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Exploiting the potential of bio-synthesized silver nanoparticles to enhance the shelf life of *Gladiolus*

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Gladiolus spikes with fully turgid petals need to have a longer shelf life to fetch a higher price as well as display value. To improve the life duration of ornamental flowers, the ability of plants to produce silver nanoparticles (AgNP) was exploited. The ability of plants to produce AgNP when treated with silver nitrate solutions was juxtaposed by using *Gladiolus* (cut flowers): (i) To find the appropriate concentration of $AgNO_3$ suitable for increasing shelf-life of *Gladiolus* cut flowers; (ii) To prepare silver nanoparticle from $AgNO_3$; and (iii) To confirm the formation of silver nanoparticle using UV-vis spectrophotometry. Two different reductants (dehydrogenases present at the surface of the plant cells and sucrose) demonstrated the reduction of Ag+ to generate AgNPs. DLS (Dynamic Light Scattering) study revealed the presence of NPs in the $AgNO_3$ salt solution incubated with *Gladiolus* cut flowers. The DLS data also suggested that the size of AgNPs decreased with increasing concentration of $AgNO_3$. In the present study, along with silver nitrate, sucrose was also used. The shelf life and display value of the cut *Gladiolus* can be increased and optimized by incubating it in sucrose solution in combination with $AgNO_3$.

Keywords: *Gladiolus*, Green synthesis, Shelf life, Silver nanoparticles (AgNPs), Sucrose

Flowers have socio-cultural and religious importance in life of people across the globe. Carnations, Chrysanthemums, Roses, *Gladiolus* and Solidago are very important flower crops with an economical value in international floral industry. Among cut flower, *Gladiolus* is very popular and has huge demand in cut flower trade because of diverse shapes, colors and hues, and ease of culture[1]. It is a bulb flower of the Iridaceae family and is also known as "all-purpose champion"[2].

Unfortunately, the cut flowers are destined to die, and shelf life of cut flowers is usually short, due to rapid wilting [3].Cut flowers wilt and floral axis become bent (bent-neck) just below the flower head[4]. The most common factor for deterioration of cut flower is trapping of air bubble and rapid growth of microorganisms in the xylem leading to blockage in the xylem vessels and hence no water supply to twig[5]. The blockage due to trapping of air can be overcome by making fresh incision of the cut end with sharp blade of knife in water, however, inhibition of growth of microorganisms is an arduous task.

The longevity of cut flowers is affected by composition of vase water and the holding solutions must contain essentially two components, sugar and germicides[1,5]. Sugars provide source of energy and bactericide or germicide prevent microbial growth and blockage of xylem vessels. Acidification of the solution for indirectly supressing the growth of microorganisms and preventing wilting and premature fall of flowers is well known. Using *Gladiolus* flowers, Singh and Sharma (2003) observed that different combination of sucrose with metal salts promoted the vase life[6]. Anti-microbial agents such as silver salts and Ag NPs have also been used to increase the shelf life of various plants such as rose, chrysanthemum, Zinnia, *etc.*[7]. Amin and co-workers (2020), recently studied the effect of sucrose and nano-silver on cut Zinnia flowers[5].

It has been reported earlier that the intact terrestrial plants when incubated in silver nitrate ($AgNO_3$) salt solution can produce AgNPs on their own. It was demonstrated that plasma membrane of the cells of root surface have dehydrogenases/reductases which reduced Ag^+ to generate $Ag0/Ag_2O$ NPs[8]. Therefore, report the ability of (i) cut flowers of *Gladiolus* pink can generate AgNPs from $AgNO_3$ and increase the shelf life; (ii) the effect of combination of sucrose and $AgNO_3$on the shelf life.

Materials and Methods

Plant material

The cut flowers were purchased from a local Florist at North Delhi Region. Care was taken to choose

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similar looking spikes with almost same number of open and closed flowers.

Experimental set up

Freshly cut spikes of *Gladiolus* Pink were incubated in 125 mL of (i) $AgNO_3$ salt solutions with concentrations *viz*. 0, 0.1, 0.25 and 0.5 mM; (ii) 2% Sucrose; and (iii) a combination of 2% sucrose with0, 0.1, 0.25 and 0.5 mM $AgNO_3$. The spike of cut plants were incubated at room temperature (25±2°C) with a 16/8h light/dark cycle. The flasks were sealed by using parafilm to avoid loss due to evaporation and covered to avoid photo-oxidation of $AgNO_3$. All the experiments were done in triplicates.

Evaluation of formation of silver nanoparticles from $AgNO_3$ by *Gladiolus and* Sucrose

The generation of Ag NPs was initially suggested by colour change, and confirmed by UV-Vis spectra. The average size of the silver nanoparticles synthesized was measured by Dynamic Light Scattering (DLS) instrument.

Results and Discussion

Shelf Life and Display Value

Assessment of the visual characteristics and display value of flowers (Fig. 1) suggested that the plants treated with a combination of sucrose and $AgNO_3$ remained better even upto 7 days compared to other treatments used during present investigations *viz*. only $AgNO_3$ or only sucrose or distilled water.The combination treatment of sucrose and $AgNO_3$showed majestic and fully expanded flowers. The flowers treated with sucrose and $AgNO_3$ alone or in combination remained turgid even upto day 5 when compared to those kept in distilled water. In case of only double-distilled water the spike did not show any significant flower opening even upto Day 5, and completely shrivelled by 7th day. *Gladiolus* is an ethylene insensitive flower and it has been demonstrated earlier that in ethylene-insensitive flowers sugars prevent a decrease in osmoticum and delay cell death[9,10]. The florets are the most active organs for sucrose accumulation[11].

In general, various studies suggest that the shelf life of cut flowers of *Gladiolus* is around 10 days or more, whereas in the present study the flowers could retain the display value only upto 7 days. It is also known that the temperatures also effect the longevity of the cut flowers, and temperatures as high as 27°C decrease the longevity[12].The present investigations were carried out in the month of August 2021, the monthly mean

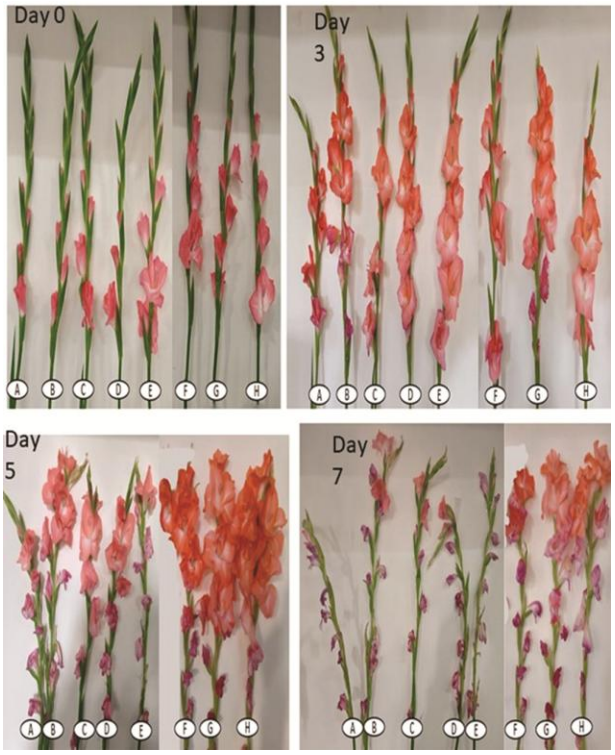

Fig. 1 — Visual characteristic and display value of cut *Gladiolus* incubated in different vase solutions *viz*. A – 0 $AgNO_3$, B – 2% Sucrose, C – 0.1 mM $AgNO_3$, D – 0.25 mM $AgNO_3$, E – 0.5 mM $AgNO_3$. F –0.1 mM $AgNO_3$+2% Sucrose, G –0.25 mM $AgNO_3$+2% Sucrose, H –0.5 mM $AgNO_3$ + 2% Sucrose for different time intervals

temperature of Delhi was 31.16°C (calculated from https://www.accuweather.com/en/in/delhi/202396/august-weather/202396?year=2021). Though the experiments were carried under controlled conditions, but the cut flowers used for the investigations had to bear this temperature in the flower market or at the local vendors, thus affecting their shelf life.

Biosynthesis of silver nanoparticles

Generation of Ag NPs from the ionic solution ($AgNO_3$) is abottom-up approach. Synthesis of NPs by reduction of metal ions include distinct stages: (a) reduction of metal ions to atoms *via* a chemical/biological reductant; (b) growth of the atoms to nuclei; and (c) growth of the nuclei into seeds which subsequently form the nanoparticles/nanocrystals. In the present study two different reductants (dehydrogenases present at the surface of the plant cells and sucrose) are demonstrated to bring about the reduction of Ag^+ to generate Ag NPs.

Incubation of cut flowers in $AgNO_3$ solution

The present studies revealed that the cut flowers when incubated in $AgNO_3$ solution of different

concentrations turned brown colloidal. However, the $AgNO_3$ salt solutions without cut flowers remained colourless. The UV-Vis spectra of these brown-colored colloidal solutions on Day 5 showed peak at around 430-450 nM (Fig. 2A). The peak in this region arises due to surface plasmon resonance in Ag NPs[13,14]. The intensity of peak increased with increasing concentration. Pardha-Saradhi and co-workers (2014) showed that the roots of intact plants of various plants possess potential to generate Ag NPs when incubated with $AgNO_3$solutions[8]. They demonstrated that the dehydrogenases/reductases (associated with plasma membrane of cells) are involved in the reduction of metal ions and generation of metal NPs.

DLS study revealed the presence of NPs in the $AgNO_3$ salt solution incubated with *Gladiolus* cut flowers. The Ag NPs were in the size range of ~100-152 nM and ~122-182 nM on Day 3 and Day 5, respectively, (Fig 2A). In the present study, DLS data suggested that the size of the NPs decreased with increasing concentration of $AgNO_3$. As dehydrogenases/reductases are involved in the reduction of Ag^+ to Ag NPs, we presume that decreased size could be due to decreased enzymatic activity, as higher concentration of $AgNO_3$is toxic to plants.It is well documented that dehydrogenases show different sensitivity to various metals[15]. The reductant in this case is the dehydrogenases which is getting limited as it is loses the enzymatic activity due to toxic effect of silver. In the present study with the passage of time the size of the nanoparticles is increased. Jena and co-workers (2014) also made similar observations where increase in reaction time caused increase in the particle size, due to agglomeration[16].

Incubation of cut flowers in $AgNO_3$and Sucrose solution

During the present studies the cut *Gladiolus* flowers when incubated in 2% sucrose solution in combination with different concentrations *viz.* 0.1, 0.25 and 0.5 mM $AgNO_3$showed the best results. The flowers remained robust and in bloom even upto 7 days. The sucrose provided the osmoticum to keep the flowers turgid and robust and $AgNO_3$ acted as an antimicrobial agent.

Interestingly, incubation solution of 2% sucrose along with different concentrations of $AgNO_3$but without cut flowers and incubated under similar conditions also turned colloidal and brown on day 3, suggesting the generation of Ag NPs in these solutions. The brown-colored colloidal solutions showed the presence of NPs in the size range of 110-166 nM (on day 3) and 121-172 nM (on day 5). This same is presented in (Fig. 2B). This change in colouration of $AgNO_3$ salt solution and sucrose without cut flowers is due to the reducing capacity of sucrose.

Sucrose and many other biomolecules are well known to bring about the reduction of metal ions and the synthesis of metal NPs[17]. The reducing action of sucrose occurs due to units of α-glucose and units of β-fructose after sucrose hydrolysis. As sucrose is a weaker reducing agent it gives initially larger nanoparticles as compared to the incubation of $AgNO_3$ with cut plants[18].

It is also observed from the DLS data that % increase in the size of the NPs (a sign of agglomeration) even after 5 days is insignificant. It is well known that sugars (Sucrose) are oxidized to the corresponding acids, and the metal ions are reduced to corresponding metal NPs. Thus, here sucrose reduced Ag ions to Ag atoms and also acted as a capping agent for the Ag atoms. These atoms form oligomeric clusters which increase in size *via* condensation reactions. At a critical size, clusters stop to grow *via* this mechanism, and behave like typical colloidal particles. They build up a repelling layer of sugar. Carboxylic acids formed as a product of sugar oxidation, generate a negative surface charge density, and prevent the particles from coalescence. Interestingly, the negative surface charge density is not effective when the NPs are very small. In such cases the layer of carboxylic acid/sugar immediate to NPs is not able to overcome the attraction among NPs and particles agglomerated[19]. But as the NPs increase in size, repulsive force is sufficient to avoid agglomeration[18].

In a way similar to the incubation of cut flowers with $AgNO_3$ solution alone, in the combination treatment ($AgNO_3$ + Sucrose) also the solutions turned brown colloidal when incubated at room temperature. The UV-vis spectra of these brown colored colloidal solutions on Day 5 showed peak at around 430-450 nM (Fig. 2C) that arises due to SPR in Ag NPs[13,14]. The intensity of peak increased with increasing concentration. As discussed above, in case of cut flowers alone probably, the enzyme dehydrogenases are involved in the reduction of Ag ions and the generation of NPs, and sucrose itself also act as a reducing agent. So, in this case the generation of Ag NPs is due to the combined reducing effect of

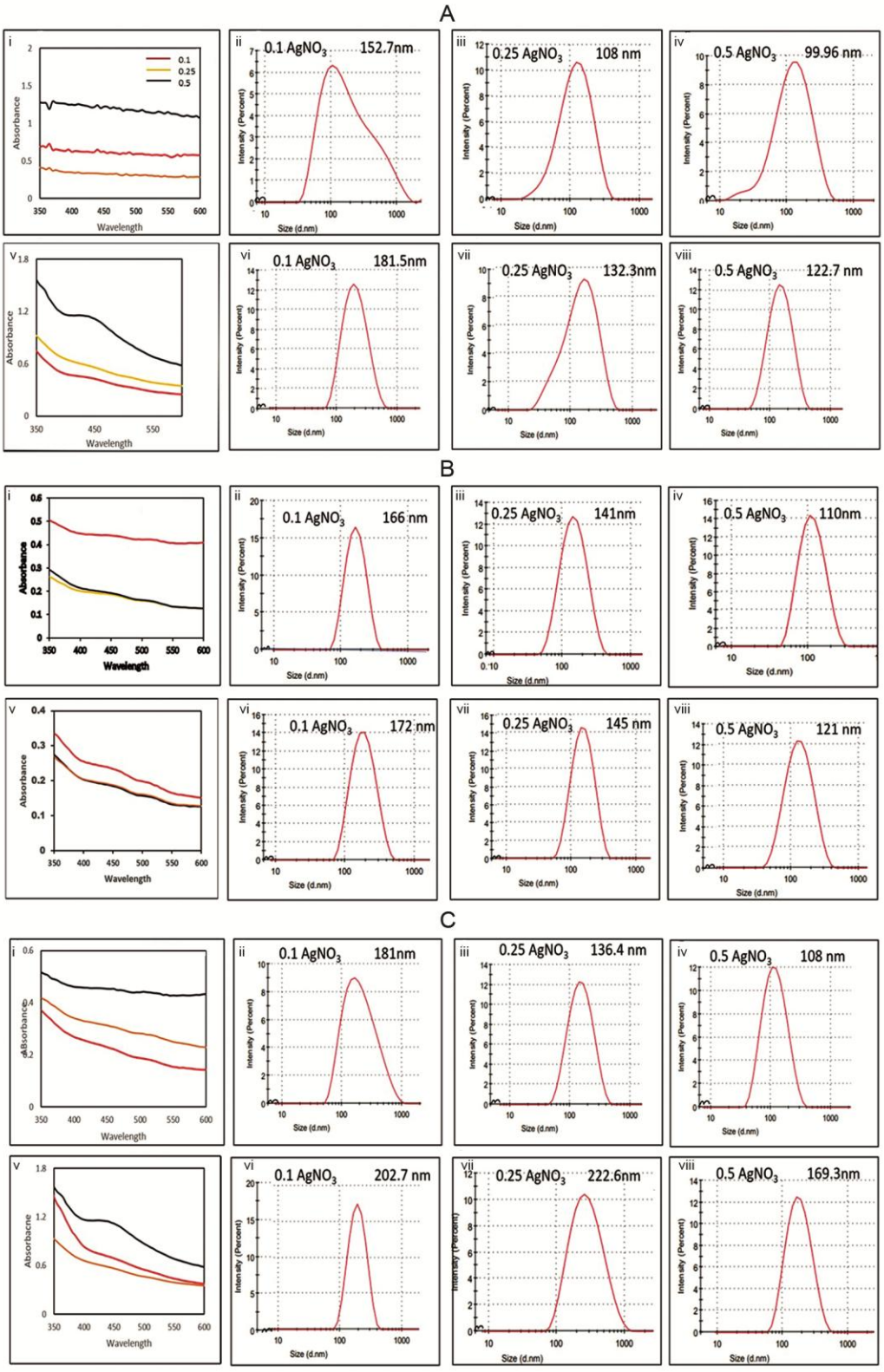

Fig. 2 — Incubation of (A) *Gladiolus* cut flowers; (B) 2% Sucrose; and (C) *Gladiolus* cut flowers in 2% sucrose in combination with 0.1, 0.25 and 0.5 mM AgNO3 solutions: (i-ii) UV-vis spectra of brown coloured colloidal solutions; (iii-viii) DLS data of colloidal solution

dehydrogenases and sucrose. With increase in the incubation time the size of the NPs increased.

Conclusion

In summary, our results demonstrated for the first time that the cut flowers alone or in combination with sucrose can also be used for the generation of Ag nanoparticles. Usage of sucrose/$AgNO_3$ alone as a vase solution is not appropriate as former leads to microbial infections, and the latter is not able to provide the osmoticum to the flowers, reducing its display value. The shelf life and display value of the cut *Gladiolus* can be increased and optimized by incubating it in sucrose solution in combination with $AgNO_3$.

Conflict of interest

All authors declare no conflict of interest.

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