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Foliar Application of Zinc Improves Agronomical and Quality Parameters and Biofortification of Cowpea (*Vigna sinensis*) under Deficit Irrigation

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Abstract: Due to climate changes, we encounter irregular and low rainfall. It is important to effectively use groundwater and to select crops that can be grown with deficit irrigation in the summer period. Restricted irrigation reduces water consumption but it may cause losses in terms of yield and quality. Different agronomic practices can be used to minimize these losses. One of these practices is the application of foliar zinc fertilizer. In previous studies, zinc application was found to increase the bioavailability of cowpea grain. In this study, the effects of the application of zinc fertilizer on yield, some yield components, physiological traits, and grain quality characteristics of three different cowpea genotypes (Akkız, Karagöz, and a Local variety) were investigated under full (100%) and deficit (50%) irrigation. The field experiment was conducted using a randomized complete block split–split plot design with irrigation rates (100% and 50%) and foliar zinc application (0 and 60 kg ha^{-1}) with three replicates used each season (2020 and 2021 growing seasons of cowpea) in the field crops trial fields of the Aydin Adnan Menderes University, at the Faculty of Agriculture, located in the western region of Turkey. Yield and quality characteristics such as grain yield, some yield components, grain protein content, grain mineral matter content, and grain amino acid content were measured. According to the data obtained, a 40% yield reduction was observed under restricted irrigation in the first year of the study. It was determined that zinc application under restricted irrigation increased the yield by approximately 10%. The second-year results found that the amount of essential amino acids such as histidine, phenylalanine, valine, and lysine increased with the zinc application. This study highlights that deficit irrigation conditions caused stress in the plant and caused losses in the yield and quality. Still, the severity of this stress was reduced by foliar zinc application, and it was determined that it positively affected grain yield and bioavailability in cowpea.

Keywords: cowpea; deficit irrigation; zinc; amino acid; biofortification

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1. Introduction

Cowpea is a legume that is grown in tropical and subtropical areas, especially in Africa. Of the approximately 15 million ha of cultivation area in the world, 14.8 million ha are located on the African continent, and 8.6 million tons of cowpea production of 8.9 million tons are obtained from the African continent [1]. Cowpeas can adapt to low fertile soils and arid conditions. In this way, they can adapt to high temperatures and low rainfall due to recent climate changes.

The Aegean Region, which has a Mediterranean climate with mild and rainy winters and dry and hot summers, is the region with the largest cultivation area in Turkey.

Drought is one of the most important abiotic stress factors that negatively affects grain yield [2,3]. The adaptation of plants to water deficits and survival may occur with different responses [4,5]. Many morphological, physiological, and molecular changes may occur in drought stress [6,7]. The most important physiological responses are decreased chlorophyll content, photosynthesis, and evaporation [8–10]. Among legumes, the cowpea is a plant that can tolerate these adverse conditions [5,9–12]. In addition, the cowpea experiences little change in its leaf water potential through osmotic adjustment under drought conditions [13]. Because of these characteristics, it is a product that can be utilized, especially in Mediterranean climate conditions where water scarcity is caused by high temperatures, irregular and low rainfall amounts, and global climate changes [13,14].

In recent years, longer drought periods and higher maximum temperature values have been observed in Mediterranean climate conditions compared to previous years. Climate change is felt more effectively in the region. Studies have predicted that the earth's average temperature will increase by 2–4.5 °C [15]. Long temperature periods and extremely hot conditions in semi-arid regions cause water scarcity in crop production [16]. Water scarcity negatively affects the plant's growth and development and thus, significantly reduces the grain yield. Under drought conditions, water is the most important factor determining the yield. It has been observed that water scarcity, especially during flowering and pod formation periods, negatively affects a plant's yield by causing a decrease in the number of pods in the plant due to a decrease in the flowering rate and inhibition of nitrogen fixation [17–19].

Cowpea plants tend to turn their leaves vertically to reduce the amount of light coming in and lessen the effect of sunlight on the leaves when there is drought in the field [20]. In this way, cowpea, unlike many other plants, can survive the vegetative period under drought conditions. This is achieved using as much soil water as possible by deepening the roots of some varieties that can handle not getting enough water [21].

One of the most important characteristics of the cowpea is that it consists of plant parts with high nutrient content [22–25]. The grain is rich in protein content (23–32%) and amino acids such as lysine (427 mg g^{-1} N) and tryptophan (68 mg g^{-1} N). The grain contains protein, fiber, and minerals such as zinc, iron, and magnesium [26]. The mineral substances contained in the cowpea gain importance in meeting the nutritional needs of the rapidly increasing world population. Zinc is the most important element associated with human health, especially in developing countries [27]. In previous studies, while the grain yield and grain zinc content increased with the zinc fertilizer application in cereals [28–31], it was also determined that the amount of zinc needed for nutrition was met [28,29,32–34]. It is known that the tryptophan content of plants decreases, protein synthesis stops, and free amino acids accumulate in a zinc deficiency. This situation naturally causes yield and quality losses. The zinc intake of plants may affect their sensitivity to drought stress. Zinc is involved in detoxifying reactive oxygen species (ROS) and it is also important for reducing the production of free radicals by superoxide radical-producing enzymes [35]. Under drought stress, the plant may die due to the production of reactive oxygen species during photosynthesis [36]. In addition, in zinc deficiency, photosynthetic carbon turnover is impaired, oxygen production and activity are reduced, and sensitivity to drought increases [37].

Zinc is an essential element in human nutrition [38]. It is known that 17% of the world's population suffers from inadequate zinc intake [39]. Many people do not receive adequate amounts of zinc in their diets [31,40]. Biofortification is the elimination of deficiencies in humans by increasing the concentrations of vitamins and minerals, which are widely deficient in society, in the products most consumed by society [41]. Increasing the amount of zinc in grains through fertilization has been observed in cereals and other crops [31,42]. Biofortification aims to improve zinc fertilizers' application and develop crop varieties that take up more zinc from outside and accumulate it in the edible parts [31]. The bioavailability of zinc varies between 18 and 34%. Compensating for this low bioavailability is important for biofortification [39], because zinc application is associated with cowpea's nitrogen metabolism and protein content [43]. Thus, zinc biofortification increases the zinc content in the cowpea grain, which is beneficial for human nutrition. As a result, exposure of the plant to zinc deficiency during drought may cause the drought sensitivity in the plant to become more pronounced.

Zinc is given to plants in two ways, through the soil, and through foliar application. Foliar application minimizes groundwater pollution [44–46]. In addition, zinc uptake from the soil is deficient in soils with high pH [47]. For these reasons, the foliar application of zinc becomes more attractive.

This study will provide information on deficit irrigation and the arid conditions that are expected to be experienced in many countries. The effect of zinc fertilizer applied under these conditions on grain yield and nutritional value will be determined. Some previous studies have been carried out on this subject, but detailed quality analyses, such as grain amino acid measurements in cowpea, have been lacking. This study aimed to determine the effects of foliar zinc application on grain yield, grain mineral matter content, and grain amino acid content under deficit irrigation conditions.

2. Materials and Methods

The study was conducted in 2020 and 2021 in the experimental field (27°51′ E, 37°51′ N, altitude 50 m) of the Department of Field Crops, at the Faculty of Agriculture, Adnan Menderes University, Aydin/Turkey.

According to Table 1, it has a sandy loamy characteristic in soil properties. The table shows that soil organic matter and potassium are low, while phosphorus and calcium are high. According to the results of soil analyses, it is observed that soil pH is high.

Table 1. Soil properties of the trial site.

Soil Texture			pH	Organic Matter (%)	Phosphorus (ppm)	Potassium (ppm)	Calcium (ppm)	Sodium (ppm)
Sand (%)	Silt (%)	Clay (%)						
72	16.7	11.3	8.0	2.0	21	176	2978	101
Sandy loam			High	Low	High	Low	High	Low

Figure 1 shows the experimental years' average temperature and total precipitation values. It can be observed that the average temperature values exceeded the long-term average in both years. In the first year of the experiment, the average temperature in June exceeded all previous years. Notably, the average temperature in July and August of the second year was higher than in the other years. When the total rainfall values were analyzed, it was determined that the total rainfall in the trial years was lower than the total of the long years, and the lowest rainfall was obtained from the second year of the trial.

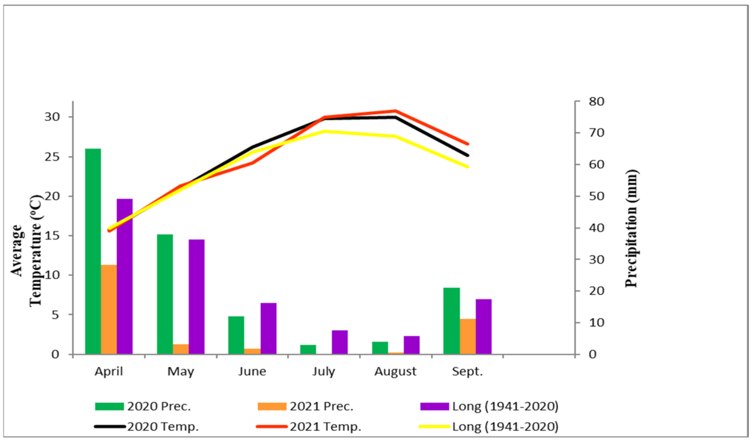

Figure 1. Climatic data for the years of the experiment.

Three cowpea varieties (Karagöz, Akkız, and a local variety) were used in the experiment. Akkız and Karagöz varieties are registered in Turkey; while the local variety is not a registered variety, it is a variety that farmers in the region have been planting in their fields for many years to meet their consumption. The local variety was supplied by farmers producing in the region because it is not commercially produced. Two varieties (Akkız and Karagöz) were widely cultivated in the region. Local variety was supplied by farmers producing in the region. The experiment was conducted under normal irrigation (100%) and restricted irrigation (50%) conditions, with and without zinc (kg Zn ha^{-1}, $ZnSO_4$ as foliar spraying). Irrigation factor was used as the main plot, cultivar as sub-plot, and Zn treatments as split–split plot.

Before sowing, 40 kg ha^{-1} of nitrogen, phosphorus, and potassium were applied as 15-15-15 fertilizer. The weeds were controlled twice, at the beginning and post-flowering stages, by hand. After sowing, nitrogen fertilization (20 kg N ha^{-1}) was applied at a 10–15 cm plant height.

Sowing was performed by regina model sowing machine manufactured by the Italian company Maschio-Gaspardo on May 9 of the first year and on 10 May of the second year. Each plot was 70 cm wide with 6 rows (5 m long). The randomized complete block split-split plot design with three replicates was used each season. Before sowing, 40 kg ha^{-1} of nitrogen, phosphorus, and potassium were applied as 15-15-15 fertilizer. The weeds were controlled twice, at the beginning and post-flowering stages, by hand. After sowing, nitrogen fertilization (20 kg N ha^{-1}) was applied at a 10–15 cm plant height. Foliar zinc applications were applied at the beginning of the flowering period. Foliar treatments were applied with a portable, hand-held field plot sprayer at 250 kPa pressure using a water carrier volume of 400 L·ha^{-1}.

The irrigation water to be applied to the plots was calculated based on the cumulative evaporation amount from the class A evaporation container multiplied (located near the experimental field) by different coefficients. In the study, 2 irrigation doses (full irrigation and deficit irrigation) were determined, in which different levels of cumulative evaporation were applied.

These are as follows:

1. Full irrigation (100%): Irrigation water applied for 7 days as much as possible for the cumulative evaporation measured with a screened US Weather Bureau Class A pan located at the meteorological station near the experimental field.
2. Deficit irrigation (50%): It was established that irrigation water was applied as 50% of full irrigation.

The equation given in [48] was utilized to apply to the plots.

$$I = Kpc \cdot Ep \cdot P \cdot A$$

I = amount of irrigation water to be applied to the plot (L); Kpc = evaporation container coefficient 100%; Ep = cumulative evaporation amount (mm); P = Plant cover (%); and A = Plot area (m^2) and drip irrigation method was used [49].

In the study, irrigation started when flowers were seen on the plants, and irrigation was carried out with a drip irrigation system every 7 days.

Plant height, number of pods per plant, number of grains per pod, leaf area index, leaf chlorophyll content, 100 grain weight, grain yield, grain protein content, grain mineral content, and grain amino acid content were measured.

Approximately 500 g samples were dried at 70 °C for 48 h. 100-seed weight (g) was determined by counting from dry seeds and weighing four replicates of 100 seeds.

Seed yield was calculated from 11.4 m^2 (4 rows × 4 m × 0.7 m) harvested area in each plot (kg da^{-1}).

Seed protein content (%), seed ash content (%), seed fiber content (%), and seed oil content (%) were measured by using Near Infrared Reflected SpectroscopyNIRS method Bruker MPA, German at Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center (ADÜ-TARBİYOMER) [50].

The seed mineral content (mg kg^{-1}) was determined by atomic absorption spectrometry after ashing samples at 550 °C and dissolving ash in 3.3% HC1 [51] (Aydın Adnan

Menderes University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Laboratory).

Seed amino acid content (g/100 g) and cowpea dry grains were ground after harvest, and random samples were prepared from each plot. A total of seventeen amino acids were measured.Seed. Amino acid analyses (high-performance liquid chromatography or high-pressure liquid chromatography, HPLC) were performed at Research and Application Center of Drug Development and Pharmacokinetics Laboratory in Ege University.

Statistical Analysis

Statistical analyses were conducted using JMP Statistical Analysis Software (version Pro 13) in the split–split plot design. The experimental data about each study parameter were subjected to statistical analysis using the variance analysis technique, and their significance was tested by the "F" test (Steel and Torrie, 1980). When differences were found in ANOVA, means were compared using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$.

3. Results

The heat map (Figure 2) was improved, including the averages of agronomic traits of three cowpea cultivars treated with zinc fertilizer under different irrigation conditions for two years of the experiment.

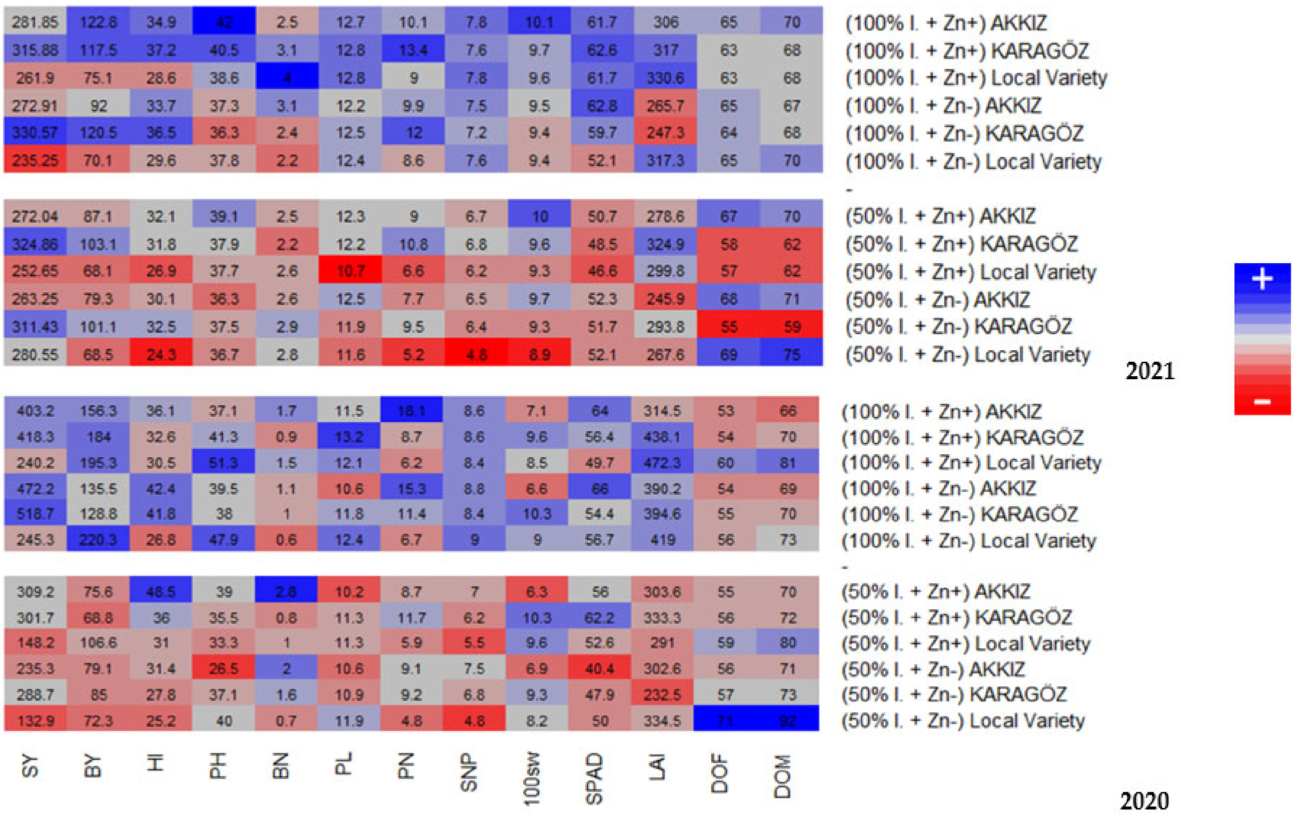

Figure 2. Mean values of grain yield and yield components.

Based on the results of the analysis of variance (Table 2), there was a significant difference between the years, so the averages for each year were evaluated separately. In the same way, the results of the analysis of variance showed that the mean values of the grain yield, biological yield, plant height, pod length, number of pods, number of grains in pods, 100 grain weight, and leaf area index were significantly better than those under deficit irrigation conditions in the first year.

Table 2. ANOVA of grain yield and yield components.

SOV	Df	Seed Yield		BY		HI		Plant H.		BN		Pod Lenght		PN	
Years	1	*		*		*		*		*		*		*	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I	1	*	-	*	*	-	-	-	-	-	-	-	-	-	-
Zn	1	-	-	*	-	*	-	-	-	-	-	-	-	-	-
C	2	*	-	*	*	*	*	*	-	-	-	-	-	*	-
I*Zn	2	-	-	-	-	*	-	-	*	-	*	*	-	-	-
I*C	2	-	-	*	-	-	-	-	*	-	-	*	*	*	*
Zn*C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I*Zn*C	11	-	*	*	-	-	-	*	-	-	-	-	-	*	-

SOV	Df	Seed Number		100 Seed Weight		SPAD		LAI		DOS		DOM	
Years	1	*		*		*		*		*		*	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I	1	-	-	-	-	-	*	*	-	-	-	-	-
Zn	1	-	-	-	-	-	-	-	-		-	-	-
C	2	*	-	*	-	-	-	-	-	*	-	*	-
I*Zn	2	-	-	-	-	*	-	-	-	-	-	-	
I*C	2	*	*	-	*	-	-	-	-	-	-	-	*
Zn*C	2	-	-	-		-	-	-	-	-	-	-	*
I*Zn*C	11	-	-	-	-	-	-	*	-	-	*	*	-

SY: seed yield, BY: biological yield, HI: harvest index, PH: plant height, BN: branch number, PL: pod length, PN: pod number, SNP: number of seeds per pod, 100 SW: 100 seed weight, SPAD: chlorophyll meter, LAI: leaf area index, DOF: days of flowering, and DOM: days of maturity. (*; significant at 5% probability level *: significant -: nonsignificant).

The first-year irrigation dose × zinc and variety interactions were significant for a plant's height. While 51.30 cm of the plant's height was obtained under full irrigation conditions, 26.51 cm was observed under restricted irrigation conditions. It was concluded that a limitation in the irrigation dose significantly decreased the plant height. Similarly, in our study, the plant heights were ranked as local variety > Karagöz > Akkız at a statistically significant level. The plant height performance of the local variety was observed to be higher than the other two varieties. In the second year of our study, irrigation dose × zinc and variety × irrigation interactions were significant regarding the plant's height. The difference between zinc application (40.37 cm) under full irrigation conditions and plant height (36.84 cm) of zinc-free plots under deficit irrigation conditions was significant. It was concluded that the response of plant height to zinc fertilization with full irrigation was positive. When the variety × irrigation interaction was analyzed, the difference between irrigation doses was insignificant in Karagöz and local varieties. In contrast, the difference between both irrigation doses was significant in the Akkız variety. Therefore, weobserved that deficit irrigation significantly affected the Akkız variety.

In the first year, the interactions did not make a difference in the number of side branches. However, in the second year, the irrigation dose × zinc interaction made a difference in the number of side branches, and the plots with a zinc application and full irrigation had the most side branches (3.23).

In the first year, there was a significant interaction between the irrigation dose, zinc level, and variety in terms of the number of pods. Under conditions where there was not enough water, the number of pods went down by a lot. The number of pods of Akkız (8.88) and the local variety (5.20) decreased significantly under restricted irrigation conditions. Zinc application was not effective under full irrigation conditions, but it positively affected Karagöz and local varieties under deficit irrigation conditions and increased the number of pods.

In the second year, the variety × irrigation interaction was found to be significant in terms of the pod number. The number of pods decreased significantly in Akkız, Karagöz, and local varieties under deficit irrigation conditions.

In the first year, the pod length was affected by the interactions between variety and irrigation and between irrigation and zinc. Using the variety × irrigation interaction, it was found that the Karagöz variety (12.05) responded significantly to water doses, and the pod length decreased significantly when there was not enough water. There was no significant difference between irrigation doses of other varieties. Pod length was higher under full irrigation × zinc application conditions than irrigation × zinc interactions. In the second year, the variety × irrigation interaction was determined to be significant. Among the varieties, the local variety was significantly affected by irrigation doses. The decrease in pod length of the local variety was found to be significant under deficit irrigation conditions.

The irrigation × cultivar interaction significantly increased the number of grains in the pod in the first year. The number of grains in pods of all varieties decreased significantly under the restricted irrigation conditions. Zinc fertilizer application had no significant effect on the number of grains in the pods. While there was no significant difference between the mean values of the varieties under full irrigation conditions, the number of grains in the pods of the Akkız (7.23) variety was higher than the others under restricted irrigation conditions. In the second year, the variety × irrigation interaction was significant. It was determined that the restricted irrigation conditions affected the local variety (5.17), which had lower grain numbers in the pods.

In terms of the weight of 100 grains, it was seen that the variety factor was important in the first year. The highest 100 grain weight among the varieties was observed in Karagöz (9.99 g) under full irrigation conditions. It was followed by local variety (8.79 g) and Akkız (6.81 g). The variety × irrigation dose interaction was significant in the second year. The responses of the varieties to irrigation doses were found to be significant. In both irrigation doses, the order of the varieties changed to Akkız > Karagöz > local variety. The lowest value in the deficit irrigation was measured for the local variety (9.10 g).

When the SPAD values were analyzed, it was observed that cultivars had a significant effect in the first year, and irrigation had a significant effect in the second year. Looking at the average values, it is observed that the SPAD values decreased under deficit irrigation conditions.

The variety factor was significant in terms of days to flowering in the first year. It was observed that the local variety flowered later among the varieties under both irrigation conditions. Akkız and Karagöz varieties flowered earlier than the local variety. However, the average number of days of flowering for the local cultivar (65 days) under deficit irrigation conditions was longer than full irrigation (59.0 days). In the second year, cultivar × irrigation × zinc application was found to be significant. It was observed that the varieties generally flowered faster under deficit irrigation conditions, especially Karagöz (55 days), which flowered faster under deficit irrigation conditions, and the local variety (69 days), which was the variety with the latest flowering period.

When the number of days to maturity values was analyzed, it was determined that the cultivar × irrigation × zinc application interaction was significant in the first year. The earliest variety was determined to be Akkız under full irrigation and zinc application conditions. However, the variety most affected by deficit irrigation conditions was the local variety, and the days to flowering and ripening were found to be longer. In the second year, the variety × irrigation × zinc fertilizer interaction was found to be significant in days to the flowering values. Akkız was found to be earlier than the other varieties, and the local variety was found to be later than the others. Variety × irrigation and variety × zinc fertilizer interactions were found to be significant in the second year in terms of days to maturity. Among the varieties, Karagöz (59 days) was significantly affected by restricted irrigation and showed a shorter ripening period under restricted irrigation conditions. This was followed by the Akkız variety and then the local variety.

First-year irrigation × zinc interaction was found to be significant for SPAD. According to the interaction, the zinc factor was significant under deficit irrigation conditions. The SPAD values of the zinc-free plots decreased significantly under restricted irrigation conditions. The irrigation factor was found to be significant in the second year. It was revealed that the drought conditions affected the plants.

In the first year, the interaction of variety × irrigation × zinc was found to be significant for the leaf area index. According to this interaction, the leaf area index of thr local variety (472.3) and Karagöz (438.1) was higher than Akkız (314.5) under full irrigation conditions. The responses of the varieties to restricted irrigation conditions were different, the local variety was significantly affected by restricted irrigation, and the leaf area index decreased. In addition, among the varieties, Karagöz was significantly affected by the zinc application, and the leaf area index increased with the zinc application in both irrigations. The variety × irrigation interaction was found to be significant in the second year. Variety responses to irrigation were found to be different, and the local variety was significantly affected by deficit irrigation. For this reason, the leaf area index of the local variety decreased significantly under deficit irrigation.

The first-year irrigation × zinc × variety interaction was found to be significant in biological yield values. It was observed that deficit irrigation conditions significantly affected the biological yield values. Water limitations caused a decrease in biological yield values. Karagöz (68.84) gave the lowest biological yield value under restricted irrigation conditions. Under full irrigation conditions, the biological yield value of the local variety (220.3) was the highest. In the second year, the irrigation and variety factors were statistically significant. Deficit irrigation caused a decrease in the biological yield. Among the varieties of the Karagöz, 110 had a higher biological yield than the others.

In the harvest index values, the irrigation × zinc application interaction was found to be significant in the first year. Under full irrigation conditions, the harvest index values were higher in zinc application (178.5). Under deficit irrigation and zinc-free conditions (78.8), the harvest index values decreased significantly. The variety factor was found to be significant in the second year. It was revealed that there was a ranking among the varieties, which was as follows: Karagöz > Akkız > local variety.

The variety and irrigation factors were significant in grain yield values in the first year. While 382.98 kg/da of yield was obtained under full irrigation conditions, 236.0 kg/da yield was measured under restricted irrigation conditions. Water limitations caused a significant decrease in the grain yield. All the varieties in the study showed a decrease in the grain yield under deficit irrigation conditions. However, Karagöz (292.2 kg/da) had the highest grain yield among the varieties, followed by Akkız (272.2 kg/da), and the local variety (140.5 kg/da). It was determined that the local variety had a lower yield than the other varieties at both irrigation doses. While zinc application was not found to be statistically significant, it was observed that the average grain yield values under deficit irrigation conditions increased with zinc application. Under stress conditions, the effect of zinc application on grain yield was found to be more significant. In the second year of the study, the irrigation × zinc × variety interaction was found to be significant. A decrease in the grain yield was observed under restricted irrigation conditions. The highest yield was obtained from Karagöz under restricted irrigation conditions. It was found that restricted irrigation conditions affected Akkız and local cultivars more. The yield values of the varieties under full irrigation and deficit irrigation conditions were ranked as Karagöz > Akkız > local variety. Zinc application had a positive effect on the grain yield under deficit irrigation conditions.

Figure 3 shows the mean of quality values. According to the results of variance analysis (Table 3), it was determined that grain copper, zinc, iron, potassium, magnesium, calcium, phosphorus, fiber, ash, oil, and protein contents were significantly superior to deficit irrigation under full irrigation conditions.

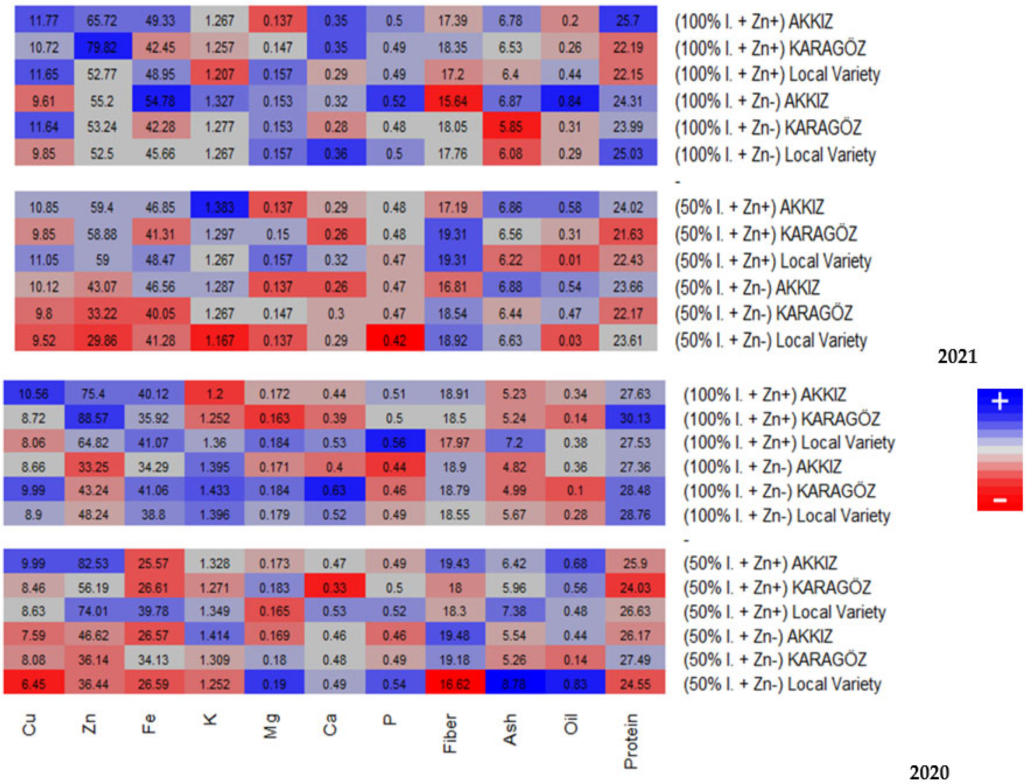

Figure 3. Mean values of grain quality components.

Table 3. ANOVA of grain quality components (*: significant -: nonsignificant).

SOV	Df	Cu		Zinc		Fe		Potassium		Magnesium		Calcium	
Years	1	*		*		*		*		*		*	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I	1	*	-	-	-	*	*	-	*	-	*	-	*
Zn	1	*	-	*	*	-	*	-	*	-	-	-	*
C	2	-	-	-	*	-	*	-	*	-	*	-	*
I*Zn	2	*	-	-	*	-	*	-	*	-	*	-	-
I*C	2	-	-	-	-	-	*	-	*	-	-	-	*
Zn*C	2	*	-	-	-	-	*	-	*	*	*	-	*
I*Zn*C	11	-	-	-	-	-	*	-	*	-	-	-	*
SOV	**Df**	**Phosporus**		**Fibre**		**Ash**		**Oil**		**Protein**			
Years	1	*		*		*		*		*			
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021		
I	1	-	*	-	-	-	-	*	-	-	*		
Zn	1	*	*	-	-	-	-	-	-	-	-		
C	2	*	*	-	*	-	*	-	-	-	*		
I*Zn	2	*	*	-	-	-	-	-	-	-	-		
I*C	2	-	*	-	-	-	-	-	-	-	-		
Zn*C	2	-	-	-	-	-	*	-	-	-	-		
I*Zn*C	11	-	*	-	*	-	-	-	-	*	-		

(cu: copper, zn: zinc, fe: iron, k: potassium, mg: magnesium, ca: calcium, and p: phosphorus). (*; significant at 5% probability level *: significant -: nonsignificant).

Regarding grain copper content, irrigation × variety, and zinc × variety interactions, were found to be significant in the first year. According to the irrigation × variety interactions, the grain copper content of the varieties was higher under full irrigation than under deficit irrigation. The varieties were ranked as Akkız > Karagöz > local variety in terms of the grain copper content. According to the zinc × variety interactions, zinc application positively affected the grain copper content in Akkız and local varieties. In the second year, the factors in the experiment were not found to be significant. However, according to the average results, it can be observed in Table 2 that the values obtained from restricted irrigation are lower.

In terms of the grain zinc content, zinc application was a significant factor in both years of the study. It was observed that zinc application significantly affected the grain zinc content in both years of the study. The grain zinc content of all varieties with zinc application under full and deficit irrigation conditions was higher than the plots without zinc application.

In the first year of the study, the irrigation factor had a big effect on the amount of iron in the grain. It was determined that deficit irrigation negatively affected the grain's iron content. In the second year, the irrigation × zinc × variety interaction was found to be significant. The iron content was found to be higher under full irrigation conditions. Among the varieties, the order was Akkız > local variety > Karagöz. Zinc application was effective in the Akkız (39.78) variety under deficit irrigation conditions.

Regarding grain potassium content, interactions, and factors, they were insignificant in the first year. When the averages were evaluated, the data obtained from full irrigation were higher than from deficit irrigation. The local variety and Karagöz had the highest average grain potassium contents among the varieties. In the second year, irrigation × zinc × variety interactions were significant.

Regarding magnesium content, irrigation × variety zinc interaction was found to be significant in the first year of the study. In the second year, irrigation × zinc and irrigation × variety interactions were significant. For the grain calcium content, the zinc × irrigation interaction was found to be significant in the first year, while the irrigation × zinc × variety interaction was found to be significant in the second year. For the grain phosphorus content, the irrigation × zinc interaction was significant in the first year, while the irrigation × zinc × variety interaction was significant in the second year. The interaction of the irrigation × zinc × variety was significant for the grain fiber content in the first year.

In contrast, the irrigation factor was significant in the second year. For the ash content, the irrigation × zinc × variety interaction was found to be significant in the first year. In the second year of the study, the zinc × variety interaction was found to be significant.

Regarding the grain oil content, the irrigation factor was significant in the first year, while the irrigation × variety interaction was significant in the second year. Regarding the grain protein content, the irrigation × zinc × variety interaction was found to be significant in the first year. In the second year, the interaction and all factors were found to be insignificant.

The average data of grain amino acid contents are indicated in Figure 4. Eight of the seventeen amino acids measured are essential (histidine, threonine, valine, methionine, phenylalanine, isoleucine, lysine, and leucine), which are not synthesized in the body and are only taken in from food. The essential amino acids in the study were evaluated. According to ANOVA, the experiment factors were found to be insignificant in the first year, while the irrigation and zinc factors were found to be significant in the second year (Table 4). In the first year, it was determined that restricted irrigation significantly affected histidine values. Histidine values were higher under full irrigation (0.86) than under deficit irrigation (0.80). However, it was determined that the histidine value increased with the zinc application.

The negative effect of restricted irrigation on histidine values in the second year was observed significantly. When the threonine values were analyzed, it was determined that the factors in the experiment in the first year were not effective on threonine, and the values obtained ranged between 0.78 and 0.85. In the second year, the water factor significantly affected threonine. It was determined that the amount of threonine obtained from full irrigation conditions (0.85) was higher than from deficit irrigation (0.78).

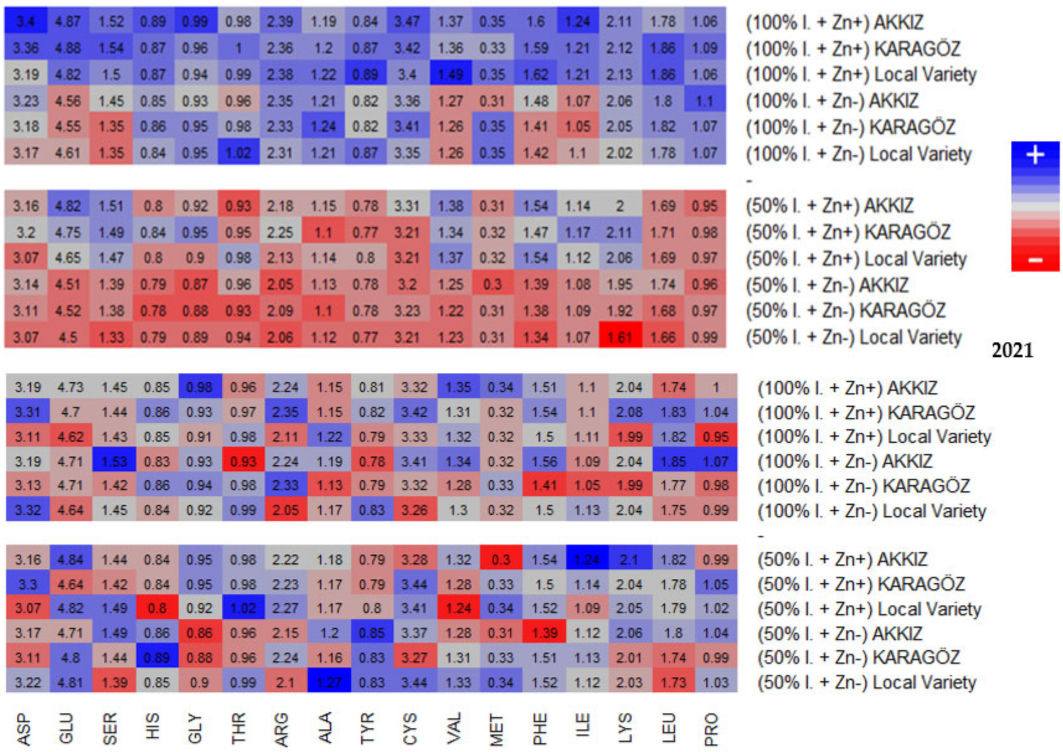

Figure 4. Mean values of grain amino acid contents.

Table 4. ANOVA of amino acids of cowpea cultivars (*: significant -: nonsignificant).

SOV	Df	ASP		GLU		SER		HIS		GLY		THR		ARG		ALA		TYR	
Years	1	*		*		*		*		*		*		*		*		*	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I	1	-	*	-	*	-	*	-	*	-	-	-	*	-	*	-	*	-	*
Zn	1	-	*	-	*	-	*	-	*	-	-	-	*	-	*	-	-	-	-
C	2	-	*	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
I*Zn	2	-	*	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-
I*C	2	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
Zn*C	2	*	*	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-
I*Zn*C	11	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-

SOV	Df	CYS		VAL		MET		PHE		ILE		LYS		LEU		PRO	
Years	1	*		*		*		*		*		*		*		*	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I	1	-	*	-	-	-	-	-	*	-	*	-	-	-	*	-	*
Zn	1	-	-	-	*	-	*	*	*		*	-	*	-	-	-	-
C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I*Zn	2	-	-	*	-	-	-	-	-	-	*	-	-	-	-	-	-
I*C	2	-	-	-	-	*	-	-	-	-	*	-	-	-	-	-	-
Zn*C	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I*Zn*C	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(ASP: aspartic acid, GLU: glutamic acid, SER: serine, HIS: histidine, GLY: glycine, THR: threonine, ARG: Arginine, ALA: alanine, TYR: tyrosine, CYS: cysteine, VAL: valine, MET: methionine, PHE: phenylalanine, ILE: isoleucine, LYS: lysine, LEU: leucine, and PRO: proline). (*; significant at 5% and probability level *: significant -: nonsignificant).

Regarding the valine values, the irrigation × zinc application interaction was found to be significant in the first year. The highest value of valine (1.33) was obtained under full irrigation conditions and from zinc-treated plots. In the second year, the zinc factor was found to be significant. Zinc-treated plots (1.39) were higher than those without zinc treatment (1.25). It was observed that the values of the varieties were close to each other.

The irrigation × variety factor was found to be significant for methionine. In the second year, zinc application was found to be significantly effective.

Regarding phenylalanine, the zinc factor was significantly effective in the first year. Phenylalanine values of zinc-treated samples (1.52) were higher than those of zinc-free samples (1.48). The irrigation × zinc application interaction was significantly effective in the second year.

In terms of isoleucine values, it was observed that the treatments were not significant in the first year. In the second year, the irrigation × variety and irrigation × zinc application interactions were significantly effective.

Regarding lysine values, treatments were not significant in the first year, but the zinc application factor was found to be significant in the second year. It was determined that zinc-free plots had lower lysine values.

Regarding leucine values, treatments were not significant in the first year. In the second year, the irrigation factor was found to be significant.

The results obtained in non-essential amino acids were evaluated. In the first year, aspartic acid was found to be significantly affected by the cultivar × zinc application interaction. In contrast, the effects of the treatments on glu, ser, gly, threonine, ala, cys, and pro were found to be insignificant, and the cultivar factor had a significant effect on arg. In the second year, the zinc × variety, irrigation × zinc application, and the variety × irrigation application interactions on asp were found to be significant. The zinc application × irrigation interactions on glutamic acid and SER were found to be significant. Irrigation factors significantly affected ala, thr, cys, and pro. For arg and gly, the irrigation × variety interactions were found to be significant.

4. Discussion

When the traits in the study were analyzed, the results were evaluated. The varieties were found to be significant in terms of days of flowering in the first year, and it was observed that the local variety flowered and matured later than the others. In the second year, the irrigation conditions significantly affected these traits, and flowering and physiological maturity periods were prolonged under full irrigation conditions.

The amount of irrigation had a significant effect on the leaf area index values, and in both years, when irrigation was limited, the leaf area index values were lower. Additionally, zinc application was found to be effective in the second year, and zinc-free plots had lower leaf index values. Under full irrigation conditions, plants grew better, and the leaf area index was higher because they did not compete [46,52]. Ref. [52] stated that the most critical period for the leaf area index is between the pod-setting and grain-filling periods. When the plant is exposed to zinc deficiency, auxin deficiency in the plant causes defoliation [53].

SPAD values decreased under deficit irrigation conditions in this study. However, according to some researchers, drought stress changes the leaves' anatomy, so leaves become smaller and thicker, and the SPAD value increases due to concentrated chlorophyll pigments in smaller cells [54,55]. Maintaining the chlorophyll concentration under drought stress helps stabilize photosynthesis [56]. The decrease in the chlorophyll content is associated with an increased production of oxygen radicals in the cell. The authors of [57] reported that with the decrease in chlorophyll concentration, the green color of the leaves decreases, and premature senescence of the plant occurs. The lowest SPAD values were obtained from a local variety under deficit irrigation conditions. In SPAD measurements made during the grain-filling period under high temperature and water stress conditions, 35.48 values were obtained under control conditions, 36.84 under high-temperature stress conditions, and 22.38 under water stress conditions, and it was determined that the chlorophyll content of the leaves decreased significantly in cases of water scarcity. Readings of the chlorophyll content (SPAD) during the grain-filling period in soybeans were said to be the best way to figure out how water stress hurts the plant's ability to use nitrogen [58]. It has been reported that SPAD measurements made at grain filling showed a significant positive correlation in yield estimation [59].

It was observed that pod length, number of pods per plant, number of grains per pod, and 100 grain weight among yield components were affected by deficit irrigation. It was determined that the mean values of these traits decreased under deficit irrigation conditions. Cowpea is generally sensitive to drought during pod-set and grain-setting periods [10]. In terms of pod length and pod number, the zinc × irrigation interaction was significantly effective in some years. Zinc applied under restricted irrigation conditions had a positive effect on these traits. Concerning the grain yield, irrigation and variety factors in the first year and irrigation × zinc × variety interaction in the second year were found to be significant.

Drought applied five weeks after sowing was found to cause a flower drop and significantly reduce the grain yield by 67% [60]. Under post-flowering drought stress, the grain yield and forage yield of cowpea grown in the field both dropped by 65% and 40%, respectively [61]. It was also found that drought applied during the vegetative and flowering stages reduced the leaf area and affected the grain quality [62]. Drought stress reduces biomass production in many crops, including cowpea, by reducing photosynthesis, stomatal conductance, transpiration, and plant water status [63]. Drought reduces biomass production in cowpea [63]. Drought stress caused a reduction in the leaf area in cultivars [64]. Under drought conditions, cowpea is most susceptible to yield loss during flowering and grain filling [65]. Yield loss occurs due to reduced assimilation by the grain due to the inhibition of photosynthesis by closing stomata (stomatal limitations) [66]. In the cowpea water deficit, a 44.3% yield loss was determined [66]. Some studies determined that the soil zinc application did not affect the grain zinc content, and different cowpea cultivars responded differently in the grain yields, Melo et al., 2017.

The grain yield decreased under deficit irrigation conditions in the first year, and the lowest yield was obtained from the local variety.The highest average yield was obtained from the Karagöz variety in both years. In both years, zinc application under restricted irrigation conditions positively affected the grain yield of the varieties. Previous studies have shown that crop loss is higher in zinc deficiency and deficit irrigation conditions [67]. Oxidative stress conditions of plants under stress conditions such as drought and deficit irrigation limit the agricultural productivity. The inability to fully reduce oxygen causes active oxygen species to appear in the environment. Their damage to cellular structures is called "oxidative stress." Active oxygen species resulting from oxidative stress have toxic effects and can cause crop losses. Under stressful circumstances, the plant's antioxidant defense mechanisms that normally reduce active oxygen species start to increase them.

The scavenging effect of zinc on active oxygen species plays a key role here [35,67]. An adequate zinc supply under restricted irrigation conditions may cause the important components of the plant's antioxidative defense system, which contains zinc, to tolerate these conditions effectively [35]. The average values of the second-year grain yield were observed to be lower than the first year. The fact that the average temperature values in the second year were higher than the long-term average and the average of the second year may have caused a decrease in crop loss. In cowpea, increases in night temperatures cause a 4–14% decrease in pod set and grain setting for each 0 °C above the threshold of 16 °C [68,69].

The measured quality traits—irrigation × zinc × variety interaction—significantly affected the protein ratio. It was observed that the protein ratio was negatively affected under restricted irrigation conditions. Protein synthesis may be suppressed in legumes under restricted irrigation conditions, and protein accumulation in legume grains may decrease when nitrogen breakdown and fixation are prevented under deficit water conditions [70]. Ref. [67] determined that zinc promoted seedling growth in durum wheat under drought conditions. In another study, the N, P, Fe, and Zn levels and, thus, the total protein content decreased with drought in bean seeds [71].

Protein quality decreased under high temperatures or poor irrigation conditions [72]. Genotypic factors and the environment are effective together in improving the grain's protein content. In this study, it was observed that the average protein values increased with zinc application. Most proteins in biological systems require zinc in their structures. Foliar application of zinc fertilizer is a fast and effective method to increase the grain's zinc content [73]. In cereals, the grain's zinc concentration was found to be correlated with the grain's protein content [70–72]. Foliar zinc applications have been observed to increase protein synthesis [74].

Increasing the micronutrient elements in the grain is the first step to making these foods a richer food source for humans. Foliar zinc application, it was found that the grain's zinc content, which is one of its micronutrients, was significantly increased in both years. There are differences among crop species in the zinc uptake and tissue utilization efficiency [75]. In general, the zinc content of cereal grains is lower than that of legumes. For this reason, zinc deficiency may occur in cereal-based diets [76]. It is important to include legumes in the diet. Zinc application significantly affected the grain iron and phosphorus contents in the second year of the experiment. This study supports the importance of foliar zinc application in reducing yield loss and contributing toward bioavailability by increasing the grain's micronutrient concentration, especially under restricted irrigation conditions.

In this study, a total of 17 amino acids were measured. Among these, eight are essential; that is, the eight amino acids that our body needs to take from outside. These are histidine, threonine, valine, methionine, phenylalanine, isoleucine, lysine, and leucine. When the results, especially those related to the essential amino acids, were analyzed, leucine and threonine were significantly affected only by deficit irrigation. All other essential amino acids were significantly affected by zinc application. In general, cowpea is low in sulfur-containing amino acids, and their consumption with cereals will make up for this deficiency. The low level of methionine in cowpeas can be overcome with such a diet. The relatively higher lysine content in cowpea will also help compensate for the cereals' deficiency [77].

Among other amino acids, cysteine is known to favor zinc bioavailability. In this study, deficit irrigation had a significant effect on cysteine.

Iron and zinc have many biological functions. More than half of the iron in the human body is bound to hemoglobin, so the most obvious consequence of an iron deficiency is anemia. Zinc is a cofactor with various structural and catalytic functions in 10% of human proteins.

The bioavailability of iron and zinc in basic foods is low because of some complicated interactions. Some minerals are thought to be absorbed by humans at about 5% for iron and 25% for zinc [40]. Some amino acids, such as cysteine and histidine, can increase zinc absorption. For this reason, they are also called fortifiers [78].

Drought, spreading over an ever-expanding area and affecting an increasing number of countries, has become a factor limiting agricultural production. To solve this problem, different farming systems or crops with an unknown value should be used in new ways. This study shows that cowpea are a very valuable crop because they are naturally tolerant to drought and they can be grown with less water. They are also very nutritious and bioavailable.

5. Conclusions

When looking at the study results, it was found that limited irrigation had a significantly effect on grain yield and the components of yield. In the first year of the study, there was a 40 percent decrease in yield with limited irrigation (382.9 kg da^{-1}) compared to full irrigation (235.0 kg da^{-1}). However, the application of zinc fertilizer was effective in the manage stress, and yield increases were observed in zinc-applied plots under limited irrigation conditions. The yield performances of the varieties showed differences depending on the genotype. Among the cultivars, Karagöz has the highest yield in both years. This was followed by Akkız and local varieties, respectively. Local varieties developed vegetatively better than the other varieties.

In the second year of the study, the amount of zinc, iron, and copper in the grain changed because of the foliar zinc application. Grain bioavailability enriches the product in terms of nutrients; it is also possible to say that grain bioavailability may be affected by the amount of essential amino acids.

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