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Influence of plant growth stimulants on nutrients concentration and yield responses of corn (*Zea mays*)

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ABSTRACT

To evaluate the influence of different plant growth stimulants on yield and yield components of corn (*Zea mays* L), a greenhouse experiment was conducted as a factorial arrangement based on a completely randomized design. The studied treatments (T) were in 8 levels: (T0, control, T1, foliar application of amino acid, T2, foliar application of seaweed, T3, fertigation of humic acid, T4, foliar application of fulvic acid, T5, foliar application of humic acid, T6, inoculation of plant growth-promoting bacteria, T7, foliar application of humic acid+ seaweed+ amino acid). Also, fertilizers (F) were applied in 3 levels (F0, control, F1, 75 kg, F2, 100 kg). The results showed that the studied factors significantly affected the yield and chemical composition of the plant. The highest average of grain number per rows (39.89) happened in F2+T0. The highest average of 100-grain weight (23.31 g) and ear number (8) were respectively belonged to F1+T1 and F2+T2. The highest average of Zn (52.33 mg kg^{-1}) concentration was measured under F2+ T5. Under the application of F1+ T3 the highest average of catalase (4.54 mg/Protein) was measured but the highest superoxide dismutase (105.89 mg/Protein) happened in the control treatment. Generally, application of bio-stimulants helps to increase the yield and yield components of corn by affecting the nutrients availability and plant enzymes.

Key words: Bio-stimulants, Corn, Nutrients availability, Plant enzymes

Application of plant growth stimulants in cereals nutrition is one of the useful solutions in getting to the sustainable agriculture (Zahir *et al.* 2004). Humic substances, indirectly by providing nutrients to roots, improving soil structure, increasing the soil microbial population, increasing soil cation exchange capacity (CEC) and the ability to buffer the soil pH or nutrient solution, etc., improve soil fertility (Sharif *et al.* 2002). Humic acid application through chelating calcium and magnesium elements in the soil, increases root access to these elements (Mackowiak *et al.* 2001). Recently, some fertilizers have been marketed that contain various amino acids and sometimes micronutrients. These fertilizers usually are used as foliar application (Cao *et al.* 2010). Seaweed extract has beneficial effects on plants due to the growth hormones of Cytokinin, IAA and IBA, nutrients such as iron, copper, zinc, cobalt, molybdenum, manganese, nickel, vitamins and amino acids (Taghadosi *et al.* 2012). The use of seaweed extract increases plant growth, stimulates root growth, delays aging and improves resistance to environmental stresses such as drought, salinity and temperature (Taghadosi *et al.* 2012).

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Plant growth promoting bacteria are used as inoculant, biological fertilizer, plant growth stimulants and biological control (Saadat and Ehteshami 2016). As was stated by Singh *et al.* (2004), plant growth-promoting bacteria improve vegetative growth through producing various vitamins, amino acids and plant growth stimulating hormones such as auxin, cytokinin and gibberellin, as well. Yazdani *et al.* (2009) showed that the ear weight, the number of rows, the number of grain per row and finally the yield of corn grain were increased affected by plant growth-promoting and phosphate solubilizing bacteria. Other experiments have shown that inoculation of corn seeds with azotobacter and azospirillium bacteria increased grain and biological yield (Soleymanifard *et al.* 2013). Considering the importance of corn as one of the most important crops in nutrition and industry, this study was conducted in order to study the effect of different plant growth stimulants on nutritional and yield responses of corn (*Zea mays* L.).

MATERIALS AND METHODS

In order to study the effect of different plant growth stimulants on yield and yield components of corn , Single Cross 704 cultivar, a greenhouse study was conducted as a factorial arrangement based on completely randomized design with 3 replications in Soil and Water Research Institute (SWRI), Karaj, Iran. The studied factors were:

Table 1 The initial properties of the soil

Mg	Cu	Mn	B	Zn	Fe	Soil Texture	Sand	Silt	clay	OC	pH	Ec (Ds/m)	Sp (%)	Available potassium (%)	Available phosphorus (%)	N total (%)	Depth (cm)
(mg/kg)							(%)										
181.2	1.32	4.76	0.8	0.36	4.76	Clay loam	32	42	26	0.56	7.81	1.16	32	221	5.6	0.06	0-30

treatments (T) in 8 levels (T_0 control, T_1 amino acid, T_2 Seaweed, T_3 fertigation of humic acid, T_4 fulvic acid, T_5 foliar application of humic acid, T_6 inoculation of the plant growth promoting bacteria, T_7 humic acid+ seaweed+ amino acid) and fertilizers (F) in 3 levels (F_0 control, F_1= 75 kg, F_2 100 kg) (Table 2). According to the semi-detailed soil science studies of Alborz province, 19 surface soil samples (0-25 cm) of agricultural lands, under corn cultivation, were prepared. After determining some physicochemical properties of the soil (Table 1), 5 kg of the soil was transferred to plastic pots (22×21×21 cm). They were irrigated until they reached field capacity (FC) and kept in FC for one week. Four seeds of corn were planted in each pot which was reduced to two plants after germination. During the growing season, agricultural cares were taken and the nutritional requirements were provided based on the soil testing results. Chlorophyll content (SPAD-502 chlorophyll meter), To select an appropriate sample for enzymatic assays of CAT, SOD activities, the second or third leaves from the top were mowed and placed instantly in liquid nitrogen to stop the activity. To determine the activity of the enzymes, the soluble protein (using bovine serum albumin as standard) was calculated according to Bradford (1976). The samples of fresh leaves (0.1 g) were placed into a 2 mL tube and frozen in liquid nitrogen. The samples were homogenized with a mortar in 100 mM phosphate buffer (pH 6.8) containing 0.1 mM EDTA and 1% PVP (polyvinylpyrrolidone). After that the homogenate was centrifuged at 12,000×*g* at 4 °C for 20 min, the supernatant was employed for rating the enzymatic activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), (CAT) (Dhindsa *et al.* 1981) and superoxide dismutase (SOD) (Alexieva *et al*. 2001) were measured. Eight weeks after planting, the plants were harvested to determine the yield and nutrients concentration (Amani 1996), Finally, the data were analyzed by using SPSS and the means comparison was performed by Duncan's test method ($P<0.05$).

RESULTS AND DISCUSSION

Yield and yield components

The results showed that the treatments and fertilizer levels significantly affected the yield and yield components. The interaction effects between the treatments and fertilizer levels were not significant only for the row number per ear, dry and fresh weight of grain (data not shown).

The highest average number of grains per row was happened under the fertilizer level of 100 kg + control treatment and the lowest average belonged to the fertilizer level of control + combination treatment of humic, seaweed and amino acid (Table 3). Khan *et al.* (2012) and Doroodian *et al.* (2016) reported that humic acid significantly increased the number of grain in spike and 1000-grain weight. Therefore, foliar application of humic acid under water deficiency condition acts as a supplementary agent and increases the grain weight and number of capsules per plant through maintaining of rhizosphere humidity (Zhang and Meng 2014) and providing sufficient nutrients for the plant (Sun *et al.* 2013).

The highest average of ear length was measured in fertilizer level of 75 kg + seaweed treatment and the lowest average was belonged to the control level of fertilizer + combination treatment of humic, seaweed and amino acid (Table 3). These results coincide to findings of Taghadosi *et al.* (2012).

Table 2 The studied treatments

Treatment	Fertilizer level	Seeds	Second irrigation	Eight weeks	Before flowering
T0	control				
T_1	AA foliar spray			5/1000 l	5/1000 l
T_2	SW foliar spray			5/1000 l	5/1000 l
T_3	HA Application soil		5kg/ha		5kg/ha
T_4	FA Foliar spray			5/1000 l	5/1000 l
T_5	HA foliar spray			5/1000 l	5/1000 l
T6	Inoculation of growth-promoting bacteria (fluvitis)	2% of seed weight			
T_7	HA + SW + AA	Seeds	HA 5 kg/ha	SW 5/1000+AA 5/1000 l	SW 5/1000 l + AA 5/1000 l

T_0: control; T_1: Amino acid; T_2: Seaweed; T_3: Fertigation of humic acid; T4: Fulvic acid; T_5: Foliar application of humic acid; T_6: Inoculation of plant growth promoting bacteria; T_7: combination of humic acid, seaweed and amino acid

Table 3 Means comparison interaction effects of treatment × fertilizer level on the studied traits.

Treatment	Fertilizer level	Ear length (cm)	100-grain weight (gr)	Ear number	Tassel	Chlorophyll (SPAD)	Ear weight (cm)
T_1	Control	18.63 c-f	26.87 bcd	1 e	4.66 hi	18.63 k	2.17 ef
T_1	75	19.51 a-f	29.55 a	1 e	13.66 d	41.56f	2.66 bcd
T_1	100	18.34 def	25.46 f-j	1 e	20.33 a	47.2 cd	2.36 de
T_2	Control	19.94 a-f	29.96 a	0 f	8f	41.56 f	2.8 a-d
T_2	75	21.05 a	24.66 ij	0f	11.33 e	45.76 de	2.97 ab
T_2	100	20.23 a-d	27.61 bc	8a	20.33 a	48c	2.56 b-e
T_3	Control	19.06 a-f	24.5 jk	0f	4.66 hi	33.13 i	2.8 a-d
T_3	75	18.74 b-f	26.65 cde	0f	14.66 cd	44.76 e	2.78 a-d
T_3	100	20.72 ab	25.8 d-i	4 c	15.66 bc	54.66 a	2.91 abc
T_4	Control	19.6 a-f	26.81 bcd	0 f	6.33 g	38.1 gh	2.98 ab
T_4	75	20.12 a-e	26.59 c-f	1 e	8.66 f	41.56 f	2.78 a-d
T_4	100	19.7 a-f	25.35 g-j	0 f	15.33 bc	47.2 cd	2.62 bcd
T_5	Control	19.05 a-f	26.49 c-g	0 f	5.66 gh	36.86 h	2.51 cde
T_5	75	18.2 ef	23.44 kl	0 f	3.66 i	48.23 c	2.41 de
T_5	100	19.43 a-f	26.10 d-h	2.66 d	16b	39.73 fg	2.96 ab
T_6	Control	18.41 def	23.30 l	0 f	2j	30.73 j	2.40 de
T_6	75	17.94 f	25.52 e-j	0 f	7.66 f	47.56 cd	2.35 de
T_6	100	20.7 ab	25.99 d-h	5.33 b	13.66 d	38.8 gh	3.11 a
T_7	Control	14.04 g	21.75 m	0 f	1.66 j	39.7 fg	1.85 f
T_7	75	20.2 a-e	27.94 b	3.33 cd	10.66 e	49c	2.76 a-d
T_7	100	20.47 abc	25.02 hij	5.33 b	15.33 bc	51.46 b	2.99 ab

Different letters in each column indicate statistically significant differences according to DMRT ($P \leq 0.05$); Details of treatments are give in Table 1.

Under fertilizer level of 75 kg + amino acid treatment highest increase in 100-grain weight was happened (Table 3). It has reported that the soil application of these fertilizers improves the condition of microorganisms in the soil, whose activity facilitates the absorption of some nutrients and ultimately increases the growth and yield of the plant (Junmardi and Sattar 2016). The highest average number of ear was related to the fertilizer level of 100 kg + seaweed treatment (Table 3). The increase in plant growth has been proven by the foliar application of seaweed extract (Taghadosi *et al.* 2012). The importance of seaweed is due to its nutrients content and having direct and indirect effects on chemical, physical and biological properties of soil (Duan *et al.* 2019).

By applying the fertilizer level of 100 kg + seaweed treatment, the maximum average of tassel number was recorded while the lowest was belonged to the fertilizer level of control + combination treatment of humic, seaweed and amino acid (Table 3). Increase in flowering and fruit formation affected by seaweed fertilizer application may be due to the increase in root volume and nutrients absorption (Crouch and Staden 1992). In order to justify the obtained results, the plant growth stimulants (Zhang 1997), cytokinins of trans-zatin (Stirk and Staden 1997), auxinic compounds and betaine that increase chlorophyll content or prevent chlorophyll degradation may be the cause of increased yield by increasing photosynthesis and assimilates production by chlorophyll (Blunden *et al.* 1996).

Based on the measurements, the highest average of chlorophyll content was recorded in the fertilizer level of 100 kg + fertigation of humic acid treatment and the lowest average was recorded in the fertilizer level of control + amino acid treatment (Table 3). Iron is involved in the formation of chlorophyll and its deficiency in the plant causes leaf chlorosis (Rahii *et al.* 2012; Vatankhah *et al.* 2015). The increase in iron accumulation by humic compounds can be attributed to the release of phenolic substances in the rhizosphere and increase of its reduction and therefore, further absorption by the plant (Poozshi *et al.* 2011).

The highest average weight of ear was measured in the fertilizer level of 100 kg + control treatment (Table 3). It has been reported that seaweed application increases fruit formation in many crops (Kingman and Moore 1982). Seaweed can play an important role in the production of auxins by the plant itself. The cytokinin in seaweed extract stimulates growth more vigorously because they translocate micronutrients to the leaves (Sasikumar *et al.* 2011).

Status of nutrients concentration in shoot

The results showed that different treatments and

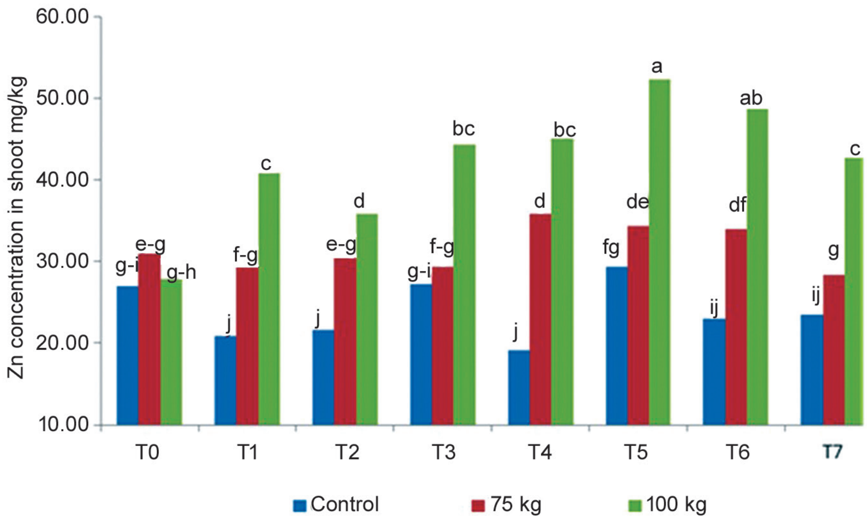

Fig 1 Effect of different fertilizer levels and treatments on Zn concentration in shoot. Details of treatments are given in Table 1.

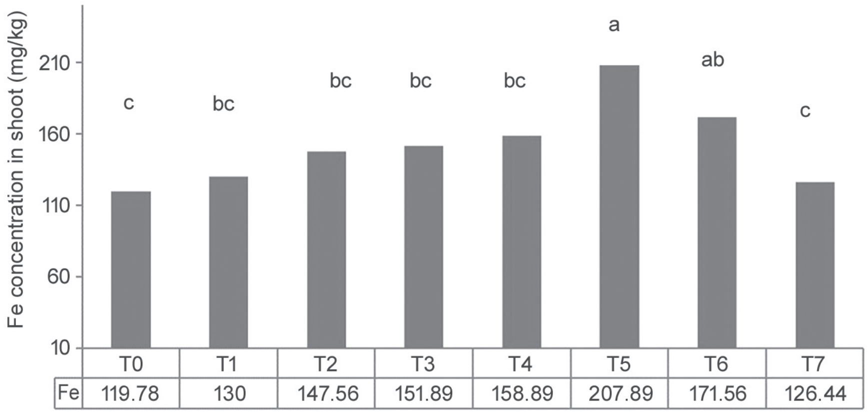

Fig 2 Effect of different treatments on concentration of Iron in shoot. Details of treatments are given in Table 1.

fertilizer levels significantly affected zinc and iron concentrations in shoot. The interaction effects of fertilizer levels and treatments were significant for zinc (data not shown).

The highest average of zinc concentration was recorded under 100 kg fertilizer level + foliar application of humic acid treatment and the lowest average was happened under fertilizer level of control + fulvic acid treatment (Fig 1). Ardakani *et al.* (2001) reported an increase in absorption of micro and macro nutrients in the inoculating wheat seeds with biofertilizers. On the one hand, humic acid dissolves and absorbs insoluble elements in the soil, and on the other hand, it keeps and maintains these elements in itself and transfers them to the plant roots at the right time, so applying of humic acid with the highest level (100 kg) caused the highest zinc concentration in plant tissue. The highest iron concentration in shoot was measured under foliar application of humic acid and the lowest concentration was happened under the control treatment (Fig 2).

The main effect of fertilizer levels showed that the highest average of iron concentration in shoot was belonged to the fertilizer level of control which had not significant difference with the fertilizer level of 100 kg (Fig 3).

One of the important benefits of humic acid is its ability to chelate different nutrients to overcome nutrient deficiencies (Ghorbani *et al.* 2010). Humic acid is able to convert soil iron into a form that can be absorbed and metabolized. This ability can be effective in alkaline and calcareous soils, which usually has iron and organic matter deficiency. The increase in iron accumulation by humic compounds can be attributed to the release of phenolic substances in the rhizosphere and the improvement of reduction reaction and further absorption of iron affected by these materials (Pouzshi *et al.* 2011). Decrease in soil pH and production of organic acids by biofertilizers play an important role in the access and transport of micro-elements such as iron (Sandra 2002).

Status of superoxide dismutase (SOD) and catalase (CAT) concentrations

The results showed that the highest and the lowest average concentration of SOD were recorded when the treatment of inoculation with plant growth promoting bacteria respectively was used with the fertilizer level of control and 100 kg (Fig 4).

The highest and the lowest average concentration of CAT were recorded when the fertilizer level of 75 kg was respectively applied with the treatment of humic acid fertigation and combination treatment of humic+ seaweed+ amino acid (Fig 4). Cordeiro *et al.* (2011) reported that humic acid stimulates CAT production and reduces reactive oxygen species (ROS), causing antioxidant effects on plant defense mechanisms. In a research, Garcia (2012) reported that humic acid can play an important role in resistance to oxidative stress by

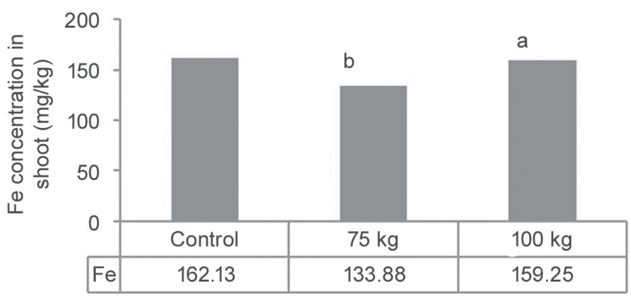

Fig 3 Effect of different fertilizer levels on iron concentration in shoot.

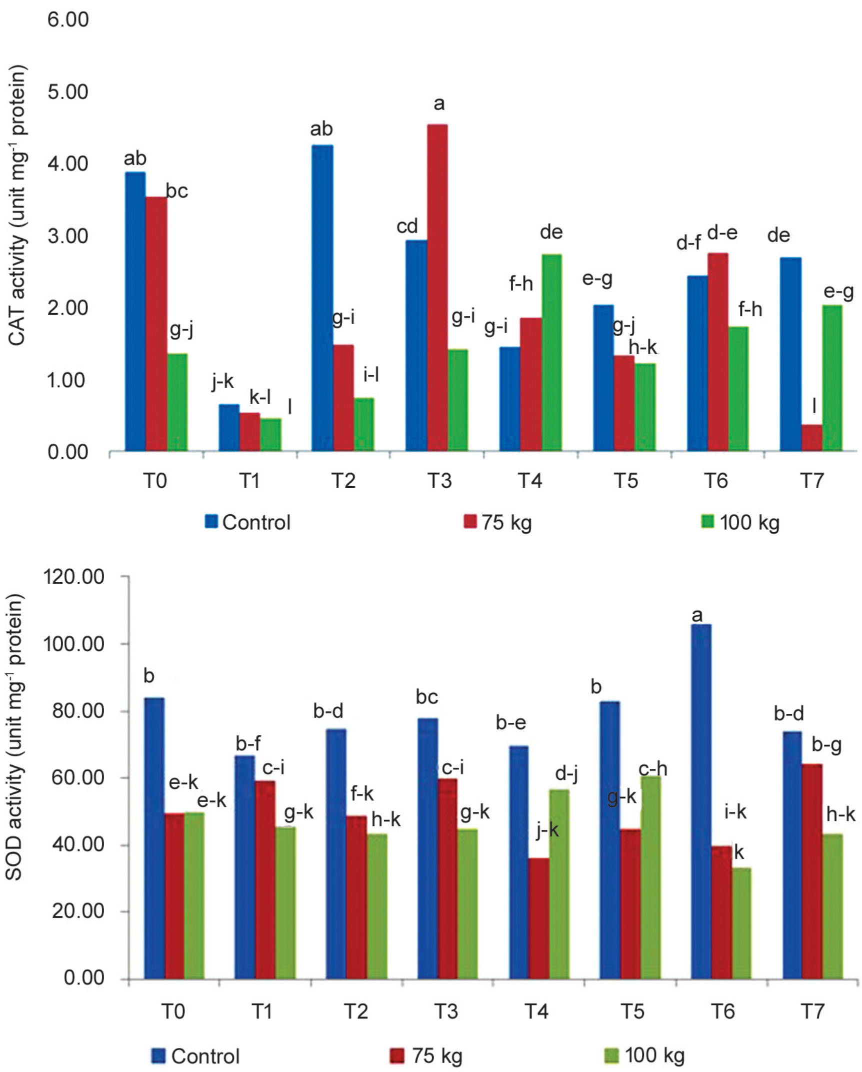

Fig 4 Effect of different fertilizer levels and treatments on catalase (up) and superoxide dismutase (down) concentrations. Details of treatments are given in Table 1.

increasing antioxidant activity and improving membrane stability. Considering the fact that SOD neutralizes hydroxyl radicals, CAT is one of the major H_2O_2 detoxification enzymes in plants, and according to the reports, the SOD enzyme in corn is directly related to iron deficiency Tewari (2005), as the concentration of iron increases, the SOD activity decreases.

Due to the fact that zinc is involved in the structure of this enzyme (Cu/Zn SOD) among the various SOD enzymes in the corn plant, it can affect its activity. Therefore, zinc (Zn) deficiency in plants can cause ROS production and prevent protein synthesis (Zand *et al.* 2010). Application of zinc improved the activity of CAT, POD, SOD and PPO enzymes, which seems to be associated with increased concentrations of this element in the leaves of corn. This point has also been considered by some researchers regarding the relationship between increasing the concentration of iron and the level of activity of the mentioned antioxidant enzymes (Kumawat *et al.* 2006). According to our results, foliar application of iron and zinc improves biochemical parameters (CAT, SOD) in cumin under drought stress (Amirinejad *et al.* 2015). According to Tewari *et al.* (2005) SOD enzymes in corn plants are directly related to iron deficiency, in fact, with increasing iron concentration, the amount of SOD activity decreases, as well.

Conclusion

The findings of this study showed that using of plant growth stimulants through improving of fertilizer use efficiency increases the nutrients availability and their uptake by the plant. The studied treatments also, affect the status of plant enzymes and finally affect the growth and yield of corn. Among the applied fertilizer levels, the level of 75 kg and among the bio-stimulants, the seaweed treatment showed the highest effect on improving the growth and yield responses of corn which in order to confirm these results more studies are recommended.

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