Foliar application of nano-chelate and non-nanochelate of zinc on plant resistance physiological processes in cotton (*Gossipium hirsutum* L.)

Mohammadali Rezaei* **and Hossein Abbasi**

Department of Biology, Islamic Azad University, Gorgan Branch, Gorgan, Iran

Abstract

Nano-fertilizers are designed in order to gradually release their food contents. They are also manufactured in such a way that the time of their liberation matches with the food product requirement. The use of nano-fertilizers leads to an increase in the efficiency of the consumption of food elements. With regard to the strategic importance of cotton plant and the effects of zinc in increasing plant resistance, the purpose of this research study was comparing the effects of mineral fertilizer of zinc (Mi), chelate of zinc (Ch) and nan-ochelate of zinc (NCh) on the growth and some physiological and biochemical process of the cotton plant. To achieve this goal, the cotton plants in field were cultivated in a completely random design. The first phase of the foliar application of fertilizer was at the square stage or squaring (S) and the second and third stages were squaring-flowering (SF) and a week after flowering (F). Results showed the maximum height, fresh weight, and dry weight were obtained in treatments with nano-chelate and chelate of zinc and there were no significant difference in squaring, squaring-flowering, and flowering application stages. The height, fresh weight, and dry weight of cotton plant increased by application of fertilizers Co, Mi, Ch, and NCh in that order. Chlorophyll a and b increased in nano-chelate and chelate of zinc at squaring and flowering stages. Application of chelate and nano-chelate zinc fertilizer in the present study improved physiological processes in cotton as it increased the amount of chlorophyll and antioxidant activity of peroxidase, catalase, and polyphenol oxidase as a result of increase in fresh and dry weight. Findings suggest that application of zinc chelate and specially the nano-chelate of zinc can improve cotton performance by increasing the number of bolls per plant and mean weight of 20 bolls.

Keywords: flowering; number of boll per plant; nano-fertilizers; zinc; nanochelate; squaring; zinc

Rezaei, M. and **H. Abbasi .** 2014. 'Foliar application of nanochelate and non-nanochelate of zinc on plant resistance physiological processes in cotton (*Gossipium hirsutum* L.)'. *Iranian Journal of Plant Physiology* ,4 (4),1137-1144.

Introduction

Cotton, because of its widespread uses in today's world, is of great economic and a commercial importance and therefore, the area under cultivation of this crop is ever increasing. Generally, cotton fiber as the main product and seeds of cotton as the by-product play an important role in industry and commerce.

Zinc is an essential element for plants and animals and plays an important role in plants metabolic system. This element activates enzymes and involved in protein, lipids, carbohydrates and nucleic acid metabolism (Khan

*Corresponding author
E-mail address: mohalirez@yahoo.com
Received: May. 2014
Accepted: July, 2014

et al., 2002; Zlatimira, 2002; lqbal, 2000). Zinc has a major role in cell defenses against ROS and as a protective factor against several chemical compositions of oxidation such as membrane lipids, protein, chlorophyll, and enzyme having SH and DNA (Cakmak, 2000). Zinc is an essential micronutrient that plays many important roles in various physiological and metabolic processes in all living organisms. It functions as a cofactor for over 300 enzymes and proteins involved in cell division, nucleic acid metabolism, and protein synthesis and it is critical in the controlling gene transcription and the coordination of other biological processes regulated by proteins containing DNA-binding Zn-finger motifs, RING fingers, and LIM domains (Kobayashi, et al., 1998; Broadley, et al., 2007). Zinc also plays a key role in controlling the production and toxicity of free radicals that can damage membrane lipids and sulphydryl groups (Alloway, 2004). It also plays a role in photosynthesis, protein synthesis, cell division, maintaining integrity of the membrane structure (Marschner, 1995), resistance against the pathogen infection (Sawar, 2011), and sexual reproduction through affecting production and shape of pollen and changes in the stigma.

It has been demonstrated that Zn deficiency is one of the most widespread mineral nutritional problems affecting normal development in plants (Pinton, et al., 1993; Mullins et al., 1992). Moreover, zinc deficiency increases the content of soluble sugar, chlorophyll, and protein (Gurmani et al., 2012).

Zinc prolonged deficiency symptoms include decrease in vegetative growth, sexual development (Pathak et al., 2012), zinc deficiency chlorose of small lamina, and a range of auxin deficiency-like responses such as internode shortening, epinasty, inward curling of leaf lamina, and reduction of leaf size (Broadley et al., 2007 ; Volschenk et al., 1996). In grapevines, deficit of Zn results in the development of leaves that are smaller than normal and/or mottled, and shortened internodes. It has been suggested that this reduction in shoot growth results from the fact that Zn is essential for the synthesis of tryptophan, a precursor of the phytohormone indoleacetic acid (IAA) (Mullins, et al., 1992). In this way, vineyards commonly correct Zn deficiency with both soil and foliar application of fertilizers. Under conditions of Zn deficiency, application of foliar Zn fertilizer shortly before anthesis increases the number of flowers that set fruit (Mullins, et al., 1992).

Zn deficiencies can be encountered in neutral to alkaline soils (Roy,et al.,2006) and Zn solubility decreases markedly above pH 6.0-6.5 (Sims, 2000). Zn uptake is positively correlated with the amount of organic matter in the soil and negatively correlated with the phosphorus (P) concentration in the soil (Hamilton et al., 1993; Ahlawat et al., 2007). Soils that have a higher concentration of sand and a lower concentration of organic matter produced lower crop yields which lead to poor Zn utilization (Singh and Ram, 1996). If elements availability in soil is less than the amount required for plant growth, plants will suffer from elements deficiency stress. This may be the result of the inherent deficiency of soil nutrients, low mobility of these elements or chemical elements within soil. Nutrients deficiency is a problem for many plants in agricultural regions of the world and leads to reduction in growth and yield of crop species.

Performance reduction caused by environmental stresses such as drought and element deficiencies is a serious problem. In the U.S. 75 percent of agriculture are potentially faced with these tensions (Bennet and Breen, 1991).

The effect of zinc on the synthesis of auxin is also shown by some researchers and this is the cause of some disorders in growth and development of the plants suffering zinc deficiency (Sarwar, 2011).

As early as 2012, Hanafy Ahmed et al., showed that the growth stimulation after zinc application may be due to the increase in IAA content. Also, Marschner (1995) reported that, in tomato plants, Zn-deficient correlated with a decrease in IAA level and resumption of IAA level takes place after zinc is resupplied , which would be expected if growth was a response of increased supply of auxin caused by application of zinc.

Low levels of IAA in zinc-deficient plants are probably the results of inhibited synthesis of IAA and tryptophan foliar application at the rates of 25, 50 and 100 ppm and IAA at the rates of 100, 200 and 300 ppm, to some extent, can be replace zinc foliar application at the recommended dose

(37.5 ppm Zn) (Hanafy Ahmed et al., 2012). Spiegel-Roy and Goldschmidt (2008) working on citrus, reported that, zinc is essential for the functioning of many enzymes, as well as the synthesis of trptophan, a precursor of indole acetic acid (IAA) and zinc deficiency causes a reduction in RNA synthesis and ribosome stability.

Nanotechnology as a new powerful technology has the ability to create a great revolution and transformation in food supply system in a global scope (Andreta, 2003; Sharma et al., 2005). This technology is particularly applied in chelate fertilizers such as zinc chelate. The present study aims at investigating the impact of zinc chelate fertilizer in two forms of nanochelate and non-nanochelate on growth and some biochemical processes of cotton plant. The study also probes into the effect of these zinc treatments on some physiological parameters of cotton.

Materials and Methods

This research was performed as a field experiment in Bagh Golbon, Gorgan, north of Iran based on a completely random design with four replications in crop year 2013. Certified cotton seeds cv: Golestan were obtained from Iranian Cotton Research Institute in Gorgan. The seeds were planted in 8 plots (3×4m) which were subsequently of assigned to the control (Co), mineral fertilizer of zinc (Mi), chelate of zinc (Ch) and nano-chelate of zinc (NCh) treatments.

The first phase of the foliar application of fertilizer was the stage of square stage or squaring (S); the second phase was at squaring-flowering stage (SF), and the third stage was a week after flowering (F). The 5^{th} and 6^{th} full leaf laminas were collected from plants of 85-90 days age for laboratory assays.

Chlorophyll assay

Chlorophyll content was determined in 1g leaves of a plant, homogenized in 10 ml of acetone 80% and the amount of chlorophyll a and b were measured at wavelengths of 645 and 663 nm by spectrophotometer (Spectronic 20 Genesys TM) (Jensen, 1987).

Catalase assay

Catalase (CAT, EC 1.11.1.6) activity was assayed by measuring the rate of H_2O_2 disappearance at 240 nm. The final volume of reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0) and 2% H_2O_2. The reaction was started by adding 100 µL leaf crude extract to the solution and the activity was calculated as units (µmol H_2O_2 consumed per minute) per mg of protein (Bergmeyer, 1974).

Peroxidase assay

Total peroxidase activity was determined by Cunningham (1969) method. The absorbance was measured spectrophotometrically (Spectronic 20 Genesys TM) at 530 nm wavelength against blank samples. The enzyme activity was estimated based on OD $min^{-1}g^{-1}FW$ (Nickel and Cunningham, 1969).

Polyphenol oxidase assay

Polyphenol oxidase was estimated using Manoranjan and Bandhumishra (1975) method and the absorbance was measured spectrophotometrically at 430 nm wavelength. The enzyme activity was calculated based on OD $min^{-1}g^{-1}FW$.

Statistical analysis

All data were analyzed using SPSS software for Windows: Release 19.0 standard version. When analysis of variance showed significant difference between means, Duncans multiple range test was applied to compare the means at $p<0.05$ and the mean differences of four replicates were compared by the Lowest Standard Error of Means test (SE).

Results

Plant growth parameters

The maximum height, fresh and dry weight were observed in treatments of nano-chelate and then in chelate of zinc. There were no significant differences between treatments at squaring, squaring-flowering, and flowering stages. Also, the impact of the stages in other foliar application of fertilizers, i.e., Control (Co) and mineral zinc (Mi) were not significant. The height, fresh, and dry weights of treated cotton plants increased in Co, Mi, Ch and NCh groups in that order with the lowest levels recorded for control (Table 1).

Chlorophyll a and b content

The highest amount of chlorophyll a was recorded in nano-chelate (N) and chelate of zinc (C) fertilizers at squaring (ChS) and flowering (ChF) stages; maximum level of chlorophyll b was observed in chelate of zinc treatment at squaring (ChS) and flowering (ChF) stages (Table 1). Maximum total chlorophyll content was obtained from chelate of zinc at flowering stage and this treatments made significant difference in total chlorophyll content in comparison with the other treatments (data not shown). Leaf phenolic compounds increased considerably in plants treated with nano-chelate of zinc at squaring stage (NChS), and it was the lowest in control plants at flowering stage (CoF).

Antioxidant activity

Comparison of the means showed the highest levels of activity of peroxidase, catalase, and polyphenol oxidase enzymes in the application of nano-chelate of zinc fertilizer while the lowest levels were in recorded in control where no significant differences were observed at various stages (Table 2).

Weight and number of bolls in plant

The results of the impact of nano and non-nanofertilizers (Tables 1 and 2) revealed that application of zinc particularly caused a significant increase in growth as well as leaf fresh and dry weight index in comparison with control. These fertilizers also increased the activity of antioxidant enzyme and thus enhanced the cotton plant products by increasing the weight of 20 bolls and number of bolls per plant.

Discussion

The maximum amount of height, fresh, and dry weight as growth parameter were observed in treatments of nano-chelate and then in chelate of zinc. Also, similar changes were recorded in the content of chlorophyll a and b, and total chlorophyll content (Table 1). Similar results are reported for zinc deficiency in chickpea (Valenciano et al., 2010; Farooq et al., 2012; Pathak et al., 2012), mung bean (Abd-El-

Table 1
Influence of nano-chelate and non-nanochelate fertilizer and plant stage on cotton plant growth and physiological characteristics

Treatments		Plant Height (cm)	Fresh Weight (g)	Dry Weight (g)	Chl a mg g^{-1}FW ($\times 10^{-3}$)	Chl b mg g^{-1}FW ($\times 10^{-3}$)
Kind of Fertilizer	Stage of Application					
Co	S	91.0±13f	3.8±0.29ef	0.92±0.2f	7.6±1.2e	6.7±0.14e
	SF	87.6±07f	4.5±0.33def	1.15±0.2ef	10.1±1.9d	8.9±1.5de
	F	97.3±04ef	3.7±0.63f	0.80±0.3f	6.2±1.9f	8.8±1.6de
Mi	S	123±07cd	5.7±0.35bcd	1.45±0.2de	12.1±1.4c	10.8±0.7cd
	SF	132±17c	6.0±0.88bc	1.5±0.2cde	11.7±0.4c	12.1±2.1c
	F	112±07de	5.2±0.42cde	1.4±0.2de	11.8±0.2c	12.1±1.7c
Ch	S	139±06bc	6.1±0.81bc	1.8±0.3bcd	14.5±0.1b	12.9±0.7bc
	SF	140±02bc	7.01±1.3ab	2.1±0.6ab	13.6±0.7b	12.1±1.9c
	F	143±04bc	6.4±0.9abc	1.7±0.3bcd	14.6±1.5b	12.3±1.9c
NCh	S	157±12ab	7.6±0.18a	2.0±0.1abc	17.7±0.5a	13.0±1.4bc
	SF	158±12ab	7.8±1.5a	2.5±0.2a	16.4±0.4a	16.0±0.2a
	F	164±20a	7.8±0.36a	2.4±0.1a	17.5±0.8a	15.0±0.3ab

Four kind of fertilizer application on foliars, Co: Control plant, Mi: Mineral fertilizer of zinc, Ch: Chelate of zinc, NCh: nano-chelate of zinc. Three phase of foliar application of fertilizer included the first phase: squaring (S), the second phase: squaring-flowering (SF), and the third phase: flowering(F). Values in the table are mean ± SD (n = 3) and ($P < 0.05$).

Lateef et al., 1998; Thalooth et al., 2006; Ali and Mahmoud, 2013), groundnut (Gobarah, 2006), and tomato (Ejaz et al., 2011; Gurmani et al., 2012).

Zinc is an essential element for the normal and healthy growth and reproduction of plants, animals, and humans. When the supply of plant available zinc is inadequate, crop yields are reduced and the quality of crop products is frequently impaired (Sarwar, 2011). This is attributed to the positive effect of zinc fertilizer on auxin biosynthesis that can stimulate cell division and better absorption of minerals and thus increase the plant growth (El-Tohamy, and El-Greadly, 2007; Cakmak, 2000). The higher growth promoting hormone contents in response to the application of Zn may be due to the fact that zinc has an effect on building up the natural auxin (IAA) and consequently activating the cell division and enlargement (El-Tohamy, and El-Greadly, 2007). In plants, zinc plays a key role as a structural constituent or regulatory co-factor of a wide range of different enzymes in many important biochemical pathways and these are mainly concerned with carbohydrate metabolism (both in photosynthesis and in the conversion of sugars to starch), protein metabolism, auxin (growth regulator) metabolism, pollen formation, maintaining the integrity of biological membranes, and the resistance to infection by certain pathogens (Alloway, 2004).

In the present research application of chelate and nano-chelate of zinc lead to an increase in the rate of photosynthesis pigments. This fits with the results of Akay (2011) for the content of chlorophyll in chickpea. The results of the correlation analysis revealed significant relationships between Zn content in the leaves and total chlorophyll concentration (Akay, 2011). Maximum activity of peroxidase, catalase, and polyphenol oxidase enzymes were obtained in nano-chelate of zinc fertilizer and the minimum activity was recorded in control with no significant differences between various stages

Table 2
Influence of nano-chelate and non-nanochelate fertilizers and plant stage on cotton plant growth and physiological characteristics.

Treatments		Peroxidase Activity (OD $min^{-1}g^{-1}FW$) ($\times10^{-3}$)	Catalase Activity (OD min^{-1} g^{-1} FW)	Polyphenol Oxidase Activity (OD min^{-1} $g^{-1}FW$) ($\times10^{-3}$)	Weight of 20 Boll	Number of Boll per Plant
Kind of Fertilizer	Stage of Application					
	S	**31±4e**	**0.44±0.036f**	**137±6 f**	**0.33±0.02f**	**12±2.64e**
Co	SF	**28±3e**	**0.46±0.026e**	**127±4 f**	**0.33±0.05f**	**12.33±1.5d**
	F	**25±4e**	**0.521±0.010e**	**133±2.8f**	**0.36±0.02ef**	**12.3±1.4 d**
	S	**48±10d**	**0.53±0.022e**	**164±1.4ef**	**0.40±0.01e**	**19±2.6d**
Mi	SF	**33±1e**	**0.55±0.010e**	**153±1f**	**0.45±0.02d**	**20±1.7c**
	F	**45±2d**	**0.62±0.0215d**	**189±1de**	**0.40±0.017e**	**22±1.5bc**
	S	**50±3cd**	**0.69±0.011c**	**219±5cd**	**0.52±0.036bc**	**26±3.5b**
Ch	SF	**66±4b**	**0.66±0.026cd**	**250±1.9bc**	**0.51±0.01bc**	**27±1.5b**
	F	**59±2bc**	**0.64±0.042d**	**198±2de**	**0.48±0.01cd**	**26±4.3b**
	S	**78±1a**	**0.87±0.017b**	**261±1b**	**0.54±0.01ab**	**36±3a**
NCh	SF	**81±2a**	**0.87±0.026b**	**309±11a**	**0.57±0.01a**	**36±6a**
	F	**77±2a**	**0.95±0.011a**	**328±2.8a**	**0.58±0.03a**	**33±1.7a**

Four groups of foliar fertilizer applications, Co: Control plant, Mi: Mineral fertilizer of zinc, Ch: Chelate of zinc, NCh: nano-chelate of zinc. Three phases of foliar application of fertilizer included the first phase: squaring (S), the second phase: squaring-flowering (SF), and the third phase: flowering (F). Values in the table are means ± SD (n = 3)and ($P < 0.05$).

(Table 2). Increase in the catalase and peroxidase activity with application of zinc was also reported in *Jatropha curcas* seedlings (Zeng–binm et al., 2010). Weisany et al. (2012) found that application of zinc on shoot and root of soybean increased peroxidase, catalase, and polyphenol oxidase enzymes activity, significantly. In the present study, the application of zinc fertilizer in cotton plant in two forms of chelate and nano-chelate influenced cotton performance through improving physiological processes including increase in the amount of chlorophyll and antioxidant activity of peroxidase, catalase, and polyphenol oxidase as well as increase in fresh and dry weight. In fact, the application of zinc chelate and specially the nano-chelate of zinc can be considered as a measure for cotton crop improvement by increasing weight of 20 bolls and number of bolls per plant.

Acknowledgements

The researcher is highly thankful to Mr. Alishah for kindly providing him with seeds that were first produced and patented in 2013 at Iranian Cotton Research Institute in Gorgan, Golestan, Iran.

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