

IMPROVEMENT OF GROWTH AND SOME METABOLIC COMPONENTS OF *ZEA MAYS* AND *HELIANTHUS ANNUUS* BY PRE-SOWING SEED TREATMENTS

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Abstract

Zea mays and *Helianthus annuus* are considered most important oil crops in the world due to the favorable fatty acid composition of the oil for human consumption, so efforts must be done to increase the yield and the desirable constituents in the two plants. In the present study the seeds of the plants were primed by soaking in two different concentrations of ascorbic acid solution or yeast extract (1% and 2%) for 12 hour. The plants were grown under greenhouse conditions and irrigated with tap water. It was found that, all these treatments significantly increased germination percentage, shoot growth parameters, photosynthetic pigments, soluble proteins and phytohormones in both *Zea mays* and *Helianthus annuus*. It was observed that, 1% yeast extract was the most promotive treatment for *Zea* while for *Helianthus annuus* it was 2% ascorbic acid. The protein profile showed the induction of several new protein bands with all treatments. These new bands revealed a changed pattern of gene expression after yeast and ascorbic acid treatments. Finally, the applied treatments decreased the percentage of saturated fatty acids and increased the unsaturated fatty acids in both the tested plants.

Key words: *Zea mays*, *Helianthus annuus*, Ascorbic acid, Yeast, Seed priming.

Introduction

Ascorbic acid (vitamin C) is synthesized in higher plants and plays an important role in plant growth and development. Ascorbic acid is considered as a coenzyme in the electron transport system and metabolism (El-Kobisy *et al.*, 2005). Bolkhina *et al.*, (2003) mentioned that ascorbic acid was the most abundant antioxidant in plant cells. In both plant and animal systems ascorbic acid interacts enzymatically and non-enzymatically with damaging reactive oxygen radicals and their derivatives, named reactive oxygen species (ROS). The ability of ascorbic acid to interact with physiologically formed ROS implicates ascorbic acid in the modulation of processes such as cell division, lignification and the hypersensitive response. The biological importance of the antioxidant activity of ascorbic acid is that unlike other low molecular weight known antioxidants (carotenoids, α -tocopherol, flavonoids, etc.), ascorbic acid is able to terminate radical chain reactions by disproportionation to non-radical, non-toxic products. Furthermore, ascorbic acid is only mildly electronegative so, it can donate the electrons to a wide range of substrates. One of the most important features of non-enzymatic antioxidant behavior of ascorbic acid is its participation in the regeneration of the lipophilic, membrane-associated α -tocopherol (vitamin E) radical (Wheeler *et al.*, 1998). Farahat *et al.*, (2007) found a pronounced increase in vegetative growth and chemical constituents of *Cupressus sempervirens* L. plants by foliar application of ascorbic acid. Eid *et al.*, (2010) stated that ascorbic acid application significantly increased oil yield of *Jasminum grandifolium* L.

Several studies indicate that yeast is one of the richest source of high quality protein, called essential amino acids like tryptophan and lysine, contain the essential minerals and trace elements namely calcium, iron, cobalt etc. and is considered the best sources of the vitamin B-complex such as B₁, B₂, B₆ and B₁₂. Yeast

extract is a rich source of bio-constituents especially phytohormones as cytokinins which improve plant production Ghoname *et al.*, (2009). Mahmoud *et al.*, (2013) found that yeast extracts improved all the tested vegetative growth parameters of pea plant. Pods quality and yield were significantly increased with application of 2% yeast extract. Moreover, El-Desuki & El-Greadly, (2006) proved that, the vegetative growth, photosynthetic pigments, carbohydrates, free amino acids and cytokinins were increased in plants which were sprayed with 3% yeast extract. It has also an important role during stress conditions due to its cytokinins content (Barnett *et al.*, 1990).

Seed priming or soaking is a simple and low cost hydration technique in which the seeds can absorb nutrients, protectants, growth regulators, etc. by immersing them in convenient solutions for extended periods (Scott, 1998). This technique is used for better crop stand and higher yields in a wide range of cultivated plants (Farooq *et al.*, 2006; Kaymak *et al.*, 2009). It has been successfully demonstrated that seed priming improve seed germination, emergence, uniform, and vigorous crops of many field plants such as vegetables, wheat, maize, sugar beet, sunflower and soybean (Parera & Cantliffe, 1994).

The aim of the present study was to evaluate the efficiency of seed priming with different concentrations of ascorbic acid or yeast extract on improving the growth and some physiological aspects of maize and sunflower plants.

Materials and Methods

Seeds of *Zea mays* and *Helianthus annuus* were obtained from the Egyptian Ministry of Agriculture, Giza, Egypt. The seeds were surface sterilized with 0.01% HgCl₂ for one minute and then washed several times with distilled water. The seeds of plants were divided into five sets. The first set was soaked in

distilled water (control), the second in 1% ascorbic acid, the third in 2% ascorbic acid, the fourth in 1% yeast extract and the fifth in 2% yeast extract for 12 hour. After each treatment, the seeds were left to grow in plastic pots (5 seeds / pot) 16 cm height and 13 cm diameter containing 600 gram soil which was a mixture of clay and sand (2:1). Each treatment was replicated 5 times and completely random design was used. The plants were left to grow under greenhouse conditions and irrigated with tap water. The number of germinated seeds were counted and the germination percentage was calculated. After 2 weeks plants were harvested and for each treatment shoot length, fresh and dry weights were determined. Fresh plants were used for protein gel electrophoresis and estimation of phytohormones. Fresh leaves were kept for assaying of pigments. Oven dried plant samples (60°C) were used for the determination of soluble proteins and fatty acids.

Determination of photosynthetic pigments: The pigments from the fresh leaves were extracted with 85% cold acetone. The absorbance of the acetone extracts was measured at 663, 644 and 452.5 nm respectively by using a spectrophotometer for the estimation of chlorophyll a, chlorophyll b and carotenoids contents according to the method described by (Metzner *et al.*, 1965).

Estimation of endogenous phytohormones: Phytohormones were quantitatively estimated in plant samples using gas chromatography (GC. Hewlett Packer D. HP 6890 series) according to Shindy & Smith, (1975).

Qualitative characterization of protein using SDS-PAGE: Fresh plant samples were homogenized with one ml of extraction buffer (25 mM Na-acetate, pH 4.5 and 1 mM phenyl methyl sulphonyl fluoride). After that, they were vortexed and left for 2 hours at 4°C. The extracts were centrifuged at 10,000 rpm at 0°C for 15 minute and the clear supernatants were used as the total protein extract. Characterization and molecular mass determination of proteins were carried out using one dimensional SDS-polyacrylamide gel electrophoresis as described by Laemmli, (1970).

Estimation of total soluble proteins: The total soluble proteins were quantitatively measured in the borate buffer extract. The protein content was calculated as mg/g dry weight using a calibration curve of Bovine Serum Albumin protein (Bradford, 1976).

Estimation of fatty acids: Transmethylation of lipids and extraction of fatty acids methyl esters in dry plant samples was carried out as described by Garaces & Mancha, (1993). Fatty acids methyl esters were analysed using gas chromatography (GC, Hewlett Packer D, HP 6890 series).

Statistical analysis

The results were expressed as mean \pm SD of three replicates. They were statistically analysed by using the analysis of variance test described by Bishop, (1983).

Results and Discussion

The growth parameters of maize and sunflower plants such as germination percentage, shoot length, shoot fresh weight and shoot dry weight were significantly increased by all the applied treatments as shown in (Table 1). For maize maximum stimulatory effect was observed in plants primed with 1% yeast extract. On the other hand, sunflower seeds treated with 2% ascorbic acid or 2% yeast extract showed a maximum increase in all the measured growth parameters compared with the control and the other treatments. Such enhancement effect of ascorbic and yeast may be attributed to their adequate influence on metabolism and biological activity which was reflected on vegetative growth. In this respect, Athar *et al.*, (2009) demonstrated that ascorbic acid as antioxidant played a beneficial role on cell metabolism, growth, division and differentiation in plants. Several authors found a significant stimulative effect of ascorbic acid on morphological characters (Emam *et al.*, 2011; Nassar *et al.*, 2016; Aziz *et al.*, 2018). Yeast a natural bio-substance has nutritional, stimulating and protective functions when used on plants. It has a promotive effect on cell division, enlargement, and vegetative growth due to its high contents of phytohormones, enzymes, vitamins, amino acids and minerals (Mahmoued, 2001).

Results shown in (Table 2) revealed a significant increase in the photosynthetic pigments content in *Z. mays* and *H. annuus* in response to the different applications. The priming treatment with 1% ascorbic acid in maize plant proved produced higher total pigment content over the other tested treatments and the untreated control. In contrast, the most promotive treatment on the total pigment in sunflower plant was 2% yeast extract. Sofy *et al.*, (2016) and Hafez & Gharib, (2016) found that application of ascorbic acid had a pronounced effect on the accumulation of the photosynthetic pigments. Ascorbic acid improves metabolism, cell division and growth which reflects on the synthesis of photosynthetic pigments (Dolatabadian & Jouneghani, 2009; Kasim *et al.*, 2019). Yeast contains elevated levels of phytohormones which may induce pigments biosynthesis and increase photosynthetic activities (Wanas, 2002).

The soluble protein content in the plant cells is considered an important indicator of their physiological state in response to different external treatments. In the present study, shoot soluble protein content of both maize and sunflower was increased by all the applied treatments compared with the control. The maximum value of the shoot soluble protein was recorded in maize plant treated with 1% yeast extract and in sunflower plant treated with 2% ascorbic acid (Fig. 1). These results are considered an important indicator of the enhancement effect induced by yeast and ascorbic acid on the growth of the two tested plants. In this respect, Wanas, (2006) and Marzauk *et al.*, (2014) found a stimulatory effect of yeast on the protein synthesis. This enhancement effect of yeast may be attributed to its high auxin and cytokinins content. There are number of reports on the increase in the plant protein content with application of ascorbic acid (Emam *et al.*, 2011; El-Hak *et al.*, 2012; Sofy *et al.*, 2016).

Table 1. Effect of different concentrations of ascorbic acid and yeast extract on some growth parameters of *Zea mays* and *Helianthus annuus* plants (Mean \pm SD, n=5).

Treatment	Germination percentage		Shoot length (cm)		Shoot fresh weight (mg)		Shoot dry weight (mg)	
	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>
Control	82.22 \pm 4.1	88.33 \pm 4.1	26.88 \pm 1.6	18.44 \pm 1.2	1318 \pm 95	1400 \pm 98	91 \pm 5.2	49 \pm 2.0
1% ascorbic acid	88.89 \pm 6.3	91.67 \pm 5.3	29.92 \pm 1.1	21.3 \pm 2.0	1500 \pm 139	1480 \pm 112	105 \pm 8.3	53 \pm 2.7
2% ascorbic acid	95.56 \pm 5.4	93.33 \pm 4.6	26.70 \pm 1.1	22.04 \pm 1.5	1434 \pm 130	1698 \pm 122	105 \pm 7.6	62 \pm 3.8
1% yeast extract	97.56 \pm 6.5	88.67 \pm 3.2	36.68 \pm 1.9	21.5 \pm 1.4	1882 \pm 123	1418 \pm 111	123 \pm 8.3	50 \pm 3.6
2% yeast extract	95.78 \pm 5.7	93.33 \pm 5.1	27.03 \pm 2.1	21.3 \pm 2.0	1344 \pm 110	1838 \pm 131	95 \pm 6.1	74 \pm 4.2

Table 2. Effect of different concentrations of ascorbic acid and yeast extract on pigments content (mg/g fresh wt.) of *Zea mays* and *Helianthus annuus* plants (Mean \pm SD, n=3).

Treatment	Chlorophyll a		Chlorophyll b		Carotenoids		Total pigments	
	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>	<i>Zea</i>	<i>Helianthus</i>
Control	0.99 \pm 0.05	0.61 \pm 0.05	0.36 \pm 0.02	0.24 \pm 0.04	0.24 \pm 0.02	0.15 \pm 0.01	1.59 \pm 0.08	1.00 \pm 0.09
1% ascorbic acid	1.58 \pm 0.09	0.63 \pm 0.04	0.63 \pm 0.04	0.30 \pm 0.01	0.39 \pm 0.05	0.14 \pm 0.01	2.60 \pm 0.21	1.07 \pm 0.10
2% ascorbic acid	1.36 \pm 0.11	0.68 \pm 0.04	0.47 \pm 0.03	0.32 \pm 0.02	0.34 \pm 0.01	0.14 \pm 0.01	2.17 \pm 0.16	1.14 \pm 0.09
1% yeast extract	1.29 \pm 0.10	0.67 \pm 0.03	0.45 \pm 0.01	0.28 \pm 0.02	0.32 \pm 0.01	0.16 \pm 0.01	2.06 \pm 0.12	1.11 \pm 0.08
2% yeast extract	1.39 \pm 0.12	0.73 \pm 0.06	0.52 \pm 0.05	0.27 \pm 0.04	0.33 \pm 0.02	0.18 \pm 0.02	2.24 \pm 0.18	1.18 \pm 0.11

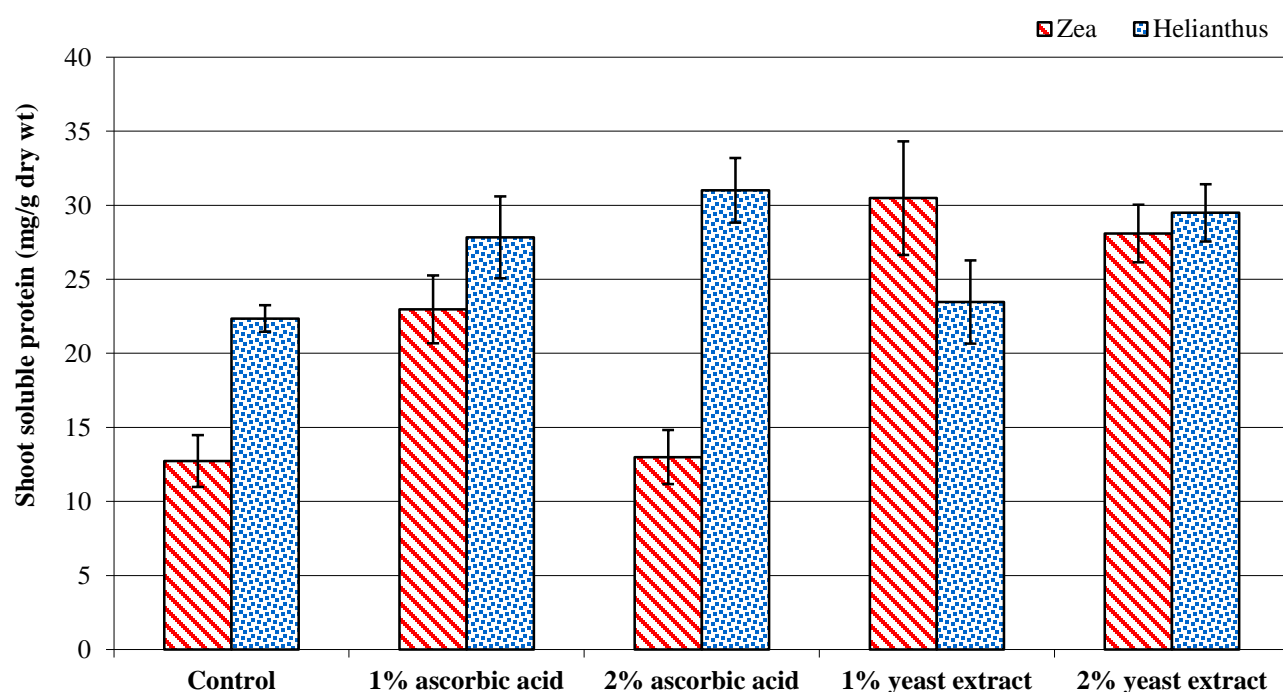


Fig. 1. Effect of different concentrations of ascorbic acid and yeast extract on shoot soluble protein (mg/g dry wt) of *Zea mays* and *Helianthus annuus* plants.

The protein profile of both maize and sunflower plants showed the induction of several new bands with all treatments compared with the controls (Fig. 2). These new bands revealed a changed pattern of gene expression after yeast and ascorbic acid treatments. In maize plant, three unique bands appeared which were not found in control with molecular masses of 97 KDa and were induced after treatment with 1% ascorbic acid, 100 KDa after treatment with 2% ascorbic acid and 101 KDa after treatment with 2% yeast. In sunflower plant 17 new bands ranging from 26-63 KDa appeared with the applied treatments and were not found in the control. Such newly formed protein bands may be used as an adaptive mechanism for application of a biofertilizers to give a maximum yield (Selvakumar *et al.*, 2012). In this connection Bassuony *et al.*, (2008) found that treatment of maize plants with vitamin C induced the synthesis of new protein bands.

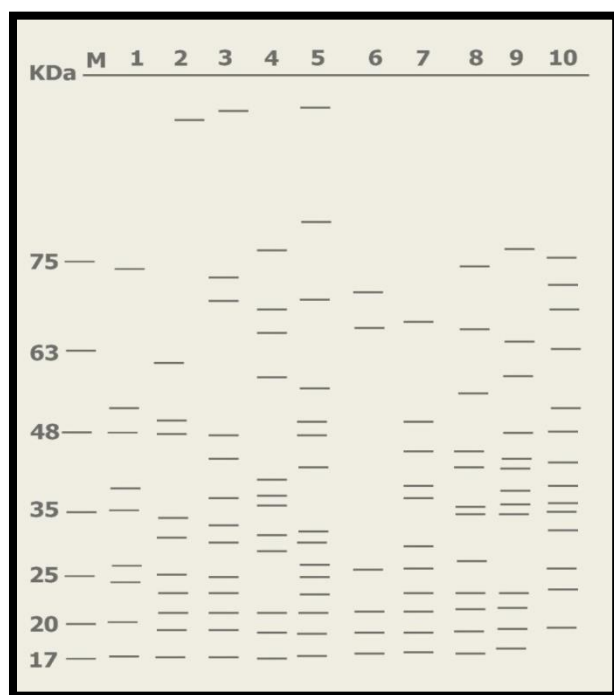


Fig. 2. Computer generated banding for SDS-PAGE protein pattern of *Zea mays* and *Helianthus annuus* plants under different concentrations of ascorbic acid and yeast extract (M= marker; 1= control *Zea*; 2= 1% ascorbic *Zea*; 3= 2% ascorbic *Zea*; 4= 1% yeast *Zea*; 5= 2% yeast *Zea*; 6= control *Helianthus*; 7= 1% ascorbic *Helianthus*; 8= 2% ascorbic *Helianthus*; 9= 1% yeast *Helianthus*; 10= 2% yeast *Helianthus*).

Endogenous phytohormones of maize and sunflower plants as affected by application of yeast and ascorbic acid are shown in (Fig. 3). According to these results, gibberellic acid and cytokinin were significantly increased in both the tested plants with all the applied concentrations compared with the control. Indoleacetic acid showed a minor increase compared with the other hormones. In this respect, Amer, (2004) and Wanas, (2006) reported that yeast was considered a natural source of auxins and cytokinins and was found to have a stimulatory effects on cell division and enlargement, nucleic acids and protein synthesis and consequently the increase in the endogenous phytohormones of the tested plants was observed. Also, ascorbic acid has a regulatory role in promoting productivity in plants by acting as a cofactor for several enzymes and regulates the phytohormones-mediating signaling processes (Barth & Mario 2006; Farooq *et al.*, 2013).

Sunflower and maize are considered as the most important oil crops in the world due to the favorable fatty acid composition of the oil with regard to human consumption. From the results (Tables 3a and 3b), it was found that five main fatty acids predominated in both sunflower and maize. These fatty acids are grouped into saturated (palmitic and stearic) which comprise 20.6% in maize and 17.2% in sunflower and unsaturated (oleic, linoleic and linolenic) which comprise 79.3% in maize and 82.8% in sunflower. All the treatments decreased the percentage of saturated fatty acids and increased the unsaturated fatty acids in both the tested plants. The highest percentage of unsaturated fatty acids was recorded in maize with 1% ascorbic acid (85.8%) and in sunflower with 1% yeast extract (91.1%). Generally, the unsaturated oils tend to decrease blood cholesterol levels in the human body and the plants which contain unsaturated oils are safer for human consumption. In this connection, El-Kady, (2010) and Nassar *et al.*, (2016) reported an increase in the unsaturated fatty acids especially oleic and linoleic acids in flax seed oil after ascorbic acid application. Nasiri *et al.*, (2018) showed that ascorbic acid could be used for improving or enhancing the content and composition of the essential oils in dragonhead plant.

Table 3a. Effect of different concentrations of ascorbic acid and yeast extract on fatty acids percentage of *Zea mays*.

Treatment	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Saturated fatty acids	Unsaturated fatty acids
Control	17.1	3.5	15.0	62.6	1.7	20.6	79.3
1% ascorbic acid	11.1	3.1	20.2	63.5	2.1	14.2	85.8
2% ascorbic acid	11.5	2.8	15.2	65.5	5.0	14.3	85.7
1% yeast extract	15.3	3.7	20.6	56.5	3.7	19.0	80.8
2% yeast extract	13.9	3.4	22.3	59.0	1.4	17.3	82.7

Table 3b. Effect of different concentrations of ascorbic acid and yeast extract on fatty acids percentage of *Helianthus annuus*.

Treatment	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Saturated fatty acids	Unsaturated fatty acids
Control	12.0	5.2	10.5	71.1	1.2	17.2	82.8
1% ascorbic acid	7.9	4.2	10.9	75.4	1.5	12.1	87.8
2% ascorbic acid	10.8	2.4	25.7	60.5	0.6	13.2	86.8
1% yeast extract	6.9	2.0	11.0	77.9	2.2	8.9	91.1
2% yeast extract	11.8	4.0	10.2	72.2	1.8	15.8	84.2

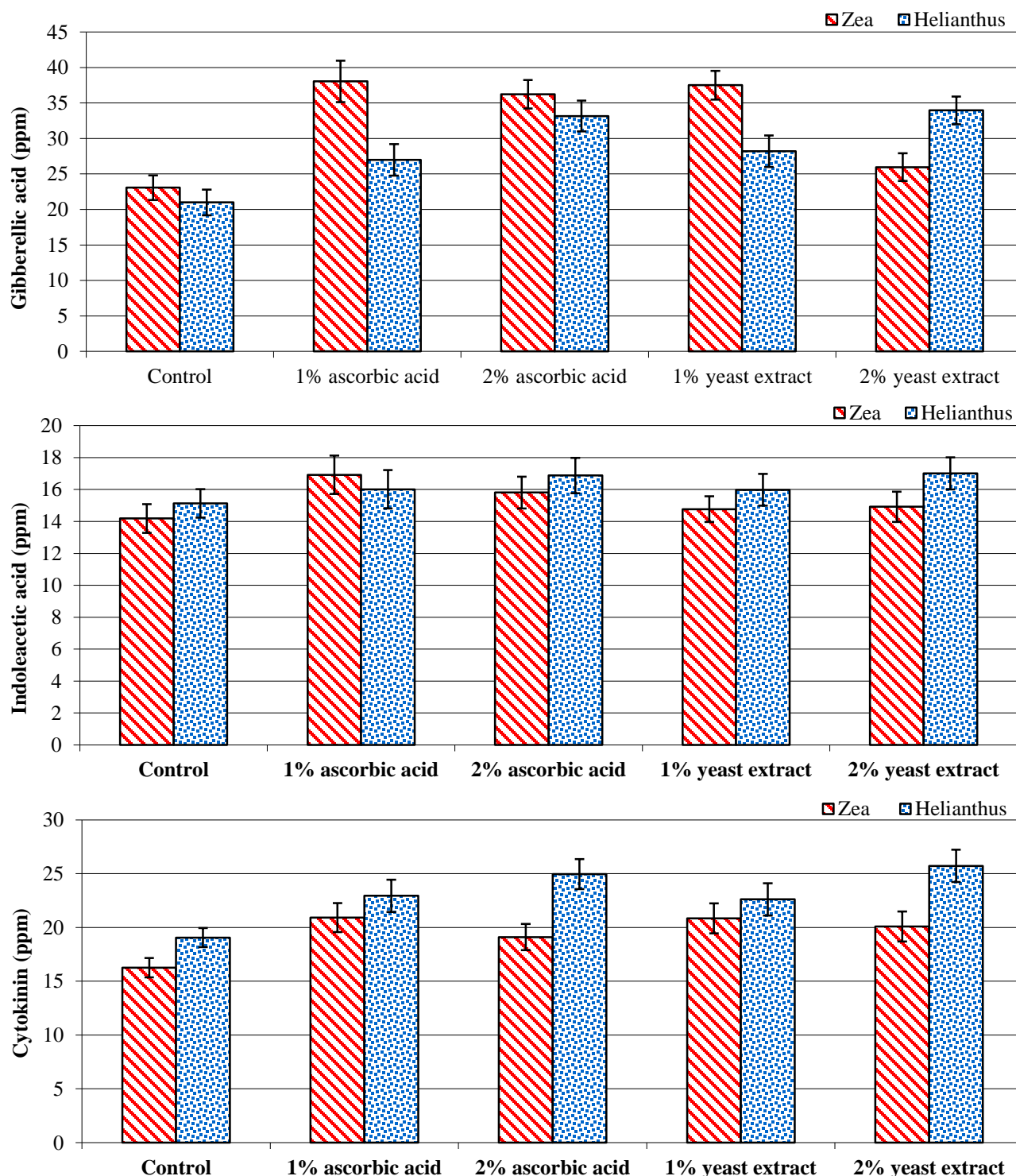


Fig. 3. Effect of different concentrations of ascorbic acid and yeast extract on endogenous phytohormones of *Zea mays* and *Helianthus annuus* plants.

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