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Effects of different treatments of silver nanoparticles (AgNPs) on the growth & physiological characteristics of lotus (*Nelumbo nucifera*)

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Abstract. Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic perennial plant with various values, such as ornamental flowers, vegetables, food, and herbal medicine. It is cultivated and consumed throughout the different regions in Vietnam as a symbol associated with local culture. This study was conducted to evaluate the effects of four other treatments of silver nanoparticles (AgNPs) on the growth of lotus in crop 2021. In the present study, we examined the effects of 4 treatments: control - no AgNPs (CT), treating the soil with AgNPs 4mg/L 5 days before planting (T1), treating plants with AgNPs 4mg/L before planting (T2), and a combination of soil treatment, plant treatment, and periodic foliar application at 4mg/L (T3). The results show that AgNPs application by different methods significantly increased plant height, leaf diameter, fresh leaf weight, dry leaf weight, and some biochemical aspects compared with the control. Furthermore, exposure to AgNPs elevated the activities of peroxidase (POD), superoxide dismutase (SOD). Among the different of AgNPs applications, plants treated with T3 showed the highest efficiency. In addition, the chlorophyll content and diameter of floating and upright leaves were positively correlated with dry leaf mass. Thus, the current use of AgNPs in agricultural sciences offers the prospect of researching their impact on various plants in the future.

1. Introduction

Lotus *(Nelumbo nucifera* Gaertn.*)* is an aquatic perennial growth throughout Asia such as China, Japan, Korea, India, Thailand, and Vietnam [23]. Lotus is a beautiful flower, which is a symbol associated with Buddhism and Vietnamese culture and history. Besides the ornamental value, lotus also has many high economic deals with local specialty products and ecological landscape factors. All parts of the lotus plant: leaves, stems, tubers, seeds, and flowers are edible, have been used for medicinal purposes, food, or beverage [8, 10]. Highly bioactive compounds such as alkaloids, flavonoids, triterpenoids, vitamins found in flowers, leaves, seeds, and lotus roots significantly improve human health problems such as anti-inflammatory, high antioxidant activity also noted in recent reports [11, 13].

Unpredictable global climate change, contamination of soil caused by fertilizers and pesticides, and ensuring a continuous supply of food and nutrition with a growing global population are the biggest challenges that agriculture faces. To deal with these issues, newer techniques and solutions are continuously provided and evolving. Nano-biotechnology is a promising strategy to ensure global agricultural food security for sustainable agriculture. Among the various types of metal nanoparticles, the silver nanoparticles (AgNPs) with unique physiochemical properties are widely used in textile, medical science, household products, water remediation, and especially agriculture. Many previous studies have reported the positive impacts of silver nanoparticles on the growth and development, crop yield, and quality of plants through changes in biochemical, physiological, and molecular aspects [12]. Krishnaraj *et al.* (2012) showed significant effects on seed germination and root growth of *Bacopa monnieri* of AgNPs, enhanced the synthesis of carbohydrates and protein, and reduced the phenols, catalase, and peroxidase activities [6]. In addition, the positive effects of AgNPs in enhancing seed germination and the growth of *Boswellia ovalifoliolata* are also reported in the study of Savithramma *et al.* [20]. The reports of Salama and Sharma *et al.* recorded an improvement in plant morphological growth parameters, including length of root and shoot and leaf area and biochemicals properties including chlorophyll content, carbohydrate, protein, and antioxidant enzymes in *Brassica juncea, Phaseolus vulgaris*, and *Zea* mays [17, 19]. Nair and Chung reported that after exposure to 0.5 and 1mg/L AgNPs for 7 days found that significantly more significant reductions in total chlorophyll and increases in anthocyanin content compared to equivalent concentrations of silver ions. Thuesombat et al. observed AgNPs-mediated dose and size-dependent decrease in shoot and root fresh and dry weight of rice. It has been reported that lower AgNPs treatments, up to 30 mg/L, accelerated the rice root growth while higher concentration, at 60 mg/L, reduced the root growth and caused root death [21]. However, literature on the interaction between plants and silver nanoparticles and their optimized use in agriculture to improve yield and quality is not studied well and is limited. The impacts depend on plant species, growth stage, experimental conditions, nutrition, and method of experimental applied nanoparticles. To increase lotus growth and quality, a novel eco-friendly and natural product-based technique is required.

Therefore, the primary aim of our study was to (i) investigate the growth parameters of *Nelumbo nucifera* caused by different methods of AgNPs exposure; (ii) evaluate the changes in physiological characteristics of *Nelumbo nucifera*.

2. Materials and methods

2.1. Plant material and Experimental design

The studies were performed on the "Cao san" Lotus (*Nelumbo nucifera* Gaertn.) - a high-yielding lotus variety. The field experiment was conducted using the Randomized Complete Block Design (RCBD) model with three replications in Phong Hien commune, Phong Dien, Thua Thien Hue, Vietnam in the summer-autumn season 2021. This study began in March 2021 and ended in August 2021. The area of each experimental plot is 64 m^2 (8 m x 8 m). Plant density is 6m^2/plant (3m x 2m). Silver nanoparticles solution is treated by spraying directly on the leaves. The treatment concentration was 4 mg/L. To control weeds, hand weeding was used.

Silver nanomaterials (AgNPs) provided by the Department of Solid State Physics, Faculty of Physics, Hue University of Science. AgNPs are synthesized from $AgNO_3$, and the reducing agent is an extract from *Aloe vera* leaves. The concentration of stock silver nanoparticles solution is 40 mg/L. The stock solution was dissolved in deionized water and used at the following concentrations: 0 (control), 4 mg/L.

In this study, we designed the following 4 treatments of application of AgNPs:

T1: soil treatment with AgNPs at 4 mg/L before planting 5 days.

T2: plant treatment with AgNPs at 4mg/L before planting

T3: the combination of soil application with AgNPs 4mg/L + plant treatment with AgNPs 4mg/L before planting + periodic foliar treatment with AgNPs 4mg/L at 10, 40 days of planting.

T4 (Control): non-treated soil and plant with AgNPs. In the control treatment, distilled water was sprayed.

2.2. Data collection

2.2.1. Growth parameters.

Data were collected on the following parameters: Floating leaf diameter (cm) and standing leaf diameter were recorded a few 2 weeks from the planting stage to the flowering stage (full blossom). In addition, the height of standing leaf, flower stalk height (cm) was recorded after 70 days of the first application of AgNPs.

2.2.2. Determining of Chlorophyll content.

The content of chlorophyll was estimated according to the method of Lichtenthaler (1987). First, 20 mg fresh lotus leaves were taken from control and treated seedlings. Leaves were then subjected to crushing in 80% acetone, followed by pigment extraction and centrifugation. The absorbance was measured at 663 and 646 nm by using Eppendorf UV-VIS Spectrophotometer [9].

2.2.3. Determining the activity of antioxidant enzymes.

100 mg of fresh leaves were grounded in 10 mL pre-cooled potassium phosphate buffer (PBS, 100 mM, pH 6.8) and centrifuged at 13,000 g for 15 min at 4ºC. The supernatant was collected and used to examine the activity of the antioxidant enzymes.

The catalase (CAT, EC 1.11.1.6) activity was measured using the method of Aebi (1984) by monitoring the degradation of H_2O_2 at 240 nm (extinction coefficient was 39.4 mM^{-1} cm^{-1}). The reaction mixture consisted of 75 µL of the crude enzyme was combined with 800 µL of 50 mM potassium phosphate buffer, pH 8.0 and 125 µL of 100 mM H_2O_2. The change in absorbance at 240 nm was measured for 1 minute, and the rate of H_2O_2 breakdown by CAT was estimated (µmol H_2O_2 consumed min^{-1} mg $protein^{-1}$) [1].

The superoxide dismutase (SOD, EC 1.11.1.7) activity was quantified using the method described by Beauchamp and Fridovich (1971) by measuring 50% of the inhibition of nitroblue tetrazolium (NBT) at 560 nm. The reaction mixture contained sodium phosphate buffer (50 mM, pH 7.8), EDTA (0.1 mM), methionine (13 mM), NBT (75 µM), riboflavin (2 µM), and 50 µL of crude enzyme [3].

The activity of guaiacol peroxidase (POD, EC 1.15.1.1) was determined by monitoring the formation of guaiacol dehydrogenation (extinction coefficient 6.39 mM^{-1} cm^{-1}) at 470 nm for 1 minute, and POX activity was calculated in µmol min^{-1} mg of $protein^{-1}$ [2].

2.3. Statistical analyses

The data are shown as Mean ± standard deviation (SD), then subjected to One-way Analysis of Variance (ANOVA) to determine the significance of individual differences. The significance level was $p < 0.05$. Duncan's multiple range tests were used to compare significant means. Correlation analysis was carried out using Pearson correlation in the IBM SPSS Statistics 22.0 software (SPSS, Chicago, IL, USA).

3. Results and Discussion

3.1. Effects of different applications of silver nanoparticles on the growth of Nelumbo nucifera

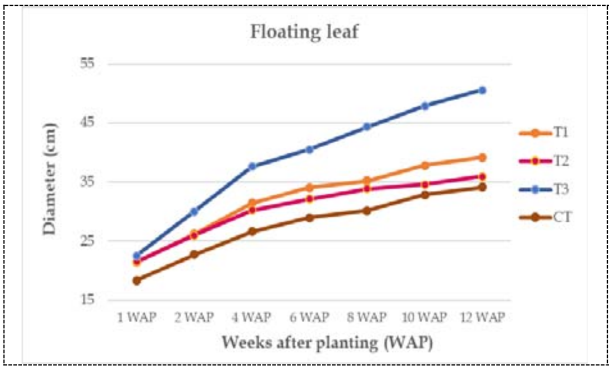
3.1.1. Leaf growth.

Lotus is an aquatic plant with subterranean stems, that develops horizontally, therefore development is primarily dependent on the growth of leaves on the water's surface. The grow-up lotus leaves are divided into two stages: floating leaf stage and standing leaf stage. The floating leaf stage is the first growth period of the lotus plant. The plant begins to generate standing leaves when the floating leaves have covered the majority of the water's surface. The effect of silver nanoparticles on the diameter of the floating leaf and the standing leaf of the lotus were recorded and presented in Table 1, Figure 1, Figure 2.

Table 1. Effects of different methods of AgNPs application on the growth of floating lotus leaf.

Treatment	Diamter of floating leaf (cm)						
	Weeks after planting						
	30/03 (1 week)	06/04 (2 weeks)	20/04 (4 weeks)	08/05 (6 weeks)	22/05 (8 weeks)	05/06 (10 weeks)	20/06 (12 weeks)
T1	21.45 ± 2.37^{b}	26.23 ± 2.39^{b}	31.47 ± 1.76^{b}	34.06 ± 2.38^{c}	35.18 ± 3.63^{b}	37.83 ± 2.04^{b}	39.15 ± 2.09^{b}
T2	21.52 ± 2.59^{b}	26.03 ± 2.65^{b}	30.26 ± 1.52^{b}	32.16 ± 2.30^{b}	33.90 ± 3.00^{b}	34.60 ± 2.33^{a}	35.92 ± 2.49^{a}
T3	22.56 ± 2.34^{b}	30.05 ± 3.72^{c}	37.60 ± 2.35^{c}	40.50 ± 2.45^{d}	44.31 ± 2.04^{c}	47.89 ± 4.23^{c}	50.58 ± 4.59^{c}
CT	18.42 ± 1.79^{a}	22.72 ± 2.39^{a}	26.65 ± 3.97^{a}	28.99 ± 2.09^{a}	30.21 ± 2.08^{a}	32.86 ± 2.84^{a}	34.11 ± 3.19^{a}

Notes: T1: soil treatment; T2: plant treatment before plating; T3: combination soil treatment + plant treatment. Values are the means ± SD. The values followed by the different letters show statistically significant differences at P ＜0.05 level.



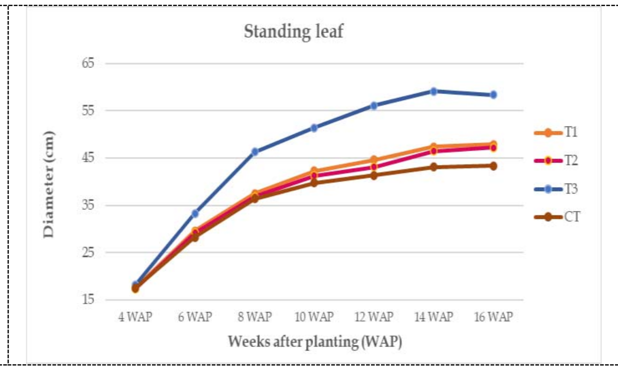


Figure 1. Effects of different methods of silver nanoparticles treatments on floating leaf of lotus *(Nelumbo nucifera).*

Figure 2. Effects of different methods of silver nanoparticles treatments on standing leaf (B) of lotus *(Nelumbo nucifera).*

The results showed that all the treatments present silver nanoparticles, including T1, T2, and T3, significantly increased in floating leaf diameter compared with the control plants, increasing by 18.08%, 13,5%, and 41,08%, respectively. In addition, the three different silver nanoparticles treatments improved the growth of the floating leaf, with significant differences among T1, T3, and CT. The highest growth of floating leaves was obtained by T3. At 4 weeks, and 8 weeks after planting, the diameter of floating leaves was treated with formula T3 with leaf diameters 37.6 cm, 44.31 cm, increasing by 41.08%, and 46,7% compared with the control plants, respectively. However, the difference between T2 and CT was not significant at 10, 12 weeks after planting.

Table 2. Effects of different methods of silver nanoparticles application on the growth of standing lotus leaf.

Treatments	Diamter of standing leaf (cm) Weeks after planting						
	20/04 (4 weeks)	08/05 (6 weeks)	22/05 (8 weeks)	05/06 (10 weeks)	20/06 (12 weeks)	04/07 (14 weeks)	18/07 (16 weeks)
T1	17.61 ± 1.72^a	29.60 ± 2.98^a	37.47 ± 2.28^a	42.28 ± 1.69^b	44.59 ± 2.31^b	47.42 ± 2.18^b	47.87 ± 2.35^c
T2	17.43 ± 1.19^a	29.25 ± 3.38^a	36.82 ± 2.42^a	41.23 ± 1.83^b	43.10 ± 2.59^b	46.50 ± 1.99^b	47.25 ± 2.37^b
T3	18.21 ± 1.32^a	33.31 ± 3.89^b	46.26 ± 2.50^b	51.41 ± 2.19^c	56.06 ± 3.91^c	59.09 ± 2.65^c	58.39 ± 3.19^d
CT	17.55 ± 1.04^a	28.32 ± 2.41^a	36.39 ± 1.63^a	39.74 ± 2.41^a	41.33 ± 2.06^a	43.12 ± 3.14^a	43.35 ± 3.38^a

Notes: T1: soil treatment; T2: plant treatment before plating; T3: combination soil treatment + plant treatment. Values are the means ± SD. The values followed by the different letters show statistically significant differences at $P < 0.05$ level.

According to data in Table 2 and Figure 2, the diameters of the standing leaf increased rapidly from the 4th week to the 10th week after planting. Following this period, the growth of these leaves remains steady. However, there were no significant differences between T1, T2, and control at 4 weeks, 6 weeks, and 8 weeks after planting. Treating with T3 had the most effective potential to promote the growth of standing leaf, and there was a substantial difference with other applications. Notably, in the T3 treatment, the largest diameter of standing leaf was 59.09 cm, increased approximately by 37%, 24%, and 27% than CT (43.12 cm), T1 (47.42 cm), T2 (46.50 cm), respectively.

3.1.2. Fresh weight, dry weight and height of plant.

Table 3 shows the influence of silver nanoparticles on the fresh weight and dry weight of floating leaves and standing leaves. When treated with different application methods of AgNPs, the fresh weight of floating leaves was observed significantly higher than that of the control leaves, ranging from 49.14 to 52.46g. T3 was remained the most efficient treatment solution, increasing by 16% in fresh weight floating leaf compared to the control. However, no significant differences were observed in the fresh weight and dry weight of floating leaf between T1 and T2 treatment. Compared with CT, T1 treatment increased 9.5% fresh leaf weight, while T2 treatment recorded an increase of 8.6% of fresh weight of floating leaf. Between T2 and CT was not a significant difference in the weight of fresh standing leaf. There was no significant difference in the dry weight obtained among the three different modes, including T1, T2, and CT.

Table 3. Effect of different treatments on some growth parameters of *Nelumbo nucifera.*

Treatments	Growth parameters					
	Height of standing leaf (cm)	Height of stalk (cm)	FW of floating leaf (g)	DW of floating leaf (g)	FW of standing leaf (g)	DW of standing leaf (g)
T1	46.50 ± 2.00^{b}	55.25 ± 3.14^{b}	49.72 ± 1.32^{b}	10.68 ± 0.98^{b}	53.92 ± 1.46^{b}	12.29 ± 0.52^{a}
T2	46.52 ± 1.18^{b}	53.52 ± 3.08^{ab}	49.14 ± 1.33^{b}	10.36 ± 0.54^{b}	52.32 ± 1.00^{ab}	12.02 ± 0.22^{a}
T3	51.94 ± 2.87^{c}	62.42 ± 3.38^{c}	52.46 ± 1.84^{c}	13.23 ± 0.63^{c}	61.12 ± 2.71^{c}	14.36 ± 0.57^{b}
CT	45.18 ± 1.51^{a}	53.03 ± 3.32^{a}	45.22 ± 1.15^{a}	8.36 ± 1.14^{a}	51.40 ± 1.14^{a}	11.80 ± 0.55^{a}

Notes: FW: Fresh weight. DW: Dry weight. Different letters in the same column indicate a significant difference ($p < 0.05$) among treatments.

3.2. Effects of silver nanoparticles on physicochemical parameters and plant metabolism

Among treatments to further understand the physiological impacts of various silver nanoparticles treatments on the growth of *Nelumbo nucifera*, we investigated the content of chlorophyll and enzyme activity in the leaves. As shown in Table 4 the content of chlorophyll treated with T1, T2, and T3 was markedly higher than that of control, increasing from 5.1% - 9.5%. In addition, leaves of the lotus cv. Cao san treated with T3 treatment accumulated the most outstanding amounts of chlorophyll a. However, no noticeable differences in the amount of chlorophyll b in lotus leaf were observed in leaves treated with T1, T2, and T3.

The results of this study are similar to the recent report of Farghaly and Nafady (2015); Latif *et al*. (2017) or Sadak (2019) reported an increase in the photosynthetic pigment content when exposed to AgNPs on tomato (*Solanum lycopersicum*), wheat (*Triticum aestivum*), and fenugreek (*Trigonella foenum-graecum)* [4; 7; 16]. In other reports Salama (2012), Sharma (2012) both recorded positive signals on morphological growth (bud height, root length, leaf area) and biochemical characteristics such as chlorophyll, carbohydrate, protein, antioxidant enzyme content of *Brassica juncea*, *Phaseolus vulgaris,* and *Zea mays* [14, 19].

Table 4. Chlorophyll and antioxidant enzymes of lotus under different methods of AgNPs applications.

Treatments	Biochemical parameters				
	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	SOD (Units mg^{-1})	POD (Units mg^{-1})	CAT (Units mg^{-1})
T1	0.95 ± 0.018^{b}	0.31 ± 0.012^{ab}	121.33 ± 1.15^{b}	1.52 ± 0.03^{a}	76.44 ± 0.62^{a}
T2	0.95 ± 0.016^{b}	0.30 ± 0.011^{ab}	133.33 ± 5.77^{c}	1.48 ± 0.03^{a}	119.54 ± 0.77^{c}
T3	0.99 ± 0.019^{c}	0.32 ± 0.01^{b}	151.50 ± 1.32^{d}	1.51 ± 0.03^{a}	120.47 ± 1.35^{c}
CT	0.904 ± 0.02^{a}	0.29 ± 0.01^{a}	98.67 ± 1.15^{a}	1.50 ± 0.05^{a}	81.61 ± 0.77^{b}

Notes: FW: Fresh weight. SOD: Superoxide dismutase. POD: Guaiacol peroxidase. CAT: catalase. Different letters in the same column indicate a significant difference ($p < 0.05$) among treatments

Reactive oxygen species (ROS) function as signaling molecules, regulating plant adaptive pathways and activities and the controlled interactions between them. ROS plays an essential part in plant stress adaption. The parameters of antioxidant enzymes were evaluated to clarify the extent of plant impact under the influence of different methods of silver nanoparticles application, as shown in Table 4. Many reports suggest that catalase is involved in maintaining oxidative stress in cells by 'scavenging' excess H_2O_2. In different treatments, the Guaiacol peroxidase (POD) leaf activity unremarkably changed from 1.48 units mg^{-1} protein to 1.52 units mg^{-1} protein. Superoxide dismutase (SOD) is one of the most antioxidant enzymes, capable of rapidly converting ·OH to H_2O_2, and the generated H_2O_2 is then converted to water and dioxygen by peroxidase and CAT. In this study, exposed AgNPs showed that SOD content increased significantly by 22.9%, 35.1%, and 53.5% in lotus leaves treated with T1, T2, and T3, respectively ($p < 0.05$).

Similarly, the highest catalase (CAT) activity was found in plants treated with T3, increasing 47.61% compared to the control plant. Rastgoo and Alamzadeh (2011) reported that in *Aeluropus littoralis*, CAT activity initially increased at low concentration of Ag treatment and then decreased at high concentration [14]. A previous report by Tripathi et al in *Pisum sativum* showed that AgNPs (100 - 300 μM) significantly increase superoxide dismutase and ascorbate peroxidase activity while suppressing glutathione reductase and dehydroascorbate reductase activity [22].

3.3. Correlation between growth parameters

In this study, the relationships between the independent variable exposure to different methods of Ag NPs application (growth parameters) were established. The correlation analysis results are shown in Figure 3. We observed a link between chlorophyll a content - growth of lotus leaf diameter - fresh weight and dry weight of floating leave when we compared it to the development of leaf diameter using Pearson correlation analysis. The chlorophyll a content had a significant positive correlation with the floating leaf diameter from week 4th to week 14th ($r = 0.728 - 0.824$; $p < 0.01$), standing leaf diameter from week 10th to week 16th ($r = 0.743 - 0.805$; $p < 0.01$), fresh weight of floating leaf ($r = 0.793$; $p < 0.01$), dry weight of floating leaves ($r = 0.793$; $p < 0.01$), fresh weight of standing leaf ($r = 0.722$, $p < 0{,}01$), and dry weight of standing leaf ($r = 0.735$; $p < 0{,}01$). The relationship between the fresh weight of floating leaves and floating leaf diameter from week 10th to week 14th showed a strong positive correlation (r = 0.800 – 0.835; p < 0,01). Meanwhile, the fresh weight of standing leaves was strongly correlated with the diameter of standing leaves at the 8th, 10th, and 12th week (r = 0.856; 0.902; 0.889; p < 0.01).

According to Pearson correlation research and data collected, it is important to enhance yield by ensuring the growth of floating leaves from week 6th to week 14th, standing leaf from 8th to 16th week after planting. Therefore, we suggested that the application of AgNPs could be considered as a solution for promoting the growth's leaves, increasing biomass accumulation, and preventing the development of harmful pests and diseases in these stages.

Parameter	Chla	Chlb	DF1w	DF2w	DF4w	DF6w	DF8w	DF10w	DF12w	DF14w	DS4w	DS6w	DS8w	DS10w	DS12w	DS14w	DS16w	FWF	DWF	FWS	DWS	HSL	HSF
Chla	1																						
Chlb	0.596**	1																					
DF1w	0.471*	0,108	1																				
DF2w	0,283	0,295	0,469	1																			
DF4w	0.728**	0.544*	0.483**	0.641**	1																		
DF6w	0.685**	0,428	0.401**	0.619**	0.806**	1																	
DF8w	0.786**	0.54*	0.481**	0.578**	0.801**	0.816**	1																
DF10w	0.714**	0.51*	0.471**	0.611**	0.807**	0.757**	0.778**	1															
DF12w	0.804**	0.538*	0.387**	0.604**	0.759**	0.758**	0.791**	0.866**	1														
DF14w	0.824**	0.647**	0.481**	0.571**	0.744**	0.763**	0.792**	0.823**	0.845**	1													
DS4w	-0,32	-0,81	0,048	0,146	0,107	0,085	0,188	0,12	0,146	0,15	1												
DS6w	0,426	0,56	0.327**	0.401**	0.458**	0.451**	0.500**	0.510**	0.557**	0.455**	0,208	1											
DS8w	0.671**	0.468*	0,18	0.511**	0.703**	0.764**	0.732**	0.716**	0.773**	0.711**	0,124	0,355	1										
DS10w	0.743**	0.583**	0.374**	0.552**	0.821**	0.795**	0.858**	0.780**	0.806**	0.834**	0,191	0.404**	0.802**	1									
DS12w	0.734**	0.465*	0.372**	0.527**	0.762**	0.774**	0.823**	0.774**	0.824**	0.787**	0,166	0.430**	0.783**	0.879**	1								
DS14w	0.805**	0.515*	0.422**	0.591**	0.836**	0.803**	0.850**	0.837**	0.840**	0.818**	0,183	0.503**	0.796**	0.840**	0.823**	1							
DS16w	0.781**	0.511*	0.486**	0.645**	0.802**	0.764**	0.832**	0.789**	0.831**	0.816**	0,195	0.506**	0.746**	0.809**	0.784**	0.938**	1						
FWF	0.793**	0.651**	0.51*	0.509*	0.731**	0.733**	0.788**	0.800**	0.817**	0.835**	-0,148	0,357	0.607**	0.678**	0.783**	0.744**	0.748**	1					
DWF	0.893**	0.577**	0.524*	0.456*	0.751**	0.839**	0.832**	0.854**	0.863**	0.904**	-0,24	0,246	0.807**	0.805**	0.846**	0.858**	0.852**	0.834**	1				
FWS	0.722**	0.493*	0,389	0,091	0.712**	0.782**	0.834**	0.747**	0.741**	0.797**	0,047	0,197	0.856**	0.902**	0.889**	0.890**	0.885**	0.628**	0.800**	1			
DWS	0.735**	0.703**	0,395	0,365	0.712**	0.681**	0.737**	0.724**	0.728**	0.794**	0,006	0,286	0.828**	0.872**	0.855**	0.821**	0.777**	0.732**	0.786**	0.842**	1		
HSL	0,391	0.478*	0.431**	0.584**	0.660**	0.650**	0.705**	0.758**	0.725**	0.710**	0.287**	0.508**	0.610**	0.721**	0.697**	0.728**	0.697**	0,402	0,356	0,277	0.585**	1	
HSF	0.51*	0.547*	0.283*	0.561**	0.635**	0.614**	0.622**	0.708**	0.696**	0.682**	0.229*	0.469**	0.633**	0.688**	0.688**	0.673**	0.655**	0.512*	0.734**	0.719**	0.726**	0.717**	1

Figure 3. Pearson's correlation coefficients among growth parameters of lotus (*Nelumbo nucifera* Gaertn.) affected by different methods AgNPs treatments. * p <0.05, **p <0.01.

4. Conclusions

In this field experiment, the three silver nanoparticle treatments significantly affected lotus leaves' growth and development compared with the control. T3 treatment had the best effects, enhanced both the diameter of floating leaf and standing leaf, fresh weight, and dry weight of leaf. From the perspective of biochemical characteristics, exposed silver nanoparticles induced higher chlorophyll content and levels of antioxidant enzymatic activities. The efficacy of the T3 proved that the integration of soil application, planting treatment, and periodic foliar treatment with AgNPs 4mg/L is highly effective for increasing the plant growth of lotus. Our study findings suggest further understanding the interactions between silver nanoparticles and plants through growth and physicochemical parameters. The current use of silver nanoparticles in agricultural sciences opens up the possibility of studying their effects on the different plants in the future to enhance quality and yield.

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