

## Effect of fertilizing with different levels of phosphorous and zinc on the botanical characteristics of table beet (*Beta vulgaris* L.)

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### Abstract

The interaction of phosphorus (P) and zinc (Zn) is a crucial factor affecting crop yield in agricultural production called a P-induced Zn deficiency. The application of Zn and P together reduces deficiencies and increases plant growth by more than the sum of the increases from Zn and P alone. This experiment was carried out during two seasons, in consecutive years, to study the effect of P and Zn levels on the physical, physiological and anatomical response in table beet plants. Treatment one was as control; the second treatment was 35 P units with 5, 10, and 20 Zn units; the third treatment was 40 P units with 5, 10, and 20 Zn units; and the fourth treatment was 45 P units with 5, 10, and 20 Zn units. The data showed that the number of leaves and the root diameters were high with the addition of 40 P units and 10 Zn units, and the roots fresh and dry weights were high under 40 P units and 10 Zn units in both seasons. The contents of TSS, AA, TS, ACY, N, P, and K were significantly increased by the use of 40 and 45 P units combined with 5 and 10 Zn units. The anatomical alterations in both leaf blade, epidermal layers, midrib zone, vessel diameter, vascular bundle area, palisade, and spongy tissues were studied. The results recommend that fertilizing table beet plants with 40 P units and 10 Zn units is suitable.

**Keywords:** anatomical alterations; chemical contents; fertilizing; P; table beet; Zn

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### Introduction

Table beet (*Beta vulgaris* subsp. *vulgaris* L.) is a vegetable from the family Chenopodiaceae. Table beet is also known as garden beet or red beet in the U.S. scientific literature, and as beetroot in Europe and many other countries around the world (Goldman and Navazio, 2003). Table beet is a member of a crop complex from the genus *Beta* that includes Swiss chard, mangle, and sugar beet. All three of these crops are derived from

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the same species, *vulgaris*, and are often represented with different subspecies designations. Table beet and Swiss chard are primarily used as vegetables, mangel and its derivatives are used as animal feed, and the sugar beet is used as a source of sucrose (Goldman and Navazio, 2008).

Phosphorus (P) is an essential macronutrient for plant growth and development, and participates in a set of physiological and biochemical processes, such as photosynthesis, respiration, carbon (C) and nitrogen (N) assimilation, energy metabolism and cell elongation (Razaq *et al.*, 2017). Furthermore, P is also a component of biomolecules in plant cells such as adenosine triphosphate (ATP), phospholipids, and many key enzymes (Mora-Macias *et al.*, 2017).

The molecules of P constitute the structural skeleton of other biomolecules such as NADPH, nucleic acids, and sugar-phosphates for primary and secondary plant metabolisms (Lambers and Plaxton, 2015; Stigter and Plaxton, 2015). At the plant cellular level, P is a crucial element for various physiological and biochemical functions. It enters a wide range of metabolic processes, specifically, the synthesis of nucleic acids and energy generation of plants which makes it hard for plants to grow under P starvation (Carstensen *et al.*, 2018; Malhotra *et al.*, 2018; Powers *et al.*, 2020). The deficiency of inorganic phosphate (Pi) in soil impairs fruit production and quality traits during the plant vegetative growth cycle (Li *et al.*, 2021). Moreover, P plays a role in vigorous root system formation and development, and ultimately crop yields (Béné *et al.*, 2015; Sun *et al.*, 2016). (Arif *et al.*, 2021) found strong positive effects of integrated P fertilizer on the biomass and grain yield of maize above the sole application of either organic or inorganic P fertilizers.

Lambers *et al.*, (2008) mentioned that, amount of carboxylates are exuded from roots of many plants under P deficiency and the amount often decreases with increasing P availability in the soil.

Low availability of P in calcareous soils is a major problem for sustainable improvement in cereal crop yield (Wahid *et al.*, 2020). Also, Malhotra *et al.*, (2018) found that, P deficiency reduces the crop yields on 30-40% and necessitating the use of a large amount of p fertilizers to correct that deficiency.

Micronutrients are also essential for plant growth. Zinc (Zn) is an essential micronutrient for plant biological systems. It has a vital physiological role in enzyme functions associated with essential biochemical pathways, photosynthetic processes, and enzyme activation. Zn application increases the photosynthetic rate and chlorophyll content (Tahir *et al.*, 2018; Bibi *et al.*, 2020; Malik *et al.*, 2021), and enhances photochemical reactions in thylakoid membranes and electron transport in PSII (Roach and Krieger-Liszkay, 2014). It plays an important role in various enzymatic reactions, metabolic processes, redox reactions, and plant hormone metabolism, promoting the development of plant reproductive organs and improving plant resistance to stress (Shemi *et al.*, 2021; Suganya *et al.*, 2020).

Zn has a vital role in plant growth. Among all metals, the largest number of proteins require Zn for their catalytic function. Zn-binding proteins make up nearly 10% of all protein in biological systems. Zn plays a vital role in biological systems like the structural integrity of membranes and contribution to protein synthesis and gene expression (Vadlamudi *et al.*, 2020).

A common problem in the soil is the low content of available Zn, which is unevenly distributed and difficult to move. However, information on the foraging strategies of roots in response to heterogeneous Zn supply is still very limited (Xu *et al.*, 2021).

Zn deficiency is probably the most prevalent micronutrient deficiency in soils (Rehman *et al.*, 2021). The lack of Zn causes a deficiency in RNA formation and protein (Singh *et al.*, 2018) and leads to lower yields (Aziz *et al.*, 2017) also, affecting the nutritional quality of crop plants (Cakmak and Kutman, 2018). Furthermore, it causes damage to plant cells mainly at the cell membrane level (Candan *et al.*, 2018) and can also alter mitochondrial ultrastructure (Chen *et al.*, 2014).

Zn deficiency is expected in alkaline calcareous soil. Contrarily, Zn toxicity is also becoming an environmental concern due to increasing anthropogenic activities (e.g., metal smelting and the copper industry). Therefore, balanced Zn application is necessary to save resources and achieve optimum crop growth and yield (Saboor *et al.*, 2021).

The treatments receiving no P application had the highest Zn uptake in both genotypes, whilst a single P application tended to reduce the Zn uptake (Imran *et al.*, 2016).

P and Zn interaction is commonly defined as P-induced Zn deficiency. It has also been stated that P may cause Zn deficiency by reducing the translocation of Zn from roots to shoot (Soltangheisi *et al.*, 2013). Also, the physiological requirement of Zn increased with increasing P concentration in plant tissues due to the inactivation of the Zn in some way (Soltangheisi *et al.*, 2014a, b). Zn rarely reduces P availability, its absorption, and translocation in plants, but higher P availability in the soil leads to Zn deficiency because higher amounts of P fertilizer are used by growers as compared to Zn fertilizer (Soltangheisi *et al.*, 2014). Therefore, balanced fertilization of P is rather essential to prevent Zn deficiency in agricultural production. Many experiments have been conducted to study the P and Zn interaction effects on wheat (Vafaei *et al.*, 2014), rice (Amanullah and Inamullah, 2016), maize (Saleem *et al.*, 2016) and cowpea (Kumar *et al.*, 2016). Gupta *et al.* (2016) mentioned that, crosstalk of Zn with P can modify the physiological functions of plants. The application of either Zn or P considerably decreased the stress factor of the other nutrient (Imran *et al.*, 2016). The bioavailability of both Zn and P can affect Root-mediated changes, including rhizosphere pH and release of carboxylates (Hacisalihoglu and Kochian, 2003).

Low P availability activates a series of morphological, anatomical and physiological responses that maximize P acquisition (Raghothama, 1999). The cell size and thickness of epidermal cells were reduced under P-deficient conditions in soybean leaves (Chiera *et al.*, 2002). In wheat plant leaves, P deficiency limited the leaf size by producing fewer cells of mesophyll tissue per leaf or limiting cell elongation (Cui *et al.*, 2003). At present, the general trend around the world is to reduce agricultural production costs to achieve sustainable agriculture management using fertilizers, so it is necessary to supplement the nutrients to maintain production. Zn nanoparticles at a concentration of 100 ppm cause a significant increase in the most studied traits such as epidermis thickness, vascular bundle length and width, vessel diameter, cortex thickness, and vascular bundles number (Al-Dhalimi and Al-ajeel, 2020). In recent years, Zn and P deficiency has been considered as one of the most widespread nutrient disorders resulting in severe losses in crop yield in agricultural production (Amanullah *et al.*, 2016). Table beet plant has not been covered by any research or studies regarding its relationship to fertilization. This is the first publication dealing with its relationship to fertilization. Evaluate the effects of P and Zn interaction on growth and the botanical characteristics of table beet plant was aim of this study.

## Materials and Methods

Two experiments were carried out during the 2018-2019 seasons in pots at the experimental farm, Faculty of Agriculture, AL-Azhar University Nasr City in Cairo to study the effect of P and Zn levels on the physiological and anatomical changes in table beet plants.

### *Experimental design and pot experiment*

Table beet seeds were sown on October 2<sup>th</sup> and 5<sup>th</sup> for the first and second seasons respectively, 10 seeds were sown in each pot at equal distance and depth in clay loam soil of pH 6.58. Thinning was done three weeks after sowing resulting in three plants left in each pot. The design of the experiment was completely in randomized blocks. Forty pots were arranged in four replicates of 10 treatments. Each pot was fertilized with the recommended fertilization with ammonium sulfate (3.75 g) and potassium sulfate (0.80 g). The fertilizer was divided into two equal parts. The first was added immediately after thinning, while the second was added three weeks later.

The treatments examined were:

Unfertilized plants (Control), 35 P units with 5, 10 and 20 Zn units, 40 P units with 5, 10 and 20 Zn units and 45 P units with 5, 10 and 20 Zn units.

### *Experimental measurements*

Data were recorded after 60 days from seeds cultivation and were recorded as follows:

#### Morphological traits

Leaf number per plant, fresh weight of roots (g) was determined in g by a balance and **root diameter** by vernier caliper.

Root dry weight (g) was determined by drying 100 g in an oven at 70 °C till constant weight was reached (Official, 2000).

#### Biochemical traits:

Total soluble solids (TSS)

TSS were determined as percentage by Abbe refractometer at room temperature. (Official, 2000).

Ascorbic acid (AA)

AA was determined using the dye 2,6 dichlorophenol indophenol method (Official, 2000). 2, 6 dichlorophenol indophenol dye 50 mg and 42 mg sodium bicarbonate (NaHCO<sub>3</sub>) were taken in the beaker and then dissolved in distilled water. The volume was made up to 250 ml. This solution was filtered and kept in a clean bottle and place in a cool place. Standard AA (50 mg) was taken in 50 ml volumetric flask and the volume was made up with oxalic acid solution (0.4%). This solution was kept in a cool place for 24 hours before use.

Oxalic acid (4 g) was taken in a volumetric flask and volume was made up to 1 liter with distilled water.

Standard AA solution (5 ml) was taken in a conical flask and was titrated against dye solution till light pink colour persisted for 15 seconds.

Dye factor (f) = ml of ascorbic acid solution taken / Volume of dye used.

Dye factor was determined separately for each determination.

10 ml of sample was taken in a volumetric flask and volume was made up to 100 ml with 0.4% oxalic acid solution. Sample solution (10 ml) was taken in a conical flask and titrated against the dye solution till light pink colour appeared which persisted for 15 second. Three consecutive readings were taken for each sample. A blank titration was also carried out.

Calculation: AA content was calculated by using the following formula

$$\text{AA (mg / 100 g)} = \text{L} \times \text{F} \times 100 \times 100 / \text{D} \times \text{P}$$

L = Volume of dye (ml) used; F = dye factor; D = Wt. (g) of jam taken for dilution; P = Volume (ml) of sample taken for titration.

#### *Total sugars (TS)*

TS contents were determined by the phenol method. The extraction consisted in pouring 0.5 g of powder into 10 ml of ethanol (80%, v/v) under vortexing. The tubes were immersed to boiling water bath for 90 min and then cooled under tap water. After centrifugation at 3000 rpm for 10 min, the supernatant was recovered and the pellet was washed once again with hot ethanol (80%). The supernatants were then mixed together. Subsequently, about 0.1 mL of supernatant was sampled and 0.9 mL of distilled water, 1 ml of 5% (w/v) phenol and 5 ml of concentrated sulphuric acid were successively added and vortexed. The tube was then let to stand 30 min. Then the absorbance was measured at 490 nm using a spectrophotometer against a blank prepared in the same conditions with 1 ml of distilled without extract. The total sugar contents expressed as glucose equivalent were determined from a glucose calibration curve (R<sup>2</sup> = 0.998). The results were expressed in g/100 g dry weight were determined according to the method reported by (Dubois *et al.*, 1956).

#### *Anthocyanins (ACY)*

ACY content was determined according to the method of (Ranggana, 1997). 5 g fresh weight was mixed 100 ml of ethanolic HCl (95 % ethanol – 1.5 NHCl) (85:15) in a blender at full speed, and completed to the volume at 500 ml with ethanolic HCl, then was store overnight in a refrigerator at 4 °C. the extract was filtered in a No. 1 Whatman paper using a Buchner funnel. The bottle and the residue were washed on the filter paper

repeatedly with ethanolic HCl until approximately 500 ml of extract is collected, 25 ml of the filtrate was filtered through a fine porosity sintered glass funnel, 2 ml of the filtrate was mixed with ethanolic HCl to 100 ml and store in dark for 2 h the color was measured at wave length of 535 nm using Spectrophotometer.

#### *Calculation*

Total OD per 100 g fresh weight (T) =  
 $100 \times \text{absorbency at } 535 \times \text{volume of diluted extract used for color measurement} \times \text{total volume / ml of the extract used} \times \text{Wt of sample taken}$   
Total anthocyanin content in mg/100 g of fresh weight = T/98.2

#### N content

was determined according to the micro-Kjeldahl method (Official, 2000).

#### P content

was determined colorimetrically using the hydroquinone and sodium sulphate method (Official, 2000).

#### K content

were determined using flamephotometer according to the method of (Dewis and Freitas, 1970).

#### *Anatomical characters in Beta vulgaris L. leaves*

Thickness of leaf blade ( $\mu\text{m}$ .), Thickness of upper and lower epidermal layers ( $\mu\text{m}$ .), thickness midrib zone ( $\mu\text{m}$ .), Length and width of vascular bundle ( $\mu\text{m}$ .), thickness palisade tissue ( $\mu\text{m}$ .), Thickness spongy tissue ( $\mu\text{m}$ .) and diameter of vessel diameter ( $\mu\text{m}$ .) were studied (Each value means of 10 sections, 10 readings per each).

Tested material included lamina of the leaves which were taken throughout the growing season of 2019 season at the age of 60 days from the sowing date. The execution of micro technique was carried out according to the method described by (Nassar and El-Sahhar, 1998).

The obtained data were statistically analyzed using the analyses of variance method according to (Snedecor and Cochran, 1980).

## **Results**

#### *Effect of P and Zn on the physical characteristics*

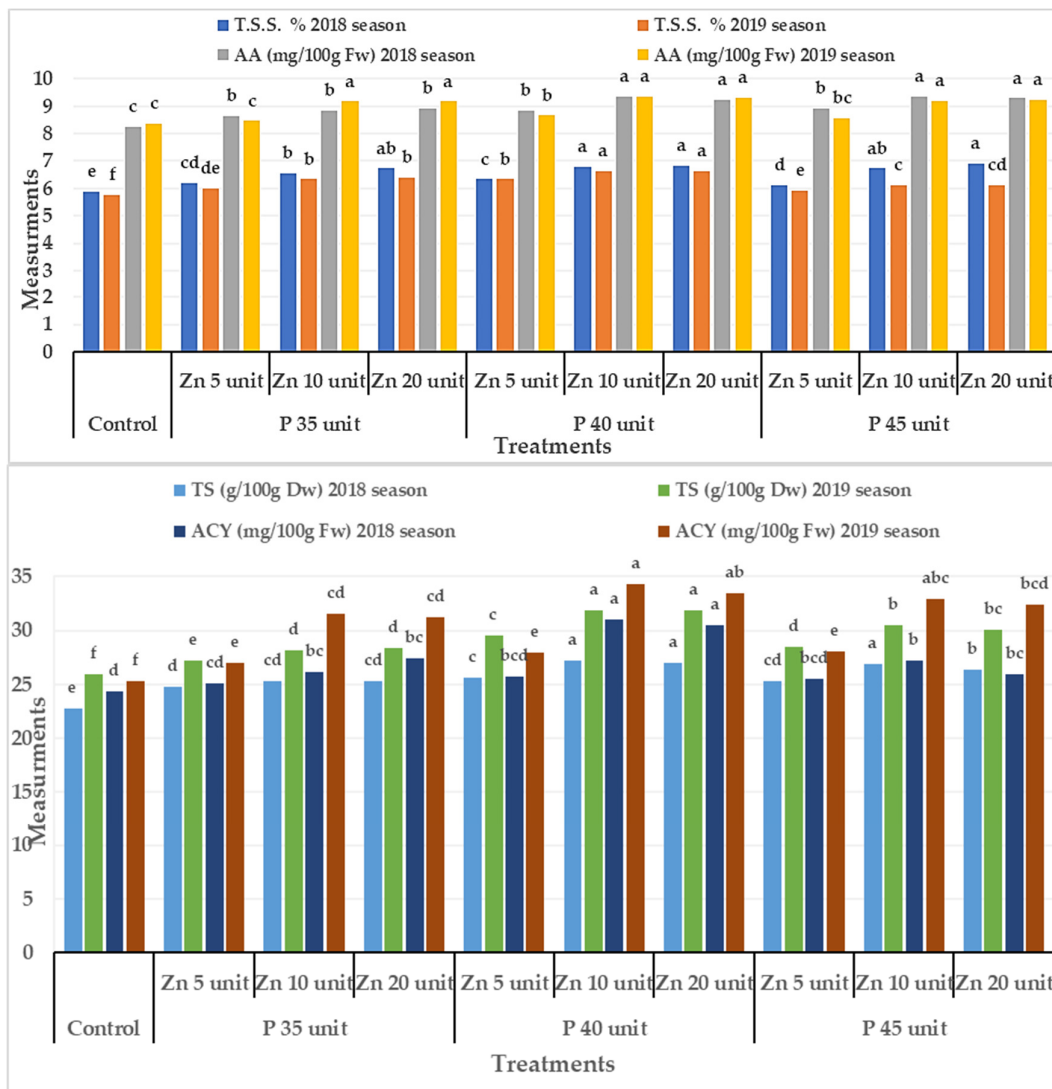
Data in Table 1 shows the effect of P and Zn on the growth parameters (leaf number, root fresh and dry weight, and root diameter) in table beet plants during the two seasons, 2018 and 2019. There were significant differences between most treatments and the control and also between the treatment's groups in both seasons. Increasing the P and Zn doses caused significant increase in the physical characteristics compared to the control; the highest number of leaves, the fresh and dry weight, and root diameter were recorded with 40 P units with 10 Zn units followed by the treatment of 40 P units with 20 Zn units.

#### *Effect of P and Zn on table beet quality*

Figure 1 (a and b) shows the effect of adding P and Zn fertilizer on TSS, AA, TS content, and ACY content in the 2018 and 2019 seasons. The increased P and Zn levels increased the TSS, AA, TS content, and ACY content compared to the control. Significant increases in TSS, AA, TS content, and ACY content were recorded from the treatment of 40 P units with 10 and 20 Zn units in both seasons. The second point indicated that the results of the chemical content reflected an increase in the TSS and AA with 40 and 45 P units + 10 and 20 Zn units. On the other hand, the roots content of TS and ACY increased with the use of 40 P units + 10 Zn units, and these contents also increased with 40 P units + 20 Zn units in both seasons.

**Table 1.** Effect of P and Zn levels on leaf number, root fresh and dry weight, and root diameter in table beet

P Units	Zn Units	Leaf number		Root fresh weight (g)		Root dry weight (g)		Root diameter (cm)	
		2018 season	2019 season	2018 season	2019 season	2018 season	2019 season	2018 season	2019 season
Control		7.56	8.93	93.99	110.22	6.96	7.66	5.31	5.50
35	5	8.67	9.63	98.71	112.61	8.71	8.87	5.68	5.67
	10	9.70	11.13	100.78	119.41	9.58	10.17	6.10	5.93
	20	9.70	11.07	102.37	122.75	9.45	10.29	6.46	6.30
40	5	9.07	10.40	111.39	116.80	9.30	9.25	6.50c	6.37
	10	10.63	11.55	134.28	140.36	10.96	11.94	6.97	6.77
	20	10.40	11.50	133.54	140.00	10.60	11.64	6.83	6.73
45	5	8.80	10.67	109.34	116.46	9.43	9.35	6.37	6.30
	10	10.33	11.37	133.95	139.22	10.45	10.78	6.77	6.77
	20	10.27	11.30	133.29	136.92	10.42	10.76	6.73	6.63
L.S.D at 5%		1.15	0.33	2.04	4.12	0.41	0.46	0.46	0.17

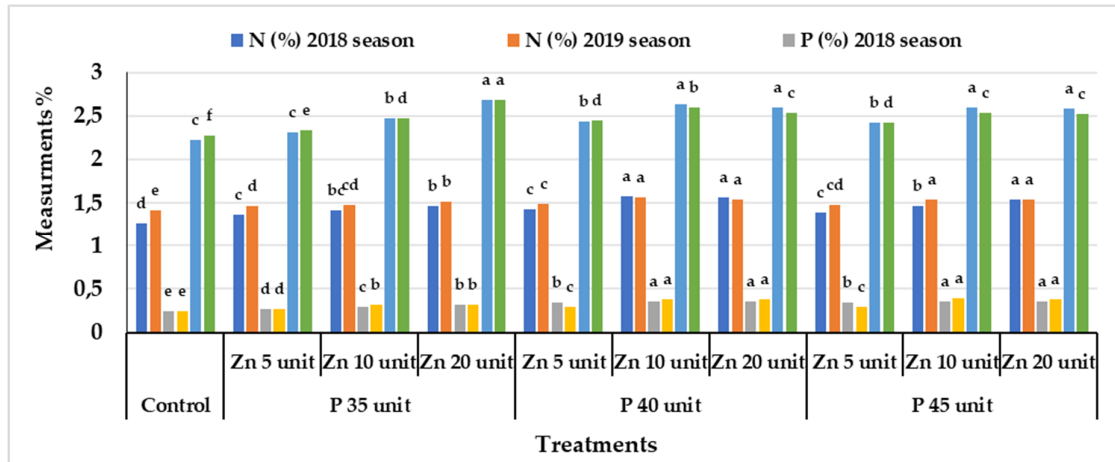


**Figure 1.** Effect of P and Zn levels on TSS and AA (a) and on TS and ACY in table beet (b)

*Effect of P and Zn on NPK uptake*

Figure 2 shows the effect of soil fertilizers on N, P, and K content in table beet, with P and Zn levels showing that adding 40 P units + 10 or 20 Zn units increased the N contents approximately 25 to 23%, respectively, in roots compared to the control. In addition, P and Zn levels showed that adding 40 and 45 P units + 10 or 20 Zn units increased the P contents approximately 37 to 39%. On the other hand, no significant differences were seen in both seasons

The K content of table beet roots were significantly increased with 35 P units + 20 Zn units approximately 19.5% in the two seasons, followed by the use of 40 P units + 10 Zn units, approximately 16.5%, respectively, compared with the control.



**Figure 2.** Effect of P and Zn levels on N, P and K content of table beet roots in the two seasons of 2018 and 2019

*Effect of P and Zn on the anatomical structure in table beet leaves*

Microscopic measurements of certain anatomical characters in transverse- sections in table beet leaves studied the effect of fertilization of P with Zn levels as compared to the control.

Table 2 and Figure 3 illustrate that the mixture of P at 35 units with 5, 10, and 20 separated units of Zn caused a slight increment in leaf blade thickness more than the untreated plants. The increase corresponds to the increase in both upper and lower epidermal layers such as palisade and spongy tissues compared to the control.

A cardinally, the same fertilizer mixture of P at 35 units with 5, 10, and 20 separated units of Zn levels led to an increase in midrib zone thickness up to +5.47 %, +1.46 %, and 5.11%, respectively, compared to the control. Its increment is related to the increased vascular bundle both in lengths up to +11.71 %, +8.82%, +5.88%, respectively, and in diameter of the xylem vessel up to +16.6 %, +26.67%, +20.0% respectively more than those of the control.

Also, Table 2 and Figures 3 and 4 show that a fertilizer mixture of P at 40 units and 5, 10, and 20 separated Zn units led to increments in leaf blade thickness up to +11.4%, +18.4%, and +14.91%, respectively, and the increases corresponded to the increases in the upper epidermal layer up to +8.57%, +14.2%, and +5.71%, respectively, in the lower epidermal layer up to +25.0%, +18.2%, and +6.82%, respectively, and increments in leaf blade related to the increases in the palisade tissue up to + 15.3%, +23.7%, and +3.85%, respectively, and spongy tissue up to +9.38%, +15.6%, and +12.51%, respectively, compared to the control.

**Table 2.** Effect of P and Zn levels on the anatomical structure of table beet leaves during the season of 2019

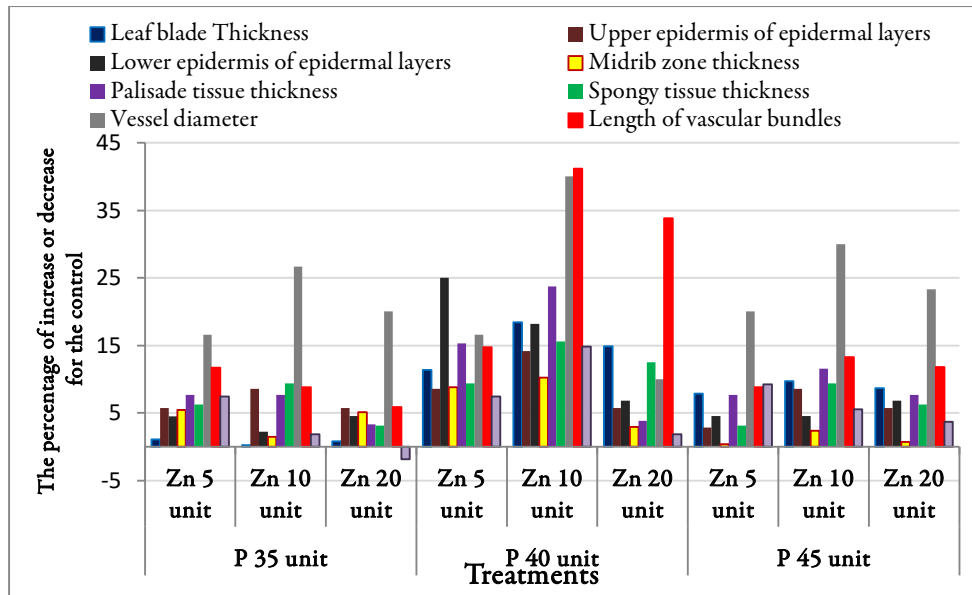
Characters ( $\mu\text{m}$ )		Palisade tissue Thickness		Spongy tissue Thickness		Vessel diameter		Vascular bundles Measurements			
Treatments		Absolute value	$\pm$ % of control	Absolute value	$\pm$ % of control	Absolute value	$\pm$ % of control	Length		Width	
P units	Zn units							Absolute value	$\pm$ % of control	Absolute value	$\pm$ % of control
Control		130	0.00	160	0.00	30	<b>0.00</b>	340	0.00	270	0.00
35	5	140	+7.69	170	+6.25	35	<b>+16.6</b>	380	+11.71	290	+7.4
	10	140	+7.69	175	+9.38	38	<b>+26.67</b>	370	+8.82	275	+1.85
	20	135	+3.3	165	+3.13	36	<b>+20.00</b>	360	+5.88	265	-1.85
40	5	150	+15.3	175	+9.38	35	<b>+16.6</b>	390	+14.71	290	+7.4
	10	160	+23.7	185	+15.6	42	<b>+40.00</b>	480	+41.17	310	+14.8
	20	135	+3.85	180	+12.51	33	<b>+10.00</b>	455	+33.82	275	+1.85
45	5	140	+7.69	165	+3.13	36	<b>+20.00</b>	370	+8.82	295	+9.25
	10	145	+11.54	175	+9.38	39	<b>+30.00</b>	385	+13.23	285	+5.55
	20	140	+7.69	170	+6.25	37	+23.33	380	+11.76	280	+3.70
Characters ( $\mu\text{m}$ )		Leaf blade thickness		Epidermal layers thickness				Midrib zone thickness			
Treatments		Absolute value	$\pm$ % of control	Upper epidermis		Lower epidermis		Absolute value	$\pm$ % of control		
P units	Zn units			Absolute value	$\pm$ % of control	Absolute value	$\pm$ % of control				
Control		369	0.00	35	0.00	44	0.00	1370	0.00		
35	5	373	+1.08	37	+5.71	46	+4.51	1445	+5.47		
	10	370	+0.27	38	+8.57	45	+2.27	1390	+1.46		
	20	372	+0.81	37	+5.71	46	+4.55	1440	+5.11		
40	5	411	+11.4	38	+8.57	55	+25.00	1490	+8.81		
	10	437	+18.4	40	+14.2	52	+18.2	1510	+10.21		
	20	424	+14.91	37	+5.71	47	+6.82	1410	+2.92		
45	5	398	+7.85	36	+2.85	46	+4.55	1395	+0.36		
	10	405	+9.75	38	+8.57	46	+4.55	1403	+2.40		
	20	401	+8.67	37	+5.71	47	+6.82	1380	+0.73		

The fertilizer mixture of P at 40 units with 5, 10 and 20 separated Zn units led to an increase in midrib zone thickness up to +8.81%, +10.20 % and 2.92% respectively, and its increment is related to the increase in both length of vascular bundle up to +14.71 %, +41.17%, +33.82%, respectively, and diameter of xylem vessel up to +16.6 %, +40%, +10.0%, respectively, more than those of the control.

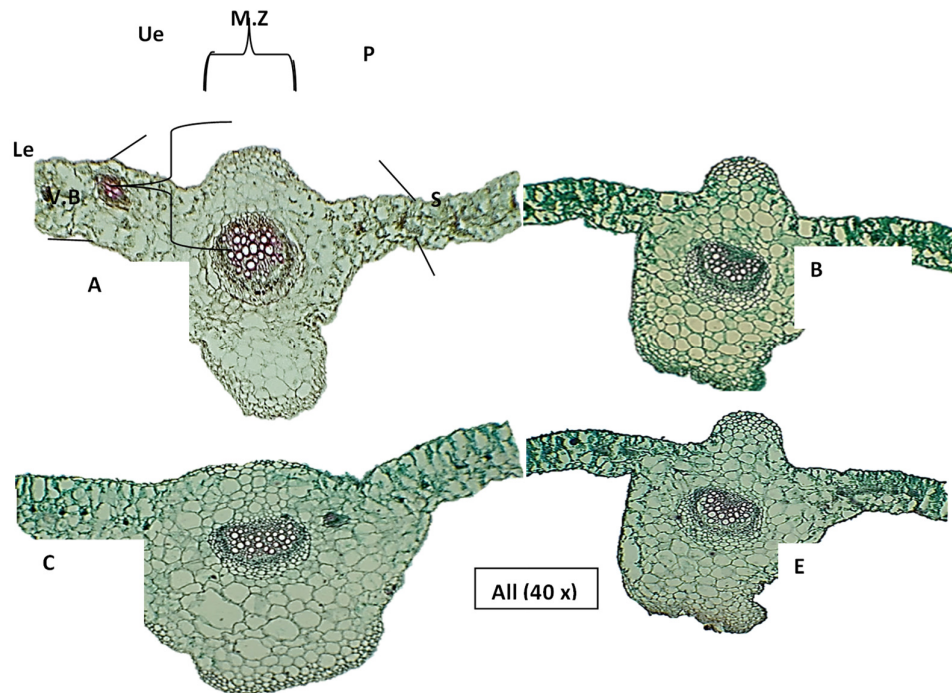
Increment availability P up to 45 units with 5, 10, and 20 separated Zn units led to increments in leaf blade thickness up to +7.85 %, +9.75 %, and +8.67%, respectively, and its increase corresponds to the increase of both upper and lower epidermal layers such as palisade and spongy tissues, compared to the control.

Combined exogenously applied 45 units of P and 5, 10, and 20 separated Zn led to a slight increase in midrib zone thickness up to +0.36%, +2.40 % and 0.73%, respectively, with its increment related to the increase

of both length of vascular bundle up to +8.82 %, +13.23%, +11.76%, respectively, and diameter of xylem vessel up to +20.0 %, +30.0%, +23.33%, respectively, more than those of the control.



**Figure 3.** The relationship between the anatomical structure in table beet leaves, combined exogenously applied of P and separated Zn units



**Figure 4.** Microphotographs of cross-sections through the leaf blade in *Beta vulgaris* L. cv. 'Detroit' dark red, aged 60 days as affected by the fertilizer mixture P and separated Zn units

A- Control, B- From plant grown under the fertilizer mixture P at 35 units and 10 Zn units, C- From plant grown under the fertilizer mixture P at 40 units and 10 Zn units and E- From plant grown under the fertilizer mixture P at 45 units and 10 Zn units.

## Discussion

### *Effect of P and Zn on the physical characteristics*

Fertilizers are important to induce the growth and production of table beet. Increasing the P and Zn doses caused a significant increase in the physical characteristics compared to the control. Applying P and Zn in combination with limited Zn availability is therefore essential to increasing growth and production. The yield increases because P helps in the development of the root system (Kamara *et al.*, 2011) and thus enables plants to absorb more water and nutrients equally. Zn application helps in the absorption of water and nutrients, which promotes growth and yield of the plant, indicating the flowering and fruiting process is greatly improved under severe Zn application. Also, Imran *et al.* (2016) found that application of Zn and P led to increasing in the growth and yield of both maize genotypes and Zn with P was more effective compared with the control.

Korkmaz *et al.* (2021) found that the effect of P and Zn interaction was significant on dry matter, P and Zn concentration and their uptake. Dry matter did not respond to Zn application at low P doses; however, chia plants produced eight-fold higher dry matter at high levels of P and Zn compared to the control. Application of P or Zn alone restricted maize growth (plant height, stem diameter, or plant biomass) and reduced yield by 8 to 85% relative to control plants in soils with medium to high P contents and low Zn contents (Sánchez-Rodríguez *et al.*, 2021). (Pandey, 2015) found that the deficiency of P leads to a higher root/shoot ratio as shoot growth is relatively more affected in comparison to root growth. It also causes stunted growth, and the foliage turns a dark green colour with reddish-purple tips and leaf margins due to the accumulation of starch and anthocyanin in the leaves (Chen *et al.*, 2014). Amjad *et al.* (2021) found that, treatments Zn in wheat caused a significant increase in shoot fresh and dry weight and also root dry weight.

These increases may be due to P as an important phytonutrient as it constitutes about 0.2% of the dry weight in the plant (Alori *et al.*, 2017). Regarding the positive effect of P in increasing vegetative parameters, P causes an increase in length and density of primary and lateral roots (Wissuwa *et al.*, 2005; Barker and Pilbeam, 2007; Farahani *et al.*, 2009) which increases water and nutrient uptake. These in turn enhanced the whole vegetative growth and chlorophyll content and lead to an increase in carbohydrate synthesis, consequently increasing total plant fruit production (fruits number and weight). It is a component of key molecules such as nucleic acids, phospholipids, and ATPs. It is one of the essential macronutrients required for the synthesis of nucleic acid, membrane build-up and stability, energy metabolism, and many other critical physiological and biological processes during plant growth and development (Hasan *et al.*, 2016).

P application may mobilize the photosynthates from growing organs to grains, consequently increasing their number and size (Singh *et al.*, 2017). P caused a higher rate of dry matter accumulation, which might be due to the increase in vegetative development and reproductive attributes under proper availability of P and better physical condition of the soil. Positive responses in terms of the yield attributes of P and Zn have also been reported by (Gangaiah and Ahlawat, 2008; Gupta *et al.*, 2006a; Kumar *et al.*, 2012; Patel *et al.*, 2013; Patil *et al.*, 2011). Also, Ova *et al.*, (2015) showed that P and Zn have positive effects on each other in wheat yield and Zn application did not enhance dry matter at low P doses, whereas plants produced more yield in response to increasing Zn at high P levels. Similar results were obtained in cowpea (Kumar *et al.*, 2016), wheat (Vafaei *et al.*, 2014), rice (Amanullah and Inamullah, 2016), and maize (Aboyeji *et al.*, 2019; Korkmaz *et al.*, 2021), indicating that combined P and Zn applications increased dry matter yield.

P also enhances the activity of rhizobia, and increases the formation of root nodules, and helps fix more of atmospheric N in root nodules (Das, 2017). The results agree with those of (Hafeez, 2013). The increase in the growth of plants under Zn treatment may also be due to its effect on the metabolism of growing plants, which may effectively explain the observed response of Zn application. Favorable responses of Zn application on plant height are similar to those found by (Shanti *et al.*, 2008).

Zn improved chlorophyll synthesis, acting as a catalytic and structural protein component and co-factor of various enzymes. Zn has a protective and stabilizing effect on cell membranes, which causes improvement in

the photosynthetic process (Ma *et al.*, 2017). Zn plays an important role in the activities of enzymes like dehydrogenases, tryptophan synthetase, aldolases, isomerases, transphosphorylases, superoxide dismutase, and DNA and RNA polymerases (Faizan *et al.*, 2021). Rengel (2001) showed that Zn fertilizer application causes root and shoot growth during the growing season and, therefore, lead to increased seed yield. Also, Sarhan *et al.* (2018) found that Zn application on the development of *Swietenia macrophylla* increased of some growth parameters (stem length, stem diameter, leaf area, number of leaves, root length and fresh weight, dry weight chlorophyll, and total carbohydrates for plant parts). Also, Z application ( $10 \text{ mg kg}^{-1}$ ) improved biomass production, chlorophyll index, and nutrient acquisition traits under normal as well as saline conditions of Basil (*Ocimum basilicum* L.) (Tolay, 2021).

Zn may be bound to cell walls, or sequestered in the cytoplasm or cellular compartments after uptake it by the roots, thus restricting translocation it from roots to shoot, and protecting photosynthetic organs from potential Zn toxicity. Zn may also be associated with glutathione (GSH), metallothioneins, nicotianamine, and carboxylates such as malate, oxalate, tartrate, and citrate in root cells without being further translocated upward (Broadley *et al.*, 2007; Gupta *et al.*, 2016; Palmgren *et al.*, 2008).

On the other hand, (Aboyeji *et al.*, 2019) found that the effect of Zn was not significant on the vegetative parameters, but the application of  $8 \text{ kg of Zn-ha}^{-1}$  significantly increased the number of seeds the weight of seeds, seed yield per hectare, and seed quality. It is estimated that almost half of the soils in the world are deficient in Zn. Also, excessive fertilization of P results in reduced shoot and Zn contents in grain and crop (Akhtar *et al.*, 2019; Barben *et al.*, 2011; Zhang *et al.*, 2012).

Zn application on yields of green gram might be due to its direct influence on auxin production, which in turn enhances the elongation processes of plant development (Patel *et al.*, 2013).

#### *Effect of P and Zn on table beet quality*

Figure 1 (a and b) shows the effect of adding P and Zn fertilizer on TSS, AA, TS content, and ACY content in the seasons 2018 and 2019. Increased P levels and average Zn levels increased the TSS, AA, TS and ACY content compared to the control. The obtained results indicated that the increases of these characteristics with the addition of P might be due to P enhancing or stimulating the vegetative growth in table beet plants. Amjad *et al.* (2021) found that Zn treatments in wheat caused a significant increase for ACY, AA and soluble sugars compared to the control.

The ACY content increase could be attributed to the potential activity of the photosynthesis process (Ibrahim *et al.*, 2012).

There are those who demonstrated that the addition of P resulted in a significant increase in the reduced and non-reducing sugar and TS as well as the carbohydrate, and starch contents of wheat grains and potatoes, respectively. The positive effect of P on the chemical composition in table beet plants such as AA, TSS, dry matter, and TS may be attributed to the presence of P, which agreed with those reported by (Abdel-Aziz *et al.*, 2016).

Zn and P deficiency often co-occurs in calcareous soils (Akhtar *et al.*, 2019; Duffner *et al.*, 2012; Schjoerring *et al.*, 2019). Zn plays an outstanding role in the synthesis of chlorophyll, protein and also regulates water absorption. Moreover, it also plays a role in carbohydrates metabolism and activation of various enzymes which helps in inducing alkalinity tolerance in crops by enhancing the Na/K and Na/Ca ratio (Chethan *et al.*, 2018). Therefore, increasing the Zn concentration could enhance the plant potential of scavenging ROS molecules by positively inducing SOD gene expression, thus elevating SOD activities (Ma *et al.*, 2017). The application of Zn increased the accumulation of total flavonoids and phenols in the berry plant (Song *et al.*, 2015). This increase in antioxidant contents was mainly due to Zn's ability to improve the biosynthesis of antioxidants.

*Effect of P and Zn on NPK uptake*

The N, P and K contents in roots is significantly influenced by the application of various P and Zn treatments compared to the control Figure 2 and these results were in agreement with (Amjad *et al.*, 2021) who found that Zn treatment in wheat caused a significant increase in Ca, P, and K content compared to the control; (Izhar Shafi *et al.*, 2020) also found that plant P concentration and its uptake in wheat were also significantly improved with the addition of P

Deshpande and Lakhdive (1994) reported that P application increased P uptake and content in leaf, stem, and roots. P uptake enhances photosynthesis and roots absorption efficiency, and thus increases P concentrations in the roots (Siam *et al.*, 2008). Korkmaz *et al.* (2021) found that the P content shows significantly higher values with increasing P doses as compared to the control and no significant impact of Zn applications on P content was noticed in plant tissues. Higher P fertilizer rates are generally recommended to increase P uptake, P use efficiency (PUE) and crop yields (Swaney and Howarth, 2019). On the other hand, high Zn application decreased P concentration of shoots compared with its low application rate under low or high soil P applications. Sánchez-Rodríguez *et al.* (2021) found that applying Zn without P to the soils had an adverse effect on P gain over the control plants. Also, increasing K content was in agreement with (Sarhan *et al.*, 2018) who found that Zn application of *Swietenia macrophylla* increased NPK, Ca, Mg, and Zn in plant parts. Z application (10 mg kg<sup>-1</sup>) in Basil plants improved K uptake under normal as well as saline conditions (Tolay, 2021). Benáková *et al.* (2017) found increased K content in the shoots of plants grown in the medium with Zn alone. High K of Zn-treated plants also confirm previously reported (Barker and Pilbeam, 2015; Fageria, 2015).

On the contrary, some previous studies found that K content decreased in roots after heavy metal treatment (Abu-Muriefah 2008; Siddiqui *et al.*, 2012).

*Effect of P and Zn on the anatomical structure in table beet leaves*

The mixture of P at 35 units with 5, 10, and 20 units separated of Zn caused increments in leaf blade thickness, both upper and lower epidermal layers, such as palisade and spongy tissues. In addition to increasing midrib zone thickness, its increments were related to the increase in vascular bundle length and diameter of xylem vessels more than the control, The fertilizer mixture of P at 40 unit with 5, 10 and 20 separated Zn units led to an increase in midrib zone thickness, its increment related to the increase in length of vascular bundle and diameter of xylem vessel more than those of the control. Combined exogenously applied 45 units of P and 5, 10 and 20 separated Zn led to a slight increase in midrib zone thickness, its increment related to the increase of lengths of the vascular bundle and diameter of the xylem vessel more than those of the control. In this regard (Mahdi and Sadoon, 2020) investigated the combination between irrigation periods and Zn nanoparticles and showed that the irrigated plants treated with nano Zn resulted in maximum cortex thickness in stems, while irrigated plants which were not treated with Zn recorded the lowest. Also, the nano Zn significantly exceeded the rest of the treatments and gave the highest value of vascular bundles number, vessel diameter phloem of sunflower leaves. The positive effect of the use of Zn Nano-particles at a concentration of 100 ppm was observed in all studied traits, as well as the role played by normal Zn in improving traits under a study compared to untreated plants. (Sarker *et al.*, 2010) found that, the anatomical response of maize (*Zea mays* L.) plants to P deficiency grown in sand culture and half-strength Hoagland solution revealed that P deficiency caused a decrease in the diameter of the root and stem as well as thickness of the leaf, P-deficient leaves revealed smaller vascular bundles with smaller size of the metaxylem vessel cavity. Phloem also occupied less area in P-deficient soybean plants compared to the control. Kavanová *et al.* (2006) found that, P deficiency reduced the leaf elongation rate due to decreases in the cell production rate and final cell length. The former was solely due to a lower average cell division rate and, thus, a lengthened average cell cycle duration. P deficiency did not affect the general controls of cell morphogenesis, but by slowing down the rates of cell division and expansion, it slowed down its pace.

## Conclusions

According to the findings obtained in this current study, the improvement in the growth, physiological, chemical composition, crop yield and anatomical characteristics of table beet corresponded to the exogenous application of the studied fertilizers levels of P and Zn.

## Authors' Contributions

All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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