

## MORPHOLOGICAL AND BIOCHEMICAL STUDY OF ALLIUM SATIVUM L. UNDER THE SELECTED MICRONUTRIENTS

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#### Abstract

The current study was conducted to estimate the effect of Zinc and Boron on some morphological and biochemical aspect of *Allium sativum* L. (Gralic) plants samples (leaves). The effect of different Zinc and Boron concentrations of (0.5mg and 0.10mg) on garlic was investigated in this study. A pot experiment was performed on a randomized complete block design with 7 treatments in 21 blocks to investigate the effectiveness of the combined application of boron (0.5mg and 0.10mg) and Zinc (0.5mg and 0.10mg) as well as when each one was applied separately on vegetative, reproductive, physiochemical attributes in garlic at KP, Pakistan during 2020. The current experiment result indicates that the significant incensing of biochemical indexes was documented as chlorophyll a (0.730), chlorophyll b (1.2313), protein, proline, sugar, and carotenoid contents under the application of zinc in highest concentration as (9.2753>1.2313>0.7307) and their synergetic (13.003> 9.7667> and 6.6342) as compared to the control group. The increase in plant height, leaf size and weight by the application of zinc and boron was significant in all the variants and was higher by 18.60 cm>17.167cm>13.83cm<sup>2</sup>, fresh weight 7.400g>dry weight 6.266g and %germination as 84.33% and 84.00% respectively. The presence of a high amount of boron and zinc in the soil has a positive correlation and increases as well. The addition of zinc and boron to the garlic plant helped to increase its marketability. Nevertheless, there was an interaction between addition of micronutrient into soil addition (fertilizer) application, suggested that zinc and boron application alone and as well as synergetic had a profound influence on improving biochemical, morphological and as well as physiochemical parameters and increased the leaf size, weight (fresh and dry) and crop productivity in stress affected soils etc.

**Keywords:** Zinc, Boran, and Allium

## 1. INTRODUCTION

Garlic (*Allium sativum* L.) is a centuries-old crop that was first cultivated in Central Asia [1]. Garlic (*Allium sativum* L.) belonging to Alliaceae family with chromosome number  $2n=16$  [2]. Garlic's name may have derived from the Celtic word 'all,' which means pungent. [3]. The origin of garlic is reported from Central Asia (Afghanistan, West China, Russia, India etc.) and then spread throughout the world [2]. Garlic is one of the most important allium crops grown worldwide for its flavor and medicinal value, and global production has been steadily increasing in recent decades [1]. Garlic (*Allium sativum* L.) is one of Pakistan's principal spice crops. It is equally essential for its medical and cooking uses. The second important *Allium* crop after onions bulb is garlic [3]. It is an upright annual herb, 75-90 cm tall.

One and the best grown in dry and mild winters. The garlic bulb, normally containing of 8–15 cloves [4]. The cloves are supported by a disc-shaped stem that is enclosed by the dried basal sheaths of the foliage leaves [5]. The garlic contains 33 “S” compounds, 17 R-CH (NH<sub>2</sub>)-COOH (amino acid), many enzymes, and minerals like selenium. Sulfur compounds are found in higher concentration in garlic than any other *Allium* species. It has pungent smell as well as many medicinal properties are due to sulfur compounds [6]. In vegetables and particularly meat dishes, garlic is widely used to add flavor. It is often used in refined form or fresh as well as fried. The spicy taste makes it valuable [7]. Garlic (*Allium sativum* L.) is highly morphological diversified asexual crop [8]. It is used to reduce cholesterol and blood pressure as well as to combat diseases and prevent cancer [9]. It includes an amino acid that helps to lower the cholesterol level in the blood. Furthermore, the aqueous extract of garlic cloves lower cholesterol level in humans [10]. It helps in the removal of waste products and harmful free radicals from the body [11]. Boron is the most essential micro-nutrients, but it is necessary in very limited amounts. The nutrient is important in the growth of plants in cell division, carbohydrate and nitrogen metabolism and water relationship [12]. Boron application will increase the size of the bulb, the amount of bulb and the garlic yield [13]. In fresh market fruits, boron deficiency is not recognized by growers. However, deficiency of boron predominant and can cause extreme diminution and erratic fruit maturation [14]. As soil pH increases, Boron becomes less accessible to plants. Boron deficiency may also be caused by the process of adding lime to boost the absorption of other nutrients [15]. One of the seven micronutrients that are essential for crop growth is zinc. In addition to altering chlorophyll, Carbohydrate and protein synthesis, zinc plays an important role in several physiological and enzymatic activities and performs multiple catalytic functions in the plant system. Zinc deficiencies are becoming so high that in many states they are located next to P and N. In metabolic processes, the enzymatic structure, seed development and the rate of maturity in plants, zinc is also an essential micronutrient. It is important for tryptophan synthesis, which is the initiator of indole acetic acid. It also plays a major role in the metabolism of starch in plants [16].

## 2. MATERIAL METHOD

### 2.1. Treatments and experimental design

The experiment led at Botany Department, Bacha Khan University Charsadda. The soil was also collected from the experimental field. The experimental soil site was silt loamy, silt 80% and 17.5%.

Seven seeds of garlic (*Allium sativum*) were sown in pots having 2kg of soil. The diameter of the pot was 3.5 inches, placed in open environment. Zinc and boron were applied at different concentration. The seed were sown on 22 Dec 2020 and after 2 days growth were shown. The randomized complete block design (RCBD) experiment was arranged with seven treatments and three replicates. The experiment was composed of 21 pots which were divided in 7 rows and 3 columns. The detail of the pots in field was as follow.

The variable doses of zinc, boron and zinc+boron in different concentration were used.

T0 = Control (3 replicates)

For Boron

T1= Boron 0.5mg (3 replicates)

T2= Boron 0.10mg (3 replicates)

For zinc

T1= Zinc 0.5mg (3 replicates)

T2 =Zinc 0.10mg (3 replicates)

Combination of Zinc and Boron

T1 =Boron 0.5mg+Zinc 0.5mg (3 replicates)

T2= Boron 0.10mg+ Zinc 0.10mg (3 replicates)

Boron and Zinc were applied on selected plants in single form but they were applied at the time of sowing.

Plants were allowed to reach at maturity phase. Different yield and growth parameters (height of plant, (leaf length, width and area) No of leaves and % germination of plant) were verified. Statistics 8.1 was used to evaluate the collected data.



**Fig.1. Plant growth recorded after 30 days Fig.2. Plant growth recorded after 1 week**

## **2.2 Determination of chlorophyll (a, b)**

The chlorophyll content of leaves was obtained by the approach of (Maclachlam and zalik 1963). [17].

## **2.3 Determination of protein content**

Protein content of leaves was find out by the method of Bradford (1976) [18].

## **2.4 Determination of proline content**

The nin-hydrin method was used to separate free proline from plant tissue using aqueous  $C_7H_6O_6S$  (bates et al. 1973) [19].

## **2.5 Determination of total soluble sugar content**

Calculation of soluble sugar was carried out as per the protocol of N. M. khani and R. Heidari (2008) [20].

## **2.6 Determination of carotenoid content**

Carotenoid content was determined using the method given by (K. Seth 2014) [21].

## **2.7 Elemental analysis**

As per protocol of (Model Perkin Elmer AA Analyst 700) the samples were measured by using Atomic Absorption Spectrometer at Chemistry section in Tobacco Research Station Mardan.

## **2.7.1 Plant elemental analysis**

### **2.7.2 Zinc, Iron, Copper, Magnesium, Sodium, Calcium**

Zn, Fe, Cu, Mg, Na and Ca was determined in plant we used the method of (Chapman and Pratt 1961) [22].

### **2.7.3 Boron (B)**

Boron was analyzed in plant samples via dry ashing (Chapman and Pratt, 1961), followed by colorimetry using Azomethine –H. (Bingham, 1982) [25].

### **2.7.4 Soil elemental analysis**

### **2.7.5 Zinc, Iron, Copper, Calcium Boron and Magnesium**

Zn, Fe and Cu Ca B and Mg were determine in soil we used the method of (Norvell and Lindsay 1978) [24].

## **3. RESULTS AND DISCUSSION**

### **3.1 Morphological parameters**

#### **3.1.1 Height of plant (cm)**

The highest plant was noted in treatment T4 (Zn 0.10mg) (18.600 cm) which was significantly higher than the T0 (control) (11.333cm). The result showed similarity with work of [28] that increases in zinc concentration with increase in plant height.

#### **3.1.2 Leaf length (cm)**

The highest leaf length was recorded in T4 (Zn 0.10mg), (17.167cm), which was higher than the control (T0) (11.433 cm). The results are accordance with work of [28] that leaf length significantly increased with increasing levels of zinc.

#### **3.1.3 Leaf width (cm)**

The highest leaf width was recorded in T5 (Zn + B 0.5mg) (0.8000cm), which was higher than the T0 (control) (0.4333cm). Our results showed similarity with [29]

#### **3.1.4 Leaf area (cm<sup>2</sup>)**

In T4, (Zn 0.10mg) the maximum leaf area was achieved (13.833cm<sup>2</sup>), which was considerably larger than the control T0 (4.8833cm<sup>2</sup>). Our results were according to the results of [30] that the concentration of zinc increase leaf area.

#### **3.1.5 No of leaves**

Results showed that maximum no of leaves in treatment T4 (Zn 0.10mg)(6.6667), while the T0 (control) showed the minimum value (4.6667). Our results were according to [28].

#### **3.1.6 Fresh weight (g)**

Maximum fresh weight was shown in T4 (Zn 0.10mg), (7.4000g) while in T0 (control) the fresh weight was (3.5667g). Our results were according to [31].

### 3.1.7 Dry weight (g)

The highest increase in dry weight was observed in T4 (Zn 0.10mg), (6.2667g), which was significantly higher than the control T0 (2.7000g). Our consequences were according to [31].

### 3.1.8 Percent germination

The rate of germination were highest at treatment T1 (B 0.5mg),(84.333%) and T4 (Zn 0.10mg), (84.000%) While minimum germination in T0 (control) (42.000%). Our finding showed that boron and zinc has positive effect on percent germination [32].

**Table: 1 Allium sativum L. morphological data**

Treatments	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	No of leaves	Fresh weight (g)	Dry weight (g)	% germination
T1 (B 0.5 mg)	18.000 AB	16.333 AB	0.6000 BC	10.867 ABC	6.0000 AB	6.1000 AB	5.7667 A	84.333 A
T2 (B 0.10 mg)	16.900 AB	14.400 AB	0.5333 BC	7.2600 ABC	6.0000 AB	2.8333 B	3.9000 C	56.000 C
T3 (Zn 0.5 mg)	13.333 BC	12.167 BC	0.4667 C	5.6333 BC	6.3333 A	4.2667 AB	4.0333 C	56.000 C
T4 (Zn 0.10 mg)	18.600 A	17.167 A	0.7000 AB	13.833 A	6.6667 A	7.4000 A	6.2667 A	84.000 A
T5 (B +Zn 0.5 mg)	16.667 AB	15.000 AB	0.8000 A	11.633 AB	5.6667 AB	3.8000 AB	5.9000 B	71.000 B
T6 (B +Zn 0.10 mg)	13.833 ABC	11.433 BC	0.5000 C	5.3000 BC	6.0000 AB	4.4333 AB	3.1000 D	72.000 B
T0 (control)	11.333 C	9.0000 C	0.4333 C	4.8833 C	4.6667 B	3.5667 AB	2.7000 E	42.000 D
LSD value	2.4240	2.3216	0.0816	3.1234	0.6424	1.8732	0.1155	0.9920

## 3.2 Results of biochemical analysis

### 3.2.1 Chlorophyll a and b" content (mg/g)

According to the statistical data the chlorophyll "a" and chlorophyll "b " content varied significantly. The highest concentration of chlorophyll "a" in treatment T4 (Zn 0.10 mg) (0.7307), which were significantly higher than the T0 control (0.1217). Where as in chlorophyll "b" the highest concentration was also obtained in T4 (Zn 0.10mg) (1.2313), which was also higher than the T0 control (0.2957). It means that Zn 0.10mg increased both chlorophyll "a" and "b". Our results were according to the results of [33].

### 3.2.2 Protein content (µg/g)

The result showed that the maximum protein content has been reported in T4 (Zn 0.10mg) (9.2753) and minimum protein content has been reported in T0 (control), (3.4057). The results was supported by [30]

### 3.2.3 Proline content ( $\mu\text{g/g}$ )

The highest proline content was obtained in T5 (B+Zn 0.5mg) (9.7667), while the lowest protein content was observed in T0 (control) (3.1010). According to [108].

### 3.2.4 Sugar content ( $\mu\text{g/g}$ )

According to the statistical outcomes maximum sugar concentration in T5 (B+Zn 0.5mg), (13.003) while the minimum sugar content in T0 (control) (8.5340). Our results supported by [30]

### 3.2.5 Carotenoid content ( $\mu\text{g/g}$ )

The statistical results described that maximum Caritenoid content in T4 (Zn 0.10mg) (6.6342), while minimum Caritenoid content was detected in T0 (control) (3.1633). According to [35].

**Table 2: Biochemical analysis data of *Allium sativum* L.**

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Protein ( $\mu\text{g/g}$ )	Proline ( $\mu\text{g/g}$ )	Sugar ( $\mu\text{g/g}$ )	Carotenoid ( $\mu\text{g/g}$ )
T1(B 0.5mg)	0.3000 B	0.4037 D	8.0900 B	3.9460 D	10.117 D	4.3793 CD
T2(B 0.10mg)	0.6570 A	0.3047 E	3.7280 C	9.1283 B	9.0867 E	4.1343 D
T3(Zn 0.5mg)	0.2973 B	1.0983 B	3.4400 D	3.5590 DE	11.570 C	4.6960 BC
T4(Zn 0.10 mg)	0.7307 A	1.2313 A	9.2753 A	9.7533 AB	9.1450 E	6.6342 A
T5(B+Zn 0.5mg)	0.3660 B	0.3127 E	3.3297 D	9.7667 A	13.003 A	4.5550 C
T6(B+Zn 0.10 mg)	0.3990 B	0.9470 C	3.4607 D	5.4873 C	12.433 B	4.9613 B
T0 (control)	0.1217 C	0.2957 E	3.4057 D	3.1010 E	8.5340 F	3.1633 E
LSD value	0.0677	0.0277	0.0751	0.2953	0.1901	0.1707

## 3.3 Effect of different doses of zinc and boron on nutrients composition of dry *Allium sativum* L.

### 3.3.1 Zinc (Zn)

Evaluation of zinc content in dry garlic was recorded in T4 (Zn 0.10mg), (0.1073) while minimum in T0 (control) (0.0601), According to [36].

### 3.3.2 Calcium (Ca)

The statistical analysis data showed that higher calcium concentration was recorded in T6 (B+Zn 0.10mg), (1.8026) while T0 (control) shows lowest result (0.1049). Our results were also supported by [34]

### 3.3.3 Magnesium (Mg)

The statistical examination shows that lowest magnesium was recorded in T3 (Zn 0.5mg), (2.1629) while highest was recorded in T0 (control) (2.9114). Our results were according to [37] [38].

### 3.3.4 Boron (B)

Highest value was recorded in T2 (B 0.10mg), (0.8763), while T0 show the lowest value as compared to other treatment (0.0810). Our results were according to [31] [39].

### 3.3.5 Sodium (Na)

The positive response showed by T0 (control), (2.6675) while the lowest result showed by T4 (Zn 0.10mg), (2.2131). The same result was reported in [40]. The concentration of zinc increases with the decreased sodium concentration in plant.

### 3.3.6 Iron (Fe)

Statistically analyzed data showed that highest result in T1 (B 0.5mg), (0.4903) while the lowest value in T0 (control) (0.1893). This result shows that increasing boron concentration increase iron content. The same results were found [41] that boron increases the iron content in plant.

**Table 3: Elemental analysis of dry garlic (*Allium sativum* L.)**

Treatments	Zinc	Calcium	Magnesium	Boron	Sodium	Iron
T1(B 0.5mg)	0.0604 C	0.8083 C	2.3724 C	0.3340 C	2.5136 AB	0.4903 A
T2(B 0.10mg)	0.0586 C	1.5898 B	2.6237 B	0.8763 A	2.5143 AB	0.4229 AB
T3(Zn 0.5mg)	0.0566 C	0.8160 C	2.1629 D	0.5721 B	2.4337 B	0.4253 AB
T4(Zn 0.10mg)	0.1073 A	0.7251 C	2.6998 B	0.3169 CD	2.2131 C	0.3927 AB
T5(Zn+B 0.5mg)	0.0892 AB	0.7232 C	2.5803 B	0.6559 A	2.4683 B	0.4011 AB
T6 (Zn +B 0.10mg)	0.0666 BC	1.8026 A	2.3478 C	0.8577 A	2.5726 AB	0.3961 AB
T0 (control)	0.0601 C	0.1049 D	2.9114 A	0.1580 D	2.6675 A	0.1893 B
LSD value	0.0124	0.0671	0.0601	0.0810	0.0823	0.1381

## 3.4 Effect of zinc and boron on physiochemical properties of soil

### 3.4.1 P.H

Result indicates that the maximum PH has been recorded in T4 (Zn 0.10mg), (8.4000) while minimum PH has been recorded in T0 (control) (7.9667). The results showed similarity with [46] the influence of pH on Zn adsorption indicated that low pH decreased Zn adsorption more in sandy soils than in soils high in colloidal-size components.

### 3.4.2 Electrical conductivity (E.C)

The results show that the highest E.C has been recorded in T4 (0.10mg), (1728.0) while the lowest E.C has been recorded in T1 (B 0.5mg), (2.2600). Our result supported by [42]



### **3.4.3 Soil organic matter**

According to the results, the highest organic matter c was found in treatment T4 (Zn 0.10mg), (1.1720) and minimum in T0 (control) (0.0343). According to our outcomes [43].

### **3.4.4 Zinc (Zn)**

The highest zinc content in treatment T6 (Zn+B 0.10mg), (1.2044) while the lowest value in treatment T0 control (0.0423). (Table: 4). the amount of Zn in the soil has a substantial impact on the amount of Zn accessible. These results were in accordance to [44].

### **3.4.5 Calcium (Ca)**

The statistical data show that maximum calcium was recorded in treatment T6 (Zn+B 0.10mg), (2.4138) while minimum in treatment T0 (0.0574). Outcomes showed that the treatment of graded level of boron and zinc at both stages had a considerable impact on calcium status [44].

### **3.4.6 Magnesium (Mg)**

The highest result showed in T6 (Zn + B 0.10mg), (2.7048) and the lowest result showed in T0 (control) (2.0347). According to [44].

### **3.4.7 Boron (B)**

The statistical analysis data show that the highest result was observed in T2 (B 0.10mg), (1.7162) and the lowest result was observed in T0 (control) (0.3017). Our results indicate that increase boron concentration in soil increase the boron content in plant [39] [44].

### **3.4.8 Sodium (Na)**

The sodium concentration of the soil decreases as micronutrients are applied, with the highest in T0 control (2.8082), while the lowest value in T4 (Zn 0.10mg), (1.5343). Zinc application reduces sodium levels in soil, according to [38].

### **3.4.9 Iron (Fe)**

The most iron was found in T5 (B+Zn 0.5mg), (0.9051), while the least was found in T0 (control) (0.3265). Our results were against to [45].

**Table 4: Effect of zinc and boron on physiochemical properties of soil**

Treatments	P.H	E C	Organic Matter	Zinc	Calcium	Magnesium	Boron	Sodium	Iron
<b>T1 (B 0.5mg)</b>	8.2000 ABC	2.2600 E	0.0694 B	0.2663 B	1.2334 BC	2.3576 B	1.4156 B	2.5143 B	0.7017 B
<b>T2 (B 0.10mg)</b>	8.1000 BC	2.4633 E	0.0345 B	1.1865 A	1.3402 B	2.2782 B	1.7162 A	2.6643 AB	0.6045 BC
<b>T3 (Zn 0.5mg)</b>	8.2000 ABC	933.00 C	0.0345 B	0.0878 CD	1.1153 C	2.3118 B	0.6469 C	2.5317 B	0.5206 BC
<b>T4 (Zn 0.10mg)</b>	8.4000 A	1728.0 A	1.1720 A	0.1862 BC	0.7600 D	2.3427 B	0.5706 C	1.5343 B	0.6803 B
<b>T5 (Zn +B 0.5mg)</b>	8.3667 AB	927.00 D	0.0345 B	1.1744 A	2.3706 A	2.2706 B	0.7648 C	2.6516 AB	0.9051 A
<b>T6 (Zn +B 0.10 mg)</b>	8.3000 AB	1243.0 B	0.8387 A	1.2044 A	2.4138 A	2.7048 A	0.6496 C	2.6451 AB	0.6375 B
<b>T0 (control)</b>	7.9667 C	3.6200 E	0.0343 B	0.0423 C	0.0574 E	2.0347 C	0.3017 D	2.8082 A	0.3265 D
<b>LSD value</b>	0.1369	0.6417	0.1782	0.0414	0.0627	0.0558	0.0824	0.0716	0.0264

#### 4. CONCLUSION

On the basis of results obtained from the present investigation, it can be concluded that various parameters of *Allium sativum* L. have been increases under zinc and boron stress; plant height, leaf length, leaf area, no of leaves, fresh weight, dry weight and percent germination increases with zinc stress as compared to boron. The biochemical data shows that chlorophyll a and b, protein and carotenoids increases with zinc stress, while the combination of zinc and boron increases sugar and proline concentration. The elemental analysis shows that application of zinc increases zinc concentration, while the treatment of zinc and boron increases calcium and boron and the application of boron (0.5mg) increase iron in garlic plant. It was concluded that as compared to boron, zinc increases many parameters.

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