



ZINC-ASPARTATE-MEDIATED DROUGHT AMELIORATION IN MAIZE PROMISES BETTER GROWTH AND AGRONOMIC PARAMETERS THAN ZINC SULFATE AND L-ASPARTATE

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SUMMARY

In developing countries, food scarcity and malnutrition are predominant problems encountered by indigenous people due to the exponential growth of the population and the unavailability of good-quality food. These problems are exacerbated by an increase in the homozygosity of crops, climate change, water stress, resistant pathogens, and the application of unnecessary fertilizers. The current study was designed to compare the effects of the modulations induced by zinc sulfate ($ZnSO_4$), zinc aspartate (Zn-Asp), and aspartate (L-Asp) in water-stressed maize in Punjab, Pakistan. This experiment was designed with a split-plot factorial pattern involving two main plots (control and stressed plants). Each plot comprised eight rows of plants that corresponded to the following foliar treatments after 30 and 45 days: no spray; water spray; and foliar application of $ZnSO_4$ (0.25%), $ZnSO_4$ (0.50%), Zn-Asp (0.25%), Zn-Asp (0.50%), L-Asp (0.5%), and L-Asp (1.0%). Drought stress was imposed for 3 weeks after germination, and samples were collected periodically after 45 days after germination and up to 110 days to maturity of the crop. Various growth attributes, such as nutrient acquisition and agronomic parameters, were examined. Biofortification with Zn-Asp proved to be highly promising in terms of improved growth, increased yield, and induced drought tolerance. This study proved that the biofortification of crops by using mineral-chelated amino acids, such as Zn-Asp, was more fruitful than that with the traditionally used $ZnSO_4$ and ameliorated drought. Hence, this experiment proved the beneficial effects of Zn-Asp on maize; these effects can also improve the nutritive quality of flour, grains, and other maize food products for better human health.

Keywords: Biofortification, drought stress, foliar spray, kernel yield, malnutrition, mineral chelated amino acids, *Zea mays* L.

Key findings: This research provides the solution to water-stress problems caused by water scarcity in rainfed areas. It provides the mechanism of the yield enhancement of maize for improved food security and provision.

INTRODUCTION

Food is the most prime among all of the life sustenance factors required for the continuation of life activities, and the provision of balanced food is paramount for the health of people. Scarcity and malnutrition are the cause of many infirmities and crimes occurring around the globe, particularly in developing countries. Half of the world's population is facing malnutrition due to the shortage of micronutrients, such as Zn, Br, Cu, and Fe, in food (Stein, 2010). The scarcity and low quality of food have mostly affected the health of children and the female population of the world in the form of anemia (Sangeetha and Premakumari, 2010), stunted growth, digestive issues, high blood pressure, hormonal disturbances (Hart, 1928), and diabetes mellitus (Shankar, 2000).

Approximately one-third of the globe comprises arid and semiarid areas for cultivation (Hadi *et al.*, 2013). Plants encounter various abiotic and biotic stresses due to their static and sessile life pattern, and their growth is directly related to water and nutrient availability. Water is a key factor for the sustenance of life, particularly for crop plants that cannot survive without plenty of water (Babaeian *et al.*, 2011). As a result of climate changes, water scarcity is intensifying worldwide. The conservation of water has become a major challenge in dry surroundings, affecting the general overall homeostasis of plants. On average, approximately 50% or more yield losses occur due to the drought constraints faced by terrestrial plants (Anjum *et al.*, 2011; Aziz *et al.*, 2019).

Some researchers also point out that drought is the biggest culprit in reducing agronomic parameters in crop plants (Ali and Ashraf, 2011; Aziz *et al.*, 2019). Water stress induces various adaptations in plant morphology

necessary to overcome stress-related damages. Succulent and desert plants develop waxy leafy surfaces and exhibit sunken stomata. Similarly, the development of narrow leaves in maize and millet confirms that plants undergo adaptation to overcome evapotranspiration losses due to climatic changes (Khan *et al.*, 2016). A total of 60% of the world's food resources is present in the form of food grains produced by food crops (Ali and Ashraf, 2011; Aziz *et al.*, 2019). Maize (*Zea mays* L.) is the third major food crop sown in Pakistan on a hectare basis (Ehsanullah *et al.*, 2015). It ranks third among all cereals and millets and is thus used in manufacturing a variety of foodstuffs, such as corn starch, flakes, gluten, grain cakes, and various popcorn-type foodstuffs. A careful estimation shows that global corn demand reached 784 million tons per year in 2020 (Aslam *et al.*, 2014).

In maize plants, water stress imposed at the early stages of flowering leads to the improper development of endospermic tissues due to reduced cell division (Artlip *et al.*, 1995; Aziz *et al.*, 2019). The protoplasmic contents of water are approximately 80% to 90%; thus, low water availability leads to gradual yield losses that affect the general growth and development of plants. The seed germination pattern in terms of seed vigor indexes leads to the improper development of crops, resulting in limited oil quantity and quality (Babaeian *et al.*, 2011; Aziz *et al.*, 2019). The water-stressed environment is the main cause of average yield losses, particularly in food crops. A maize plant has a life cycle duration of approximately 80–110 days. During this period, the maize plant requires 500 mm to 800 mm of water. Only 25%–30% of the area of Pakistan receives proper rainfall. Nonmechanized farming and injudicious resource use

result in the depletion of approximately 40% of water. Irrigation water resources are expected to decrease by half due to unwise practices (Aslam *et al.*, 2014). The growth of female maize plants is negatively influenced; thus, the general process of fruit setting and fruit retention is being affected at an increased magnitude (Hajibabae *et al.*, 2012). This situation leads to an overall reduction in the number of kernel grains per fruit case produced by a maize plant (Nasri, 2010). For homeostasis and osmoregulation purposes, plants accumulate a variety of osmolytes, including soluble sugars, polyols, and free amino acids, in their protoplasm to minimize losses from dehydration. These osmoprotectants enable the internal defense system of the plants to combat ROS-induced damages, thereby limiting yield losses. Various amino acids, such as proline; osmolytes, like trehalose; and glycinbetains, are accumulated by the plants to tolerate ROS-induced damages (Ali and Ashraf, 2011). Approximately 30% of the world's land resources is deficient in zinc (Ram *et al.*, 2016). Zinc is an important micronutrient that is not only an important activator in enzymatic functions but is also an immune booster for the living worlds of plants, animals, and microbes. Zinc fingers are important motifs in translating genetic messages while helping in transcriptomic and translational modifications during protein synthesis (Kleckerova *et al.*, 2011). Zinc deficiency retards normal osmoregulation in plants, thus deteriorating water use efficiency and subsequently reflecting dehydration (Hadi *et al.*, 2013). Approximately 60 important enzymes require zinc as a cofactor in the human body, thus proving that zinc is an important component of human metabolism (Ali *et al.*, 2011; Zhang *et al.*, 2012). A normal human should take approximately 15 mg of zinc in their diet for stable metabolism. Several metabolic disorders may result in fetuses and children due to losses in zinc dietary intake. People obtain these micronutrients

through consuming food crops. Therefore, biofortifying crops with essential micronutrients to avoid any deficiency is important (Fang *et al.*, 2008; Aziz *et al.*, 2019).

Zinc sprinkling on crop plants has been proven to be fruitful by various plant researchers (Mohsin *et al.*, 2014). Exogenously sprayed zinc enhances crop yields and is a good short-term solution for mitigating micronutrient-related deficiencies (Ram *et al.*, 2016). Crop fertigation with zinc provides growth-friendly and yield-friendly results (Mohsin *et al.*, 2014). Micronutrients should be applied in chelated form to improve agronomic vigor because they may transform into an insoluble form if supplied alone. Given that chelating agents secure metal ions from extra chemical pathways, such as precipitation, they are used abundantly (Datir *et al.*, 2012; Aziz *et al.*, 2019).

The key aims of the present study were to a) investigate the potential of zinc sulfate (ZnSO₄), zinc aspartate (Zn-Asp), and aspartate (L-Asp) to induce ameliorations in maize plants in response to a water-scarce environment and b) analyze various growths and yield parameters. We hypothesized that Zn-Asp provided better osmoprotection than ZnSO₄ in drought mitigation and enhanced morphological growth, yield, and nutritive composition of grains.

MATERIALS AND METHODS

Various agronomic traits were recorded along with root and shoot allometric statistics by subjecting the maize plants to conventional zinc salt treatment in the form of ZnSO₄ and synthesized chelates in the form of Zn-Asp and L-Asp for experimental purposes. Maize plants were cultivated under natural climatic conditions in the botanical garden of the Government College University Faisalabad, Pakistan (latitude 30°30'N, longitude 73°10'E and altitude 213).

Experimental design

This experiment was designed with a split-plot factorial pattern involving two main plots (i.e., control and stressed). Two major plots were selected for control and stress treatments. Each major plot was designed with eight rows that were subjected to the differential application of treatments. Twenty plants of *Z. mays* L. were planted in each row. Shallow plant roots require moisture. The soil was slightly moistened to provide an atmosphere that was conducive to seed germination.

Field capacity was determined by collecting three soil samples each weighing 200 g. The samples were placed in an oven at 105 °C for 24 h. After oven-drying, the samples were weighed for the analysis of soil moisture contents at sowing time. Saturation percentage was quantified, and field capacity was studied in accordance with the following formula:

$$\text{Field Capacity} = \frac{\text{Saturation Percentage}}{2}$$

Seed germination ratio

Seeds were planted during the first week of August 2016. Maize seeds were collected from Ayub Agriculture Research Institute, Faisalabad, Punjab, Pakistan. Surface sterilization with 0.1% mercuric chloride was performed as a presowing treatment. In accordance with the experimental design, 20 seeds were planted in each row in moisture-rich soil with the help of a hoe. The seedlings were keenly and scientifically monitored and observed to obtain the healthy progeny of crop plants for analysis. Thinning was done to allow for the abundant growth of plants. A healthy soil atmosphere was thus established for good gaseous exchange. Five plants from each row were removed through periodic thinning to maintain a plant spacing of 20 cm. The seedlings were subjected to the first water treatment during the second week of

germination. The subplot under water shortage was irrigated twice only when plants were in their vegetative and flowering stages. The other subplot was irrigated in accordance with normal irrigation requirements. Drought stress was imposed on the plants after 3 weeks from the time of the germination of maize seeds. Meanwhile, the soil was regularly fertilized with manure at the rate of 130 kg NPK ha⁻¹.

Growth parameters

The method given by Leu (1991) was performed to synthesize chelated Zn-Asp. Briefly, 260 g of ZnSO₄·7H₂O was added and dissolved in H₂O. Then, L-Asp monohydrochloride (146.12 g) was added and warmed well at 95 °C for 3 h. Zn-Asp treatments were prepared with various concentrations, including 0.25% and 0.50%. The row-wise treatment applied included no spray; water spray; and foliar sprinkling of ZnSO₄ (0.25%), ZnSO₄ (0.50%), Zn-Asp (0.25%), Zn-Asp (0.50%), L-Asp (0.5%), and L-Asp (1.0%). Foliar sprays were fertigated after 20 days upon the induction of drought stress. Samples were collected periodically from 45 days after germination up to 110 days at the maturity of crops. All the collected samples were stored in liquid nitrogen at -80 °C--85 °C. Various agronomic and physiological attributes, including the biochemical parameters of the plant samples, were studied. Soil physio-chemical characteristics were analyzed, and the soil was identified as sandy loam with the organic content of 1.13%, available total N of 0.74% and P of 8.5 ppm, and a saturation percentage of 33%. The soil solution had the following properties: HCO₃⁻ (4.93 meq L⁻¹), SO₄⁻² (1.98 meq L⁻¹), Cl⁻ (8.52 meq L⁻¹), Ca²⁺⁺Mg²⁺ (14.3 meq L⁻¹), Fe (0.041 meq L⁻¹), Na (2.98 meq L⁻¹), and SAR (0.086 meq L⁻¹). Soil analysis was performed by following the method given by Davis and Freitas (1970). The pH and EC of the soil were 7.5 and 2.51 ds m⁻¹, respectively.

Experimental trial

Fully matured leaf samples were taken from each plant in correspondence with the specific treatment, and their fresh biomass was quantified. The leaves were placed in distilled water for 8 h, and the turgid weight of each leaf sample was recorded. All the samples were oven-dried at 70 °C for the calculation of dry weight. The leaf relative water content was studied by using the following equation:

$$\text{Leaf relative water content (\%)} = \frac{\text{Leaf Fresh Weight} - \text{Leaf Dry Weight}}{\text{Leaf Turgid Weight} - \text{Leaf Dry Weight}} \times 100$$

A manual electronic balance was used to determine the weight of the freshly uprooted shoots of the plants. The calculation was recorded in grams and noted for further analysis. The roots of the uprooted plants were separated with a sharp cutter. The roots were cleaned, and soil entities were removed. Root fresh weight was measured in grams by using an electronic balance. Shoot moisture and water content were completely evaporated. For this purpose, the shoots were placed in the open air under a sun-bath. Dried samples were placed on an electronic balance for the calculation of dry weight. A similar practice was performed with the root samples. Root and shoot dry weights were noted in grams for further statistical analysis. Shoot length was measured with the help of a meter rod and taken in units of cm. The shoot samples were labeled carefully, and their length was recorded. Similarly, the root length of the samples was measured by using a meter rod and recorded for calculations. The flag leaf area (cm²) of each separate sample was calculated with the following formula:

$$\text{Leaf Area} = \text{Length} \times \text{Width} \times 0.7$$

(Correction factor)

The obtained data of leaf area were used for the determination of the plant leaf area index. The total number of leaves was counted for each plant sample

and subjected to statistical analysis to measure the effect of drought and the effectiveness of the specific treatment given.

After complete development upon maturity, the plant fruit cases (cobs) were collected and preserved for yield analysis. Maize was harvested from a 6 m² area. Clean kernels were exposed to sun and air for yield estimation. Cob length was measured from the base to the tips of the fruit case. Random samples were collected with differential treatments from each row. Length was measured in cm. Samples were averaged separately for analytical purposes. The yield of kernels was directly dependent on the number of rows containing grains within a fruit case. A high number of rows indicates high kernel yield and vice versa. For yield estimation, the numbers of grain-containing rows were counted and noted. The total number of ripened kernels was calculated from each sample randomly collected from each row. Ten cobs from each row were selected and averaged separately for analysis.

A hand-held electronic balance was used to evaluate 100-grain weight, and the samples were averaged for 100-grain weight. Starch contents were analyzed via iodine test in randomly selected maize kernels with glucose as a standard in accordance with the procedure given by Sullivan (1935). Each sample to be evaluated was mixed with 1 mL of iodine solution (4 g of potassium iodide and 1.27 g of iodine) for 10 min. Absorbance was noted at 660 nm with a spectrophotometer. Maize kernels were crushed into powder to analyze protein contents. Kernel protein contents were studied by using the method given by Gornall *et al.* (1949) and Aziz *et al.* (2019). In this method, bovine serum albumin was taken as the standard protein. Burette reagent was prepared by mixing 0.3 g of CuSO₄·5H₂O, 0.5 g of KI, and 0.9 g of sodium potassium tartrate in up to 100 mL of distilled water. The same concentration of reagent was mixed with standards, as well as maize kernels, and subjected to spectrophotometric analysis

at 540 nm in accordance with the procedure. The maize kernels were subjected to proximate analysis for crude fiber by using method 32-10 as depicted in AACC (Anonymous, 2000).

Statistical analysis

The proximate analysis for the kernel inorganic and organic matter was performed and the obtained data were analyzed by using LSD test.

RESULTS

Plants play an important role in human lives. In particular, crops, i.e., maize, have a paramount role in life sustenance. This study was designed to enhance the yield of maize for coping with the food needs of the increasing population of this country. In this work, the useful effects of different chelated fertilizer combinations on water-stressed maize crops were observed in terms of morphological and yield effects.

The compiled data on the morphological parameter of shoot fresh weight are presented in Figure 1, which depicts that the shoot fresh weight of the corn plants decreased upon subjecting the plants to a drought environment. The figure also shows that root fresh weight was affected significantly by various treatments. However, the increasing or decreasing effect was treatment-specific. Under controlled and drought environments, among all treatments, the foliar sprinkling of 0.5% Zn-Asp increased shoot fresh weights.

Shoot dry weight decreased in a water-scarce environment (Figure 2). Shoot dry weight was affected under all different levels of the given treatments. The best results were produced by Zn-Asp treatments, proving their superiority over the conventional zinc salt treatment. Different foliar sprays increased root fresh weight. The Zn-Asp foliar spray induced morphological modulations that ameliorated drought-induced damages (Figure 3).

The experimental data indicated that a water-stressed environment caused a significant reduction in the root dry weights of maize plants (Figure 4). Each foliar treatment showed a significant effect on grain quality and weight. A maximum increase in root dry weight was obtained with 0.5% Zn-Asp and zinc sulfate solution treatment. The shoot length of the maize plants was significantly affected under water shortage and exogenous treatments (Figure 5). The Zn-Asp treatments proved beneficial in alleviating the drought-induced reduction in the shoot lengths of maize plants under a water-stressed environment and controlled condition.

The experimental data demonstrated that drought-induced treatments decreased the root lengths of the corn plants (Figure 6). Generally, the foliar spraying of various treatments positively affected root length and increased the root length of corn plants, even though the effect of each treatment was specific. Overall, the best-pronounced effect was shown by the Zn-Asp treatment. Water stress decreased the number of leaves of maize plants. The foliar sprinkling of various treatments regulated the number of leaves produced per plant. Zn-Asp treatments increased the number and length of leaves compared with simple ZnSO₄ and L-Asp treatments (Figure 7).

Leaf area indexes obtained by measuring the length and width of flag leaves indicated that the imposition of drought significantly reduced the lamina of maize plants (Figures 8 and; 9). Exogenous treatments with various chemicals significantly affected flag leaf area indexes. However, each treatment had different effects. Zn-Asp treatments applied through foliar sprinkling increased the length and width of flag leaves, thereby proving to be beneficial in combating drought-induced decreases in leaf area. Figure 10 illustrates the decrease in lengths of cobs produced by water-stressed maize plants under the influence of drought. Cob length is considered an important criterion for assessing

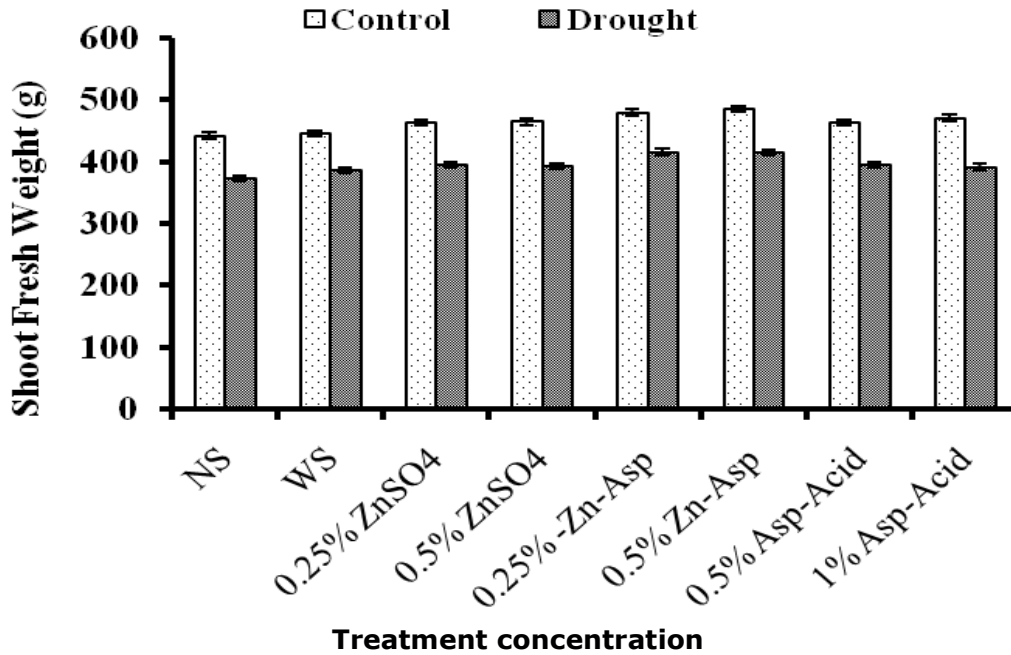


Figure 1. Increase in the shoot fresh weights (g) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

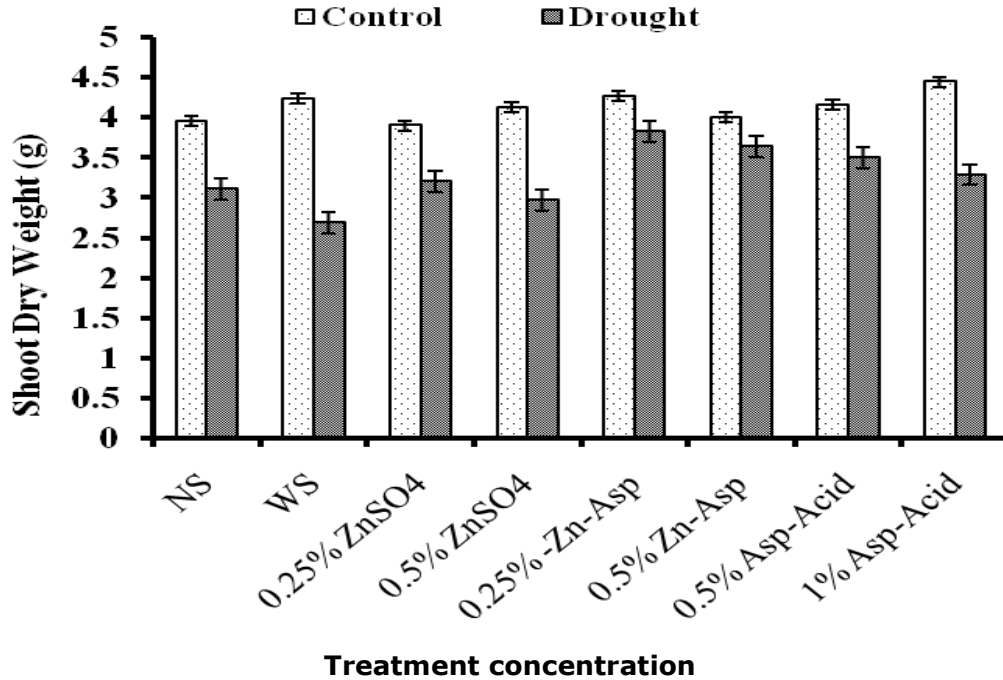


Figure 2. Increases in the shoot dry weights (g) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

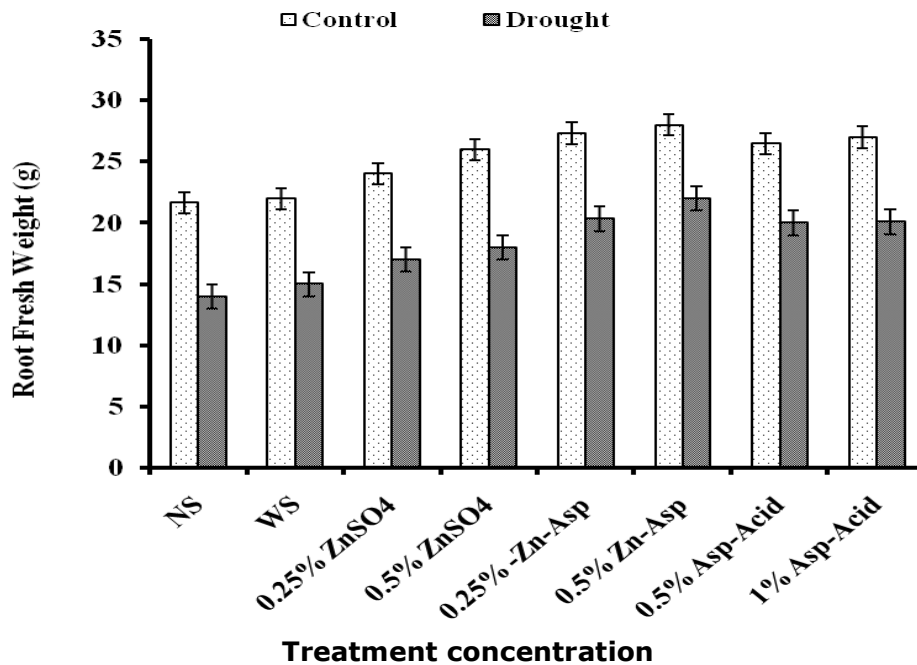


Figure 3. Increases in the root fresh weights (g) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

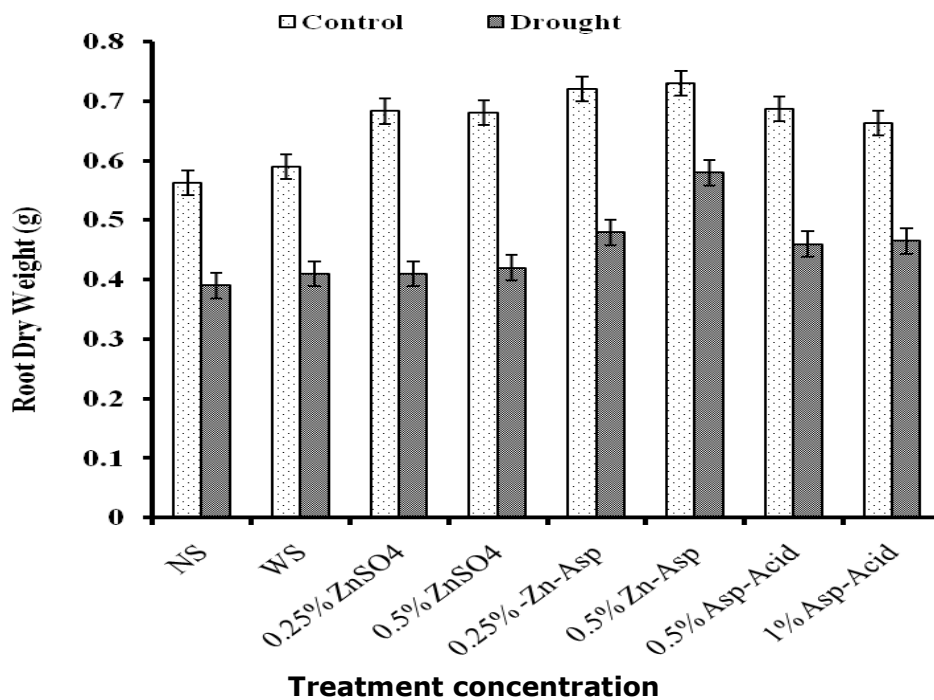


Figure 4. Increases in the root dry weights (g) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

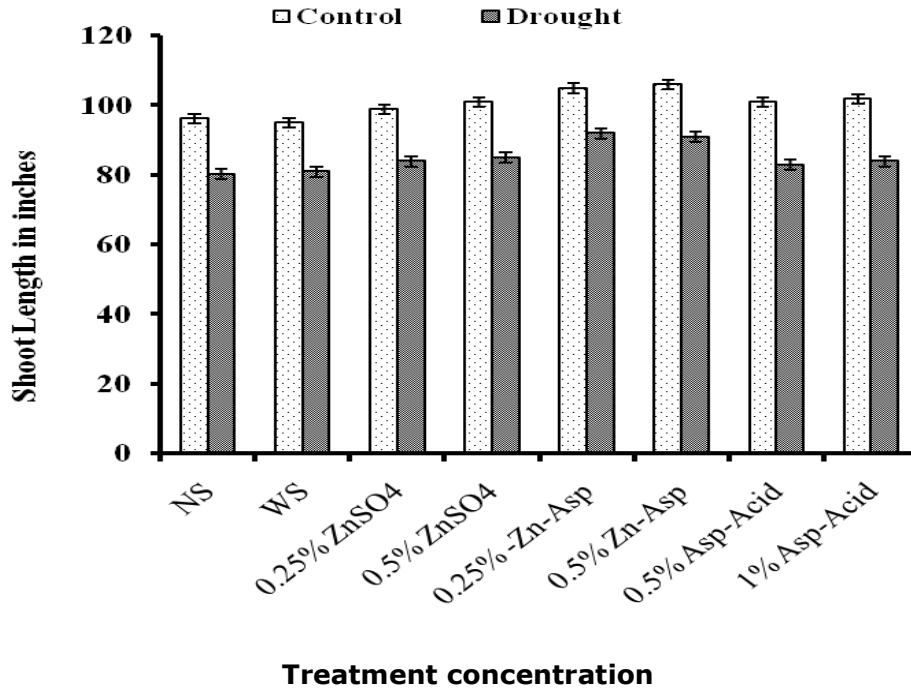


Figure 5. Increases in the shoot lengths (cm) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

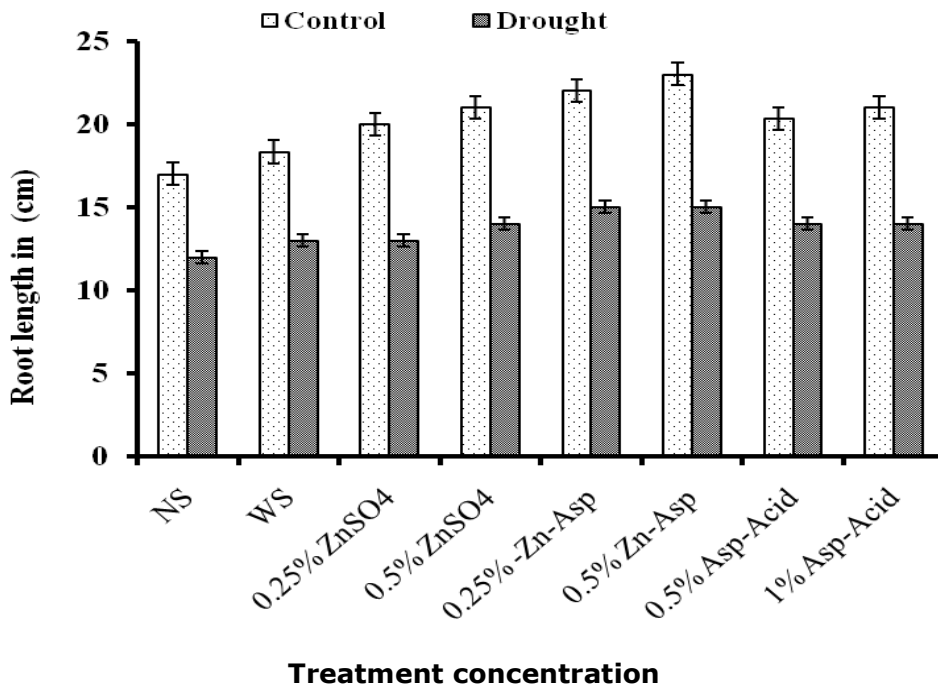


Figure 6. Increases in the root lengths (cm) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

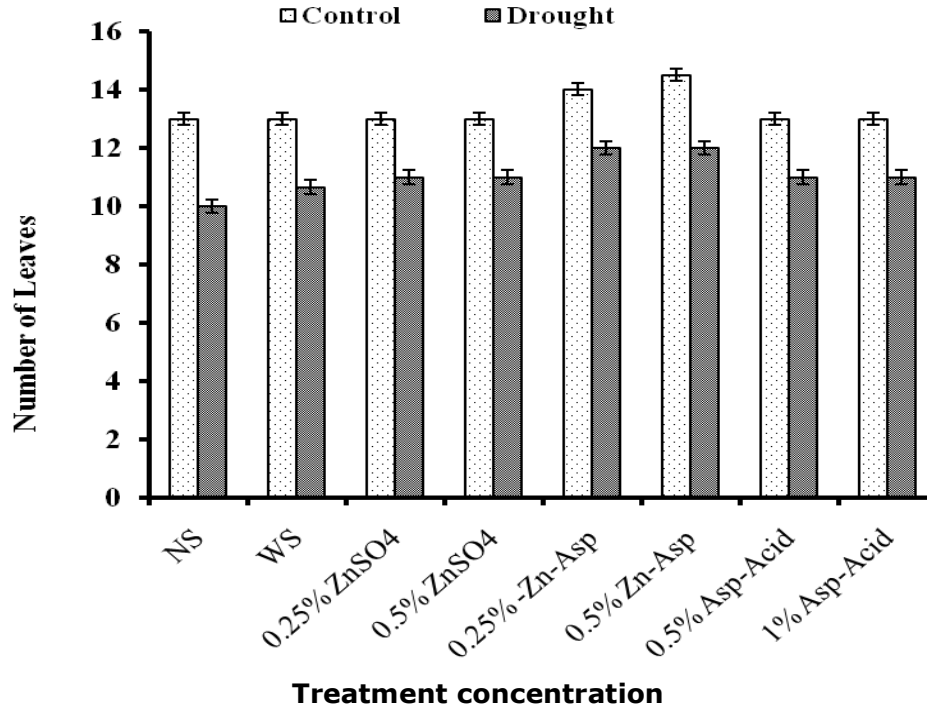


Figure 7. Increases in the numbers of leaves of water-stressed maize plants under the foliar application of Zn-Asp (mean ± SE; n = 3).

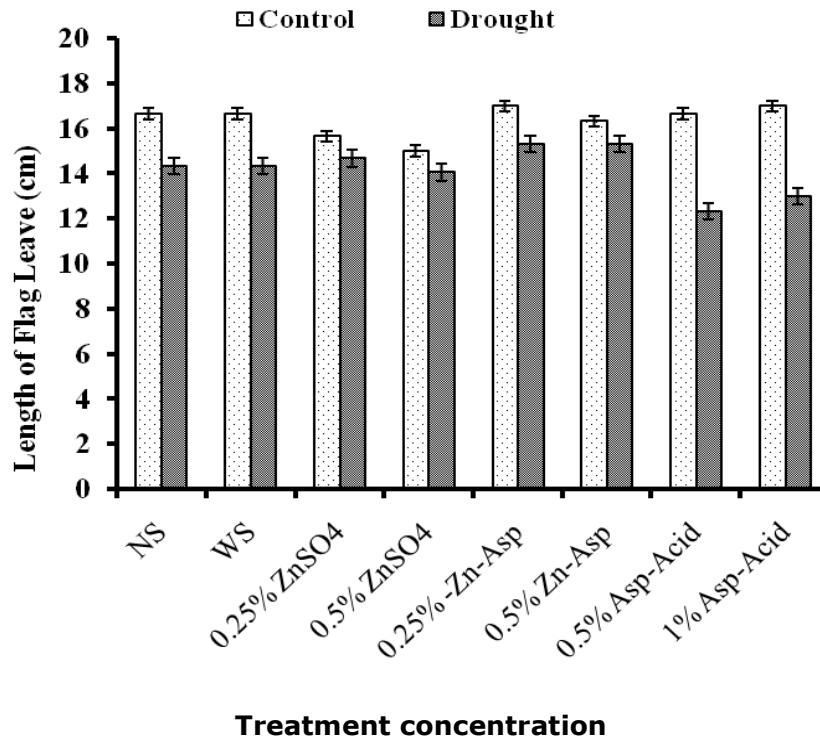


Figure 8. Increases in the lengths of flag leaves (cm) of water-stressed maize plants under the foliar application of Zn-Asp (mean ± SE; n = 3).

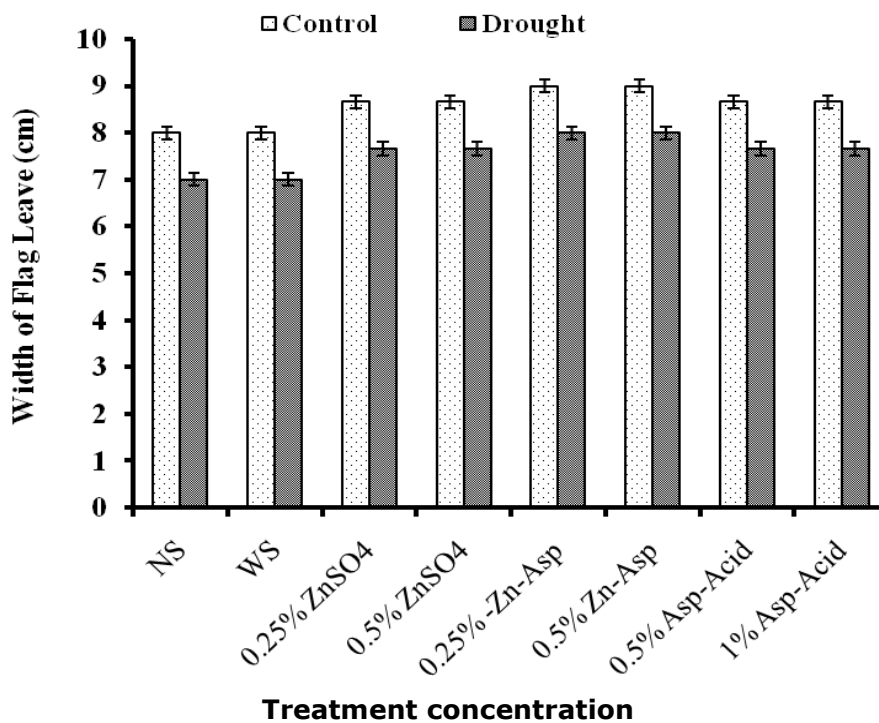


Figure 9. Increases in the widths of flag leaves of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

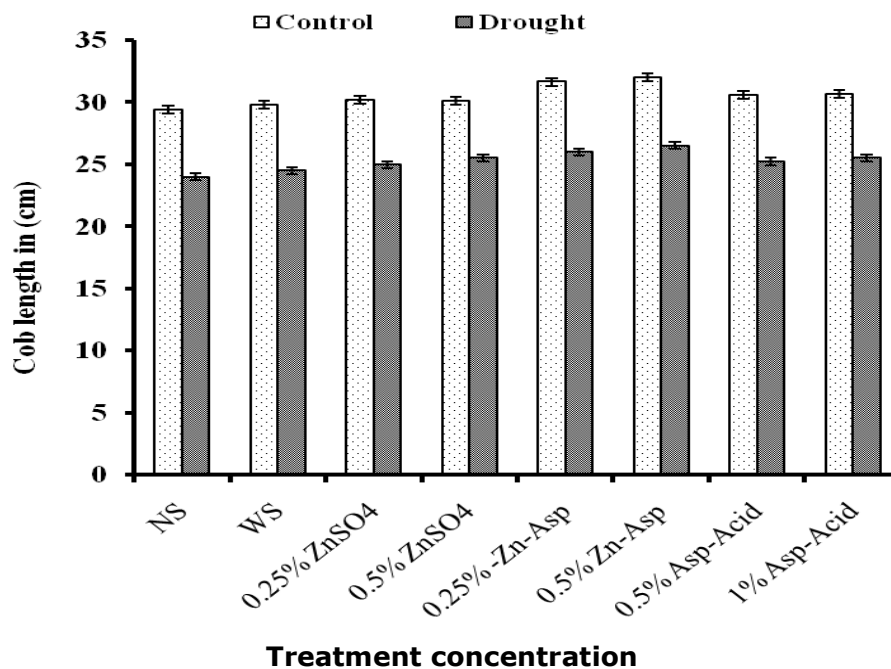


Figure 10. Increases in the cob lengths (cm) of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

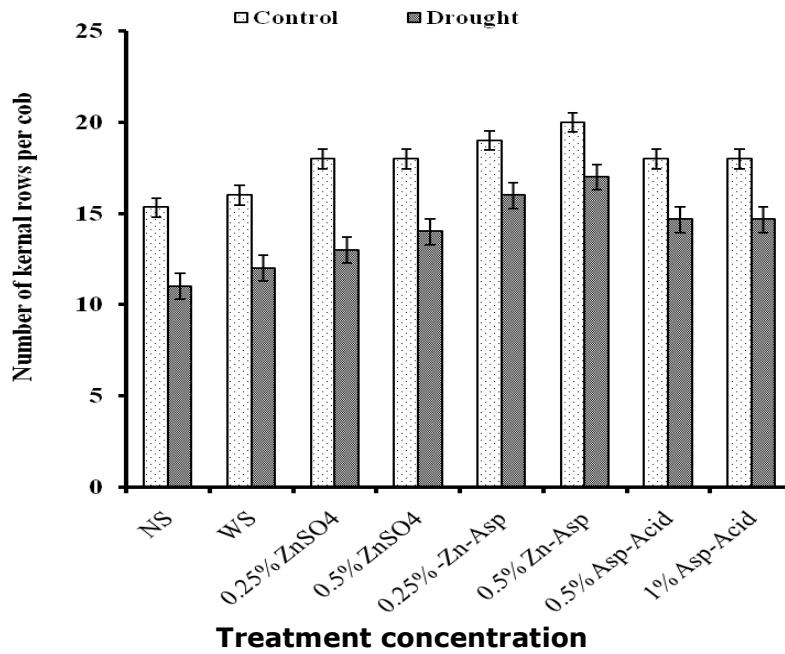


Figure 11. Increases in the numbers of kernel rows per cob of water-stressed maize plants the foliar application of Zn-Asp (mean ± SE; n = 3).

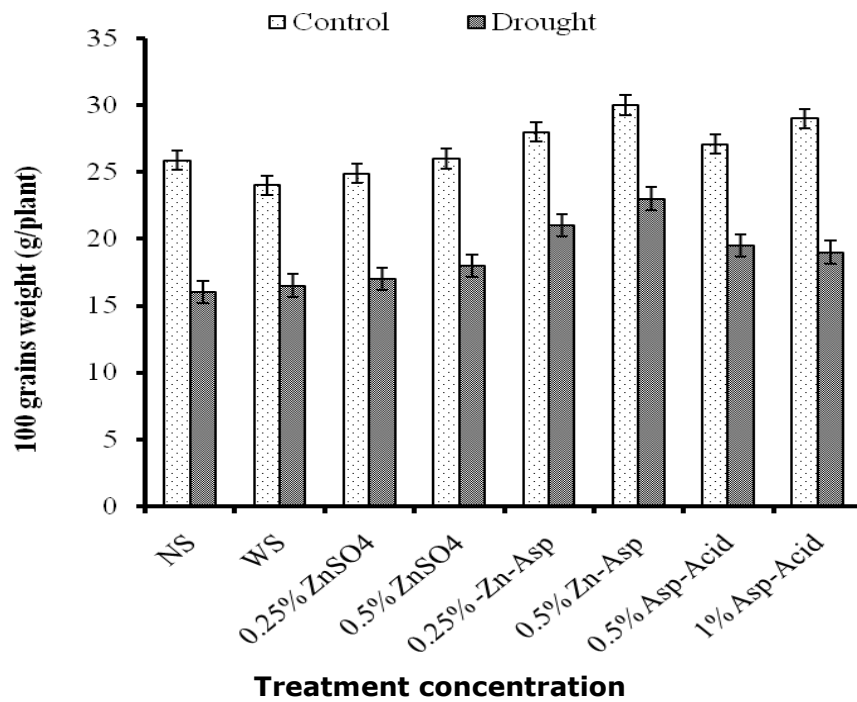


Figure 12. Increases in the 100-grain weight (g) of water-stressed maize plants under the foliar application of Zn-Asp (mean ± SE; n = 3).

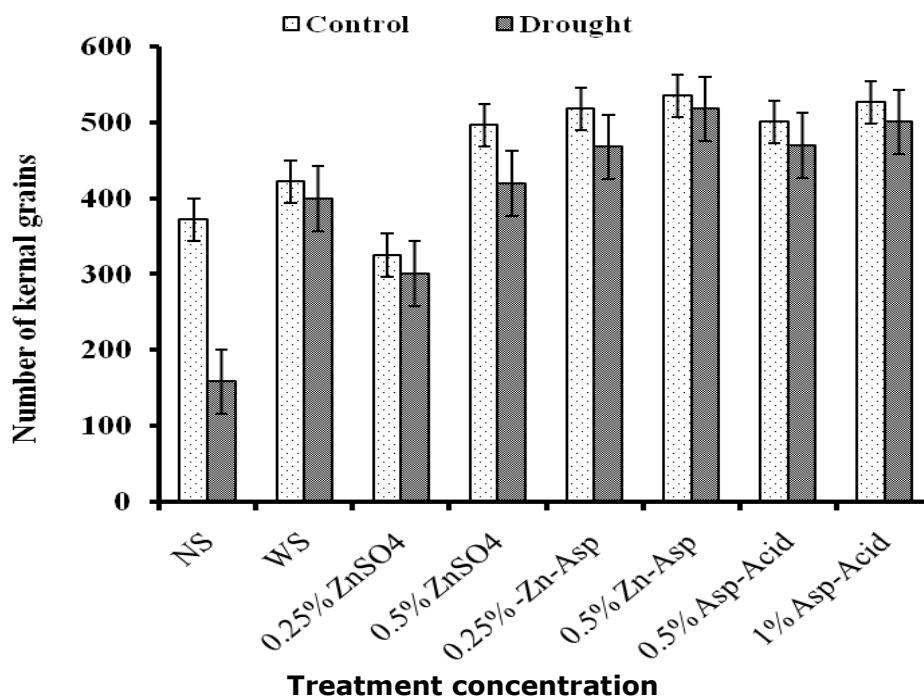


Figure 13. Increases in the numbers of kernels per cob of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; $n = 3$).

agronomic vigor. The details in the figure indicated that all treatments affected cob lengths. However, Zn-Asp treatments showed the most pronounced effect. The results obtained in this study favored the use of metal amino acid complexes for the biofortification of maize plants. The data given in Figure 11 are for the number of kernel rows within a fruit case. The graphic indicates that the number of kernel rows in a fruit case decreased in a water-stress environment. The foliar sprinkling of Zn-Asp proved to be the best short-term solution in optimizing the number of kernel rows within a fruit case. Although all the treatments affected the number of kernel rows within a fruit case, the increasing or decreasing effect was specific to specific treatments. Figure 12 presents the data on 100-kernel weight, which was calculated by separating the kernels from the cobs of maize plants. Water stress significantly reduced the

100-kernel weight due to an increase in fiber production rather than endospermic contents. As inferred in the current study, drought stress under various levels of treatments affected the kernel weights of maize plants. The best results were obtained with Zn-Asp treatments under stressed and controlled environments.

A water deficit environment resulted in a decrement in the number of kernel rows within a fruit case (Figure 13). Zn-Asp treatment significantly enhanced the number of grain rows; this effect thus caused an increase in the yield of maize crops (Table 1). The detailed data presented in Figure 14 proved that the number and length of crude fibers originating from maize kernels increased with treatment dose. The data showed that fiber contents increased under drought stress. Various treatments exerted different effects on the amount of fiber produced. However, among all

Table 1. Analysis of variance of various growth parameters, yield, and nutrients in the roots and shoots of water-stressed maize plants treated with foliar sprinkles of ZnSO₄ Zn-Asp, and L-Asp.

Source	d.f.	CL (cm)		NOKPC		NOKRC	
Stress	1	6.2262402*	0.0179	0.1034483 ns	0.7498	0.0856317 ns	0.7717
Treatment	7	1.3026506 ns	0.2808	1.364532 ns	0.2539	6.9434941***	0
Interaction							
Stress	7	2.1438394 ns	0.067	3.3875205**	0.0081	2.1315038 ns	0.0685
*Treatment							
Error	32						
Total	47						
Source	Df	100 GW (g)		SDW (g)		RDW (g)	
Stress	1	0.6928515 ns	0.4114	0.0011***	0.000	162.80141***	0
Treatment	7	1.0260913 ns	0.4324	1.1187528 ns	0.3758	0.00001*	0.021
Interaction							
Stress	7	0.009**	0.003	3.0882685*	0.0133	2.2371719 ns	0.057
*Treatment							
Error	32						
Total	47						
Source	Df	NOL		RFW (g)		SFW (g)	
Stress	1	1.1636364***	0.000	0.0004 *	0.020	0.013***	0.000
Treatment	7	1.7766234 ns	0.1266	2.1303669 ns	0.0686	0.360 ***	0.000
Interaction							
Stress	7	0.8727273 **	0.00412	1.2561615 ns	0.3027	1.4027393 ns	0.2384
*Treatment							
Error	32						
Total	47						
Source	Df	LOFL (cm)		WOFL (cm)		RL (cm)	
Stress	1	34.240741***	0	4.515625*	0.0414	20.375814***	0.0001
Treatment	7	1.457672 ns	0.2176	0.2120536 ns	0.9801	1.128505 ns	0.3702
Interaction							
Stress	7	6.2619048***	0.0001	1.7299107 ns	0.1372	2.3218605*	0.0492
*Treatment							
Error	32						
Total	47						
Source	Df	SL (cm)		KSC		KF	
Stress	1	0.2203623 ns	0.6419	286.54517***	0	7591.3279***	0
Treatment	7	0.7945425 ns	0.5974	5.5445616***	0.0003	13.118345***	0
Interaction							
Stress	7	2.9185639*	0.0177	2.5902711*	0.031	7.2242682***	0
*Treatment							
Error	32						
Total	47						
Source	Df	KOM		KIM		KPC	
Stress	1	3373.1765***	0	2167.0737***	0	507.00324***	0
Treatment	7	29.867947***	0	8.1280215***	0	15.904195***	0
Interaction							
Stress	7	30.837935***	0	6.4500396***	0.0001	3.6131184**	0.0056
*Treatment							
Error	32						
Total	47						

ns nonsignificant, d.f. degree of freedom, CL cob length, NOKPC number of kernels per cob, NOKRC number of kernel rows per cob, 100 GW (g) 100-grain weight in grams, SDW shoot dry weight, RDW root dry weight, NOL number of leaver per plant, RFW root fresh weight, SFW shoot fresh weight, LOFL length of flag leaf, WOFL width of flag leaf, RL root length, SL shoot length, KSC kernel starch contents, KF kernel fiber, KOM kernel organic matter, KIM kernel inorganic matter, KPC kernel protein contents

*, **, and ***=significant at the 0.05, 0.01, and 0.001 levels, respectively.

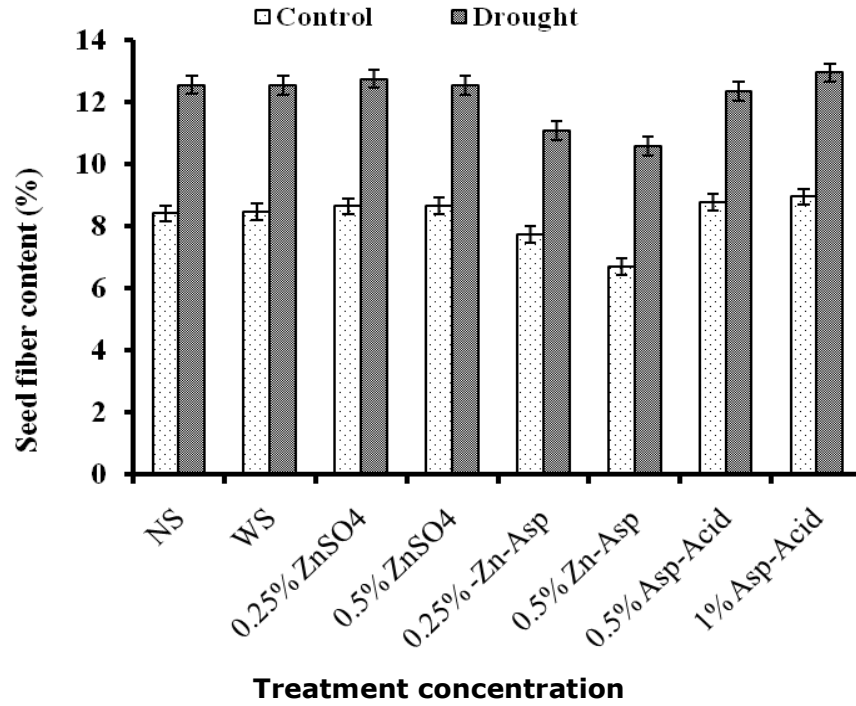


Figure 14. Reduction in the fiber contents of maize kernels upon foliar treatment with Zn-Asp (mean \pm SE; n = 3).

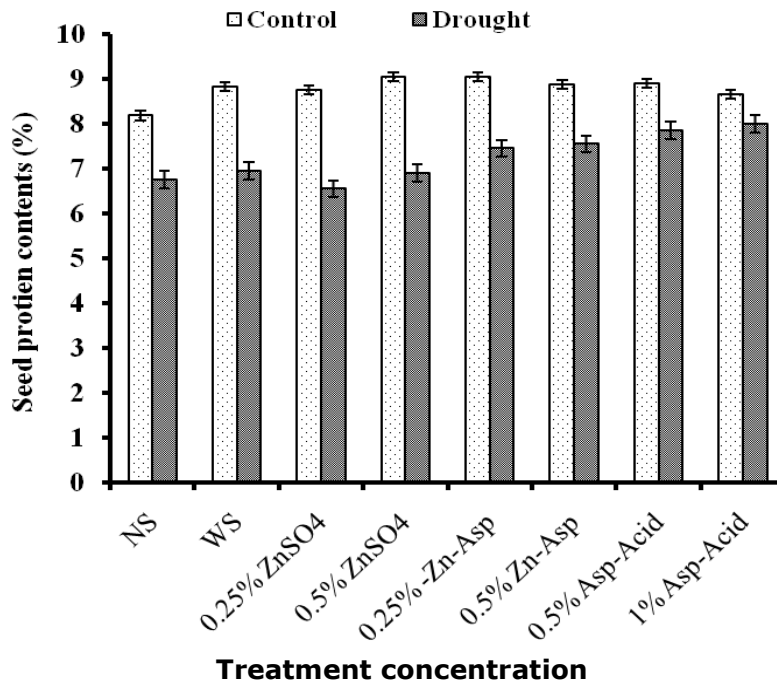


Figure 15. Increases in the percentages of protein contents in the rows of water-stressed maize plants under the foliar application of Zn-Asp and L-Asp (mean \pm SE; n = 3).

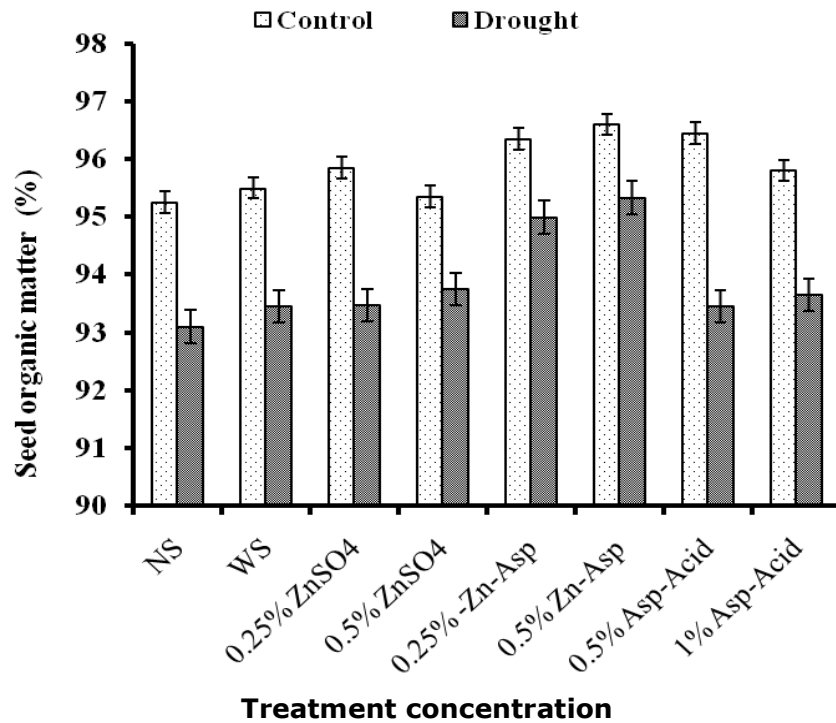


Figure 16. Percentages of the kernel organic matter of water-stressed maize plants under the foliar application of Zn-Asp (mean \pm SE; n = 3).

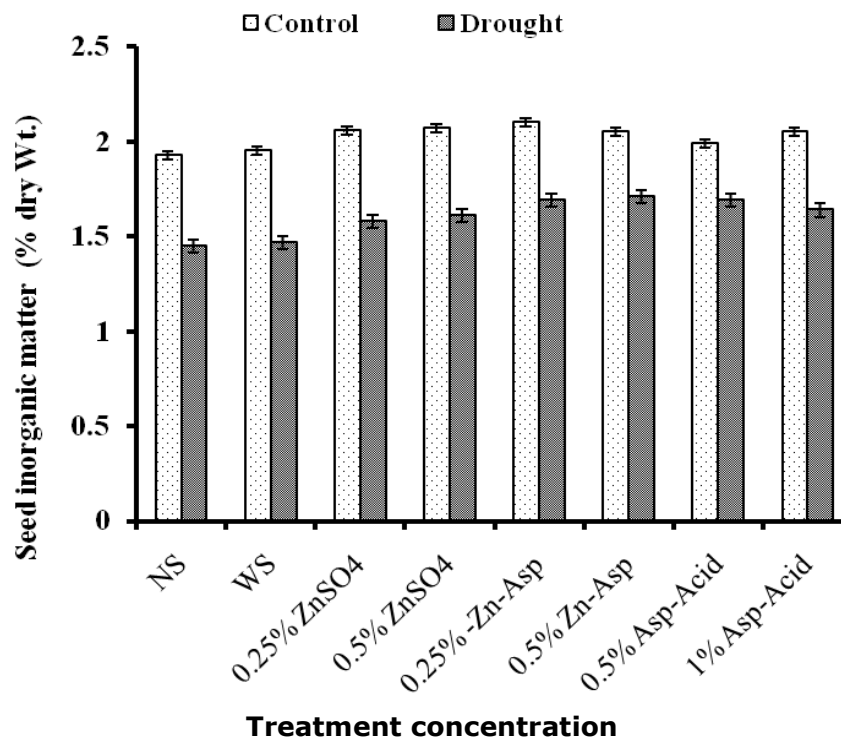


Figure 17. Increases in the total inorganic matter percentages of maize kernels under the foliar application of Zn-Asp (mean \pm SE; n = 3).

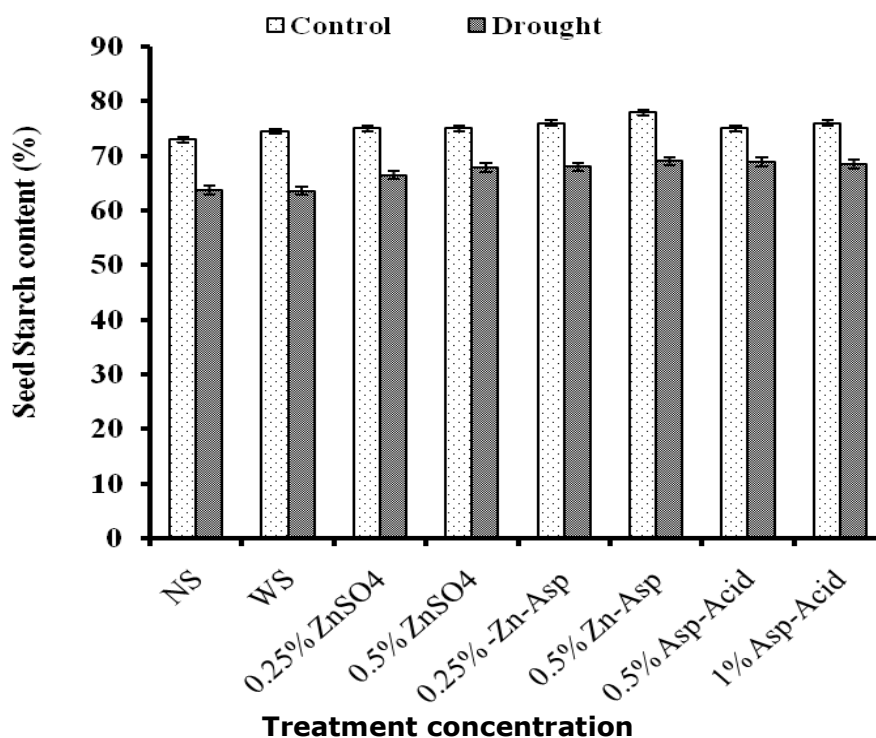


Figure 18. Increases in the percentages of the starch contents of maize kernels under the foliar application of Zn-Asp (mean \pm SE; $n = 3$).

treatments, L-Asp treatment had the best effect on decreasing the number of fibers. The results also supported the fact that Zn-Asp mediated losses in kernel fibers. The data given in Figure 15 and Table 1 demonstrate that water stress decreased the protein contents of maize kernels. Endospermic protein content increased under exogenous ZnSO₄, Zn-Asp, and L-Asp treatments, which modulated the hazardous effects of drought on maize plants. These data indicated that the Zn-Asp treatment was superior to the other treatments and resulted in the maximum enhancement in kernel protein contents. The results obtained with L-Asp treatments were also encouraging.

The organic and inorganic contents of maize kernel endosperms decreased under water deficit conditions (Figures 16 and 17). Drought induced the enhanced production of the crude fiber, thereby reducing kernel endospermic contents. Compared with all other treatments, the exogenous application of Zn-Asp as a

foliar sprinkle significantly helped enhanced the kernel's endospermic contents in terms of organic and inorganic reserves (Table 1). Figure 18 provides the data related to seed starch contents. The figure depicts that drought decreased starch food reserves in the maize kernels under water deficit conditions. Compared with other treatments, treatment with various levels of Zn-Asp enhanced the stored food reserves of starch.

DISCUSSION

Food security, availability, and hygienic quality are global challenges that forever exceed the population of the world. Climate changes and water scarcity trigger this menace by posing further threats to maize crops, culminating in low yield production. The changes in weather patterns and precipitation quantity also compromise the yields of maize, wheat, and millet (Thornton *et al.*, 2018). Low

yield or other constraints in the nutritive profile of maize may be mitigated or addressed by using transgenic varieties of maize crops; however, the introduction of genetically modified organisms (GMOs) threatens ecosystem integrity and brings biosafety-related risks (Bawa and Anilakumar, 2013), as well as poses risks to health and other ecosystem services. Therefore, the application of osmolytes in the form of mineral-chelated amino acids as a foliar spray is the superior option for the farmers to increase their yield and agronomic values of maize and other crops. Metal chelates with amino acids are proving their worth, and although conventional salt treatments increase yield in Ajwain (*Trachyspermum ammi* L.) to some extent, they are capable of creating a saline environment (Ali *et al.*, 2020).

The current study focused on various nutrient acquisition patterns via grain endospermic analysis involving seed starch, organic contents, inorganic matter, and total protein analysis. The present work found that all of the attributes related to kernel nutritional contents were affected in a water-scarce environment. A decrease in seed protein contents has also been reported in various occasions, such as those in the works of Mohsin *et al.* (2014) and Aziz *et al.* (2019). The amount of crude fiber was also studied as part of the analysis. Drought decreased all endospermic contents and increased crude fiber amounts. These results were in correspondence with previous results (Ali and Ashraf, 2011). The present work highlighted that the foliar sprinkling of synthesized mineral amino acid chelates (Zn-Asp) decreased the kernel crude fiber amount and enhanced grain endospermic values by increasing protein, starch, organic and inorganic contents, thus improving nutrient acquisition by maize plants. Treatments in the form of zinc amino acids chelates are organic, and the worth of organic treatments has been reported by several studies, including the study of Jjagwe *et al.* (2020). The increase in kernel protein values might be attributed to the increased synthesis of asp family amino acids, such as threonine,

isoleucine, methionine, and lysine (Jander and Joshi, 2009). An increase in the free amino acid pool later serves as the building block of various endospermic proteins. Notably, the results obtained under Zn-Asp-mediated treatments are better than those obtained under various levels of L-Asp treatments because chelated forms enable the improved absorption and utilization of amino acids (Datir *et al.*, 2012). Compared with orthodox salt treatments, amino acids serve as important candidates in delivering the metal cations required for active utilization by plants and help as superior catalysts in provoking risk-free drought mitigation (Ali *et al.*, 2020). Agronomic attributes, such as the number of kernels per cob, number of kernel rows per cob, length of fruit case, and 100-grain weight, were also studied in the present work. The present findings indicated a reduction in the number of kernels rows per cob and subsequently affected the number of kernels per cob under a drought-stressed environment. Foliar sprinkling with Zn-Asp ameliorated the drought-induced reduction in the yield of kernels by improving the number of kernel rows per cob and ultimately enhancing yield. The lengths of fruit cases produced by maize plants under drought were also analyzed and were shown to decrease in a water-scarce environment. The length of the cob is directly related to crop yield. Exogenous treatment with Zn-Asp positively affected cob length and promoted increased kernel yield as compared with all other treatments, again proving that metals chelated with amino acids were the superior treatment. Previous research works involving the application of zinc foliar sprays in maize and other cereals have provided improved results (Mohsin *et al.*, 2014). The use of metal chelates with amino acid solutions produced a far better enhancement in the yield of maize than the use of conventional salt treatments (Ghasemi *et al.*, 2013). The yield graphs of cereal and maize have been explored on several occasions, and the efficacy of zinc foliar spraying has proven its worth in

increasing 100-grain weight and the number of grains per spike. Spike length and plant height have been reported by Yavas and Unay (2016). Similar results with zinc foliar spray in the form of Zn-Asp were found in the current study.

In this study, 100-kernel weight was analyzed after separating the kernels from the cobs of random samples. The kernels were washed, air-dried under sunlight, and then weighed. Drought resulted in a decrement in 100-grain weight in line with previous results (Shahri *et al.*, 2012; Mosavifeyzabadi *et al.*, 2013). Zn-Asp application increased 100-kernel weight and alleviated drought-induced reductions in crop yield. The flag leaves of maize plants subjected to drought stress were studied. Lamina characteristics in terms of length and width were recorded in cm² and found to have decreased under water stress. These results favored the previous results of Ali *et al.* (2011). The foliar sprinkling of Zn-Asp increased leaf length and width. The numbers of leaves per plant reduced under drought stress. Exogenous Zn-Asp treatments proved to be beneficial in maximizing the food factories of the maize plants under water stress. Growth parameters regarding shoot fresh and dry weights, root fresh and dry weights, shoot length, and root length were also measured. The results showed reductions in all of these mentioned parameters under the influence of water stress and followed previously reported outcomes (Ali *et al.*, 2011; Hajibabae *et al.*, 2012). The decrement in shoot length might be attributed to the shortening of internodes (Ali *et al.*, 2011). All treatments were compared, and Zn-Asp treatments were inferred to be better than conventional salt treatments in optimizing all of the studied growth parameters. The treatments also proved to ameliorate drought stress.

CONCLUSIONS

On the basis of all the agronomic and growth parameters, Zn-Asp was inferred

to be more effective than all other conventional treatments, including the traditional use of ZnSO₄, and showed that the conventional use of zinc salts should be discouraged. This study proved that Zn-Asp and Zn-Asp amino acid-mediated foliar usage produced better results and will be better than GMO practices. The current study recommends Zn-Asp foliar application in maize crops to increase yield, nutritive quality, and mineral constituents to meet the global food challenges of human beings, particularly in developing countries.

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REFERENCES

- Ali Q, Ali S, El-Esawi MA, Rizwan M, Azeem M, Hussain AI, Wijaya L (2020). Foliar spray of Fe-Asp confers better drought tolerance in sunflower as compared with FeSO₄: Yield traits, osmotic adjustment, and antioxidative defense mechanisms. *Biomolecules* 10(9): 1217-1245.
- Ali Q, Ashraf M (2011). Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (*Zea mays* L.) under water deficit conditions. *Environ. Exp. Bot.* 2: 249-259.
- Ali Q, Ashraf M (2011). Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations, and oxidative defense mechanism. *J. Agron. Crop Sci.* 4: 258-271.
- Ali Z, Basra SM, Munir HA, Mahmood AR, Yousaf SH (2011). Mitigation of drought stress in maize by natural and synthetic growth promoters. *J. Agric. Soc. Sci.* 7(2): 56-62.
- Anjum S, Wang L, Farooq M, Hussain M, Xue L, Zou C (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic

- antioxidants and leaf gas exchange. *J. Agron. Crop Sci.* 3: 177-185.
- Anonymous, (2000). Approved methods of the American Association of Cereal Chemists, 10th ed. AACC, St. Paul, MN, USA.
- Artlip TS, Madison JT, Setter TL (1995). Water deficit in developing endosperm of maize: cell division and nuclear DNA endoreduplication. *Plant Cell Environ.* 18: 1034-1040.
- Aslam M, Zeeshan M, Maqbool MA, Farid B (2014). Assessment of drought tolerance in maize (*Zea May L.*) genotypes at early growth stages by using principle component and biplot analysis. *The Experiment.* 1: 1943-1951.
- Aziz MZ, Yaseen M, Abbas T, Naveed M, Mustafa A, Hamid Y, Saeed Q, Ming X, (2019). Factors influencing hybrid maize farmers' risk attitudes and their perceptions in Punjab Province, Pakistan. *J. Integr. Agric.* 6: 1369-1378.
- Babaeian M, Tavassoli A, Ghanbari A, Esmaeilian Y, Fahimifard M (2011). Effects of foliar micronutrient application on osmotic adjustments, grain yield, and yield components in sunflower (Alstar cultivar) under water stress at three stages. *Afr. J. Agric. Res.* 5: 1204-1208.
- Bawa AS, Anilakumar KR (2013). Genetically modified foods: safety, risks, and public concerns-a review. *J. Food Sci. Technol.* 50(6): 1035-1046.
- Datir RB, Apparao BJ, Laware SL (2012). Application of amino acid chelated micronutrients for enhancing growth and productivity in chili (*Capsicum annum L.*). *Plant Sci. Feed.* 7: 100-105
- Dewis J, Freitas F, (1970). Physical and chemical methods of soil and water analysis. *FAO Soils Bulletin.* (10).
- Ehsanullah D, Tariq A, Randhawa M, Anjum S, Nadeem M, Naeem M (2015). Exploring the role of zinc in maize (*Zea Mays L.*) through the soil and foliar application. *Univers. J. Agric. Res.* 3: 69-75.
- Fang Y, Wang L, Xin Z, Zhao L, An X, Hu Q (2008). Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. *J. Agric. Food Chem.* 6: 2079-2084.
- Ghasemi S, Khoshgoftarmanesh A, Afyuni M, Hadadzadeh H (2013). The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat. *Eur. J. Agron.* 45: 68-74.
- Gornall AG, Bardawill CJ, David MN (1949). Determination of serum protein by means biuret reaction. *J. Biol. Chem.* 2: 751-766.
- Hadi MHRS, Bazargani P, Darzi MT (2013). Effects of irrigation treatment and zinc foliar application on yield and yield components of chickpea (*Cicer arietinum*). *Int. J. Farm Alli. Sci.* 19: 720-724.
- Hajibabae M, Azizi F, Zargari K (2012). Effect of drought stress on some morphological, physiological, and agronomic traits in various foliage corn hybrids. *Am. Eurasian. J. Agric. Environ. Sci.* 7:890-896
- Hart TS (1928). The diagnosis of chronic myocarditis without cardiac insufficiency. *Am. Heart J.* 3: 430-432.
- Jander G, Joshi V (2009). Aspartate-Derived Amino Acid Biosynthesis in *Arabidopsis thaliana*. *The Arabidopsis Book* 7: e0121.
- Jjagwe J, Chelimo K, Karungi J, Komakech AJ, Lederer J (2020). Comparative performance of organic fertilizers in maize (*Zea mays L.*) growth, yield, and economic results. *Agron.* 10(1): 69.
- Khan R, Gul S, Hamayun M, Shah M, Sayyed A, Ismail Begum HA, Gul H (2016). Effect of foliar application of zinc and manganese on growth and some biochemical constituents of *Brassica Juncea* grown under water stress. *Am. Eurasian. J. Agric. Environ. Sci.* 16(5): 984-997.
- Kleckerova A, Sobrova P, Krystofova O, Sochor J , Zitka OM , Babula P , Adam V, Docekalova H , Kizek H, (2011). Cadmium (II) and zinc (II) ions effects on maize plants revealed by spectroscopy and electrochemistry. *Int. J. Electrochem. Sci.* 6: 6011-6031.
- Leu M (1991). U.S. Patent No. 5,061,815. Washington, DC: U.S. Patent and trademark office.
- Mohsin AU, Ahmad AUH, Farooq M, Ullah S (2014). Influence of zinc application through seed treatment and foliar spray on growth, productivity and grain quality of hybrid maize. *J. Anim. Plant Sci.* 5: 1494-1503.
- Mosavifeyzabad S, Vazin F, Hassanzadehdelouei M (2013). Effects

- of nitrogen and zinc spray on yield of corn (*Zea mays* L.) in drought stress. *Cercet. Agron. Mold.* 46(3): 29-38
- Nasri M (2015). Effect of zinc foliar, potassium elements, and irrigation terms of concentrations of nitrogen, phosphorus, and potassium in grain and some quantitative characteristics of corn (KSC704). *Int. J. Biosci.* 2: 15-23.
- Ram H, Rashid A, Zhang W, Duarte A, Phattarakul N, Simunji S (2016). Biofortification of wheat, rice, and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. *Plant Soil.* 1(2): 389-401.
- Sangeetha N, Premakumari S (2010). Effect of micronutrient supplementation on the nutritional and immune status of school-going children with iron deficiency anemia. *Int. J. Nutr. Metab.* 2:45-55.
- Shahri ZB, Zamani GR, Sayyari-Zahan, MH (2012). Effect of drought stress and zinc sulfate on the yield and some physiological characteristics of sunflower (*Helianthus annuus* L.). *Adv. Environ. Biol.* 2: 518-525.
- Shankar B (2000). The science of chemical discovery. Probing the unknown with new technologies. *Drug Discov. Today.* 5: 533-534.
- Stein AJ (2010). Global impacts of human mineral malnutrition. *Plant soil.* 335(1): 133-154.
- Sullivan GT (1935). Estimation of starch. *Ind. Eng. Chem. Anal. Ed.* 5: 311- 314.
- Thornton P, Dinesh D, Cramer L, Loboguerrero AM, Campbell B (2018). Agriculture in a changing climate: Keeping our cool in the face of the hothouse. *Outlook Agric.* 47(4): 283-290.
- Yavas, Unay A, (2016). Effects of zinc and salicylic acid on wheat under drought stress. *J. Anim. Plant Sci.* 26(4): 1012-1018.
- Zhang Y, Sun Y, Ye Y, Karim M R, Xue Y, Yan P, Meng Q, Cui Z, Cakmak I, Zhang F, Zou C (2012). Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Res.* 125: 1-7.