INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 10, p. 6-12, 2014

RESEARCH PAPER OPEN ACCESS

Effects of different concentrations of zinc on chlorophyll, starch, soluble sugars and proline content in *Cucurbita pepo*

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Key words: Medicinal plant, chlorophyll, proline, soluble sugars and zinc.

http://dx.doi.org/10.12692/ijb/4.10.6-12 Article published on May 18, 2014

Abstract

Naked pumpkin (*Cucurbita pepo*) is planted as a medicinal plant in Iran. This herb is effective against prostate cancer and its seeds are a rich source of zinc. It has been demonstrated anti-parasitic and anti-fungal effects. Zinc is one of the micronutrients essential for plant growth and development. In addition, zinc is a heavy metal and too much zinc can cause toxicity in most plants. Currently, environmental pollution, especially pollution from heavy metals due to industrial activities and indiscriminate use of chemical fertilizers cause irreparable damage to the plants. In this study examined the effect of different concentrations of Zn^{2+} [control (zero), 30, 60, 90, 120 and 150 mM] on chlorophyll a, chlorophyll b, starch, soluble sugars and proline in naked pumpkin (*Cucurbita pepo*). Plants were treated for 14 days and after this time will be tested. Experimental design was a completely randomized design with four repetitions. Data were analyzed using software Mstatc, Spss and Excel and mean comparisons were performed using the least significant difference test (LSD). The results showed that that high zinc concentrations decrease of photosynthetic pigments such as chlorophyll a and chlorophyll b. However, soluble sugars and proline increased. These compounds are used in various stress for osmotic adjustment, preserve the enzymes and membranes in plant cells. Reduced starch is related to degrade it to soluble sugars. Correlations between traits showed a positive correlation exists between photosynthetic pigments and starch and their correlation with soluble sugar and proline is negative.

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Introduction

Naked pumpkin (*Cucurbita pepo*) of the oldest medicinal plants in Iran and is owned cucurbitaceae. Annual plant with creeping stems and very fluffy. Ingredients needed in modern pharmaceutical naked pumpkin seed there (Hosseini and Emami, 2008). On naked pumpkin seeds are effective materials needed in modern medicine, which can be phytosterols, flavonoids, fatty acids, vitamin E and minerals such as zinc mention (Kumara *et al*, 2012). This herbal medicine as an anti-sugar, salt provides the body, such as prostate cancer treatment and fungicide applications (Van and Clijsters, 1990; Broadly *et al*, 2007).

Zinc is an element with atomic number 30 and the twenty-third most abundant element on Earth (Weiss *et al*, 2005). Zinc form divalent metal (Zn^{2+}) is absorbed by plants and transfers it to be essential amino acids (Kafi and Mahdavi Damgani, 2001). Zinc concentration in the phloem is low and it is unmoved element. Zinc an microelements essential for the growth and development of plants and is involved in many metabolic processes also, the structure of many enzymes, such as oxide reductase, transferase, hydrolase, isomerase and ligase (Broadly *et al*, 2007; Maleki *et al*, 2014). Zinc is involved on the activity of enzymes such as chlorophyll biosynthesis, auxin, protein, carbohydrate and lipid metabolism, nucleic acid and membrane integrity (Yadav, 2010).

Nevertheless, it is a heavy metal and like other heavy metals, too much of it in most plants is poisonous. Zn^{2+} ions in high concentrations due to the overproduction of reactive oxygen caused oxidative damage to the plants (Lin and Mark, 2012; Khan *et al*, 2014). Zinc toxicity in agricultural soils irrigated with sewage and indiscriminate use of fertilizers to occur (Yadav, 2010; Muntean *et al*, 2012). Toxicity symptoms on plants include reduced productivity, stunted growth, leaf chlorosis due to iron deficiency, reduction in chlorophyll synthesis, analysis of chloroplast and interfere with the absorption of phosphorus and iron (Nagaiyoti *et al*, 2010; Nadi *et al*, 2013).

Research carried out by Lira (1995) and Muntean *et al* (2012), showed the chlorophyll content of leaves, especially upper leaf canopy in pumpkin (*Cucurbita pepo*) has been significantly increased with increasing zinc. Reported that increasing zinc in pumpkin, starch content decreased significantly ,but it has increased the amount of soluble sugars (Decker, 1988 and Ondigi *et al*, 2008).

The aim of this study was to evaluate the effect of various concentrations of zinc on chlorophyll, starch, soluble sugars and proline in Naked pumpkin (*Cucurbita pepo*).

Materials and methods

The study was conducted in a research laboratory of Islamic Azad University of Miandoab in 2013. Experimental design was a completely randomized design with four repetitions. To sterilize the seeds of Naked pumpkin (*Cucurbita pepo*) for 15 minutes in a solution of 10% sodium hypochloride was placed and the seeds were washed several times with distilled water. Sterilized seeds were transferred to plastic pots containing perlite. The first four days were irrigated with distilled water and then during the six days of distilled water with Hoagland solution was used. After ten days the growth of the seedlings added various amounts of zinc. Treatments included [control (zero), 30, 60, 90, 120 and 150 mM] of zinc. Duration of treatment was 14 days. Light and dark periods were 16 and 8 hours respectively, and temperature was adjusted to 20 °C. After 14 days, plants outside of the treatment setting and experiments have been done on them.

chlorophyll content

Measurement of chlorophyll a and b were performed using fresh leaves. Different parts of the plant including the shoots and roots dried in the oven for 48 hours at 70 °C in order to measure the amount of soluble sugars and starch. Some of them to measurement of proline were frozen in liquid nitrogen and transferred to a freezer. Calculate the concentration of chlorophyll a and chlorophyll b using method Lichtenthaler and Welburn (1994) were performed. In this method, the rate of photosynthesis pigments absorb wavelengths of 663 and 647 nm using a spectrophotometer model Biochrom S 2100 were read and using equations Lichtenthaler and Welbrum was measured.

soluble sugars and starch content

The amount of soluble sugars and starch were measured by phenol-sulfuric acid method (Yang *et al*, 2001). In this method, the 0/1 g of dried plant material was added 10 ml of 70% ethanol and was stored in the refrigerator for a week until the release of soluble sugars. After a week of supernatant samples was removed for shoot 0.5 ml and for root 0.1 ml and adding distilled water, their volume was 2 ml.

After adding 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid, the absorbance was read by a spectrophotometer at a wavelength of 485 nm and finally the sample was calculated using a standard curve of glucose.

Proline content

Proline assay method was conducted (Bates *et al*, 1973). In this method was pulverized 0.5 g of fresh plant with 10 ml of 3% salicylic acid sulfosuccinates. The homogeneous mixture obtained after filtration, 2 ml was removed and after the addition of 2 ml of acid ninhydryn and 2 ml of pure acetic acid were placed in a water bath with temperature of 100 ° C for one hour. Then put them in the ice water bath, followed by addition of 4 ml of toluene, absorbance was read at a wave length of 520 nm using a standard curve obtained proline.

Data were analyzed using Mstatc and Spss softwares and the charts from Excel software. Mean comparisons were performed using least significant difference test (LSD).

Results and discussion

In the ANOVA table revealed the effect of zinc on chlorophyll a, chlorophyll b, starch of root and proline of root at the 5% level and the starch of shoot, soluble sugars of shoot, soluble sugars of root and proline of shoot were significant at the 1% level (Tab 1).

Tab 1. Variance analysis of characters.

SV	df	Chlorophyll (a)	Chlorophyll (b)	Soluble sugars of shoot	Soluble sugars of root	Starch of shoot	Starch of root	Proline shoot	of Proline of root
Treatment	5	13,81*	8,97*	94.85**	62.33**	81.47**	50.62*	11.87**	6.21*
Error	18	0.38	0.240	53.61	18.25	14.35	38.21	0.54	0.19
CV %		4.34	7.62	11.23	8.58	5.60	7.99	5.78	6.14

* and ** : significant at the 5% and 1% levels of probability respectively.

chlorophyll

The zinc concentration increased to 30 mM increase the amount of chlorophyll a and b, but higher concentrations of zinc decreased their amount. According to least significant difference test, photosynthetic pigments decrease in the levels of 60, 90, 120 and 150 mM zinc was significant. Figures 1-A and 1-B can be seen that the reduction in chlorophyll a is greater than chlorophyll b.

In this study it was observed with increasing zinc, the amount of chlorophyll a and chlorophyll b decreased. Studies show that the influence of heavy metals on chlorophyll and other them through direct inhibition of the enzyme and induced deficiency of essential nutrients that interfere with the synthesis of photosynthetic pigments (Kafi and Mahdavi Damgani, 2001; Saeedipour, 2014).

Soluble sugars and starch

Zinc concentrations caused soluble sugars in roots and shoots increased compared to control while the starch content decreased. According to least significant difference test soluble sugars in shoots, in all treatments except 30 mM zinc and the root in treatments of 90, 120 and 150 mM zinc was significantly increased (Fig 2- A, B). The starch in roots and shoots increased significantly only at 30 mM and the levels of 60, 90, 120 and 150 mM decreased significantly (Fig 3- A, B).

The results of measurement of soluble sugars and starch in roots and shoots of wheat plants with the right amount of zinc concentration, the concentration of soluble sugars increased while the starch content decreased. Research indicates that the salinity, waterlogging, frost and heavy metal increase soluble sugars (Van and Clijsters, 1990; Sthanadar *et al*, 2014). Accumulation of soluble sugars helps regulate osmotic stress in plant cells and leads to preservation of biological molecules and membranes (Irannejad and Shahbazian, 2004;).

Table 2. Correlation coefficients between traits affected by zinc.

Choloropgill (a)							
0.317*	Cholorophill (b)						
0.952**	0.234*	starch of shoot					
0.532*	0.489*	0.913**	Starch of root				
-0.095ns	-0.031ns	-0.765**	-0.128*	Soluble sugare of shoot			
-0.076ns	-0.015ns	-0.209*	-0.640**	0.266*	Soluble sugar of root		
-0.905**	-0.629**	-0.941**	-0.119*	0.918**	0.373*	Proline of shoot	
-0.250*	-0.141*	-0.881**	-0.273**	0.442*	0.804**	0.195*	Proline of root

ns, * and **: non-significant and significant at the 1% and 5% levels of probability respectively.

The plant with the increase in soluble sugar in addition to maintaining the osmotic potential in stress conditions, will be able to store their carbohydrate metabolism of the cell is kept at an optimum level (Gibson, 2005). Reduction of starch can be decomposed into smaller units that it causes the accumulation of soluble sugars in plant cells (Ashraf and Harris, 2004). Also, the stress of large amounts of heavy metals such as zinc on the activity of key enzymes in starch synthesis inhibition and therefore prevents the synthesis of starch (Yadav, 2010).

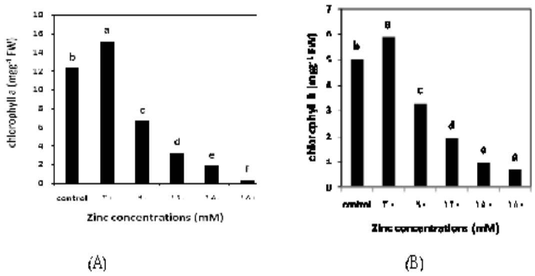

Fig. 1. Effects of zinc on: A- cholorophyll **a** and B- cholorophyll **b** in (*Cucurbita pepo*).

Proline

The results of the measurement of proline in roots and shoots showed an increasing trend. According to least significant difference test increased proline in the root at concentrations of 120 and 150 mM and shoot at concentrations of 90, 120 and 150 mM zinc was significant (Fig 4- A, B).

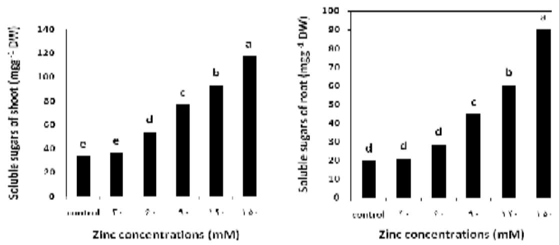

Fig. 2. Effects of zinc on: A-Soluble sugare of shoot and B-Soluble sugar of root in (*Cucurbita pepo*).

Proline accumulation in plants caused by exposure to heavy metals is common (Alia and Saradhi, 1991). Increased proline in plant stress is a defensive mechanism (Koocheki *et al*, 2004). Proline by several mechanisms, such as removal of hydroxyl radicals, osmotic adjustment, protect enzymes and maintains protein synthesis, plant tolerance and resistance against stress increases (Mattioli *et al*, 2009; Motavaseel *et al*, 2014). Proline accumulation was studied in resistant and non-resistant varieties of *Silen vulgaris* to increasing concentrations of zinc (Schat *et al*, 1997). The study also showed that Naked pumpkin (*Cucurbita pepo*), like other plants for defense against stress caused by heavy metals such as zinc, increased levels of proline and soluble sugars.

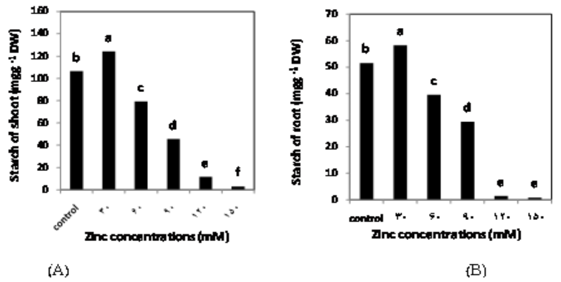

Fig. 3. Effects of zinc on: A- Starch of shoot and B- Starch of root in(*Cucurbita pepo*).

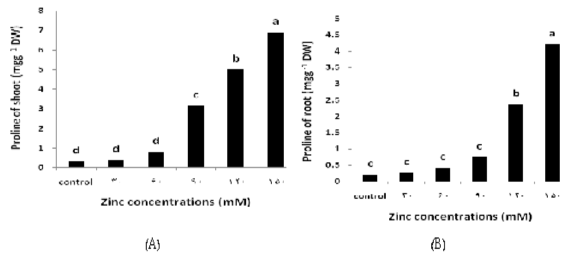

Fig. 4. Effects of zinc on: A- Proline of shoot and B- Proline of root in Naked pumpkin (*Cucurbita pepo*).

Correlation traits

Correlation analysis showed that the photosynthetic pigments with the amount of starch and proline were significant positive and negative correlations, respectively. Negative correlation between soluble sugar and chlorophyll pigments was non-significant. The relationship between starch with soluble sugar and proline in shoot and root was a significant negative correlation. Correlation between soluble sugars and proline was also positive and significant (Tab 2).

Conclusion

In this study it was found that high zinc concentrations decrease of photosynthetic pigments such as chlorophyll a and chlorophyll b. Reduced chlorophyll had a negative effect on plant photosynthesis and plant growth in the results is diminished. However, soluble sugars and proline increased. These compounds are used in various stress for osmotic adjustment, preserve the enzymes and membranes in plant cells. Reduced starch is related to degrade it to soluble sugars. Correlations between traits showed a positive correlation exists between photosynthetic pigments and starch and their correlation with soluble sugar and proline is negative.

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