Potential benefits of foliar application of chitosan and Zinc in tomato

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Abstract

The current study was carried out to investigate the efficiencies of foliar supplementations of Zinc and/or chitosan on the growth and physiology of tomato (*Lycopersicon esculentum* L.) and clarify the involved mechanisms. Seedlings were sprayed with three concentrations (0, 50, and 100 mgL^{-1}) of chitosan and/or three levels of Zinc sulfate (0, 50 and 100 mgL^{-1}). The application of Zn and/or chitosan led to increases in shoot fresh mass, about 31%, over control. In comparison with the control, enhancement (approximately 28.5%) in shoot dry mass resulted from the single application of chitosan or zinc while this percentage reached to about 45% for seedlings simultaneously treated with the chitosan and Zn supplements. About 29% improvements in the plant height resulted from the chitosan and/or zinc. Higher amounts of chlorophyll contents were recorded in the chitosan and/or zinc-treated plants, among which the highest levels were found in the combined treatments. Simultaneous applications of chitosan and zinc were the most effective treatments to induce PAL (phenylalanine ammonia lyase) activity (about 64%) when compared to the control. Chitosan and/or zinc treatments, especially the latter, significantly promoted the activity of (SOD) superoxide dismutase enzyme (about two folds), over the control. Also, increases in proline contents was provoked by applying the treatments. Foliar supplementations of these compounds as an eco-friendly solution may have considerable potential to act as exogenous elicitors and trigger various physiological traits, thereby improving plant growth and resistance under abiotic stress conditions.

Keywords: Bio-fortification; Chitosan; elicitor; foliar; *Lycpersicon*

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Introduction

Chitosan is regarded as a biodegradable, low toxic, and cost effective substance formed by deacetylation process of chitin (Iriti et al., 2009), applied in various food, agriculture, and medicine industries (Pichyangkura and Chadchawan, 2015). Antimicrobial characteristics of this biopolymer have been well known. Interestingly, it may act as an exogenous elicitor to improve plant immunity (Pirbalouti et al., 2017). Various strategies have been investigated to find the eco-friendly solutions for improving crop protection, among which chitosan is a suitable candidate, taking into account sustainable agriculture and environmental issues (Asgari-Targhi et al., 2018).

Zinc (Zn) deficiency in soil, especially in calcareous soils has been proposed as a global concern (Subramanian et al., 2014) and an important limiting factor affecting plant growth

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and yield. There are evidences that about two billion people are indirectly influenced by this deficiency (Subramanian et al., 2014). Zinc is known as a vital microelement in various living organisms (Cakmak, 2008). Implications of Zn in some crucial plant process, including cell division, DNA, and RNA metabolism, as well as protein biosynthesis has been well documented (Ahmed et al., 2012). Zinc as a cofactor of many enzymes plays crucial roles in various aspects of plant metabolism (Simoglou and Dordas, 2006). In addition, Zn deficiency affects plant growth and development mainly due to its involvements in the synthesis of auxin precursor, tryptophan (Taiz and Zeiger, 2010). Zinc is known as a critical micronutrient and its implications in different aspects of cellular metabolism like protein synthesis and nucleic acid metabolism have been well documented (Ahmed et al., 2012). Moreover, there are growing concerns worldwide regarding Zn deficiency in humans which is recorded among the top five micronutrient deficiencies (Hotz and Brown 2004), and crops (Zhang et al., 2012). Key tools of biofortification are breeding, biotechnology, and fertilization. So, foliar fertilization of crops with Zn is considered as a short-term and complementary way, which is required for making Zn pool and compensating Zn deficiencies (Zhang et al., 2012). Obviously, micro-elements have narrow boundaries between requirement and toxicity levels and play critical roles in quality and quantity of yield. So, more convincing studies are required to determine the effects of various levels of exogenously applied substances.

Foliar supplementations possess suitable potential as a high efficiency eco-friendly method for achieving different biological aims, including improving plant growth, affecting biosynthesis of vital secondary metabolites, and improving plant resistance against various biotic and abiotic stress conditions. There are limited research studies on the simultaneous application of Zn and chitosan and their possible interactions.

The current was carried out with the aim of evaluating the efficiencies of foliar supplementations of Zn and/or chitosan in the growth and physiology of tomato plants and clarifying the involved mechanisms in this important crop.

Material and Methods

Experimental design and treatments

A complete randomized study was employed as the experimental design. Tomato seeds were purchased from a reliable center. The seeds were sterilized with NaOCl solution for ten minutes and washed completely with distilled water and planted in pots of 3 Kg containing peat and perlite (1:1). The seedlings were grown in natural condition (relative humidity of 56 % and mean light intensity of 90 µM photon $m^{-2}s^{-1}$). Thirty five-day old seedlings were sprayed with three concentrations (0, 50, and 100 mgL^{-1}) of chitosan ($C_{56}H_{103}N_9O_{39}$; 110 kDa; de-acetylation 85%; Solarbio Life Sciences Company) and/or three levels of Zinc sulfate (0, 50, and 100 mgL^{-1}).

Foliar applications were done three times with one-week interval. Two weeks after the last treatment, plants were harvested for different physiological analysis. Plants were grouped in nine different groups and called C = Control, CS50 = foliar treatment with chitosan 50 mgL^{-1}, CS100 = foliar treatment of chitosan 100 mgL^{-1}, Zn50 = seedlings treated with Zinc 50 mgL^{-1}, Zn100 = seedlings treated with Zinc 100 mgL^{-1}; $Zn_{50}CS_{50}$ = simultaneous supplementations with chitosan and Zinc 50 mgL^{-1}, $Zn_{50}CS_{100}$ = simultaneous application of chitosan of 100 mgL^{-1} and Zinc of 50 mgL^{-1}; Zn100CS50 = combined treatment of chitosan 50 mgL^{-1} and Zinc 100 mgL^{-1}, $Zn_{100}CS_{100}$ = simultaneous application of chitosan 100 mgL^{-1} and Zinc 100 mgL^{-1}.

Determination of biomass and photosynthetic pigments

Total shoot fresh and dry mass and plant height were measured. Chlorophyll was extracted using acetone (80% (v/v)) as a solvent and spectrophotometrically assayed according to the equation presented by Arnon (1949). Chlorophyll contents were expressed in milligrams per gram leaf fresh weight ($mgg^{-1}FW$).

Table 1
Effects of the various concentrations of Chitosan and/or Zinc on the different physiological characteristics related to the photosynthetic pigments, plant growth and biomass

Treatments	Shoot fresh mass (g)	Shoot dry mass (g)	Height (cm)	Chla ($mgg^{-1}FW$)	Chlb ($mgg^{-1}FW$)	Total chlorophyll ($mgg^{-1}FW$)
C**	7.00 ^{f*}	1.79^{e}	28.67^{d}	0.47^{d}	0.25^{e}	0.72^{f}
CS_{50}	8.27^{e}	2.16^{d}	31.07^{d}	0.58^{c}	0.28^{de}	0.87^{e}
CS_{100}	8.65^{de}	2.30^{c}	35.66^{c}	0.60^{c}	0.29^{cd}	0.89^{de}
Zn_{50}	8.94^{cd}	2.3^{6c}	37.33^{c}	0.62^{bc}	0.31^{bcd}	0.93^{cde}
Zn_{100}	9.50^{bc}	2.43^{bc}	37.00^{c}	0.63^{abc}	0.32^{abc}	0.96^{bcd}
$Zn_{50}CS_{50}$	9.92^{ab}	2.54^{ab}	38.66^{bc}	0.66^{ab}	0.34^{abc}	1.00^{abc}
$Zn_{50}CS_{100}$	9.71^{ab}	2.58^{a}	40.33^{abc}	0.67^{ab}	0.35^{ab}	1.02^{ab}
$Zn_{100}CS_{50}$	10.20^{a}	2.67^{a}	43.66^{a}	0.69^{a}	0.37^{a}	1.05^{a}
$Zn_{100}CS_{100}$	9.78^{ab}	2.61^{a}	42.33^{ab}	0.62^{bc}	0.33^{abc}	0.95^{bcd}

***** Mean values followed by different letters are significantly different at P<0.05 according to Duncan's multiple range test.
** C: Control; CS_{50}: foliar treatment with chitosan of 50 mgl^{-1}, CS_{100}: foliar treatment of chitosan of 100 mgl^{-1}, Zn_{50}: seedlings treated with of Zinc of 50 mgl^{-1} Zn_{100}: seedlings treated with of Zinc of 100 mgl^{-1}, $Zn_{50}CS_{50}$: simultaneous supplementations with chitosan and Zinc of 50 mgl^{-1}, $Zn_{50}CS_{100}$: simultaneous application of chitosan of 100 mgl^{-1} and Zinc of 50 mgl^{-1}, $Zn_{100}CS_{50}$-: combined treatment of chitosan of 50 mgl^{-1} and Zinc of 100 mgl^{-1}, $Zn_{100}CS_{100}$: simultaneous application of chitosan of 100 mgl^{-1} and Zinc of 100 mgl^{-1}

Enzyme extraction and protein determination

Enzymes were extracted at 4° C with a mortar and pestle using 0.1 M phosphate buffer at pH of 7.5, containing 0.5 mM Na_2-EDTA and 0.5 mM ascorbic acid as an extraction buffer. The homogenates were centrifuged for 15 min at 4° C and the supernatants were applied as enzyme extracts. Protein contents in the prepared extracts were determined according to the method of Bradford (1976).

Determination of phenylalanine ammonia lyase (PAL) activity

PAL activity was determined according to the method previously described by Beaudoin-Eagan and Thrope (1985). PAL activity was assayed by measuring the amount of produced cinnamic acid using the standard curve of cinnamic acid and expressed in micromole cinnamate per minute per milligram protein ($\mu molCinMin^{-1}mg^{-1}pr$).

Measurement of superoxide dismutase (SOD) activity

SOD activity was analyzed by the rate of NBT (nitro blue tetrazolium) reduction according to the method of Giannopolitis and Ries (1977). SOD activity was expressed in unit enzyme per milligram protein (Unit E $mg^{-1}pr$).

Measurement of proline content

Proline content was carried out according to the method of Bates et al. (1973). Proline was extracted using sulfa salicylic acid (3% w/v). The mixtures of extract, glacial acetic acid, and ninhydrin reagent were kept at 100° C for one hour. After cooling and mixing with toluene, the toluene phase was used for measuring absorbance at 520 nm. Proline contents were assayed based on the proline standard curve and expressed in milligrams per gram leaf fresh weigh ($mgg^{-1}FW$).

Statistical Analysis

The obtained data was analyzed using SPSS software. Mean separation was performed with Duncan's multiple range test at P≤0.05.

Results

In comparison with the control, shoot fresh masses were increased by 21%, 31%, and 41% for the chitosan, Zn, and combined treatments of chitosan and Zn, respectively (Table 1). Over the control samples, significant enhancements (approximately 24% and 33%) in

Table 2
Effects of the various concentrations of Chitosan and/or Zinc on the different physiological characteristics, including leaf PAL activities, SOD activities, and proline contents.

Treatments	PAL ($\mu molCinMin^{-1}mg^{-1}pr$)	SOD (Unit $Emg^{-1}pr$)	Proline ($mgg^{-1}FW$)
C**	0.98^{e*}	20.97^{e}	0.45^{f}
CS_{50}	1.39^{cd}	30.83^{d}	0.52^{e}
CS_{100}	1.56^{abc}	31.75^{d}	0.56^{d}
Zn_{50}	1.22^{d}	37.32^{cd}	0.54^{de}
Zn_{100}	1.34^{cd}	46.05^{ab}	0.6^{bc}
$Zn_{50}CS_{50}$	1.45^{bc}	41.47^{bc}	0.57^{cd}
$Zn_{50}CS_{100}$	1.67^{ab}	44.13^{abc}	0.61^{bc}
$Zn_{100}CS_{50}$	1.62^{ab}	47.53^{ab}	0.66^{a}
$Zn_{100}CS_{100}$	1.7^{a}	49.56^{a}	0.63^{ab}

***** Mean values followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.
** C: Control, CS_{50}: foliar treatment with chitosan of 50 mgL^{-1}, CS_{100}: foliar treatment of chitosan of 100 mgL^{-1}, Zn_{50}: seedlings treated with of Zinc of 50 mgL^{-1}, Zn_{100}: seedlings treated with of Zinc of 100 mgL^{-1}, $Zn_{50}CS_{50}$: simultaneous supplementations with chitosan and Zinc of 50 mgL^{-1}, Zn50CS100: simultaneous application of chitosan of 100 mgL^{-1} and Zinc of 50 mgL^{-1}; $Zn_{100}CS_{50}$: combined treatment of chitosan of 50 mgL^{-1} and Zinc of 100 mgL^{-1}: $Zn_{100}CS_{100}$- simultaneous application of chitosan of 100 mgL^{-1} and Zinc of 100 mgL^{-1}

shoot dry mass resulted from the single applications of chitosan or Zn while these percentages improved and reached to about 45% for the seedlings simultaneously supplemented with chitosan and Zn. Similarly, significant improvements in plant height by 16%, 29.5%, 42% were respectively found in the seedlings treated by chitosan, Zn or combined treatments, over the untreated control (Table 1). Chitosan and/or Zn supplementations led to the significant increases in Chla contents by 25%, 33%, and 40%, compared with the control (Table 1). The highest amount of Chlb was observed in the $Zn_{100}CS_{50}$ treatment group (Table 1). Individual applications of chitosan or Zn resulted in 11% and 31% promotions in total chlorophyll contents while the combined treatments led to 39% enhancements of this trait (Table 1). In comparison with control, single treatments of chitosan or Zn significantly induced PAL activity by 50% and 30%, respectively (Table 2). The simultaneous supplementations of chitosan and Zn were the most effect treatment to induce PAL activity (64%) when compared to the control (Table 2). As shown in Table 2, the foliar applications of these compounds led to the significant inductions in SOD activities. Chitosan and/or Zn treatments significantly promoted activity of SOD enzyme (about two fold). Similarly, in comparison with the control, 19.5%, 26.5%, and 37% increases in proline contents caused by individual treatments of chitosan, Zn, and combined treatments, respectively (Table 2). The highest amounts of proline contents were recorded in $Zn_{100}CS_{50}$ treatment group.

Discussion

The assessment of the effects of single or combined applications of chitosan and Zn on the growth-related characteristics revealed that the foliar supplementations of these fertilizers, especially the simultaneous applications, have considerably improved the growth and biomass accumulation. In addition, some critical physiological changes were recorded. Accordingly, increases in photosynthetic pigments and possibly photosynthesis, regulations in enzyme activity, controlling the transpiration rate, and improvements in plant nutritional status may be regarded as the involved mechanisms. The potential benefits of chitosan have been reported in some plant species such as orchid (Nge et al., 2006), maize (Suvannasara et al., 2011), okra (Mondal et al., 2012), turmeric (Anusuya et al., 2016), and pepper (Asgari-Targhi et al., 2018). Studies have shown that the exogenous application of chitosan influences the net photosynthetic rate, stomatal conductance, and transpiration rate of soybean and maize (Khan et al., 2002**).** However, the high levels of chitosan may inhibit growth and even lead to death

(Vasil'ev et al., 2009; Zuppini et al., 2004; Asgari-Targhi et al., 2018).

Findings revealed that Zn and chitosan had a considerable potential to trigger critical physiological alterations, including key enzymes (PAL and SOD), proline contents, as well as photosynthetic pigments. Based on the findings, chitosan was more effective than Zn to induce PAL activity, whereas Zn potential to enhance SOD activity was more than that of the chitosan.

Results clearly indicated that the simultaneous supplementations of chitosan and Zn were the most effective method to trigger SOD and PAL (two key enzymes implicated in defense related responses). It has been stated that chitosan may act as an efficient elicitor to trigger signaling pathways (El Hadrami et al., 2010; Asgari-Targhi et al., 2018). Chitosan activates the synthesis and accumulation of defense-related proteins among which PAL is regarded as a critical key enzyme (El Hadrami et al., 2010). Application of carboxymethyl chitosan induced three key enzymes activities involved in nitrogen metabolism, including nitrate reductase, glutamine synthetase and protease, resulting in enhanced growth rate in rice plants (Li et al., 2001). With respect to Zn implications in the activity of Cu/Zn-SOD, Zn detoxifies superoxide radicals, thereby protecting membranes against oxidative destruction (Simoglou and Dordas, 2006). Also, there is evidence that the exogenous application of Zn ameliorated the adverse effects of drought stress on wheat via regulating the activities of antioxidant enzymes, including SOD, peroxidase, and catalase (Yavas and Unay, 2016).

Another important dependent variable studied in the current research is proline content. The observed increases in leaf proline contents caused by chitosan and/or Zn supplementations may be due to their ability to trigger signaling pathways, thereby affecting metabolism. Increase in the proline content is regarded as a crucial mechanism, thereby improving plant resistance against various environmental stress conditions. Proline acts as an important protein and cell membrane stabilizer, antioxidant compound, and compatible osmolyte (Ardebili et al., 2015). Hence, it should be regarded as a critical metabolism index. Therefore, it seems that the applied treatments not only had desirable effects on the growth rates, but also may have improved the cellular potential resistance against environmental changes which is of importance.

In conclusion, the foliar applications of Zn and/or chitosan, especially the simultaneous treatments (an eco-friendly way) may be considered for their potential as exogenous elicitors to trigger various physiological traits, thereby improving plant growth and resistance.

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