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ORIGINAL ARTICLE

The Effect of Phosphorus and Sulfur Nanofertilizers on the Growth and Nutrition of *Ocimum basilicum* in Response to Salt Stress

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KEYWORDS

Basil;

Eutrophication;

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ABSTRACT: Eutrophication is one of the most serious ecological threats to aquatic environments. It is defined as the enrichment of water bodies by organic matter or surface runoff containing nitrate and phosphate that directly control the growth of algae and other water plants. The use of nanofertilizers increases nutrient use efficiency and consequently reduces soil toxicity and minimizes the adverse effects of the over application of chemical fertilizers. This study was conducted in factorial form of a completely randomized design with four replications to evaluate the effect of phosphorus nanoparticles on the growth and nutrition of basil under salt stress. The first factor was three levels of salt stress, namely, 1, 3, and 6 dS m^{-1}. The second factor was three levels of phosphorus fertilizer, namely, without phosphorus fertilizer (P1), ammonium phosphate (P2), and phosphorus nanoparticles (P3). Powdered elemental sulfur with a particle diameter of <0.6 mm at two rates, namely, 0% (S0) and 20% (S2), was utilized in the experiment. Physiological traits (i.e., chlorophyll content, P uptake, and proline content of leaves) were investigated in this study. Plant growth and P uptake decreased with the increase in salinity ($P < 0.05$). The application of phosphorus nanoparticles significantly increased P uptake in response to salt stress. Phosphorus nanoparticles significantly increased photosynthetic activity and plant weight in response to salt stress. Leaf proline content increased significantly in response to salt stress.

INTRODUCTION

One of the effects of salinity on plants is the specific ion effect. This effect is due to the high concentration of one ion compared to other ions in the soil solution. As such, ion competition disturbs the absorption of other ions and affects the growth and health of plants.

High concentrations of Na^{+} and Cl^{-} in the soil solution

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may suppress nutrient ion activities and produce extreme ratios of Na^{+}/Ca^{2+}, Ca^{2+}/Mg^{2+}, and Cl^{-}/No^{3-}. Salinity causes ion imbalance. An imbalance in the salt content may result in the competition between elements, called the antagonistic effect between ions. For example, high levels of chloride in the soil solution limit nitrate uptake by plants [1].

Plant resistance to salinity depends largely on plant species and plant growth stage. The salinity level during P uptake by plants is significantly reduced. In saline soil solution, phosphate ion activity significantly decreased with the decrease in ionic strength. Phosphates in calcareous soils with calcium ions combine to create compounds with low solubility [2]. Therefore, phosphate concentrations in field-grown agronomic crops decreased with the increase in salinity (NaCl + $CaCl_2$) [1]. Champagnol [3] concluded that competition between Cl^{-} and $H_2PO_4^{-}$ ions is unlikely during plant uptake.

Eutrophication is defined as the enrichment of water bodies by organic matter or surface runoff containing nitrate and phosphate that directly control the growth of algae and other water plants. Eutrophication naturally and gradually occurs in cycles of more than 100 years; however, human activities have accelerated the process to an uncontrolled level [4]. The use of fertilizers resulted in numerous problems when runoff from agricultural land infiltrated streams and groundwater resources. Agricultural activities are a major cause of water pollution in the world.

The use of nanofertilizers increases nutrient use efficiency and consequently reduces soil toxicity and minimizes the adverse effects of the overapplication of chemical fertilizers [1, 6]. The use of phosphate nanoparticle fertilizers instead of chemical fertilizers can increase crop production, P use efficiency, economic efficiency, and food security and decrease water pollution. When the mineral nutrients required for plants are produced at the nanoscale, they become more absorbable and their fixation in soil decreases. As a result, the absorption of insoluble nutrients in soil increases [5]. Generally, several benefits of nanofertilizer application include 1) the increase in efficiency and food quality due to accelerated absorption, 2) the prevention of fertilizer loss by leaching and complete absorption by plants due to availability and controlled release during the growth period, and 3) the reduction in soil and water pollution and consequently food product contamination through the reduction of fertilizer leaching [7]. In Canada, nanofertilizers could prevent the loss of 200 million dollars caused by the low efficiency of common chemical fertilizer application [8].

Nanofertilizer application, such as zinc oxide, is effective in improving soil quality and increasing land fertility [9]. Liu and Rattan [10] have created a new type of phosphorus fertilizer to increase the growth and yield of soybean. Transmission electron microscopy images showed that the diameter of nanosized hydroxyapatite is in the range of 15 nm to 67.4 nm.

Nanosilica particles absorbed by roots have been shown to form a film that adheres to the cell walls, which can enhance the resistance of the plant to stress and lead to improved yield [11, 12].

This study was conducted in factorial form of a completely randomized design with four replications to evaluate the effect of phosphorus nanoparticles on the growth and nutrition of basil under salt stress.

MATERIALS AND METHODS

This experiment was conducted in 2013-2014 at the Agricultural Experiment Research Station of Islamic Azad University, Damghan Branch, Iran. The first factor was three levels of salt stress, namely, 1, 3, and 6 dS m^{-1}. The second factor was three levels of phosphorus fertilizer, namely, without phosphorus fertilizer (P1), ammonium phosphate (P2), and phosphorus nanoparticles (P3). Powdered elemental sulfur with a particle diameter of <0.6 mm at two rates, namely, 0% (S1) and 20% (S2), was utilized in this experiment. In May 2010, soil samples were collected from calcareous soil (20%) at a depth of 0 cm to 30 cm. Soil samples were dried in open air and passed through a 4 mm sieve. Then, 10 kg of soil was used to fill each pot. Basil (*Ocimum basilicum*) seeds were surface-disinfected in 1% NaOCl for 5 min and washed thrice with sterile distilled water. Then, eight seeds were planted in each pot. After four weeks, the seedlings were treated with saline water, ammonium phosphate, and phosphate nanoparticle. The salinity levels of plants were randomly increased every other day at six stages during two weeks.

Table 1 shows the chemical and physical properties of the soil samples. The organic carbon content of the soil samples was determined using the method described by Walkey and Black [14]. Nitrogen (N) content was determined using the micro-Kjeldahl method [14]. The pH of rock phosphate and electrical conductivity (EC) were measured using a pH meter and an EC meter, respectively. Then, the effect of different treatments on basil growth and physiological traits were assessed. The chlorophyll content of new and developed leaves was assayed [15]. The proline content of leaves was measured using the method proposed by Bates [16]. The fresh weight of leaves was immediately recorded using a digital scale. Then, the leaves were kept in an 80 °C oven for 48 h to obtain the dry weight. Data were analyzed using the SAS software, and means were compared according to Duncan's multiple range tests at $P \leq 0.05$.

Table 1. Chemical and physical properties of soil sample

Soil Texture	ECe(dsm^{-1})	pH	Total N (%)	Organic C (%)	P ($mgkg^{-1}$)	TNV (%)	K($mgkg^{-1}$)
Loam	2.1	7.8	0.004	0.05	7	15	350

RESULTS

Analysis of variance showed significant differences between salt stress levels. Various concentrations of NaCl had a significant effect on all the investigated traits (P<0.05). Analysis of variance also showed that the effects of the interaction of phosphorus and salt were significant for all the investigated traits (Table 2).

Table 2. Analysis of variance of treatments

Source	Degree of freedom	Mean square				
		Chlorophyll a	Chlorophyll b	Proline	*P*	Weight
N	2	95^{**}	126^{**}	0.24^{**}	0.013^{**}	106^{**}
P	2	43^{**}	42^{**}	0.108^{**}	0.006^{**}	63^{**}
S	1	2.6^{ns}	3.6^{ns}	0.001^{ns}	0.00081^{ns}	1.08^{ns}
P*N	4	7.8^{*}	7.01^{*}	0.017^{ns}	0.001^{**}	17.5^{*}
N*S	2	0.003^{ns}	0.002^{ns}	0.0001^{ns}	0.0001^{ns}	0.006^{ns}
P*S	2	0.71^{ns}	0.72^{ns}	0.001^{ns}	0.0001^{ns}	0.013^{ns}
P*N*S	4	0.75^{ns}	0.71^{ns}	0.0002^{ns}	0.00008^{ns}	0.004^{ns}
Error		27	26	0.06	0.005	67
CV (%)		5.7	7.6	7.1	7	9.3

* Significant differences at $P < 0.05$

** Significant differences at $P < 0.01$

Chlorophyll *a* content in plants treated with different levels of NaCl (1, 3, and 6 dS m^{-1}) and phosphorus fertilizer exhibited a significant difference ($P< 0.01$). The highest content of chlorophyll *a* was achieved during N1S1P2 treatments. By contrast, the lowest content of chlorophyll *a* was achieved during N3S2P0 treatments. A significant difference between N3S3 and N3S2 was observed. Chlorophyll *a* content in response to severe salt stress (6 dS m^{-1}) significantly increased during the application of phosphorus nanoparticles in comparison with the application of ammonium phosphate. The use of phosphorus moderated salt stress to a large extent. Sulfur has no significant effect on chlorophyll *a* content in basil. Chlorophyll *a* content decreased with the increase in salt stress (Fig. 1). The highest content of chlorophyll *b* was observed during N1S1P2 treatments. By contrast, the lowest content of chlorophyll *b* was observed during N3S2P0 treatments. The application of phosphorus nanoparticles increased chlorophyll *b* content during nonsaline treatments in comparison with 3 dS m^{-1} treatments (Table 2).

Table 3. Mean comparison of main effects using Duncan multiple range test

Treatment	Weight (gr)	Chlorophyll *b*	Chlorophyll *a*	Prolin (μggr^{-1} fresh weight)	P (%)
N1S1P2	25.96a	16.42a	19.8267a	0.366a	0.23a
N1S2P2	26.68a	16.20a	19.6a	0.374a	0.23a
N1S1P0	21.12a	13.24b	16.64b	0.471b	0.19b
N2S2P2	20.01b	12.57bc	15.97bc	0.504bc	0.19b
N2S1P2	20.61bc	12.4033bc	15.8033bc	0.514bc	0.18bc
N1S1P1	20.61bc	12.3967bc	15.7967bc	0.514bc	0.18bc
N2S1P1	20.13bcd	12.0433bc	15.4433bc	0.562bc	0.18bc
N1S2P1	20.09bcd	12.01bcd	15.41bc	0.588bc	0.18bc
N1S2P0	19.77bcd	11.7667bcde	15.1667bce	0.601bc	0.18bc
N2S2P1	19.23bcde	11.3667ced	14.7667ced	0.602ce	0.17bce
N3S1P2	18.06efd	10.48def	13.88efd	0.620cef	0.16ce
N2S1P0	17.70efd	10.2867def	13.6867ef	0.620cef	0.16ef
N3S1P1	17.89ef	10.2767ef	13.6767ef	0.629ef	0.16ef
N3S2P1	16.76ef	9.5033fg	12.9033fg	0.662f	0.15efg
N3S1P0	16.64ef	9.4133fg	12.8133fg	0.710gh	0.15fg
N3S2P0	15.86fg	8.6467gh	12.0467gh	0.771gh	0.15fg
N3S1P0	13gh	7.4767h	11.32gh	0.820gh	0.13h
N3S2P0	13h	7.3233h	10.97h	0.811h	0.12

Means with similar letter(s) in each trait was not significantly different at 5% probability level according to Duncan's Multiple Range Test.

The variation of the fresh weight of basil was significant ($P < 0.01$) during different treatments. The fresh weight of basil significantly decreased with the increase in salt stress level. The lowest weight was observed during N3S1P1 treatments. By contrast, the highest weight was observed during N1S2P2 treatments. A significant difference between control and N3S1 treatments was observed. Shoot fresh weight of basil treated with different levels of NaCl decreased (Table 3). The results indicated that sulfur had no significant effect on any of the investigated traits.

Salt stress significantly ($P < 0.01$) increased the proline content of *O. basilicum*. Salt stress significantly ($P < 0.05$) decreased the shoot P content of *O. basilicum*. The effect of the P × Salinity interaction on this trait was also significant ($P < 0.05$). The highest and lowest proline contents were observed during N3S1P0 and N1S1P2 treatments, respectively, with significant differences (Table 3). Proline content increased with the increase in the severity of salt stress. Proline content also significantly increased when phosphorus nanoparticles were applied in response to severe salt stress (6 dS m^{-1}) in comparison with ammonium phosphate. Salt stress significantly affected and decreased P uptake.

Plants sprayed with silicon nanoparticles under severe salt stress had higher proline content than plants treated with the common silicon fertilizer. Proline content was significantly the same in interactions containing the common silicon fertilizer (Figure 2).

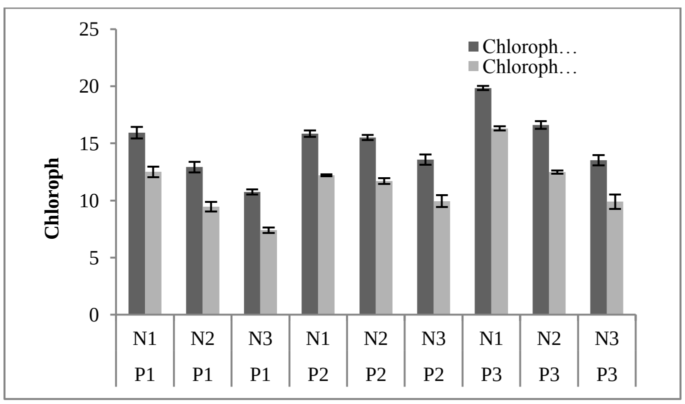

Figure 1. Effect of the treatments on Chlorophyll *a* and Chlorophyll *b*

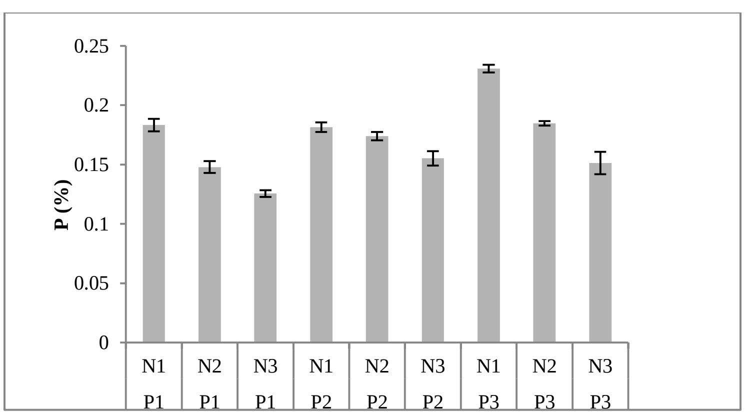

Figure 2. Effect of the treatments on P uptake

DISCUSSION

Salt stress is one of the most important abiotic stresses that affect plant growth, yield, and quality. When plants are under salt stress, photosynthetic activity is impaired. Thus, the chlorophyll content of plants is reduced. The lowest chlorophyll content and fresh weight were observed during N3S2P0 treatments. The decline in productivity observed for many plant species subjected to excess salinity is often associated with the reduction in photosynthetic capacity. Salts absorbed by plants may indirectly control their growth by affecting turgescence, photosynthetic activity, or enzyme activity rather than the buildup of salt in old leaves, which may hasten leaf death [17]. The chlorophyll content in plants decreased with the decrease in photosynthetic activity under saline condition. Salinity reduced the growth of radish at high salinity levels, which could be attributed to the reduction in leaf area expansion and resulted in low light interception [17]. The lowest chlorophyll content and fresh weight were observed during N3S2P0 treatments.

P uptake was strongly affected by all salt concentrations (Figure 2). P concentrations in plant tissues decreased with the increase in salt concentration. A marked reduction in P uptake was observed at 5 dS m^{-1} NaCl (Figure 2). Most of the studies [2, 3, and 17] that reported salinity-reduced P concentrations in plant tissues were conducted in soils. Phosphate availability is reduced in saline soils not only because of ionic strength effects that reduce phosphate activity but also because phosphate concentrations in soil solution are tightly controlled by the sorption process and low solubility of Ca–P minerals. In many cases, tissue P concentration decreased by 20% to 50%. However, no evidence of P deficiency in crops was observed [3].

Competition between Cl^- and $H_2PO_4^-$ ions is unlikely during plant uptake [3]. Reduced P activity in the soil solution resulted in salinity-reduced P concentration in plant tissues caused by the high ionic strength of the media and low solubility of Ca–P minerals. Twelve weeks after planting, the results showed that the application of phosphorus nanofertilizer as P source promoted *O. basilicum* growth at a rate of 20%, which is higher than that of *O. basilicum* grown under regular P fertilizer. Phosphorus particles could be used as P fertilizer to enhance crop yield and biomass production [3]. The application of phosphorus nanoparticles increased the fresh weight, P concentration, and chlorophyll content of plant tissues under saline conditions. These results are consistent with the findings of Liu and Rattan [10].

The use of these nanofertilizers increases nutrient use efficiency, reduces soil pollution, minimizes the potential negative effects associated with overdosage, and reduces the frequency of application. Nanofertilizers mainly delay the release of nutrients and extend the fertilizer effect period. Notably, nanotechnology significantly influences energy consumption, the economy, and the environment by improving fertilizers [10]. Thus, nanotechnology has the potential to revolutionize agriculture to achieve sustainability, particularly in developing countries [7].

CONCLUSIONS

The use of nanofertilizer benefits plants under saline conditions. Thus, nanotechnology has the potential to revolutionize agriculture to achieve sustainability, particularly in developing countries.

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