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Farah Deeba
 Division of Floriculture and Landscape Architecture, SKUAST, Kashmir, Shalimar, Jammu and Kashmir, India

ZA Bhat
 Division of Floriculture and Landscape Architecture, SKUAST, Kashmir, Shalimar, Jammu and Kashmir, India

MH Khan
 Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

Nelofar
 Division of Floriculture and Landscape Architecture, SKUAST, Kashmir, Shalimar, Jammu and Kashmir, India

Barkat Hussain
 Division of Entomology, SKUAST-Kashmir, Shalimar, Jammu and Kashmir, India

Nageena Nazir
 Division of Agricultural Statistics, SKUAST-Kashmir, Shalimar, Jammu and Kashmir, India

FA Khan
 Division of Agricultural Statistics, SKUAST-Kashmir, Shalimar, Jammu and Kashmir, India

ZA Rather
 Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

Sonober Mushtaq
 Division of Floriculture and Landscape Architecture, SKUAST, Kashmir, Shalimar, Jammu and Kashmir, India

Corresponding Author:
Farah Deeba
 Division of Floriculture and Landscape Architecture, SKUAST, Kashmir, Shalimar, Jammu and Kashmir, India

Growth and flowering of cut roses (*Rosa hybrida*) as influenced by salicylic acid and sodium silicate under protected conditions

Farah Deeba, ZA Bhat, MH Khan, Nelofar, Barkat Hussain, Nageena Nazir, FA Khan, ZA Rather and Sonober Mushtaq

Abstract

This experiment was conducted to investigate the effects of different concentrations of Salicylic acid and Sodium silicate as a foliar spray on greenhouse rose (*Rosa hybrida* var. Avalanche) to improve growth, flower quality and yield of rose. A total of 10 treatments T_1 = control, T_2 = Sodium silicate @ 1mM and Salicylic acid @ 1mM, T_3 = Sodium silicate @ 1mM and Salicylic acid @ 3mM, T_4 = Sodium silicate @ 1mM and Salicylic acid @ 5mM, T_5 =Sodium silicate @ 3mM and Salicylic acid @ 1mM, T_6 = Sodium silicate @ 3mM and Salicylic acid @ 3mM, T_7 = Sodium silicate @ 3mM and Salicylic acid @ 5mM, T_8 =Sodium silicate @ 5mM and Salicylic acid @ 1mM, T_9 = Sodium silicate @ 5mM and Salicylic acid @ 3mM, T_{10} = Sodium silicate @ 5mM and Salicylic acid @ 5mM, were tested in randomised complete block design with three replications. All the treatments improve growth, flower quality and yield as compared to control treatment. The results of the study revealed that T_{10} significantly improved vegetative and flowering parameters of rose (*Rosa hybrida*). The parameters viz. maximum plant height (90.41cm), plant spread (45.44cm), number of basal shoots (3.64), diameter of basal shoots (1.05cm), number of compound leaves per flowering shoot (14.28). Minimum number of days to first flower bud appearance (105.33) and minimum number of days to colour break stage (20.57) was also recorded in T_{10} and flowering parameters viz. maximum flower bud diameter (1.40cm), neck girth (0.73cm), length of cut stems (74.47cm), girth of cut stems (0.77 cm), flower diameter (7.25cm), duration of flush (43.22days), number of cut stems plant⁻¹ (6.63) and number of cut stems m⁻² (30.00) maximum stem dry matter (8.15g) was recorded in T_{10} . Maximum total fresh weight of cut stems harvested per plant (177.82g) and maximum vase life (11.41days) was recorded in T_8 .

Keywords: Rose, salicylic acid, sodium silicate, growth, flowering

Introduction

Roses belong to the family Rosaceae, the third-largest plant family including many ornamental landscape plants, such as berries and fruits, apples, raspberries, cherries, etc., however, the rose is considered the most important plant in this family (Hummer and Janick, 2009)^[29]. The *Rosa hybrida* L. is mostly used as valuable decorative flowers in the world (Boskabady *et al.*, 2011; Mirjalili and Kavoosi, 2018)^[9, 42]. The rose has been called the 'Queen of Flowers' as well as the 'King of Flowers'. No other flower surpasses its beauty, colour and fragrance which is why it is universally considered a favourite flower (Gitonga *et al.*, 2014)^[23]. Roses are considered important floral crops in the world and used as raw material in various industries like cosmetics and perfume (Ghadimian and Danaei, 2020)^[21]. The number of species of genus Rosa varies among 200 species and it contained more than 35,000 commercial cultivars most of which are hybrids. The perfume, medicine and floriculture industries are mainly depended on the commercial cultivation of Roses (Gil *et al.*, 2019)^[2]. There is great diversity in plant growth, colour of flowers, flower shape, fragrance, slow opening of flowers and good keeping quality which made roses so popular that roses are grown commercially to meet the demand of cut blooms.

Plant growth regulators (PGRs) play key role in life cycles of plants and these can be produced naturally by plants or synthetically by chemists (Davies, 2013)^[15]. Amanullah *et al.* (2010)^[4] noticed that PGRs enhance the source-sink relationship and translocation of photo-assimilates to the sink, thereby helping effective flower formation, fruit and seed development and enhancing the productivity of crops. Plants quickly respond to change in hormonal balance due to growth substances' (PGS). Various PGRs are being used commercially in cut flower industry to modify plants behavior like compactness of growth, number and aesthetic value of

flowers (Pal, 2019) [48]. PGRs modify growth and development in different ways under natural and stressful environmental conditions. The role of PGRs on various physiological processes in plants is well known and they enable a change in the phenotype of plants within one season to achieve desirable results. They are now being widely used to increase the production of many crop types (Barrett, 2001) [6]. The most important factor for success is that application of PGRs at a stage in growth where the physiological processes are at their peak. Some characteristics such as height and number of branches are important morphological parameters contributing to increase yields (Darasteanu *et al.*, 2005) [14]. Floriculture industry has been revolutionized through identification of various growth regulators which control growth and flowering for high quality end product (Sajid *et al.*, 2015) [53]. With advancements in technology, grower's main objectives in the cut flower industry are the quality of flowers and increased flower production. Various chemicals are now being trialed to control the growth and flowering of roses with a view of creating plants that are compact as well as stretching out or retarding the rates of plant growth (Hashemabadi, 2010) [26].

Salicylic acid (SA) or orthohydroxybenzoic acid is a phenolic compound known as plant growth regulator and affects various processes, such as inhibition of ethylene synthesis and glycolysis (Martín-Mex *et al.*, 2015) [40]. It is known as a regulator of several physiological and biochemical processes such as thermogenesis, plant signaling or plant defense, and response to biotic and abiotic stress (Chen *et al.*, 2009; Wani *et al.*, 2017) [12, 60]. Studies show that many phenolic compounds are involved in regulating physiological processes, including plant growth, stomatal closure, photosynthesis, and ion uptake (Kabiri *et al.*, 2014; Mohajeri *et al.*, 2018) [43]. SA is an important regulator of photosynthesis and affects leaf structure, chloroplast, stomach closure, chlorophyll and carotenoid content, and activity of enzymes, such as rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase, resulting in changes in leaf area (Afshari *et al.*, 2013) [2]. Salicylic acid is a tool to increase plant tolerance against the adverse effect of biotic and abiotic stresses (Bosch *et al.*, 2007) [8] either by foliar application or seed or plant treatment. Since, it has a regulatory effect on activating biochemical pathways associated with tolerance mechanisms in plants (Najafian *et al.*, 2009) [46]. In some studies, the flower inducing factor is known as SA, which is consistent with reports of the use of SA in the induction of organic tobacco flowering (Muthulakshmi and Lingakumar, 2017) [44]. However, the precise mechanism of the SA inducer property has not yet been investigated. Therefore, it can be concluded that SA can act as a regulator that affects plant growth and productivity (Hayat *et al.*, 2010; Khatiby and Shadmehri, 2019) [27]. SA is involved in stimulating specific responses against various biotic and abiotic stresses (Kareem *et al.*, 2017) [30] and many physiological processes of plants, flowering, control of root ions absorption and stomatal closure (Shahmoradi and Naderi, 2018) [56]. This molecule is a plant regulator that has a protective effect against oxidative damage.

Silicon (Si) is a non-toxic, useful, and abundant element that participates in a wide range of plant activities (Pozza *et al.*, 2015) [50]. Taking plant physiology as an example, Si can reinforce the photosynthesis at the cost of lower transpiration, thus benefiting the nitrogen metabolism and carbon accumulation. Silicon is absorbed and forms a double silicate layer on the leaf epidermis, allowing better leaf architecture

and greater light assimilate capacity (Campos *et al.*, 2020) [10]. One of the most beneficial roles of Si that has been demonstrated is that it helps plants manage biotic and abiotic stresses (Li *et al.*, 2020) [34]. Moreover, many studies have suggested that Si plays an important role in increasing the rigidity of the cell walls of plants, leading to a more erect plant (Feng *et al.*, 2010) [19]. Equally as important, Si has a great potential in mitigating the symptoms caused by Fusarium wilt, which is believed to destroy water translocation, inducing drooping foliage, leaf chlorosis, and other wilt symptoms (Fortunato *et al.*, 2012) [20]. Si also reduces stomatal conductance in relation to turgor loss of guard cells resulting from Si deposition and modified cell wall properties (Zhu and Gong, 2014) [64]. When plants are grown under conventional environments (i.e., not subject to stress), Si probably makes plants more efficient in responding to environmental cues by activating different metabolic processes (Luyckx *et al.*, 2017) [35] with crucial cascading effects on plant structure and function (Guntzer, Keller and Meunier, 2012; Coskun *et al.*, 2019) [25, 13]. Si has biostimulant effects on plants (Epstein, 2009; Gómez-Merino and Trejo-Téllez, 2018) [18] by modifying physiological processes in a way that provides benefits to growth, development or stress responses (Savvas and Ntatsi, 2015) [54]. Silicon has the potential to be used in the production of floriculture crops to increase flower and stem size, accelerate flowering and improve resistance to stresses including drought. Silicon is a non-essential nutrient for most plants, however in floriculture production, it is known to affect plant growth and quality, photosynthesis, transpiration and enhance plant resistance to stresses such as drought. Reduction of the transpiration rate could further benefit floriculture crop production. A study conducted to see the effect of supplemental silicon on stomatal conductance, mechanism of plants used to open and close "water vapor" valves. Under normal greenhouse conditions, leaf resistance (reduction of transpiration) increased with a high rate of sodium silicate foliar sprays. There is an indication that sodium silicate foliar spray applications can act as a film-forming anti-transpirant that increases leaf resistance (Wang *et al.*, 2017) [61]. The present investigation was undertaken to find out the use of growth regulators *viz.*, Salicylic acid and Sodium silicate for improving growth, flower quality and yield in cut roses.

Materials and Methods

A field experiment was conducted at the experimental farm of Division of floriculture and Landscape Architecture, Sher-e-Kashmir University of Agriculture Sciences and Technology of Kashmir during 2020-21. The experiment was laid out in randomized complete block design (RCBD) with ten treatments and three replications. The treatments were as follows: T₁= control, T₂= Sodium silicate @ 1mM and Salicylic acid @ 1mM, T₃ = Sodium silicate @ 1mM and Salicylic acid @ 3mM, T₄ = Sodium silicate @ 1mM and Salicylic acid @ 5mM, T₅ =Sodium silicate @ 3mM and Salicylic acid @ 1mM, T₆ = Sodium silicate @ 3mM and Salicylic acid @ 3mM, T₇ = Sodium silicate @ 3mM and Salicylic acid @ 5mM, T₈ =Sodium silicate @ 5mM and Salicylic acid @ 1mM, T₉ = Sodium silicate @ 5mM and Salicylic acid @ 3mM, T₁₀ = Sodium silicate @ 5mM and Salicylic acid @ 5mM. Land area inside the polyhouse was thoroughly ploughed up to 40 cm deep prior to planting. Weeds stubbles were removed completely and brought the soil into a fine tilth. Raised beds of 5 m length, 1.5 m width and 40 cm in between two beds for passage were prepared for

path way. Rooted cuttings were first dipped for about 6 hours in different concentrations of salicylic acid *viz.*, 1mM, 3mM and 5mM. First spray of salicylic acid and sodium silicate was given at 30 days after transplanting and second, third spray was given after 30 days interval from the first spray date. After one month of the last spray observations of various parameters on growth and flowering were recorded by using standard methods. The data regarding various characters were statistically analyzed using SAS software with analysis of variance.

Results and Discussion

a) Effect on vegetative parameters

In the present investigation, data recorded on growth parameters presented in Table 1 revealed that all treatments of salicylic acid and sodium silicate significantly affected plant height, plant spread, number of basal shoots per plant, diameter of basal shoots, number of compound leaves, girth of cut stems, length of cut stems, number of cut stems plant^{-1} and number of cut stems m^{-2} . All the growth parameters increase with increase in the concentration of salicylic acid and sodium silicate. However, maximum plant height (90.41cm), plant spread (45.44cm), and length of cut stems (74.47 cm) was observed in treatment combination of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum plant height (65.34 cm), plant spread (32.10 cm) and length of cut stems (56.90 cm) was recorded in control. This increase may be due to the reason that Salicylic acid could be involved in the regulation of cell enlargement and division in synergy with other substances such as auxin, IAA and phenolics which cause stimulatory effect to increase plant height, plant spread and length of cut stems (Padmapriya and Chezhiyan, 2002) [47]. Salicylic acid increased plant height by increasing Rubisco activity and photosynthetic rate (Nagasubramaniam *et al.*, 2007) [45]. The observations and findings of the present investigation are in conformity with the findings reported earlier by Al-Abbas *et al.* (2015) [3] and Zeb *et al.* (2017) [63] in zinnia and Anwar *et al.* (2014) [5] in tuberose. Similarly, sodium silicate may increase the plant growth due to the stimulation by silicon. It may be either indirect, owing to the protective effects of silicon against pathogens (Belanger *et al.*, 1995) [7] or direct originating from implications of silicon to both morphological changes and physiological processes in plants. The number of basal shoots per plant, number of cut stems plant^{-1} , and number of cut stems m^{-2} increased with the increase in the concentration of salicylic acid and sodium

silicate. Maximum number of basal shoots per plant (3.64), number of cut stems plant^{-1} (6.63) and number of cut stems m^{-2} (30.00) was observed in treatment combination of Sodium silicate @ 5mM and Salicylic acid @ 5mM. Minimum number of basal shoots per plant (1.67), number of cut stems plant^{-1} (4.05) and number of cut stems m^{-2} (12.00) was recorded in control. Salicylic acid increased immunity endorsed plant through continuous supply of nutrients and well-acted response to environmental stresses, an important signal molecules altered physiological regulation. Eventually it was documented that potentially the plant regulator engendered extensive range of metabolic response in plants and finally photosynthetic factors of plant was positively affected (Zeb *et al.*, 2017) [63]. Also, increase in number of shoots per plant might be due to stimulation of growth by the silicon application. These results were in accordance with Voogt and sonneveld (2001) [59], Seung (2005) [55] in rose. Diameter of basal shoots and girth of cut stems increased with increase in concentration of salicylic acid and sodium silicate. Maximum diameter of basal shoots (1.05 cm) and girth of cut stems (0.77 cm) was observed in treatment combination of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum diameter of basal shoots (0.75 cm) and girth of cut stems (0.56 cm) was recorded in control. It might be due to increase in photosynthetic activity and accumulation of more carbohydrates in the shoots. Similar results were reported by Saeed *et al.* (2009) in rose, Seung *et al.* (2005) [55] in rose, Kamenidou *et al.* (2010) [29] in gerbera. Maximum number of compound leaves per flowering shoot (14.28) was observed in treatment combination of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum number of compound leaves per flowering shoot (10.00) was recorded in control. Anwar *et al.* (2014) [5] found that the number of leaves increased by increasing of internodes distance and reduction of leaves due to the decreasing of internodes distance. Numbers of leaves were more at high salicylic acid concentrations and minimum leaves were obtained at low level of salicylic acid and control. It means that number of leaves increased by increasing concentration of salicylic acid. These findings are corroborated by zeb *et al.* (2017) [63] in zinnia cultivars; Mahroof *et al.* (2017) [38] in *zinnia elegans* and Chaudhary *et al.* (2015) [11] in African marigold. It is involved in regulation of growth processes, such as in ornamental plants young shoots stimulate leaves (Singh, 1993) [58].

Table 1: Effect of salicylic acid and sodium silicate on vegetative parameters of Rose (*Rosa hybrida*)

Notation	Treatment	Plant height (cm)	Plant spread (cm)	Length of cut stems (cm)	Number of basal shoots per plant	Number of cut stems plant^{-1}	Number of cut stems plant^{-2}	Diameter of basal shoots (cm)	Girth of cut stems (cm)	Number of compound leaves per plant
T ₁	NoS ₀	65.34	32.10	56.90	1.67	4.05	12.00	0.75	0.56	10.00
T ₂	N ₁ S ₁	66.96	38.12	61.02	2.01	4.19	14.00	0.87	0.69	11.20
T ₃	N ₁ S ₂	69.15	38.96	61.96	2.02	4.41	16.00	0.90	0.64	12.18
T ₄	N ₁ S ₃	82.86	39.90	67.52	2.64	4.86	18.00	0.93	0.73	12.74
T ₅	N ₂ S ₁	73.96	39.45	64.91	2.32	4.52	26.66	0.92	0.65	11.75
T ₆	N ₂ S ₂	85.02	41.64	70.75	2.83	5.75	22.00	0.96	0.75	12.20
T ₇	N ₂ S ₃	88.72	42.86	72.90	2.99	5.97	24.00	0.98	0.73	12.97
T ₈	N ₃ S ₁	84.14	40.55	69.04	2.72	5.08	20.00	0.94	0.74	11.95
T ₉	N ₃ S ₂	89.52	43.79	72.96	3.22	6.19	26.00	1.00	0.750	13.94
T ₁₀	N ₃ S ₃	90.41	45.44	74.47	3.64	6.63	30.00	1.05	0.77	14.28
C.D		6.31	2.37	0.552	0.102	0.007	0.631	0.013	0.060	0.059
SE(d)		2.98	1.12	0.261	0.048	0.003	0.298	0.006	0.028	0.020

Table 2: Effect of salicylic acid and sodium silicate on flowering parameters of Rose (*Rosa hybrida*)

Notation	Treatment	Days to first flower bud appearance (day)	Days to colour break stage (day)	Flower bud diameter (cm)	Flower diameter (cm)	Neck girth (cm)	Duration of flush (day)	Total fresh weight of cut stems harvested per plant (g)	Stem dry matter (g)	Vase life (day)
T ₁	N ₀ S ₀	108.56	23.11	1.18	5.5	0.53	33.56	104.96	4.70	9.33
T ₂	N ₁ S ₁	107.66	22.66	1.20	6.10	0.54	40.55	128.88	5.23	10.62
T ₃	N ₁ S ₂	106.69	22.54	1.240	6.30	0.56	40.88	119.58	5.32	10.32
T ₄	N ₁ S ₃	105.99	21.43	1.27	6.68	0.58	42.00	117.08	6.32	9.80
T ₅	N ₂ S ₁	107.09	22.47	1.247	6.62	0.57	41.00	161.48	5.46	10.82
T ₆	N ₂ S ₂	106.83	21.70	1.32	7.06	0.61	41.77	156.20	6.90	10.58
T ₇	N ₂ S ₃	105.66	21.24	1.33	7.13	0.67	42.55	116.00	7.40	9.79
T ₈	N ₃ S ₁	106.68	22.25	1.30	7.05	0.58	41.47	177.82	6.95	11.41
T ₉	N ₃ S ₂	105.55	21.15	1.36	7.20	0.68	42.88	170.55	7.70	11.13
T ₁₀	N ₃ S ₃	105.33	20.57	1.40	7.25	0.73	43.22	114.08	8.15	9.52
C.D		0.438	0.056	0.005	0.045	0.033	2.190	0.568	0.454	0.028
SE(d)		0.207	0.026	0.002	0.021	0.016	1.034	0.268	0.215	0.013

V₁: Avalanche; V₂: Top secret; N₁: Sodium silicate at 1mM; N₂: Sodium silicate at 3mM; N₃: Sodium silicate at 5mM; S₁: Salicylic acid at 1 mM; S₂: Salicylic acid at 3mM; S₃: Salicylic acid at 5mM

b) Effect on flowering parameter

In the present investigation, data recorded on growth parameters presented in Table 2 and Fig. 1-3 revealed that salicylic acid and sodium silicate significantly affected flowering parameters. Minimum days to first flower bud appearance (105.33 days) and days to colour break stage (20.57 days) was observed in treatment combinations of Sodium silicate @ 5mM and Salicylic acid @ 5mM and maximum days to first flower bud appearance (108.56 days) and days to colour break stage (23.11 days) was recorded in control. Early flowering and floral bud sprouts have been induced by salicylic acid concentrations because this stimulating agent accelerates biosynthesis of secondary metabolites. Salicylic acid as a manager of blooming time, interacts with both photoperiod-dependent and self-governing pathways (Martinez *et al.*, 2004) [39]. Salicylic acid stimulated initiation of flowers subsequently involved in the physiological processes (Hayat *et al.*, 2010) [27]. The present results are agreed with the findings of Padmapriya and Chezhiyan (2002) [47] in chrysanthemum and Pawan Kumar (2019) [49] in rose. Also, the mechanism involved in accelerated anthesis remains unclear, even though several studies associated with Si supplementation with increased photosynthesis, decreased transpiration and phytochrome changes especially on agronomic crops (Ma and Takahashi, 2002) [36]. Increase in the concentration of salicylic acid and sodium silicate increases flower bud diameter, flower diameter and neck girth. Maximum flower bud diameter (1.40 cm), flower diameter (7.25 cm) and neck girth (0.73 cm) was observed in treatment combinations of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum flower bud diameter (1.18 cm), flower diameter (5.57 cm) and neck girth (0.53 cm) was observed in control. Salicylic acid has positive effect on photosynthesis in leaves that result in accumulation of more carbohydrates in the shoots, due to this reason, flower bud size, flower diameter and neck girth increased. The increase in flower bud diameter, flower diameter and neck girth could be due to the synergism between SA and auxins. These results are in conformity with Zeb *et al.* (2017) [63] in zinnia. Silicon application has been found to reduce evapotranspiration (McAvoy and Bernard, 1996) [41] which could have contributed to increased turgor pressure within the flower, resulting in cell swelling and thus larger flower bud diameter, flower diameter and neck girth. Maximum duration of flush (43.22 days) was observed in treatment combinations

of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum duration of flush (33.56 days) was recorded in control. Increased levels of ethylene is synthesized several days after full opening of the flower during natural senescence (Woodson *et al.*, 1992) [62]. This increased ethylene production accelerates in rolling of petals resulting in wilting of the flower. PGR's are attributed to the improvement of vegetative growth in plants that leads to increased absorption of nutrient and promotion of photosynthetic activities and finally higher carbohydrate assimilation. This physiological mechanism increased both vegetative and floral development by maintaining hormonal balance that leads to extensive duration of flush of plants. These results are in conformity with Abbass *et al.* (2016) [1] in *Antirrhinum majus* and zeb *et al.* (2017) [63] in zinnia. Maximum stem dry matter (8.15 g) was observed in treatment combinations of Sodium silicate @ 5mM and Salicylic acid @ 5mM and minimum stem dry matter (4.70 g) was recorded in control. The effectiveness of salicylic acid can be due to water relations enhancement, prevent vascular occlusion due to antimicrobial effect, anti-ethylene effect which reduces respiration rate of cut flowers and increased dry matter (Edrisi, 2009) [17].

Maximum total fresh weight of cut stems harvested per plant (177.82 g) was observed in treatment combinations of Sodium silicate @ 5mM and Salicylic acid @ 1mM and minimum total fresh weight of cut stems harvested per plant (104.96 g) was recorded in control. The increases in water uptake and subsequently cut flower fresh weight may be due to the acidifying and stress alleviating properties of SA. Maximum vase life (11.41 days) was observed in treatment combinations of Sodium silicate @ 5mM and Salicylic acid @ 1mM. Minimum vase life (9.33 days) was recorded in control. At high concentrations of salicylic acid, vase life of flowers decreased. This could be due to the addition of low level of salicylic acid which delayed senescence while higher level promoted abscission and also significantly enhanced senescence in cut flowers (MacKay *et al.*, 2000). Application of salicylic acid could reduce the synthesis of ethylene and make the plant capable to block the synthesis of auxins (Shekari *et al.*, 2003) [57]. Kazemi *et al.* (2011) [31] reported that SA treatment extended the vase life with maintaining chlorophyll content. The present results are agreed with the findings of Dumitras *et al.* (2002) [16] in Gerbera and Gladiolus.

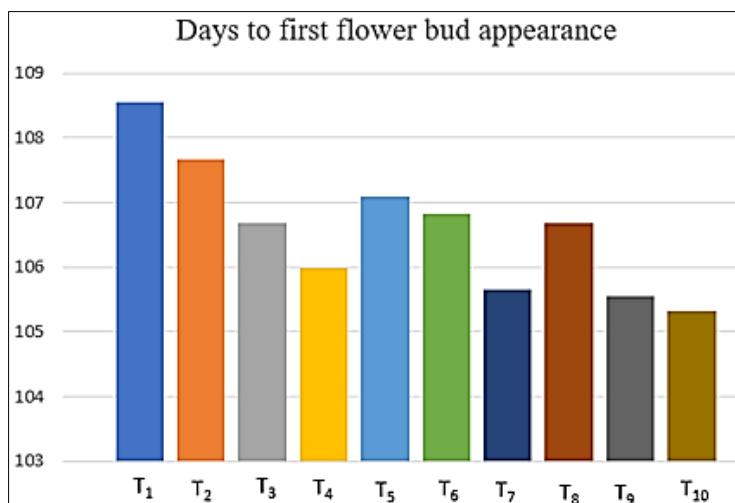


Fig 1: Effect of different concentrations of salicylic acid and sodium silicate on days to first flower bud appearance

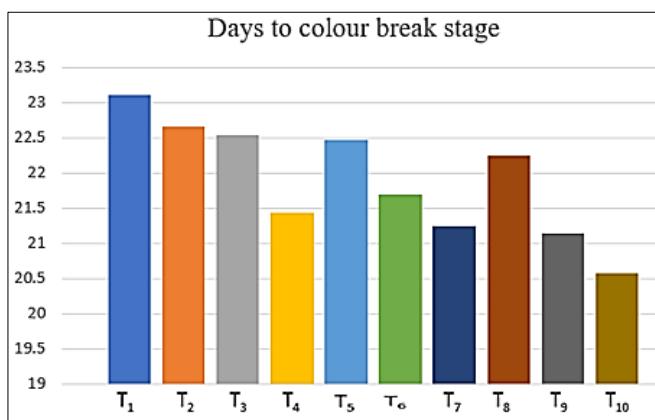


Fig 2: Effect of different concentrations of salicylic acid and sodium silicate on days to colour break stage

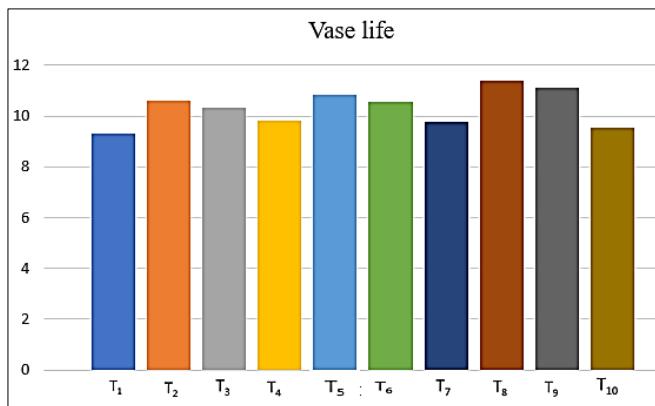


Fig 3: Effect of different concentrations of salicylic acid and sodium silicate on vase life.

Conclusions

In conclusion, an attempt was made to investigate the effect of salicylic acid and sodium silicate, exogenously applied on rose plants under protected conditions. All treatments combinations of salicylic acid and sodium silicate levels affected significantly growth and flowering of rose. The results have shown that quantity and quality of rose production could be increased significantly through exogenous application of sodium silicate and salicylic acid and it could be a useful option to improve vegetative growth and hence leads to flowering of rose. Among different treatments, the application of sodium silicate @ 5mM + salicylic acid @ 5mM was found most effective in improving

growth and flowering of rose except vase life and total fresh weight of cut stems harvested per plant which was found best with the treatment combinations of sodium silicate @ 5mM + salicylic acid @ 1mM.

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