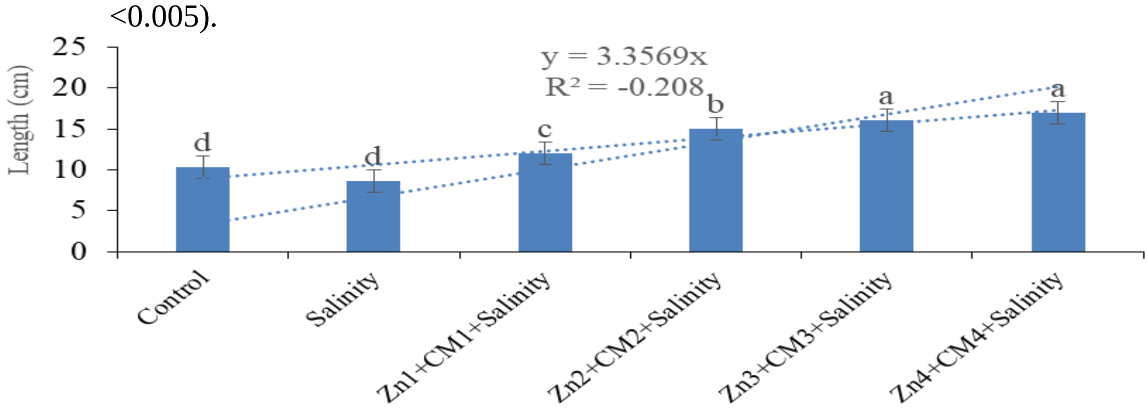

Figure 4. Effect of different zinc treatments on the length (cm) of Spanish leaves. Different letter on error bars represents the difference at significance level (*P* <0.005).

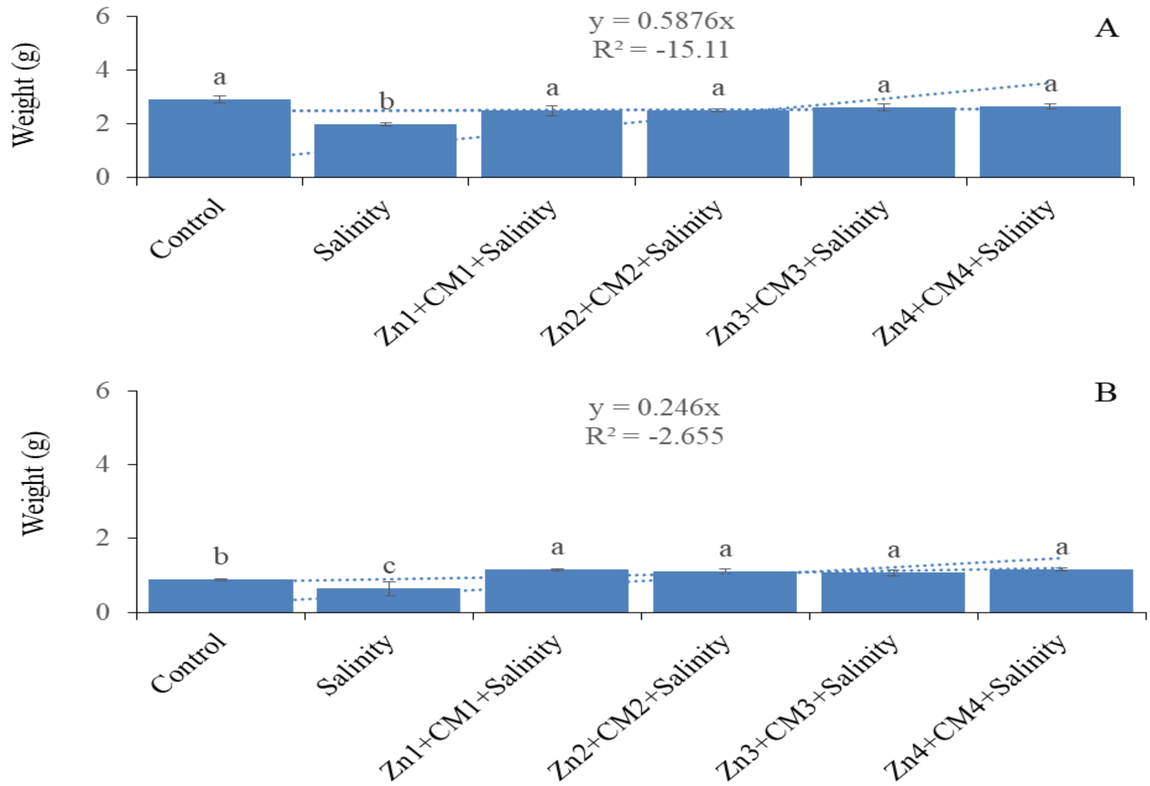

Figure 5. Effect of different zinc treatments on the fresh (A) and dry (B) weight (g) of Spanish leaves. Different letter on error bars represents the difference at significance level (P <0.005).

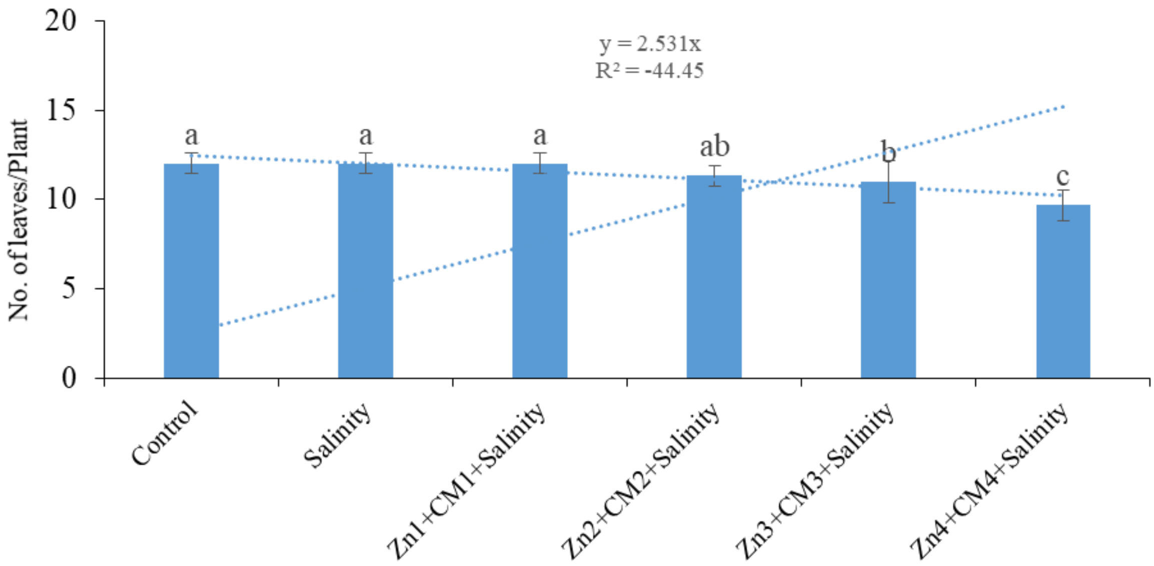

Figure 6. Effect of different zinc treatments on the length (cm) of Spanish leaves. Different letter on error bars represents the difference at significance level (*P* <0.005).

3.2. Leaf Area, Length and Width

High doses of Zinc and cow manure can significantly increase the size of the leaves thus increases production, as in leafy vegetables main component is leaf as compare to the salinity stress. Comparing the leaf size of (0.20g Zinc + 150g (60 t/ha) cow manure + salinity) T6 and T2 (salinity stress) there is significant difference in Leaf area in salinity stressed conditions, however control treatment T1 (control) performed better as compare to T2 (salinity stress) but not as well as T6 (0.20g Zinc + 150g (60t/ha) Cow manure + salinity).

3.3. Leaf Fresh and Dry Weight

The highest leaf fresh weight was recorded in T1 Control, T6 (0.20g zinc + 150g (60 t/ha) cow manure + salinity) was also nearly equal to that of T5 (0.20g Zinc + 75g (60t/ha) + Salinity) t/ha) cow manure + salinity) while least was recorded in salinity stressed T2 (salinity stress) treatment. But the dry weight in T6 (0.20g zinc + 150g (60 t/ha) cow manure + salinity) was recorded as the highest while least in salinity stressed T2 treatment.

3.4. Number of leaves per plant

Number of leaves were the same and higher in number for 3 treatments T1 (control), T2 (salinity stress) and T3 (0.10g Zinc + 75g cow manure + salinity) respectively. While were the least in T6 (0.20g Zinc + 150g (60 t/ha) cow manure + salinity) treatment.

4. DISCUSSION

The research was performed to check the implication of Zinc and Cow manure for the enhancement of biomass production of spinach (*Spinacia oleracea*) leaf with under stress of saline water.

The most important and salt-sensitive stage of plant growth, seed germination is negatively impacted by rising salinity. The process of germination of seeds starts with their imbibition. When salinity stress impairs seed germination, harmful ion effects, a lack of water absorption, and a reduction in nutrient mobilization typically follows it (Gul et al., 2013). In the current study, the effect of Salinity stress on germination percentage of *Spinacia oleracea* seeds was compared. Seeds of spinach germinated the most were under T6 (0.20g Zinc + 150g (60 t/ha) Cow manure + Salinity) whereas the least germinated seeds were under T2 (Salinity stressed). The difference in percentage of germination of spinach seeds was by 20% as shown in Figure 1. These results are in pipelines with the study of (Ibrahim et al., 2019) who studied influence of stress caused by salinity on water spinach (*Ipomoea aquatica*) Germination. as a result, it was found that high salinity caused a delay in seed germination, requiring more time for the seeds to sprout. Salt stress prevents seeds from absorbing water, which lowers the percentage of seeds that germinate overall (Sarker et al., 2014). Reduction in salinity-induced seed germination stress is brought on by osmotic-toxic salts found in salty environments or by physicochemical impacts (Pradheeban et al., 2014). Furthermore, by making seeds poisonous, excessive environmental concentrations of sodium and chloride ions -delay seed development (Moghaieb et al., 2004).

When compared to non-water stress treatment, the leaf area reduced during water stress (Ors and Suarez, 2017). Which is quite similar to our findings as shown but high doses of zinc and manure (0.20g Zinc + 150g (60 t/ha) Cow manure + Salinity) performed the best in terms of leaf area which may be due to zinc which minimized the effect of salinity and simultaneously cow manure provided the nitrogen which is required for vegetative growth.

(Sanni, 2016) revealed that the availability of N, which encouraged leaf area throughout the vegetative stage and contributed in maintaining a functional leaf area throughout the growth stage, is probably in charge of the soil supplemented with organic fertilizer's increased leaf area, which is consistent with present findings that high doses of cow manure have high leaf area in saline conditions. Comparing high doses of zinc and manure (0.20g Zinc + 150g (60 t/ha) Cow manure + Salinity) with Control and Salinity stressed there is a great difference which may be due to availability of nitrogen (provided by high dose of cow manure) required for vegetative growth.

Salinity shock reduced the physiological characteristics and development of spinach plants as indicated by (Ibrahim, Abas et al. 2019). Salinity severely inhibits the germination and development of spinach by lowering the fresh and dry weight of the plants. Highest fresh weight was recorded in Control and then in High doses of zinc and manure (0.20g Zinc + 150g (60 t/ha) Cow manure + Salinity) and was least in salinity stressed, While more dry weight was recorded by high doses of zinc and Manure (0.20g Zinc + 150g (60 t/ha) Cow manure + Salinity) and then in control while the least was recorded in Salinity stressed treatment which is due to salinity stress as shown in figure 5. This means that fresh weight and dry weight was greatly affected salinity, osmotic stress and causes growth inhibition under salt stress. Because of the decreased metabolic activity of plants, salt stress significantly affects the uptake of nutrients and water. According to (Xu et al., 2016) fresh and dry biomass declined in situations of salinity stress which is quite similar to present study. Since nitrogen promotes plant vegetative growth, the application of organic fertilizers most likely increased the amount of nitrogen in the soil, which had a favorable impact on leaf fresh weight as reported by (Gülser, 2005). But in the current study control in normal conditions has high fresh weight after that high dosed zinc and cow manure performed well in saline conditions means that in saline conditions cow manure increased fresh weight as reported by (Gülser, 2005) which may be due to increase in nitrogen content provided by cow manure.

(Ors and Suarez, 2017) indicated that with increasing salinity, initially leaf number increased greatly and subsequently drastically fall. Additionally, when water stress increased across all salinity levels, the number of leaves reduced somewhat (but significantly). There is less difference in number of leaves in current study, which is consistent in control, Salinity stressed as well as other treatments but comparatively decreased in high doses of zinc and cow manure. (Sanni, 2016) investigated that application of cow dung has a major favorable impact on *A. hybridus's* vegetative performance. Since amaranth leaves are the plant's primary photosynthetic organ, variations in their quantity will inevitably have an impact on the plant's overall performance but in the current study high dose of cow manure doesn't affect in saline conditions which means that in saline conditions cow manure does not have significant effect on number of leaves.

5. Conclusion

Our results demonstrate that the application of zinc and cow manure induced resistance to salinity stress and positively impacted on Spinach growth. Future research can focus on how zinc affects enzymatic activities and various mechanisms under salt stress.

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7. Conflict of Interest

The Authors declare that there is no conflict of interest.

8. Authors' Contribution Statements

MAA, MAA, MS executed the field activity and write up and polished the article, SK, BA and JI, MI conceived the idea and supervised the work, AA carried out statistical analysis.

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