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Effect of foliar application of zinc and boron on growth, yield and quality attributes in chickpea (*Cicer arietinum* L.)

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Abstract
The present study was conducted in the Division of Plant Physiology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu to evaluate the effect of zinc and boron fortification on yield and quality in chickpea variety GNG-1958. Foliar application of boron and zinc were applied at different concentration with treatment T1-Control, T2-Zn (0.5%), T3 - Zn (1.5%), T4-B (0.5%), T5-B (1.0%), T6-Zn (0.5%) B (0.5%), T7-Zn (0.5%) B (1%), T8-Zn (1%) B (0.5%), T9-Zn (1%) B (1%), T10-Zn (1.5%) B (0.5%) and T11-Zn (1.5%) B (1%). Maximum plant height was recorded in T6 (32.33 cm) in comparison to control (22.80 cm). Highest chlorophyll content was observed in T6 (2.56 mg/g) and lowest was found in control (1.18 mg/g). Maximum grain zinc and boron content were recorded in T6 (43.63 mg/Kg), (40.55 mg/Kg) and minimum were observed in control (39.42 mg/Kg) and (35.99 mg/Kg) respectively. The highest harvest index was found in T6 (33.11%) and lowest in control (30.01%). Foliar application of Zn (0.5%) + B (0.5%) combination were found best for quantitative yield and quality production of chickpea crop.

Keywords: Chickpea, zinc, boron, fortification, quantitative yield and harvest index

Introduction
Chickpea (*Cicer arietinum* L.) is considered as the largest food legume in South Asia. India is the largest producer of chickpea with 65% of global production. Chickpea is naturally a good source of protein (20-22%), fibre, minerals (phosphorus, calcium, magnesium, iron and zinc) and β-carotene. Nutrient deficiency in soil is the major cause for poor productivity of pulses. The nutrient deficiency in the soil has risen due to intensive agriculture and indiscriminate use of plant nutrients (Quddus *et al*. 2011) [23]. Among pulses, chickpea is an important source for food and fodder throughout the world. It is regarded as an excellent whole food and as a source of dietary proteins, carbohydrates, micronutrients, and vitamins (Jukanti *et al*. 2012) [14]. It has been reported by Kayan *et al*. (2015) [15] that the important micronutrient that limits legumes productivity is zinc and its deficiency is found common among various parts of the world. Chickpea is generally found sensitive to zinc deficiency. It has been proved that fertilization is one of the agronomic management strategies to enhance nutritional quality of chickpea grains and also has an important role in raising their productivity (Pathak *et al*. 2012) [22]. Application of Zn had a significant positive effect on seed Zn concentrations and grain yield, especially under Zn deficient conditions (Wei *et al*. 2007) [28]. According to analysis of soil and plant samples, it has been indicated that 49% of soils in India are potentially deficient in zinc, 12% in iron, 5% in manganese, 3% in copper, 33% in boron and 11% in molybdenum. Boron ranks third place among micronutrients. Boron plays a significant role in cell division and also accounts for seed and pod formation. An increase in deficiencies of micronutrients in soils and crops making the burden of disease, hunger globally, increased susceptibility of illness and loss of working time.

Johnson *et al*. (2005) [13] explained that foliar method influences the extent of micronutrient concentration both directly and indirectly in the treated plant and the parent plant through enrichment of the seeds. In order to encounter domestic demand of pulse requirement and ensure independence in pulses, a sustainable production and productivity approach has to be validated by fortification of chickpea crop. Fortification gives us a new mile stone to encounter all three kinds of hunger like calorie shortage, protein deficiency and micronutrient deficiency to our end users. With the aim of making the condition better, work has been done on small scale regarding micronutrient deficiencies in chickpea whereas it is a major limiting factor in chickpea production and productivity.

Materials and Methods

The purpose of this study was to determine the influence of foliar application of different concentrations of zinc and boron on growth, yield and quality attributes of chickpea (*Cicer arietinum* L.) variety, GNG-1958. The seeds of chickpea were obtained from Pulses Research Station Samba (PRSS). The experiment was conducted under pot condition and the laboratory work was carried out in the Division of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu-180009, J&K. The seeds of chickpea were germinated in plastic pots having 30 cm in height and the ratio of soil to vermicompost was kept as 6:1 i.e the mixture was made up to 6kg in each pot. The first foliar application of zinc and boron was given at the vegetative stage (40 DAS) of the crop in the form of zinc sulphate monohydrate and borax respectively. Final application of treatment was given to the crop just before flowering at 90 DAS. A number of observations pertaining to morphological, physiological and biochemical changes in plants were made, e.g., plant height, number of branches, relative water content, chlorophyll content, total carotenoids at 140 DAS and at harvest (168 DAS). When plants attained their maturity level, the observations on yield and quality, e.g., number of pods per plant, protein content, grain zinc and boron content, nitrogen content and harvest index were done at harvest.

Methodology

Growth characters

Plant height of the tagged plants were measured in centimetres as the distance from ground level to the tip of the plant at harvest. The number of branches emerging directly from the main shoots were counted at the time of maturity on three randomly selected plants and the average was recorded.

Quantitative analysis of pigment content

The chlorophyll estimation was done by acetone method (Arnon, 1949) [3] and expressed in mg g^{-1} FW. 200 mg of fresh plant leaf sample was placed in a test tube and crushed with 10 ml of 80% acetone in mortar using pestle and was collected in centrifuge, centrifuged at 5000 rpm for about 15 minutes, the supernatant was collected from centrifuge tubes into conical flask. The remnant was extracted again with 80% acetone until the colour of the remnant turns white and the final volume was made upto 25 ml with 80% acetone. The concentration of chlorophylls a, b and total chlorophyll were quantified in samples by reading the optical density at 663, 645 and 480 nm by using the formula. The chlorophyll content was calculated in mg chlorophyll per g F.W tissue using the following equations: mg chlorophyll a g^{-1} of tissue = 12.7 (A663) − 2.69 (A645) x V/ 1000 × W. mg chlorophyll b g^{-1} of tissue= 22.9 (A645) − 4.68 (A663) x V/ 1000 × W. mg total chlorophyll g^{-1} of tissue = 20.2 (A645) + 8.02 (A663) x V/ 1000 × W. mg total carotenoids g^{-1} of tissue = 0.114 + 480 (A663) – 0.638 (A645) x V /1000 x W. The RWC was calculated using the formula (Weatherley 1950) [27]. RWC% = [(fresh weight - dry weight)/ (turgid weight - dry weight)] x 100.

Biochemical analysis

Nitrogen estimation was done by Kjeldahl Method developed by Kjeldahl in 1883 [17]. 200 mg of homogenous sample of leaves was finely powdered, weighed using weighing balance and put into 30 ml digestion flask and to it 3g of K2So4 + $CuSO_4$ was added in 10:1 ratio in solid tube with 10 ml concentrated H_2SO_4 and mixed well. Glass beads were added and the sample were digested over digestion rack for 40 min at 170 °C. Minimum quantity of water was added along the sides of the flask to dissolve solids and transfer quantitatively to the distilled apparatus. 100 ml conical flask was placed in containing 5 ml of boric acid solution with few drops of mixed indicator. 10 ml of sodium hydroxide was added to the digest in the apparatus through the funnel and thoroughly rinsed with water. Ammonia was distilled and collected in boric acid. The colour of the solution changed from violet to green was an indication of ammonia absorbed. Finally, the tip of the condenser was rinsed with water and the distilled sample was titrated against standard hydrochloric acid until the appearance of original violet colour as the end point. Nitrogen% was calculated as ml HCL in sample-ml HCL in blank x Normality of acid x 14.01 x 100/ weight of sample in grams. Crude protein content was determined was then converted to protein by multiplying the corresponding nitrogen content in the chickpea leaves with a factor 6.25 (A.O.A.C., 1990) [2].

Quality parameters

Grain boron content in plant samples was measured by dry-ashing (Ahmad *et al.* 2012) [1] and subsequent measurement of B by colorimetry using Azomethine-H (Bingham, 1982) [5].1g dried and ground plant material was weighed in porcelain crucible. Ignited in a muffle furnace by slowly raising the temperature to 550 °C. Continued ashing for 6 hours after attaining 550 °C. and ash was wetted with five drops DW , and 10 mL 0.36 N H_2SO_4 solution was added into the porcelain crucibles. Steam bath was heated for 20 minutes and placed at room temperature for 1 hour, stirring occasionally with a plastic rod to break up ash. Filter through Whatman No.1 filter paper into a 50 ml polypropylene flask and bring to volume. Filtrate was ready for B determination. 1ml an aliquot of the extract was pipetted out into a 10ml polypropylene tube. 2 ml buffer solution was added. Added 2 ml azomethine-H solution and mixed well. Standard curve was prepared as follows: 1 ml of each standard (0.5 – 3.0 ppm) was pipetted out and proceeded as for the samples. Made a blank with 1 ml DI water, and proceeded as for the samples. Absorbance of blank was read out, standards, and samples after 30 minutes on the spectrophotometer at 420 nm wavelength. Calibration curve was prepared for standards, plotting absorbance against the respective B concentrations. B concentration was read out in the unknown samples from the calibration curve as mg/g B from calibration curve x volume of extract/ weight of dry plant.

Grain zinc content

The zinc content in grain and straw of chickpea was analysed by Atomic Adsorption Spectrophotometer Model (Lindsay and Norvell, 1978) [18]. 1000 mg of dried and crushed plant material was weighed, and then transferred into a 100 ml pyrex digestion tube. 10 ml (2:1 ratio) nitric acid and perchloric acid mixture was added to it, and kept overnight or until the vigorous reaction phase was over. Small and short stemmed funnels was placed in the mouth of the tubes to reflux acid. After the preliminary digestion, the tubes in a cold block digester was placed, and then raised temperature upto 150 °C for an hour. Placed the U shaped glass rods under each funnel to permit exit of volatile vapours. Increased temperature slowly until all traces of HNO_3 disappear, and then remove U shaped glass rods. Temperature was raised to

235 °C. When the dense white fumes of $HClO_4$ appeared in the tubes, continued digestion for 30 minutes more. Lifted the tubes rack out of the block digester, cooled for few minutes, and a few drops of DW water cautiously added through the funnel. After condensation of vapours, distilled water was added in small increments for washing down walls of tubes and funnels. Brought to volume, the solution was mixed of each tube, and leave undisturbed for a few hour. Each batch of samples for digestion contained at least one reagent blank. Operated Atomic Absorption Spectrophotometer or Flame Photometer according to the instructions provided for the equipment. Series of suitable standards was run and calibration curve was drawn. Supernatant liquid was decanted and analysed Zn in the aliquots by Atomic Absorption Spectrophotometer. Calculation of the supernatant liquid concentrations according to the calibration curve was made as mg/g Zn from calibration curve x volume of extract/ weight of dry plant. The harvest index of each treatment was calculated by dividing the economic yield (grain yield) by the biological yield (seed + stover yield) of the same net treatment and multiplied by 100.

Statistical analysis

Data were analysed using Completely Randomized Design (CRD). Treatments were compared using critical difference (CD) at 5% level of significance. All the data recorded were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar 125004, Haryana, India).

Result and Discussion

Plant Height (cm)

A significant increase in plant height and number of branches per plant were recorded in zinc treated plant in alone @ 1.5% and boron @ 0.5% followed by Boron 1% and Zinc @ 1.5% at harvest in comparison to control. As evident from figure,1 in various combinations of treatments, a significant increase in plant height and number of branches per plant were observed in Zn 0.5% + B 0.5% followed by Zinc 1.5% + B 0.5% Zn 1% + B 0.5% Zn 0.5%+ B 1% Zn 1% + B 1% and Zinc 1.5% + B 1%. As described by (Egamberdieva *et al*. 2017) [10] that the increase in plant length might be due to the role of Zn foliar application in the synthesis of IAA, metabolism of auxins, biological activity, stimulation of an enzyme activity and photosynthetic pigments because of that, encourage vegetative growth of plants. B plays a crucial role in multiplication of cell in meristematic tissues in legumes (Dashadi *et al*. 2013; Kayan *et al*. 2015) [9, 15]. Zn is also involved in the hormone synthesis, as a result, it's indirectly linked to carbohydrate translocation and metabolism, which contributes to more growth in comparison to control (Blesseena *et al*. 2019) [6].

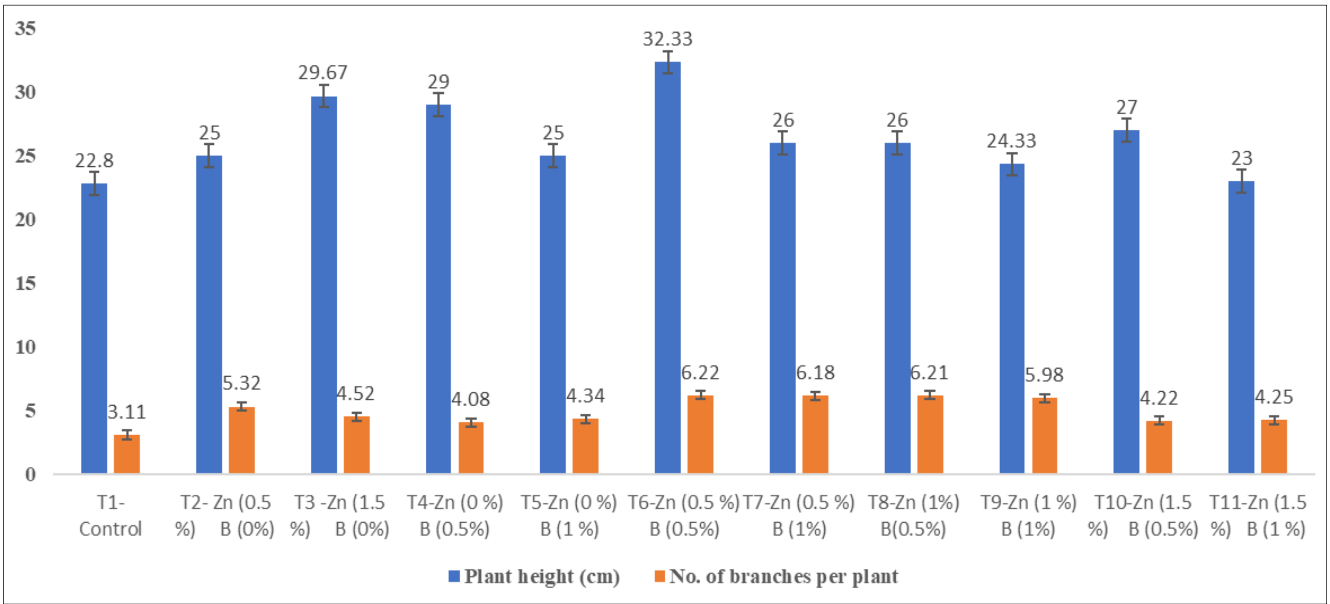

Fig 1: Effect of Zinc and Boron fortification on plant height (cm) and number of branches per plant in chickpea at harvest

	Plant height (cm)	No. of branches per plant
S.E. m.±	1.66	0.02
CD at 5%	4.91	0.06

S.E. m.± shows error bar mean

Chlorophyll content (mg/g)

A significant increase in chlorophyll *a* and chlorophyll *b* and total carotenoids were observed in zinc treated plant in alone @ 1.5% and boron @ 0.5% followed by Boron 1% and Zinc @ 1.5% at harvest in comparison to control. As evident from table 1. in various combinations of treatments, a significant increase in chlorophyll and carotenoids were found in Zn 0.5% + B 0.5% followed by Zinc 1.5% + B 0.5% Zn 1% + B 0.5% Zn 0.5%+ B 1% Zn 1% + B 1% and Zinc 1.5% + B 1%. Both Zn and B are essential for basic plant processes such as photosynthesis, protein synthesis, and chlorophyll synthesis (Cakmak, 2008) [7]. Our results are supported by (Bellaloui, 2011 and Nandan *et al*. 2018) [4, 20]. They found that foliar application of B enhance leaf chlorophyll content. These micronutrients, Zn and B are involved in root growth, synthesis of proteins and carbohydrates, increase flower setting (Wahid *et al*. 2011) [26].

Relative water content (%)

A significant increase in relative water content were recorded in zinc treated plant in alone @ 1.5% and boron @ 0.5% followed by Boron 1% and Zinc @ 1.5% at harvest in comparison to control. As evident from table 1. in various combinations of treatments, a significant increase in chlorophyll and carotenoids were found in Zn 0.5% + B 0.5%

followed by Zinc 1.5% + B 0.5% Zn 1% + B 0.5% Zn 0.5%+ B 1% Zn 1% + B 1% and Zinc 1.5% + B 1%. Similar results were also noted by (Sultana *et al*., 2016) that Zn defended the adverse effect of drought and remarkably increased wheat productivity. The study of (Chattha *et al*. 2017) [5] noted that Zn application enhanced maize yield and harvest index in drought stress. Moreover, Hera *et al*. (2018) [12] concluded that foliar applied Zn reduced the adverse impacts of water shortfall and enhanced growth and yield of wheat. Consequently, Zn application defends drought by making the membrane more stable, hormone synthesis, the photosynthetic process and the scavenging of Reactive Oxygen Species.

Table 1: Effect of Zinc and Boron fortification on chlorophyll *a* (mg g^{-1} FW), chlorophyll *b* (mg g^{-1} FW), total carotenoid content (mg $100g^{-1}$ FW) and relative water content (%), leaves in chickpea at 140 DAS

Treatments	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total carotenoid content (mg g^{-1} FW)	Relative leaf water content (%)
T_1- Control	1.18	0.68	0.18	63.63
T_2- Zn (0.5%) B (0%)	2.21	1.20	0.36	64.55
T_3 -Zn (1.5%) B (0%)	2.36	1.48	0.39	80.22
T_4-Zn (0%) B (0.5%)	2.49	1.55	0.43	79.45
T_5-Zn (0%) B (1%)	2.43	1.49	0.42	73.12
T_6-Zn (0.5%) B (0.5%)	2.56	1.64	0.46	89.55
T_7-Zn (0.5%) B (1%)	2.31	1.43	0.37	76.89
T_8-Zn (1%) B(0.5%)	2.18	1.48	0.38	76.40
T_9-Zn (1%) B (1%)	2.26	1.51	0.40	69.97
T_{10}-Zn (1.5%) B (0.5%)	2.30	0.98	0.43	74.85
T_{11}-Zn (1.5%) B (1%)	2.14	1.41	0.39	67.15
S.E. m.±	*0.08*	*0.12*	*0.03*	*2.39*
CD at 5%	*0.22*	*0.36*	*0.10*	*7.04*

S.E. m.± shows error bar mean

Nitrogen content and Crude protein content (%)

Nitrogen and crude protein content of leaves was increased in plant treated with zinc @1.5% followed by boron @ 0.5%, Zinc 0.5% and boron @1% at 140 DAS in comparison to control. As evident from Table 2. In various combinations of zinc and boron a significant increase in nitrogen content of leaves was recorded in Zinc 0.5% + B 0.5% (3.51) followed by Zinc 1.5% + B 0.5% (3.31), Zn 0.5%+ B 1% (3.17), Zn 1% + B 1% (3.21), Zinc 1% + B 0.5% (3.22) and Zinc 1.5% + B 1% (3.33) at harvest. Incorporating Zn into seeds may result in a large boost in protein production (Singh *et al*. 2005) [24]. The combined application of Zn and B at the dose of 0.5% each had significant effect on crude protein content of chickpea grains (Murthy, 2006) [19]. Protein synthesis and protein levels are markedly reduced in Zn deficient plants, but amino acids and amides are accumulated, as Zn is the structural component of the protein synthesizing polymerase enzyme. Hence, in Zn deficient plants, the protein synthesis of Ribonucleic Acid ceases (Fageria, 2009) [11].

Grain zinc and boron content (mg kg^{-1})

There was a significant increase in grain zinc content of plants treated with Zinc @1.5% and Boron @ 1% (46.18 and 52.78) followed by zinc @ 0.5% (37.10) and boron @ 0.5% (38.62) at harvest in comparison to control (41.26). As evident from table 2 in various combinations of zinc and boron a significant increase in grain zinc content was recorded in Zn 1% + B 1% (47.34) followed by Zn 1.5% + B 0.5% (45.45), Zn 1% + B 0.5% (47.13), Zn 0.5%+ B 1% (43.49), Zn 0.5% + B 0.5% (47.32), Zn 1.5% + B 1% (44.83) at harvest. There was a significant increase in grain boron content of plants treated with Boron @ 1% (41.98) followed by Zinc @ 0.5% (38.19) and Boron @ 0.5% (40.26) at harvest in comparison to control (36.72). As evident from table 2 in various combinations of zinc and boron a significant increase in grain boron content was recorded in Zn 0.5% + B 1% (46.79) followed by Zinc 1.5% + B 0.5% (38.96), Zn 1% + B 0.5% (46.28), Zn 1%+ B 1% (46.08), Zn 0.5% + B 0.5% (46.52) and Zn 1.5% + B 1% (38.05) at harvest. The current findings corroborate the findings of (Yadav *et al*. 2010; Pathak *et al*. 2012 and Nandaniya *et al*. 2016) [29, 21, 22]. Pathak *et al*. (2012) [22] revealed the amount of Zn in chickpea seeds and leaves had increased after foliar fertilization of Zn deficient as well as Zn sufficient plants. The use of micronutrients in a variety of pulses has boosted the concentration of nutrients in grains. The findings agree with those of Nandaniya *et al*. (2016) [21] who discovered that Zn, B, and Mo significantly boosted chickpea and lentil straw yield.

Table 2: Effect of Zinc and Boron fortification on grain Zn content, grain B content, nitrogen content and crude protein content in chickpea at harvest

Treatments	Grain Zinc (mg kg^{-1})	Grain Boron (mg kg^{-1})	Nitrogen content (%)	Crude protein content(%)
T_1- Control	41.26	36.72	2.99	18.70
T_2- Zn (0.5%) B (0%)	37.10	38.19	3.16	19.75
T_3 -Zn (1.5%) B (0%)	46.18	37.28	3.31	20.69
T_4-Zn (0%) B (0.5%)	38.62	40.26	3.00	18.74
T_5-Zn (0%) B (1%)	52.78	41.98	3.07	19.19
T_6-Zn (0.5%) B (0.5%)	47.32	46.52	3.51	21.96
T_7-Zn (0.5%) B (1%)	43.49	46.79	3.17	19.83
T_8-Zn (1%) B(0.5%)	47.13	46.28	3.22	20.11
T_9-Zn (1%) B (1%)	47.34	46.08	3.21	20.08
T_{10}-Zn (1.5%) B (0.5%)	45.45	38.96	3.31	20.71
T_{11}-Zn (1.5%) B (1%)	44.83	38.05	3.33	20.83
S.E. m.±	0.21	0.36	0.12	0.23
CD at 5%	0.62	1.06	0.35	0.67

S.E. m.± shows error bar mean

Test weight and Harvest index (%)

A significant increase in test weight and harvest index were found in zinc treated plant in alone @ 1.5% and boron @ 0.5% followed by Boron 1% and Zinc @ 1.5% at harvest in comparison to control. As evident from figure. 2 in various combinations of treatments, a significant increase in test weight and harvest index were observed in Zn 0.5% + B 0.5% followed by Zinc 1.5% + B 0.5% Zn 1% + B 0.5% Zn 0.5%+ B 1% Zn 1% + B 1% and Zinc 1.5% + B 1%. B is involved in the retention of flowers, pollen tube growth, seed formation, seed setting, and also plays an important role in transportation of metabolites from source to sink. Application of Zn enhance quality and yields of chickpea reported by Khan *et al*. (2003) [16]. Crop responses in terms of productivity have been assorted through micronutrient fertilization. A significant increase in Harvest index with the application of Zn and B was also found by Valenciona *et al*. (2010) [25].

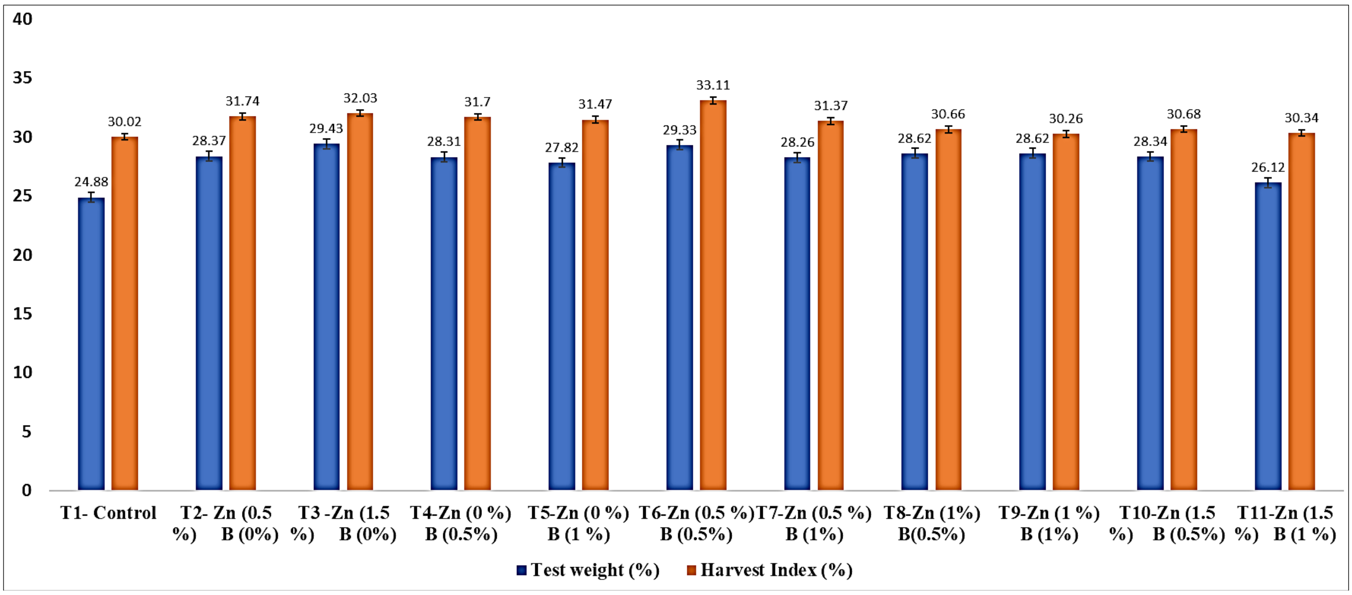

Fig 2: Effect of Zinc and Boron fortification on Test weight (%) and Harvest Index (%) in chickpea at harvest

S.E. m.±	0.72	0.43
CD at 5%	2.12	1.27

Conclusion

Application of Zn (0.5%) B (0.5%) by foliar spray is beneficial for better yield and quality production of chickpea crop. Fortification of zinc and boron also gives better performance when applied in their combination. Therefore, it is suggested that application of Zn (0.5%) B (0.5%) commands a great significance in maintaining a number of physiological, biochemical, yield and quality parameters by maintaining growth and relative water content, increasing nitrogen and protein content, increasing the grain zinc and boron content that deals with mineral deficiency problems. Application of Zn and B had a significantly positive effect on seed Zn and B concentrations and grain yield, especially under Zn deficient conditions. Fortification of zinc and boron increased quantity of minerals in plants, in addition to being a fascinating approach for promoting beneficial phytochemical compounds to plants.

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