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ORIGINAL ARTICLE

Effect of silica Nanoparticles on Basil (*Ocimum basilicum)* Under Salinity Stress

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KEYWORDS

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ABSTRACT: Application of nanofertilizers is one of the promising methods for increasing resources use efficiency and reducing environmental pollutions. Uncontrolled application of chemical fertilizer and pesticides has caused many problems to human health and domestic animals. Nanofertilizers application could be a suitable way to reduce these problems. Accordingly, in order to assess the silicon nanoparticles effect on some vegetative features of basil under salinity stress, a factorial experiment based on a completely randomized design with three replications was conducted in greenhouse condition. Treatments included different levels of silicon fertilizer (without silicon, normal silicon fertilizer and silicon nanoparticles) and salinity stress (1, 3 and 6 ds/m). Physiological traits (chlorophyll and proline content of leaves) and morphological traits (shoot fresh weight and dry weight) were investigated in this study. Results showed a significant reduction in growth and development indices due to the salinity stress. Leaf dry and fresh weight reduced by increment in NaCl concentration while significantly ($P \leq 0.01$) increased with silicon nanoparticles application. The chlorophyll content reduced in salinity stress, but increased by silicon nanoparticles treatment. Proline content increased under salinity stress which was a response to stress. Moreover, proline increased by silicon nanoparticles which was due to tolerance induction in plant. Silicon nanoparticles application reduced the pollution effects originated from salinity in Basil.

INTRODUCTION

Salinity is one of the major factors reducing plant growth in most parts of the world. There are 24 million ha of saline soil in Iran, which is equal to 15% of Iran's agricultural lands [1]. Uncontrolled application of chemical fertilizers has affected the world water resources and has increased swamps. The common

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chemical fertilizers have low application efficiency and cause pollution in the environment. Moreover, increased saline lands results in the enhancement of zoonotic diseases. Nanotechnology which studies the nanometer scales is able to change the food and agricultural sectors, medicine, environmental, sanitation and safety, water resource and etc. [2]. Mineral consumption of silicon is a mechanism of alleviation of salinity stress effects on plants. Silisium is the second most abundant element in soil that constitutes to 28% of the earth's crust [3] and alleviates the salinity stress. Investigations have shown the positive effect of silisium on plants yield and growth [4, 5, and 6]. Silisium sediments in cell wall and forms complexes with macromolecules (Cellulose, pectin, glycoproteins and lignin) to produce colloidal compounds with high absorption surface. One gram of silisium particles with 7 nm diameter has absorption surface equal to 400 m^2. So, the nanoparticles of silisium could have an effect on xylem and water translocation which consequently increases the water use efficiency [7]. Silica is the second most abundant element in the earth's crust and is a beneficial element for plants. Silica also has an important role in plant tolerance to environmental stresses such as salinity stress. Plant photosynthesis also increases by silisium which results in leaf number and leaf area increment. Moreover, fresh and dry weight in leaf and root significantly increases by silisium application under salinity stress [8].

Use of nanofertilizers in plant nutrition is one of the major roles of nanotechnology in agriculture, soil and water sciences [9]. Fe nanoparticles as catalyzer accelerate the oxidation process and convert the pollutant materials (3-chloro ethane and dioxins) to carbon compound with a lower toxicity level. In addition, researches have shown that nanofertilizer application such as zinc oxide is effective in soil improvement and increasing land fertility in industrial countries [10].

Many of researchers have been able to produce silica nanoparticles from natural sources. These particles were produced from rough rice for the first time which was a cheap method. Nutrients are absorbed by plant root or leaves; nanofertilizers are absorbed by both organs due to their gradual and controlled releasing. So, nanofertilizers are preferred to other fertilizer types. The nanofertilizers application is most effective in comparison with common fertilizers due to more efficient absorption by plants and fast releasing [11]. Nanotechnology has been used in nanofertilizers as an instrument to synchronize the releasing and absorption of phosphorous and nitrogen in plant and also inhibit the interaction between nutrients, microorganisms, water and air. Nanofertilizers could prevent a huge money loss due to low efficiency of common chemical fertilizer application. In addition, nanoparticles can increase the yield through light reduction of nitrogen gas [12]. Generally, some benefits of nanofertilizers application include: (1) increase of efficiency and food quality due to accelerated absorption, (2) prevention of fertilizers loss by leaching and complete absorption by plant due to availability and controlled release in the growth period, and (3) reduction in soil and water pollution and consequently food products through reduction of fertilizer leaching. Silica nanoparticles absorbed by root produce a layer in cell wall which helps to tolerate stresses and improve yield in plants [12].

Basil (*Ocimum basilicum*) belongs to Labiatae family with high variation in morphologic traits among the ecotypes [13]. This genus has more than 30 species [14]; *O. basilicum* L. is the most commercial one and it is being cultivated all over the world. This plant needs warm climate and sufficient sunlight in the growth period. The optimum temperature for basil seed germination is 18-20 °C. Basil also needs enough water in its growth period. The soil with medium texture or sandy loam texture containing high humus is suitable for basil cultivation.

It seems that there is little information about silica nanoparticles effect on basil under salinity stress. Therefore, the purpose of this research was studying the silica nanoparticles effect on basil traits under salinity stress.

MATERIALS AND METHODS

This research was conducted in the Research Greenhouse of the Faculty of Agriculture, Islamic Azad University of Damghan, Iran, in 2012. The experiment was conducted in factorial based on a completely randomized design with two factors and three replications. The first factor was three levels of salinity stress (1, 3 and 6 ds/m) and the second factor was three levels of silica fertilizer were dissolved in water. (S_1, without silica fertilizer; S_2, common silica fertilizer; S_3, silica nanoparticles). Soil samples were prepared from 30 cm depth and after drying, passed through a 4 mm sieve. Plastic pots with 30 cm diameter were filled with sandy-loam soil and were used as the cultivation medium.

First, eight seeds were planted in each pot. After four weeks, the seedlings were treated with saline water and Silica. Salinity levels were applied to random plants every other day at 6 stages during two weeks. After this, the plants were irrigated by distilled water for one week in order to protect plants from severe salinity. NaCl was used to produce salinity treatments. Then, the plants were irrigated with different levels of silica was dissolved in water, to find their effects on saline stressed plants. Therefore, plants were irrigated with three levels of silica (without silica fertilizer, common silica fertilizer and silica nanoparticles) for two weeks. In this regard, 10 ml silica nanoparticles was dissolved in 1 L of distilled water and sprayed on plants. Similarly, 10 ml of common silica fertilizer was dissolved in 1 L of distilled water and sprayed on other plants. The treatments were applied every other day during two weeks. Then, the effect of treatments on basil growth and physiological traits were assessed. Chlorophyll content was assayed by Arnon [15] method in new and developed leaves. Proline content of leaves was measured by Bates et al. [16] method. Fresh weight of leaves was immediately recorded by digital scale and then leaves were kept in an 80°C oven for 48 h to obtain the dry weight. Data were analyzed by SAS software and means were compared according to the Duncan's multiple ranges test at $P \leq 0.01$.

RESULTS

Chlorophyll a

When pigments were assayed by Arnon [1] method, a significant difference ($P<0.01$) was observed in chlorophyll a content in plants treated with different levels of NaCl (1, 3 and 6 ds/m) and silica (S_1, without silica fertilizer; S_2, common silica fertilizer; S_3, silica nanoparticles). The highest content of chlorophyll a was achieved in N_3S_3 treatments while the lowest amount was observed in N_3S_2. There was a significant different between N_3S_3 and N_3S_2. This result shows that chlorophyll a content in severe salinity stress (6 ds/m) significantly increased by the application of silica nanoparticles, in comparison with the common silica fertilizer. Because no salinity stress was applied in N_1S_1, N_1S_2 and N_1S_3, there was no significant different between these treatments regarding to chlorophyll a content; chlorophyll content in these three treatments were increased in a similar trend. Generally, the chlorophyll a content in basil reduced due to different NaCl treatments, but increased by silica nanoparticles application (Figure 1).

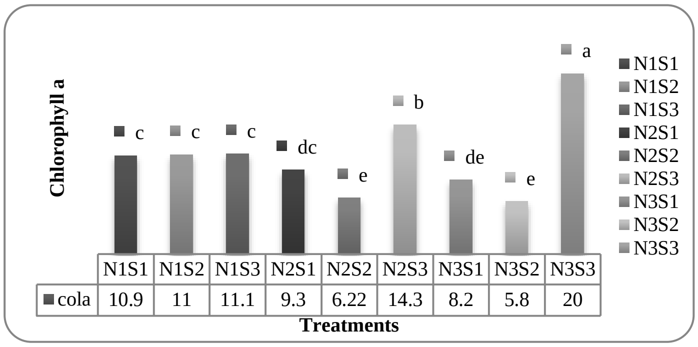

Figure 1. Effect of the treatments on chlorophyll a content

Chlorophyll b

Studying pigments by Arnon [15] method, a significant difference (P<0.01) was observed in the chlorophyll b content in plants treated with different levels of NaCl (1, 3 and 6 ds/m) and silica (S_1, without silica fertilizer; S_2, common silica fertilizer; S_3, silica nanoparticles). The highest content of chlorophyll b was observed in N_1S_3 treatments while the lowest content was observed in N_3S_1. Silica nanoparticles application increased chlorophyll b content in non-saline treatments, in comparison with 3 ds/m treatment; meaning that an increasing trend was observed between "N_1S_1, N_1S_2 and N_1S_3", "N_2S_1, N_2S_2 and N_2S_3", and "N_3S_1, N_3S_2 and N_3S_3" treatments. The highest content of chlorophyll b was achieved in treatments containing silica nanoparticles; however, in salinity × silica fertilizer interaction, no significant differences were observed between N_3S_3, N_2S_3 and N_1S_3 (Figure 2).

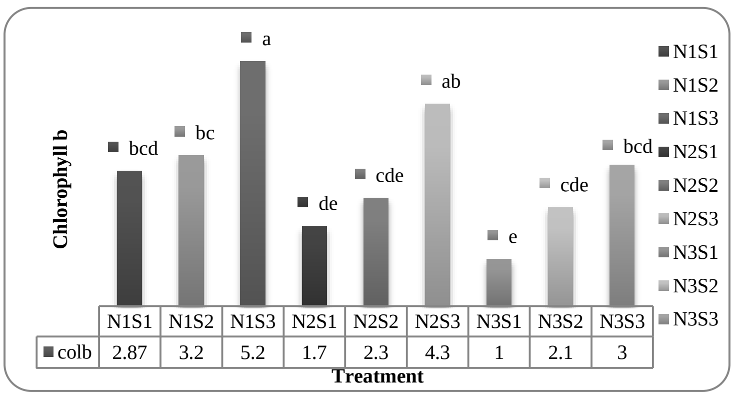

Figure 2. Effect of the treatments on chlorophyll b content

Proline

Analysis of variance showed the significant effect of NaCl (1, 3 and 6 ds/m) on proline content. Proline assay showed that the application of different levels of silica (S_1, without silica fertilizer; S_2, common silica fertilizer; S_3, silica nanoparticles) and NaCl (1, 3 and 6 ds/m) significantly increased proline content. The highest and the lowest content of proline were observed in N_3S_3 and N_1S_1, respectively. The proline content increased with increasing severity of salinity stress. Moreover, proline content significantly ($P \leq 0.01$) increased when silica nanoparticles were applied in severe saline stress (6 ds/m), in comparison with common silica fertilizer. The interaction of salinity × silica fertilizer for proline showed that plants sprayed with silica nanoparticles under severe salinity stress had more proline content in comparison with plants treated by the common silica fertilizer. Proline content was the same in interactions containing the common silica fertilizer (Figure 3).

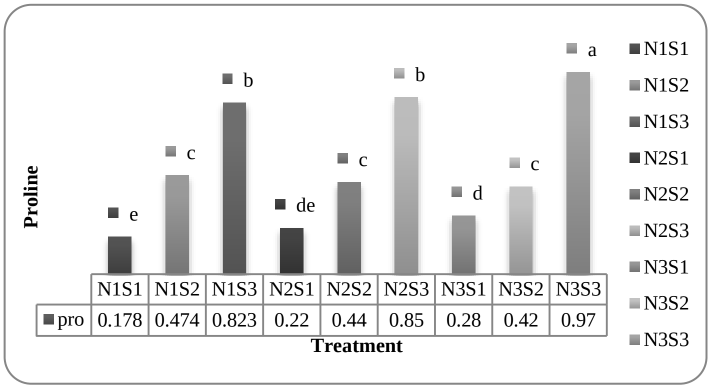

Figure 3. Effect of the treatments on proline content (Mg/g F.W)

Fresh and dry shoot weight

The variation of fresh weight of basil was significant (P<0.01) in different treatments and fresh weight significantly decreased with increasing salinity stress level. The lowest fresh weight was observed in N_3S_1 (6 ds/m × without fertilizer). There was a significant (P<0.01) difference between control and N3S1 treatments. Shoot fresh weight decreased in different levels of NaCl but as shown in Figure 4, significantly (P<0.01) increased by the application of silica nanoparticles, even after one month of being treated with saline water. After silica nanoparticles application, the fresh weight increased in N_1S_3, N_2S_3 and N_3S_3 while this increment did not occur in N_1S_2, N_2S_2 and N_3S_2 (Figure 4). Shoot dry weight was calculated after samples were dried in 80°C oven (Figure 5).

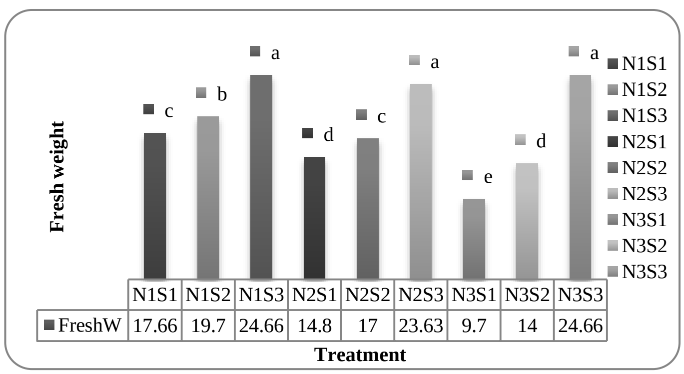

Figure 4. Effect of the treatments on fresh weight (g)

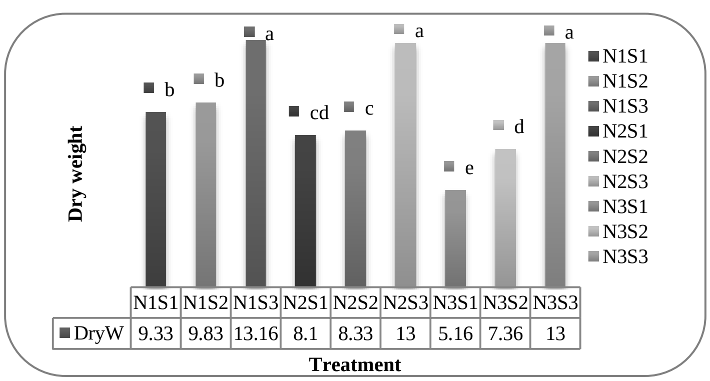

Figure 5. Effect of the treatments on dry weight (g)

DISCUSSION

Growth reduction caused by salinity stress and growth improvement caused by silica nanoparticles application can be observed in Table 1. Analysis of variance showed that salinity × silica nanoparticles interaction not only affected chlorophyll b, but was also effective on other traits. The silicon nanoparticles treatment had a significant effect on all traits and caused a significant increase in fresh and dry weight; however, a significant improvement was not observed when common silica fertilizer was applied. Leaf chlorophyll content reduced by salinity stress in three levels (1, 3 and 6 ds/m). In fact, reduction in leaf growth and chlorophyll content is the fastest response of plants to salinity [17]. Salinity increases the required energy for preservation of normal cell condition; lower energy remains to support plant growth [18]. Leaf area development and chlorophyll content increase as the result of silica availability to plants under saline stress and consequently give more fresh and shoot dry weight. Silica nanoparticles increase turgor pressure and plant size by improving water use efficiency and leaf relative water content [19]. In this experiment, shoot fresh and dry weight was affected by salinity stress. Osmotic potential increases in the plants due to enhancement of salt concentration under salinity stress and turgor pressure reduces through the reduction of water absorption. Water loss in cells inhibits their growth. Moreover, the mineral obtained by cells remarkably reduces; resulting in cell size and number reduction [19]. Salinity inhibits growth, elongation and division of cells and results in cell death, through changes in water status in plants [20]. Although Na concentration increased in shoot as the result of salinity stress; however, applying silica nanoparticles reduced Na concentration in plant tissues. Salinity stress interferes with plant growth due to reduction of osmotic potential and toxicity of Na ion. Silica nanoparticles reduce Na toxicity by reducing Na absorption; resulting in the improvement of plant growth [21].

Application of silica nanoparticles increased shoot fresh and dry weight under saline condition. These results are in agreement with the findings of Gao et al. [8]. It seems that silisium increases sustainability of cell wall by forming a layer [22]. One gram of silica nanoparticles with 7 nm diameter has an absorption surface equal to 400 m^2. So, silica nanoparticles application affect xylem humidity and water translocation which results in water use efficiency improvement [7]. Although chlorophyll b content decreased by increasing salinity concentration and thereafter increased with silica nanoparticles application; however, this interaction was not significant for chlorophyll b while was significant for chlorophyll a. According to analysis of variance in this experiment, proline content increased in the leaves under salinity stress (1, 3 and 6 ds/m) which may be due to increase of proline synthesis or reduction of proline degradation in response to saline condition. The proline content in leaves decreased when silica nanoparticles were applied. In other word, salinity and silica and their interaction had significant effect on proline content in basil. Generally, results of this research demonstrated that application of silica nanofertilizer benefits plants under saline conditions. It seems that the beneficial effects of silica nanofertilizer are more remarkable for stressed plants. So, silisium application must be considered especially in plants faced with stresses. Results of this experiments showed that silica nanoparticles application under saline stress significantly increased basil growth and yield. However, common silica fertilizer did not show a remarkable effect on basil growth and yield.

CONCLUSION

In this experiment, silica nanoparticles application (S_3) was as the best treatment for plant growth and improvement, regarding the salinity levels, plant type and environmental conditions.

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