EVALUATION OF THE PHYSIOLOGICAL RESPONSE OF ORNAMENTAL SUMMER SEASON ANNUALS TO SALINITY STRESS

ABSTRACT

Over the past several decades, the southwest zone of Punjab is encountering various problems such as waterlogging, high use of surface canal water for irrigation and swift rise in water demandlead to stagnation in saline zone due to salinity ingression. Due to salinization, most of crops failed to grow, therefore, identification of salt tolerant plants and to check the level of tolerance to salinity in order to prevent mortality plants in salt affected areas is highly required for environment sustainability. To understand these salinity issues, the present study was conducted at Punjab Agricultural University,Ludhiana, Punjab, India to screen four ornamental summer season annual species ideal for salt affected areas of Punjab. Screening was done on the basis of physiological changes in leaves of plants. The one month old seedlings of four different annual species were grown in 8 inch earthen pots were subjected to five levels of sodium chloride salinity stress (control, 30, 60, 90, and 120 mM). Thefactorial completely randomized block design was followed in the experiment with three replicates in each treatment. The physiological parameters viz., chlorophyll, carotenoid, relative leaf water content, electrolyte leakage and proline content were calculated to depict the salt tolerant ornamental summer season annual species.It has been concluded on the basis of physiological response, the maximum salt tolerance was observed in *P. grandiflora*followed by *B. scoparia*, *T. erecta* and *Z.elegans*.

Key words: Salinity, Physiological changes, Summer annuals, Chlorophyll

Introduction

Soil salinity is emerging as a global environment problem and a major threat to agriculture sustainability and productivity particularly in arid and semi-arid regions where rainfall is insufficient to meet the water requirements of the crops, and leach mineral salts out of the root-zone(Hossain*et al.* 2012).Saline environment is commonly found in seashore areas, drought lands and temperate inlands with higher evaporation.Identification of salt-tolerant crop varieties in arid and semi- arid regions is highly important due to salt accumulation on soil, restrictions on groundwater use, and saltwater intrusion into groundwater. Salt-tolerant plants have the ability to minimize these detrimental effects by producing a series of morphological, physiological, and biochemical processes

It has been estimated thatmore than 800Mha of land area are affected by salinity in the world, i.e. 10% of the world's total arable land area (Munns and Tester *et al* 2008). According to the soil map of the world, the regional estimate of the saline and sodic soils was between 397 and 434 mha, which constitute about 15% of the global land area (FAO 1997). These were distributed essentially in the Asia and the Pacific Australia (8%), Europe (3.6%), Latin America (2.5%), Africa (1.8%), Near East (0.8%) and North America (0.8%). In India, out of total geographical area (329Mha), salt-affected soils accounts for 6.73Mha i.e. 2.10% of the geographical area (Arora*et al* 2017).In Punjab, 6.40% of the total geographical area is affected by salinity (Sharma *et al* 2011). Ground water in many regions of Punjab contains high amount of dissolved salts having EC 2-7 ds/m (Shakya and Singh 2010).It has been estimated that the area under salinity keeps on increasing annually at a rate of 10% due to less precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water and poor cultural practices. Because of high salinity,plants lose their turgor as plant growth is more influenced by salt than other toxic substances (Xiong and Zhu 2002). Moreover, crops grown on saline soils suffer on an account of high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions and reduced crop productivity. Researchers has analyzed that more than 50% of the arable land would be salinized by the year 2050 (Jamil et al., 2011).

The ability to survive at a particular salinity is important ecologically in influencing the natural distribution of plant species in salt-affected soils.Salinity and poor quality irrigation water is most prevalent issue in arid and semi-arid regions, so there is requirement to select the plants that can make arid and semi-arid regions green.

FloweringAnnual plants complete their life cycle in one year or one season. Summer season annuals are those which grow luxuriantly and produce flowers under high temperature. The four summer season annual species selected for the present investigation were Marigold (*Tageteserecta*), Zinnia (*Zinnia elegans*), Portulaca (*Portulacagrandiflora*), Kochia (*Bassiascoparia*). Marigold (*Tageteserecta*) is a summer season annual plants belong to family Asteraceae and is native to Central and South America. It is grown for beautification of garden as well as for loose and cut flowers. The essential oil extracted from few species of *T. erecta* can find use in perfumery industry (Bhattacharjee*et al* 2006). Zinnia (*Zinnia elegans*) is a summer annual plants belongs to family Asteraceae and is native to Mexico. Both annual as well as perennial species are available but in India zinnia is popularly grown as a summer annual. It can be grown in garden for edging, bedding, rock gardens and hanging baskets etc.Portulaca (*Portulacagrandiflora*) is a colorful annual flowering plant belongs to family Portulacaceae and native to Argentina and Southern Brazil. It is easy to grow in rock gardens. It makes a lovely ground cover and edger and is perfect for container or hanging baskets. Kochia (*Bassiascoparia*) belongs to family Chenopodiaceae and is native to Eurasia. Thin and finely cut leaves of Kochia add its ornamental value and grown in almost any soil except very acidic soils. It is helpful to control soil erosion by growing at a mass level.

The objective of the present investigation was to study the relative tolerance of four summer season annual plants, which has been compared on different levels of induced salinity.There are many reports that depict that salt-tolerant species can be categorized using physiological criteria such as chlorophyll content, carotenoid content, relative leaf water content, electrolyte leakage and proline content (Percival and Fraser 2001).An understanding of growth and survival of plants under saline habitat conditions is needed for

(i) Screening the plant species for growing in saline affected areas for ornamental purpose

(ii) To understand the physiological phenomenon being used by the salt tolerant plantsto tolerate salt stress.

Material and method

Seeds of *Zinnia elegans*, *Bassia*scoparia, *Portulacagrandiflora* (Moss-rose Purslane) and *Tageteserecta* (African marigold) were sown during first week of March 2019 at Landscape Nursery, PAU Ludhiana. Seedlings of plants were transplanted in 8 inchearthen pots on first week of April 2019 by using Soil: FYM (2:1) as growing media. After transplanting different concentration of NaCl, i.e. 30mM (1.4g/800ml of water/pot), 60mM (2.80g/800ml of water/pot), 90mM (4.2g/800ml of water/pot) and 120mM (5.60g/800ml of water/pot) were given with irrigation water and EC of media was maintained according to different concentration of salt. The irrigation water volume was determined by adding the leaching amount of water consumed by the plants. These NaClconcentrations were given till the end of season i.e. July 2019. The data was recorded after one month of transplanting. The following physiological analysis was performed: Chlorophyll and Carotenoid were determined in leaves according to method described by Hiscox and Israelstam 1979 and Kirk and Allen 1965, Electrolyte leakage and Proline were determined in the leaves by the method Deshmukh*et al* 1991and Bates *et al* 1973. Relative leaf water content was also measured in the leaves of plants.

Statistical analysis

The experiment was arranged in a Completely Randomized Design (CRD) with 3 replications and 10 plants per treatment per replication. SAS software version 9.3 was used and the mean were compared using Duncan’s New Multiple Range test (DMRT).

Results and discussion

Chlorophyll and Carotenoid (mg/g fresh weight)

Photosynthetic pigments such as chlorophyll and carotenoid content are significantly affected by salt stress. The results in the fig.1 showed that among species, maximum chlorophyll content was observed in *T. erecta*(2.45) and minimum in *P. grandiflora*(0.19). Maximum chlorophyll was recorded under T1 control (non-saline condition) in *Z. elegans* (1.14), T4 (90mM) in*T. erecta* (3.01), T5 (120mm) in *P. grandiflora* (0.35) and T3 (60mM) in *B. scoparia* (1.54) as shown in Table 1. During the months from April to July 2019, there was slight increase in total chlorophyll content in *P. grandiflora*(41.3%) and *B. scoparia* (44.5%).Increase in chlorophyll content in these plants indicates that the plants managed to survive under salt stress conditions. However, in *Z. elegans* and *T. erecta* the photosynthetic pigment i.e. total chlorophyll content was decreased with increasing concentration of salt solution i.e. 52.8% and 41.2% as compared to control.The depressing effect of salt stress on chlorophyll biomass may be due to the formation of proteolytic enzyme such as chlorophyllase, which is responsible for the chlorophyll degradation (Kaya *et al* 2003) and damaging the photosynthetic apparatus (Singh and Jain 1981).

Among these four plant species, maximum carotenoid content was observed in *T. erecta* (0.09) and minimum was in *P.* grandiflora (0.02) as in fig.2. Among interaction of plant x month x salt treatment, it was found that carotenoid content maintained in *P. grandiflora*.Whereas, in *B.* scoparia, *Z. elegans*, *T.erecta*, carotenoid content slightly decreased as salt concentration increased. The maximum percent decrease was observedin *T. erecta* (45.4%) followed by *Z. elegans*and *B. scoparia*, shown in Table 2.Sayyed*et al* (2014) reported that carotenoid content of *Tageteserecta* decreased with increased concentration. Our results agree with several reports of decrease content of chlorophyll and carotenoids by salinity as reported in a number of glycophytes. Carotenoidplays an important role in plants as antioxidants and protects chlorophyll pigment from photo damage (Alexander 1999). Havaux (2014) studied a new function of carotenoid, which relates to the response of plants to environmental stresses.

Relative leaf water content (%)

Relative water content in plants is probably the most appropriate measure of plant water status in terms of physiological consequence of cellular water deficit. The present results revealed that RLWC varies significantly with salt treatments, different plant species, months and salt treatment x plant x month, interaction. The maximum RLWC was found in *P. grandiflora* (90.89) and minimum was in *Z. elegans*(35.98). The relative water content reduced significantly with the increase in the salinity stress and reduce maximum under 120mM of NaCl. Under non-saline condition, the RLWC was maximum in variety *Z. elegans* and *T. erecta*. But in *P. grandiflora*and *B. scoparia*maximum RLWC was observed under 30mM, 60mM and 30mM, 120mM concentration of NaCl respectively (Table 3). The relative water content (RWC) did not differ significantly in *C. paludosum*because plants saved a large amount of water in the leaf discs and not affected by salt stress (Yasemin*et al* 2017). The maximum reduction was observed in *T. erecta* while minimum reduction was observed in *P. grandiflora* followed by *B. scoparia*. Karimi*et al* (2005) reported that in *Kochiaprostrata*, the relative leaf water content decreased with the increased concentration of salt in soil. Thus, *P. grandiflora*and *B. scoparia*had the highest value of salt tolerance index. Whereas, *Z. elegans* had the lowest value for salt tolerance index for RWC among all other varieties.

Electrolyte leakage (%)

Electrolyte leakage permits cell membrane injury to be evaluated when plants are gone through salinity stress. Tuna *et al* 2007 and Tiwari*et al* 2010 reported that plasma membrane permeability has been an effective selection criterion for salt tolerance in different crops. Among different species, maximum electrolyte leakage was observed in *P. grandiflora*

(40.97) and minimum was observed in *T. erecta*(14.55). Among months, during July 2019, electrolyte leakage was slightly increased in all plants except *Z. elegans* because *Z. elegans* survived up to May 2019. In *Z. elegans*, *T. erecta* and *P. grandiflora*, electrolyte leakage were more under T4 and T5, while in *B. scoparia* electrolyte leakage was more under non saline conditions and decreased with the increased concentration of salt (Table 4). Guan *et al* (2012) reported that electrolyte leakage increased in *Chrysanthemum okiense* and *C. ornatum* under salt stress.

Proline (mg g^{-1} dry wt.)

The observations in the fig.5 revealed that proline was accumulated more in *P. grandiflora* (90.89) due to increased salt concentration that helps the plants to tolerate it and less in *Z. elegans*. Plants accumulate compatible solutes such as proline to with stand salt stress, which helps to decrease the osmotic potential, facilitating water absorption and scavenges reactive oxygen species (ROS) molecules (Qureshi*et al* 2016). In *Z. elegans*, increased concentration of salt cause lowproline level due to which plants did not tolerated salt stress and become withered. In *T. erecta*and *B. scoparia*, the results showed at par observations. Among treatments, proline was more accumulated under non saline conditions in *Z. elegans* and *T. erecta*, however in *P. grandiflora*, it was observed more under T2 (30mM) and T3 (60mM) and in *B. scoparia*, it was more observed under T5 (120mM) i.e. higher dose of NaCl in the experiment as shown in Table 5. Similar results were observed by Mazhar*etal* (2012) that proline content in *Chrysanthemum indicum* increased as a result of increasing salinity. Thus, *P. grandiflora* and *B.* scoparia had the highest value of salt tolerance index as compared to other two species. The results of present study agreed with Chen *et al* (2003) and Rahdari*et al* (2011), they observed that Proline accumulated significantly with consequent increase in level of salinity. Don *et al* (2010) evaluated that the proline content increased in gerbera with increased salinity levels and this proline depict the adaptive response of gerbera to short term salinity.

Conclusion

This experiment was conducted to contextualize the possibility about saline irrigation water may be used to grow ornamental flowers in pots under sub-tropical condition of Punjab for summer season. The results of the experiment depicted that the increased salinity concentration significantly reduced the chlorophyll, carotenoid, relative leaf water content and proline in *Z. elegans* and *T. erecta*, due to increased cell membrane injury during the season. In *P. grandiflora*and *B. scoparia*chlorophyll content, relative leaf water content and proline level increased and carotenoid content was maintained with increase in salinity level during the experiment. The electrolyte leakage increased with the increased concentration of salt in all annual species except *B.* scoparia. In *B.* scoparia, electrolyte leakage decreased with increased concentration of salt. It has been concluded that depending on the physiological responses, viz. total chlorophyll content, carotenoid content, relative water content, electrolyte leakage and proline concentration, maximum salt tolerance was observed in *P. grandiflora* followed by*B. scoparia*up to 60mM to 90mM of NaCl followed by B. scoparia, further it decreased at 120mM of NaCland minimum salt tolerance was observed in *Z. elegans*followed by *T. erecta*.

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Table1. Effect of different concentration of NaCl on chlorophyll content (mg/g fresh weight) of summer season annuals

Treatments	**Species**

	V1	V2	V3	V4
T1	1.14a	2.80ab	0.21b	1.10b
T2	1.04b	2.50b	0.16b	1.04bc
T3	0.67b	2.49b	0.10c	1.54a
T4	1.11a	3.01a	0.12c	1.15b
T5	1.01b	1.42c	0.35a	0.96c

Table2. Effect of different concentration of NaCl on carotenoid content (mg/g fresh weight) of summer season annuals

Treatments	**Species**			
	V1	**V2**	**V3**	**V4**
T1	0.05a	0.11a	0.02a	0.06a
T2	0.03b	0.10b	0.02a	0.05b
T3	0.05a	0.08b	0.01b	0.06a
T4	0.03b	0.11a	0.02a	0.05b
T5	0.04b	0.06c	0.02a	0.05b

Table3. Effect of different concentration of NaCl on relative leaf water content(%) of summer season annuals

Treatments	**Species**			
	V1	**V2**	**V3**	**V4**

T1	39.38a	89.00a	89.53b	84.80b
T2	37.52b	83.11b	92.69b	86.35a
T3	36.29b	80.97c	94.29a	80.59c
T4	34.33c	83.44b	88.98c	81.78c
T5	32.38c	49.80d	88.95c	87.13a

Table4. Effect of different concentration of NaCl on electrolyte leakage (%) of summer season annuals

Treatments	**Species**			
	V1	**V2**	**V3**	**V4**
T1	15.99c	8.79c	30.59d	53.80a
T2	16.90c	13.27b	37.35c	35.79c
T3	17.83b	18.01a	42.10b	38.05b
T4	20.14a	19.17a	48.14a	25.64d
T5	20.88a	13.53b	46.69b	33.53cd

Table5. Effect of different concentration of NaCl on proline (mg g^{-1}dry wt.)of summer season annuals

Treatments	**Species**

	V1	**V2**	**V3**	**V4**
T1	0.23a	0.77a	1.64b	0.42c
T2	0.23a	0.66ab	2.38a	0.50bc
T3	0.19b	0.58b	2.18a	0.59b
T4	0.14c	0.54bc	1.53b	0.68ab
T5	0.16bc	0.27c	0.76c	0.77a

Note: The different letters in each column are significantly different at $P \leq 0.05$ by Duncan's Multiple Range test (DMRT)

Salt concentration: Ornamental summer season annual:

T1: Control (Tap water) V1: *Z. elegans*

T2: 30 mMNaCl V2: *T. erecta*

T3: 60 mMNaCl V3: *P. grandiflora*

T4: 90 mMNaCl V4: *B. scoparia*

T5: 120 mMNaCl

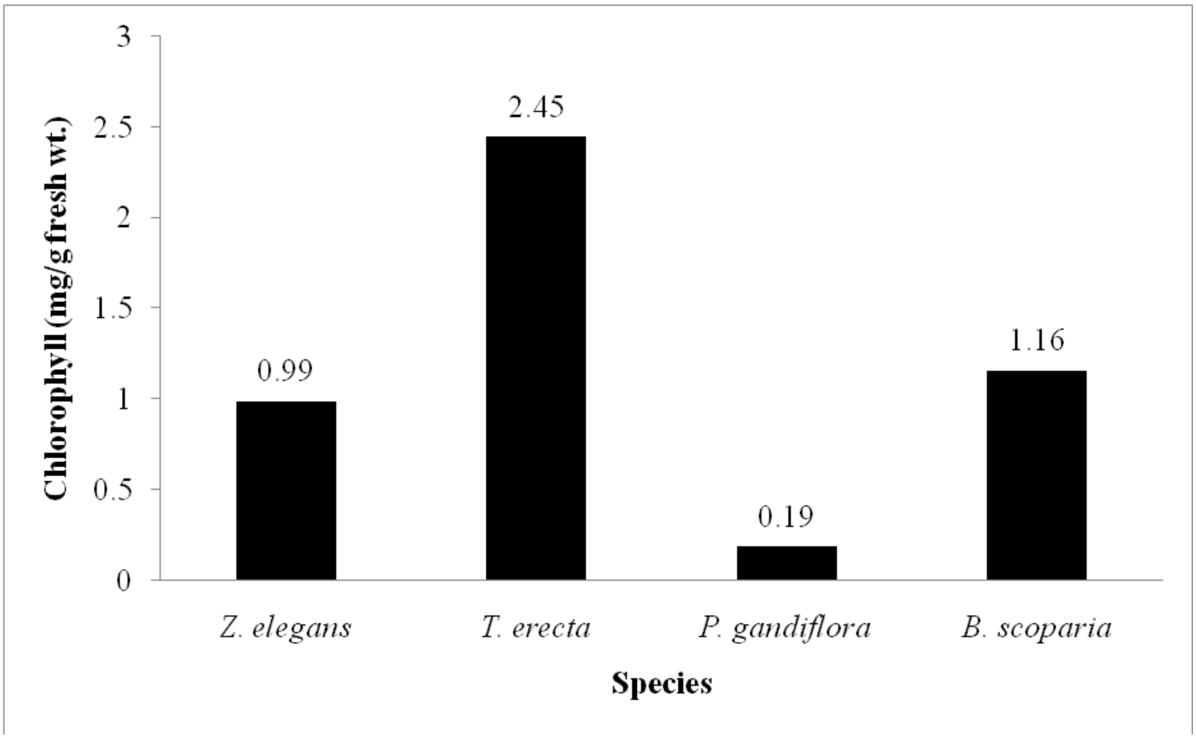

Figure1. Effect of NaCl on mean chlorophyll content (mg/g fresh weight) of four ornamental annual species.

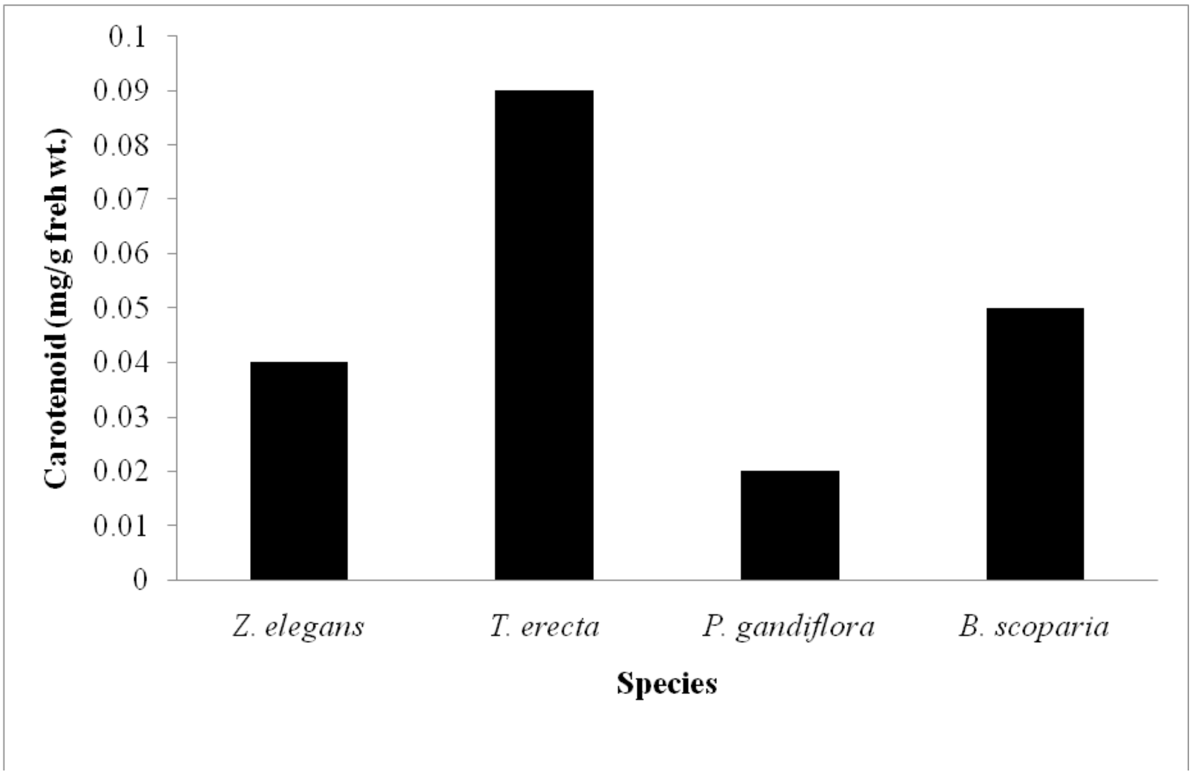

Figure2. Effect of NaCl on mean carotenoid content (mg/g fresh weight) of four ornamental annual species.

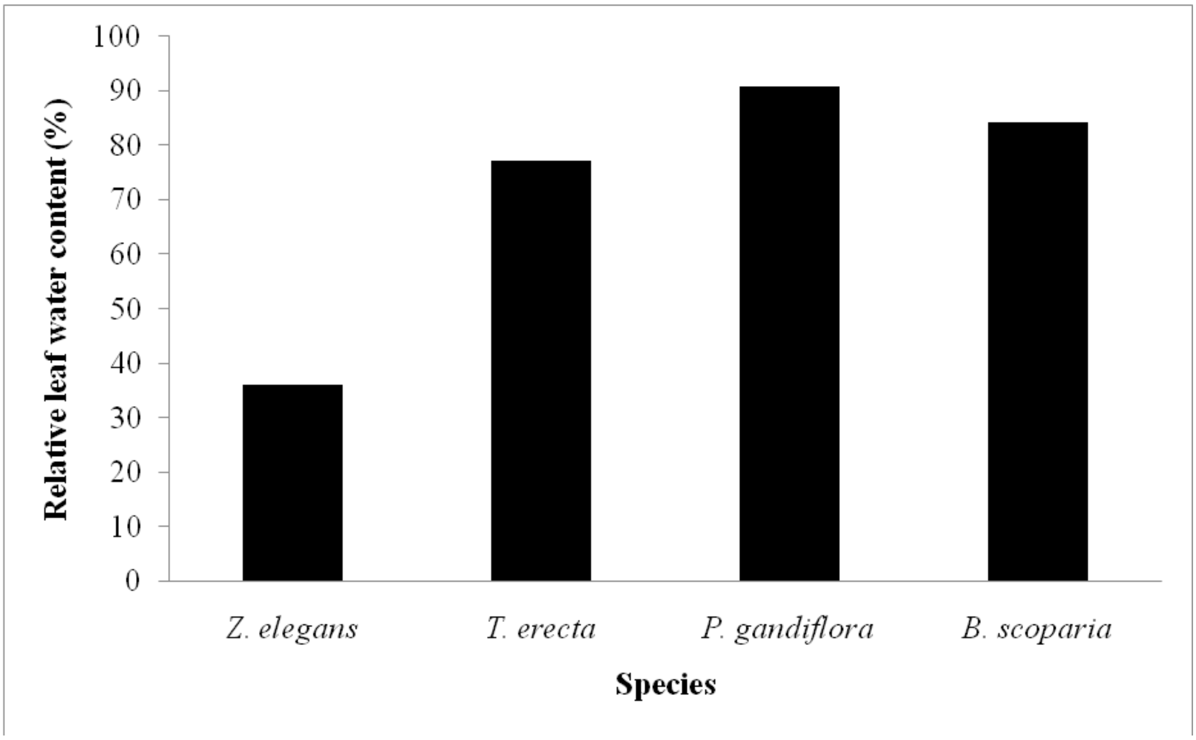

Figure3. Effect of NaCl on mean relative leaf water content (%) of four ornamental annual species.

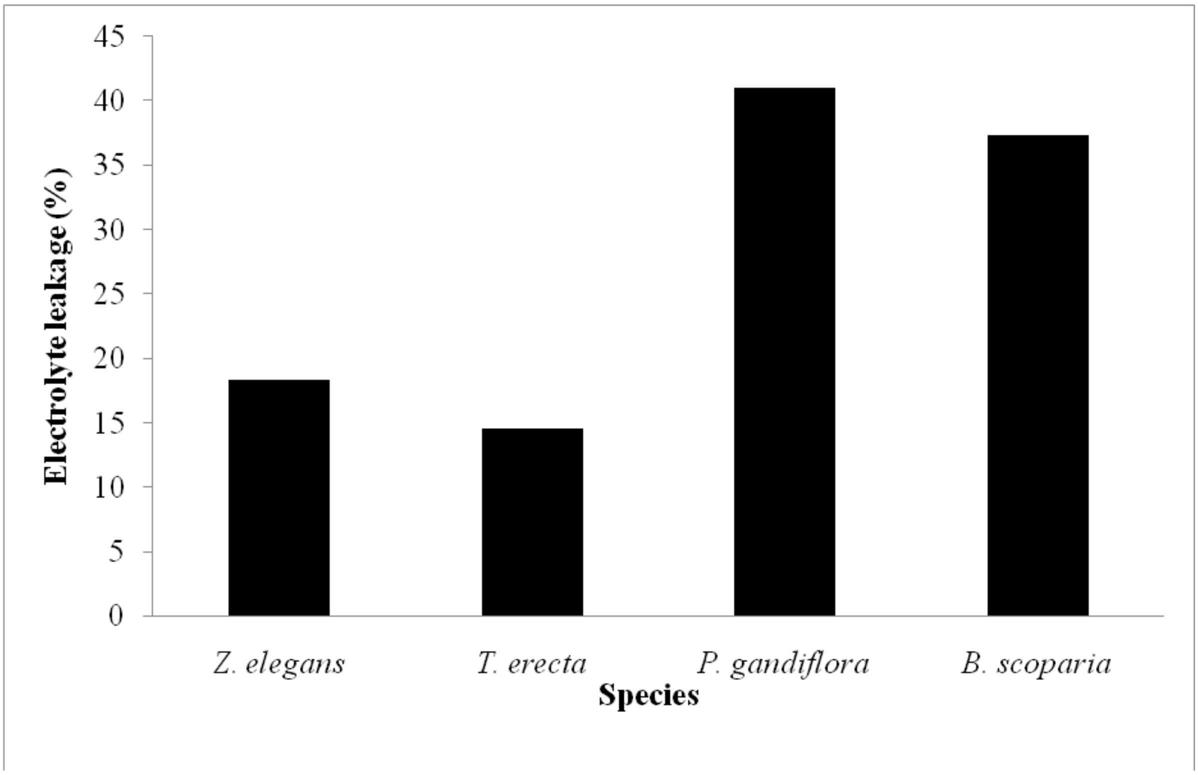

Figure4. Effect of NaCl on mean electrolyte leakage (%) of four ornamental annual species.

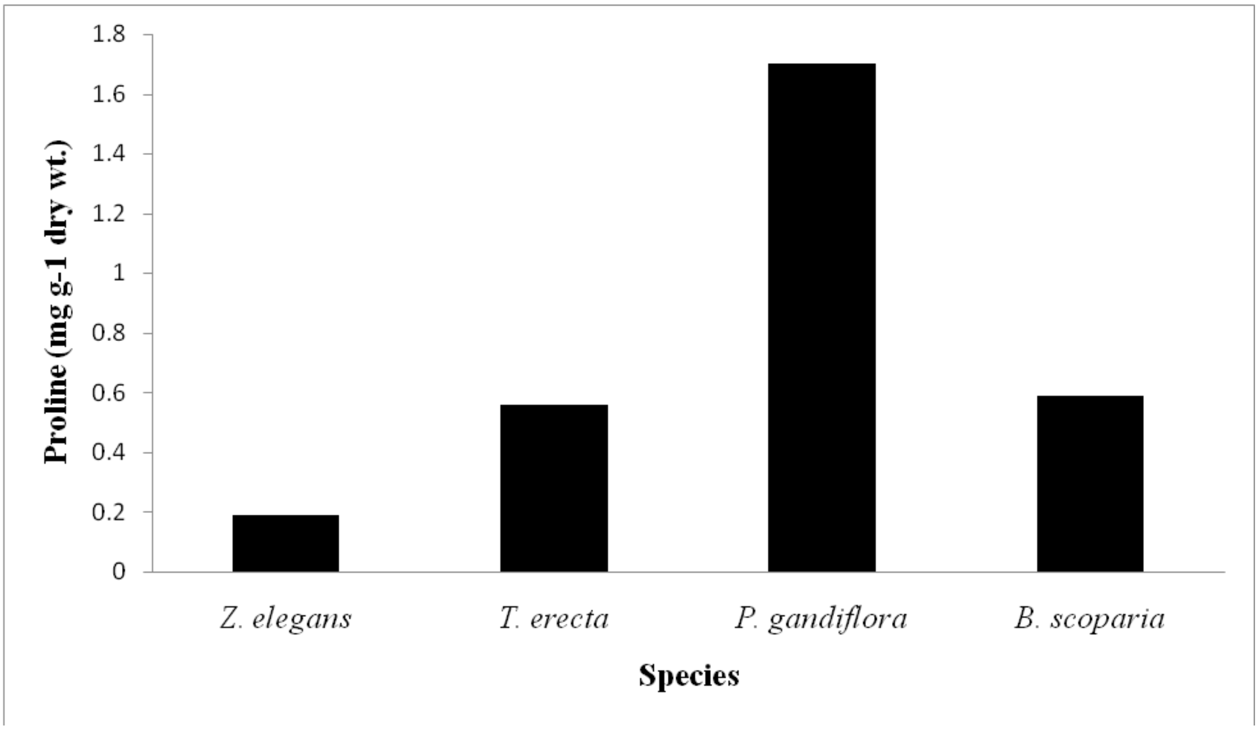

Figure5.Effect of NaCl on mean proline content (mg g^{-1} dry wt.) of four ornamental annual species.

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