Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2020, 10(4), 33-42, doi: 10.15421/2020_164

ORIGINAL ARTICLE

Effect of iron, zinc and boron on the growth, physiological state, productivity and storability of *Allium Sativum* L.

V. Yatsenko*, O. Ulianych, Y. Yanowskiy

Uman National University of Horticulture, Faculty of Horticulture, Ecology and Plants Protection, Department of Vegetable Growing, 1 Instytutska St, UA20300 Uman, Ukraine

**Corresponding author E-mail: slavikslavin16@gmail.com*

Received: 13.07.2020. Accepted 14.08.2020

An experiment was conducted to examine the effect of different micronutrients on garlic yield. We use the Iron (10, 20 and 30 kg ha^{-1}), Zinc (1, 2, and 3 kg ha^{-1}), and Boron (2, 4, and 6 kg h^{-1}) for the fertilizing patterns. Our results showed the significant increasing of chlorophyll content under the application of iron in 10 and 20 kg ha^{-1} by 12.0 and 16.4% respectively. The content of chlorophyll was the least significant (10.9%) at boron application of 6 kg ha^{-1}. The activity of the enzyme was the highest with optimal norms of micronutrients. We registered the highest increases for the catalase (CAT) and glutathione S-transferase (GST). Zinc has the greatest effect on the formation of bulb mass. The increase in bulb weight by the application of zinc was significant in all the variants and was higher by 14.3–20.0% to control. Fertilization with microelements in the minimal and optimal norms contributed to a significant increase in yield, whereas the application of maximal norms led to a decrease in productivity in general, except boron, where plant productivity increased with increasing norms, from which it can be concluded that garlic needs were insufficient 6 kg ha^{-1} is not the maximum. With fertilization with Fe of 10 and 20 kg ha^{-1} the yield increased by 4.26 and 4.85 t ha^{-1}; the application of Fe in 30 kg ha^{-1} caused increase in garlic yield by 3.70 t ha^{-1}. Zinc fertilization had the best effect on the yield increase (4.25–5.12 t ha^{-1}). A significant increase in the yield (3.26 t ha^{-1}) was observed after maximum norm of boron fertilization. The application of zinc and boron contributed to the extension of the marketability of garlic bulbs to 210 and 220 days in warm storage and up to 240 and 260 days in cold storage. We registered cloves mass germination after 210 days in warm storage and after 260 days in cold storage. Bulbs from control and Fe fertilzied groups germinated after 120 and 180 and 190–210 days towards the warm and cold storage regime respectively.

Key words: antioxidant enzyme activity, chlorophyll, garlic, micronutrients, weight, yield.

Introduction

Garlic (*Allium sativum* L.) has accompanied mankind along his long history as a vegetable and medical plant for more than 4,000 years (Maas & Klaas 1995, Lallemand et al. 1997, Fritsch & Friesen 2002, Simon & Jenderek 2003, Volk et al. 2004). It is commonly used as a spice or in the medicinal purposes. In Ukraine, it has been generally cultivated for both local consumption and export. The area and production of garlic in Ukraine are about 25×10^3 ha, with an average yield of 9.6 t ha^{-1} (https://www.tridge.com/intelligences/garlic/UA/production/).

Micronutrients play an active role in the plant metabolic process from cell wall development to respiration, photosynthesis, chlorophyll formation, enzymes activity, nitrogen fixation etc. Micronutrients work as a co- enzyme for a large number of enzymes (Ding et al. 2004, Lawrence et al., 2011, Poldma et al., 2011, Ameri et al., 2012, Aminifard et al., 2012). It also plays an essential role in improving yield and quality and highly required for better plant growth and yield of many crops (Alam et al., 2010). Soil application of micronutrients during crop growth (onion) was successfully used for correcting their deficits and improving the mineral status of plants as well as increasing the crop yield and quality (Jawaharlal et al., 1986; Thakare et al., 2007; Ali, 2013).

Boron deficiency in fresh fruit market is often not documented by the growers. Boron deficiency, however, is widespread and can serious yield diminution and irregular ripening of fruit (Adams, 1978). Boron becomes less accessible to plants as soil pH increases (Bunt, 1956). Therefore, the practice of applying lime to improve the uptake of other nutrients can cause B deficiency (Fleming, 1980).

Zinc is one of the seven micronutrients vital for the crop growth. Zinc plays a considerable role in various enzymatic and physiological activities and performs many catalytic functions in plant system besides alteration of carbohydrates, chlorophyll and protein synthesis. Deficiencies of zinc become so extensive that it ranks next to N and P (Takkar and Randhawa, 1980). Zinc is also an important micronutrients concerned in metabolic processes, enzymatic system, seed production and rate of maturity in plants. It is essential for synthesis of tryptophan, which is originator of indoleacetic acid. It also plays an important role in starch metabolism in plants (Alloway, 2008). Zinc is crucial for plant growth because it controls the synthesis of indoleacetic

acid, which noticeably regulates plant growth and also active many enzymatic reactions which is necessary for chlorophyll synthesis and carbohydrate formation (Vitosh, 1994).

The decisive role in increasing the plant productivity belongs to the biochemical protection systems. Among them, much attention is paid to elucidating of antioxidant enzymes in metabolism and the formation of plant resistance to abiotic factors (Polesskaya et al, 2006; Turpaev, 2002). Antioxidant enzymes are involved in the neutralization of reactive oxygen, the accumulation of which in the plant cell initiates the oxidative destruction of membrane structures (Polesskaya et al, 2006, Tarchevskiy, 2002).

The aim of our study was to determine the optimal microelement norms for the garlic in Right Bank Forest-Steppe of Ukraine and to qualify the impact of iron, zinc and boron different concentrations on the growth, physiological processes, yield, and storage quality of garlic.

Materials and methods

Our research was carried out in 2017–2019 on the experimental field of the Department of Vegetable Growing of Uman National University of Horticulture (Right-Bank Forest Steppe of Ukraine) in accordance with national methods (Bondarenko and Yakovenko, 2001; Ulianych et al., 2019; 2020).

The soil was black, puddle, heavy loam with a well developed humus horizon (about 2.9 % of humus) in the deep of 40–45 cm. Soil pH was determined in water (soil water ratio 1:1). Electrical conductivity (ECe) of the soil suspension was measured using the conductivity meter. The P, K and Zn were determined by AB-DTPA method (Ryan et al, 2001).

Table 1. Chemical properties of soil

Parameter	Value
organic carbon, %	2.2
pH	6.0–6.2
Extractable P (AB-DTPA, mg/kg	102
Extractable K (AB-DTPA), mg/kg	123
NO_3-N, mg/kg	64
Fe (AB-DTPA), mg/kg	0.43
Zn (AB-DTPA), mg/kg	0.79
B (AB-DTPA), mg/kg	0.28

Planting was carried out by the scheme of 45×6 cm within 05.10-10.10. The total area for the experiment was 400 m^2, for the plot it was 100 m^2, and for the sampling – 10 m^2. The experiment was perfprmed as a Factorial Randomized Block Design with four replications.

Single factor experiment consisted of fertilization Iron sulphate ($FeSO_4$), Zinc sulphate (Zn_2SO4), Boric acid (H_3BO_3), and the control (without fertilizer). Fertilizers were applied before plowing. Micronetrients were used according to recommended norm of macronutrients ($N_{110}P_{70}K_{70}$) for the Right-Bank Forest-Steppe of Ukraine (Likhatsky et al., 2017).

This experiment included the following fertilization:

1. Control (without fertilizer).
2. Iron sulphate ($FeSO_4$). Fe 10, 20, 30 kg ha^{-1}.
3. Zinc sulphate (Zn_2SO_4). Zn 1, 2, 3 kg ha^{-1}.
4. Boric acid (H_3BO_3). B 2 , 4, 6 kg ha^{-1}.

The leaf length and width, leaf blade area and total leaf area per plant on the 60th day after the planting (DAP) were determined; the plant height and the number of leaves per plant were calculated, and the leaf blade area was determined by a calculated (linear) method, using the parameters of length and width of the leaf by the formula:

$$Sn=0.67\times ab$$

Where: Sn – single leaf area, cm^2, a – the largest leaf width, cm, b – leaf length, cm, 0.67 is the leaf configuration coefficient.

We studied the effect of different norm of micronutrients on the enzymes activity, productivity of plants, pigments contents in leaf, vitamins B complex and vitamin C of garlic cloves, and storability.

Plant material. Garlic (*Allium sativum* L.) cv. Lyubasha. **Assimilating pigment content** was determined by spectrophotometric method (Ermakov et al., 1987).

Activity measurements of antioxidant enzymes.

Enzyme activities were determined, 10 days after spraying plants by organic acids solutions. A one g of plant tissue from control and treated plants was homogenized on ice in 4 ml extraction buffer (50mM^{-1} phosphate buffer pH 7.0, containing 1mM EDTA, 1mM phenylmethylsulfonyl fluoride and 1% polyvinylpolypirrolidone). The homogenate was centrifuged for 25 min at 15,000×g^{-1} and 4 °C. The supernatant was used for enzyme activity assays. The means ± SD were calculated from the data of at least 3 independent measurements. SOD activity was determined spectrophotometrically by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa et al. 1981). One unit (U^{-1}) of SOD was the amount that causes 50% inhibition of NBT reduction in light. The enzyme activity was expressed in terms of specific activity (U mg $protein^{-1}$). CAT activity was determined by the decomposition of H_2O_2 which, in turn, was measured by the decrease in absorbance at 240 nm (Upadhyaya et al. 1985). One U equals the amount of H_2O_2 (in μmol)

decomposed in 1 min. POD activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Upadhyaya et al. 1985). The amount of enzyme producing 1 µmol min^{-1} of oxidized guaiacol was defined as 1 U. GR activity was determined by measuring the absorbance increment at 412 nm when 5.5 dithiobis(2–nitrobenzoic acid) (DTNB) was reduced by GSH, generated from glutathione disulfide (GSSG) (Smith et al. 1988). The specific activity was calculated as the amount of reduced DTNB, in µmol min^{-1} protein mg, ε_{420} = 13.6 mM cm^{-1}. GST activity was determined spectrophotometrically by using an artificial substrate, 1–chloro–2.4–dinitrobenzene (CDNB), according to Habig et al. (1974). One U is the amount of enzyme producing 1 µmol conjugated product in 1 min, ε_{340} = 9.6 mM cm^{-1}. The protein contents of the extracts were determined by the method of Bradford (1976).

Bulb dry matter (%). The average dry matter weight (g^{-1}) of bulbs after curing were measured by drying 10 randomly sampled bulbs in an oven with a forced hot air circulation at 70°C until a constant weight was obtained. The percentage of bulb dry matter was calculated by taking the ratio of the dry weight to the fresh weight of the sampled bulbs and multiplying it by 100.

Determination of content of vitamins B complex. A 50 g sample was cut into small pieces and extracted with 0.1 NHCL (sodium chloride) on the water bath at suitable temperature and time. All extracts were filtered through 0.40 micron filter and taken into 100 ml^{-1} volumetric flask which was added up for mobile phase.

The standard preparation: stock of standard (Sigma Aldrich Analytical grade Reagent) prepared by dissolving 0.01 g^{-1} of each standard in 100 ml of mobile phase followed by successive dilutions.

High-performance liquid chromatography (HPLC). *Analysis* of HPLC (Shimadzu, Model Prominence 20A) equipped with UV detector and Supelco Discovery Cis18 column (25 -cm^{-1} in length and 0.45-cm^{-1} internal diameter) was used. Mobile phase was 50 m MK_2HPO_4 and MeOH (70:30) at 1 ml min^{-1} flow rate and 10µL^{-1} of each sample/standard was injected and monitored at UV 254 nm.

Analysis of vitamin C. Lyophilized samples (each 0.2 g) were ground and added to 30 mL of 3 % metaphosphoric acid solution and homogenized at 11,000 rpm for 2 min using a T25 basic ULTRA–TURRAX homogenizer (IKAWerke GmbH & Co. KG, Staufen, Germany). The volume was made up to 50 mL with 3 % metaphosphoric acid solution. The extract (2 mL) was centrifuged at 12,000 rpm for 3 min, and the supernatant filtered through a 0.45 lm polyvinylidene difluoride (PVDF) membrane filter (Whatman I nternational Ltd., Maidstone, UK). All samples were immediately analyzed using an HPLC system, equipped with a PU (2089 pump), an AS (2057 auto injector), and a MD (2010 UV) with variable wavelength detector (JASCO Corp., Tokyo, Japan). Separation was carried out in a Crest Pak C18S column (15094.6 mm, i. d., 5 lm, JASCO Corp.), and the isocratic elution was carried out with 0.1 % trifluoroacetic acid in distilled water as a mobile phase for 15 min (flow rate 0.8 mL min^{-1}). The peak was read at 254 nm using an UV detector and quantification was determined via external calibration against ascorbic acid.

Garlic storage regimes. During the storage period, the indicators of air temperature and relative humidity in the warm storage regime were relatively stable, and in the cold – strictly controlled and remained at the same level throughout the period (Fig. 1).

Statistical analysis. The validity of the research and significance of the differences between the mean values of the variables examined were evaluated by the dispersion and correlation analysis (Ehrmantraut et al., 2000). For the food and chemical composition, three samples were analayzed in three replicates. The results were presented as averages and standard deviations. The antioxidant activity and chemical composition were analyzed and considered significant at $p \leq 0.05$ (for the yield and bulb weight), and 0.01 (for enzyme activities, pigments content in leaf, vitamins B complex and vitamin C in cloves, dry matter).

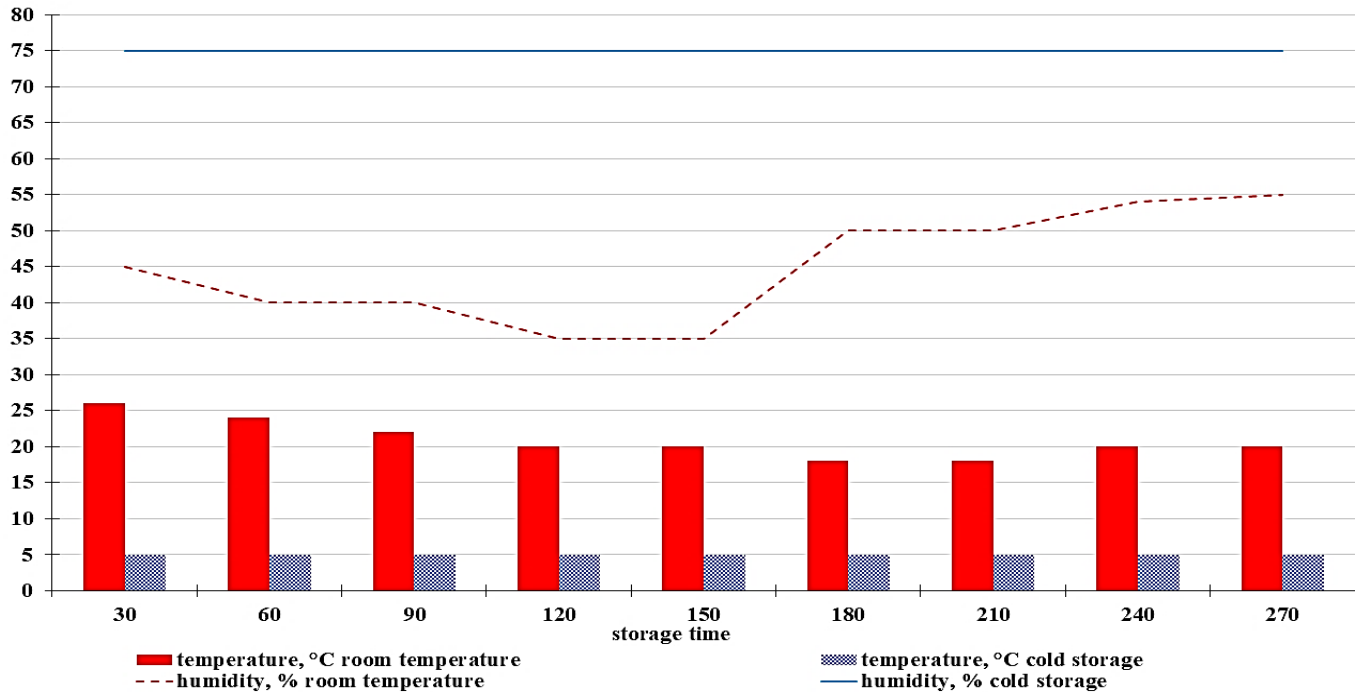

Fig. 1. Air temperature and humidity under the room conditions and cold storage

Results

Our results proved that iron application of 20 kg ha^{-1} is optimal for the garlic growth (Table 2). Thus, after the iron application of 30 kg ha^{-1} the height of plants was higher by 7.8 %. The same tendency was observed for the application of zinc, but the increase in the garlic height was 17.2; 22.1 and 15.1 % respectively towards the rates of 1, 2 and 3 kg ha^{-1}. The application of boron caused a lower increase in plant height (by 13.0, 14.6, and 16.6% to control). Application of iron at 10 kg/ha increased the leaf area by 21.8%, application of zinc at 2 kg ha^{-1} – up to 28.4 %, boron at 6 kg ha^{-1} – up to 24.2 %. At the same time, the application of iron caused the increase in the leaf index by 27 %, zinc – by 38.4%, and boron – by 38.1%. A significant increase in chlorophyll content was registered after the application of iron at 10 and 20 kg/ha – by 12.0 and 16.4% respectively; whereas the application of zinc caused the increase in chlorophyll content by 15.1, 48.0, and 30.4% regards the experimentally accepted norms. We also registered the increase of chlorophyll content by 10.9% after the application of boron.

Table 2. Plant morphological indicators under the action of different micronutrients' norms (garlic cultivar Lyubasha, here and then the average for 2017–2019)

Variant		Plant height (cm)	Number of leaves (pcs)	Leaf area (cm²)	Area of the leaves per plant, cm²	Leaf area index	Leaf's total chlorophyll content, % d.w.
Control (without fertilizer)		63.21	9.12	93.87	506.62	1.87	1.63
	10 kg/ha	68.57	9.31	108.82	599.21	2.22	1,82*
Iron	20 kg/ha	70.31*	9.51	114.33	643.51*	2.38*	1.89*
	30 kg/ha	68.13	9.31	106.52	586.53	2.17	1.76
	1 kg/ha	74.11*	9.61	117.43*	668.17*	2.47*	1.87*
Zinc	2 kg/ha	77.16*	9.82*	120.53*	700.57*	2.59*	2.41*
	3 kg/ha	72.74*	9.41	115.63*	643.76*	2.38*	2.12*
	2 kg/ha	71.43*	9.82*	109.02	633.66	2.34*	1.76
Boron	4 kg/ha	72.43*	9.82*	111.83	649.95*	2.40*	1.76
	6 kg/ha	73.68*	10.13*	116.63*	699.29*	2.59*	1.80*
LSD (0.05; 0.01**)*		*6.56**	*0.52**	*21.05**	*134.22**	*0.46*	*0.17***

* and ** statistically similar at p≤0.05 and p≤0.01.

The dynamics of the activity of antioxidant enzymes was similar to the dynamics of growth processes. The activity of enzymes was the highest at optimal norms of micronutrients. The increase in SOD activity was significant in all variants by the application of iron (4.9–11.3%), zinc (8.4–11.3%), and boron in the maximal rate (9.4%). CAT activity (Fig. 3) increased most significantly among the studied complex; CAT activity increased by 32.2–42.0 % after the application of iron, by 37.8–49.1% after the zinc and by 1.0–11.9% after the boron application. POD activity (Fig. 4) was slightly lower than the previous ones, but the most significant activation of the enzyme was registered after the application of zinc at 2 and 3 kg ha^{-1} – 33.7 and 27.2% to the control respectively. Glutathione reductaze (GR) sharply increased its activity after the application of zinc at 2 kg ha^{-1} by 51.1%, while in all experiment, this value ranged from 1.6% (boron at 2 kg ha^{-1}) to 23.5% (zinc at 3 kg ha^{-1}) (Fig. 5). The activity of glutathione S-transferase (GST) was more consistent, but we recorded the most significant increase at optimal norms of micronutrients. Among the studied enzyme complex, the activity of catalase (CAT) and glutathione S-transferase (GST) increased most significantly.

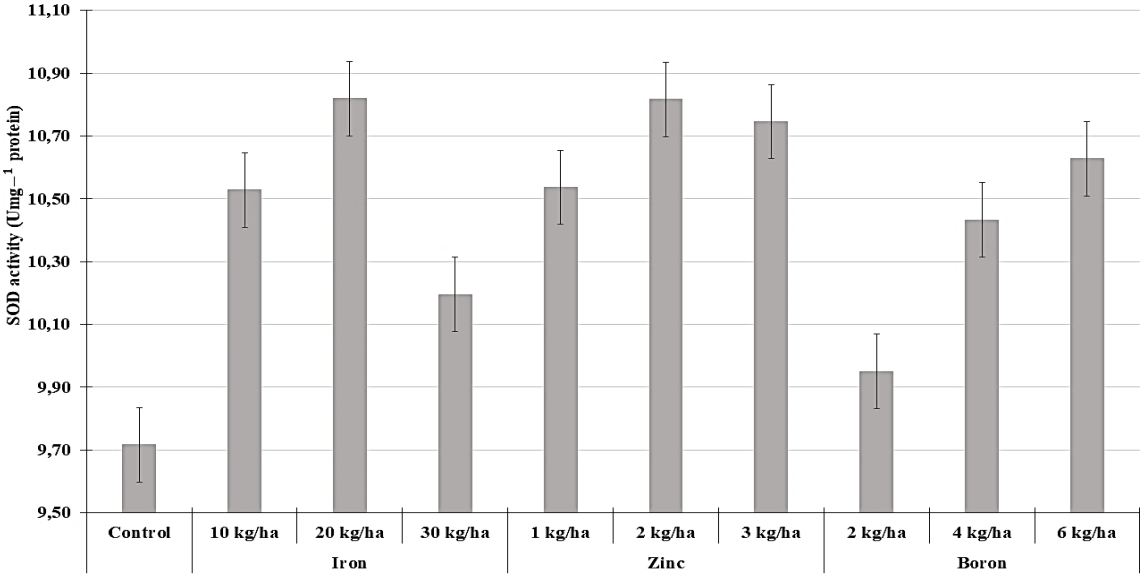

Fig. 2. Superoxide dismutase activity in leaves of the garlic plant (here and then average for 2017–2019),*LSD (0.01) = 0.84*

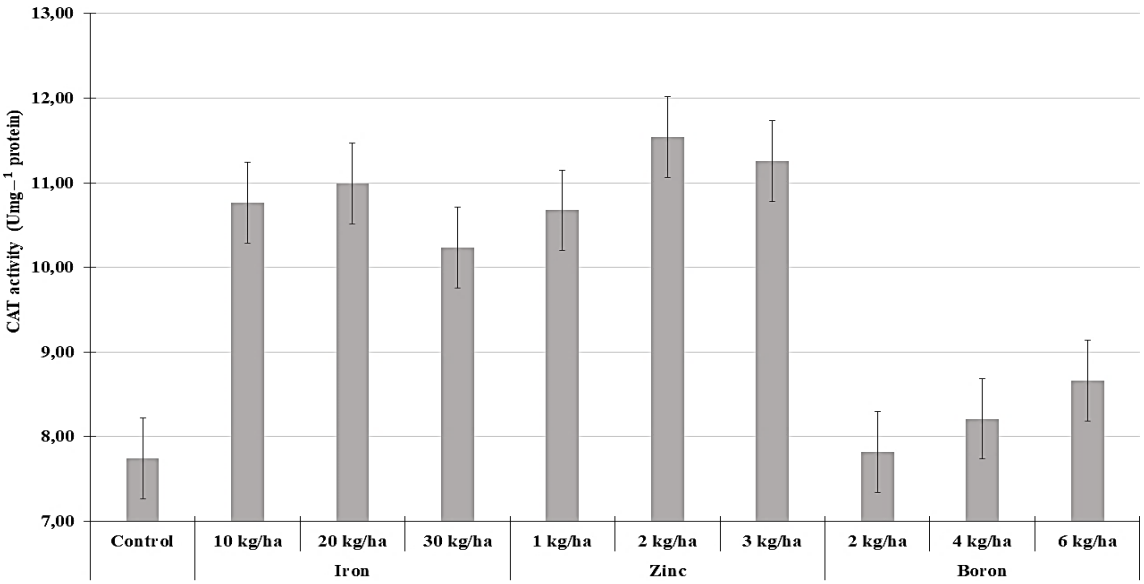

Figure 3. Catalase activity in leaves of the garlic plant, *LSD (0.01) = 1.97*

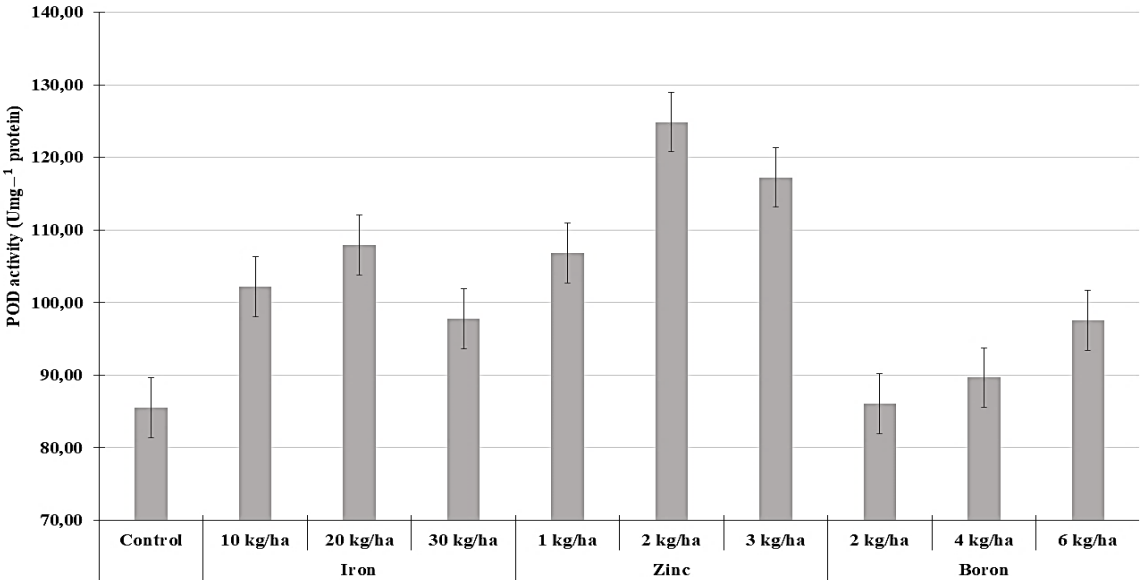

Fig. 4. Guaiacol peroxidase activity in leaves of the garlic plant, *LSD (0.01) = 11.63*

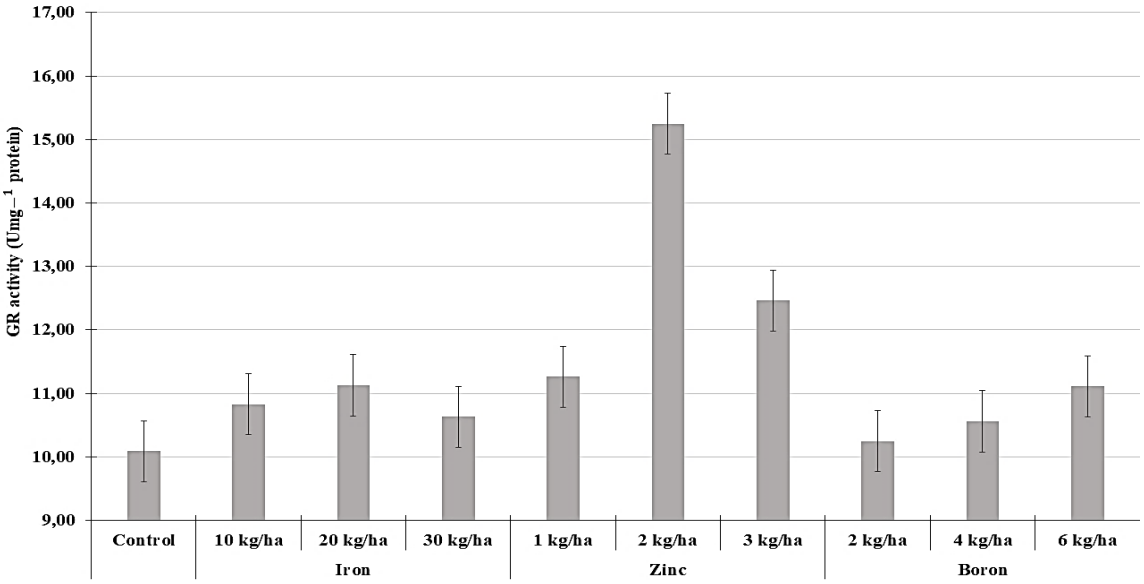

Fig. 5. Glutation reductaze activity in garlic leaves, *LSD (0.01) = 1.02*

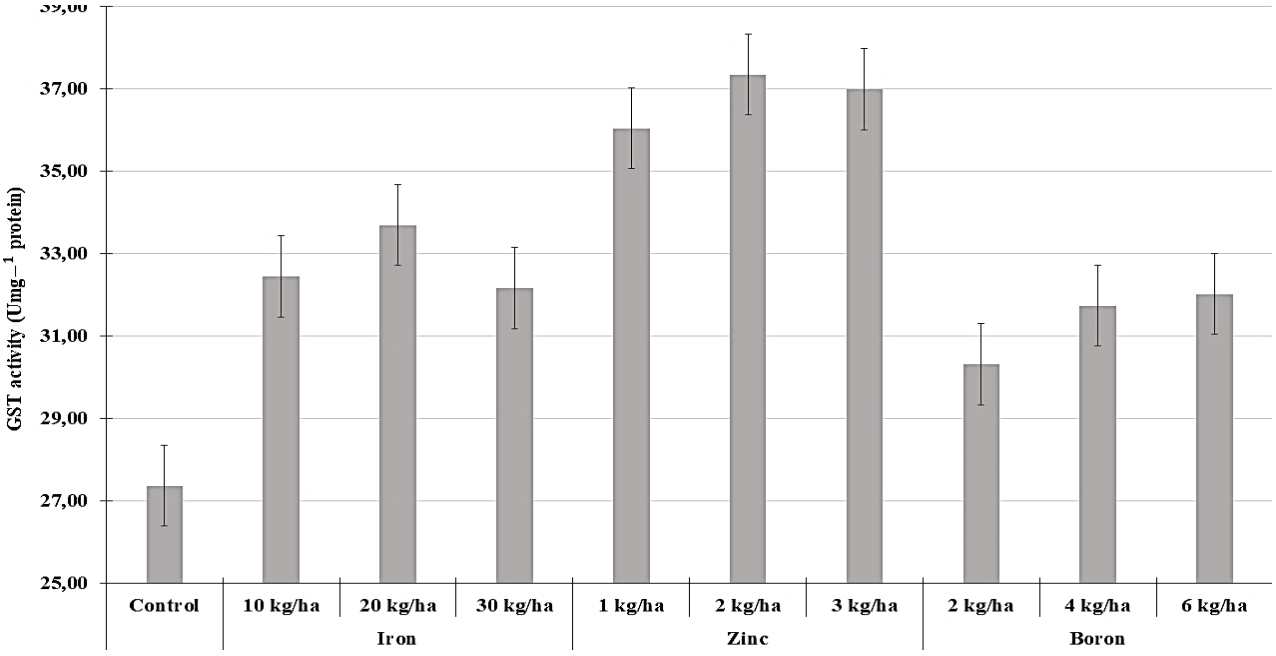

Fig. 6. Glutathione S-transferase activity in garlic leaves, *LSD (0.01) = 4.08*

The size and weight of the bulb are core values for garlic market and crop production. We revealed that zinc mostly influenced the bulb mass formation (Fig. 7). The increase in bulb weight cause by zinc application was significant in all variants (14.3–20.0% to control). Application of iron increased the bulb weight by 6.9 to 14.6%, while the application of boron increased the bulb weight insignificantly and significant increase was only at boron rate of 6 kg ha^{-1}. We determined that enzymatic activity was the highest with the use of iron and zinc in optimal norms, whereas this activity increased along with the boron rate.

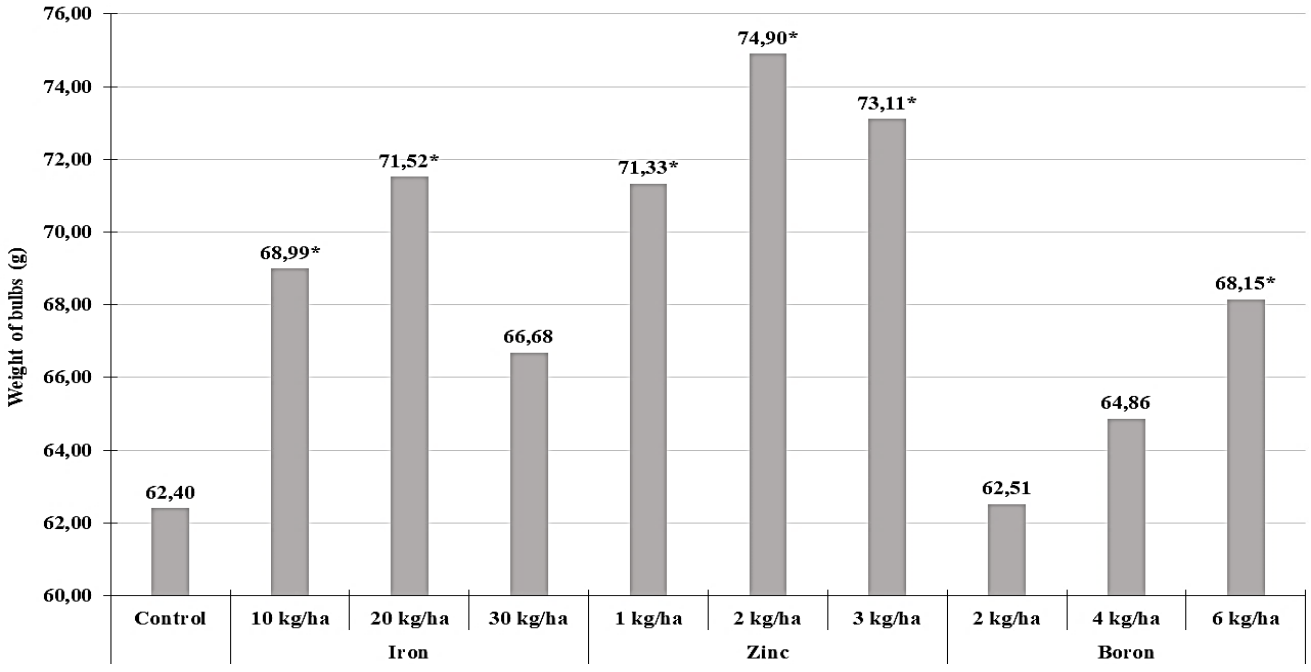

Fig. 7. Weight of garlic bulbs (g), *LSD (0.05) = 5.22.* Here and then values marked by * are statistically similar at p≤0.05)

Yield is the main parameter of crop cultivation. The application of iron at 10 and 20 kg/ha caused the yield increase by 27.9 and 31.8 %, the application of 30 kg/ha of iron caused the increase by 24.2 % (Fig. 8). The application of zinc had the most significant effect on the yield increase (27.9–33.6 % regards the control). A significant increase in the yield after the boron application was also registered (21.4%).

We noticed that along with the increase of the micronutrients' norms, the dry matter content also increased. We also registered the significant increase in dry matter after application of zinc and boron in all the variants, the application of iron, however caused less significant increase (Table 3). A significant increase in protein content was observed only after the application of zinc (11.9–15.8 %). We calcaulated the medium power connection between the dry matter content and ascorbic acid (R^2 = 0.6192) and a close relationship between dry matter content and B vitamins (R^2 = 0.8335) (Fig. 9).

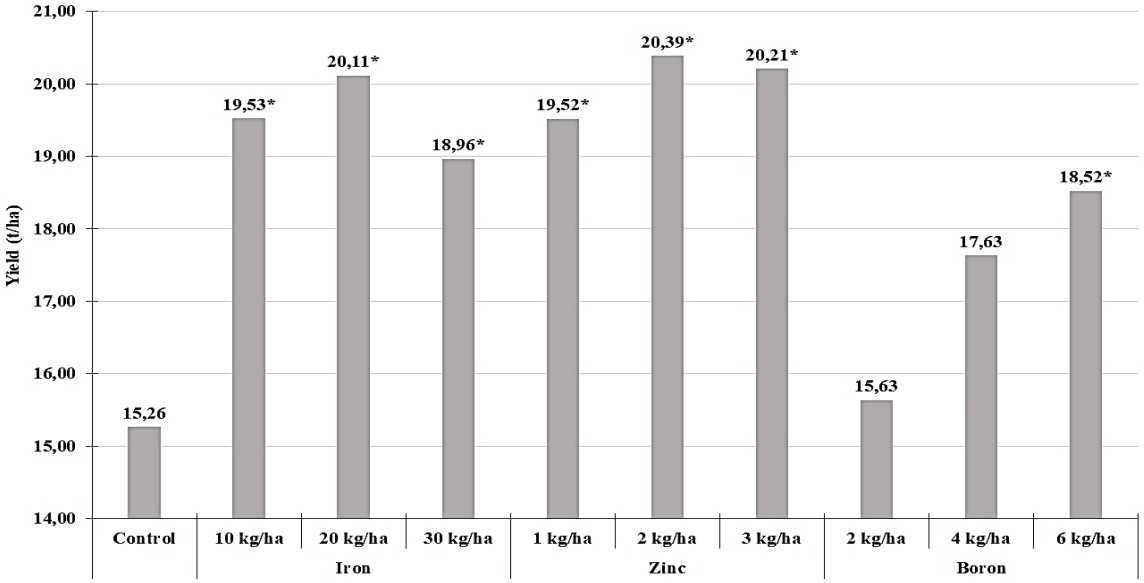

Fig. 8. Yield (t ha^{-1}) of garlic, *LSD (0.05) = 3.03.*

Table 3. Biochemical parameters of garlic cultivar Lyubasha vs. different micronutrients' norms.

Variant		Bulb's dry matter content, %	Protein content, mg/100 g	Ascorbic acid, mg/100 g	B vitamins complex, mg/100 g
Control		30.29	5.87	7.43	20.18
	10 kg/ha	30.38	6.43	7.52	21.45
Iron	20 kg/ha	30.86	6.25	7.61	21.72
	30 kg/ha	31.35	6.17	7.66	22.56
	1 kg/ha	32.95*	6.57*	7.87*	23.21*
Zinc	2 kg/ha	33.34*	6.64*	7.78	23.01*
	3 kg/ha	32.73*	6.79*	7.56	22.88
	2 kg/ha	33.10*	6.17	8.10*	23.32*
Boron	4 kg/ha	33.30*	6.21	8.33*	23,35*
	6 kg/ha	33.59*	6.28	8.36*	24,46*
LSD (0,01)		*2,12*	*0.57*	*0.42*	*2.74*

* in each column are statistically similar at p≤0.01.

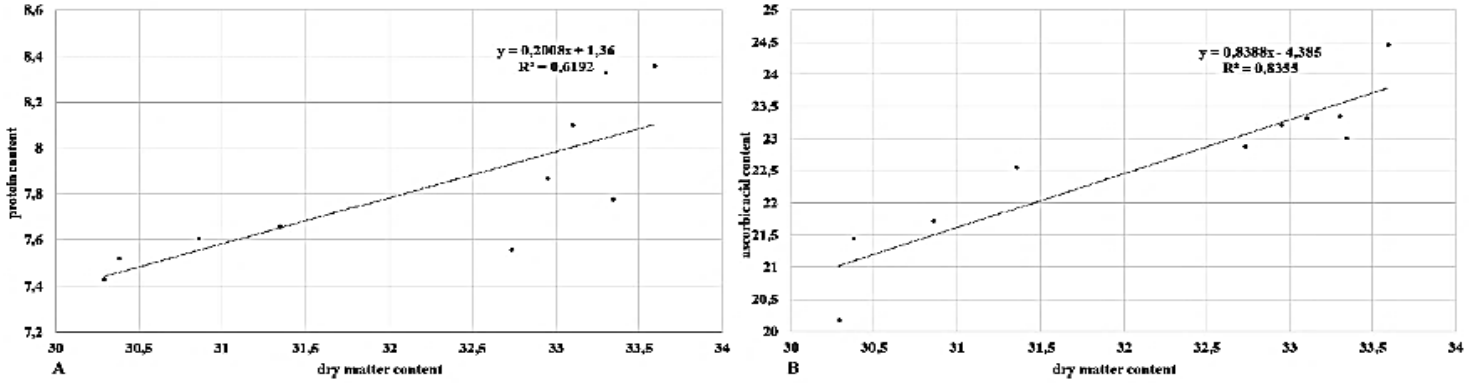

Fig. 9. Dependence of protein on the dry matter (A) and ascorbic acid content (B).

The marketability of garlic cloves depends on the duration of the dormancy period (before germination). During the storage, the marketability of garlic bulbs was on average over the years of research by 140–150 days for warm storage and up to 180–200 days for cold storage the by the application of iron. The use of zinc and boron contributed to the extension of the marketability of garlic bulbs to 210 and 220 days for warm storage and up to 240 and 260 days for cold one. After the 210 days of warm storage and after 260 days of cold storage there was a mass germination of cloves. Bulbs of the control variant and by the application of iron germinated after 120-180 and 190–210 days respectively to the variant and storage regime. Further storage (up to 270 days) shows only theoretical data on the weight loss of bulbs.

The total weight loss of the bulb during warm storage in the control was 39.8 % for cold storage – 14.7 %. By the application of iron, the percentage of weight loss decreased to 31.7–36.3% during warm storage and 10.6–13.0 % during cold storage (with an increase in the microelement norm, the weight loss of the bulb decreases). By the application of zinc in the optimal norm noted the lowest weight loss of the bulb (28.2 % for warm storage and 9.0 % for cold storage). By the application of boron, the weight loss of the bulb decreased respectively with the increase in the norm of micronutrients (Fig. 9 and 10).
There be a close relationship between dry matter content and weight loss of the bulb at the beginning and end of the storage period, where the coefficient of approximation ranged from R^2 = 0.8703–0.9286 at the beginning of storage and R^2 = 0.8815–0.8788 at the end storage period (Fig. 12).

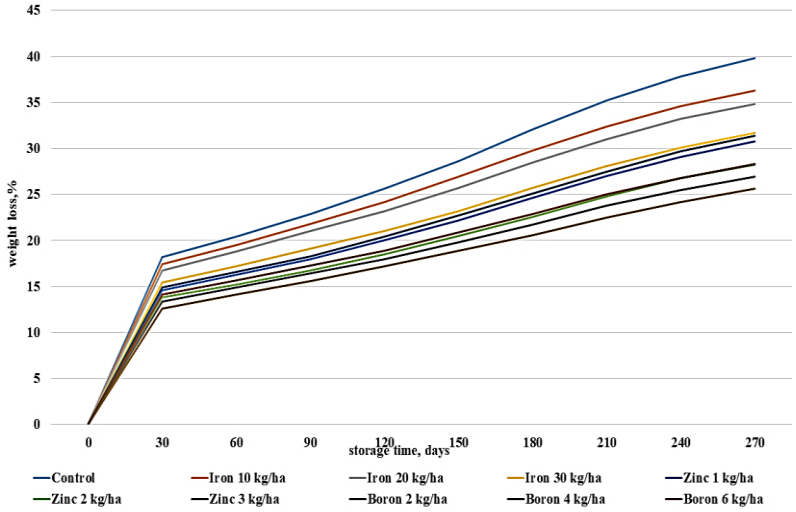

Fig. 10. Effect of different micronutrients norms on the weight loss (%) of garlic cultivar Lyubasha during 270 days of storage under the room temperature

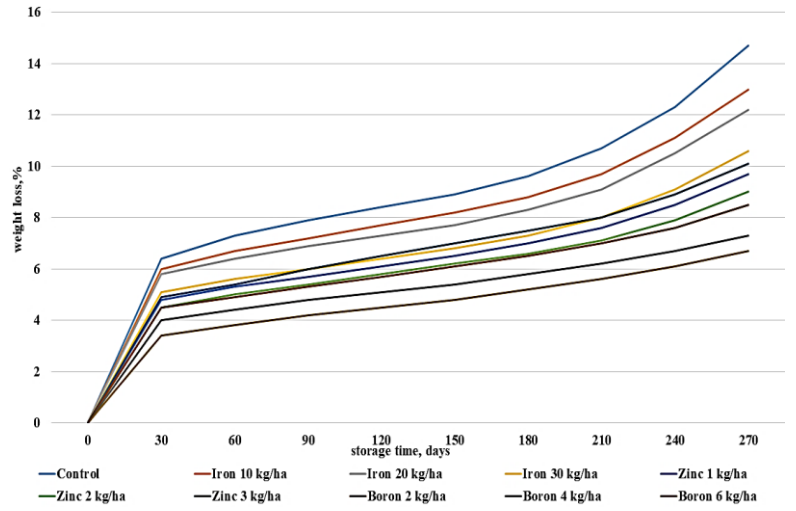

Fig. 11. Effect of different micronutrients norms on the weight loss (%) of garlic cultivar Lyubasha during 270 days of storage under the cold storage regime

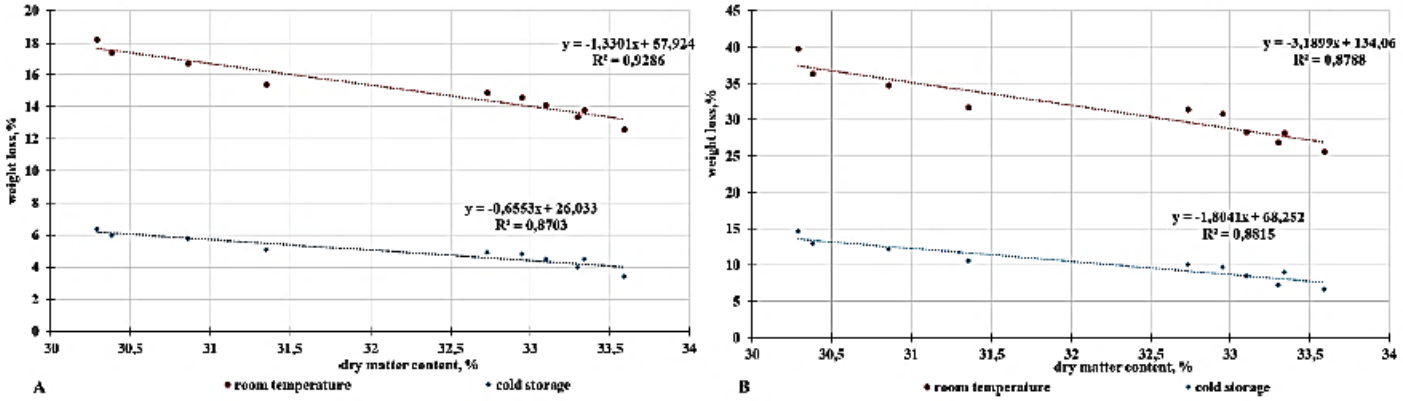

Fig. 12. Dependence of bulb weight loss during the 30 days (A) and 270 days (B) storage on the dry matter content

Disscussion

The results are in conformity with the findings of Srivastava et al. (2005), Rahohida et al. (2010), Yousuf et al. (2015) and Choudhary et al. (2014) in garlic crop. The application of micronutrients soil or foliar spray significantly influenced bulb yield of onion crop (Pramanik and Tripathy, 2017; Singh et al., 2015). Micronutrients takes active part in photosynthesis, which ultimately helps towards increase in number and weight of bulbs. Similarly, significant influence of micronutrients mixture on growth and yield parameters of garlic as reported by Srivastava et al. (2005), Rahohidas et al. (2010), Yousuf et al. (2015), and Choudhary et al. (2014) in garlic crop. Pramanik and Tripathy (2017) reported that application of micronutrients mixture have marked influence on growth and yield attributing characters of onion like plant height, number of roots per plant, diameter of bulb and bulb weight as well as bulb height.

The improvement in the nutrients use efficiency could be attributed to an enhancement in absorption and assimilation of the micronutrients which provided balanced nutrition to the crops for higher growth and thereby nutrients uptake which ultimately resulted into higher yield of the crops. The increase in content of micronutrients and their uptake by garlic crop due to use of multi-micronutrients fertilizers have also been reported by El Sayed et al. (2015) and El-Tohamy et al. (2009) in garlic, and Hamid and Mohsen (2013) in tomato crop.

Conclusion

We established that zinc and boron have the highest influence on plant growth and formation of the leaf apparatus, whereas the pigment complex reaches its maximum under application of zinc. After the boron application at maximum norm of 6 kg/ha the increase in chlorophyll content was the least significant – 10.9 %. We also found that the activity of key antioxidant enzymess (SOD, CAT, POD, GR, and GST) differed significantly and depended on micronutrient norm. The dynamics of antioxidant enzymes activity was similar to growth dynamics. The activity of enzymes was the highest at optimal norm of micronutrients. We registered that the activity of catalase (CAT) and glutathione S-transferase (GST) increased most significantly.

The most significant increase in bulb weight was influenced by the zinc, where the increase was 8.93–12.50 g. The use of zinc and boron contributed to higher accumulation of dry matter and extended the marketability of garlic bulbs up to 210 and 220 days in warm storage and up to 240 and 260 days in cold storage.

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Citation:

Yatsenko, V., Ulianych, O., Yanowskiy, Y. (2020). Effect of iron, zinc and boron on the growth, physiological state, productivity and storability of *Allium Sativum* L. *Ukrainian Journal of Ecology, 10*(4), 33-42.