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Responses of *Allium cepa* L. exposed to silver nanoparticles

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Abstract

The study was aimed to determine the gallic acid, rutin and quercetin contents and yield of Narli onion genotype (*Allium cepa* L.,) exposed to four different doses (0, 25, 50, 75, 100 mg L^{-1}) of silver nanoparticles (AgNPs)for30 days, after planting the onion bulbs, attwo-week intervals. Quercetin, rutinand gallic acid contents in the leaves and bulbs of onion plants were determined.While the quercetin content was the highest in 25 mg L^{-1}ofAgNPs treatment (575.0 ± 10.39 µg g^{-1})in the bulb parts, gallic acid content reachedtothe highest rate in 50 mg L^{-1} of AgNPs(3605.8 ± 90.96µg g^{-1}), inthe onion bulb, compared to the control (2819.3 ± 65.72µg g^{-1}).The content of rutinwere enhanced in 25 (19.72 ± 0.28µg g^{-1}), 50 (21.66 ± 0.57µg g^{-1}) and 75 mg L^{-1}(31.08 ± 0.53 µg g^{-1}) of AgNPs treatments, but it was significantly close to the control (7.15 ± 0.93µg g^{-1}), in100 mg L^{-1}(10.92 ± 0.38 µg g^{-1}), in bulb parts.Chlorophyll content showed reducesin all doses, except for25 mg L^{-1} of AgNPs treatment. Total yield enhanced in treatments of AgNPs, but the highest increase was obtained in treatment of 50 mg L^{-1} of AgNPs (97.49 ± 0.92 µg g^{-1}). The analysis of quercetin, rutin and gallic acid contents were performed by high performance liquid chromatography (HPLC), and Chlorophyll was determined by SPAD.

Keywords: Chlorophyll, Onion, Quercetin, Rutin, Silver Nanoparticle

Introduction

Onion (*Allium cepa*L.), a member of *Liliaceae* family, is consumed asdry, fresh and cooked. The onion contains A,B and C vitamins and rich in sulfurand antibiotic; thus,itis considered a medicinal plant(Morimitsuet al.,1992).China was the largest producer of onion in the world in 2017 with 1056139 tons fresh onion. The production of fresh onion in Turkey was138993 ton in the same year (FAO, 2020). Nanotechnology has been developing rapidly. Nanoparticles differ from the bulk products due to their physical and chemical characteristics. Different responses have been reported regarding the commercial and scientific applications of nanoparticles (Oberdorsteret al., 2005). The word nanooriginatesfromGreek, meaningvery small and indicates one billion of any physical size (Tegart, 2003). Silver is used in different stages of plant production due to the nanomaterial antibacterial characteristics (Kimetal., 2007). Nanotechnology is a field of applied science deals with biological or non-biological particles less than 100 nm diameters (Cıracıet al., 2005). Nanotechnology is the use and examining of materials at the atomic and molecular sizes (a scale ranging from 10 to 100 nm) (Kaphleet al.,2018). Nanoparticles are defined as organic and inorganic materials. The organic nanoparticles contain carbon and inorganic nanoparticles contain titanium, zinc, silver, gold and copper (Xuet al., 2006).

Significant effects of nanoparticle applications at various doses on morphological and biochemical growth and development of plants have been reported (Maetal.,2010). The silver nanoparticles are the most commonly used commercialized nanoparticles among the different nanoparticles (Ahmed et al., 2008), which have been used in biological synthesis due to rapid disintegration, low cost and potential of compatibility (Vermaet al.,2014). Babu et al. (2008) reported that applications of silver nanoparticle at various concentrations (10, 20, 40 and 5 mg L^{-1}) caused significant decrease in mitosis index and structural deviation in chromosome *Allium* plants. Similarly, the cytotoxic and geotoxic effects of silver nanoparticles were reported in exposure todifferent

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silver doses (20, 25, 75, 100 mg L^{-1}) *Allium* plants (Mamta Kumariand Mukherjee, 2009). Theresearchers indicated no irregularity in the control plants, while the mean mitotic index was 60% in the silver treatments. Onion contains different flavonoids such as quercetin. Kamferol, quercetin, rutin, resveratrol, isorhamnetin and myelicinare the examples of important flavonoids (Sefer, 2000).The flavonoids are generally found in shells of different onion genotypes along with red, purple and brown anthocyanins(Griffiths et al.,2002). Buckwheat plants contain high amounts of protein, vitamins, minerals and also rich in important phenolic components such asrutin and quercetin. Similar to the other germinated plants, buckwheat shoots were rich in lysine, minerals, raw fiber, phenolic substances, vitamin C, etc. compared to the usual seeds (Hsu et al.,2008; Kim et al., 2004). The rutin is the only flavonoid which is capable of chelating metal ions such as iron and causes the formation of oxygen radicals with its high antioxidant activities. Park and Cha (2008) reported the presence of 19 flavonoids (6 quercetin derivatives, 6 isorhamnetine derivatives and 7 camherol derivatives) in the leaves of 30 different grape varieties and the amounts of phenolic compounds varied between the grape varieties. The aim of this study was to determine the effects of different silver nanoparticleapplication doses on yield and quality traits of onion plants.

Materials and Methods

The study was carried out in Şırnak University Faculty of Agriculture, Department of Horticulture, at research laboratory. Narli onion genotypewas used as the plant material in theexperiment. Four different silver nanoparticle doses (0, 25, 50, 75, 100 mg L^{-1}) in addition to 0 mg $L^{-1}$of pure water were used as the treatments of the experiment. The silver nanoparticles, purchased from Gute Chemie-abcrGmbh, Deutschland, were applied between 8-10 hours intervals during the experiment. Silver nanoparticles used in the experimentwereof80 nm in size, 99.995% purity and metal basis. pHand EC values of the nutrient solution onion cultivation was set at 5.5-6.1 and 1.9 dS m^{-1}, respectively. In the onion growing stage (in mg L^{-1}): N (200), P (60), K (300), Ca (170), Mg (60), Fe (3.0), Mn (0.8 -1.0), Cu (0.1), Zn (0.3), B (0.3) and Mo (0.05) nutrient solutions were used.Plant height, chlorophyll (SPAD), stem diameter and plant width were measured on November 20 and December 14, 2018. The plants were harveste don January 14, 2019.

Calibration curves of quercetin, rutin and gallic acid compounds

The standard solutions of quercetin, rutin and gallic acid were prepared at four different concentrations (0.25, 0.5, 1.0, 2.5, 5.0 µg mL^{-1}) by dissolvingtheir stock solutions in dimethyl formamide (DMF, 1 mg mL^{-1}). Each standard solution was injected in triplicate to obtain the calibration curves (Figure 1). The concentrations of quercetin, rutin and gallic acid in the extracts were calculated using the calibration curves of the compounds.

Extraction of quercetin, rutin and gallic acid

The leaves and bulbs of onion plants were dried in an oven and ground into powder using a blender. Then, 0.2 mg of powder sample was accurately weighed, extracted using10 mL of DMFkeptat overnight. The final volume of extracts was adjusted to 80 mL with DMF. The solutions were filtered through a 0.45 mm nylon filter membrane prior to analysis.

QuercetinandrutinanalysisinHigh Performance Liquid Chromatography (HPLC)

The quercetin, rutin and gallic acid contentsin plant samples were determined by using a High Performance Liquid Chromatography (HPLC) system (Shimadzu Corporation, Japan). The HPLC system was equipped with an Inertsil ODS-3 C18 column (5µm x 4.6 mm x 250 mm), LC-20AT pump, DGU 20A5R degasser, SIL 20A-HT auto sampler and SPD M-20A PDA detector. The mobile phase consisted of 60% water and 40% acetonitrile with 0.1% TFA for quercetin and gallic acid and 80% water and 20% acetonitrile for rutin. The mobile phase was filtered through a 0.45 mm filter and then degassed by ultrasonification. An isocratic elution profile was used for both compounds, and the column temperature and flow rate wereadjusted to 30 ^{0}C and 0.6 mL min^{-1}. Separation process was carried out at room temperature. Quercetin, rutin and gallic acid contents were detected at wavelengths of 320, 256 and 266 nm, respectively. Retention times of quercetin, rutin and gallic acid were 13.4, 10.6 and 6.5 min, respectively. Injection volume was set to20 µl. The correlation coefficients (R) of the standards were 0.998 for quercetin, 0.997 for gallic acid, and 0.9997 for rutin. Quantification of the three compounds was performed by comparing the retention time of the samples with the standards.

Chlorophyll measurements

Chlorophyll measurements were carried outon3 leaves at outside of the plant in a sunny weather and around 10 a.m. SPAD meter (502 Minolta brand) was used in the chlorophyll measurements.

Yield

The plants were harvested on January 14, 2019. Onion roots were weighed after cutting from the leaves.

Results and Discussion

Quercetin, rutin and gallic acid contentsin onion bulbs

Quercetin, rutin and gallic acid contents of onion bulbs exposed to AgNPs are shown in table 1and HPLC chromatogram of quercetin, rutin and gallic acid compounds on different silver nanoparticle doses were presented in figure 2. a-b.While the quercetin content enhanced in 25 and 50 mg L^{-1} AgNPs treatments, it was reduced in 75 and 100 mg L^{-1} AgNPs, in onion bulbs.The highestquercetin was obtainedinbulbs treated with 25 mg L^{-1} AgNPs treatments (575.0 ± 10.39 µg g^{-1}), comparedtothecontrol (285.3 ± 3.86µg g^{-1}). Rutin content enhanced in increasing doses of AgNPs, but it was the lowest in 100 mg L^{-1} (10.92 ± 0.38µg g^{-1}).The content of rutin was the highest in bulb treated with75 mg L^{-1} AgNPs (31.08 ± 0.35 µg g^{-1}). Gallic acid content was the highest in 50 mg L^{-1} of AgNPs (3605.8 ± 90.96 µg g^{-1}), but in the other treatments, it was reduced or closetothe control group (2819.3 ± 65.72 µg g^{-1}) (Table 1).

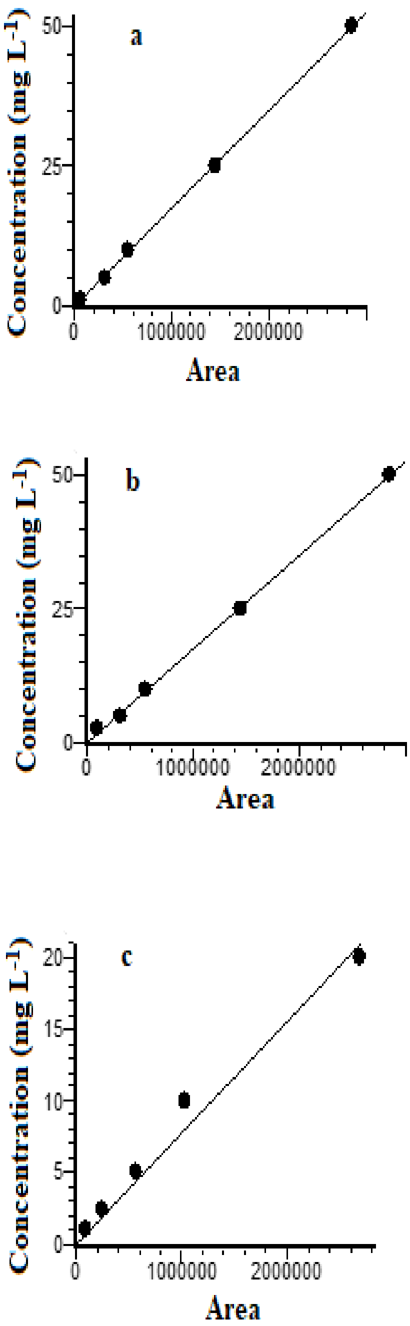

Fig1. Calibration curves, (a) quercetin, (b) gallic acid, (c) rutin

Similar to our findings, several authors stated an increase in some flavonoids and phenolic compounds (rosmarinic and salvanolic acids) intreatment with a certain doses of silver nanoparticles (GeandWu 2005; Xing et al.,2015; Zhang et al.,2004). Chung et al. (2018) was reported that it was found an enhancement in Total Phenolic and flavonoid content contents in hairy bulb cultures of *cucumisanguria*treated with the AgNPs and $AgNO_3$. In another study, it was found thatthe Total phenolic content was increased in the tissue culture of *Vanilla planifolia* Jacks. Ex Andrews, with the application of 25 and 50 mg L^{-1}AgNPs (Spinoso-Castilloet al.,2017).The silver nanoparticles induced some phytochemical production of in the bulb parts of *Cucumisanguria*(Chung et al.,2018). Zhang et al. (2013) reported that treatment with Ag-SiO_2core-shell nanoparticle increased artemisin content in *Artemisia annua*plant.

Quercetin, rutin and gallic acid contents in onion leaves

The quercetin, rutin and gallic acid contents in the leavesof onion exposed to AgNPswere significantly different (Table 2). The highest quercetin (10.99±0.12µg g^{-1}) and gallic acid contents (3562.05±112.3µg g^{-1}) in leaf samples was recorded in 50 mg L^{-1}AgNPsapplication. The highest rutincontent (16.31±0.78) was detectedin 75 mg L^{-1} application, compared to the control plants (6.55 ± 0.81µg g^{-1})(Table 2). Similar changes in quercetin content of onion have been reported by Patil et al.(1995).The oxidant and anti-oxidant contents in arabidopsis plants exposed to silvernanoparticleswere higher compared to the control (Qian et al., 2013). Silver nitrate accumulation in the cell walls of *Cucumissativus* (Zhang et al.,2012)and pumpkin(Corredoret al.,2009) have been reported absorptionby plants and accumulated in leaf tissues.In this study, phenolic acid contents were significantly affected byapplication of silver ions. The nanoparticle may increase activation of enzymatic pathways; thus may contribute to the production of metabolites. Xinget al. (2015) reported significant increases of rosmarinic acid (Ra), cafeic acid and ferulic acid in in *Salvia miltiorrhiza*, while salvanolic acid, danshensu and cinnamics significantly decreased with the application of silver nanoparticles.

Chlorophyll Content, Yield and Leaf Dry Matter Ratio

The effects of different silver nanoparticle doses on yield and leaf dry weight of onion were significantly important ($P \leq 0.05$). The onion yield in control (74.64g $plant^{-1}$) insignificantly increased in 25 mg L^{-1}(75.44 g $plant^{-1}$) application; however, application of50 mg L^{-1} dose significantly increased the onion yield (97.49 g $plant^{-1}$). Further increase in silver nanoparticle doses caused a significant decrease in onion yield which was 85.42 g $plant^{-1}$in

75 mg $L^{-1}$and 73.18 g $plant^{-1}$ in 100 mg L^{-1} doses (Table 3).

While thechlorophyllcontentin 25 mg L^{-1} AgNPs application (63.50 ± 0.10) was close to the control (62.25 ± 0.22), treated with 50, 75 and 100mg L^{-1} AgNPsreducedchlorophyll content in the onion leaves. Qian et al. (2013) also reported a decrease in chlorophyll content of*Arabidopsis*treated with AgNPs. The inhibitor effect of AgNPsonphotosynthesis pigment contents reported of *Spirodelapolyrhiza*and *Dunaliellatertiolecta*, in the study ofJiang et al.(2012).

Table 1. Quercetin, rutin and gallic acid contents in onion bulb (μgg^{-1})

AgNPs Doses	Quercetin	Rutin	Gallic acid
0 mg L^{-1}(Control)	285.3 ± 3.86 ^{c}	18.27 ± 0.49 ^{d}	2819.3 ± 65.72^{b}
25 mg L^{-1}	575.0 ± 10.39 ^{a}	19.72 ± 0.28 ^{c}	2411.5 ± 60.51^{c}
50 mg L^{-1}	477.0 ± 68.97^{b}	21.66 ± 0.57 ^{b}	3605.8 ± 90.96^{a}
75 mg L^{-1}	245.0 ± 7.43^{c}	31.08 ± 0.53 ^{a}	2821.6 ± 72.22^{b}
100 mg L^{-1}	179.3 ± 5.73^{d}	10.92 ± 0.38 ^{e}	2310.2 ± 53.62^{c}
Mean	352.3	20.33	2793.68
$LSD_{0.05}$	47.51	0.70	105.17

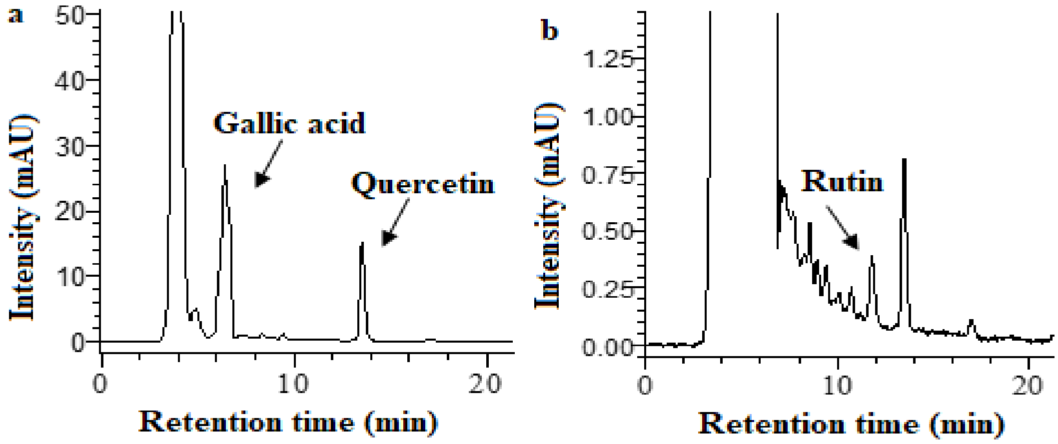

Fig 2. HPLC chromatograms of the onion extracts (a) gallic acid and quercetin (b) rutin

Table 2. The quercetin, rutin and gallic acid contents in onion leaves (μg g^{-1})

AgNPs Doses	Quercetin	Rutin	Gallic acid
0 mg L^{-1}(control)	3.19 ± 0.12^{d}	6.55 ± 0.81^{d}	2432.86 ± 58.06^{d}
25 mg L^{-1}	9.44 ± 0.07^{b}	8.07 ± 0.29^{c}	2793.57 ± 101.31^{c}
50 mg L^{-1}	10.99 ± 0.12^{a}	11.80 ± 0.22 ^{b}	3562.05 ± 112.3^{a}
75 mg L^{-1}	5.92 ± 0.30^{c}	16.31 ± 0.78^{a}	3343.47 ± 95.84^{c}
100 mg L^{-1}	6.08 ± 0.39^{c}	7.15 ± 0.93^{cd}	2292.86 ± 35.03^{e}
Mean	7.12	9.97	2884.96
$LSD_{0.05}$	0.35	1.01	129.07

Table 3. Yield, Chlorophyll and Leaf dry matter ratio of onions

Silver Np Doses	Total yield (g $plant^{-1}$)	Chlorophyll (SPAD)	Leaf dry matter (%)
0 mg L^{-1}(Control)	74.64 ± 0.35^{cd}	62.25 ± 0.22^{a}	8.78 ± 0.95^{d}
25 mg L^{-1}	75.44 ± 0.17^{c}	63.50 ± 0.10^{a}	9.03 ± 1.82^{c}
50 mg L^{-1}	97.49 ± 0.92^{a}	55.75 ± 0.1^{bc}	9.78 ± 2.38^{a}
75 mg L^{-1}	85.42 ± 2.95^{b}	57.00 ± 0.02 ^{b}	9.33 ± 1.70^{b}
100 mg L^{-1}	73.18 ± 0.99^{d}	54.00 ± 0.06^{c}	8.67 ± 0.81^{d}
Mean	81.23	58.50	9.11
$LSD_{0.05}$	2.21	2.47	0.18

Conclusion

This study revealed that the interaction of onion with silver can be optimized by promoting the yield and chemical compounds testedin the onion without damaging the plant. Accordingly, the use of silver nanoparticles in different plant species will be commercially successful in future studies. In Statistically significant difference were obtained between different silver nanoparticle doses. The results indicated that the best useful doses of AgNPs on onion are25 and 50 mgL^{-1}.

Compliance with Ethical Standards

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

Author contribution

The contribution of the authors is equal. All the authors' readand approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before

Ethics committee approval

This article does not contain any studies with human or animal subjects. Ethics committee approval is not required.

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Data availability

Not applicable

Consent for publication

Not applicable.

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