

USING *ARACHIS PINTOI* LEAF EXTRACTS IN BIOSYNTHESIS OF SILVER NANOPARTICLES FOR IMPROVING THE VASE LIFE OF CUT CARNATION *DIANTHUS CARYOPHYLLUS* L.

Tran Lap Xuan, Le Ba Le, Le Thi Anh Tu[✉]

Da Lat University, Lam Dong, Viet Nam

[✉]To whom correspondence should be addressed. E-mail: tulta@dlu.edu.vn

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SUMMARY

A biological method for synthesizing silver nanoparticles (SNPs) using the leaf extracts of *Arachis pintoii* Krapov. & W.C. Greg. was developed. The optimum conditions of input materials were found with leaf autoclaving in 15 min, 20 g fresh leave, and 4 mM of silver nitrate (AgNO₃). To study the role of time, temperature, and solution pH of the reaction, varying time reaction (5, 30, 60, 90, 120, 150, and 180 min), temperature reaction (10, 20, 30, 40, and 50°C) and pH of the solution (1, 3, 5, 7, 9, and 11) were investigated. The optimal biosynthesis conditions were achieved in 180 min of reaction time at 50°C and pH 11. The obtained nanoparticles have spherical and oblong in shape with average size of 26.4 nm. The SNPs in 4 concentrations (5, 15, 25, and 35 ppm) combined with and without 2% sucrose extended vase life, enlarged flower diameter, and maintained increase the relative fresh weight with vase solution uptake rate. SNPs inhibited significantly the bacterial growth in the stem end and vase solution, reduced the blockage in stems and therefore promoted the postharvest quality of carnation cut flowers. Out of the treatments, administration of 5 ppm SNPs with 2% sucrose of vase solution gave the best results for all parameters. The biosynthesis SNPs could be applied as a promising preservative solution for carnation cut flowers.

Keywords: *Arachis pintoii*, biosynthesis, carnation, cut flowers, preservative, silver nanoparticles

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is one of the most popular cut flowers in the world. It's a member of the family Caryophyllaceae and belongs to the genus *Dianthus* (Galbally, Galbally, 1997; Jurgens *et al.*, 2003). Carnations own the excellent quality, wide range of colors, forms, ability to withstand long distance transportation and rehydrate after continuous shipping (Basavaraj, Hermla, 2014). The balance between water uptake and water loss determine the quality and longevity of cut flowers (Lu *et al.*, 2010a; Da Silva, 2003; He *et al.*, 2006). There are three types of stem end blockage including physical air emboli (Van Meeteren, 2006), physical wound -induced (Williamson *et al.*, 2002; He *et al.*, 2006; Loubaud, Van Doorn, 2004), and microbial contamination (Liu *et al.*, 2009b; Put, 1990). Microbes can also secrete extracellular virulence factors including enzymes, hormones or toxic compounds (Buttner, Bonas, 2010; Salmond, 1994)

produce ethylene (Williamson *et al.*, 2002) to accelerate senescence. Vascular occlusions and associated wilting in cut carnation (*Dianthus caryophyllus* L.) usually occurred when the number of bacteria in vase water reached 10^7 - 10^{11} colony forming units (CFU/ml) (Edrisi *et al.*, 2011; Rahman *et al.*, 2012).

There is a need to extend vase life in the economically significant cut flowers. One of the proper ways to extend the vase life of cut flowers is to treat them with various chemicals instantly after harvest such as silver thiosulphate (STS) (Nowak, Rudnicki, 1990), gibberellins (GA) (Hamidimoghadam *et al.*, 2014) and cytokinins (CK), accel (BA+GA4+7) (Mutui *et al.*, 2001) and thidiazuron (TDZ) (Ferrante *et al.*, 2002). Supply of chemicals associated with alleviation of bacterial accumulation at the stems (Hamed *et al.*, 2013; Van Doorn *et al.*, 1991) and inhibition of suberin synthesis (Williamson *et al.*, 2002), then increased

the vase life. A drawback of this supply is the high concentrations that lead to the phytotoxic (Nell, 1992; Van Doorn, 2012). As the novel antiseptic, silver nanoparticles (SNPs) have become common use in pharmaceuticals, cosmetics, textile, water purification, and vegetable disinfection applications (Jiang *et al.*, 2006; Patra, Baek, 2017; Usha, Rajasekharreddy, 2011). Morones *et al.* (2005) reported that SNPs can kill 650 species of bacteria in water. SNPs caused the decrease of cell membrane permeability by releasing mono valent silver ions (Ag^+) which replace the hydrogen cation (H^+) of sulfhydryl or thiol groups (-SH) on surface proteins in bacterial cell membranes (Feng *et al.*, 2000). SNPs have been an effective antimicrobial agent (Solgi *et al.*, 2009; Liu *et al.*, 2009a), an ethylene inhibitor (Kim *et al.*, 2005) and/or a regulator of stomatal aperture (Lu *et al.*, 2010b). SNPs inhibited the growth of bacteria-related blockage in cut roses (Li *et al.*, 2012; Lu *et al.*, 2010b; Ohkawa *et al.*, 1999), cut gerbera flowers and cut *Acacia holosericea* (Liu *et al.*, 2009b) and extended their vase life.

There have been some reports on treatments of SNPs to prolong the vase life of carnation cut flowers (Hamidinoghadam *et al.*, 2014; Hashemabadi *et al.*, 2014). However, most of the study uses the commercial SNPs or chemically synthesized particles. A little has been reported on SNPs synthesized with plant extracts on the vase life (Solgi *et al.*, 2011). The biosynthesis of SNPs from plant extracts has been reported to be economically efficiency and nontoxic to the environment (Yasin *et al.*, 2013). SNPs can be produced at low concentration of variety of leaf extracts without using any additional harmful chemical/physical methods (Ahmed *et al.*, 2015) such as particularly neem leaf broth (*Azadirachta indica*), *Pelargonium graveolens*, geranium leaves, *Medicago sativa* (Alfalfa), *Aloe vera*, and *Embllica officinalis* (Amla, Indian Gooseberry) (Hamed *et al.*, 2013). Till date, no study was reported for the synthesis of SNPs using the Pinto peanut (*Arachis pintoii* Krapov. & W.C. Greg.)

Arachis pintoii Krapov. & W.C. Greg. is grown as a permanent pasture in intensive grazing systems and in very shaded situations under plantation crops where annual rainfall is above 1100 mm. It is cultivated as a ground cover or as an ornament and improving soils and degraded pastures (Cab Jiménez *et al.*, 2008; Cook *et al.*, 2005). The presence of

flavonoids, tannin, and phytosterol has been detected in *Arachis* species extracts (Grosso *et al.*, 2000; Lopes *et al.*, 2011). These phytocompounds play a role of reduction of silver ions into silver nanoparticles and stabilizing SNPs to prevent agglomeration (Medda *et al.*, 2015; Raja *et al.*, 2017; Yugandhar *et al.*, 2016). Many study revealed that the extract from the leaves have high antioxidant capacity and antibacterial activity (De Sousa-Machado *et al.*, 2018; Sang *et al.*, 2014). The aim of this study was to investigate the *Arachis pintoii* leaf extracts-mediated biosynthesis of SNPs and their effects on some bacteria which are involving in decreasing the flower longevity in order to find the proper method to extend the vase life and keep postharvest quality of carnation cut flowers.

MATERIALS AND METHODS

Materials

The fresh leaves of *Arachis pintoii* Krapov. & W.C. Greg. were collected from Dalat, Lamdong, Vietnam. The leaves were washed thoroughly 3 times with tap water and twice with sterile water. The fresh leaves were dried at room temperature and finely ground for synthesis SNPs. The voucher specimen is available from the resoures unit of Herbarium of Dalat Univeristy, Lamdong, Vietnam.

The carnation (*Dianthus caryophyllus* L.) cut flowers were obtained from a commercial greenhouse at their optimum developmental stage with the uniformity size, color and lack of defects. Flowers were placed immediately in water bucket, covered with a plastic film and transported to the laboratory. Stems were re-trimmed to a length of 20 cm (under deionized water). The experiments were carried out at the same day.

Synthesis of SNPs

Fresh leaves of *A. pintoii* were finely ground and added to 200 mL of distilled water. The different methods to prepare leaf extracts including heating at the different time period of 5, 10, 15, 20, 25 min at 60°C with continuous stirring or autoclaving at 121 °C at 15 lbs psi for 15 min. The mixture was cooled down and then filtered with the Whatman paper number 1. Filtrate was collected. The varied initial weight of fresh leave on synthesis of SNPs (5, 10, 15, 20, 25 g) were prepared. Effect of time on the biosynthesis of SNPs under the optimum conditions (including the initial weight of fresh leave and the method to extraction) was measured at the different

time interval of 5, 30, 60, 90, 120, 150 and 180 min. The efficiency of the bio-synthesis also was investigated under various conditions including changing AgNO₃ concentration (1, 2, 3, 4 mM), the pH (1, 3, 5, 7, 9, 11), and temperature of the reaction (5, 20, 30, 40, and 50°C).

The silver ions reduced to SNPs can be observed by the gradual change in color of the solution. The final reaction mixture was purified by centrifugation at 9000 rpm for 30 min. Supernatants were discarded and the pellet was redispersed in de-ionized water to eliminate any contaminating plant material before centrifuge at 9000 rpm for 60 min. This wash step was repeated twice to remove water-soluble biological residues. The pellet was dried at 37°C for 24 h to determine the dry mass of SNPs for yield analysis. The relationship between the corresponding the dry mass and the volume of mixture of the SNPs synthesized at the optimum conditions were determined.

Characterization of SNPs

The bio-reduction of SNPs was determined using UV-Vis spectrophotometer. The absorbance spectrum of the sample was obtained in the range of 400 - 700 nm wavelength, using a UV-Vis spectrometer (Specord 200 plus, Jena, Germany) with distilled water as a reference. SNPs prepared under optimal conditions were centrifuged at 10000 rpm for 20 min and the pellet was collected, freeze-dried to obtain a dried powder which was subjected to study the size and shape of the nanoparticles by a transmission electron microscope (TEM). TEM measurements were done by JEOL JEM-1010, operating at 100 kV. The TEM grid was prepared by placing a drop of the bio-reduced diluted solution on a carbon-coated copper grid and later drying it under a lamp.

Effects of SNPs on vase life

The flowers were placed in the bottles containing 100 mL of preservative solution SNPs. The mouths of bottles were covered with non-absorption cotton to minimize evaporation loss and prevent contamination. Solution contains the following treatments and remains until the end of vase life: T1: SNPs of 5, 15, 25, 35 ppm, T2: SNPs of 5, 15, 25, 35 ppm + sucrose 2%, T3: Water (filtered through the membrane filter with the pore size of 0.2 μm) - the control, T4: Water (filtered through the membrane filter with the pore size of 0.2 μm) + sucrose 2%.

Measurement

Vase life: Vase life was considered to have ended when visible of 20% petal color fading

Vase solution uptake: Vase solution uptake was calculated in the vase containing 100 mL of solution after 10 days. The weights of vase without the cut flowers were recorded after 10 days. The average of 5 replication values was used. The vase solution uptake after 10 days was calculated by the formula:

$$WU_{10} \text{ (g/stem)} = (S_1 - S_{10}) - (S_{1c} - S_{10c})$$

Where S₁ is the weight vase of solution (g) at the initial day, S₁₀ is the weight of vase solution (g) on the 10th day, S_{1c} is the weight vase of solution (g) without flowers at the initial day, and S_{10c} is the weight of vase solution (g) without flowers on the 10th day (He *et al.*, 2006).

Relative fresh weight: The relative fresh water (RFW) of cut flowers was calculated using the following formula:

$$\text{RFW (\%)} = (FW_t / FW_0) \times 100$$

Where FW_t is the fresh weight of stem (g) at t = days 0, 1, 2, etc., and FW₀ is the fresh weight of stem (g) at t = day 0 (He *et al.*, 2006).

Flower diameter: The flower diameter was measured as an index for blossom expanding rate. The outer diameter of opened flowers was measured by a caliper in millimeter.

Bacterial counts: Bacterial solution populations were determined by spread the aliquots of vase solutions on LB agar and incubated at 30°C for 48 hours (Liu *et al.*, 2009b) to count the total colony. To determine bacterial population in the stem-end, 3 cm long stem-end segments were trimmed, washed with distilled water twice and chopped to small pieces with sterile secateurs. These pieces were placed in the sterilized tube containing 1 mL of sterile 0.9% saline and vortexed in 1 min. The aliquot of the extracts was spread onto LB agar plates. Bacterial colonies were determined as described above.

Statistical analysis

One-way analysis of variance (ANOVA) and t-test were performed using Excel 2011 statistical tools. A P-value < 0.05 was used as a criterion for significance level. ANOVA was used to determine whether bactericidal activity of SNPs and

preservative ability from the different conditions (concentrations and with/without sucrose) are statistical different.

RESULTS AND DISCUSSION

Effect of methods to prepare leaf extracts on synthesis of SNPs

The bio-reduction of silver ions to SNPs was optically approved by color changes to yellow or brown (Rani *et al.*, 2011). The formation of colloidal SNPs was monitored by measuring the UV-Vis spectrum that showed strong evidence of colloidal metal particle formation, and the productivity growth in the synthesis medium was indicated by the gradual increase in the absorbance values (Bogireddy *et al.*, 2016). The different methods to prepare leaf extracts were optimized for biosynthesizing SNPs including heating at 60°C and autoclaving at 121 °C at 15 lbs psi (Figure 1). The

absorption observed at the range of 433 - 436 nm in UV-Vis spectrum which falls between a typical SPR band of spherical SNPs (400 - 450 nm) for all treatments. No other measurable peak was observed in the spectrum which confirms that the synthesized products are SNPs only. Appearance of this peak, assigned to a surface plasmon, is well-documented for various metal nanoparticles with size less than 100 nm (Henglein, 1993). The absorbance increased with the increasing heating time to autoclaving treating.

The color of the solution changed from yellow to brownish color on increasing heating time to autoclaving treating due to the number of Ag^+ ions that have been reduced to zero-valent Ag^0 atoms and the number of SNPs of smaller sizes increased (Saion *et al.*, 2013). The absorption intensity demonstrated that autoclaving treating yielded a larger amount of SNPs.

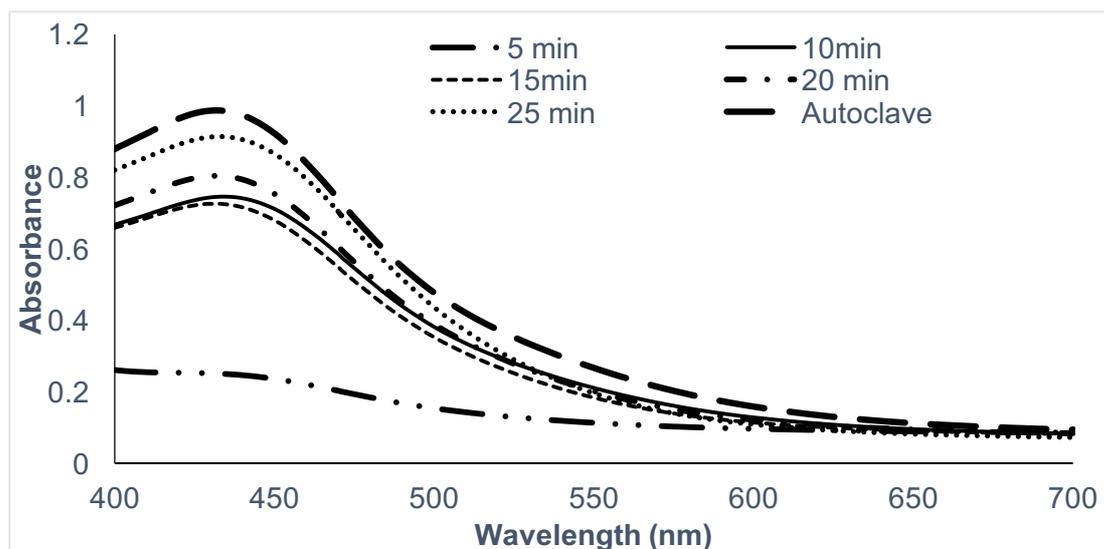


Figure 1. Effects of the heating methods of 5, 10, 15, 20 and 25 min at 60°C and autoclaving at 121 °C at 15 lbs psi on synthesis of SNPs using UV-Vis spectroscopy measurement.

Effect of the initial weight of fresh leave on synthesis of SNPs

The UV-Vis spectroscopy is a sensitive method to detect the formation of the SNPs (Gao *et al.*, 2016; Kim *et al.*, 2003). The different initial fresh leaf weight to prepare leaf extracts was also characterized for biosynthesis. The peak positions of the surface plasmon resonances (SPRs) were 436, 434, 439, 437 and 438 nm of wavelength and the maximum absorbance were 0.6112, 0.7874, 1.5801,

1.6765, and 1.4947 in the experiments of 5, 10, 15, 20 and 25 g fresh leave for extraction, respectively. The formation of SNPs was confirmed by the SPRs in the range of 350 nm to 600 nm (Henglein, 1993). No SRPs at more than 500 nm in Figure 2 indicated that most obtained SNPs have small size and similar shape (Saion *et al.*, 2013). Birla *et al.* (2013) reported that the intensity of the plasmon peak increased with the increasing concentration of SNPs. The similar trend was observed by Mukherjee *et al.*

(2008). Figure 2 shows a gradual increase in the absorption intensity when increasing the initial weight of fresh leave. The localised SPR peak is

more pronounced for SNPs prepared using 20 g fresh leave.

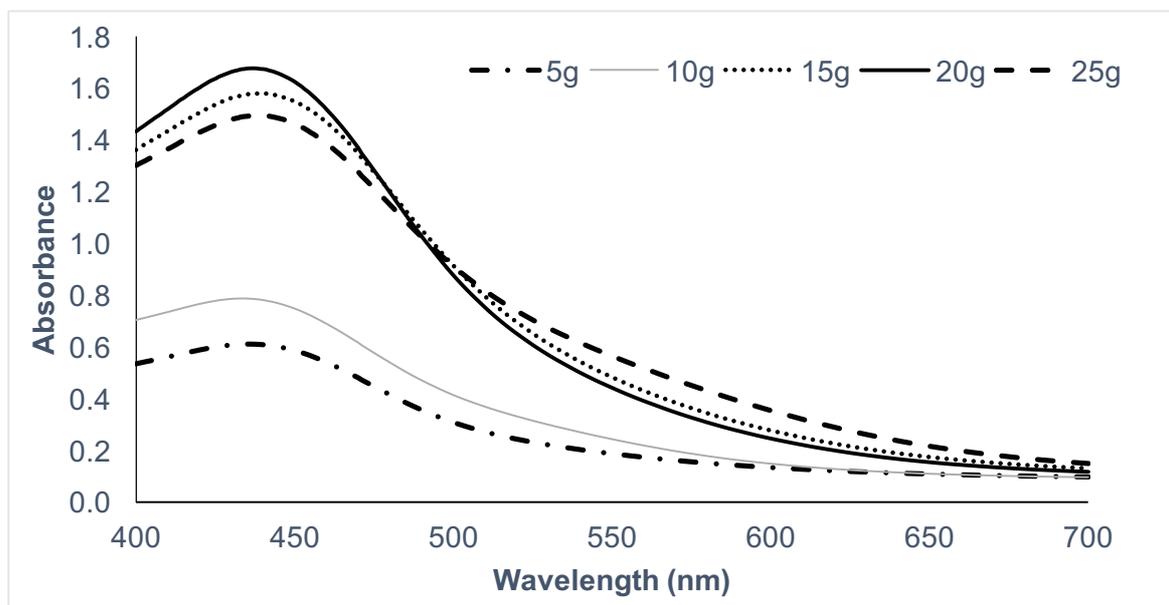


Figure 2. Effects of the initial weight of 5, 10, 15, 20 and 25 g fresh *A. pintoii* leave on synthesis of SNPs using UV-Vis spectroscopy measurement.

Effect of time on synthesis of SNPs

Figure 3 depicts the UV-Vis spectra of SNPs with a range of the time required for the completion of the reaction. Change in color of the reaction mixture was observed after 5 min. Accordingly, all the spectra except for 5 min duration have an intense peak ranging from 434 to 470 nm. The absorption peak blue shifted toward the lower wavelength with increasing the time reaction to decrease particle size (Saion *et al.*, 2013). During the whole reaction process, the shift of the peak positions and the shape of the absorption spectra show that at the beginning a small volume of small size particles were formed and then the small particles aggregated to the large particles. After 120 min the large particles decomposed to the small ones. The optimum time for the reaction from this study is 180 min and no further change in color was observed after this time (Figure 3). Dinesh *et al.* (2012) reported the completion reduction of AgNO_3 using the aqueous park extracts of *H. antidysenterica* after 120 min, while the reduction reaction of *Azadirachta indica*

leaf extracts with AgNO_3 completed after half of hour (Shankar *et al.*, 2004). This also shows that the plant species had significant influences on the time reaction to synthesize SNPs.

Effect of the metal ion concentrations on synthesis of SNPs

The effect of metal ion concentrations was investigated by varying AgNO_3 concentrations from 0.5 to 4 mM. The change of the mixture to brownish color was found in all experiments. At the low silver ion concentration, the absorbance was less than the higher metal ion concentrations (Figure 4). Sharp plasmon peaks displayed in the Figure 4 is observed from 430 nm to 453 nm, as expected for SNPs. The UV-Vis spectra indicated that increasing AgNO_3 concentrations led to form more SNPs.

Effect of pH on synthesis of SNPs

pH is one factor which affects the synthesis of SNPs. Change of the pH of the reaction mixture affects the charge of biomolecules which may alter the formation and stability of SNPs (Verma, Mehata, 2016). The peak position and intensity on varying the

pH of the solution show in the Figure 5. At low pH (pH 1), a flat UV-Vis spectrum was observed. When pH increased from 3 to 7, absorption peaks increased. The UV-Vis spectra at pH 3, 5, 7, 9, and 11 were 442 nm, 434 nm, 432 nm, 434 nm, and 438 nm, respectively. As the pH increased from 3 to 7, the size of SNPs decreased. However, when increase the pH from 9 to 11, the energy required for excitation of surface plasmon electrons decreases, thus the absorption maximum shifted towards the longer wavelength regions. This was the evidence that the size of the SNPs increased when the pH shifted from 9 to 11. Gane *et al.* (2012) demonstrated that a large number of functional groups in lightly acidic pH mixture enhances the number of Ag ions to bind and then formed a larger number of SNPs with smaller diameters. The negative ions in a basic medium amplify the reduction of Ag^+ into SNPs. The diffusion between adjacent adsorption sites on a

surface of Ag atoms at a higher ion density increases and lead to form bond with nearest neighbor atoms (Ball *et al.*, 1987). This leads to form the SNPs with a larger size. However, there was a lightly different in the absorption peak.

The absorption intensity increased with increasing pH from 3 to 11. At the pH 11, the highest absorption intensity indicated that a largest number of SNPs were synthesis. Thus, pH 11 was the optimum pH for biosynthesis of SNPs from *A. pinto* leaf extracts to obtain the appropriate SNPs in term of size and yield for later application. Khalil *et al.* (2013) reported that the absorbance of the SNPs synthesized from olive leaf extracts increased with the increasing pH from 2 to 8 while Vanaja *et al.* (2012) stated that the alkaline pH was more favorable for forming SNPs. The inconsistency may be the results of the leave resources and conditions to synthesis.

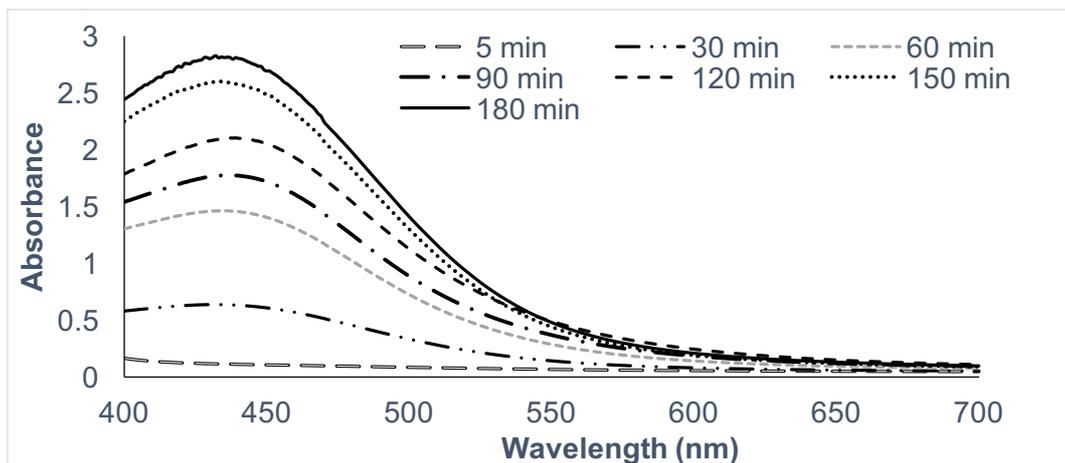


Figure 3. Effects of the time reaction of 5, 30, 60, 90, 120, 150 and 180 min on synthesis of SNPs using UV-Vis spectroscopy measurement.

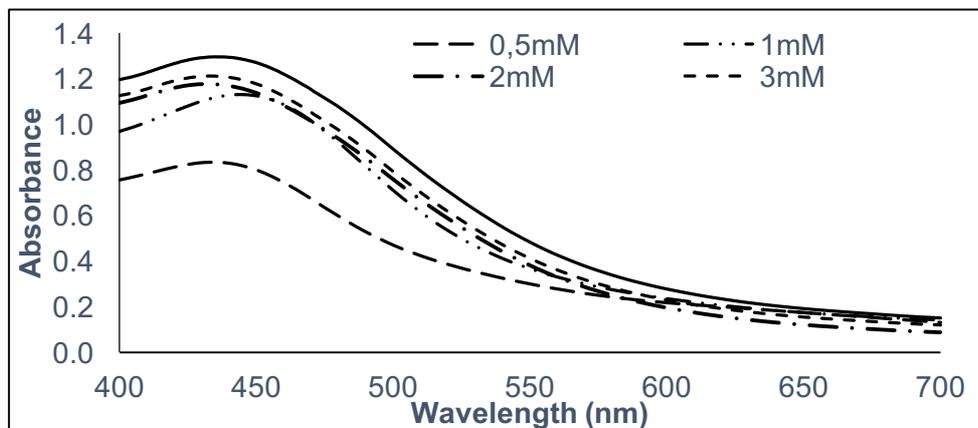


Figure 4. Effect of metal ion concentrations (0.5, 1, 2, 3 and 4 mM) on synthesis of SNPs using UV-Vis spectroscopy measurement.

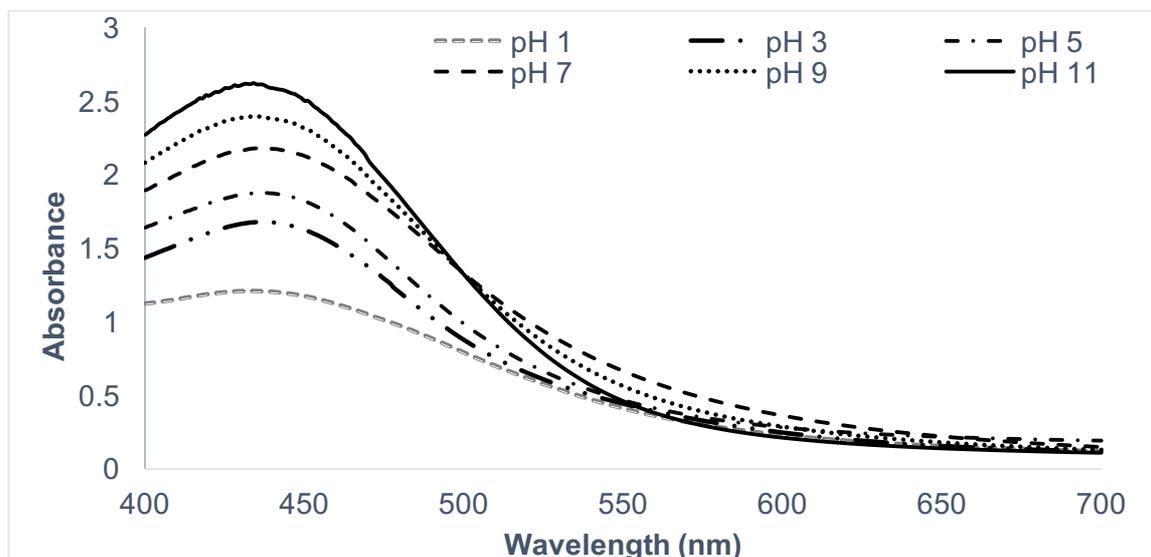


Figure 5. Effect of pH of 1, 3, 5, 7, 9, and 11 on synthesis of SNPs using UV-Vis spectroscopy measurement.

Effect of temperature on synthesis of SNPs

Another parameter which affects the formation of SNPs is the temperature. The absorption peaks of the SNPs prepared at different temperatures (10 to 50°C) while keeping the above optimum conditions constant display in Figure 6. The absorption peaks observed at 10°C to 50°C shifted toward blue from 451 to 426 nm. UV-Vis spectra shifted toward lower wavelength as the temperature increased as the results of the formation of smaller size SNPs. As the increase the temperature, the increasing kinetic energy of the molecules may lead to the consuming silver ions faster, and then smaller particles of the uniform size are formed (Verma, Mehata, 2016). The rapid nucleation process of metallic nanoparticles involving the enhanced consumption of the metal ions with least secondary reduction of the preformed nuclei tends to occur at the high reaction temperature (Dwivedi, Gopal, 2010). This results are agreement with the previous studies (Verma, Mehata, 2016; Ibrahim, 2015; Dinesh *et al.*, 2012; Traiwatcharanon *et al.*, 2017). Further, the higher temperature enhanced the rate of the biosynthesis as the color change was observed after few minutes when AgNO₃ mixed with the leaf extracts.

Yield analysis

The concentration of SNPs synthesized at the optimum conditions was determined on a mg/ml from the final reaction mixture. The final reaction

mixture volume is linearly related to the yield of the SNPs dry mass with $R^2 = 0.9893$. The yield of bio-synthesis SNPs at the optimum conditions was 0.5195 mg/mL.

Morphology of SNPs

Transmission electron microscopy (TEM) technique was used to visualize the size and shape of synthesized SNPs through leaf extracts of *A. pinto* under optimum conditions. The TEM image reveals a mixture of small and bigger synthesized SNPs (Figure 8A). The biosynthesized SNPs were spherical and oblong in shape. The size of particles distributed in the range of 5 to 65 nm with the average particle diameter of 26.4 nm (Figure 8B).

Vase life

Results in Table 2 show that all SNP treatments enhanced the vase life of carnation flowers ($P \leq 0.05$). With the treatments without sucrose, the 5 ppm SNP treatment gave a significant difference compared to the control (filtered water). Comparing the treatments with only SNPs, the 35 ppm SNP treatment had the longest vase life (16.8 days). Increasing SNP concentrations led to prolong the vase life of carnation cut flowers. This result is in agreement with the previous studies on other cut flowers such as Polianthes tuberosa (Hutchinson *et al.*, 2004), 'Cherry Brandy' rose (Jowkar *et al.*, 2013) and Tulipa spp. (Bowyer *et al.*, 2003). However, Carrillo-López *et al.* (2016) reported that,

the low concentrations of SNPs favored for vase life. The inconsistency may be the results of different types of SNPs including the size and shape.

The combination of SNPs with 2% sucrose increased the vase life. Sucrose is generally used to sustain metabolic activity. The efficacy of sucrose in prolonging vase life and delaying cut flower senescence has been reported for rose, lily, peony, sweet pea, orchid, and carnation (Ichimura, Suto, 1999; Chen *et al.*, 2001; Verlinden, Garcia, 2004; Hoerberichts *et al.*, 2007; Arrom, Munne-Bosch, 2012; Zhang *et al.*, 2012). The treatments of SNPs with 2% sucrose exhibited statistically significant

differences compared to the control. Safa *et al.* (2012) reported that SNPs have the potential to extend vase life and enhance the postharvest quality of cut *Gerbera* cv. "Balance" flowers. Nevertheless, there was no significant difference between all treatments with SNPs plus 2% sucrose in term of the vase life. The data shows that the efficiency of the solution included the antibacterial activity of SNPs and sustaining metabolic activity of sucrose. Hatami *et al.* (2013) revealed that SNPs plus sucrose significant extended the vase life because of increasing hydraulic conductance related to the high leaf water content.

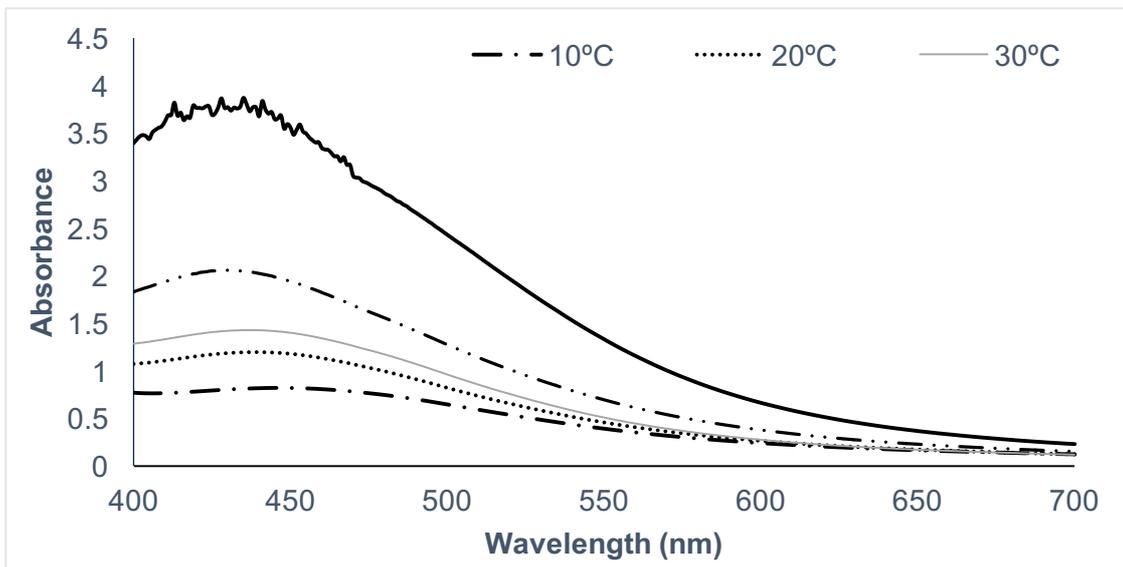


Figure 6. Effect of temperature (10, 20, 30, 40, and 50°C) on synthesis of SNPs using UV-Vis spectroscopy measurement.

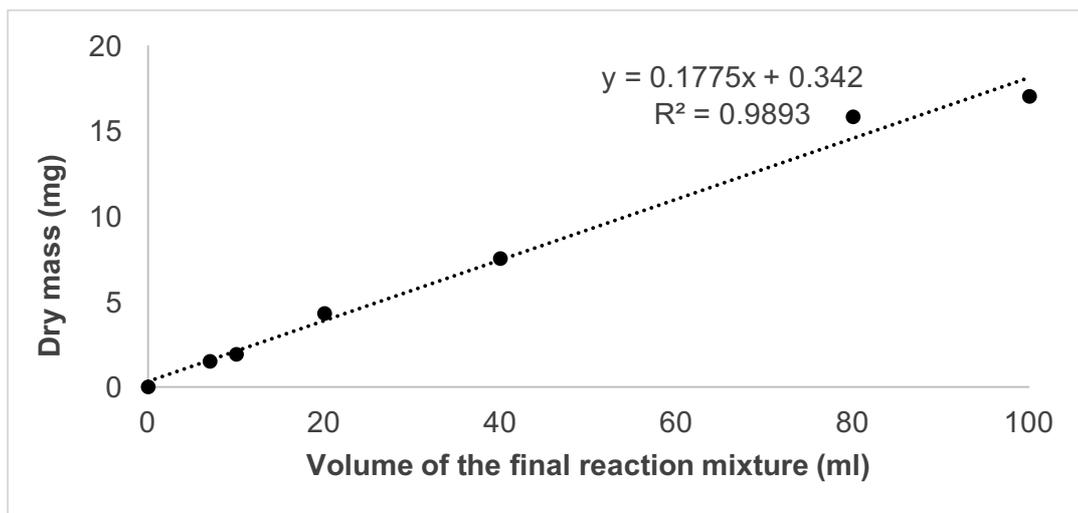


Figure 7. Relationship between the volume of the final reaction mixture and SNPs dry mass.

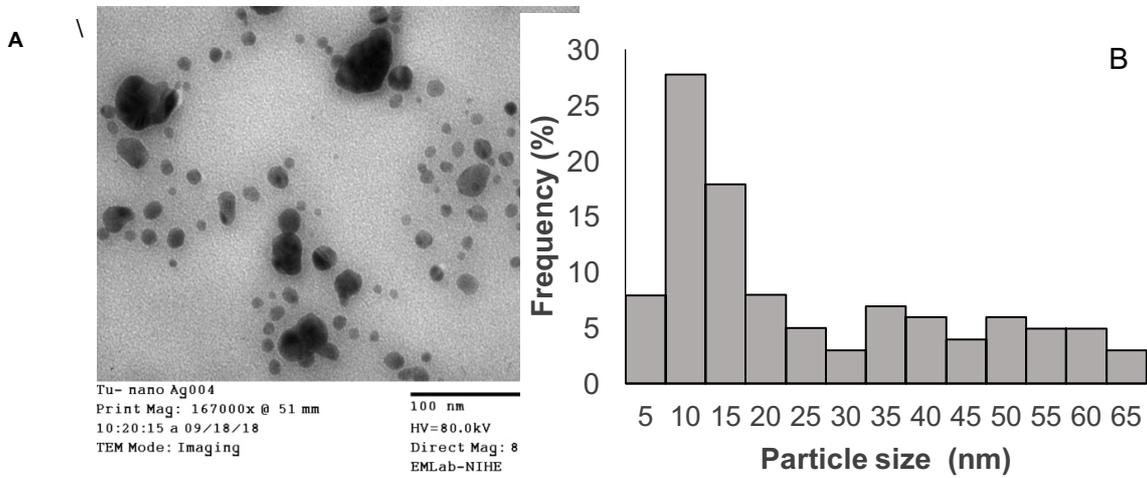


Figure 8. (A) TEM image of the SNPs; (B) Particle size distribution histogram of SNPs.

Table 3. Effect of different treatments of SNPs on vase life.

Treatments	Vase life (days)	Treatments	Vase life (days)
Filtered water	9 ± 0.3 a	Filtered water + Sucrose 2%	11 ± 0.2 f
5 ppm SNPs	12.4 ± 0.2 b	5 ppm SNPs + Sucrose 2%	19 ± 0.2 g
15 ppm SNPs	13.6 ± 0.3 c	15 ppm SNPs + Sucrose 2%	19 ± 0.1 g
25 ppm SNPs	15.4 ± 0.1 d	25 ppm SNPs + Sucrose 2%	19 ± 0.1 g
35 ppm SNPs	16.8 ± 0.3 e	35 ppm SNPs + Sucrose 2%	19 ± 0.15 g

Relative fresh weight and water uptake

The relative fresh weight (RFW) increased at the beginning of the experiments, and later decreased (Figure 9). Similar patterns of changes were also reported for cut *Chrysanthemum* cv. Puma (Carrillo-Lopez *et al.*, 2016), cut rose (Lu *et al.*, 2010b; Alaei *et al.*, 2011). The control had significantly lower weight than treatments. The RFW of treatments of SNPs with sucrose were remained higher than that without sucrose and the control. Sucrose serves as a substrate for respiration and cell wall synthesis and it maintains water balance, all of which should prolong vase life (Carrillo-Lopez *et al.*, 2016; Weiss, 1997).

The antimicrobial effect of SNPs minimized vase life disorders. A variation in term of the evolution of the fresh weight was observed. The control and sucrose treatments gained the highest weight for the first 5 days, while treatments with 5 and 15ppm SNPs, 25 and 35 ppm SNPs, 5 and 15 ppm SNPs plus sucrose, 25 and 35 ppm SNPs plus

sucrose occupied the highest weight after 6, 7, 7, and 8 days, respectively. The highest relative fresh weight (122.6%) was obtained with the SNPs of 5 ppm plus sucrose at the 7th day. The relative fresh weight were 118.9%, 119.5%, and 120.8% for treatments of 2% sucrose with SNPs of 15, 25, 35 ppm, respectively.

The result of the water consumption after 10 days was displayed in the Figure 10 ($P \leq 0.05$). SNPs enhanced the absorption of vase solutions. There is significant difference between the water uptake of control and treatment of SNPs with or without sucrose. The water uptake increased when SNP concentrations increased in the experiments with only SNPs. The results are in good agreement with the study of Nemati *et al.* (2013). The introduction of SNPs as a senescence delaying compound and effective on the loss of fresh weight led to the positive impact on water uptake enhancement which ultimately keeps fresh weight.

However, the trend was different in the treatments with SNPs plus sucrose. The water uptake of treatments of 5, 15, 25, 35 ppm SNPs with sucrose was not significantly different. This result is in agreement with the results of the vase life. In addition, the xylem which is mainly responsible for water transport to the flowers were observed on the days 0

and 10 (Figure 11). The biofilm formation and bacterial blockage were observed at the xylem after 10 days of flowers in the filtered water. Meanwhile, very least bacteria were observed in the xylem vessel walls of flower stems treated with SNPs. There was no stem breakage for SNPs or the combination of sucrose with SNP treatments.

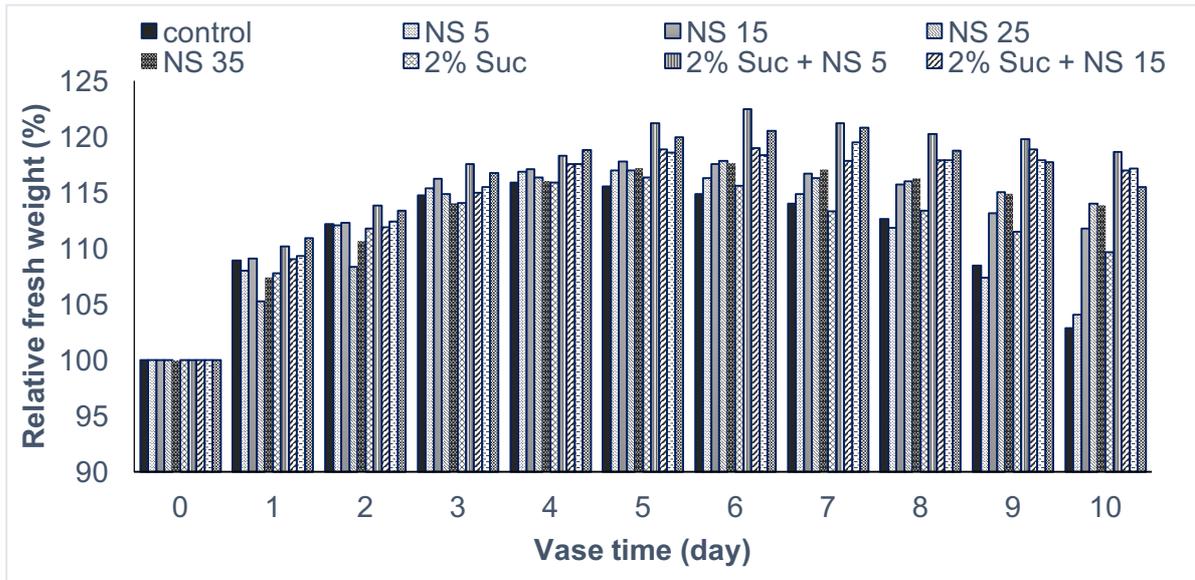


Figure 9. Relative fresh weight variation after 10 days of carnation vase life of the control (filtered water), the filtered water-added SNPs (5, 15, 25, and 35 ppm), and the filtered water-added SNPs (5, 15, 25, and 35 ppm) with 2% sucrose.

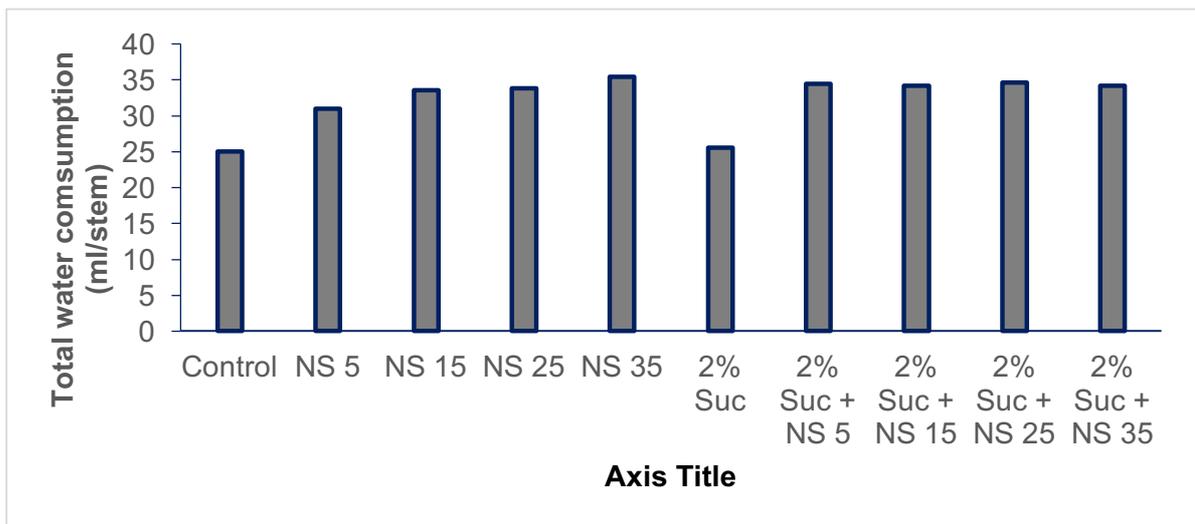


Figure 10. Vase solution uptake rate after 10 days of carnation vase life of the control (filtered water), filtered water added SNPs (5, 15, 25, and 35 ppm), filtered water-added SNPs (5, 15, 25, and 35 ppm) with 2% sucrose.

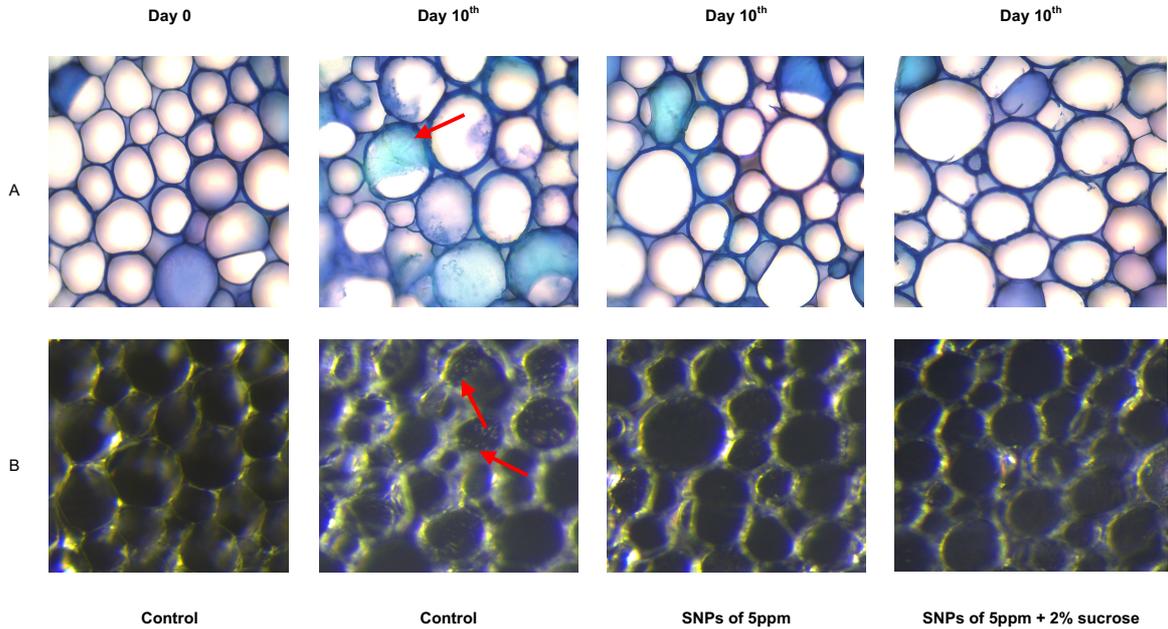


Figure 11. Morphology of the cut flower stem surface of carnation: (A) using optical microscope, (B) using stereo microscope, Red arrows: blockage.

Flower diameter

There were statistically significant differences in term of the diameter of inflorescences between the treatments with SNPs with the control ($P \leq 0.05$) (Figure 12). In this study, application SNPs with

sucrose improved the diameter of carnation cut flowers. It could be observed from the data that the low concentration of SNPs of 5 ppm with sucrose favored the inflorescence opening. When the SNP concentrations increased, the flower diameter declined. SNPs at the higher concentrations may have the toxicity effect on the inflorescence.

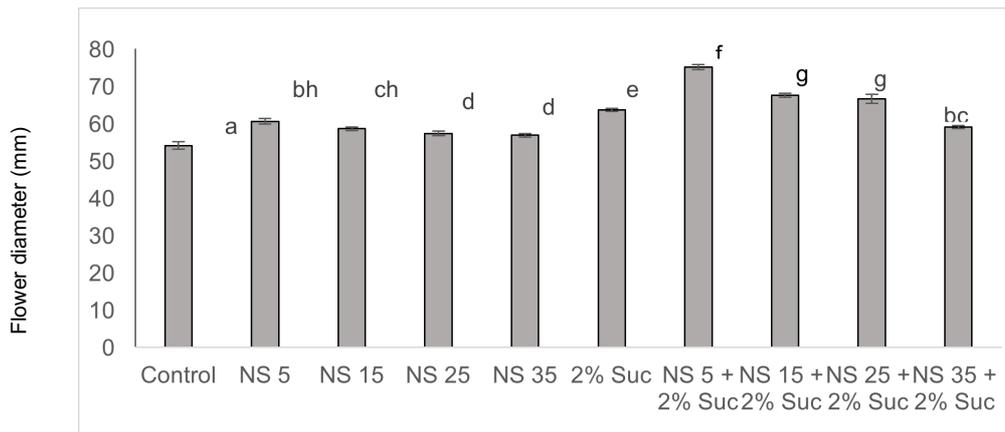


Figure 12. Effect of SNPs ($P \leq 0.05$) after 10 days on the inflorescence opening of carnation flowers of the control (filtered water), filtered water-added SNPs (5, 15, 25, and 35 ppm), filtered water-added SNPs (5, 15, 25, and 35 ppm) with 2% sucrose.

Many studies reported on the extending vase life ability of sucrose (Paulin, Jamain, 1982; Ichimura, Hiraya, 1999). Sucrose plays as an energy source (Moalem-Beno *et al.*, 1993), osmotic regulator (Bialeski *et al.*, 1993), thereby playing a role in flower opening and water balance regulation (Kuiper *et al.*, 1995). However, sucrose may promote the bacterial growth. Thus, vase solutions containing a carbohydrate supply like sucrose with an antimicrobial substance to maximize vase life is a promising method. When supplement sucrose with SNPs in the vase solutions, not only the vase time and relative fresh weight were improved, the flower diameters increased, as well. Nevertheless, when SNP concentrations increased, this efficacy altered. Even comparing to the control, all the treatments revealed a statistically greater in flower diameter, the consideration reduction in the flower diameter was observed when increased the SNP concentration from 5 to 15, 25, 35 ppm.

Bacterial counts

Bacteria in the vase solutions and in the basal 3 cm from the stem ends was investigated on the 10th

day. Significant difference in number of bacteria in the vase solutions and the stems was observed between the control and the SNP treatments with and without sucrose (Figure 13). The number of bacteria in treatment of sucrose only was the highest. This evidence proves that sucrose not only prolongs the vase life but also promotes the bacterial growth that inhibits the water uptake of cut flowers. Van-Doorn and Perik (1990) reported that bacteria in vase solutions and in stems was responsible for xylem occlusion, releasing toxic such as metabolites or enzymes, producing ethylene, thus led to inducing the senescence and shortening the vase life.

The lower bacteria in the vase solutions and stems suggested that supplement of SNPs inhibited the bacterial growth. SNPs caused cell damage, preventing cell division (Liu *et al.*, 2009, Morones *et al.*, 2005), and inhibiting the forming of biofilm (Ibrahim, 2015). Vascular occlusion has been caused by microbial proliferation (Van Doorn *et al.*, 1995). The antibacterial activity of SNPs is well established. In this study, SNPs inhibited the bacterial growth and therefore could prolong carnation cut flower longevity.

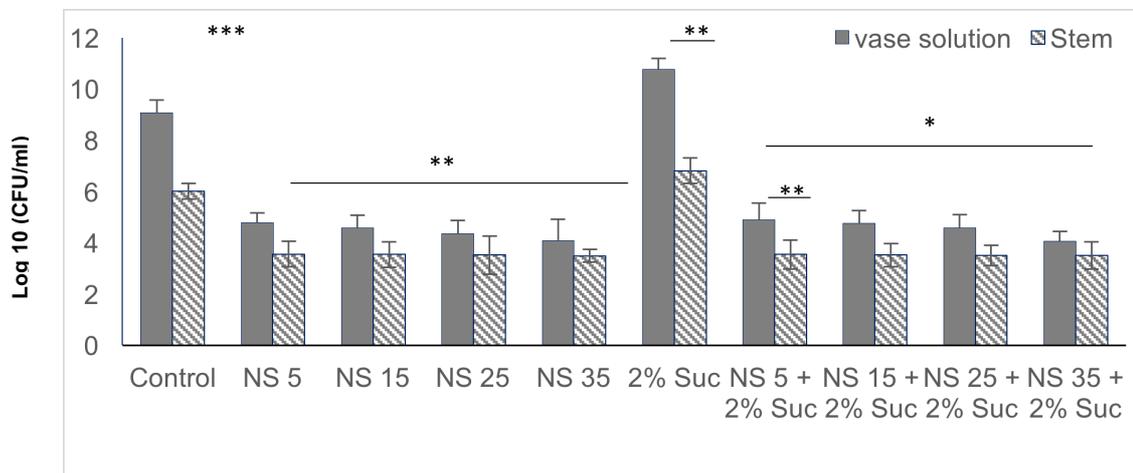


Figure 13. Changes for SNPs in number of bacteria after 10 days in the stem end and vase solutions of the control (filtered water), added SNPs (5, 15, 25, and 35 ppm), added SNPs (5, 15, 25, and 35 ppm) with 2% sucrose ; * P ≤ 0.05; ** P ≤ 0.01; ***P ≤ 0.001.

CONCLUSION

In conclusion, a simple and rapid procedure for bio-synthesis of SNPs through *A. pinto* extracts was developed. Various reaction conditions were

investigated to obtain the optimum reaction conditions including 180 min of reaction time at 50°C and pH 11. Bio-synthesis at the optimum conditions yielded 0.5195 mg/mL. The SNPs were spherical and oblong in shape. In addition, the

calculated particle size of the SNPs from the TEM histogram particles distribution ranged from 5 to 65 nm. Among the treatments, the combination of SNPs 5ppm applied with 2% sucrose was the best in term of vase life, maintaining the water uptake, relative fresh weight and also flower diameter. SNPs could be used as a promising preservative agent for improving the postharvest quality of carnation cut flowers. These results suggest the possibility of the use of SNPs as an antibacterial agent to made full advantages of sucrose in extending vase life.

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TỔNG HỢP NANO BẠC TỪ DỊCH CHIẾT LÁ CÂY CỎ ĐẬU *ARACHIS PINTOI* NHẪM NÂNG CAO CHẤT LƯỢNG SAU THU HOẠCH CỦA HOA CẨM CHƯỞNG (*DIANTHUS CARYOPHYLLUS* L.) CẮT CÀNH

Tran Lap Xuan, Le Ba Le, Le Thi Anh Tu

Đại học Đà Lạt, Tỉnh Lâm Đồng, Việt Nam

TÓM TẮT

Nano bạc được tổng hợp từ dịch chiết lá cây cỏ đậu *Arachis pintoi* Krapov. & W.C. Greg. Nguyên liệu đầu vào được chuẩn bị như sau: hấp 20g lá tươi ở 121°C, 15 psi, 15 phút, và sử dụng 4 mM bạc nitrat (AgNO₃). Điều kiện tổng hợp nano bạc được thử nghiệm với thời gian sinh tổng hợp (5, 30, 60, 90, 120, 150, và 180 phút), nhiệt độ phản ứng (10, 20, 30, 40, và 50°C) và pH của dung dịch (1, 3, 5, 7, 9, và 11). Kết quả cho thấy, hiệu quả tạo nano bạc tốt nhất tại 50°C, pH 11, sau 180 phút phản ứng sinh tổng hợp. Các hạt nano thu được có dạng hình cầu và hình thuôn dài với kích thước trung bình 26,4nm. Dung dịch nano bạc với các nồng độ 5, 15, 25 và 35 ppm có bổ sung hoặc không bổ sung 2% sucrose được thử nghiệm trên hoa cẩm chướng cắt cành cho thấy kéo dài thời gian tươi, đường kính hoa và tỷ lệ tăng trọng lượng tươi của cành hoa tương ứng với tốc độ hấp thụ dung dịch nước cắm hoa. Nano bạc đã ức chế đáng kể sự phát triển của vi khuẩn trong cành hoa và dung dịch cắm hoa, dẫn đến hạn chế sự tắc nghẽn trong cành hoa. Dung dịch cắm hoa chứa 5 ppm nano bạc có bổ sung 2% sucrose có hiệu quả tốt nhất trong bảo quản hoa cẩm chướng cắt cành sau thu hoạch. Kết quả ghi nhận, nano bạc sinh tổng hợp từ dịch chiết lá cây cỏ đậu có thể được sử dụng làm chất bảo quản cho hoa cẩm chướng cắt cành.

Từ khoá: *Arachis pintoi*, bảo quản, cẩm chướng cắt cành, nano bạc, sinh tổng hợp