

## BIOCHEMICAL ATTRIBUTES OF ZINC INDUCED STRESS ON *CICER ARIETINUM*

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### Abstract

Chickpea (*Cicer arietinum* L.) is one of the major cultivated legume crops that is used as a source of nutrition for both humans and animals. Zinc is the micronutrient highly beneficial, and its cultivation area is widely spread all over the world, but it is mainly cultivated in the arid and semi-arid areas com. The experiment was conducted at the research area of Post Graduate Agriculture Research Station (PARS), University of Agriculture Faisalabad, Pakistan. Seeds of two chickpea varieties (Bittle 2016, Noor, 2019) were collected from Ayoub Agricultural Research Institute Faisalabad, Pakistan. Zinc sulphate was used as a source of zinc and was applied foliar at (0, 100, 200 and 300ppm) under normal conditions. Physiological (chlorophyll a, chlorophyll b and carotenoids) and growth attributes (fresh weight, dry weight, shoot length, root length, leaf area and number of pods per plant) analysis were determined application at different levels also varies the results, increased the yield with the increase in Zn quantity. However, adequate supply of Zn 200-300 ppm improved the 35% water holding capacity and enzymatic activities, photosynthesis, and accumulation of free leaf proline. The increasing levels of treatments also enhanced the seed quality and production.

**Key words:** Chlorophylls, Crop, Chickpea, Zinc applications, Metal toxicity.

### Introduction

Chickpea (*Cicer arietinum* L.) is one of the major cultivated legume crops. (Murtaza *et al.*, 2022). It is used as a source of nutrition for both of the humans and animals (Hafeez *et al.*, 2023). Its cultivation area is widely spread all over the world but it is mainly cultivated in the arid and semi-arid areas com (Ramzan *et al.*, 2022). In chickpea yield and yield contributed attributes, there is less genetic information available (Malik *et al.*, 2014), more in India, Pakistan and Iran (Paolini *et al.*, 2006). Chickpea cultivated in declined moisture levels, the long roots play major role against drought (Jabeen *et al.*, 2023). Greater genetic variations noticed for heat tolerance in chickpea (Bilal *et al.*, 2021). It happened with reference set was screened against heat stress at various places in India (Krishnamurthy *et al.*, 2010). A field screening approach has been established and various sources of heat tolerance were recognized (Raza *et al.*, 2024). The status of genomic resources provided for chickpea betterment and proposed ways to develop the genome assisted breeding in chickpea (Varshney *et al.*, 2013). Evaluation of apparent line in India, Kenya and Ethiopia at different places guide to the distinguishing with consequently greater yield as compared to JG11 at every location in every condition (Gaur *et al.*, 2014).

In cultivated field crops including chickpea, deficiency of zinc (Zn) has been considered as major yield limiting factor in the world (Roy *et al.*, 2006; Ahlawat *et al.*, 2007). Zinc regulates various essential physiological

attributes in plants such as proteins, key enzymes activation and carbohydrates metabolism (Palmer & Guerinot, 2009). A sufficient Zn supply is necessary for the viability and germination of pollen grains (Pandey *et al.*, 2009). In fact, the pre-mature excision of leaves, pollens, anthers and flower buds is also due to the deficiency of Zn that enhanced the abscisic acid which lowers the seed yield (Alloway, 2004). In mesophyll cells, Zinc is involved in the regulation of carbon dioxide influx (Gupta *et al.*, 2016). Zn is involved in the maintenance and regulation of photosystem II and biosynthesis of IAA (Indole acetic acid) (Hansch & Mendel, 2009).

Foliar application of Zn in chickpea increases the Zn content in grain by 22% (Hidoto *et al.*, 2017). Zn concentration in different chickpea genotypes varies from 2.0-2.7 mg/100 g in one study (Hemelatha *et al.*, 2007) to 3.7-7.4 mg/100 g in another study (Thavarajah & Thavarajah, 2012). Plant growth promoting bacteria may also collaborate in increasing the availability of nutrients particularly for iron and zinc (Sharma *et al.*, 2013). Salinity is another major threat to the pulse production in the world including Pakistan which may knock food security (Kapoor & Sirivastava, 2010). Plant resistance to salt stress increased by Zn nutrition (Iqbal *et al.*, 2023). Cell membrane of root stabilizes by application of Zn which reduces leakage of ions and decreases root permeability (Danishbakhsh *et al.*, 2013).

The aims of this this work presented the foliar application of zinc applied foliar at (0, 100, 200 and 300ppm) under normal conditions for measuring the

physiological such as chlorophyll a, chlorophyll b and carotenoids and growth attributes (fresh weight, dry weight, shoot length, root length, leaf area and number of pods per plant) analysis were determined application at different levels also varies the results, increased the yield with the increase in Zn quantity.

## Material and Method

A potted experiment was conducted at the research area of Post Graduate Agriculture Research Station (PARS), University of Agriculture Faisalabad. Zinc sulphate was used as a source of zinc and was applied foliarly at (0, 100, 200 and 300ppm) under normal conditions on chickpea (*Cicer arietinum. L*) Completely Randomized Design (CRD).

**Experimental site and design:** The experiment was conducted to study the effect of Zinc Sulphate on growth and development of Chickpea (*Cicer arietinum*) at University of Agriculture PARS, Faisalabad. The crop was sown 13 November 2020. There were four replications and two factors. Experiment was conducted in pots. The pots have 30 cm height and 12 cm width. 5 kg soil was filled in each 24 pots. Seeds were sown with hands at recommended rate was 12 seeds per pot. Fertilizers (NPK) were applied when required in recommended dose.

**Zn sulphate (ZnSo<sub>4</sub>) treatments:** Zn treatments, 0, 100, 200, 300 ppm were foliarly applied. NPK in small amount were applied whenever needed. Treatments were applied with full strength of Hoagland's solutions (Hoagland & Arnon, 1950) spray was applied with ZnSo<sub>4</sub> solution given with full strength of Hoagland's nutrient solution once in a week and applied three times after germination of the seeds.

**Seed materiel:** Two chickpea cultivars Bittle-2016 and Noor-2019 obtained from the Ayub Agriculture Research institute Faisalabad. In Chickpea plants will be grown in sand medium 24 plastic pots are used and 5kg bath sand is filled in each pot the 12 seeds of chickpea are sown in each pot after two weeks of germination thinning practice is performed and 5 healthy plants were kept in each pot and others were discard.

**Sowing and culture medium:** Before sowing the seeds, plastic pots were washed thoroughly and filled with 5kg of Sand medium. The area of the pots was 23×29. The germinated seedlings were irrigated with tap water on daily basis. Hoagland's solutions ½ dose (Hoagland & Arnon, 1950).

**Experimental site and design:** The experiment was conducted to study the effect of Zinc Sulphate on growth and development of Chickpea (*Cicer arietinum*) at University of Agriculture PARS, Faisalabad. The crop was sown 13 November 2020. Experiment was laid out completely in randomized design with factorial arrangement. There were four replications and two factors. Experiment was conducted in pots. The pots have 30 cm height and 12 cm width. 5 kg soil was filled in each 24 pots.

Seeds were sown with hands at recommended rate was 12 seeds per pot. Fertilizers (NPK) were applied when required in recommended dose.

## Treatments

**Factor A: Verities (V);** V<sub>1</sub>= Bittle 2016, V<sub>2</sub>= Noor 2019

**Factor B: Zinc (Zn);** Zn<sub>0</sub>= Control, Zn<sub>1</sub>= 100 ppm, Zn<sub>2</sub>= 200 ppm, Zn<sub>3</sub>= 300 ppm

Replications = 4

**Plant height (cm):** Plant height is the most significant parameter for morphological studies of plant. It can be calculated as Plant height = Shoot length and Root length.

**Shoot length (cm):** Length of shoot of plant was calculated by measuring and cleaning through the action of distills water.

**Root length (cm):** Length of root of plant was calculated by measuring and cleaning through the action of distills water.

**Shoot fresh weight (FW) (g):** Fresh shoot weight of plant was calculated by measuring and cleaning through the action of distills water.

**Shoot dry weight (DW) (g):** Length of root of plant was calculated by measuring and cleaning through the action of distills water.

**Root fresh weight (FW) (g):** Fresh mass of root of 1 plant each replicate, every treatment was estimate at once after pull out the plants with the aid of weight balance and mean data were calculated.

**Root dry weight (DW)(g):** Root dry weight of plant was calculated by measuring and cleaning through the action of distills water.

**Number of leaves:** Number of leaves of each plant was counted from each selected plant of 24 pots.

**Photosynthetic pigments (chlorophyll a, b carotenoids) mg/g. FW:** The extract then filtered and kept for overnight at room temperature than absorbance of the extract was measured at 663, 645, and 470 nm using a spectrophotometer.

**Total soluble protein content (TFA):** Parameter was quantified by Hamilton method (1973). Phosphate buffer was used to extract fresh plant material. Took test tube of 25 mL and 1 mL of plant material was added. Then 1 mL of ninhydrin solution with 2% concentration and 1 mL of 10% pyridine solution was mixed. Water bath was used to heat test tubes for half hour. Volume was made up to 15 mL with distilled water. Spectrophotometer reading was taken as 570 nm.

**Carotenoid:** One of the samples was dissolved in 4 mL of acetone and centrifuged at 1.500 g for 20 min at 40C followed by Maclachlan & Zalik (1963).

### Statistical analysis

ANOVA test was designed for the validation of the analysis at 5% significant level also by using the Statistical software through a Version of 8.

### Results

**Shoot length:** Shoot length is an important aspect of plant growth which is an indirect measure of overall plant growth. Shoot length contributing a vital role in biomass production. Chickpea variety Bittle and Noor under Zn level (300ppm) have shown increased shoot length than other treatments of zinc. The interaction of zinc and varieties also show non-significant increase in shoot length (Fig. 1). The results reported by Hussain *et al.*, (2019) also clarify that zinc affects the growth and shoot length of wheat which ultimately increase the yield.

**Root length:** Root is that part of plant which connected the plant with soil surface give support and nutrition, buried in soil that provide water and essential nutrients to plant for plant growth and development with the help of root hairs and root system. Fig. 2 shows that various zinc levels affected the root length of both varieties of chickpea (Bittle and Noor). Maximum root length was observed in high zinc level plants.

**Shoot fresh weight (mg):** Figure 3 shows that the effect of different levels of zinc on shoots fresh weight of both varieties. Zinc (Zn) application significantly ( $p < 0.001$ ) affect the shoot fresh weight of chickpea plants as compared to plants under control conditions. Chickpea variety Bittle and Noor under Zn level (300ppm) have shown increased shoot fresh weight than other treatments of zinc.

**Root fresh weight:** Figure 4 shows the interaction of zinc and varieties also show non-significant increased in root fresh weight Varieties significantly ( $p < 0.001$ ) affect the root fresh weight in low levels to high levels of zinc application. Chickpea variety Bittle and Noor under Zn level (300ppm) have shown increased root fresh weight than other treatments of zinc.

**Shoot dry weight (g):** Shoot dry weight is the most reliable parameter for the determination of overall plant growth. Fig. 5 shows that shoot dry weight (g) of chickpea plants increasing trend under the increasing concentrations of zinc. Zinc (Zn) application non-significantly increased the shoot dry weight of chickpea plants as compared to plants under control conditions. Varieties non-significantly increase the shoot dry weight in low levels to high levels. Chickpea variety Bittle 2016 and Noor 2019 under level (300ppm) have shown increase shoot dry weight than other treatments of zinc.

**Root dry weight (g):** Roots dry weight is also an important growth parameter. Fig. 6 shows the zinc levels and their interaction non-significantly affected the root dry weight

(g) of chickpea plants. In general, root dry weight (g) of chickpea plants increased with the increasing concentration of zinc solution. Zinc application non-significantly increased the root dry weight of chickpea plants as compared to plants under control conditions. Varieties significantly increased the root dry weight in low levels to high levels of zinc application.

**Leaf area mm<sup>2</sup>:** Leaf area is a dimensionless that characterizes plant canopies. Fig. 7 shows the varieties of chickpea showing that zinc application affected the leaf area. It was observed that increasing levels of zinc increased the leaf area respectively. Zinc (Zn) application significantly ( $p < 0.001$ ) increased the leaf area of chickpea plants as compared to plants under control conditions. Varieties significantly ( $p < 0.001$ ) increased the leaf area in low levels to high levels of zinc application. Chickpea variety Bittle 2016 and Noor 2019 under Zn level (300ppm) have shown increased leaf area than other treatments of zinc.

**Number of leaves per plant:** Figure 8 shows the show significantly increased in number of leaf per plant as a result of higher zinc level (300ppm) that ultimately increased plant yield. Zinc (Zn) application significantly ( $p < 0.05$ ) effected the number of leaf per plant of chickpea plants as compared to plants under control conditions. Varieties significantly ( $p < 0.001$ ) increased the number of leaves per plant in low levels to high levels of zinc application. Chickpea variety Bittle 2016 and Noor 2019 under Zn level (300ppm) has shown increased number of leaves per plant than other treatments of zinc.

**Chlorophyll a:** Results regarding in Fig. 9 shows the revealed that exposure to 300ppm of zinc was showed maximum effect of chlorophyll a in both varieties (Bittle and Noor). Zinc application (Zn) non-significantly increased the chlorophyll a of chickpea plants as compared to plants under control conditions. Varieties non-significantly affected the chlorophyll a in low levels to high levels of zinc application. Chickpea variety Bittle 2016 and Noor 2019 under Zn level (300ppm) have shown increased chlorophyll a than other treatments of zinc.

**Chlorophyll b:** Fig. 10 shows the high level of zinc (300ppm) increased the Chlorophyll b in both varieties of chickpea (Bittle and Noor). Zinc application (Zn) non-significantly reduced the chlorophyll b of chickpea plants as compared to plants under control conditions. Varieties non-significantly ( $p < 0.001$ ) effected the chlorophyll b in low levels to high levels of zinc application. Mustard variety Bittle 2016 and Noor 2019 under Pb level (90ppm) have shown decreased chlorophyll b than other treatments of zinc.

**Carotenoids:** Figure 11 shows the carotenoid content of two cultivars of Chickpea as a result of zinc application. It showed different levels under zinc application in two varieties of Chickpea (Bittle, 2016 and Noor, 2019) had significant effect on carotenoid content of Chickpea. There was increased in the carotenoid content of Chickpea under zinc application in order of  $0 < 100 < 200 < 300$  (ppm). Zinc application (Zn) non-significantly reduced the carotenoids of chickpea plants as compared to plants under control conditions.

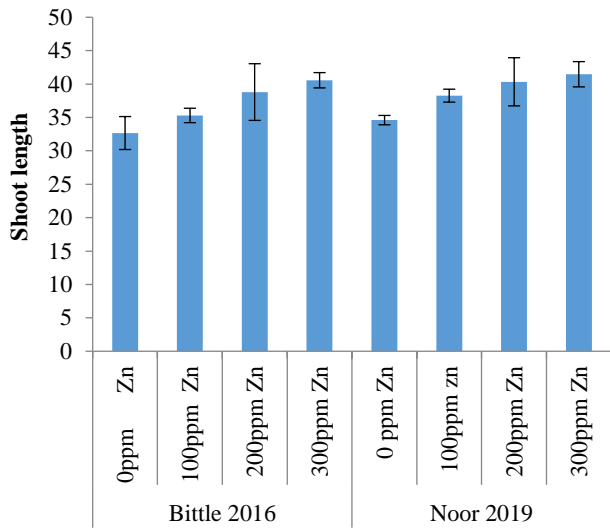


Fig. 1. Effect of zinc on shoot length of two varieties of chickpea.

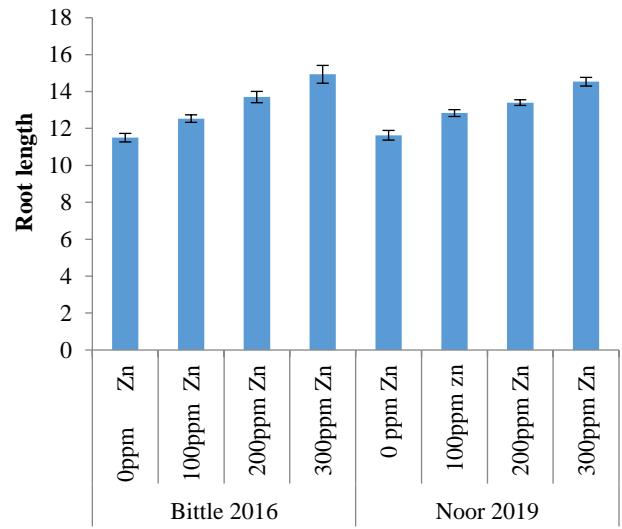


Fig. 2. Effect of different concentration of zinc on root length of two varieties of chickpea

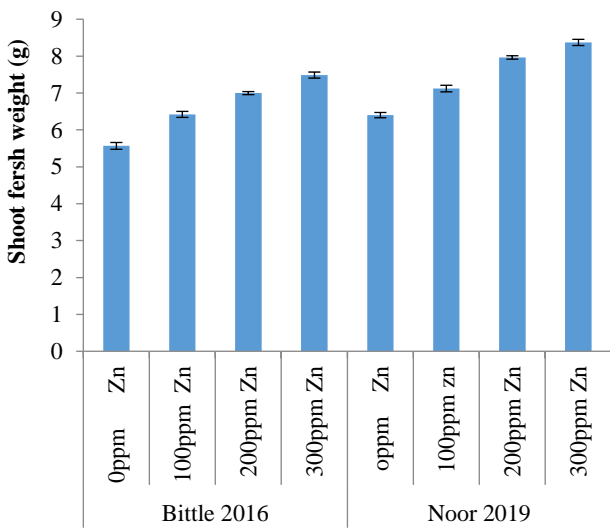


Fig. 3. Effect of different concentration of zinc on shoot fresh weight of two varieties of chickpea.

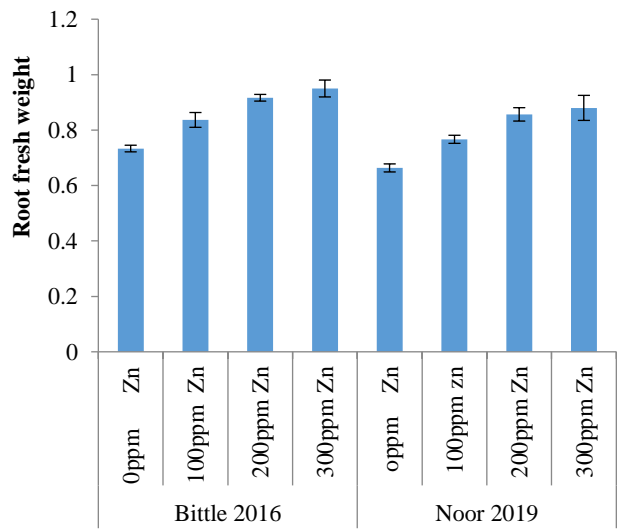


Fig. 4. Effect of different concentration of zinc on root fresh weight of two varieties of chickpea.

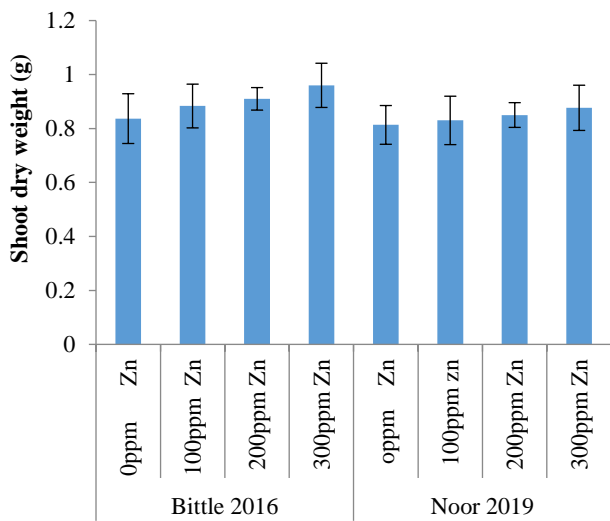


Fig. 5. Effect of different concentration of Zinc on shoot dry weight of two varieties of chickpea.

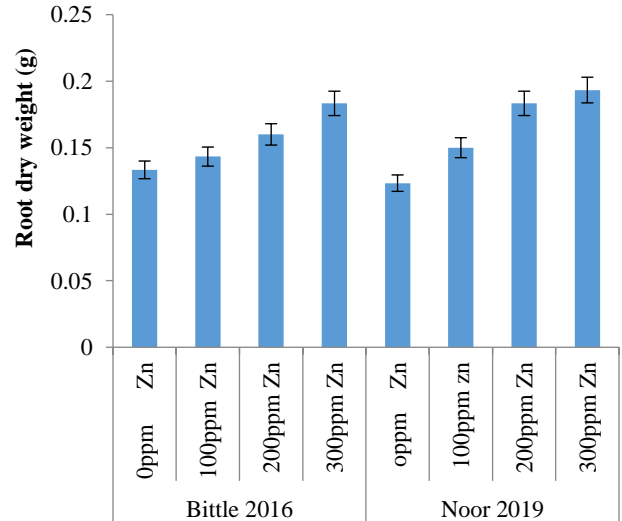


Fig. 6. Effect of different concentration of zinc on root dry weight of two varieties of chickpea.

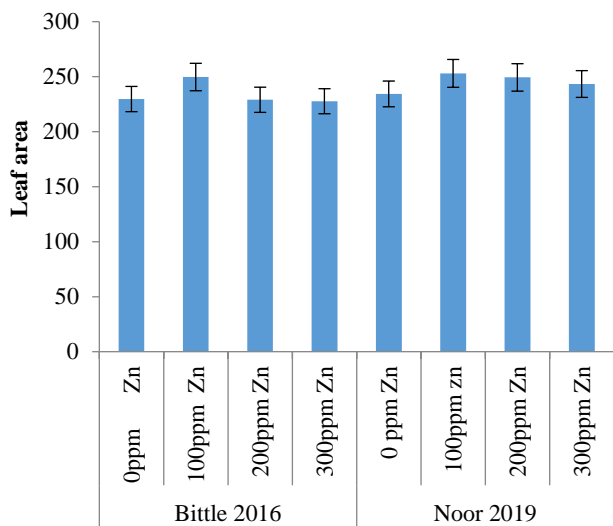


Fig. 7. Effect of different concentrations of zinc on leaf area of two varieties of chickpea.

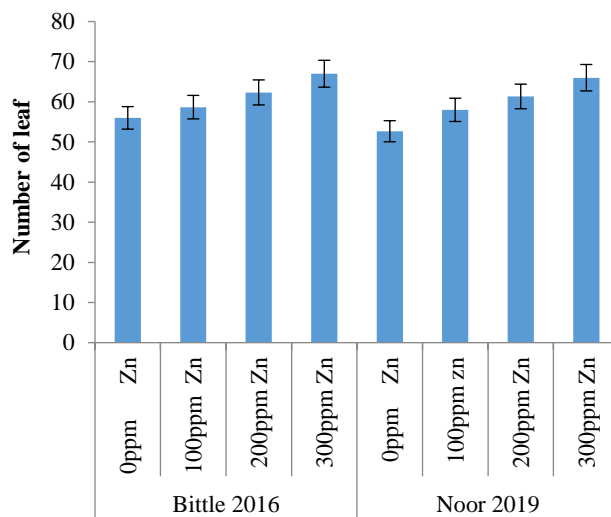


Fig. 8. Effect of different concentrations of zinc on number of leaf of two varieties of chickpea.

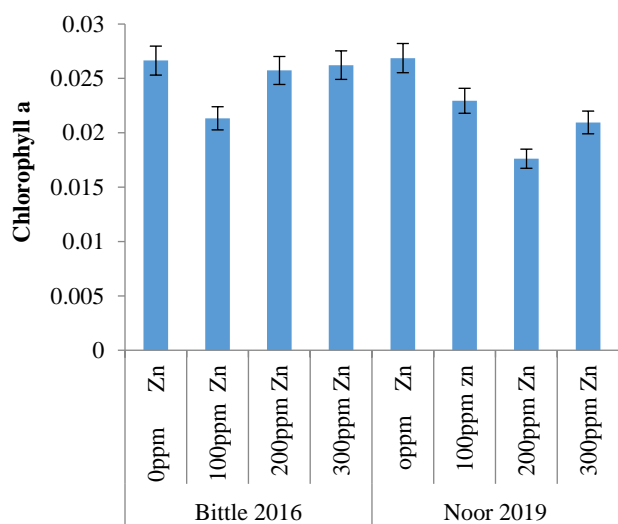


Fig. 9. Effect of different concentrations of zinc on chlorophyll a of two varieties of chickpea.

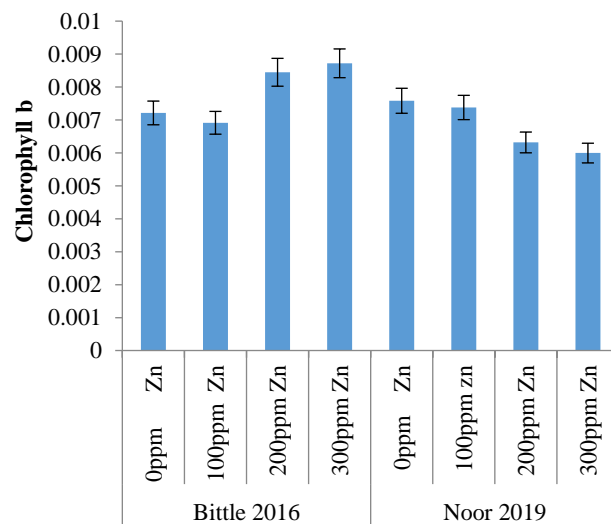


Fig. 10. Effect of different concentrations of Zinc on chlorophyll b of two varieties of chickpea.

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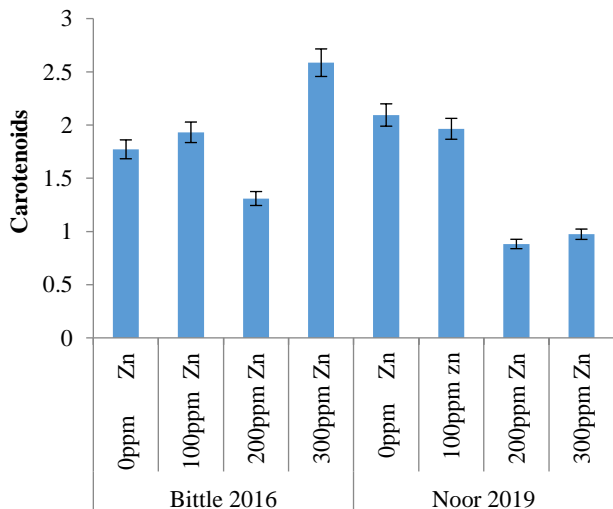


Fig. 11. Effect of different concentrations of zinc on carotenoids of two varieties of chickpea.

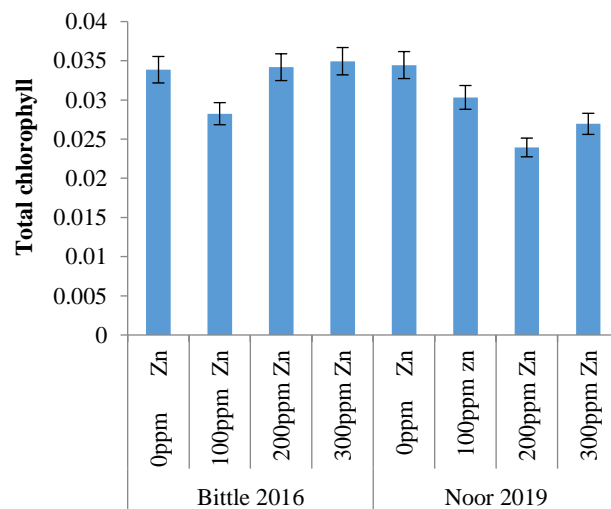


Fig. 12. Effect of different concentrations of zinc on total chlorophyll of two varieties of chickpea.

**Total chlorophyll:** Figure 12 shows the showed different levels under zinc application in two varieties of Chickpea (Bittle 2016 and Noor 2019) had non-significant effect on chlorophyll a of Chickpea. There was increased in the chlorophyll a of Chickpea under zinc application in order of 0<100< 200<300 (ppm). Zinc application (Zn) non-significantly reduced the total chlorophyll of chickpea plants as compared to plants under control conditions.

## Discussion

The data reveals root length of Chickpea under zinc increased in order of 0<100< 200<300 (ppm). Foliar application of Zn in chickpea increases the Zn content in grain by 22% (Hidoto *et al.*, 2017). Zn concentration in different chickpea genotypes varies from 2.0-2.7 mg/100 g in one study (Hemelatha *et al.*, 2007) to 3.7-7.4 mg/100 g in another study (Thavarajah & Thavarajah, 2012). Plant growth promoting bacteria may also collaborate in increasing the availability of nutrients particularly for iron and zinc (Sharma *et al.*, 2013). Zinc appears to affect the water intake and transportation capability of plants and also to reduce the negative impact of short heat and salt tension cycles. Zn is also an important growth hormone for producing auxin, since Zn is needed to synthesize tryptophan which precedes the IAA. In order to maintain the structural orientation of macromolecules and ion transport systems, Zn is essential for the integrality of cellular membranes. Its association with membrane proteins phospholipids and sulphhydryl groups leads to membrane maintenance (Sturikova *et al.*, 2018). Changing the expression of VvZIP3 during flowering and fertilization may change the dispersal and obtainability of Zn, thereby affecting common reproductive growth (Weng *et al.*, 2015).

Foliar application of Zn enhanced the concentration of grain and straw (Pathak *et al.*, 2012 and Habbasha *et al.*, 2013). Various Zn sources had different effects on yield of grain and economic of chickpea. Foliar application of Zn at 0.4% increased the grain yield by 12% and have outcome of 138 USD per hectare (Kayan *et al.*, 2015). A field study conducted application of Zn EDTA was better than the ZnSO<sub>4</sub> and foliar application was more beneficial than soil and the Zn foliar application at flowering stage has the outcome of grain yield (2.19 t/ha), biofortification of Zn (67.9 mg/kg grain), Zn uptake (149.1 g/ha). Foliar Zn application (0.1%) at the initiation if flowering stage increased the fertility of pollen and seed yield of chickpea (Pathak *et al.*, 2012). In the recent studies, it was observed the at 0.5% of Zn application improved the grain yield more than 40.9%, Zn contents (27.5%) and Zn uptake (36.8%) than at the control level (Purushottam *et al.*, 2018). Foliar application of Zn in chickpea has beneficial effects on the number of branches and the seed yield (Hadi *et al.*, 2013).

Seed treatment involves the application of fertilizer and other chemicals by seed priming and seed coating (Frooq *et al.*, 2012). It is costly and efficient as compared soil (Nawaz *et al.*, 2023). Furthermore, soil with the composition of associated with humic acids which are fundamental for soil structure, prosperity and internal

drainage (Machado and Serralheiro, 2017). Similar results were observed with the findings of Alam *et al.*, (2020), who found that Zn have positive effects on root growth and yield in specific crop species under normal conditions. The results reported by Rasheed *et al.*, (2014) also clarify that zinc application affects the growth and shoot fresh weight which ultimately increase the yield. Similar results reported by Mahmood *et al.*, (2009) that zinc application affects the root fresh weight which ultimately increase the yield. The results reported by Shafi *et al.*, (2010) also clarify that zinc application affects the growth and shoot dry weight which ultimately increase the yield. The results reported by Qin *et al.*, (2018) also clarify that zinc application affects the growth and root dry weight which ultimately increases the yield. The results reported by Khan *et al.*, (2006) also clarify that zinc sulphate affects the growth and leaf area which ultimately increases the yield. The results reported by Baryla *et al.*, (2001) also clarify that zinc application affects the growth which ultimately enhance the yield. The results reported by Sarma *et al.*, (2006) also clarify that zinc application affects the growth and chlorophyll a of chickpea which ultimately increase the yield. The results reported by Li *et al.*, (2015) also clarify that zinc application affects the chlorophyll b content of chickpea which ultimately increases the yield. Plants, algae, and photosynthetic bacteria all include carotenoids as pigments (Mahmood *et al.*, 2023). The results reported by Mishra & Ma, (2005) also clarify that zinc affects the carotenoid content of chickpea which ultimately increases the yield. The results reported by Sarma *et al.*, (2006) also clarify that zinc application affects the total chlorophyll of wheat which ultimately increased the yield.

Rise in temperature above the moderate level at the flowering stage will negatively affects pod formation and seed, resulting reduction in grain yield (Kumar *et al.*, 2013). Chickpea cultivated in declined moisture levels, the long roots play major role against drought (Upadhyay *et al.*, 2012, Gaur *et al.*, 2014). The status of genomic resources provided for chickpea betterment and proposed ways to develop the genome assisted breeding in chickpea (Varshney *et al.*, 2013). Evaluation of apparent lines in India, Kenya, and Ethiopia at different places guides to distinguish with consequently greater yield as compared to JG11 at every location in every condition (Gaur *et al.*, 2014).

## Conclusion

In conclusion, the application of Zn fertilization through the soil is the principal method of fertilizers application in chickpea crops but in some kind pros and cons in some required situations. In conclusion, foliar application betters the grainsome expertise and due to its excessive cost and usual sprays. This reseahc indicated that zinc application affects the growth and root dry weight which ultimately affected the yield.

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