AN ABIOTIC STRESS FACTOR: PHYTOTOXICITY OF HIGH DOSES OF SALICYLIC ACID ON GROWTH PARAMETERS OF *TULIPA* SP. UNDER GLASSHOUSE AND OUTDOOR CONDITIONS

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Abstract. Abiotic and biotic stresses are the major problems in agriculture alone or in combination. Salicylic acid (SA) is recognized as a plant stress tolerance enhancer chemical by including plant metabolic activities. Besides natural occurring, exogenous usage of SA and its doses depend on plant species and growth conditions. This study was carried out to reveal the positive or negative effects on tulip (*Tulipa gesneriana* L. cv. Pretty Woman) growth parameters after dipping the bulbs dipped in aqueous solutions (100, 250, 500 and 1000 mg L^{-1}) of SA under glasshouse and outdoor condition. The evaluated vegetative and flowering parameters were: emergence rate, first emergence day, duration between first-last emergence, vegetative growth success, flowering capacity, healthy flowering capacity, first flowering day, flower life, flower length and flower stalk length. The data indicated that more than 250 mg L^{-1} SA doses had a toxic effect on most of the measured vegetative and flowering characteristics (i.e. vegetative growth success, flowering and healthy flowering capacity) under glasshouse condition. While SA had no negative effect on the measured vegetative growth characteristics, high doses of it had also a negative effect on healthy flowering capacity at outdoor conditions. When considering the mentioned observed and measured parameters, before using SA against stress factors exogenously, SA application recommended after determining the toxicity level for any aimed plants and aimed growth conditions at first step.
Keywords: *Tulipa spp., salicylic acid, toxicity, tulip, geophytes, dipping method, plant abnormalities, plant growth regulators*

Introduction

Tulip (*Tulipa gesneriana* L.), which belongs to the Liliaceae family (Zonneveld, 2009; Christenhusz et al., 2013), is a famous, well known ornamental flowering bulbous plant that is used as a cut flower, a pot flower and a garden plant. One of the most important issues in flowering and bulbous ornamental plants is to ensure the health and longevity of the flower. Well-known growth factors, such as biotic or abiotic factors, play a role in plant morphology, plant health and flowering time changes. One of the vital factors is the environmental conditions in the planting area. At the same time, some applications such as exogenous usage of plant growth regulators or substances at proper concentrations encourage plant growth via reducing biotic diseases and via dealing with abiotic stresses.

Salicylic acid (SA) is a phenolic derivative (Hayat et al., 2007), probably present in all plants (Raskin et al., 1990). SA is classified within the plant hormones (Raskin, 1992a). The synthesis of SA is induced by biotic and abiotic stress factors and therefore its intracellular and extracellular transport begins (Maruri-López et al., 2019). In addition to the natural presence of SA, there are many successful studies on its use against abiotic

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stress by external applications for example salt tolerance (Kowalska and Smoleñ, 2013; Jini and Joseph, 2017; Ma et al., 2017), chilling tolerance (Min et al., 2018; Chen et al., 2020) and drought tolerance (Damalas, 2019; Haghighi et al., 2021) with different application methods. On the other hand, there are an increasing number of studies in the usage of external applications against diseases that are biotic stress factor, especially to *Fusarium* (Mandal et al., 2009; Makandar et al., 2012) and on yield with growth (Pradhan et al., 2016; Semida et al., 2017).

As well-known plant growth regulators and chemicals including salicylic acid for useful aims for avoiding biotic and abiotic harmful effects, can be toxic on plant if they do not applicate at proper concentration and proper methods. The purpose of this study was to assess the negative effects of high concentrations of Salicylic acid (SA) on the growth attributes of *Tulipa gesneriana* cv. Pretty Woman under glasshouse and outdoor environmental condition for determining the proper dose to be a pioneer in future abiotic and biotic stress studies.

Materials and Methods

Experimental site

The glasshouse and outdoor experiments were conducted at the Agriculture Faculty of Kocaeli University in Kocaeli city, Türkiye between January 31st, 2021 and May 31st, 2021. At the experimental area the altitude is 77.4 m, latitude is $40^0$71’ N and longitude is $30^0$02’ E. According to long term data (1921-2021), the average highest and lowest temperature were in August (29.7 ^{0}C) and in January (3.2 ^{0}C). The average highest and lowest sun-take durations were in July (9.5 hours) and in January (2.5 hours). The average monthly precipitations are changed between 43.2 mm in August and 109.7 mm in December. In that part of the country, winters are mostly cold, rainy and seldom snowy, summers are hot and seldom rainy. The climate is very suitable for bulbous plant growth and there are some ornamental plant growers for trade aims. In the study the sowed bulbs were placed to grow in the glasshouse without heating or cooling and in the outdoor place (open-field) covered from only six meters high, and each side was open with full sun, as it was intended to irrigate with the same amount of irrigation water.

Plant material

The tulip bulb variety (*Tulipa gesneriana* L. cv. Pretty Woman) used in the study was obtained from an officially-registered flower-bulb company in Türkiye. According to Orlikowska et al. (2018) [adaption after Van Scheepen (1996) and Okubo and Sochacki (2013) description] cv. Pretty Woman is in the Lily-flowered group. The purchased three times more than needed bulbs (since it is necessary to choose similar bulbs) were held in a cold storage warehouse at 5^0C±2 for one month immediately after purchasing on October, 19^{th} 2020. Between November, 19^{th}, 2020 and January 30^{th}, 2021 the bulbs were kept under darkness and an airy condition in the laboratory until sowing. Just before the water and SA solution application, the tunics of the bulbs were removed (*Figure 1A*) and bulbs were checked for similarity, some characters, such as circumference, diameter and weight of the bulbs were noted. According to the initial measurements a bulb was 9.36 cm in circumference, 2.98 cm was in diameter, and 16.51 g in weight according to the mean of all used naked bulbs.

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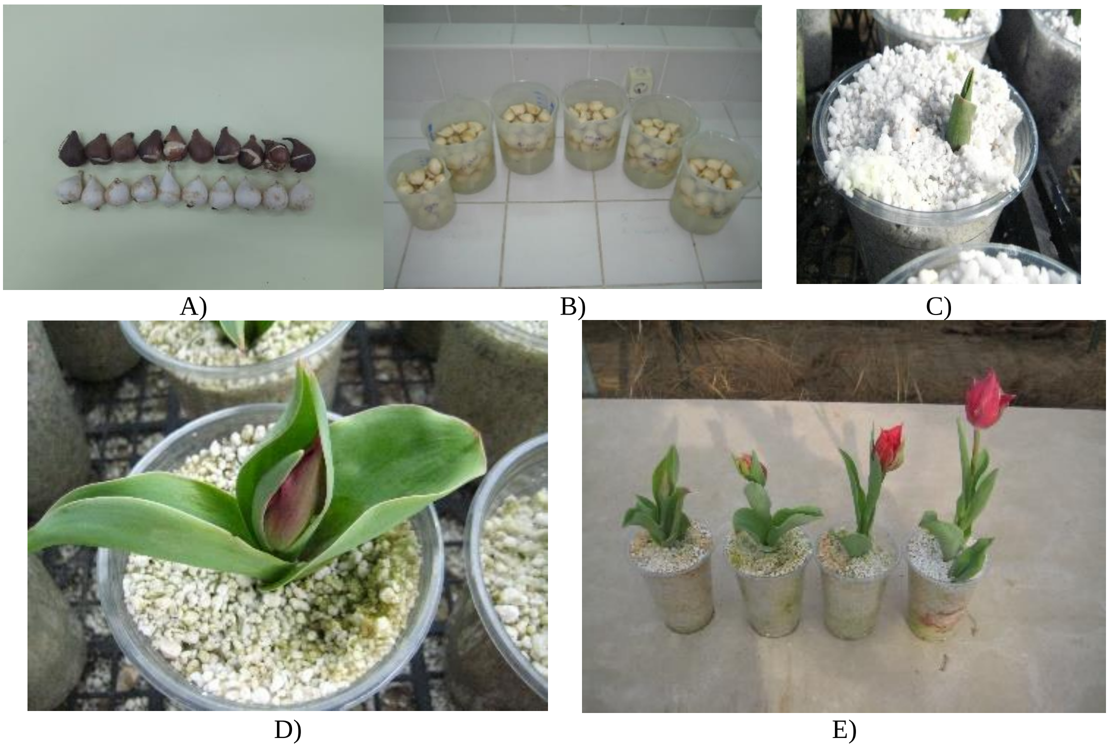

***Figure 1.** Tulipa gesneriana cv. Pretty Woman; A) The bulbs with tunic in the top row and the used bulb samples after removing tunics just before treatment in the bottom row, B) Salicylic acid application via dipping method C) An emergency sample, D) The first flowering time at which flower color is obvious, E) Growth steps of the Tulip flowers*

Salicylic acid applications

The molecular weight of the Salicylic acid (SA) ($C_7H_6O_3$) was 138.12 g mol^{-1} with in ≥99.5 purity. SA concentrations were prepared with distilled water of 100 mg L^{-1}, 250 mg L^{-1}, 500 mg L^{-1} and 1000 mg L^{-1}, besides only water treatment (0 mg L^{-1} SA) as Control-Water. The bulbs were treated for 24 hours with the dipping method (*Figure 1B*). In addition, there were Control-Dry (the bulbs were not treated any of water or SA solutions) waiting ready right next to them (*Figure 1B*). All SA and water treatments also non-treated bulbs were kept under normal laboratory conditions in darkness. After 24 hours all SA and water treated in addition to non-treated bulbs sowed under approximately 8 cm deep from the surface in the pots that volume 450 ml filled with perlite (just to reveal the effect of SA purely) immediately on January, 31st 2021. The pots and perlite have not been used before, newly purchased and opened their packaged just before the study. The dipping method was based on Atait and Qureshi (2020). The one of the chosen concentrations (100 mg L^{-1}) also based on the same study in which 0.1 g/L SA was used. Similarly, SA were used in some agricultural experiment as 50-200 mg/L in a study with onion (Amin et al., 2007), as 0-1.5 mM on cyclamen (Farjadi-Skakib et al., 2012) and as 0-300 ppm with garlic (Shama et al., 2016). The similar concentrations were chosen by us. In addition to the doses higher concentrations were prepared for obtain definite results.

Evaluations

The results of some vegetative and floral characteristics were noted daily during the experiment (*Figure 2*) until May, 31st 2021 when all leaves dried. The results were revealed in terms of both two growing sites and all treatments (Control-Dry, Control-Water, 100 mg L^{-1} SA, 250 mg L^{-1} SA, 500 mg L^{-1} SA and 1000 mg L^{-1} SA). Evaluations were made in terms of plant growth characteristics;

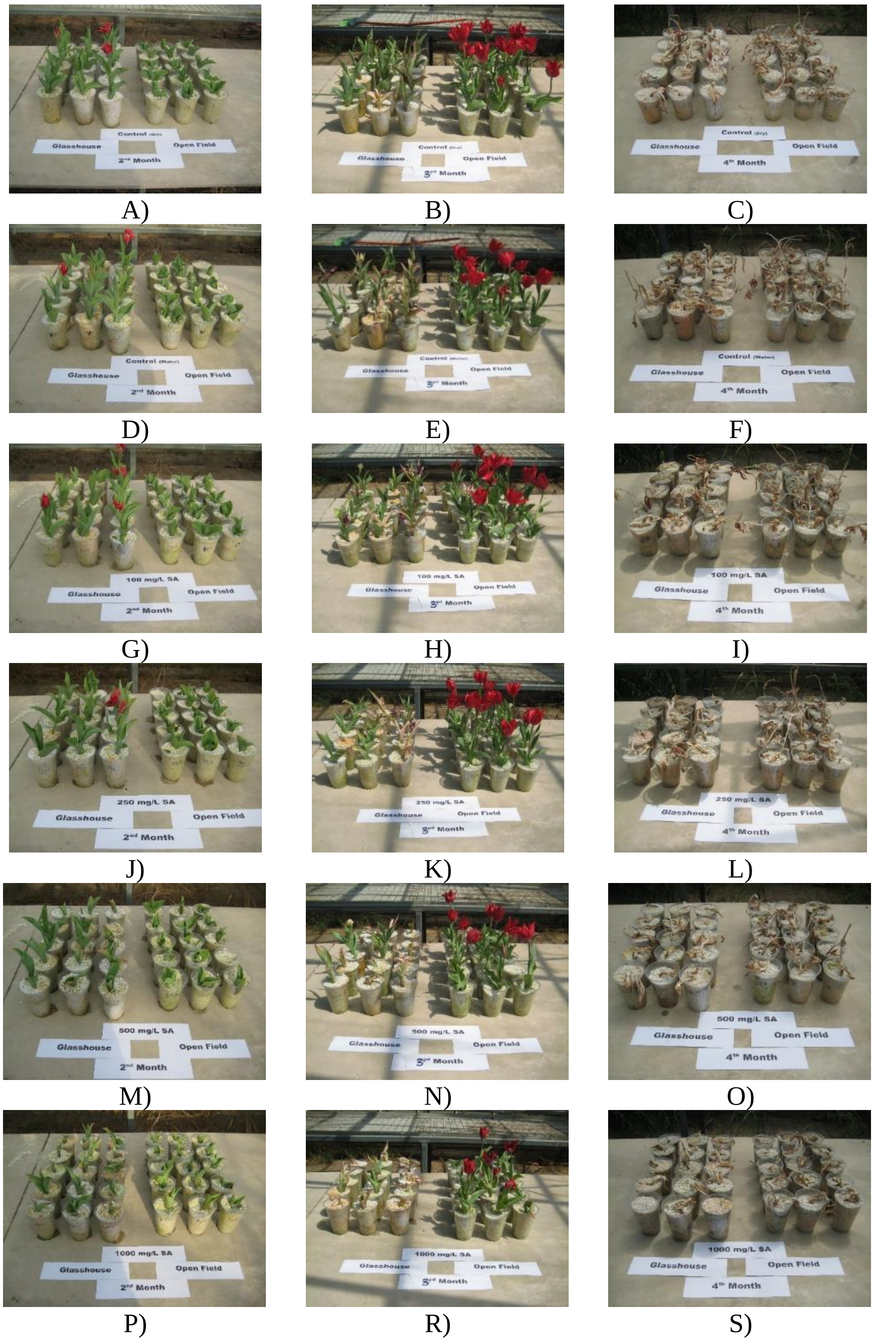

Figure 2. *Growth steps of Tulipa gesneriana L. cv. Pretty Woman in 2nd, 3rd and 4th months left to right ; A, B, C) Control-Dry , D, E, F) Control-Water treatment, G, H, I) 100 mg L^{-1} SA treatments, J, K, L) 250 mg L^{-1} treatment, M, N, O) 500 mg L^{-1} treatment, P, R, S) 1000 mg L^{-1} treatment (in each photo; left fifteen plants in the glasshouse and right fifteen plants in the outdoor place)*

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Emergence Rate (%): from all the bulbs sowed (*Figure 1C*),

First Emergence Day (day): from all emerged bulbs on which day after sowing,

Duration between first-last emergence (days): from first days to last days from all plots has emerged bulbs,

Vegetative Growth Success (%): from all the bulbs sowed that can continue its vegetative development until the end even if it does not bloom,

Flowering Capacity (%): from all the bulbs sowed, that were contained unhealthy flower (without stalk or unusual weak flower) and healthy flower (with stalk and familiar developed flower),

Healthy Flowering Capacity (%): from all the bulbs sowed (with stalk and familiar developed flower),

First Flowering Day (Day): from all healthy flowers when the flower bud color becomes slightly understood on which day after sowing (*Figure 1D*),

Flower Life (Days): from all healthy flowers from when the flower bud color becomes slightly understood to when the first tepal falls (*Figure 1E*),

Flower Length (cm): from all healthy flowers from the base of tepal to the end,

Flower Stalk Length (cm): from all healthy flowers from the substrate surface to the base of tepals.

Statistical analysis

The experiment laid out in Completely Randomized Design (CRD), consisted of six treatments with three repeats and each repeat consisted of five bulbs. Totally 6 treatments×2 growing sites×3 repeats×5 bulbs=180 bulbs were sowed in pots and observed. The recorded data were analyzed through statistical software (SPSS Statistics 16.0). A two-way analysis of variance (ANOVA) was used to test for differences in the calculated data. Multiple comparison was made using Duncan's Multiple Range Test at P≤0,05. Results were shown as mean values and standard deviation (±) in the tables.

Results and Discussion

The data showed that the emergence rate was affected by neither SA applications nor growth sites (*Table 1*). Apical meristem is controlled by endogenous factors which are under genetic control unless something unusual happens (Barton, 2010). On the other hand, first emergence day was statistically affected by SA treatments in the glasshouse. The first emergence day was shortened by the highest concentration of SA compared to Control-Dry, Control-Water and lower doses of SA gradually in the glasshouse, but this effect was seen at only the highest two concentrations whose effectiveness were only numerically in the outdoor place. Similarly, Shakirova et al. (2003) found that exogenously dipping of *Triticum aestivum* with SA as pre-sowing methods leads to the activation of germination and seedling growth. In a study (Kumar and Nanda, 1981) on *Impatiens balsamina*, SA hastening bud initiation was observed as ours. According to the data on vegetative growth success, high doses of SA (250, 500 and 1000 mg L^{-1}) in the glasshouse showed negative effect while the parameter showed no differences in the outdoor place (*Table 2*). Supportingly, Clarck et al. (2004) emphasized that plants' responses to chemical exposure results are less parallel sometimes between growing on field and glasshouse. In addition to the emphasis, the flowering capacity gradually decreased at high SA concentrations in the glasshouse, especially obtaining healthy flowers were affected negatively by again high SA concentrations both in the glasshouse

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and outdoor place in our experiment (*Table 3*, *Figure 2*). Klessig and Malamy (1994) and Raskin (1992b) stressed that flowering is variable parameter under SA applications plant to plant even species. As seen in *Tables 4* and *5* some flower parameter could not be calculated at the higher concentrations of SA in the glasshouse because of insufficient healthy flowers in each repeat. The data showed that more than 100 mg L^{-1} SA has a harmful effect on healthy flowering and related parameters which has crucial importance in flowering-ornamental plants. Nevertheless, it is possible to say that first flowering day came about earlier in the glasshouse than in the outdoor place in all treated concentrations and also the dose of 100 mg L^{-1} SA was more successful with 58^{th} day than other doses in the glasshouse. Similarly, some studies (Martin-Mex et al., 2005; Larqué-Saavedra and Martin-Mex, 2007) emphasized SA effectiveness on early appearance in flowers.

Table 1. *Emergence Rate (%) and Emergence Day of Tulipa gesneriana cv. Pretty Woman at glasshouse and outdoor conditions*

	Vegetative Characteristics*					
Salicylic Acid Concentration	**Emergence Rate (%)**			**First Emergence Day (day)**		
	Glasshouse	Outdoor	Mean	Glasshouse	Outdoor	Mean
Control-Dry	100±0,00	100±0,00	100±0,00	26,5±1,21 c*	21,5±4,11	23,9±3,86 c
Control-Water	100±0,00	93,3±11,55	96,7±8,17	19,0±5,24 b	18,9±2,86	18,9±3,78 b
100 mg L^{-1}	100±0,00	100±0,00	100±0,00	19,9±2,70 b	20,7±4,43	20,3±3,31 bc
250 mg L^{-1}	100±0,00	93,3±11,55	96,7±8,17	18,4±2,55 b	22,4±1,59	20,4±2,89 bc
500 mg L^{-1}	93,3±11,55	93,3±11,55	93,3±10,33	14,9±2,19 ab	17,3±4,49	16,1±3,41 ab
1000 mg L^{-1}	100±0,00	100±0,00	100±0,00	12,1±3,08 a	16,1±1,21	14,1±3,03 a
Mean	98,9	96,7	97,8	18,5	19,5	19,0

*Different lower case letters within a column denote significant differences at the p≤0,05 level within treatments under the each growth conditions or their mean separately

Table 2. *Duration Between The First-Last Emergence (days) and Vegetative Growth Success (%) of Tulipa gesneriana cv. Pretty Woman at glasshouse and outdoor condition*

	Vegetative Characteristics*, **					
Salicylic Acid Concentration	**Duration Between The First-Last Emergence (days)**			**Vegetative Growth Success (%)**		
	Glasshouse	Outdoor	Mean	Glasshouse	Outdoor	Mean
Control-Dry	17,0±4,00 b A	18,3±1,53 A	17,7±2,81	100±0,00 a A	100±0,00 A	100±0,00 a
Control-Water	18,0±7,55 b A	16,7±2,52 A	17,3±5,09	100±0,00 a A	93,3±11,55 A	96,7±8,17 ab
100 mg L^{-1}	17,7±6,43 b A	17,7±6,35 A	17,7±5,72	93,3±11,55aA	100±0,00 A	96,7±8,17 ab
250 mg L^{-1}	21,3±4,51 b A	16,7±10,02 A	19,0±7,40	86,7±11,55abA	93,3±11,55 A	90,0±10,95abc
500 mg L^{-1}	14,0±2,65abA	12,7±1,53 A	13,3±2,07	73,3±11,55bcB	93,3±11,55 A	83,3±15,06 bc
1000 mg L^{-1}	7,0±3,46 a A	14,7±4,04 B	10,8±5,38	66,7±11,55cB	93,3±11,55 A	80,0±17,89 c
Mean	15,8	16,1	16,0	86,7 B	95,6 A	91,1

*Different lower case letters within a column denote significant differences at the p≤0,05 level within treatments under the each growth conditions or their mean separetely.
**Different uppercase letters within a line denote significant differences at the p≤0,05 level between growh conditions under the same treatment or their mean separately

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Table 3. *Flowering capacity (%) and Healthy flowering capacity (%) of Tulipa gesneriana cv. Pretty Woman at glasshouse and outdoor condition*

Salicylic Acid Concentration	Flowering Characteristics*, **					
	Flowering Capacity (%)			Healthy Flowering Capacity (%)		
	Glasshouse	Outdoor	Mean	Glasshouse	Outdoor	Mean
Control-Dry	100±0,00 a	100±0,00	100±0,00 a	40,0±20,0 a B	93,3±11,55aA	66,7±32,66
Control-Water	100±0,00 a	93,3±11,55	96,7±8,17 a	46,7±23,09a B	86,7±11,55aA	66,7±27,33
100 mg L^{-1}	93,3±11,55 ab	100±0,00	96,7±8,17 a	46,7±50,33a B	100±0,00 a A	73,3±43,21
250 mg L^{-1}	86,7±11,55 ab	93,3±11,55	90,0±10,95a	13,3±23,09b B	80,0±20,0 a A	46,7±41,31
500 mg L^{-1}	73,3±11,55 b	93,3±11,55	83,3±15,06ab	6,7±11,55 b B	60,0±34,64abA	33,3±37,24
1000 mg L^{-1}	46,7±23,09 c	93,3±11,55	70 ±30,33 b	0,0±0,00 c B	40,0±34,64 bA	20,0±30,98
Mean	83,3 B	95,5 A	89,4	25,6 B	76,7 A	51,1

*Different lower case letters within a column denote significant differences at the p≤0,05 level within treatments under the each growth conditions or their mean separately.
**Different uppercase letters within a line denote significant differences at the p≤0,05 level between growth conditions under the same treatment or their mean separately

Table 4. *First Flowering Day (Day) and Flower Life (Days) of Tulipa gesneriana cv. Pretty Woman at glasshouse and outdoor condition*

Salicylic Acid Concentration	Flowering Characteristics*, **, ***					
	First Flowering Day (Day)			Flower Life (Days)		
	Glasshouse	Outdoor	Mean	Glasshouse	Outdoor	Mean
Control-Dry	62,1±1,72 b A	71,8±1,50 B	66,9±5,51	14,2±1,27 B	17,5±0,46 A	15,9±2,03
Control-Water	61,2±1,65 b A	73,6±2,42 B	67,4±7,02	14,9±0,17 B	16,5±0,50 A	15,7±0,94
100 mg L^{-1}	58,3±0,64 a A	72,7±1,62 B	65,5±7,99	15,4±1,98 B	17,4±0,40 A	16,4±1,69
250 mg L^{-1}	-***	72,4±1,37	-	-	18,7±0,08	-
500 mg L^{-1}	-	72,0±1,00	-	-	16,9±1,00	-
1000 mg L^{-1}	-	73,5±1,80	-	-	15,8±2,14	-
Mean	-	72,7	-	-	17,1	-

*Different lower case letters within a column denote significant differences at the p≤0,05 level within treatments under the each growth conditions.
**Different uppercase letters within a line denote significant differences at the p≤0,05 level between growth conditions under the same treatment.
***(-) indicates Insufficient data provided

On the contrary to the first flowering day, flower life in a pot which is another crucial parameter for the plant, was found statistically longer in the outdoor place than in the glasshouse in all data taken into account at calculatable concentrations (*Table 4*). Similarly, the flower length and the flower stalk length were higher in the outdoor place than in the glasshouse in all calculatable concentrations that can be compared. There are some studies about the glasshouse cultivation for flower aim (Nayeem and Qayoom, 2015; Saad, 2018), but in our conditions and used cultivar subsequent studies will be necessary in the glasshouse cultivation. In our study -with some exception in 250 mg L^{-1}

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SA- higher doses of SA damaged all flowering-related parameters in the greenhouse. Also there was no difference between 100 mg L^{-1} SA and Control-Dry or Control-Water applications in terms of the flower life, the flower length and the flower stalk length at both growing sites (*Table 5*). These results revealed the must to work on concentrations between 0-100 mg L^{-1} SA. Similarly, in a study about *Raphanus sativus* submitted to water stress, emphasized that SA or ASA showed toxicity in seeds treated at 10 mM concentration (Ferreraze et al., 2019). Rozman and Doull (2000) stressed dose and time, Clarck et al. (2004) stressed plant taxonomy, location and test protocol as variables of toxicity.

Table 5. *Flower Lenght (cm) and Flower Stalk Lenght (cm) of Tulipa gesneriana cv. Pretty Woman at glasshouse and outdoor condition*

	Flowering Characteristics*, **					
Salicylic Acid Concentration	**Flower Lenght (cm)**			**Flower Stalk Lenght (cm)**		
	Glasshouse	Outdoor	Mean	Glasshouse	Outdoor	Mean
Control-Dry	4,6±0,65 B	6,5±0,45 A	5,5±1,16	15,2±2,17 B	17,4±1,14 A	16,3±1,96
Control-Water	4,8±0,40 B	6,4±0,38 A	5,6±0,96	15,0±2,38 B	15,9±0,56 A	15,5±1,62
100 mg L^{-1}	4,4±0,33 B	6,6±0,23 A	5,6±1,20	14,7±0,52 B	18,0±0,81 A	16,3±1,89
250 mg L^{-1}	-**	7,1±0,27	-	-	18,2±1,80	-
500 mg L^{-1}	-	6,4±0,70	-	-	17,2±2,71	-
1000 mg L^{-1}	-	5,9±0,72	-	-	13,6±3,11	-
Mean	-	6,5±0,55	-	-	16,7	-

*Different uppercase letters within a line denote significant differences at the p≤0,05 level between growth conditions under the same treatment or
** (-) indicates Insufficient data provided

Conclusion

It can be possible to make a general assessment about vegetative development with SA usage. SA did not affect the emergence rate in both growth environments and did not affect the first emergence day in the outdoor place where is the usual growth place for the tulips. Similarly, the duration between the first-last emergence day was not affected by SA in both growth environments except 1000 mg L^{-1} SA in the glasshouse. On the other hand, vegetative growth success was negatively and gradually affected from 250 mg L^{-1} SA to 1000 mg/SA at glasshouse conditions while there was no difference in vegetative growth success at outdoor conditions. The flowering and the healthy flowering capacity decreased gradually in the glasshouse while only healthy flowering capacity decreased at highest doses of SA in the outdoor place. It is obvious that the marketable healthy pot flower capacity was considerably highest in control plants grown in the outdoor place. Moreover, it was higher in low SA dose (i.e. 100 mg L^{-1}) treated plants in the outdoor place than in the glasshouse. In all these manners SA application at 100 mg L^{-1} was found hopeful for some vegetative and flowering characters in tulip at glasshouse cultivation, higher doses of more than 100 mg L^{-1} showed toxicity in the plant for the two characteristics. On the other hand, new studies need to be worked with lower doses of SA effectiveness under different environmental factors such as edaphic, climatic etc. in terms of plant protection. The exposure dose and the location determine the phytotoxicity. If it

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is desired to use SA as a remedy and preventive agent for biotic and abiotic problems, it must be determined firstly the dose for each location independently that is not toxic to any aimed plants.

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