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Original Research Article

IMPACT OF DIFFERENT ZINC APPLICATION METHODS AND PHOSPHORUS DOSES ON GROWTH, YIELD AND GRAIN ZINC CONTENTS OF WHEAT

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Abstract *Wheat is a staple crop of around half of the world's population. About 30 percent of the world's population is suffering from Zn deficiency. Zn deficiency problems can be encountered by agronomic and genetic biofortification. Phosphorus (P) is an important plant macronutrient and has an antagonistic relationship with Zn. A field study was performed to evaluate the impact of different Zn application methods and P doses on growth, yield, and grain Zn contents of wheat at the Agronomic Research Farm, University of Agriculture Faisalabad, Pakistan, using a high accumulation wheat variety Zincol-2016. Three doses of P (100, 150, and 200% of recommended P) along with three Zn application methods (seed priming, soil mixing, and foliar application) were applied to wheat using randomized complete block design (RCBD) with three replicates under split-plot arrangement. All agronomic practices were kept normal and uniform for all treatments. Parameters including growth, yield, and Zn content in wheat grains were monitored using standard protocols. The results were evaluated using Fisher's analysis of variance at a 5% probability level. A comparison of means of treatments was performed using the least significant difference (LSD) test. The highest value of SPAD reading (56.38) was noted in treatment where Zn was applied as priming and P was applied at 200%. Maximum crop growth rate (63.17) was observed in P3 where Zn was applied as mix in soil. Maximum spike length (14.25), spikelets per spike (17.67), and Zn concentration were observed in P3 as a result of foliar application of Zn. Highest grain weight per spike (1.89g), no. of tillers (691.01), grain yield (4.09 t ha-1), biological yield (11.01 t ha-1), harvest index (36.54%) and NDVI (0.42 were observed in P3 where Zn was applied as soil mix.*

Keywords: *Wheat; Zn deficiency; Phosphorus; biofortification*

Introduction

Wheat is a cereal crop belonging to *Poaceae* family that is most commonly used around the world. It provides a large share of daily calories, proteins, and micro and macro-nutrients. It is commonly cultivated for grain purposes which is consumed by humans to fulfill their nutritional requirement. In world trade, the wheat crop contributes more than other crops. In China, food products based on wheat provide more than 70 percent of daily calorie requirements and about 20 percent of Zn and Fe needs (Ma *et al.*, 2008). Wheat production was recorded 25.49 million tons in the year 2017-18.

Zinc is a vital micro-nutrient that is involved in biological systems and gaining more attention on a global level (Cakmak, 2008). It plays regulatory, catalytic, and structural roles in many physiological processes of plants (Caldelas and Weiss, 2017), and optimum supply is required to improve productivity (Rameshraddy *et al.*, 2017). It plays a key role in structural components and is involved in the regulation of various enzymes (Barak and Helmke, 1993) which are essential for biochemical pathways including photosynthesis and carbohydrate metabolism.

According to an estimate, people affected by deficiency of Zn are about two billion in the world. In Pakistan, nearly 54.21% kids, 37.11% toddler (Ejaz and Latif, 2010), and 40.0% of mothers have

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Zn deficiency (Hussain *et al.*, 2010). Reports indicate that 50% of wheat is sown on Zn-deficient soils globally which is also a reason for decreased Zn concentration in wheat grains (Cakmak, 2008). Therefore, populations living in wheat-growing areas are more susceptible to Zn malnutrition (Alloway, 2009). This condition can be handled by mineral supplementation, food fortification or dietary diversification. However, these strategies have not always been successful (Gibson, 2012; Rehman et al., 2023; Rehman et al., 2024; Shahzad et al., 2022). Biofortification would be an efficient alternative to enhance Zn concentrations in crops by use of Zn fertilizers and plant genotypes that have a higher capacity to attain and assimilate Zn in their edible tissues (Velu *et al.*, 2014).

Phosphorus is an essential macronutrient affecting plant growth, yield, and quality. Zn and P are the most affected nutrients of soil problems. P is only 10-15% available for plants from the total application of P fertilizer application to soil. In contrast, the remaining P is adsorbed by soil particles or makes a precipitated compound with calcium (Ca) or magnesium (Mg) and is unavailable for plants. Plants have developed various survival mechanisms under deficiency of P. Changes in length, morphology, and architecture of roots along with exudation of organic proton acids (Aziz *et al.*, 2011).

Interaction between P and Zn has been found antagonistic in plants (Hussain *et al.*, 2015; Abbas et al. 2021; Abbas et al. 2022). Zn deficiency in crops often occurs after heavy P fertilization in soils. Enhanced P uptake is usually found to cause Zn deficiency symptoms in leaves but does not affect total Zn content in plants. However, the proportion of water-soluble Zn in plant tissues is decreased. Zn and P can bind each other forming insoluble Zn-phosphate compounds, as a result, inhibition of Zn uptake by roots and translocation in plants would occur. Therefore, increased P fertilization can reduce Zn uptake resulting in developing Zn deficiency in plants. Interaction between Phosphorus and Zn has been reported in roots because they prevent mutual translocation to the upper parts of the plant (Hussain *et al.*, 2015). This, in turn, decreases Zn movement within the plant. Enhanced P fertilization can decrease Zn absorption, but increased plant P uptake thus causes Zn deficiency (Zhao *et al.*, 2007). Zn plays a key role in the P uptake signal transduction pathway. In Zn-deficient plants, the expression of genes that encode a high-affinity P transporter protein breaks down and induces P accumulations in plants (Huang *et al.*, 2000; Junaid and Gokce, 2024; Khalid adn Amjad 2018; Raza, 2023). The purpose of this study was to assess the impact of P doses and Zn application methods on growth, yield, and grain Zn contents of wheat.

Material and methods

Location

Research was conducted to study the impact of Zn treatments on growth, yield, and Zn contents in grains of wheat as affected by phosphorus. The study was conducted at the Agronomic Research area, UAF, Faisalabad, Pakistan, during winter 2019-2020.

Soil Physicochemical Properties:

Soil samples were taken from the trial area using an augur at a depth of up to 30 cm. The collected soil samples were dried before grinding and then passed through 2mm sieve for the analysis of soil to check the physicochemical properties in the lab (table 1).

Table 1 Soil Physico-chemical Properties

Physico-chemical properties	**Units**	**Diagnostic values**
N	%	**0.050**
Available Phosphorus	Ppm	**7.9**
Available K	Ppm	**159**
Soil pH		**7.4**
Organic Matter	%	**0.1**
Electrical conductivity	Dsm	**1.41**
Capacity	%	**40**
Texture	-	**Loam**
SAR	-	**6**
ESP	**-**	**7**

Experiment and Treatments

Factor A: Zinc Application Method (Sub plot)

Following treatments were applied

Zn_0= control (no application of Zn)

Zn_1= seed priming (0.0015% $ZnSO_4.7H_2O$)

Zn_2=Mix in soil (5kg/ha $ZnSO_4.7H_2O$)

Zn_3= Foliar Application (0.3%$ZnSO_4.7H_2O$)

Factor B: Fertilizer (main plot)

Following four doses of P were applied for the study

P_0=No application

P_1=100kg ha^{-1}(100%)

P_2=150kg ha^{-1}(150%)

P_3=200kg ha^{-1}(200%)

Crop Husbandry

Before the drilling, the land was prepared using ploughing and planking, when the soil was at field capacity after rauni irrigation. Zincol-2016 was taken from Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad. Wheat was sown on 27 November 2019 at an agronomic research farm using a hand drill using the suggested seed rate i.e. 125 kg ha^{-1} in RCBD (Split plot arrangement) in 3 replications. Row x row distance was kept at 22.5 cm. To protect the wheat from different insects and weeds, standard plant protection measures were adopted. The requirement of irrigation for wheat is about 4 to 6 irrigation which depends upon the climatic condition and other factors like rainfall, soil type, and varieties. The 1st irrigation was provided to the crop 25 days after sowing and the other irrigations were provided when required by the crop.

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Wheat was harvested 23 April 2020 when it was fully matured. Threshing was done on 29th April 2020 at an agronomic research farm**.** The weather condition during the whole growing period is mentioned in table 2.

Table 2: Weather figures for full crop period of wheat 2019-20

Month	**Average temperature (^{0}C)**			**Relative Humidity (%)**	**Rainfall in growing season (mm)**	**Wind Speed (average) (Km/h)**	**Sunshine Radiation (hour)**	**Pan Evaporation(mm)**
	Max.	Min.	Avg.					
November-2019	25.4	13.5	19.4	77.0	5.3	2.7	6.2	02.1
December-2019	16.7	6.5	11.6	83.6	0.7	2.5	3.9	0.9
January-2020	16.8	6.0	11.4	80.8	30.2	3.6	5.5	05.5
February-2020	23.3	9.0	16.2	72.4	30.5	4.3	08.3	02.5
March-2020	25.0	13.6	19.3	78.9	82.8	4.8	7.4	03.0
April-2020	33.8	18.1	25.9	45.3	5.1	14	10	4.8

OBSERVATIONS

Data concerning the following observations were documented during crop growth and after crop maturity. In each subplot, a one-meter square crop area was picked during the growing season, and harvested at 15-day intervals, and their fresh and m;p;[‘[dry weight were recorded three times. The crop growth rate was calculated using the following formula.

CGR $= (W_2 - W_1) / (t_2 - t_1)$

To take SPAD readings from each plot, 3 plants were selected then SPAD meter readings were recorded. The green seeker handheld crop sensor is an energetic light source visual sensor that is used to measure plant NDVI (Normalized Difference Vegetation Index) value. When you push the button, the measured NDVI value appears on the display immediately. To estimate NDVI from each plot, five plants were selected and values were recorded using green seeker. To measure plant height, spike length, number of spikelets per spike, number of grains per spike, and grain weight per spike five plants were selected from each plot then data were recorded and averaged. 1000 grains were counted from each treatment and weighed. After harvesting from the plot, samples were tied into bundles, sun dried then the final biological yield was estimated by weighing on balance. After maturity, the entire plot was harvested and threshed manually using a mini thresher. The weight of the grains of the whole plot was taken to estimate economic yield. By taking the ratio of economic yield and the biomass yield, the harvest index was calculated.

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biomass yield}} \times 100$$

Grain Zn concentration (mg kg^{-1})

Grains were ground using the grinding mill. For digestion 0.25g of grain powder was shifted to 100mL flask and 10mL of bi-acid mixture (HNO_3-$HClO_4$) with ratio 2:1 was added. The mixture was digested using the hot plate till the residues became colorless. The mixture was cooled and then purified water was used to make the desired volume. Zn concentration was taken on an Atomic Absorption Spectrophotometer.

Statistical Analysis

Collected experimental observations were evaluated using Fisher analysis of variance and treatment means were compared by LSD test at a 5% probability level (Steel *et al.*, 1997).

Results and discussion

Results of ANOVA presented in Table 3 showed that different Zn application methods and fertilizer doses significantly affected all the attributes under study except for CGR. However, interaction among Zn application methods and P doses was non-significant for all the traits under study.

SPAD values

Among Zn treatments, highest SPAD value (56.38) was noted in treatment where Zn was applied as seed priming which was about 2% higher than control followed by mix in soil (table 4). Lowest SPAD values (55.33) was observed in control treatment. Among P doses highest SPAD values (57.44) was observed in P-200% which was about 5% higher than control followed by P-150%. While, lowest SPAD values (54.31) was observed in control where no P was applied. Zn is an essential element and has role in all photosynthetic tissues and necessary for biosynthesis of chlorophyll (Ali *et al.*, 2008). So, the positive impact of Zn on chlorophyll might be due to its role in enzyme activation (Movahhedy-Dehnavy *et al.*, 2009).

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Phosphorus is involved in many metabolic processes essential for normal growth, such as photosynthesis. This element exert influence on stability of the chlorophyll molecule (Tucker, 2004). The results of this study are in line with the finding of (Bojović and Stojanović, 2005) who reported that the chlorophyll contents were increased in fertilized soil by the different levels of phosphorus fertilizer application to the soil.

Table 3: Mean sum of squares of P doses and different Zn application methods on different attributes of wheat

SOV	DF	SPAD values	Plant height	NDVI values	spike length	CGR value	spikelets per spike	Grains /spike	wt. of grains/spike	1000 grains weight	no. of tillers	Biological yield	grain yield	Harvest index	Zn conc.
R	2	0.72	7.47	0.00021	1.38	33.51	0.0039	0.03	0.002	0.76	8350.6	2.76	0.34	1.7	0.1
P	3	25.67**	38.70**	0.00074*	1.83*	597.97*	1.59*	14.37*	0.04*	8.93*	27088.0*	2.85*	1.90**	47.82*	89.27 *
Error	6	0.31	1.8	0.00014	0.21	78.6	0.27	2.43	0.004	0.92	3451.5	0.6	0.06	9.14	16.65
Zn	3	2.80*	12.87 *	0.00164**	1.53*	78.60ns	0.53*	2.43*	0.05**	3.27*	10891.7*	3.02**	1.59**	47.42**	634.18**
Zn × P	9	1.56ns	5.28 ns	0.00018ns	0.44ns	52.35 ns	0.07ns	0.68ns	0.008ns	1.62ns	888.9ns	0.82ns	0.39ns	9.86ns	31.55ns
Error	24	0.97	2.76	0.00023	0.33	52.89	0.13	1.24	0.005	0.86	3436.6	0.45	0.21	9.02	30.5

P: phosphorus doses; Z: Zinc application methods; SOV: Sources of variations; DF: Degree of freedom; SS: Sum of squares; MSS: Mean sum of squares; ns: Non-significant

Table 4: Mean Comparison Table of P doses and different Zn application methods on SPAD value of wheat

Treatment	**P$_0$**	**P$_1$**	**P$_2$**	**P$_3$**	**Zn mean**
Zn$_0$	53.37	55.10	56.33	56.53	**55.33 B**
Zn$_1$	55.00	55.33	57.45	57.75	**56.38 A**
Zn$_2$	53.17	56.14	57.32	57.95	**56.15 AB**
Zn$_3$	55.71	54.90	57.07	57.55	**56.31 A**
P mean	**54.31 C**	**55.37 B**	**57.04 A**	**57.44 A**	

LSD at 0.05: phosphorus 0.55: Zinc 0.83; Where Zn$_0$: No Zn application -; Zn$_1$: Seed priming-; Zn$_2$: Mix in soil-; Zn$_3$:Foliar application: P$_0$:No P application-; P$_1$:100% -; P$_2$: 150% -; P$_3$:200%

Plant height (cm)

Among Zn treatments, maximum plant height (102.32cm) was noted in treatment where Zn was applied as mix in soil which was about 2% higher than control followed by seed priming. However, minimum plant height (100.23 cm) was observed in control treatment. Among P doses maximum value (102.81cm) was observed in P-200% which was about 5% higher than control followed by P-150%. While, minimum value (98.56cm) was observed in control where no P was applied (table 5). Zn is crucial component of enzymes which are required for plant height. Zn has vital role in increasing plant length, occurrence of various functional activities in plants, cell enlargement and cell partition (Kaya and Higgs, 2002). These findings are in cooperation with (Khan *et al.*, 2009) who identified the plant height of wheat increase due to soil application. Different phosphorus levels have greatly influenced plant height. Results are associated with (Hussain *et al.*, 2008) who reported that taller plants were generated by wheat crop under higher amount of P. The results of our field trail was in line, Zn present in soil with findings of (Ghulam *et al.*, 2009), who reported that increasing the rate of Zn with recommended doses of NPK over control plant height increased significantly.

Table 5 Mean Comparison Table of P doses and different Zn application methods on plant height (cm) of wheat

Treatment	**P$_0$**	**P$_1$**	**P$_2$**	**P$_3$**	**Zn mean**
Zn$_0$	96.40	99.20	102.33	103.00	**100.23 B**
Zn$_1$	98.00	101.87	100.43	100.53	**100.21 B**
Zn$_2$	99.27	103.13	102.67	104.20	**102.32 A**
Zn$_3$	103.00	101.17	100.93	103.50	**101.54 AB**
P mean	**98.56 C**	**101.34 B**	**101.59 AB**	**102.81 A**	

LSD at 0.05: Phosphorus 1.342: Zinc 1.400: Where, Zn$_0$: No Zn application -; Zn$_1$: Seed priming-; Zn$_2$: Mix in soil-; Zn$_3$:Foliar application: P$_0$:No P application-; P$_1$:100% -; P$_2$: 150% -; P$_3$:200%

Value of NDVI

Among Zn treatments, highest NDVI value (0.42) was noted in treatment where Zn was applied as mix in soil which was about 5% higher than control followed by seed priming. However, lowest NDVI value (0.40) was observed in control treatment.

Among P doses highest NDVI value (0.42) was observed in P-150% which was about 5% higher than control followed by P-100%. While, lowest NDVI value (0.40) was observed in control where no P was applied (table 6).

Table 6 Mean Comparison Table of P doses and different Zn application methods on NDVI of wheat

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Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	0.39	0.40	0.41	0.41	**0.40 B**
Zn_1	0.41	0.40	0.42	0.41	**0.41 B**
Zn_2	0.42	0.42	0.43	0.43	**0.42 A**
Zn_3	0.37	0.41	0.40	0.40	**0.40 B**
P mean	**0.40 B**	**0.41 AB**	**0.42 A**	**0.41 A**	

LSD at 0.05: Phosphorus 0.011 : Zinc 0.012: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3:200%

Spike length (cm)

Among Zn treatments, highest spike length (14.25cm) was noted in treatment where Zn was applied as foliar application which was about 5% higher than control followed by seed priming. While lowest spike length (13.47cm) was observed in control treatment. Among P doses highest spike length (14.51cm) was observed in P-200% which was about 6% higher than control followed by P-150%. While, lowest value (13.57cm) was observed in control where no P was applied (table 7). Due to contribution of Zn in growth and development process, such as cell division the spike length were increased. The positive results of spike length were shown by the application of Zn. (Habib, 2009) stated that Zn treatments are capable increasing spike length of wheat. In zinc applied plots, the individual plant performance, or more availability of other nutrients due to zinc may have increased the spike length (Tariq *et al.*, 2007). Our results are in agreement with (Curtin *et al.*, 2008) who indicated that spike length get increased with foliar application of Zn. These findings are also in line with those obtained by (Arshad *et al.*, 2016) who reported that higher P manure consumption noted maximum spike length due to maximum buildup of photosynthesis.

Table 7 Mean Comparison Table of P doses and different Zn application methods on spike length (cm) of wheat

Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	12.70	13.16	13.90	14.13	**13.47 B**
Zn_1	13.80	14.33	13.50	14.63	**14.06 A**
Zn_2	14.16	13.70	14.40	14.73	**14.20 A**
Zn_3	13.63	14.40	14.20	14.56	**14.25 A**
P mean	**13.57 B**	**13.90 B**	**14.00 B**	**14.51 A**	

LSD at 0.05: phosphorus 0.46: Zinc 0.48: Where Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Crop Growth Rate (g $m^{-2}day^{-1}$)

Among Zn treatments, highest CGR value (63.17) was noted in treatment where Zn was applied as mix in soil which was about 5% higher than control followed by seed priming. However, lowest CGR value (59.70) was observed in control treatment. Among P doses highest value (66.15) was observed in P-150% which was about 28% higher than control followed by P-100%. While, lowest CGR value (51.19) was observed in control where no P was applied (table 8). Results are associated with (Haldar and Mandal, 1981) who reported that crop growth rate increased by application of P. They also reported that application of P significantly increased root and shoot length. Similarly (Shah and Khalil, 2016) reported that crop growth rate increased due application P may due to its involvement in leaf area index. The increase in dry matter accumulation with the application of P and Zn was also reported by other researchers (Islam *et al.*, 2009).

Table 8 Mean Comparison Table of P doses and different Zn application methods on crop growth rate of wheat interval of 15 days.

Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	50.95	56.17	47.23	68.61	**59.70 A**
Zn_1	53.63	61.22	65.92	71.92	**63.17 A**
Zn_2	47.23	64.67	68.13	67.51	**63.17 A**
Zn_3	52.97	54.11	68.13	62.6	**59.30 A**
P mean	**51.19 B**	**59.04 AB**	**66.15 A**	**65.87 A**	

LSD at 0.05: phosphorus 8.856 : Zinc 6.128: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Number of spikelet's per spike

Among Zn treatments, highest spikelet's per spike (17.67) was noted in treatment where Zn was applied as foliar application which was about 2% higher than control followed by seed priming. However, lowest spikelet's per spike (17.19) was observed in control treatment (table 9). Among P doses highest spikelet's per spike (17.67) was observed in P-200% which was about 4% higher than control followed by P-100%. While, lowest spikelet's per spike (16.98) was observed in control where no P was applied. Many plant processes like dry material gathering, pollen

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feasibility pollination and tryptophan combination that can produce (IAA) in which play a vital role. Zn has sensible properties in plant breakdown process that are answerable for larger metabolites gathering in generative tissues (Kaya and Higgs, 2002). These conclusions a according to (Ali *et al.*, 2008) who determined that Zn treatments on wheat expressively increase the no. of spikelet's and also has finding similar (Cakmak, 2000) who determined that Zn application on seed meaningfully improved number of spikelet's. Phosphorus significantly increased the amount of spikelet's per spike over control. (Memon and Puno, 2005) reported that phosphorus application increased, normal plant growth and as a result number of spikelet's per spike also increased.

Table 9 Mean Comparison Table of P doses and different Zn application methods on spikelet's per spike of wheat

Treatment	P_0	P_1	P_2	P_3	Zn mean
Zn_0	16.73	17.36	17.26	17.40	**17.19 B**
Zn_1	16.83	17.66	17.80	17.76	**17.62 A**
Zn_2	17.16	17.70	17.40	18.06	**17.58 A**
Zn_3	17.20	17.76	17.60	18.13	**17.67 A**
P mean	**16.98 B**	**17.62A**	**17.51 A**	**17.67 A**	

LSD at 0.05: phosphorus 0.519: Zinc 0. 0.313: While, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3:200%

Number of grains per spike

Among Zn treatments, highest value (53.02) was noted in treatment where Zn was applied as foliar application which was about 3% higher than control followed by mix in soil. However, value (51.57) was observed in control treatment (table 10). Among P doses highest value (53.52) was observed in P-200% which was about 5% higher than control followed by P-100%. While, lowest value (50.95) was observed in control where no P was applied. If the spike length is increase, then the no. of spikelet's and number of grains were also increased. Application of Zn rises the situation of grains in wheat is present in procedures of fertilization and pollination because of its impact on construction of pollen tube (Kaya and Higgs, 2002). The formation of grains was affected by the poor fertilization which caused by Zn deficiency. These results were similar to who stated that by applying Zn on wheat crop the number of grains increased by contribution of Zn in growth and development process like cell division and cell enlargement (Thiruppathi *et al.*, 2001). When number of spikelet's per spike increased, it ultimately increased number of grains per spike. It is possible to relate an increase in number of grains per spike attributed to greater spike length and a greater number of spikelet's per spike. (Dewal and Pareek, 2004) reported that decrease in number of grains per spike at lower level of phosphorus application in wheat.

Table 10 Mean Comparison Table of P doses and different Zn application methods on No. of grains per spike of wheat

Treatment	P_0	P_1	P_2	P_3	Zn mean
Zn_0	50.20	52.10	51.80	52.20	**51.57 B**
Zn_1	50.50	53.00	53.40	53.30	**52.55 A**
Zn_2	51.50	53.10	52.20	54.20	**52.75 A**
Zn_3	51.60	53.30	52.80	54.40	**53.02 A**
P mean	**50.95B**	**52.87 A**	**52.55 A**	**53.52 A**	

LSD at 0.05: phosphorus1.558: Zinc 0.939: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Grain Weight per Spike (g)

Among Zn treatments, maximum grain weight per spike (1.89g) was noted in treatment where Zn was applied as mix in soil which was about 9% higher than control followed by foliar application. However, minimum grain weight per spike (1.73g) was observed in control treatment. Among P doses highest value (1.88g) was observed in P-200% which was about 8% higher than control followed by P-150%. While lowest value (1.73g) was observed in control where no P was applied (table 11). The grain yield directly relies on grain weights per spike and wheat qualities involvement. For determining output value, the beneficial apparatus showed undesirable or helpful correlation. Definitely, on grain yield the stem height and spike length has a undesirable effect (Waqar-Ul-Haq and Akram, 2010) although plant's tiller and grain weight per spike (Ashfaq *et al.*, 2003). The temperature had a negative impact on the plant because it decreases grain filling time, and grains were shrinkable due to early maturity which can caused by the temperature (Karam *et al.*, 2007).

Table 11 Mean Comparison Table of P doses and different Zn application methods on wt. of grains per spike of wheat

Treatment	P_0	P_1	P_2	P_3	Zn mean

Zn$_0$	1.61	1.71	1.80	1.83	**1.73 C**
Zn$_1$	1.76	1.74	1.85	1.87	**1.80 B**
Zn$_2$	1.82	1.94	1.88	1.93	**1.89 A**
Zn$_3$	1.74	1.93	1.80	1.91	**1.85 AB**
P mean	**1.73 B**	**1.83A**	**1.83 A**	**1.88 A**	

LSD at 0.05: phosphorus0.063: Zinc 0.063: Where, Zn$_0$: No Zn application -; Zn$_1$: Seed priming-; Zn$_2$: Mix in soil-; Zn$_3$:Foliar application: P$_0$:No P application-; P$_1$:100% -; P$_2$: 150% -; P$_3$:200%

Thousand Grains weight (g)

Among Zn treatments, highest 1000 grain weight (35.92g) was noted in treatment where Zn was applied as mix in soil which was about 3% higher than control followed by foliar application. While, lowest 1000 grain weight (34.82g) was observed in control treatment (table 12). Among P doses highest value (35.88g) was observed in P-200% which was about 5.5% higher than control followed by P-150%. However, lowest value (33.99g) was observed in control where no P was applied. Zn application improved the thousand grain weigh because Zn has grater phloem movement from roots to developing grains (Rengel, 2007). The marks of current investigation was in association with marks of (Harris *et al.*, 2007), who determined that Zn treatments improved the yield associated mechanisms in wheat such as thousand grain weight and organic or biomass yield. (Torun *et al.*, 2001) reported that 1000 grain weight increased particularly by seed treated with Zn. Due to improved enzymatic activity, the application of zinc might have increased photosynthetic efficiency (Martín-Ortiz *et al.*, 2010) and thus might have increased weight of thousand grains. Our results are in conformity with finding of (Abbas *et al.*, 2010) who concluded that high level of P intake gives maximum grain weight due to maximum buildup of photosynthesis. The results are identical with those proposed by (Rahim *et al.*, 2010) who reported that 1000-grain weight of wheat increased significantly with increasing P rate application

Table 12 Mean Comparison Table of P doses and different Zn application methods on 1000 grains weight per spike of wheat

Treatment	P$_0$	P$_1$	P$_2$	P$_3$	Zn mean
Zn$_0$	32.80	34.60	35.33	33.93	**34.82 B**
Zn$_1$	34.10	34.63	35.03	35.73	**34.87 B**
Zn$_2$	35.13	36.86	36.03	35.66	**35.92 A**
Zn$_3$	33.93	36.23	36.10	35.56	**35.45AB**
P mean	**33.99B**	**35.58A**	**35.62 A**	**35.88 A**	

LSD at 0.05: phosphorus0.961: Zinc 0.785: Where, Zn$_0$: No Zn application -; Zn$_1$: Seed priming-; Zn$_2$: Mix in soil-; Zn$_3$:Foliar application: P$_0$:No P application-; P$_1$:100% -; P$_2$: 150% -; P$_3$:200%

Total Tiller

Among Zn treatments, highest value (691.01) was noted in treatment where Zn was applied as mix in soil which was about 11% higher than control followed by foliar application (table 13). However, lowest value (620.75) was observed in control treatment. Among P doses highest value (692.28g) was observed in P-200% which was about 16% higher than control followed by P-100%. While, lowest value (594.45g) was observed in control where no P was applied. Number of spikes has a main factor that can be contribute in yield if spike is taking place on tiller at time of harvesting. Enhanced the no. of tillers per part zone is agreement for higher produce at collecting because increased the number of fertile tillers yield was finally improved.

Table 13 Mean Comparison Table of P doses and different Zn application methods on no. of tillers of wheat

Treatment	P$_0$	P$_1$	P$_2$	P$_3$	Zn mean
Zn$_0$	567.03	652.27	593.62	670.09	**620.75 B**
Zn$_1$	566.72	666.52	613.06	705.73	**638.01.B**
Zn$_2$	647.65	738.60	678.50	699.30	**691.01 A**
Zn$_3$	596.62	700.20	638.99	693.99	**657.39 AB**
P mean	**594.45 C**	**689.40AB**	**631.04 BC**	**692.28 A**	

LSD at 0.05: phosphorus58.688: Zinc 49.395; Where, Zn$_0$: No Zn application -; Zn$_1$: Seed priming-; Zn$_2$: Mix in soil-; Zn$_3$:Foliar application: P$_0$:No P application-; P$_1$:100% -; P$_2$: 150% -; P$_3$:200%

Biological yield (t ha^{-1})

Among Zn treatments, highest biological yield (11.01 t ha^{-1}) was noted in treatment where Zn was applied as mix in soil which was about 9% higher than control followed by foliar application. However, lowest value (10.02 t ha^{-1}) was observed in control treatment. Among P doses highest biological yield (10.95 t ha^{-1}) was observed in P-100% which was about 11% higher than control followed by P-200%. While, lowest biological yield (9.91 t ha^{-1}) was observed in control where no P was applied (table 14). These results are confirmed by (Manzar-ul-Alam *et al.*, 2005) who also reported that enhanced the dry matter yield of wheat crop by application of

Phosphatic fertilizer. These results confirm the findings (Wiatrak, 2013), who stated that increased biological yield production of winter wheat i with P fertilizer rate applications compared to control.

Table 14 Mean Comparison Table of P doses and different Zn application methods on biological yield (t ha^{-1}) of wheat

Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	9.07	10.71	9.21	11.08	**10.02 B**
Zn_1	9.53	10.26	10.32	10.15	**10.06 B**
Zn_2	10.43	11.23	11.32	11.08	**11.01 A**
Zn_3	10.60	11.62	10.04	10.83	**10.77 A**
P mean	**9.91 B**	**10.95 A**	**10.22 AB**	**10.79 A**	

LSD at 0.05: phosphorus0.779: Zinc 0.570: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Grain yield (t ha^{-1})

Among Zn treatments, highest grain yield (4.09 t ha^{-1}) was noted in treatment where Zn was applied as mix in soil which was about 24% higher than control followed by seed priming. While, lowest grain yield (3.29 t ha^{-1}) was observed in control treatment. Among P doses, highest value (4.31 t ha^{-1}) was observed in P-200% which was about 29% higher than control followed by P-100%. However, lowest grain yield (3.34 t ha^{-1}) was observed in control where no P was applied. Zn has important role in various physiological functions of plants including cell division. In addition, it plays key role in chlorophyll synthesis which is indirectly linked with different yield contributing traits including productive tillers, no. of grains spike^{-1}, test weight, harvest index and grain yield. Soil application is an established methods of Zn application; however, it requires high application rates and its efficacy depends largely upon physico-chemical properties of soil. (Aboutalebian *et al.*, 2012) reported that treatment of seed with $ZnSO_4$ improved stand establishment and enhanced yield. Significantly higher yield of grain in wheat has been obtained by many researchers with optimum use of P fertilizer. Our results also supports the findings of (Jan *et al.*, 2013) who stated that maximum grain yield may be due to the application of more phosphorous fertilizer to the soil which meets required level of P nutrient to the soil. Similarly, (Zia *et al.*, 1997) stated that grain yield of wheat enhanced with increasing rate of P over control.

Table 15 Mean Comparison Table of different Zn application methods and P doses on grain yield (t ha^{-1}) of wheat

Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	2.80	3.33	3.71	3.33	**3.29 B**
Zn_1	3.67	3.94	4.04	4.26	**3.98 A**
Zn_2	3.34	4.11	4.10	4.80	**4.09 A**
Zn_3	3.58	4.20	3.32	4.84	**3.98 A**
P mean	**3.34 C**	**3.90 B**	**3.79 B**	**4.31 A**	

LSD at 0.05: phosphorus0.251: Zinc0.392 Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Harvest Index (%)

Among Zn treatments, highest harvest index (36.54%) was noted in treatment where Zn was applied as mix in soil which was about 12% higher than control followed by foliar application. However, lowest harvest index (32.44) was observed in control treatment (table 16). Among P doses highest harvest index (36.99%) was observed in P-200% which was about 14% higher than control followed by P-150%. While, lowest harvest index (32.27%) was observed in control where no P was applied. Harvest index can link with organic ability of products to translate total dehydrated biomass into grain yield. Ratio of economic and biomass yield is called harvest index. Use of Zn for handling seed is more active in enhancing the profit associated workings in wheat such as H.I. These outcomes strongly agree with the discoveries of (Harris *et al.*, 2007) they decided that seed treated with Zn particularly improved H.I.

Table 16 Mean Comparison Table of P doses and different Zn application methods on harvest index (%) of wheat

Treatment	**P_0**	**P_1**	**P_2**	**P_3**	**Zn mean**
Zn_0	29.63	32.01	32.88	35.24	**32.44 B**
Zn_1	32.86	30.34	35.35	35.78	**33.58 B**
Zn_2	32.30	36.55	39.05	38.27	**36.54 A**
Zn_3	34.31	37.42	34.21	38.67	**36.15 A**
P mean	**32.27 B**	**34.08 AB**	**35.37 A**	**36.99 A**	

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LSD at 0.05: phosphorus3.021: Zinc2.531: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Grain Zn contents (mg kg^{-1})

Among Zn treatments, highest grain Zn contents (60.06 mg/kg) was noted in treatment where Zn was applied as foliar application which was about 22% higher than control followed by mix in soil. However, lowest grain Zn contents (43.76 mg/kg) was observed in control treatment (table 17). Among P doses highest grain Zn contents (54.85mg/kg) was observed in P-200% which was about 14% higher than control followed by P-100%. While, lowest grain Zn contents (48.48mg/kg) was observed in control where no P was applied. By the foliar application nutritious value of wheat seed increased, because it is easy and cheap techniques (Erenoglu *et al.*, 2011). Zn concentration in seed can be improved by foliar application, due to short term strategy that is cooperative to the qualification of Zn shortage health problem in developing countries (Yaseen *et al.*, 2011). These marks are in cooperation with the results of (Shi *et al.*, 2010) who determined that Zn expressively enhanced the grain Zn contents through foliar spray.

Table 17 Mean Comparison Table of P doses and different Zn application methods on Zinc concentration (mg kg^{-1}) of wheat

Treatment	P_0	P_1	P_2	P_3	Zn mean
Zn_0	36.80	43.30	45.46	49.50	**43.76 C**
Zn_1	49.30	49.43	51.63	46.26	**49.15 B**
Zn_2	50.86	58.90	55.70	59.50	**56.24 A**
Zn_3	56.96	61.96	57.20	64.13	**60.06 A**
P mean	**48.48 B**	**53.40 A**	**52.50 AB**	**54.85 A**	

LSD at 0.05: phosphorus 4.077: Zinc 4.654: Where, Zn_0 : No Zn application -; Zn_1: Seed priming-; Zn_2: Mix in soil-; Zn_3:Foliar application: P_0:No P application-; P_1:100% -; P_2: 150% -; P_3 :200%

Conclusion

Zn deficiency that is among the major causes of causalties in world, also causing malfunctioning of human body can be alleveiated better using biofotification. Various methods can be deployed for biofortication of agronomic crops especially for wheat because of its usage as staple food in various parts of the world. Use of different doses of phousphours because Phosphorus is an important macronutrient affecting plant growth, yield and quality. An antagonistic interaction between P and Zn has been found in plants. From the results obtained after the conducted study it can be concluded that application of Zn by mix in soil is helpful for increasing the growth and yield as compared to other methods and application of Zn by foliar application increasing Zn content in grains to greater extent compared to other methods. Zincol-2016 resulted better in accumulation of Zn.

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

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Authors' Contribution

All authors contributed equally.

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Conflict of interest

There is no conflict of interest among the authors of the manuscript.

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