

## Effect of application methods of phosphate and zinc sulfate fertilizers on water use efficiency and qualitative characteristics of corn under water deficit stress

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### ABSTRACT

To study the effects of phosphate and zinc sulfate fertilizers application methods on water use efficiency and qualitative characteristics of corn under water stress, an experiment was conducted in split plot factorial arrangement on randomized complete block design with three replications at research farm of the Bu Ali Sina University in 2014. Three levels of irrigation after 60, 90, and 120 mm evaporations from evaporation A pan were assigned in the main plots and two factors including phosphate fertilizer application methods (broadcasting and band placement) and zinc sulfate (non, foliar, and soil application) were assigned in sub-plots. Measured traits included protein percent in the grain, phosphorus and zinc content in the grain, and water use efficiency. Results showed that at severe water deficit stress, foliar application of zinc sulfate and band placement phosphate fertilizer compared to no zinc sulfate in the same application increased grain protein by 12%. The highest water use efficiency (1.50 kg grain per cubic meter of water) was obtained from treatment after 90 mm evaporation and band placement of phosphate and foliar application of zinc sulfate. In severe water stress conditions, band placement phosphate increased grain phosphorous content by 26%. Eventually, the highest and the lowest grain zinc concentration were 16.58 and 11.53 mg kg<sup>-1</sup>, respectively.

**Keywords:** Band placement, foliar, soil application, zinc concentration.

## تأثیر شیوه مصرف کودهای فسفات و سولفات روی بر کارایی مصرف آب و صفات کیفی ذرت تحت تنش رطوبتی

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### چکیده

به منظور بررسی تأثیر شیوه مصرف کودهای فسفات و سولفات روی بر شاخص‌های رشدی، پروتئین و کارایی مصرف آب ذرت تحت تنش رطوبتی، آزمایشی به صورت اسپلیت پلات فاکتوریل در قالب طرح بلوک‌های کامل تصادفی با سه تکرار در مزرعه دانشگاه بوعلی سینا همدان در سال ۱۳۹۳ انجام گرفت. عامل تنش رطوبتی در کرت‌های اصلی شامل سه سطح آبیاری بعد از ۶۰، ۹۰ و ۱۲۰ میلی‌متر تبخیر تجمعی از تشت تبخیر کلاس A و در کرت‌های فرعی دو عامل شیوه کاربرد کود سولفات روی در سه سطح (عدم مصرف، محلول‌پاشی، مصرف خاکی به صورت پخش) و شیوه کاربرد کود فسفات با دو سطح (مصرف پخش و مصرف نواری) به صورت فاکتوریل قرار داده شدند. صفات اندازه‌گیری شده شامل درصد پروتئین دانه، مقدار فسفر و روی دانه و کارایی مصرف آب بودند. نتایج حاصل از تجزیه واریانس نشان داد، در تنش شدید رطوبتی، محلول‌پاشی سولفات روی با مصرف نواری فسفات پروتئین دانه را نسبت به عدم مصرف سولفات روی در همین شیوه مصرف حدود ۱۲ درصد افزایش داد. بالاترین کارایی مصرف آب نیز از تیمار دور آبیاری پس از ۹۰ میلی‌متر تبخیر و مصرف نواری فسفات و محلول‌پاشی سولفات روی با میانگین ۱/۵۰ کیلوگرم دانه در متر مکعب آب به دست آمد. به هر حال محلول‌پاشی سولفات روی و مصرف نواری فسفات می‌تواند باعث بهبود مقاومت گیاه در شرایط تنش باشد، در تنش شدید رطوبتی مصرف نواری فسفات درصد فسفر دانه را نسبت به مصرف پخش فسفات حدود ۲۶ درصد افزایش داد، همچنین بیشترین و کم‌ترین میزان روی دانه با میانگین ۱۶/۵۸ و ۱۱/۵۳ میلی‌گرم بر کیلوگرم به دست آمد.

**واژه‌های کلیدی:** جای‌گذاری، محلول‌پاشی، مصرف خاکی، مصرف نواری.

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### Introduction

Maize (*Zea mays* L.) is one of the three major cereal crops in the world (FAO, 2012). Drought stress reduces nutrient uptake by roots. Naturally, the concentration of limiting nutrient elements is reduced during drought stress. Thus, the indirect effect of soil moisture stress on plants growth can be important as its direct effect. In this connection, reducing the availability of nutrient is one of the major factors that limit the plant growth under stress (Hussein *et al.*, 2009). Zinc element (Zn) is one of the elements of ribosomes and it is very important for its maintenance. Furthermore, Zn content is a critical parameter in RNA, without which enzymes are dismantled and RNA is degraded and, finally, protein is not formed. One of the most important roles of zinc in the plant is protein synthesis as lack of zinc results in the decreased protein and then increased free amino acids and amides (Whiting *et al.*, 2009). Reducing the amount of soil moisture causes decreased zinc absorption from soil and, due to the limited root growth because of drought stress, makes plants to face a severe shortage of nutrient elements (Bagci *et al.*, 2007). Ghasemian *et al.*, (2010) stated that resistance to environmental stress is increased by foliar application of micronutrients. In drought stress conditions, crop roots cannot absorb some important nutrients such as micronutrients (Heidarian *et al.*, 2011) and then foliar application of elements becomes more influential as compared to soil application (Narimani *et al.*, 2010). Among micronutrient, lack of Zn causes more problems for crop production (Cakmak *et al.*, 1999). In addition to Zn, phosphorus (P) is also effective on root growth, stem strength, improving flower formation, seed production, and uniformity in maturity (Verma & Abidi, 2009; Tariq *et al.*,

2011). Phosphorus band placement and foliar application of zinc sulfate on calcareous soils can help improve nutrients absorption because it reduces the chance of stabilization in such soils. Band placement of P compared to broadcasting method leads to close contact of developing roots with P-enriched soil. In addition, there is a little contact of applied P with alkaline carbonated substances and soil colloids through band placement (Shah *et al.*, 2006; Rahim *et al.*, 2010). Khan *et al.*, (2003) found that the use of micronutrients could increase the water use efficiency in the peas. The main objective of the present study is to select the best application method of phosphate and zinc sulfate fertilizers under water deficit stress in order to improve water use efficiency and qualitative characteristics of grain corn.

### Materials and Methods

In order to investigate the effects of phosphate and zinc sulfate fertilizers application methods on the water use efficiency and qualitative characteristics of corn under water stress, an experiment was conducted at agricultural research station of Bu-Ali Sina University in Hamedan (35°1'N, 48°31'E, 1690 m.a.s.l). According to the 55-year meteorological statistics, Hamedan city has a semiarid cold climate with mean annual rainfall of 333 mm and an average temperature of 24°C in the hottest month of the year (Aboutalebian & Moqisaei, 2016). This experiment was carried out in a split-plot factorial order based on a randomized complete block design with three replications. The main plots consist of three irrigation levels (60, 90, 120 mm cumulative evaporation from class A pan), and sub-plots consisted of two factors including phosphate fertilizer application methods (broadcasting and band placement) and

zinc sulfate (non, foliar, and soil broadcasting application). In this study, an average-maturing single cross cultivar NS640 (FAO600) was selected as the plant material, which is originated from Serbia and provided by the Seed and Plant Improvement Institute, Karaj, Iran. Tillage processes included a plow, disk, and leveling. By adding 100 kg/ha urea and 200 kg/ha phosphate, the rest of urea fertilizer 100 kg/ha was used in the two stages of 8 leaves and tassled as top-dress fertilizer. Zinc sulfate fertilizer was used 50 kg/ha. Each plot consisted of 6 rows grown just 6 m in length and row spacing 18 cm. Planting density was 75,000 plants per hectare. The distance between the main plots and replicates were 2 m while it was 75 cm between the subplots. Foliar application time was set through two stages of 4-6 leaves. Before to tasselling stage and foliar application, a ratio of 5/1000 zinc sulfate was performed in about 250 l/ha and water was sprayed to other experimental units at the time of foliar application. The physical and chemical characteristics of the soil are given in Table 1. To measure the nitrogen percentage in grains, one gram of each sample was digested using sulfuric acid and hydrogen peroxide. Next, with the aid of Kjeldahl method, its amount was determined (Sajedi, 2008) while the following equation was used for determining of protein percent in grains (Walton, 1990).

$$\text{Protein Percentage of Total Grains (\%)} = \text{Nitrogen Percentage} \times 6.12$$

Concentration of zinc in air-dried seeds was determined by atomic absorption spectrophotometer (Shimadzu AA-670, Shimadzu, Kyoto, Japan) in clear solution obtained after treating the seed flour with combination of sulfuric acid, salicylic acid, selenium, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) according

to the method described by Chapman & Pratt (1978).

To calculate water use efficiency, the ratio of grain yield (kg/ha) to consumed water volume (m<sup>3</sup>/ha) was used (Andrade *et al*, 2002). Water volume in each time of irrigation and in each water deficit stress was measured with a volumetric water counter.

Eventually, the obtained information was analyzed using SAS software. The mean comparison was made with MSTATC in the least significant difference test (LSD) at 5% probability.

## Results

### Protein percentage

The obtained results from analysis of variance (ANOVA) showed that the main effect of water stress and application methods of phosphate and zinc fertilizer were significant at 1% on grain protein. Among interactions, the interaction effect of water stress×application method of zinc sulfate was significant at 1%. Among the interaction effects, the triple effect of stress, the application method of phosphate and zinc sulfate was significant at 5% level (Table 2).

The highest amount of grain protein was related to the treatment of irrigation after 120 mm evaporation, foliar application of zinc sulfate, and phosphate band placement with average 12.39%. On the other hand, the lowest average was related to the treatment of irrigation after 60 mm evaporation and no zinc sulfate and broadcasting of phosphate with average 7.07%. Finally, under severe water stress, the foliar application of zinc sulfate increased this ratio about 12% compared with no zinc sulfate consumption plus broadcasting of phosphate (Fig. 1).

### Zinc concentration in grain

According to ANOVA results (Table 2), the main effects of water stress,

application methods of phosphate, and zinc sulfate were significant at 1%. The interaction effect of water stress  $\times$  application method of zinc sulfate was significant at 5% (Table 2). Foliar application of zinc sulfate in the absence of water stress and severe water

stress conditions significantly increased zinc concentration in grain compared with no application of zinc sulfate (Fig. 2). In this regard, the highest and the lowest zinc concentrations were 16.58 and 11.53 mg kg<sup>-1</sup>, respectively.

Table 1. Physical and chemical properties of soil (0-30cm)

O.C (%)	EC (dS/m)	pH	Zn (ppm)	N (%)	K (ppm)	P (ppm)	Soil Texture	Sand (%)	Silt (%)	Clay (%)
1.38	0.35	7.47	0.88	0.13	270	2	Loam	35	48	17

Table 2. Analysis of variance for different characteristics of corn affected by treatments

S.O.V	df	Mean Squares			
		Protein	Zinc concentration in grain	Grain phosphorus percentage	Water use efficiency
Block	2	0.32 <sup>ns</sup>	0.80 <sup>ns</sup>	0.012 <sup>**</sup>	0.001 <sup>ns</sup>
Water stress (S)	2	32.45 <sup>**</sup>	70.28 <sup>**</sup>	0.023 <sup>**</sup>	0.19 <sup>**</sup>
Error1	4	0.10	2.20	0.001	0.04
Phosphate (P)	1	3.19 <sup>**</sup>	4.08 <sup>**</sup>	0.036 <sup>**</sup>	0.05 <sup>*</sup>
Zinc (Zn)	2	13.89 <sup>**</sup>	7.85 <sup>**</sup>	0.012 <sup>**</sup>	0.09 <sup>**</sup>
S $\times$ P	2	0.04 <sup>ns</sup>	0.07 <sup>ns</sup>	0.0004 <sup>*</sup>	0.003 <sup>**</sup>
S $\times$ Zn	4	2.99 <sup>**</sup>	1.09 <sup>*</sup>	0.0002 <sup>ns</sup>	0.005 <sup>ns</sup>
P $\times$ Zn	2	0.29 <sup>ns</sup>	0.65 <sup>ns</sup>	0.0004 <sup>ns</sup>	0.001 <sup>ns</sup>
S $\times$ P $\times$ Zn	4	1.05 <sup>*</sup>	0.35 <sup>ns</sup>	0.0001 <sup>ns</sup>	0.03 <sup>*</sup>
Error2	30	0.29	0.39	0.0001	0.011
C.V. (%)	-	5.37	4.80	5.22	9.05

\*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively; ns: no significantly difference.

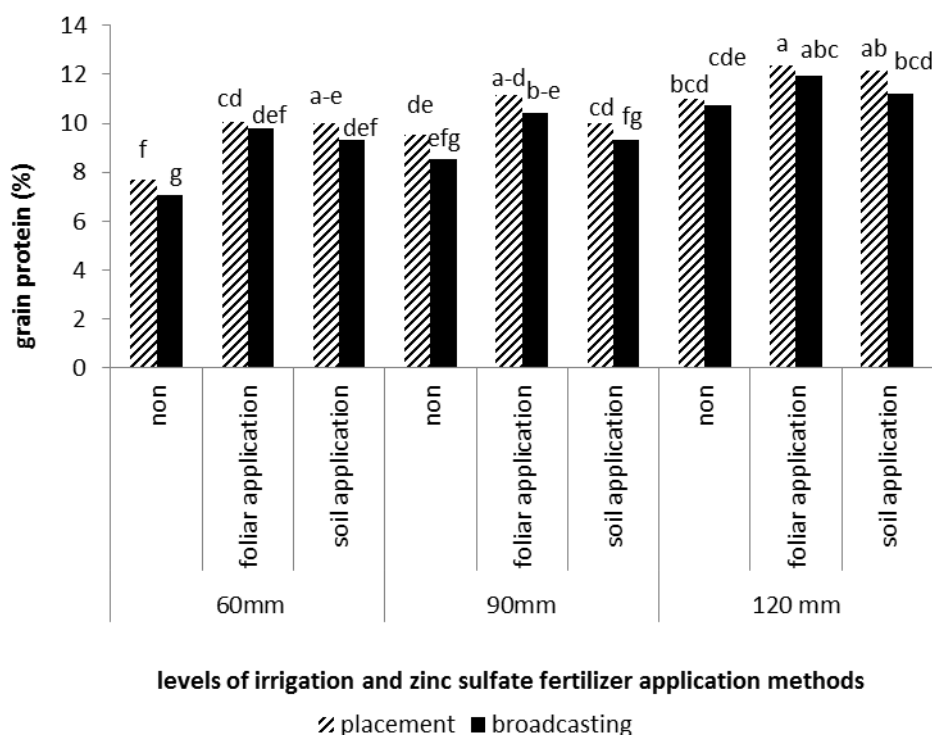


Figure 1. Effect of water deficit stress and application method of phosphate and zinc sulfate on grain protein percentage (LSD<sub>0.05</sub>=1.78)

### Grain phosphorus percentage

Based on the ANOVA results (Table 2), the main effects of water stress and application methods of phosphate and zinc sulfate were significant at 1%. The interaction effect of water stress  $\times$  application method of phosphate was significant at 5% (Table 2). The highest percentage of phosphorus was related to the treatment of 120 mm evaporation

and band placement phosphate while the minimum percentage of phosphorus was related to the treatment of 60 mm evaporation and broadcasting of phosphate (Fig. 3). Phosphate band placement under severe water stress conditions increased phosphorus content in the grain by 26% compared to the phosphate broadcasting treatment.

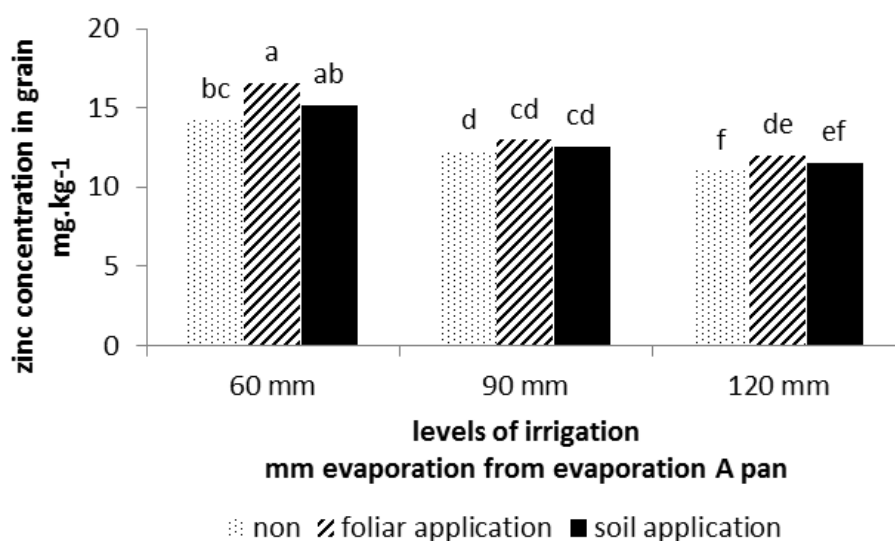


Figure 2. Effect of water stress and zinc sulfate application method on concentration of grain zinc ( $LSD_{0.05}=1.80$ )

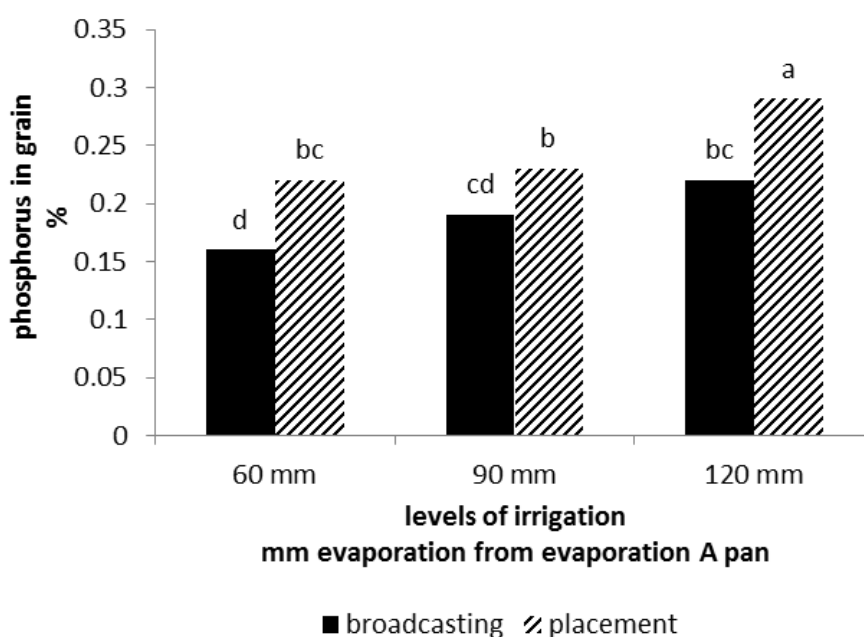


Figure 3. Effect of water deficit stress and application method of phosphate on grain phosphorus percentage ( $LSD_{0.05}=0.032$ )

### Water Use Efficiency (WUE)

The obtained ANOVA results showed that the main effects of water stress and zinc application method at 1% level and phosphate application method at 5% level were significant on water use efficiency. The interaction effect of water stress×application method of phosphate was significant at 1%. Among the interaction effects, triple effect of water stress×phosphate×zinc sulfate application methods was significant at 5% (Table 2). The highest

water use efficiency was obtained from irrigation after 90 mm evaporation and band placement of phosphate with foliar application of zinc sulfate (1.50 kg grain per cubic meter of water). In comparison, its minimum level was obtained in the lower and higher irrigations (irrigation after 120 and 60 mm evaporation, respectively) with the broadcasting of phosphate and no zinc sulfate and soil application of zinc sulfate with average 0.90 kg of grains per cubic meter of water (Fig. 4).

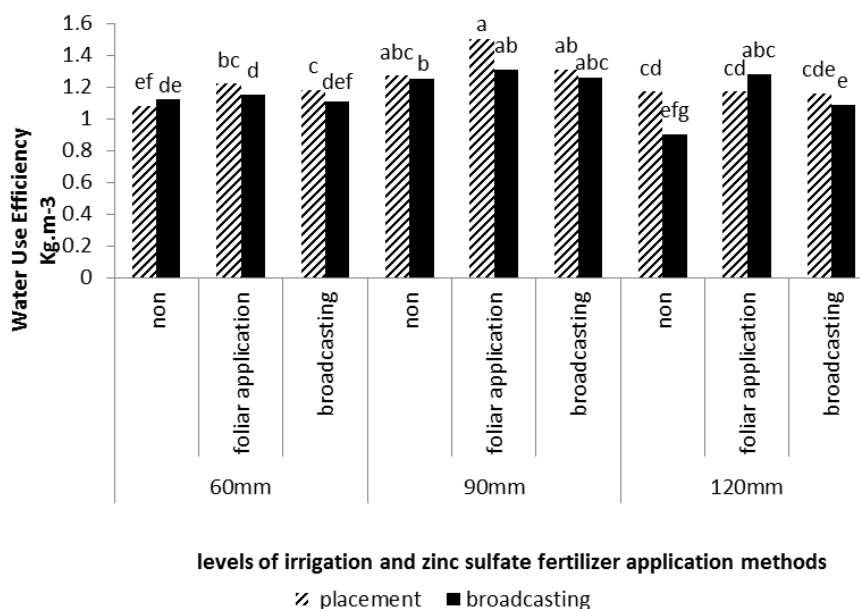


Figure 4. Effect of water stress and application method of phosphate and zinc sulfate on water use efficiency ( $LSD_{0.05}=0.28$ )

### Discussion

Some researchers have reported that seed protein content increases by zinc application. It has been shown that in zinc deficiency conditions, the activity of RNA polymerase enzyme and protein synthesis enzyme are decreased extremely while the rate of amino acids transformation is declined. Thus, by applying zinc, seed protein content will be increased due to increasing the activity of RNA polymerase enzyme and protein synthesis enzyme (Akhtar *et al.*, 2009; Rahman *et al.*, 2008; Sangwan & Raj, 2004). The higher

grain protein percentage in water deficit stress conditions can be related to the reduced grain-filling period in water-restricted treatment; which reduces the ratio of carbohydrates to protein due to the decreased abundance of starch synthesis enzymes. Thalooh *et al.* (2006) reported that water stress affects the photosynthesis, enzyme activity, and protein synthesis, leading to the changed movement of metabolites to seed. Sadeghipour & Aghaei (2012) also stated that new protein synthesis is the cause of increased bean seed protein under drought stress. Protein content is

significantly increased under water deficit (Ozturk & Aydin, 2004), mainly due to higher rates of accumulation of grain N and lower rates of accumulation of carbohydrates. Ozturk & Aydin (2004) observed that late water deficit stress increased grain protein relative to the fully irrigated treatment. Water use efficiency is one of the critical factors affecting agricultural productivity in arid and semi-arid areas around the world. Hence, various soil and crop management practices have been developed to increase crop yields (Huang *et al.*, 2005). The results of this study indicated that foliar application of zinc sulfate increased grain Zn concentration and it was more effective than soil zinc sulfate application. Foliar Zn application also reduced the ratio of Phytic acid to Zn in grain and increased Zn absorption more than soil application of Zn (Bukvic *et al.*, 2003). Low Zn diffusion coefficients may be the main factor controlling grain Zn concentrations in calcareous soils. Phosphorus uptake by the plant decreased by increasing Zn in the soil while foliar application of Zn increased phosphorus absorption of soil by plants (Bukvic *et al.*, 2003).

Foliar application is a very fast method for providing required elements for plants compared to absorption by roots and zinc transfers to grain by remobilization. Besides, it provides a higher remobilization compared to other micronutrients (Aboutalebian & Moqisaei, 2016; Ranjbar & Bahmaniar, 2007).

Allen & Musik (1993) reported an increase in water use efficiency under

drought stress and explained it by the loss of water through evapotranspiration and deep percolation in the full irrigation treatment. On contrary, reduction of water use efficiency under water stress has been reported in corn plant (Al-Kaisi & Zinhuva, 2003). These reports are consistent with obtained results of the present study because wasting water in irrigation after 60 mm evaporation treatment and reducing the water consumption in irrigation after 120 mm evaporation are the main reasons for the yield reduction and the subsequent reduction in water use efficiency, respectively. Hence, 90 mm treatment was considered as the most suitable irrigation treatment to increase water use efficiency. It seems that under water stress and shorting the reproductive growth period, there is less opportunity for storing starch. As a result, the amount of seed protein would increase (Farley & Coot, 1998).

### Conclusions

Based on the findings of this study, by increasing the severity of water stress, the protein and phosphorus percentages of grain increased but its zinc concentration decreased significantly. Moderate water stress improved corn water use efficiency. Band placement phosphate compared to its broadcasting treatment increased grain protein and phosphorus percents. In general, band placement of phosphate fertilizer along with the foliar application of zinc sulfate in conditions of water deficit stress can be applied to improve corn water use efficiency.

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