

# Research Article



# Response of Chickpea (*Cicer Arietinum* L.) to Phosphorus and Zinc Levels and Their Application Methods

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Abstract | The time, quantity and method of nutrients application are crucial factors for enhanced crop yield and economic return. This study investigated the effect of phosphorous (0, 35, 70 and 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and zinc (0, 10, 20 and 30 kg ha<sup>-1</sup>) levels applied through broadcast and banding on chickpea growth and yield during winter 2013-14 at the New Developmental Farm, The University of Agriculture, Peshawar. The experiment was carried out by following randomized complete block design procedure replicated four times. Pods number plant (PN), grain yield (GY) biological yield (BY), and harvest index (HI) were significantly increased by 9, 4, 23 and 8%, respectively over the control with fertilizer banding compared with broadcast. Plots receiving 10 kg Zn ha<sup>-1</sup> registered a significant increase of 8, 9, 4, 27 and 9%, in plant height (PH), PN, GY, BY and HI, respectively over the control, and recorded the lowest number of days to maturity (185) compared to control (188 days). Both 70 and 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> registered PH with 8% increase, PN with 8 and 7.4% increase, respectively, GY with 4% increase, BY with 25% increase and HI with 9.2 and 9.5% increase over the control. Number of days to achieve crop maturity also remained the same (185 days) for the P<sub>2</sub>O<sub>5</sub> levels and remained statistically similar with each other (185 days) compared to the control (188 days). Interaction of 70 kg P<sub>2</sub>O<sub>5</sub> with 10 kg Zn ha<sup>-1</sup> produced maximum HI (35%). This study suggested that 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 10 kg Zn ha<sup>-1</sup> should be applied in bands for enhanced yield and related growth traits of the chickpea crop. Interaction among nutrients and application methods showed that application of nutrients with band placement method gave excellent results even at lower doses emphasizing proper application method as more important than mare increasing nutrient levels for obtaining higher returns.

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#### Introduction

Chickpea (*Cicer arietinum* L.), is an important winter food legume and is grown throughout the world. Its ability to get mature within a span of time (3-6 months) based on the variability of agro ecological conditions indicate its wider adoptability in a variety of climates (Singh and Diwakar, 1995). Being less water demanding, it is widely cultivated in moisture

scarce areas of the agricultural field. In Pakistan, its share is about 75% of the pulses grown (Ali et al., 1991). Chickpea is the major and economic source of edible protein (20%), which satisfying the nutritional requirements of the rural people in the country (Ahmad et al., 1991).

The time, quantity and method of nutrient application are crucial factors for profitable chickpea crop





production. It is pertinent that amount of yield losses fluctuate due to nutrients scarcity (Ali et al., 2002). Because of poor economic position of our farmers, increase in the quantity of the applied nutrients may rarely be possible; however, their application methods can greatly affect the availability and agronomic effectiveness of the applied nutrients. Amongst the required essential nutrients, phosphorus (P) and zinc (Zn), being crucial element for optimum growth and yield of all crop (Ryan et al., 2012) throughout the world, are most important for our farmers consideration because of the sensitivity of these two nutrients to the existing soil conditions and their mutual interaction. Phosphorus availability in optimum quantities is needed for early growth stages, development of the reproductive parts, root growth, reduced disease incidence and early maturity. Compared to vegetative growth, P availability in considerable quantities is critically needed for seed formation (Gidago et al., 2012). The required quantity, may however, vary with respect to location. For example, in Peshawar, 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> has considerably increased the chickpea yield (Biser et al., 2008), tissue P accumulation and dry matter production. Likewise, the Zn importance for chickpea productivity (Ahlawat et al., 2007) and plant growth and nitrogen-fixation has also been established. Since, under most alkaline conditions, its scarcity is widespread, among the micronutrients (Roy et al., 2006), chickpea productive sensitivity is not an exception despite a range of variation among varieties (Khan et al., 2004). In cases where Zn is deficient, crop maturity is greatly delayed, water use efficiency and yield are decreased (Khan, 2010), and the important process of nitrogen fixation and nodulation (Shukla et al., 2004). Zinc deficiency can be simply overcome by foliar and soil fertilization of zinc (Roy et al., 2006). Proper placement of P fertilizer enhances yield and yield attributing traits and fertilizer use efficiency (Gidago et al., 2012). These are important especially under high soil pH conditions, since the availability of P and Zn for optimum growth and yield under alkaline conditions are largely affected through soil because it forms zinc phosphate which is not soluble in water this phenomena is called antagonistic effect of nutrients (Wijebandara, 2007). Both these nutrients, therefore, require special attention under alkaline conditions. This experiment was, therefore, planned to determine the optimum phosphorous and zinc levels under deficient soil with minimum antagonism for each other under different application method suitable to get higher yield from chickpea.

# Materials and Methods

The experiment was conducted during winter 2013-14 at the New developmental Farm, The University of Agriculture, Peshawar (34°01'N and 71°35'E), Khyber Pakhtunkhwa, Pakistan, using factorial randomized complete block design replicated four times. Broadcast and banding methods were evaluated for different doses of both phosphorus (0, 35, 70 and 105  $kg P_2O_5 ha^{-1}$ ) and zinc (0, 10, 20 and 30 kg ha<sup>-1</sup>). In band placement the fertilizer was placed near the seed row at the time of sowing. Treatments were applied in 5 m × 1.8 m plots cultivated with chickpea variety NIFA 2005 on 13<sup>th</sup> November 2013 in six rows 30 cm apart maintaining 60 kg ha<sup>-1</sup> seed rate. All zinc (Zn) and half phosphorous (P) were applied in sowing time where as remaining half phosphorous was applied after 45 days. Sources for phosphorous and zinc were single super phosphate and zinc sulphate (ZnSO<sub>4</sub>), respectively, whilst urea was used as a source of nitrogen applied as basal dose at the rate of 30 kg ha<sup>-1</sup> to facilitate initial growth of the crop. The soil was silt loam in texture, alkaline in reaction, calcareous in nature, low in soil organic matter content, poor in available P and zinc. Physical and chemical properties of experiment are given in the Table 1. The experimental sites were classified in order inceptisol, suborder ochrepts having great group is ustochrepts according to the principle as described in key of soil taxonomy. During the cropping session of chickpea mean monthly temperature and rainfall recorded from Peshawar forest institute (PFI) are present in Figure 1.

**Table 1:** Physico- chemical characteristics of the experimental site before crop sowing.

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Property	Units	Concentration				
Sand	%	41.4				
Silt	%	51.4				
Clay	%	7.2				
Textural class	-	Silt loam				
pH (1:5)	-	7.61				
Electrical conductivity(EC)	$dS\ m^{\text{-}1}$	1.12				
Lime	%	15.3				
Organic matter content	%	0.9				
Phosphorous (P)	mg kg <sup>-1</sup>	2.14				
Zinc (Zn)	mg kg <sup>-1</sup>	0.23				
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For growth data collection, five plants were randomly selected in each plot. Plant height was measured





from base to the plant top using a meter rod. Number of pods was counted manually after harvesting on 23<sup>rd</sup> may 2014 and then averaged for number of pods plant<sup>-1</sup>. Days from sowing to harvest were counted and averaged. For biological yield, four central rows were harvested. The whole material was air dried, weighed and then converted into biological yield (BY) kg ha<sup>-1</sup> as follows;

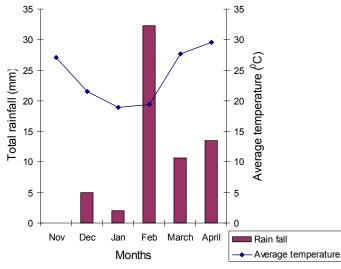
$$BY = \frac{Biological\ yield\ from\ harvest\ rows}{Row - Row\ distance\ \times No.of\ rows\ \times rows\ length}\ \times 10000\ m^2$$

Grain yield was recorded by harvesting four central rows, sun dried and threshed. Seeds from the harvested rows of each plot were obtained through threshing, and converted into grain yield (GY) kg ha<sup>-1</sup> as follows;

$$GY = \frac{Grain\ yield\ from\ harvested\ rows}{Row - Row\ distance\ \times No.of\ Rows\ \times row\ length} \times 10000\ m^2$$

Harvest index (HI) was determined as;

$$HI(\%) = \frac{Grain\ yield}{Biological\ yield} \times 100$$



**Figure 1:** Total rainfall and average temperature during the phase of chickpea growth (PFI Peshawar).

# Statistical analysis

The recorded data of factorial randomized complete block design (control Vs rest analysis of variance) were analyzed statistically using Statistix 8.1 computer software. Significantly different means were separated through least significant difference (LSD) test at the 5% probability level (Steel and Torri, 1984).

#### **Results and Discussion**

The effect of nutrients phosphorus and zinc levels significantly (p<0.05) enhanced plant height (PH) whereas application methods showed non significant effect. Increase in PH with 35 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was negligible (0.6%) over the control perhaps because of the fixation of the whole of it on soil fixing sites. However, increase in PH with both 70 and 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> were 7.9 and 8%, respectively (Table 2), indicating the sufficiency of 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> compared to other doses. Increase in PH with zinc (Zn) at the rate of 10 kg ha<sup>-1</sup> was 8% over the control whilst those attained by 20 and 30 kg Zn ha<sup>-1</sup> were 3.9 and 4.7%, respectively, and both being statistically the same (Table 2). Reduction in PH with increase in Zn level might be accredited to its increased antagonism with the applied phosphorous (P) at higher doses El-Habbasha et al. (2013). It can be deduced from these results that the both inherent P and Zn levels under the existing soil condition were below the crop requirement and external P and Zn application enhanced the availability of these nutrients to the crop and ensured maximum vegetative growth. However, this increase in vegetative growth was proportional to increase in their external supplementation up to a certain level which was 70 and 10 kg, P<sub>2</sub>O<sub>5</sub> and Zn ha<sup>-1</sup> in this case, respectively. Other soil conditions as those studied by Togay et al. (2008) showed that 80 and 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> were optimum and increased the PH and height of the first pod as compared to P<sub>2</sub>O<sub>5</sub> lower levels, respectively. This comparison confirms Ali et al. (2002) stating that yield losses fluctuate according to nutrient scarcity in soil. The effect of Zn levels on plant height was in conformity with those of Taha and El-Habbasha (2011) showing increased plant height in chickpea with external application of Zn to the crop.

Phosphorus and zinc levels as well as their application methods had significantly (p<0.05) increased pods number (PN) plant<sup>-1</sup>, while their interaction was non-significant (Table 2). Phosphorus at 70 kg ha<sup>-1</sup> showed the maximum number of pods plant<sup>-1</sup> (by 8%), however, it was at par with 105 kg ha<sup>-1</sup> (by 7.4%) whilst the minimum number of pods plant<sup>-1</sup> (by 2%) was showed increase at 35 kg  $P_2O_5$  ha<sup>-1</sup>. These findings conformed to those suggested by Bicer (2014) i.e.  $P_2O_5$  supplementation at the rate of 70 kg ha<sup>-1</sup> was superior in increasing the number of chickpea pods plant<sup>-1</sup>. Similarly, Zn application at the rate of 10, 20 and 30 kg Zn ha<sup>-1</sup> recorded 8.6, 4.5 and 4% increase in





**Table 2:** Plant height, Pods plant<sup>-1</sup> and days to maturity as influenced by phosphorous, zinc and their application methods.

Treatments		Parameters				
Phoshorous (kg ha <sup>-1</sup> )	Plant height (cm)	Pods plant <sup>-1</sup>	Days to maturity			
35.00	62.92	67.58	187.00			
70.00	67.46	71.83	185.25			
105.00	67.50	71.46	185.50			
$LSD_{0.05}$	0.42	0.55	0.30			
Zinc (kg ha <sup>-1</sup> )						
10.00	67.46	72.25	185.75			
20.00	64.96	69.54	185.92			
30.00	65.46	69.08	186.08			
$LSD_{0.05}$	0.42	0.55	0.30			
Application methods (AM)						
Broadcast	65.33	68.28	186.14			
Band placement	66.58	72.31	185.69			
$LSD_{0.05}$	0.28	0.36	0.20			
Interaction						
$AM \times P$	NS	NS	NS			
$AM \times Zn$	NS	NS	NS			
$P \times Zn$	NS	NS	NS			
$AM \times P \times Zn$	NS	NS	NS			
Control	62.50	66.50	188.00			
Rest	65.96	70.29	185.92			

AM: application method; NS: non significant.

PN over the control, respectively (Table 2) indicating the sufficiency of 10 kg Zn ha<sup>-1</sup> under the present experimental conditions compared to its higher doses. These results were in agreement with the finding of El-Habbasha et al. (2013) who stated that zinc fertilization at flowering enhanced number of pods plant<sup>-1</sup>. Results indicated significant increase in PN (9%) with band placement of fertilizer compared to the increase registered by broadcast application (3%) of fertilizer over the control. Better results with band placement of fertilizer is assumed to ensure higher concentration of nutrients in the root zone compared to the broadcast where the applied fertilizers are diluted by the large volume of soil to which it come in contact Abdel et al. (2005). Availability of nutrients for plant uptake is therefore, higher in band placement than broadcast resulting in higher number of pods per plant. Application of P and Zn and their dose adjustment require special techniques and knowledge of the behavior of each nutrient in soil in presence of the other. Better results with regards to number of pods plant<sup>-1</sup> at medium levels of P and Zn points out their better adjustment to subside their mutual antagonism at these levels, which at the highest levels of one nutrient and being antagonistic would, otherwise, have reduced the availability of the other and decreased the growth and yield of the crop.

Numbers of days to achieve maturity by the crop were significantly reduced with P fertilizer addition irrespective of its method of application. The effect of Zn levels on number of days to maturity was also non-significant. The results showed that early maturity (185 days) was observed for 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> whereas late maturity (188 days) was recorded for control (no zinc and phosphorus). The number of days to maturity at the highest P level (105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) was statistically similar to 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table 2). Applications of required phosphorus levels not only enhance the rate of crop growth from emergence to initiation of flowers but also advance the anthesis of the crop and turn it towards reproductive stage. These results were in agreement to Abdel et al. (2005) stating that P fertilization resulted in early maturity.

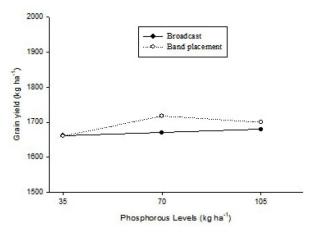


Figure 2: Interaction effect of phosphorous and application method on grain yield of chickpea.

Grain yield (GY) was positively influenced to a significant extent by P and Zn levels as well as their application methods (AM) and the interaction AM×P (Figure 2). Applied P levels of 35, 70 and 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased the GY by 2, 4.3 and 4.1%, respectively over the control (Table 2) where the GY at the two higher P levels were statistically the same. Increase in GY at Zn levels of 10, 20 and 30 kg ha<sup>-1</sup> was 4.4, 3 and 3.3% over the control, respectively. Increase in GY with band and broadcast method was 4.3 and 3%, respectively over the control. These results indicated the demand for P and Zn by the crop as well

as the superior results by the optimum dose of the applied nutrients under the prevailing soil conditions. In a similar study, Singh et al. (2012) reported higher GY after P and Zn application at the rate of 60 and 25 kg P<sub>2</sub>O<sub>5</sub> and ZnSO<sub>4</sub> ha<sup>-1</sup>, respectively. This shows that selection of optimum dose of a particular nutrient strongly depends on the prevailing soil fertility conditions which in our case were 70 and 10 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and ZnSO<sub>4</sub>, respectively. The outcome of particular nutrients depends on the way the nutrients are applied. In our study band placement of P and Zn achieve superior results. It is the band placement of P and Zn, both being much demanding for higher crop growth under the condition of this experiment, which maintain higher concentration near roots compared to the broadcast. Abdel et al. (2005) also reported superior result for 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied in bands for chickpea GY. The interaction AM×P in our experiment confirmed the findings of Abdel et al. (2005) recorded the maximum GY for 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied in bands was the most successful treatment among the other treatments to increase yield and growth of chickpea whilst plots receiving phosphorous doses 35 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> resulted in reduced GY. Similarly, application of 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> reduced grain because it was found exceeding above the optimum levels and diminishing the performance of a crop (shah et al., 2016).

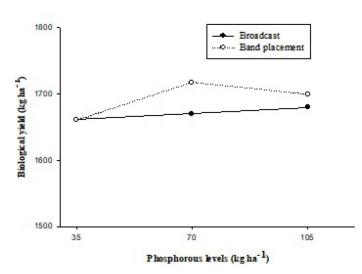


Figure 3: Interaction effect of phosphorous and application method on biological yield of chickpea.

Phosphorus (P) and zinc (Zn) levels as well as the application methods (AM) effect on grain yield were significant for biological yield (BY). Furthermore, the interaction effects AM×P (Figure 3) and P×Zn (Figure 4) on BY were also significant (Table 3). Results showed that P levels of 35, 70 and 105 kg  $P_2O_5$  ha<sup>-1</sup> increase in BY 8.5, 25 and 25.3%, respectively over

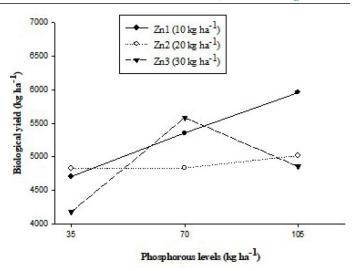


Figure 4: Interaction effect of phosphorous and zinc levels on biological yield of chickpea.

the control treatment. Results also indicating that a non-significant increase was observed in BY at 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> over the 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> level. Perhaps increase in P2O5 beyond 70 kg ha-1 negatively affected another nutrients availability for example Zn. Increase in BY with 10, 20 and 30 kg Zn ha<sup>-1</sup> was 27, 16 and 15.7% over the control, respectively, indicating the lower dose sufficiency under the present soil conditions. Increase in BY with band placement was 24% whilst with broadcast, it was 15.4% over the control plot with nutrient applications. Interaction (Figure 3) showed that the effect of application methods (AM) on BY at 35 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was statistically the same, however, growing up of the P<sub>2</sub>O<sub>5</sub> dose to 70 kg ha<sup>-1</sup> significantly improved the BY in band placement over the broadcast. The same trend in BY among the AM was observed at 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> but the different was smaller than 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The probable reason may be due to the dilution effect of P (Wijebandara, 2007). Application of 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was the optimum level through band method amongst other treatments in term of enhancing growth and yield of the crop. Interaction between P and Zn levels indicated that at highest P level, BY decreased with increasing Zn doses beyond 10 kg Zn ha<sup>-1</sup>. Singh et al. (2012) reported his results in the same style and stated that chickpea straw yield increased with P and Zn levels of 60 and 5 kg P<sub>2</sub>O<sub>5</sub> and Zn ha<sup>-1</sup>, respectively. Higher nutrients concentration near plant roots and their subsequent availability in band placement of fertilizer application resulted in higher BY with 70 and 105 kg ha<sup>-1</sup>  $P_2O_5$  and 10 kg ha<sup>-1</sup>  $Z_1$ .



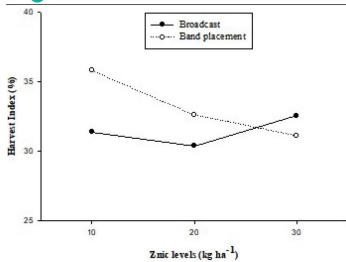
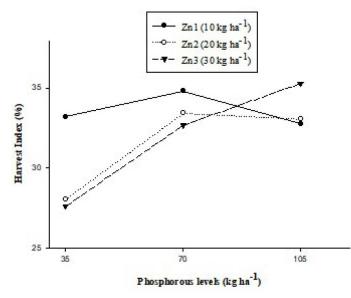


Figure 5: Interaction effect of zinc and application methods on harvest index of chickpea.



**Figure 6:** Interaction effect of phosphorous and zinc levels on harvest index of chickpea.

Significantly higher harvest Index (HI) was observed with P, Zn and their interaction (PxZn) (Figure 5). Application methods (AM) and its interaction with Zn (AMxZn) (Figure 6) also showed significant effect on HI. Lower dose of P2O5 (35 kg ha-1) resulted in 3.7% reduced HI over the control. The lower dose P<sub>2</sub>O<sub>5</sub> (35 kg ha<sup>-1</sup>) only increase the biological yield as compared to control which ultimately reduced the harvest index Ramakrishna et al. (2005) whilst 70 and 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> showed 9.3 and 9.6% improvement, respectively in HI over the control treatment (Table 3). Thus, application of 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> is more beneficial than 105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> since the quantity is being reduced by 33% and the return is almost the same. In case of Zn, HI for 10 kg Zn ha<sup>-1</sup> was 9% higher over the control whilst those for 20 and 30 kg Zn ha<sup>-1</sup> were only 2.4 and 3.6% higher over the control.

**Table 3:** Grain yield, biological yield and harvest index as influenced by Phosphorous, zinc and their application methods.

Treatments		Parameters				
Phosphorous (kg ha <sup>-1</sup> )	Grain yield (kg ha <sup>-1</sup> )	Biological yield(kg ha <sup>-1</sup> )	Harvest index (%)			
35.00	1661	4568	29.6			
70.00	1694	5257	33.6			
105.00	1690	5276	33.7			
$LSD_{0.05}$	17	83	0.35			
Zinc (kg ha <sup>-1</sup> )						
10.00	1694	5339	33.60			
20.00	1673	4891	31.50			
30.00	1678	4871	31.85			
$LSD_{0.05}$	17	83	0.35			
Application methods						
Broadcast	1671	4860	31.44			
Band placement	1693	5207	33.19			
$LSD_{0.05}$	14	55	0.24			
Interaction						
$AM \times P$	Fig2	Fig3	NS			
$AM \times Zn$	NS	NS	Fig5			
$P \times Zn$	NS	Fig4	Fig6			
$AM{\times}P{\times}Zn$	NS	NS	NS			
Control	1623	4208	30.75			
Rest	1682	5034	32.32			

AM: Application methods.

Thus, increasing Zn dose over 10 kg ha<sup>-1</sup> was resulting in decreasing grain yield compared to straw yield. For AM, the HI was 2 and 8% higher for broadcast and banding of fertilizer over the control where no fertilizer was applied. It means, not only optimization of a particular nutrient is required for higher grain yield, its proper application through adopting a suitable method is also necessary which in our case was banding compared to broadcast. This study indicated the usefulness of band application since lesser quantity produced better results compared to more quantity of the nutrient applied as broadcast. Dutta and Bandyopadhyay (2009) and Ramakrishna et al. (2005) also reported increased HI in chickpea for P and Zn application. Interaction PxZn (Figure 5) revealed that Zn at the rate of 10 kg ha<sup>-1</sup> showed higher HI with 70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> whilst 30 kg Zn ha<sup>-1</sup>showed higher HI with 105 kg P<sub>2</sub>O<sub>5</sub> ha-1 whilst both of these HI values were not much different. It means increasing nutrients dose were not returning the grain yield according to its proportion.





Thus, the best combination of P and Zn for favorable HI was  $10 \text{ kg Zn ha}^{-1}$  and  $70 \text{ kg P}_2\text{SO}_5 \text{ ha}^{-1}$ .

This is clear indication of antagonistic effect between phosphorus and zinc in soil since the performance of one nutrient is affected by increase or decrease in the quantity of the other. The interaction AM×Zn (Figure 6) revealed that HI decreased consecutively with increasing Zn level in banding. In broadcast, higher Zn level (30 kg Zn ha<sup>-1</sup>) however showed higher HI but it was still well below than HI obtained with 10 kg Zn ha<sup>-1</sup> applied through banding. Thus, application of Zn by using a proper application method may give excellent results even at lower doses. In other words, using proper application method is much more important than mare increasing nutrient levels for obtaining higher returns.

# **Conclusions**

This study suggests 70 and 10 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and Zn respectively for higher yield and yield components of chickpea and the application methods as band placement. Interaction among nutrients and application methods showed that application of nutrients by using band placement method gave excellent results even at lower doses indicating that using proper application method is much more important than mare increasing nutrient levels for obtaining higher returns.

#### **Author's Contribution**

**Saeed Ullah:** Conducted the exdperiment and collected the field data.

**Amanullah Jan:** Planned the experiment and superivsed its conductions and helped in data calculation.

**Murad Ali:** Helped in field data collection and field management, data entry into the computer.

Wiqar Ahmad: Helped in the abstract and results and discussion of the manuscript write up.

Hafeez Ur Rehman: Helped in data handling in the computer and statistical analysis.

**Muhammad Ishaq:** Helped in collection of review and write up of introduction.

**Bilal Ahmad:** Helped in laboratory analysis and calculations.

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