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Effect of Zinc Application Methods on Nutrition Uptake in Cotton

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Abstract: This study was carried out to determine the effect of different zinc application methods on cotton plant nutrient uptake and nutrition content in the soil. The study was conducted at Siirt University Faculty of Agriculture Department of Field Crops experimental area as a randomized complete block design with four replications. Seven different zinc applications were performed (Control, To Soil 200 g da^{-1}, To Soil 400 g da^{-1}, Soil + Leaves at Pre-Squaring Stage, Pre-Squaring Stage + Initial Flowering Stage to Leaves, Pre-Squaring Stage + Pre Flowering Stage + Flowering Stage to Leaves, Pre-Flowering Stage + Flowering Stage to Leaves). According to the results of leaf analysis, it was determined that there were non-significant differences in terms of N, K, Ca, Na, Mg, Fe, Zn, Mn, and Cu content, but soil analysis after harvest showed that N, K, Ca, Na, Mg and Cu content were affected from different zinc treatments, however, P, Fe, Zn, Mn were not affected from applications. It has been observed that the highest N, P, K, Ca, and Na value in the soil was obtained from zinc applications to the soil + pre- squaring stage to leaf, and the highest Mg and Fe value in the soil was obtained from control. Although there were no significant differences detected between the zinc applications in terms of leaf analysis, it was observed that the highest K, Ca, Na, Mg, Fe, Mn, and Cu values were obtained from the zinc application of 200 g da^{-1} to the soil. In the study, it was observed that the highest zinc values in the leaf and soil were obtained from the foliar application of zinc applied before squaring and beginning of flowering. This application can be recommended to increase the zinc content in the soil and leaves.

Keywords: cotton; zinc; yield; plant development; plant nutrition; fiber quality.

1. Introduction

A timely and appropriate dosage of macro and micro plant nutrients during plant development is a way to increase productivity. It is known that six micronutrients (boron, manganese, iron, copper, zinc, and molybdenum) play an important vital role in the plant.

Zinc (Zn) is one of the most important micronutrients that all living organisms need and must take in very low amounts. Zinc is taken up by plants in relatively small amounts and its uptake ability by plants is highly variable. Plants take zinc in the form of ZN^{2+} ions. Zinc is also taken in the form of chelates (Zinc EDTA, Zn-DPTA, Zn-EDDHA). Plants first take the zinc ZN^{2+} dissolved in the soil solution. They also benefit from ZN^{2+} adsorbed in exchange complexes and formed organic complexes in soil solution or soil solid phase [1].

In cases where the zinc concentration is low, especially in case of zinc deficiency in the soil, the transport of zinc and other nutrients such as phosphorus, potassium, copper, iron, and manganese from the root surface occurs by diffusion. With the mass flow, only the amount of nutrients required by the plants is transported [2].

Zinc element regulates growth hormones, root development of the plant, and metabolic activities of the plant. The role of zinc in plant nutrition is important because it plays a regulatory role in many enzyme systems, and is used in nucleic acid synthesis, chlorophyll and carbohydrate production, and plant hormone metabolism. In addition, the presence of zinc is needed for the synthesis of indole acetic acid (IAA), which is of great importance for plants. The lack of zinc to be taken up by plants in the soil severely limits the growth and yield-producing capacity of plants.

Zinc has various and important functions in plants as well as in humans and animals. It is involved in the structure of various enzymes and activates many enzymes. It plays a role in carbohydrate, protein, and auxin metabolism. For this reason, in the case of zinc deficiency, carbohydrate, protein, and auxin metabolism are adversely affected due to the decrease in enzyme activity. Stunted growth and small leaf formation, which are the most obvious signs of zinc deficiency in plants, are due to the deterioration in auxin metabolism and especially the decrease in indole acetic acid (IAA) formation. The low amount of IAA in plants with zinc deficiency is due to the regression in IAA synthesis and the rapid breakdown of the formed IAA [3].

Zinc is required for chlorophyll formation and carbohydrate production. It was determined that the chlorophyll content and RNA levels of the plants decreased significantly in zinc deficiency. In most cases, the formation of short internodes in plants and the appearance of chlorosis in the leaves are symptoms of zinc deficiency. Small yellow spots appear on the leaves. Plant growth is delayed and cell growth is disrupted. Zinc is not a very active element in plants. Although the movement of zinc in the plant is limited, it is more mobile than the other micronutrients Fe, B, and Mo. Especially when large amounts of Zn are applied to the growth medium, Zn accumulation occurs in the root tissues [1].

The Zn contents of the plants are normally between 5-100 mg kg^{-1}, and toxicities usually start after 400 mg kg^{-1}. It has been determined that Zn levels in plants with zinc deficiency are quite low (0-15 mg kg-1) [4,1]. Zinc deficiencies are effective in large regions around the world. It is naturally present in low amounts in some soils, or it cannot be taken up by the plant due to the interactions of some components in the soil or because the roots of plants are under stress and pressure due to drought (abiotic), and disease (biotic) reasons [5,6,7].

The fact the amount of Zn in the form that can be taken by the plant is generally insufficient in the soils of our country, the pH value is high due to excess lime in the soil, and the unnecessary use of P fertilizers in the soil causes Zn deficiency to occur in almost all plants. The presence of excess Ca, Fe and Mn in soils and the presence of insufficient organic matter are among the causes of Zn deficiency. Zinc element takes place in biochemical events in the plant. It plays a role in the synthesis of carbohydrates, proteins, fats, and starches. In its deficiency, the leaves cannot develop sufficiently and the internodes become shorter and the event called small-leaved (rosette) occurs.

Zinc deficiency is a common microelement problem in soils all over the world. Zinc deficiency occurs especially in semi-arid regions in areas planted with grains. The regions where deficiency is most common are the Mediterranean, Southeast and East Asian countries, and Australia. In studies, it has been reported that zinc deficiency is seen in 20 million hectares of arable land in China, 30 million in India, 14 million in Turkey, at least 10 million in Australia, and 8 million hectares in Bangladesh [8,9,10]. In other studies, it has been stated that half of the world's grain-grown soils have Zn deficiency problems [11].

Similarly, approximately 50% (14 million ha) of agricultural soils in Turkey contain Zn below the critical level (DTPA-Zn < 0.5 mg kg^{-1} soil). This problem is seen in Turkey, especially in the Central Anatolian Region, where intensive wheat farming is carried out [12; 13]. According to the analyzes made on 1511 soil samples collected from different regions of Turkey, Zn deficiency was found to be the most common microelement with 49%, followed by iron (Fe) with a rate of 27% [12].

The lack of zinc to be taken up by plants in the soil severely limits the growth and yield-producing capacity of plants. Zinc deficiency, on the one hand, limits vegetative productivity, on the other hand, it leads to a low Zn concentration in the harvested product. Li et al. [14] determined the effect of zinc on the growth, development, and yield components of the cotton plant both in the field and in pots. They reported that zinc application increased N, P, K uptake, utilization metabolism, root and green part development of the plant and dry matter production. One of the measures to be taken to eliminate Zn deficiency, which causes serious negativities in both plant production and nutrition, is the use of zinc-containing plant nutrition products against Zn deficiency or the breeding of resistant plant genotypes [13].

This research was carried out to determine the effects of zinc applications on plant nutrient uptake in cotton.

2. Materials and Methods

This research was conducted in the experimental area of the Faculty of Agriculture and Department of Field Crops at Siirt University in 2016. Stoneville 468 cotton variety and Dokto Zinc-15 leaf fertilizer were used as material (Fertilizer contain only one trace element, zinc chelate- EDTA, water-soluble zinc 15%, EDTA cheated zinc 15% and 100% water soluble). The experiment was carried out according to the randomized complete block design with four replications and 7 different zinc applications were included in the experiment.

Treatments:
1. Control;
2. 200 g da^{-1} at the soil;
3. 400 g da^{-1} at the soil;
4. Soil (200 g da^{-1}) + Foliar (200 g zinc/100-liter water before the squaring stage;
5. Foliar application at the two times (Before squaring stage + Beginning of flowering stage);
6. Foliar application at twice times (Before squaring stage + Before flowering stage + Flowering stage);
7. Foliar application at the two times (Before flowering stage + Flowering stage).

Sowing was made with a cotton drill machine on 6 May 2016, in sowing each plot consisted of four rows of 12 m in length, the distance between rows was kept constant at 0.70 m during planting, and the distance between inter-row was created by thinning which performed when plants were reached 10-15 cm high. Each parcel is 2.8 m wide and 2 m of space is left between the blocks. Before sowing, the soil samples were taken from 0-30 cm depth in the experimental area, analyzes were made at the Siirt University laboratory and the amount of fertilizer needed by the plant was determined. The results of soil analysis can be shown in Table 1.

Table 1. Soil properties of the experimental area.

Texture	Clay	
pH	7.98	Slightly alkaline
EC (mS/cm)	0.363	Saltless
Lime (%$CaCO_3$)	13.02	Limy
Organic matter (%)	1.31	Low
N (%)	0.082	Low
P (ppm)	7.47	Low
K (me/100g)	0.98	High
Fe (ppm)	5.70	Adequate
Cu (ppm)	2.63	Adequate
Zn (ppm)	0.23	Low
Mn (ppm)	6.04	Low

In the experiment, all maintenance operations were done on time. All plots received 140 kg ha^{-1} N and 80 kg ha^{-1} P_2O_5. At sowing 80 kg ha^{-1} N and 80 kg ha^{-1} P_2O_5 were applied to the band in the form of 20-20-0 compose fertilizer, and the remaining nitrogen (60 kg ha^{-1} N) was applied before the first irrigation (approximately 45 days after planting) as ammonium nitrate (33%). In addition, different applications of zinc fertilizer were applied to the trial plots. Zinc application from soil and leaves was applied with a small motorized back sprayer. In the application of zinc applied to the soil, after the zinc fertilizer was applied to the soil surface, it was mixed with a rake and a homogeneous mixture was achieved. The experiment was hoed three times by hand and two times with a machine. Herbs and insects were monitored throughout the experiment and no pesticides were necessary during the growing season.

Drip irrigation systems were used for irrigation, plots were irrigated for the first time at squaring stage, and irrigation terminated at the 10% boll opening stage. The youngest 30 leaves that have completed their development in the main stem in 30 plants from each plot were taken and N, K, Ca, Na, Mg, Fe, Zn, Mn, and Cu analyzes were made in the Central Laboratory of Siirt University (SIU). Both pre-planting soil analysis was made and the contents of N, P, K, Ca, Mg and micronutrients (Fe, Mn, Zn, Cu, and B) in soil samples taken from each plot after harvest were determined by analyzing them in the Central Laboratory of SIU.

From Table 2, it can be seen that the average and maximum temperature in the experimental year were higher than the long-term period and minimum temperature was lower than the long-term period and the average rainfall in the April and May in which sowing take place was lower than long-term periods. The harvest was done by hand and was completed two times. Statistical analysis was performed using JUMP 5.0.1 statistical software and the means were grouped with the LSD $_{(0.05)}$ test.

Table 2. Average values of temperature, rainfall, and relative humidity during the experimental and long-term period.

Month	Year	Average Temp (°C)	Max. Temp (°C)	Min. Temp (°C)	Rainfall (Kg/m^2)	Humidity (%)
April	2016	19.20	26.50	4.20	66.80	41.50
	Long term	13.80	13.90	9.10	104.30	50.40
May	2016	22.30	30.60	8.00	64.70	41.90
	Long term	19.20	25.20	13.50	66.20	41.50
June	2016	26.50	38.40	13.90	20.60	27.30
	Long term	25.90	32.20	18.90	9.20	24.10
July	2016	31.20	41.60	20.60	2.40	25.90
	Long term	30.50	37.10	23.30	1.60	18.10
August	2016	32.30	41.80	22.40	0.20	20.50
	Long term	30.00	37.00	23.10	1.00	17.20
September	2016	25.00	36.30	12.40	19.00	29.80
	Long term	25.00	32.30	18.70	5.20	24.00
October	2016	19.50	31.20	10.20	27.10	36.80
	Long term	17.90	24.50	12.70	50.90	45.30
November	2016	10.40	22.60	1.50	55.60	49.70
	Long term	10.20	15.40	6.30	80.10	57.10

Source: Turkish State Meteorological Service

3. Results and Discussion

The differences between investigated traits in terms of leaf and soil analysis test results are given in Tables 3 and 4, respectively.

Average values in terms of the nitrogen (N) content in the plant is given in Table 3. From Table 3, it is seen that there are no statistically significant differences between the applications in terms of nitrogen (N) content in the plant, the average values of the nitrogen (N) content in the plant ranged between 2.30 and 2.55 ppm and the average nitrogen content of the experiment was 2.42 ppm. The highest nitrogen content of the plant was obtained from the zinc application of soil + foliar application on a leaf in the before squaring stage which is the 4th application as 2.55 ppm, while the lowest value was obtained from the control application (2.30 ppm). Esmailnia et al. [15] reported that the nitrogen rate in leaf increase with zinc applications in cotton under salt stress conditions.

Table 3. Average values and groupings of nutrient content in the leaves.

Treatment	**N (ppm)**	**K (ppm)**	**Ca (ppm)**	**Na (ppm)**	**Mg (ppm)**	**Fe (ppm)**	**Zn (ppm)**	**Mn (ppm)**	**Cu (ppm)**
1. Control	2.30	6346.93	25116.71	1695.77	3613.17	27.89	17.95	6.01	0.74
2. To Soil (200 g da^{-1})	2.35	8428.56	29253.15	1837.96	3690.96	32.81	20.77	6.84	0.85
3. To Soil (400 g da^{-1})	2.36	7294.23	22248.49	1562.78	3125.14	26.39	20.09	5.09	0.78
4. Soil +Foliar (Before Squaring)	2.55	7765.36	25289.64	1787.32	3768.96	28.77	19.79	6.68	0.77
5. Foliar Application (Before Squaring + Beginning of Flowering)	2.52	6720.15	24990.03	1591.99	3207.65	28.73	24.09	6.56	0.85
6. Foliar Application (Before Squaring + Beginning of Flowering + Flowering)	2.48	6318.34	23129.12	1612.75	3293.42	27.94	21.12	5.04	0.82
7. Foliar Application (Before Flowering + Flowering)	2.40	8001.75	22833.88	1580.87	3145.43	30.19	20.91	6.09	0.85
Average	2.42	7267.90	24694.40	1667.06	3406.39	28.96	20.68	6.05	0.81
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns

*-Statistical significance i.e. $p<0.05$. **-Statistical significance i.e. $p<0.01$. ns-denotes not statistically significant at $p<0.05$.

It can be observed that the values obtained in terms of potassium (K) content in the plant varied between 6318.34 and 8428.56 ppm and there were no statistically significant differences between the applications. Depending on the applications, it was determined that the highest value in terms of potassium (K) content was obtained from the second application (200 g da^{-1} to soil) and zinc application (8428,56 ppm). The lowest potassium content was obtained by applying zinc to the leaf 3 times (6318.34 ppm), which is the 6th application (Before squaring + Beginning of flowering + Flowering stage). Sial et al. [16] and Ahmed et al. [17] revealed that the value of potassium (K) content in the plant increased with zinc application, these findings were not found compatible with the results of the research.

Depending on the applications, the average values of calcium (Ca) content in the plant varied between 22248.49 and 29253.15 ppm; it can be observed from Table 3 that the general average of the experiment is 24694.40 ppm, but the differences between applications are not statistically significant. With 400 g da^{-1} zinc applied to the soil the lowest value (22248.49 ppm) was obtained in terms of calcium (Ca) content in the plant, and the highest value was obtained in the plant with 200 g da^{-1} zinc application to the soil (29253.15 ppm). Previous researchers reported that the calcium content of plants decreased with zinc application [17], but these results are different from research findings. Ceylan et al. [18] findings that zinc has no significant effect on calcium content support the results of the research.

It can be observed that the average values of sodium (Na) content in the plant varied between 1562.78 and 1837.96 ppm and the general average of the experiment was 1667.06 ppm (Table 3). The lowest sodium content (Na) value (1562.78 ppm) was obtained with the application of 400 g da^{-1} zinc to the soil, while the highest value was obtained with 200 g da^{-1} zinc application to the soil (1837.96 ppm), but the differences between the applications were not statistically significant. Esmailnia et al. [15] stated that the value of Na content in the plant decreased with the zinc applications and research findings differ.

The average values of magnesium (Mg) content in the plant changed between 3125.14 and 3768.96 ppm depending on the applications; it is seen from Table 3 that the general average of the experiment is 3406.39 ppm. It was determined that the lowest magnesium (Mg) content value (3125.14 ppm) was obtained in the plant with the application of 400 g da^{-1} zinc to the soil, and the highest Mg value was obtained (3768.96 ppm) with the application of zinc applied to the soil + leaf in the period before squaring. However, the differences between the applications were not found to be statistically significant. Similar results were reported by Ceylan et al. [18] and are in line with the research findings; on the other hand, Ahmed et al. [17] reported that zinc applications reduce the Mg value in the leaf.

Iron (Fe) content values in the plant varied between 26.39 and 32.81 ppm depending on the applications. With the application of 400 g da^{-1} zinc to the soil, the lowest iron (Fe) content value (26.39 ppm) was obtained in the plant, while 200 g da^{-1} zinc application to the soil showed the highest value (32.81 ppm). However, it can be observed that the differences between applications are not statistically significant. Findings obtained from the study are supported by Ceylan et al. [18], however, Ahmed et al. [19; 17] revealed that the Fe content of the plant decreased with zinc application and these findings are different from the research findings. Sial et al. [16] reported that the value of iron content in the plant increased with the application of zinc.

The average values of the zinc (Zn) content in the leaf varied between 17.95 and 24.09 ppm and the general average of the experiment was 20.68 ppm. The lowest value in terms of zinc (Zn) content in the leaf was obtained with the control application (17.95 ppm), while the highest value was obtained (24.09 ppm) with the application of zinc applied to the leaf twice (Pre-squaring + Beginning of flowering period) which is the 5th application. When compared with the control it was determined that zinc applications increased the value of zinc content in the leaves, but the differences formed were not statistically significant. Sial et al. [16] and Ahmed et al. [17] report that the value of zinc content has increased. Menon and Rahman [20] reported that the critical deficiency level of zinc is 15-30 mg kg^{-1} in leaves, while the critical toxicity concentration is 200-500 mg kg^{-1} in leaves. It is stated that the zinc uptake of plants may vary depending on the zinc content in the soil, soil pH, organic matter amount, soil temperature, moisture in the soil, and root distribution.

Manganese (Mn) content in the plant varied between 5.04 and 6.84 ppm and the general average of the experiment was 6.05 ppm. With the application of 200 g da^{-1} zinc to the soil, the highest value was obtained in terms of Mn content in the plant (6.84 ppm); It can be observed that the lowest value of manganese content (5.04 ppm) was obtained in the plant with zinc applied to the foliar application with 3 cotton growing stages as the 6th application (Before squaring + Beginning of flowering + Flowering stage), but the differences between the applications were not statistically significant. Sial et al. [16] reported that the Mn content value of the plant increased with zinc applications; on the other hand, Ahmed et al. [19] stated that zinc applications reduce the Mn content in the plant. It is seen that parallel results cannot be obtained. It was found that zinc applications do not have a significant effect on the manganese value of the plant. Similar research findings were reported by Ceylan et al. [18].

It was found that the average values of copper (Cu) content in the plant varied between 0.74 and 0.85 ppm; Table 3 shows that the general average of the experiment is 0.81 ppm. With the control application, the lowest value (0.74) was obtained in the plant, while the highest value was obtained from the 2nd application (200 g da^{-1} to soil), the 5th application (Before squaring + Beginning of flowering), and the 7th application (Before flowering + Flowering) as 0.85. While Sial et al. [16] reported that the copper content of the plant increased due to zinc applications, Ahmed et al. [19] stated that the copper content value has decreased. Ceylan et al. [18] stated that the value of copper content in the plant is not affected by zinc application, similar results were obtained.

From Table 4, it can be observed that there are statistical differences at a 5% significance level between the applications in terms of nitrogen (N) content in the soil. It is seen that the average values of nitrogen (N) content in the soil, depending on the applications, vary between 0.08 and 0.14 ppm and the general average of the experiment is 0.11 ppm. The highest N value in the soil was obtained from the 5th application (Before Squaring + Beginning of Flowering) and the 4th application (Soil + Before Squaring) at 0.14 and 0.13 ppm and these applications were shared in the same statistical group. The lowest value in terms of N content in the soil was obtained from the 6th application (Before squaring + Beginning of Flowering + Flowering Period).

Table 4. Average values and groupings of nutrient content in the soil.

Treatment	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Na (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
1. Control	0.12 ab	4.95	381.84 a	8045.50 ab	816.63 a	**832.47** a	18.85	1.66	22.92	3.61 ab
2. To Soil (200 g da-1)	0.09 bc	4.34	176.24 c	4031.74 d	503.73 bc	480.52 b	10.21	0.91	18.00	2.35 cd
3. To Soil (400 g da-1)	0.11 abc	4.01	336.03 ab	6515.80 abc	803.39 a	770.61 a	16.94	0.98	25.21	3.34 ab
4. Soil +Foliar (Before Squaring)	**0.13** a	5.02	360.63 a	**8369.43** a	**876.75** a	797.59 a	13.50	1.54	15.98	3.80 ab
5. Foliar Application (Before Squaring + Beginning of Flowering)	**0.14** a	4.68	234.85 c	5560.87 cd	660.83 abc	647.35 ab	15.98	2.27	25.07	2.96 bc
6. Foliar Application (Before Squaring + Beginning of Flowering + Flowering)	0.08 c	4.14	242.99 bc	6215.57 bc	738.70 ab	744.55 a	14.12	1.34	20.02	**4.02** a
7. Foliar Application (Before Flowering + Flowering)	0.11 abc	4.61	205.89 c	3857.37 d	440.93 c	405.02 b	7.69	0.46	8.04	1.76 d
Average	0.11	4.54	276.92	6085.18	691.56	668.30	13.90	1.31	19.32	3.12
LSD (0.05)	0.03*	ns	94.52**	1982.06**	239.98**	246.79*	ns	ns	ns	0.97 **

*Statistical significance i.e. $P < 0.05$. **Statistical significance i.e. $P < 0.01$. ns denotes not statistically significant at $P < 0.05$.

The differences between the applications in terms of phosphorus (P) content in the soil are not statistically significant. It is seen from Table 4 that the average values of phosphorus (P) content in the soil, depending on the applications, vary between 4.01 and 5.02 ppm; and the general average of the experiment is 4.54 ppm. It can be observed from the same Table that the lowest P content value (4.01 ppm) was obtained in the soil with the application of 400 g da^{-1} zinc the soil, and the highest value (5.02 ppm) in the application of zinc applied to the soil and before squaring period which is the 4th treatment. It is reported that there is an antagonistic interaction between zinc and phosphorus in the soil [20], but such an effect was not found in the study because similar values were obtained in the research. Loneragan and Webb [21] and Sawan [22] reported that zinc deficiency occurs with a high P amount applied to the soil.

It can be observed from Table 4 that there are statistical differences at the 1% significance level between the applications in terms of potassium (K) content in the soil. The average values of potassium (K) content in the soil, depending on the applications, vary between 176.24 and 381.84 ppm; and it is seen that the general average of the experiment is 276.92 ppm. It was determined that there were significant statistical differences between the applications in terms of this trait, and the lowest potassium (K) content value (176.24) was obtained with the application of 200 g da^{-1} zinc applied to the soil, while the highest value (381,84 ppm) was obtained with the control application. Control application was included in the same statistical group as the 4th and 3rd applications.

Table 4 shows that the average values of calcium (Ca) content in the soil, depending on the applications, vary between 3857.37 and 8369.43 ppm; the general average of the trial was 6085.18 ppm and the difference between the applications was significant at the 1% level. The lowest calcium content value in the soil (3857.37) was obtained with zinc applied to the leaf twice during the Pre-Flowering Period + Flowering Period which is the 7th application, while the highest value was obtained from zinc application at the soil + Foliar (Before Squaring) which is the 4th application. In terms of this feature, the 4th application and the control application were in the same statistical group. The interaction between zinc application and Ca was reported by Araujo et al. [23].

From Table 4, it can be observed that there are statistical differences at the 1% significance level between the applications in terms of sodium (Na) content in the soil. Table 4 shows that the average values of sodium (Na) content in the soil, depending on the applications, vary between 440.93 and 876.75 ppm; and the general average of the trial was 691.56 ppm. The highest sodium content value (876.75 ppm) is obtained with the zinc application to the soil + before squaring stage which is the 4th application, and the lowest sodium content value (440.93 ppm) was recorded in the 7th application, in the Before Flowering + Flowering Period. It is seen that together with the 4th application, the 3rd application and the control application are in the same statistical group.

It can be observed that there are statistical differences at the 5% significance level in terms of magnesium (Mg) content in the soil (Table 4). It shows that the average values of magnesium (Mg) content in the soil, depending on the applications, vary between 405.02 and 832.47 ppm; and the general average of the experiment was 668.30 ppm. The lowest magnesium (Mg) value (405.02 ppm) was obtained in the soil with zinc (7th application) applied to the leaf twice, in the Pre-Flowering Period + Flowering Period, and the highest value (832.47) was obtained with the 1st application (control). In terms of this feature, it was determined that the 4th, 3^{rd,} and 6th applications, together with the control, were in the same statistical group, respectively. Prasad et al. [24] reported that positive interaction between magnesium and zinc availability. Seatz [25] observed that degree of Zn availability could be altered by selecting the lime materials based on their Mg content and the optimum growth of flax (*Linum usitatissimum*).

From Table 4, it can be observed that there are no statistically significant differences between the applications in terms of iron (Fe) content in the soil. The average values of iron (Fe) content in the soil varied between 7.69 and 18.85 ppm, and the general average of the experiment was 13.90 ppm. Among treatments, the highest Fe value (18.85 ppm) was obtained with the control, and the lowest value (7.69 ppm) was obtained with the two times of foliar application (Before flowering + Flowering period) which is the 7th application. Although there were no significant differences between the applications, it was observed that zinc applications caused some decrease in iron content. Menon and Rahman [20] stating that the increase in zinc in the soil decreases the Fe uptake of plants supports the results of this research. Similar findings were reported by Ahmed et al [17]) and Prasad et al [24].

It can be observed from Table 4 that there are no statistically significant differences between the applications in terms of zinc (Zn) content in the soil. The average values of zinc (Zn) content in the soil, depending on the applications, varied between 0.46 and 2.27 ppm; and the general average of the experiment was 1.31 ppm. The lowest value (0.46 ppm) was obtained with zinc applied to the leaf twice during the Pre-Flowering Period + Flowering Period which is the 7th application, while the highest value (2.27 ppm) was obtained from zinc applied to the leaf twice during the Before squaring stage + Beginning of Flowering Period which is the 5th application. Çakmak et al. [26] stated that the zinc level in the soil being less than 0.5 is a critical level for the plant.

From Table 4, it can be observed that there is no statistically significant difference between the applications in terms of manganese (Mn) content in the soil. It is seen from Table 4 that the average values of manganese (Mn) content in the soil, depending on the applications, vary between 8.04 and 25.21 ppm, and the general average of the experiment was 19.32 ppm. The lowest manganese (Mn) value (8.04 ppm) was obtained in the soil with zinc application applied to the leaf twice, in the Pre-flowering Period + Flowering Period, and the highest value was obtained with the application of zinc at a dose of 400 g da^{-1} to the soil (25, 21 ppm). Menon and Rahman [20] reported that manganese uptake decreases with zinc applications, and these two elements are antagonistic. Similar findings were also reported by Prasad et al [24].

It can be observed that there are statistical differences at the 1% significance level between the applications in terms of copper (Cu) content in the soil, and values varied between 1.76 to 4.02 ppm. The lowest Cu content obtained with zinc applied to the leaf twice during Pre-Flowering Period + Flowering Period (1.76 ppm), while the highest Cu content obtained with zinc applied to the leaf 3 times in Pre-Squaring Period + Pre-Flowering Period + Flowering Period (4.02 ppm). It can be observed that the differences between applications are statistically significant at the 1% level. It has been reported by Menon and Rahman [21] that the high level of zinc in the soil increases the copper deficiency, that copper and zinc are absorbed with similar metabolism, and both prevent the other's uptake in competition.

4. Conclusion

The results of soil analysis indicated that N, K, Ca, Na, Mg, and Cu values in the soil were affected by zinc application. However, there were no significant differences between zinc application methods in terms of P, Fe, Zn, and Mn values in the soil. The result of leaf analysis indicated that zinc applications did not have a significant effect on the nutrient elements in the leaves. Although there were no significant differences detected between the zinc applications in terms of leaf analysis, it was observed that the highest K, Ca, Na, Mg, Fe, Mn, and Cu values were obtained from the zinc application of 200 g da^{-1} to the soil. In the study, it was observed that the highest zinc values in the leaf and soil were obtained from the foliar application of zinc applied before squaring and beginning of flowering. It has been concluded that the application of zinc twice during the early development period of the cotton plant provides an advantage. Thus, this application can be recommended to increase the zinc content in the soil and leaves.

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