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Effect of Silver Nanoparticles on Growth and Physiological Responses of Spinach (*Spinacia oleracea* L.) under Salt Stress

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Abstract

Saline soil or water can highly affect plant physiological and biochemical responses including general disruption in the nutritional status of plants, osmotic stress, and ion-specific toxicity The rapid development and potential release of engineered nanoparticles (ENPs) have raised considerable concerns due to the unique properties of nanomaterials. Spinach is considered a model plant in hydroponic system production and is of research interest all over the world. In this study, we aim to study the physiological responses of spinach under different concentrations of both silver nanoparticles and salt stress. Spinach seedlings were exposed to 16 treatments asthreesalinity concentrations (4.0, 6.0, and 10.0 $dS.m^{-1}$),three silver nanoparticles concentrations (20, 40, 60 ppm), nine treatments as combination of salinity and silver nanoparticles and control.Relative water content (RWC), stomatal conductance (g_s), chlorophyll content index (CCI),), dry weights (DW), leaf area (LA), and specific leaf area (SLA) of 41 days old spinach seedlings were examined and recorded for 6 weeks. The application of Silver nanoparticles had affected plant growth and altered many plant physiological responses. We concluded that silver nanoparticles might have positive effects on the physiological parameters but only under non-saline stress. However, it negatively impacts plants when it was added under saline conditions.

Keywords: spinach, silver nanoparticles, hydroponic, salt stress, water relations, gas exchange, relative growth rate, net assimilation rate.

1. Introduction

Salt stress (salinity) is the most abiotic stress that extremely influences plant growth and production. Saline soil or water can highly affect plant physiological and biochemical responses, including general disruption in the nutritional status of plants, osmotic stress, and ion-specific toxicity as a result of the accumulation of sodium (Na) and chloride (Cl) ions (Nazar et al. 2011). One of the essential goals for plant researchers is to investigate the physiological responses that help to develop salt tolerance in plants. In addition, soil salinity also affected the quality of many crops, which had a negative impact on the agriculture economy.

Soil salinity in many countries is mainly a consequence of arid climatic conditions. Most crops are sensitive to salt stress that cause subsequent yield loss. To cope with salinity, plants implement many physiological and anatomical traits as adaptation strategies that reduce the effect of salt stress (Bsoul et al., 2017)

Green leafy vegetables are an important part of healthy diets. Spinach (*Spinacia oleracea* L) is an annual edible flowering plant belongs tothe Amaranthaceae. Spinach leaves are a superfood that is loaded with many nutrients in a low-calorie package (Giri et al., 2016). It was found that spinach could tolerate irrigation with salinity around 5.7 dS/m (Uçgun et al,.2020).

Hydroponics is a method of growing plants in water based nutrient rich solution. Growing with hydroponics comes with many advantages, the biggest of which is the significantly increased rate of plant growth. With the proper setup, plants will mature up to 25% faster and produce up to 30% more than the same plants grown in soil (Ritter et al. 2001). Spinach grows quickly in a hydroponic system.

Engineered nanomaterials have received a particular attention for their positive impact on improving many sections of economy, including agriculture(Nowack and Bucheli 2007).The European Union has defined a nanomaterial as a natural, incidental or manufactured material containing particles, in an unbound state or as aggregate or as agglomerate. One more external dimension is the size range 1 – 100 nm" (Rauscher et al., 2015). Nanoparticles (NPs) are used to improve agriculture production and crop protection. However, using them is relatively new and needs further exploration in field of agriculture(Lijuan et al., 2020).Nanoparticles interact with plants causing many morphological and physiological changes, depending on the properties of NPs. Research findings suggested both positive and negative effects on plant growth and development, and the impact of engineered nanoparticles (ENPs) on plants depends on the composition, concentration, size, and physical and chemical properties of ENPs as well as plant species (Xingmao et al. 2010).

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Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties (Zhang et al. 2016).Both positive and negative effects of Ag NPs on plant growth have been reported (Abdel Kareem et al. 2017). However, scarce studies have reported the role of NPs on plants under salinity (Almutairi, 2016). Our objective in this study is to investigate the physiological responses of spinach under different concentrations of both silver nanoparticles and salt stress.

2. Materials and Methods

2.1. Study location

This study was conducted in a greenhouse at The Hashemite University, Zarqa, Jordan. 32°05' N Latitude and 36°06 E Longitudes. Greenhouse day temperature, humidity and the light intensity were (24.6 ± 0.039 °C), (51%± 1.48), respectively. Mean midday photosynthetic photon flux density (PPFD) was (365± 0.71 µmol· s^{1}·m^{-2})measured by a quantum sensor (LI-250A; LICOR.)

2.2. Plant material and experimental design

Seeds of baby spinach from the local market were used for this experiment. Seeds were germinated in the greenhouse in trays containing peatmoss (KEKKILA, European Union). After the appearance of the primitive stem, spinach seedlings were then grown in a hydroponic system to prevent any interaction that can impede nanoparticles from the plants. Aftertwo weeks, uniform plants were selected and randomly assigned toexperimental treatments. Each plant was moved to 200 ml flask covered with Aluminum foil (Figure 1) and filled with sterile perlite and Hoagland's solution. Spinach seedlings were left in the flask under greenhouse conditions for four days as adaptation period (Alkhatib et al., 2019).

Uniform spinach plants were assigned randomly to one of 16 treatments ((control), (S1) 4.0dS.m^{-1}, (S2) 6.0 dS.m^{-1}, (S3)10.0dS.m^{-1}, (N1) 20 ppm AgNPs,(N2) 40 ppm AgNPs,(N3) 60 ppm AgNPs,(S1N1)4.0dS.m^{-1} + 20 ppm AgNPs, (S1N2)4.0dS.m^{-1} + 40 ppm AgNPs,(S1N3)4.0dS.m^{-1} + 60 ppm AgNPs,(S2N1)6.0dS.m^{-1} + 20 ppm AgNPs, (S2N2)6.0dS.m^{-1} + 40 ppm AgNPs,(S2N3)6.0dS.m^{-1} + 60 ppm AgNPs(S3N1)10.0dS.m^{-1} + 20 ppm AgNPs, (S3N2)10.0dS.m^{-1} + 40 ppm AgNPs,(S3N3)10.0dS.m^{-1} + 60 ppm AgNPs) for 65 days. The experimental design was Randomize Completely Block Design (RCBD). There were five experimental replications, each containing a total of 16 plants and the total number was 80 seedlings. Plants were grown in a 200 ml flask filled with perlite and the designated solution treatment

All the spinach seedlings from all the treatments had equal and appropriate amount of Hoagland's solution during the experiment period with pH = 5.9. In addition, a fungicide (Vapco Top 70 %,Thiophanate- methyl) was added to the treatment solutions(2 g L^{-1}) to prevent the growth of fungal species.

2.3. Initial seedling traits

After30 days of acclimatization, uniform plants were randomly selected as experimental units. Extra 16 seedlings were harvested to determine the initial dry weights on the same day when the treatments were applied. The harvested plants were separated into shoots and roots. Oven dry weights of shoot and root were determined at 65º C for 3 days.

2.4. Salinity and silver nanoparticles treatments

A 3:1 proportion of calcium chloride and sodium chloride was diluted in water to make a stock solution. Treatment solutions were made by adding stock solution to distilled water until the desired salinity levels were achieved. All readings were recorded using an EC meter (Milwaukee SPEM500).Silver nanoparticles (AgNPs) water dispersion was used in this study has the APS: 2 nm.

2.5. Physiological traits

Two youngest fully-expanded mature healthy leaves were selected to measure the chlorophyll Content Index by using chlorophyll content meter (Optic- Sciences, CMM 200) every two weeks. Stomatal conductance (g_s) was measured biweekly using AP4 Porometer(Delta-A Devices-Cambridge-U. K).

2.6. Final harvest

At the end of the experiment after 41 days, all plant parts were harvested and separated into leaves, shoots and roots.Leaf area was recorded using the leaf area meter (LI-3050C; LI-COR, Lincoln, Nebr.).All plant parts were oven dried at 68°C for three days.Plant stem diameter was measured at the harvest day by using digital Vernier caliper (US 7533474B2, United States).Relative growth rates were calculated using the equation of Gutschick and Kay (1995): RGR = (ln W2 - ln W1)/ (T2 - T1), where W2 was the final dry weight at day 41 (T2), and W1 was the initial DW determined from initial data harvest on day one (T1). Net assimilation rates (NAR) were calculated as: NAR = M2 - M1/T2 - T1 X log L2 - log L1/L2 - L1, where M2 was the final dry weight at day 41 (T2), and M1 was the initial DW determined from the initial recorded on day one of the experiment (T1). Leaf area ratio ($cm^2.g^{-1}$) was calculated as SLA= leaf area/leaf dry weight. Specific stem length ($cm.g^{-1}$) was calculated as SSL= stem height/ stem dry weight.

Leaf discs from five of the youngest fully expanded mature leaves from the median portion of the stem of two randomly selected plants from each treatment were selected. RWC was calculated using the equation: RWC (%) = (FW-DW/SW-DW) * (100) where: FW is the fresh weight and DW represents fresh weight sample oven dried at 68 °C and SW represents saturated weight of sample, which was immersed overnight in distilled water (Bsoul et al., 2007).

2.7. Statistical analysis

Statistical analysis was performed using SAS 9.1 software for Windows (2003). Significant differences between values of all parameters were determined at $P \leq 0.05$ using Proc Glm, PDIFF, ANOVA and Least Significant Difference (LSD).

3. Results

Salinity and silver nanoparticles had affected plant growth parameters. There were no significant differences among treatments in root DW ($P \leq 0.28$), shoot DW ($P \leq$

0.25), total plant DW ($P \leq 0.49$). However, there were significant differences among treatments in root/shoot ratio ($P \leq 0.04$) and stem diameter ($P < 0.0001$) (Table 1).

Plants treated with (S3) had the highest root/shoot DW ratio (6.7) while plants treated with moderate salinity (S2) concentration (6.0 $dS.m^{-1}$), 40 ppm of Ag nanoparticles, (S1N1), (S1N2) and plants with (S1N3) treatments had the lowest value (1.9), (1.2), (1.8), (1.8) and (1.9), respectively. Moreover, plants irrigated with 20 ppm of Ag nanoparticles had the highest stem diameter (0.29 mm), while plants with high salinity concentration (10 $dS.m^{-1}$), (S3N1), (S3N2) and (S3N3) treatments had the lowest and similar stem diameter (Table 2).

Spinach seedlings had no significant differences among treatments in their specific leaf area (SLA) ($P \leq 0.58$) and leaf area ratio (LAR) ($P \leq 0.17$). However, there were significant differences among treatments in leaf area (LA) ($P < 0.0006$), specific leaf weight (SLW) ($P \leq 0.04$) and leaf weight ratio (LWR) ($P \leq 0.03$). The highest leaf area value (5.1 cm^2) was recorded for control, but plants treated with high salinity concentration had among the lowest LA (3.1 cm^2) (Table 2).

Table 1: Effects of salinity and silver nanoparticles treatment onRoot DW, Shoot DW, Plant DW, root to shoot ratio and stem diameter (SD) subjected to irrigation treatments and harvested on day 41 of the experiment.

Treatment	Root DW (g)	Shoot DW (g)	Plant DW (g)	Root/Shoot	SD (mm)
Control	0.061[a*]	0.024 [a]	0.086 [a]	2.5 [cd]	0.33 [a]
S1 (4.0 dS/m)	0.053[a]	0.023 [a]	0.076 [a]	2.3 [cd]	0.08 [dc]
S2 (6.0dS/m)	0.042[a]	0.022 [a]	0.064 [a]	1.9 [d]	0.12 [c]
S3 (10.0dS/m)	0.134 [a]	0.020 [a]	0.155 [a]	6.7 [a]	0.01 [f]
N1(20 ppm)	0.062 [a]	0.022 [a]	0.084 [a]	2.8 [cd]	0.29 [ab]
N2(40 ppm)	0.037 [a]	0.030 [a]	0.067 [a]	1.2 [d]	0.28 [b]
N3(60 ppm)	0.061 [a]	0.022 [a]	0.083 [a]	2.7 [cd]	0.28 [b]
S1N1	0.068 [a]	0.038 [a]	0.105 [a]	1.8 [d]	0.07 [cde]
S1N2	0.066 [a]	0.036 [a]	0.101 [a]	1.8 [d]	0.07 [cde]
S1N3	0.045 [a]	0.024 [a]	0.068 [a]	1.9 [d]	0.07 [cde]
S2N1	0.080 [a]	0.013 [a]	0.093 [a]	6.2 [abc]	0.04 [ef]
S2N2	0.130 [a]	0.021 [a]	0.151 [a]	6.2 [abc]	0.05 [def]
S2N3	0.074 [a]	0.026 [a]	0.099 [a]	2.8 [cd]	0.04 [def]
S3N1	0.091 [a]	0.023 [a]	0.113 [a]	4.0 [abcd]	0.02 [f]
S3N2	0.074 [a]	0.017 [a]	0.091 [a]	4.4 [abcd]	0.01 [f]
S3N3	0.073 [a]	0.021 [a]	0.094 [a]	3.5 [bcd]	0.01[f]
Mean	0.072	0.024	0.096	03.3	0.11
P-value	0.28	0.25	0.49	0.04	<0.0001

* Means within the columns followed by the same letter are not significantly different.

Table 2: Effects of salinity and silver nanoparticles treatment onLeaf Area (LA), Specific Leaf Area (SLA), Specific Leaf Weight (SLW), Leaf Weight Ratio (LWR) and Leaf Area Ratio (LAR) of spinach seedlings subjected to irrigation treatments and harvested on day 41 of the experiment

Treatments	LA (cm^2)	SLA ($cm^2.mg^{-1}$)	SLW ($mg.cm^{-2}$)	LWR ($g.g^{-1}$)	LAR($cm^2.mg^{-1}$)
Control	5.1[* a]	1.49[a]	0.44 [abcd]	0.049 [abc]	0.86 [a]
S1 (4.0 dS/m)	4.8 [ab]	0.78 [a]	0.32 [abcde]	0.038 [abcde]	0.61 [a]
S2 (6.0 dS/m)	3.9 [defg]	0.36 [a]	0.11 [de]	0.018 [bcde]	0.32 [a]
S3 (10.0 dS/m)	3.1 [g]	1.11 [a]	0.04 [e]	0.004 [e]	0.21 [a]
N1 (20 ppm)	4.4 [abcdef]	1.39 [a]	0.58 [abc]	0.042 [abcd]	0.73 [a]
N2 (40 ppm)	4.7 [abcd]	0.96 [a]	0.39 [abcde]	0.037 [abcde]	0.55 [a]
N3 (60 ppm)	4.4 [abcdef]	2.30 [a]	0.40 [abcde]	0.024 [bcde]	0.75 [a]
S1N1	4.8 [abc]	1.61 [a]	0.66 [a]	0.051 [ab]	0.81 [a]
S1N2	4.6 [abcde]	1.38 [a]	0.49 [abcd]	0.031 [abcde]	0.52 [a]
S1N3	4.6 [abcde]	1.78 [a]	0.60 [ab]	0.065 [a]	1.15 [a]
S2N1	4.0 [cdef]	0.78 [a]	0.20 [cde]	0.010 [de]	0.19 [a]
S2N2	3.7 [fg]	1.08 [a]	0.42 [abcd]	0.011 [de]	0.18 [a]
S2N3	3.7 [fg]	0.85 [a]	0.19 [cde]	0.016 [bcde]	0.34 [a]
S3N1	4.2 [bcdef]	0.72 [a]	0.22 [bcde]	0.012 [cde]	0.21 [a]
S3N2	4.2 [bcdef]	1.22 [a]	0.14 [de]	0.011 [de]	0.38 [a]
S3N3	3.8 [efg]	0.17 [a]	0.23 [bcde]	0.009 [de]	0.08 [a]
Mean	4.3	1.13	0.34	0.027	0.49
P-value	0.0006	0.58	0.04	0.03	0.17

* Means within the columns followed by the same letter are not significantly different.

At the end of the experiment relative growthrateRGR results indicated that there were no significant differences among treatments (P= 0.6373) (Fig. 1).

significant differences among treatments (P= 0.6373) (Fig. 3).

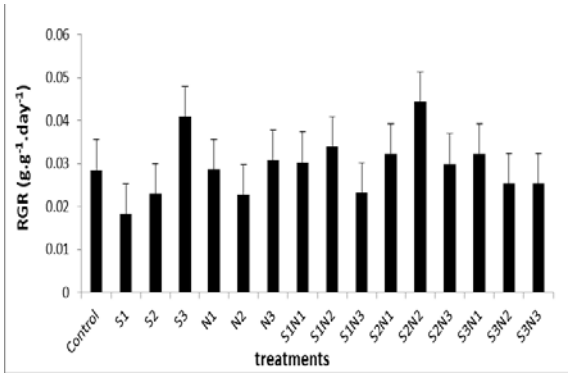

Figure 1: Relative Growth Rate($g.g^{-1}.day^{-1}$): RGR values for all treatments at the end of the experiment (41 days after planting) under the effect of salt stress and nanoparticles irrigation.

Net assimilation rate had significant differences among treatments under salt stress and nanoparticles irrigation (P<0.0469). Plants treated with high salinity (S3=10 $dS.m^{-1}$), (S2N2) had the highest NAR value (1.104, $0.8566mg.cm^{-2}.day^{-1}$) respectively, while other treatments had similar values(Fig. 2).

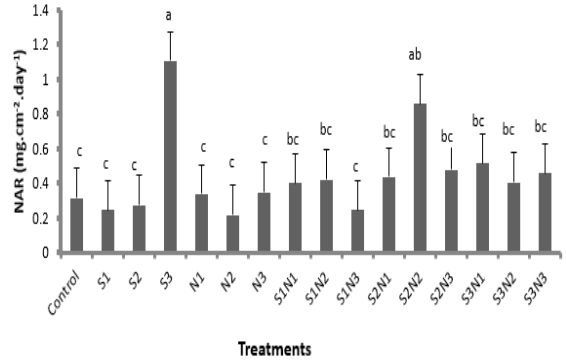

Figure 2: Net Assimilation Rate($mg.cm^{-2}.day^{-1}$): NAR values for all treatments at the end of the experiment (41 days after planting) under the effect of salt stress and nanoparticles irrigation. Data are means ± SE of 5 replicates. Means within the columns marked with the same letter were not significantly different at the $P \leq 0.05$.

Relative water contents RWC results showed significant differences among treatments (P = 0.006). (S1N3) had the highest RWC value (40%). However, the lowest RWC value was (3%) and (4%) for the plants treated with (S3) and (S3N1), respectively (Fig. 3).

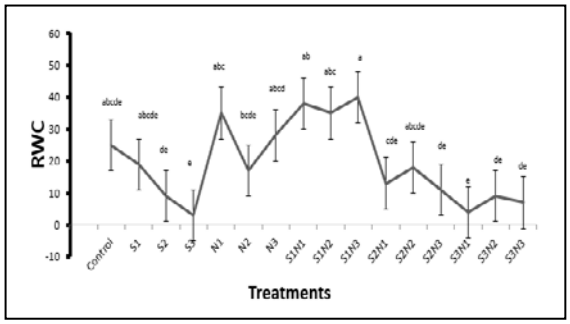

Figure 3: Relative Water Content RWC %:Treatments of nanoparticles irrigation compared with control after 41 days.Data are means ± SE of 5 replicates. Means within the columns marked with the same letter were not significantly different at the $P \leq 0.05$.

Stomatal conductance (g_s) had significant differences among treatments (P=0.0169) in the 6thweek, plants irrigated with 40 ppm of silver nanoparticles (N2) and(S1N1)had the highest stomatal conductance ($0.14cm.s^{-1}$), while S2 and S3 had the lowest stomatal conductance (0.03 and $0.02cm.s^{-1}$), respectively. However, in the 2nd and 4thweek, plants had no significant differences in g_s (P = 0.4391)**,** (P = 0.3762) (Table 3).

Table3 . Biweekly stomatal conductance (g_s) under salt stress and NPs irrigation

Treatment	(g_s) ($mmol.m^{-2}.s^{-1}$) 2nd week	(g_s) ($mmol.m^{-2}.s^{-1}$) 4th week	(g_s) ($mmol.m^{-2}.s^{-1}$) 6th week
Control	0.13 ^{a*}	0.11 ^{a}	0.14 ^{ab}
S1 (4.0 dS/m)	0.2 ^{a}	0.09 ^{a}	0.08 ^{abcd}
S2 (6.0 dS/m)	0.09 ^{a}	0.06 ^{a}	0.03 ^{d}
S3 (10.0 dS/m)	0.03 ^{a}	0.02 ^{a}	0.02 ^{d}
N1 (20 ppm)	0.15 ^{a}	0.11 ^{a}	0.08 ^{abcd}
N2 (40 ppm)	0.17 ^{a}	0.15 ^{a}	0.14 ^{a}
N3 (60 ppm)	0.12 ^{a}	0.1 ^{a}	0.11 ^{abc}
S1N1	0.19 ^{a}	0.12 ^{a}	0.14 ^{a}
S1N2	0.13 ^{a}	0.1 ^{a}	0.07 ^{abcd}
S1N3	0.13 ^{a}	0.09 ^{a}	0.07 ^{bcd}
S2N1	0.14 ^{a}	0.09 ^{a}	0.09 ^{abcd}
S2N2	0.15 ^{a}	0.05 ^{a}	0.06 ^{cde}
S2N3	0.18 ^{a}	0.1 ^{a}	0.07 ^{abcd}
S3N1	0.13 ^{a}	0.09 ^{a}	0.09 ^{abcd}
S3N2	0.15 ^{a}	0.04 ^{a}	0.04 ^{cd}
S3N3	0.09 ^{a}	0.04 ^{a}	0.04 ^{cd}
Means	0.14	0.09	0.08
P-value	0.44	0.38	0.02

*Means within the columns followed by the same letter are not significantly different.

4. Discussion

The current study showed that salinity treatments had no effect on whole plant dry weights, shoot dry weight and root dry weight but significantly affected other growth root to shoot ratio and stem diameter. Guenther et al. (1987) reported that salinity stress is a serious abiotic stress that influences the growth of spinach seedlings and they reported that the reasons were the excessive uptake of (Na^+) and (Cl^{-1}) ions, the accumulation of Na+ in the leaves and nutritional imbalance. Salinity significantly increased root/shoot ratio in spinach seedlings because plants usually invest more in roots than in shoots under salt or drought stress. The application of silver nanoparticles in the current study had no effect on root to shoot ratio that had values similar to the control. These finding were consistent with Mazumdar (2014) who reported that root fresh weight and shoot fresh weight were not affected at low concentration below of 50 µg/mL of silver nanoparticle. This implied that the effect on the root/shoot ratio was due to salinity only.

Salinity treatments reduced spinach stem diameter because plants reduce their hydrolytic conductivity when they are subjected to water stress. Similar results were reported in *Spondias tuberosa* plants (Da silva et al., 2008).

Salinity treatments significantly reducing leaf area of spinach might be attributed to the fact that plants usually reduce their leave's surface area under water deficit or unavailable water in order to reduce transpiration and prevent dehydration. In addition, salinity reduces the total plant growth in general. Beinsan et al. (2003) reported that the negative impact of salt stress on leaf area is due of the reduction in both photosynthesis rate and chlorophyll content in *Phaseolus vulgaris L* plants. The Application of silver nanoparticles had almost similar effect on spinach leaf area that was only under salt stress, and silver nanoparticles had no advantage in improving the plant response to salinity. On the other hand, the application of silver nanoparticles might have a negative impact on the plant under salinity. Almutairi(2016) reported that AgNPs play an important role in moderating the inhibition of plant growth in saline environments by inducing salt tolerance in plants. It was found that exposure to AgNPs is capable of increasing the germination percentage, the germination rate, the root length and the seedling fresh and dry weights of tomato plants under NaCl stress, if applied AgNP on seeds through seed germination. Based on that, we might conclude that it is important to consider the plant growth stage when we apply nanoparticles under saline conditions.

RGR represents the extent to which a plant invests its photosynthesis in current growth and enhances its capacity for future photosynthesis (Fitter and Hay, 2002). Current work indicated that spinach RGR were not significantly affected when treated with the different salt levels. Water availability is a factor that usually reduces NAR and growth. El-Hendawy et al. (2005) reported that the salinity stress reduced NAR values in wheat. The authors attributed the reasons behind that to the reduction in plant relative water content in some cases. Current research indicated a decrease in NAR values when Ag nanoparticles were applied and that might be because silver nanoparticles were able to decrease plant's chlorophyll content.

Relative water content RWC is an important salinity stress indicator and its response varies depending on salinity severity(Galmés et al., 2007).In this study, salinity treatments decreased RWC values. That might be because the plant roots were unable to absorb enough water from the surrounding medium. Yang et al. (2011) reported that RWCin *Medicago ruthenica*plants was decreased under salt stress, as the plants were unable to compensate for water lost by transpiration. Application of silvernanoparticles at concentration of 20 ppm of Ag nanoparticles (N1) had the highest RWC, contra the results of Çekiç et al. (2017) who reported that AgNPs did not significantly affect the water status of *S. lycopersicum*. Applying both salt and Ag nanoparticles together had a negative effect on spinach RWC because saline water might reduce the free Ag nanoparticles amount. As a result, the effect of salinity and Ag nanoparticles treatments was similar to the effect of salinity treatment alone.

Stomatal conductance had no significant differences among treatments in the 2^{nd} and 4^{th}week. However, Stomatal conductance had significant differences among treatments in the 6^{th} week and reduced under salinity. Reduction of stomatal conductance and transpiration rate are considered as adaptations to protect plants from dehydration (Romero et al., 2001). Application of silvernanoparticles had a positive effect on the 6^{th}week and the reason behind that might be the increase in the available amount of water for spinach plants under hydroponic system.

Chlorophyll is one of the major chloroplast components necessary for photosynthesis, and the chlorophyll content index has a positive relationship with the photosynthetic rate. The decrease in chlorophyll content under salinity stress has been considered as chlorophyll degradation. Decreased chlorophyll level during salinity stress has been reported and considered the main cause of inactivation of photosynthesis and loss of chlorophyll and found to be dependent on the duration and severity of salinity. Omoto et al. (2010) reported that the negative effect of salinity on plant's chlorophyll content index was due to chlorophyll deficiency. Chlorophyll deficiency is attributed to the inhibition of chlorophyll synthesis. In our study, the application of silver nanoparticles had a negative effect on spinach chlorophyll content index. Xingmao et al. (2010) reported that silver nanoparticles concentration below 20 ppm can be taken up by plants and transport from intracellular spaces to inside plant cells through plasmodesmata of root cells. These nanoparticles then pass through shoots and accumulate in the leaves which cause an adverse effect on total chlorophyll content index of tested plants. In addition, the Ag NP treatments may cause toxicity to the plants. In greenhouse experiments, Song et al. (2013) reported that mature tomato plants showed evidence of phytotoxicity due to AgNPs by exhibiting low chlorophyll contents and less fruit production. Application of both salt and AgNPs had a negative effect on spinach chlorophyll content, and this might have been attributed to increase in chlorophyllase enzyme activity. Abdel Kareem et al. (2017) reported that total chlorophyll contents decreasing under salt might be due to AgNPs toxicity in plants.

5. Conclusion

Application of silver nanoparticles up to 20 ppm concentration might be considered as a successful method for spinach seedlings under non-saline irrigation conditions and might be successfully used to enhance physiological responses of spinach seedlings. For plants under salinity stress, silver nanoparticles were not able to alleviate the salinity stress with a complement amount of specific nanoparticles concentration. In addition, the application of Ag nanoparticles had a negative effect on stomatal conductance and chlorophyll content when mixed with saline water. Ag nanoparticles did not improve the physiological parameters under salinity stress. Nanoparticles application method and the stage of application might have a great importance when it is used for spinach plants.

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References

Alkhatib, R, Alkhatib B, Abdo N, Laith AE and Creamer R. 2019. Physio-biochemical and ultrastructural impact of (Fe_3 O_4) nanoparticles on tobacco. *BMC Plant Biol* **19**:253.

Abdel Kareem SH. Mohamed FQ, Abdel-Hadi AM, Rabia AR, Shafaqat A and Muhammad R. 2017. Interactive effect of salinity and silver nanoparticles on photosynthetic and biochemical parameters of wheat, *Archives of Agronomy and Soil Science*, **63(12):**1736-1747.

Almutairi ZM. 2016. Influence of silver nano-particles on the salt resistance of tomato (*Solanum lycopersicum*) during germination. *Int. J. Agric. Biol.*, **18**: 449–457.

Beinsan, C, Camen D, Sumalan R, and Babau M. 2003. *'Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local landraces from Banat area', Faculty of Horticulture*, 119: 416-19.

Bsoul E., Hilaire RSt., and VanLeeuwen D. 2007. Bigtooth maples from selected provenances effectively endure deficit irrigation. *Hortscience*: **42:**1167-1173.

Bsoul, EY, Shahrestani SA and Shdiefat SM. 2017. Growth, nutrient acquisition, and physiological responses of three olive cultivars to induced salt stress. *Journal of Plant Nutrition* **40(14):** 1955-1968.

Çekiç, FÖ, Ekinci S, İNAL MS, and ÖZAKÇA D. 2017. Silver nanoparticles induced genotoxicity and oxidative stress in tomato plants. *Turkish Journal of Biology*, **41:** 700-707.

Da Silva EC, Nogueira RJMC, de Araújo FP, de Melo NF and de Azevedo Neto AD. 2008. Physiological responses to salt stress in young umbu plants. *Environmental and Experimental Botany* **63(1-3):** 147-157.

El-Hendawy, Salah E, Yuncai Hu, and Urs Schmidhalter. 2005. Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances. *Australian Journal of Agricultural Research*. **56:** 123-34.

Fitter A. and Hay R. 2002. **Environmental Physiology of Plants**, Academic Press, San Diego,

Galmés J, Medrano H, and Flexas J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New phytologist*, **175:** 81-93.

Giri A, Armstrong B, Rajashekar CB .2016. Elevated carbon dioxide level suppresses nutritional quality of lettuce and spinach. *Am J Plant Sci* **7**:246–258.

Guenther, J.E., Melis, A. 1987. Targets of Salinity Stress in Spinach. In*: Biggins, J. (eds) Progress in Photosynthesis Research. Springer, Dordrecht.*

Gutschick VP and KayLE.. 1995. Nutrient-limited growth rates: quantitative benefits of stress responses and some aspects of regulation. *J. Exp. Bot.***46:** 995-1009.

Lijuan Z, Li Lu, Wang A, Zhang H, Huang M, Wu H, Xing B, Wang Z, and Ji R. 2020.Nano-Biotechnology in Agriculture: Use of Nanomaterials to Promote Plant Growth and Stress Tolerance. *Journal of Agricultural and Food Chemistry* **2020** ***68*** **(7):** 1935-1947.

Mazumdar H. 2014. The impact of silver nanoparticles on plant biomass and chlorophyll content. *Int. J. Eng. Sci*, **4:** 12-20.

Nazar, R, Noushina I, Asim M, Shabina S and Nafees A K. 2011. Understanding the significance of sulfur in improving salinity tolerance in plants. *Environmental and Experimental Botany*, **70:** 80-87.

Nowack B, and Bucheli TD. 2007. Occurrence, behavior and effects of nanoparticles in the environment. *Environmental pollution,***150:** 5-22.

Omoto E, Taniguchi M and Miyake H. 2010. Effects of salinity stress on the structure of bundle sheath and mesophyll chloroplasts in NAD-malic enzyme and PCK type C4 plants. *Plant Production Science***13(2):** 169-176.

Rauscher, H., Roebben, G., Sanfeliu, A., Emons, H., Gibson, N., Koeber, R., Linsinger, T.,Rasmussen, K., Sintes, J., Sokull-Klüttgen, B., & Stamm, H. (2015). *Towards a review of the EC recommendation for a definition of the term nanomaterial*. Report by the Joint Research Centre of the European Commission, European Union. Ispra, Italy.

Ritter E, Angulo B, Riga P, Herran C, Relloso J, San Jose M. 2001. Comparison of hydroponic and aeroponic cultivationsystems for the production of potato minitubers. *Potato Research.* **44(2):**127-135.

Romero-Aranda R, Soria T. and. Cuartero J. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant science***160 (2):** 265-272.

Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J and Lee EJ. 2013. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). *Ecotoxicol Environ Saf*. **93**:60-7.

Uçgun, K., Ferreira, JFS, Liu, X, da Silva Filho, JB, Suarez DL, Lacerda CFd and Sandhu D. **2020**. Germination and Growth of Spinach under Potassium Deficiency and Irrigation with High-Salinity Water. *Plants* , ***9:*** 1739.

Xingmao M, Geiser-Lee J, Deng Y, Kolmakov A, 2010. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation', *Science of the total environment*, **408:** 3053-61.

Yang J, Zheng W, Tian Y, Wu Y, and Zhou D. 2011. Effects of various mixed salt-alkaline stresses on growth, photosynthesis, and photosynthetic pigment concentrations of Medicago ruthenica seedlings. *Photosynthetica***49(2):** 275-284.

Zhang XF, Liu ZG, Shen W, Gurunathan S. 2016. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *International Journal of Molecular Sciences*. **17(9):**1534.