A biological method for synthesizing silver nanoparticles (SNPs) using the leaf extracts of *Arachis pintoi* 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 pintoi*, 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, Hermila, 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 pharmaceutics, cosmetics, textile, water purification, and vegetable disinfection applications (Jiang et al., 2013; Patra, Baek, 2017; Usha, Rajasekarreddy, 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 sulphhydryl 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), Pelargarumum graveolens, geranium leaves, Medicago sativa (Alfalfa), Aloe vera, and Emblica officinalis (Amla, Indian Gooseberry) (Hamed et al., 2013). Till date, no study was reported for the synthesis of SNPs using the Pinto peanut (Arachis pintoi Krapov. & W.C. Greg.)

Arachis pintoi 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 Jimenez et al., 2008; Cook et al., 2005). The presence of flavonoids, tannin, and phytonsterol has been detected in Arachis species extracts (Grosso et al., 2000; Lopes et al., 2011). These phyto compounds 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 pintoi 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 pintoi 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 resources 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. pintoi 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 centrifuging 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 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 remain 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 \( S_1 \) is the initial weight of solution (g) and \( S_{10} \) is the weight of solution (g) on the 10th day, \( S_{1c} \) is the weight 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:

\[ RFW\% = \left( \frac{FW_t - FW_0}{FW_0} \right) \times 100 \]

Where \( FW_t \) is the fresh weight of stem (g) at \( t \) days 0, 1, 2, etc., and \( FW_0 \) is the fresh weight of stem (g) at \( t = \text{day 0} \) (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 seccateurs. 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 Ag0 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.

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.
more pronounced for SNPs prepared using 20 g fresh leaf.

Figure 2. Effects of the initial weight of 5, 10, 15, 20 and 25 g fresh A. pintoi 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 AgNO₃ using the aqueous park extracts of H. antidysenterica after 120 min, while the reduction reaction of Azadirachta indica leaf extracts with AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 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 slightly 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 inconsistence 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, Mehat, 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 biosynthesis 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. pintoi 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 tuberosus (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 inconsistence 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; Hoeberichts 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.
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; Alaey 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 15 ppm 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 ≤ 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 significant 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 ≤ 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 ≤ 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 (Bieleski 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.

**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 make full advantages of sucrose in extending vase life.

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Table 6: 111-119

TÔM TÀT

Nano bạc được tổng hợp từ dịch chiết lá cây cọ dầu 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 sự dùng 4 mM bac 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 đường kính trung bình 26.4nm. Dung dịch nano bạc với các nồng độ 5, 15, 25 và 35 ppm có bộ sung gốc không bộ sung 2% sucréose được thử nghiệm trên hoa cẩm chướng cát cánh cho thấy kéo dài thời gian tươi, đường kính hoa và tỷ lệ tăng trưởng lượng tử của cành hoa tương ứng với tốc độ hấp thụ dung dịch nước cẩm hoa. Nano bạc đã ức chế đáng kể sự phát triển của vi khuẩn trong cành hoa và dung dịch cẩm hoa, dẫn đến hạn chế sự tác nghiệm trong cành hoa. Dung dịch cẩm hoa chứa 5 ppm nano bạc có bộ sung 2% sucréose có hiệu quả tốt nhất trong bảo quản hoa cẩm chướng cát cánh sau thu hoạch. Kết quả ghi nhận, nano bạc sinh tổng hợp từ dịch chiết lá cây cọ dầu có thể được sử dụng làm chất bảo quản cho hoa cẩm chướng cát cánh.

 Từ khóa: Arachis pintoi, bảo quản, cẩm chướng cát cánh, nano bạc, sinh tổng hợp