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PHYSIOLOGICAL AND ANATOMICAL STUDIES ON NECK BENDING IN CUT ROSE CV. RED BELINDA

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ABSTRACT

An experiment was conducted on the 'physiological and anatomical studies on neck bending in cut rose cv. Red Belinda' during the year 2023-24, at Dr. YSRHU-COH, Venkataramannagudem with an objective of studying the influence of holding solutions on physiological and anatomical parameters in rose cv. Red Belinda. The experiment was conducted in completely randomized design with seven treatments and replicated thrice. Among the physiological parameters studied Water uptake, Transpirational loss of water and water balance were significantly high in treatment with Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1}) and control recorded the least. The results regarding the anatomical studies were analysed with microscope pictures of the bending area indicated that 9 mg/l SNPs along with sucrose@ 20 g ml^{-1} prevented vessel blockage and maintain anatomical structures such as epidermis cells, parenchyma cells and vascular bundles.

Key words : Neck bending, Silver nanoparticles, Water uptake, Transpirational loss of water, Vase life.

Introduction

Flowers have been cherished by humans for centuries for their beauty, fragrance and symbolic meanings, thus making floriculture a significant global industry. Floriculture industry generates billions of dollars annually as revenue and is particularly important in countries having favourable climate for flower production. Roses and gerberas are delightful and popular choice among the cut flowers in various occasions, adding beauty and elegance to any setting. Roses is economically the most important among the top ten cut flowers in the international market. The international rose trade is substantial in major exporter countries like Ecuador, Colombia and Kenya to meet global rose demand. The export value of fresh cut flowers (4047.63 MT) has been estimated at about 9.8 crores among this, rose trade contributes to about (125.68 MT) 3.97 crores (APEDA, 2023). Roses are known for their timeless appeal, with wide range of colours and each colour conveying distinct emotions. The classic red rose for symbolizing love and delicate pink for admiration. Roses have been cherished for centuries for their fragrant blooms and romantic connotations.

Neck or stem bending is one of the most important postharvest problems, as it reduces the commercial value and decrease vase life of rose cut flowers (Perik *et al*., 2014; Garcia – Gonzalez *et al*., 2022), it is mainly due to attack of microorganisms at cut end of the stem and in preservative solutions due to vascular blockages there by preventing water transport to the flower (Ghada *et al*., 2022). This research was conducted to evaluate the effectiveness of different chemical combinations as holding solutions including silver nano particles, 8-HQS, TGA, DTT and thymol over control in delaying neck

bending in *Rosa hybrida* cv. Red Belinda. Our results indicated a positive effect of SNPs over control in delayed in neck bending, reduced the number of bacteria and increasing the vase life of rose as silver nano particles act as an effective antimicrobial agent due to the high surface area-to-volume ratio and their physical and chemical properties (Rai *et al*., 2012 and Rashidiani *et al*., 2020), which adhere and break through to the microbial cells leads to damage the respiration chain and eventually leads to death of cell.

Materials and Methods

The present investigation was carried out at post-harvest laboratory in the Department of Floriculture and Landscaping, College of Horticulture, Dr. Y.S.R. Horticultural University, Venkataramannagudem, West Godavari district, Andhra Pradesh during November 2023 to February 2024. The experiment was carried out in a Completely Randomized Design (CRD) with three replications and 7 treatments (T_1-distilled water, T_2- 8-Hydroxyquinolinesulphate (200 mg L^{-1}) + Sucrose (20 g L^{-1}), T_3- Thioglycolic acid (TGA 0.5 mM), T_4- Dithiothreitol (DTT 0.2 mM), T_5- Thymol (0.2 ml L^{-1}) + Sucrose (20 g L^{-1}) +Tween 20 (0.1 ml L^{-1}), T_6- Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1}), T_7- Pulsing with SNPs (20 mg L^{-1}) for 24 h + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1}). Flowers of rose (*Rosa* sps.) cultivar 'Red Belinda' obtained from commercial floriculture farm *i.e.*, Om Sri Sai Ram farms, which is close to Bengaluru. Observations like bent neck score, water uptake (WU), transpirational loss of water (TLW), microbial counts, vase life and anatomical studies. Physiological parameters were recorded at every alternate day.

Results and Discussion

The neck bending scores were significantly lower in (T_6) Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1}) from day 2 (0.00°) to 8th (75.93°), followed by (T_7) Pulsing with SNPs (20 mg L^{-1}) for 24 hours + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1}). Meanwhile, the control treatment (T_1) exhibited the highest level of bending by the 4th day (90.00°), and the other treatments showed differing degrees of bending for various days as mentioned in Table 1. The treatments with silver nano particles recorded least bent neck score *i.e.* no bent neck symptoms were observed up to 3 days of experiment period later very low bent neck score was observed compared to other treatments. The reasons may include bending degree results were correlated with improvement in water relations, a lower number of bacteria in the vase solution as bending is mostly caused by an air embolism or the

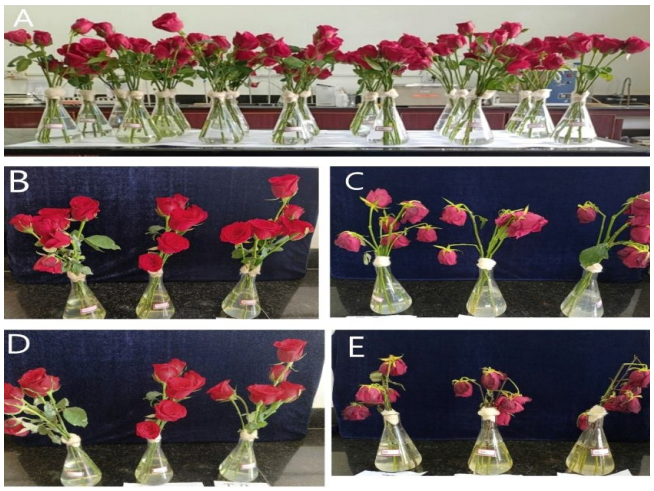
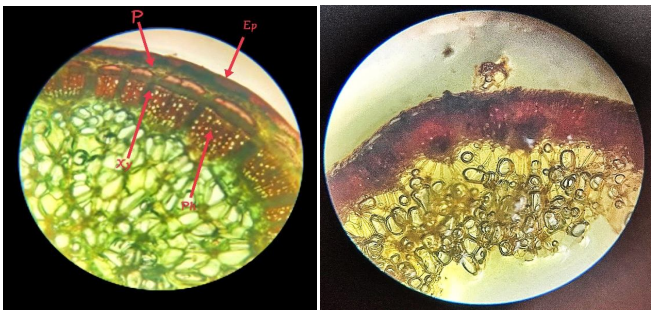


Fig. 1 : (A): Cut flowers of rose cv. Red Belinda kept in T_6 on initial day of the experiment **(B):** Cut flowers of rose cv. Red Belinda in T_6 on 8th day of the experiment **(C):** Cut flowers of rose cv. Red Belinda kept in T_1 on initial day of the experiment **(D):** Cut flowers of rose cv. Red Belinda kept in T_1 on 4th day of the experiment.



T_6:SNP (9mg L^{-1}) + Sucrose (20g ml^{-1}) **T_1:** Control

Fig. 2 : The anatomical structure of Rosa *hybrida* cv. Red Belinda cut flowers. (A): SNPs at 9 mg/l along with sucrose, (B): Control. Ep: epidermis, P: parenchyma, Vb: vascular bundle (Xy: xylem, Ph: phloem).

growth of bacteria in the vase solution and cumulating of microorganisms at or inside the stem end, which leads to the clogging of vessels and prevent water absorption (Ghada *et al*., 2022). Silver nano particles and 8-HQS acts as anti-microbial agents and silver acts as anti-ethylene agent which might have played significant role in reducing the neck bending (Prashanth *et al*., 2011).

The water uptake of the cut roses significantly varied across the different treatments. The data revealed that flowers placed in (T_6) Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g L^{-1}) exhibited the highest water uptake compared to the other treatments from day 2 to 8th day followed by Pulsing with SNPs (20 mg L^{-1}) for 24 hours + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1}) (T_7) from 2nd day to 6th day. This water uptake increased steadily from 2nd (26.70g) day until the 4th day (33.80g) and then gradually declined to the end of the vase life (18.04g).

Table 1 : Effect of holding solutions on bending stem score (degree) of cut rose cv. Red Belinda.

Treatments	Days			
	2	4	6	8
T_1- Control (distilled water).	15.50^{o}	90.00^{o}	-	-
T_2- 8-Hydroxyquinoline sulphate (200 mg L^{-1}) + Sucrose (20 g L^{-1})	0.00^{o}	48.13^{o}	90.00^{o}	-
T_3- Thioglycolic acid (TGA 0.5 mM)	0.00^{o}	19.16^{o}	90.00^{o}	-
T_4- Dithiothreitol (DTT 0.2 mM)	2.50^{o}	31.33^{o}	90.00^{o}	-
T_5- Thymol (0.2 ml L^{-1}) + Sucrose (20 g L^{-1}) +Tween 20 (0.1 ml L^{-1})	0.00^{o}	29.53^{o}	90.00^{o}	-
T_6- Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1})	0.00^{o}	4.56^{o}	36.53^{o}	75.93^{o}
T_7- Pulsing with SNPs (20 mg L^{-1}) for 24 h + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1})	0.00^{o}	9.58^{o}	42.77^{o}	90.00^{o}
Mean	**2.57**	**33.19**	**62.42**	**84.78.**
SE (m)	0.23	0.52	0.46	-
C.D at 5%	0.69	1.57	1.40	-

Table 2 : Effect of holding solutions on water uptake (g $flower^{-1}$) of cut rose cv. Red Belinda.

Treatments	Days			
	2	4	6	8
T_1- Control (distilled water).	12.88	12.94	-	-
T_2- 8-Hydroxyquinoline sulphate (200 mg L^{-1}) + Sucrose (20 g L^{-1})	29.04	33.24	-	-
T_3- Thioglycolic acid (TGA 0.5 mM)	22.96	26.82	-	-
T_4- Dithiothreitol (DTT 0.2 mM)	27.01	31.21	-	-
T_5- Thymol (0.2 ml L^{-1}) + Sucrose (20 g L^{-1}) +Tween 20 (0.1 ml L^{-1})	20.73	24.95	-	-
T_6- Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1})	39.26	45.65	29.35	18.04
T_7- Pulsing with SNPs (20 mg L^{-1}) for 24 h + 8-HQS (200 mg L^{-1}) + Sucrose (20 gL^{1})	35.02	40.94	25.62	-
Mean	**26.70**	**33.80**	**27.48**	**18.04**
SE (m)	0.73	0.69	-	-
C.D at 5%	2.22	2.11	-	-

whereas, the control treatment (T_1) had the lowest water uptake on the 2nd (12.88g) day comparatively and reached the end of its vase life by the 4th day (12.94g) of the experiment. Enhanced water absorption could be attributed to the synergistic impact of the silver nano particles along with sucrose. Sucrose serves as a source of carbohydrate and a favourable respiratory substrate for sustaining the osmotic potential in flowers, thereby enhancing tissue's water absorption capability, and maintaining turgidity. Silver nano particles play an important role in improving the water uptake in cut rose cv. Red Belinda, because of their distinct physical and chemical properties, as well as their high surface area-to-volume ratio, silver nanoparticles have potent antibacterial effects due to Ag^{+} ions within SNP, which may influence the regulation of water channel activity by inhibiting sulfhydryl-containing proteins in bacteria, thereby enhancing solution uptake (Varun *et al.*, 2017). Therefore, in cut flowers, these nanoscale silver particles successfully prevent vascular occlusion and bacterial development (Liu *et al.*, 2021).

The transpirational loss of water of the cut roses significantly varied across the different treatments. The data revealed that flowers placed in (T_6) Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g L^{-1}) exhibited the highest transpiration loss from 2nd (26.00g) to 8th day (16.56g) of the experiment compared to the other treatments followed by Similarly, (T_7) Pulsing with SNPs (20 mg L^{-1}) for 24 hours + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1}). This water loss through transpiration increased steadily from 2nd day (19.04g) to 4th day (22.35g) and then gradually declined from 6th day (19.41g) to 8th day (12.56g) *i.e.*, the end of the vase life. whereas, the control treatment (T_1) had the least transpirational loss on the 2nd day (10.06g) comparatively and reached the end of its vase life by the 4th day of the experiment. Nanoparticles have the potential to have a notable impact on cellular biochemical processes. They can serve as innovative agents that stimulate and enhance the production of bioactive compounds in plants (Tymoszuk *et al.*, 2022). The application of nano silver treatments led to a reduction in the transpiration rate and stomatal conductance of the cut flower, likely caused by the closure of stomata induced

Table 3 : Effect of holding solutions on transpirational loss in water (g flower^{-1}) of cut rose cv. Red Belinda.

Treatments	Days			
	2	4	6	8
T_1- Control (distilled water).	10.06	11.41	-	-
T_2- 8-Hydroxyquinoline sulphate (200 mg L^{-1}) + Sucrose (20 g L^{-1})	21.35	23.75	-	-
T_3- Thioglycolic acid (TGA 0.5 mM)	17.35	20.57	-	-
T_4- Dithiothreitol (DTT 0.2 mM)	19.99	23.43	-	-
T_5- Thymol (0.2 ml L^{-1}) + Sucrose (20 g L^{-1}) +Tween 20 (0.1 ml L^{-1})	16.39	19.67	-	-
T_6- Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1})	26.00	30.06	22.32	16.56
T_7- Pulsing with SNPs (20 mg L^{-1}) for 24 h + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1})	22.19	27.33	19.50	-
Mean	**19.04**	**22.35**	**19.41**	**12.56**
SE (m)	0.75	0.89	-	-
C.D at 5%	2.26	2.71	-	-

Table 4 : Effect of different holding solutions on microbial count (cfu/ml) and vase life (days) of cut rose cv. Red Belinda.

Treatments	Microbial count (cfu ml^{-1}) (x 10^5 dilutions)		Vase life
	Initial	Final	
T_1- Control (distilled water).	5.26	132.34	4.00
T_2- 8-Hydroxyquinoline sulphate (200 mg L^{-1}) + Sucrose (20 g L^{-1})	2.92	74.61	5.83
T_3- Thioglycolic acid (TGA 0.5 mM)	4.12	88.76	4.50
T_4- Dithiothreitol (DTT 0.2 mM)	3.65	87.24	5.00
T_5-Thymol (0.2 ml L^{-1}) + Sucrose (20 g L^{-1}) +Tween 20 (0.1 ml L^{-1})	4.89	99.02	4.13
T_6- Silver Nano Particles (9 mg L^{-1}) + Sucrose (20 g ml^{-1})	1.35	62.91	8.10
T_7- Pulsing with SNPs (20 mg L^{-1}) for 24 h + 8-HQS (200 mg L^{-1}) + Sucrose (20 g L^{-1})	1.96	69.40	7.21
Mean	**3.45**	**87.73**	**5.23**
SE (m)	0.11	0.47	0.23
C.D at 5%	0.34	1.43	0.69

by nano silver ions (Devi Priya *et al.*, 2021).

The observed data indicates that flowers placed in silver nano particles @ 9 mg L^{-1} along with sucrose (T_6) recorded the least microbial growth through initial phase (1.35×10^{-5} cfu/ml) as well as final count (62.91×10^{-5} cfu/ml) which was followed by treatment (T_7) Pulsing with SNPs @ 20 mg L^{-1} for 24 h along with 8-HQS @ 200 mg L^{-1} and Sucrose @ 20 g L^{-1} with initial microbial count 1.96×10^{-5} cfu/ml and final microbial count was 69.40×10^{-5} cfu/ml. The highest microbial count during initial (5.26×10^{-5}cfu/ml) and final count (132.34×10^{-5} cfu/ml) was found with flower kept in control (T_1). The reasons may include Ag^+ has antimicrobial efficacy and antiviral and algicidal effects as silver nanoparticles (SNPs) possess the capability to attach to the bacterial cell wall and then enter it, leading to alterations in the structure of the cell membrane which eventually leads to cell death (Sushma *et al.*, 2023). The results obtained were similar with the findings of Solgi *et al.* (2009) in gerbera cv. 'Dune', Ghada *et al.* (2022) and Sushma *et al.* (2023) in *Rhynchostylis retusa*.

The vase life period of cut rose cv. Red Belinda showed significant variation across the treatments. The vase life period ranged between 4.00 days and 8.10 days. Among different treatments, Significantly the maximum vase life period (8.10 days) was obtained with flowers kept in silver nano particles @ 9 mg L^{-1} along with sucrose (T_6) followed by Pulsing with SNPs @ 20 mg L^{-1} for 24 h along with 8-HQS @ 200 mg L^{-1} and Sucrose @ 20 g L^{-1}(T_7) (7.21days). Control (T_1) reported considerably the lowest vase life period (4.00days) while, the remaining all other treatments recorded intermediate values whereas, these research findings indicated that the longevity of cut rose cv. Red Belinda flowers was

prolonged by postponing bending, reducing bacterial presence in the vase solution, enhancing water uptake. the longest vase life of flowers was achieved by utilizing 9 mg/l SNPs along with sucrose (T_6). These results correlated with Ghada *et al*. (2022) in rose and gerbera, Atefepour *et al*. (2021) and Skutnik *et al*. (2021) on Lisianthus.

Anatomical studies

Differences in anatomical structures were noted in the neck bended portions of cut rose cv. Red Belinda flower stalks between the control group and the other treatments. The untreated rose flower stalks exhibited undulation and ruptured cells in the epidermal layer, along with more noticeable shrinkage of parenchyma cells. Comparatively, the transverse sections of rose flowers treated with SNPs at 9 mg/l showed denser parenchyma layers distinct from those treated with alternative substances. Additionally, there was a slight disturbance in the vascular bundle cells of the untreated cut rose flowers. Notably, a clear blockage was observed in the xylem vessels of the control sections, with a minor blockage present in the other treatments, except for the SNPs at 9 mg/l along with sucrose treatment in cut rose flowers. In conclusion, the anatomical structure of stems placed in silver nano particles @ 9 mg L^{-1} along with sucrose (T_6) showed very slight disturbance in vascular bundle cells and xylem vessels were open with least blockage for solution uptake and denser parenchyma with least undulations in the epidermis comparatively.

Conclusion

SNPs have been found to outperform remaining chemicals in enhancing the quality and preventing bending by suppressing bacterial growth, regulating the water balance in flowers. This helps maintain the anatomical structure, ultimately extending the vase life of *Rosa hybrida* cv. Red Belinda cut flowers, especially those more prone to bending. It is suggested that using a solution containing 9 mg/l of SNPs along with 20 g/l sucrose as holding solution can effectively delay bending in the preservation of cut rose.

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