



# Evaluation of the Physiological Response of Ornamental Summer Season Annuals to Salinity Stress

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## 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 demand lead to stagnation in saline zone due to salinity ingress. 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 to screen four ornamental summer season annual species suitable for salt affected areas of Punjab. Selection of plant species was done on the basis of physiological changes in plant leaves. The one month old seedlings of four different annual species were grown in 8 inch earthen pots were exposed to five different concentrations of sodium chloride salinity stress (control, 30, 60, 90, and 120 mM). The factorial completely randomized block design was

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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*.

**Keywords:** Salinity; physiological changes; summer annuals; chlorophyll.

## 1. 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 insufficient rainfall does not fulfill the water requirements to irrigate the crops and mineral salts leach out of the root-zone (Hossain et al. 2012). Identification of salt-tolerant crop varieties in such regions is highly important to improve the yield and quality of crops being grown in naturally salt affected areas and areas having limited water resources.

Salt accumulation on soil, restrictions on groundwater use, and saltwater intrusion into groundwater.

In saline environment, salts in the soil consume more water and make the plants water deficient that leads to root dehydration, less availability of water to plants and consequently weak plant growth. However, salt-tolerant plants are blessed with natural mechanisms such as morphological, physiological, and biochemical processes to mitigate these detrimental effects through controlled distribution of salt concentration in the plants.

It has been estimated that more than 800 Mha of land area are affected by salinity in the world, i.e. 10% of the world's total arable land area [1]. It is estimated by soil map of the world that Saline and sodic soil ranges 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%). India constitutes around 6.73Mha salt-affected soils, out of total geographical area (329 Mha) i.e. 2.10% of the geographical area [2]. In Punjab, 6.40% of the total geographical area is affected by salinity [3]. Ground water in many regions of Punjab contains high amount of dissolved salts having EC 2-7 ds/m [4]. It has been estimated that the area under salinity keeps on increasing annually

at a rate of 10% due to low rate of precipitation, high surface evaporation, weathering of native rocks, saline water irrigation and poor cultural practices. Under the influence of high salinity, plants lose their turgor as plant growth is more influenced by salt than other toxic substances [5] that further leads to high osmotic stress, nutritional deficiencies and toxicities, poor soil structure and low crop productivity. Researchers has analyzed that more than 50% of the arable land would be salinized by the year 2050 [6].

The growth and development of plants tend to suppress when grown in salt stressed soil [7], therefore their ability to extract water from saline soils to survive at a particular salinity is ecologically important corresponding to the natural occurrence of plant species in salt-stressed soils. Salinity and poor quality irrigation water is most prevalent issue in arid and semi-arid regions, so there is need to select the plants that can be grown to make arid and semi-arid regions green.

Flowering Annual 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 (*Tagetes erecta*), Zinnia (*Zinnia elegans*), Portulaca (*Portulacagrandiflora*), Kochia (*Bassiascoparia*). Marigold (*Tagetes erecta*) 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 current investigation was to study the comparison of relative tolerance of four summer season annual plants on the basis of different concentration of induced salinity. This experiment was conducted after citing literature that highlights that salt-tolerant species can be classified using physiological criteria such as chlorophyll content, carotenoid content, relative leaf water content, electrolyte leakage and proline content [8]. The basic information about the plant growth and survival under saline environmental conditions is required 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 plants to tolerate salt stress.

## 2. MATERIALS AND METHOD

Seeds of *Zinnia elegans*, *Bassia scoparia*, *Portulaca grandiflora* (Moss-rose Purslane) and *Tagetes erecta* (African marigold) were sown during first week of March 2019 at Landscape Nursery, PAU Ludhiana. Seedlings of plants were transplanted in 8 inch earthen pots containing media Soil: FYM (2:1) in first week of April 2019. The transplanted seedlings were irrigated with water containing different concentration of NaCl, i.e. 30 mM (1.4 g/800 ml of water/pot), 60 mM (2.80 g/800 ml of water/pot), 90mM (4.2 g/800 ml of water/pot) and 120 mM (5.60 g/800 ml of water/pot) and EC of growing media was adjusted according to different salt concentration. The amount of water given to plants for irrigation was determined corresponding to the leached amount of water consumed by the. These NaCl concentrations were applied 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

[9] and Kirk and Allen [10], Electrolyte leakage and Proline were determined in the leaves by the method Deshmukh et al. [11] and Bates et al. [12]. Relative leaf water content was also measured in the leaves of plants.

## 2.1 Statistical Analysis

The experiment was chalked out 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).

## 3. RESULTS AND DISCUSSION

### 3.1 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 (90 mM) in *T. erecta* (3.01), T5 (120 mM) in *P. grandiflora* (0.35) and T3 (60 mM) 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 declined with high concentration of salt solution i.e. 52.8% and 41.2% as compared to control. It has been found that a proteolytic enzyme i.e. chlorophyllase causes the chlorophyll degradation [13] formed in response to salt stress and damaging the photosynthetic apparatus [14].

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 observed in *T. erecta* (45.4%) followed by *Z. elegans* and *B. scoparia*, shown in Table 2.

Sayed et al. [15] reported that carotenoid content of *Tagetes erecta* 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. Carotenoid plays an important role in plants as antioxidants and protects chlorophyll pigment from photo damage [16]. Havaux [17] studied a new function of carotenoid, which relates to the response of plants to environmental stresses.

### 3.2 Relative Leaf Water Content (%)

Relative water content in plants is considered as one of the accurate measures to evaluate the amount of water in a plant leaf in terms of physiological response of plant to 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 notably with the increase in the salinity stress and reduces 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) of *C. paludosum* did not show variations because plants saved a large amount of water in the leaf discs and not affected by salt

stress [18]. The maximum reduction was observed in *T. erecta* while minimum reduction was observed in *P. grandiflora* followed by *B. scoparia*. Karimi et al. [19] 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.

### 3.3 Electrolyte Leakage (%)

Electrolyte leakage is evaluated during cell membrane injury when plants have gone through salinity stress. Tuna et al. [20] and Tiwari et al. [21] reported that permeability of plasma membrane is found to be a productive criterion to evaluate 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. [22] reported that electrolyte leakage increased in *Chrysanthemum okiense* and *C. ornatum* under salt stress.

**Table 1. 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

**Table 2. 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

**Table 3. 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

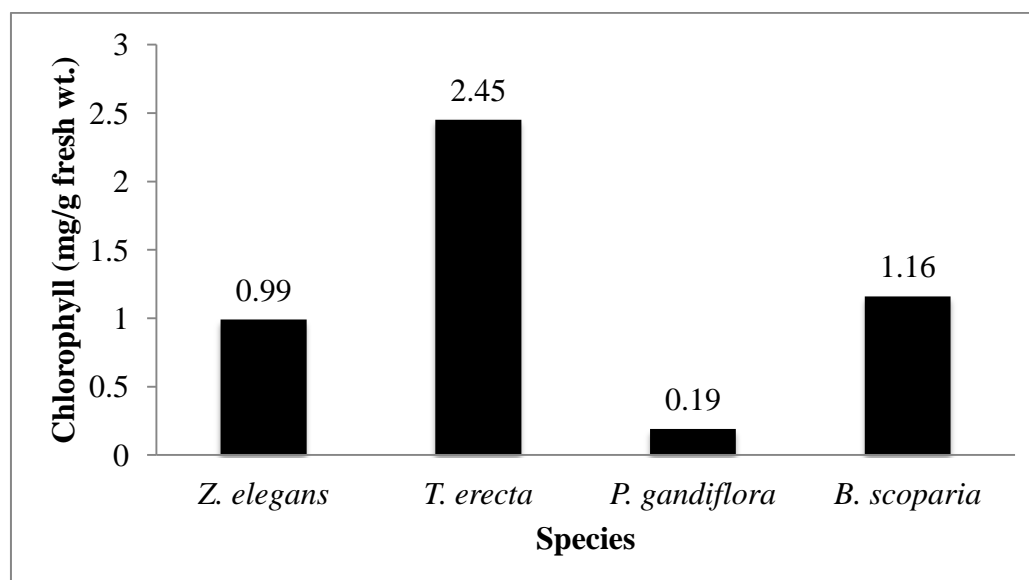
**Table 4. Effect of different concentration of NaCl on electrolyte leakage (%) of summer season annuals**

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

**Table 5. Effect of different concentration of NaCl on proline (mg g<sup>-1</sup> 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



**Fig. 1. Effect of NaCl on mean chlorophyll content (mg/g fresh weight) of four ornamental annual species**

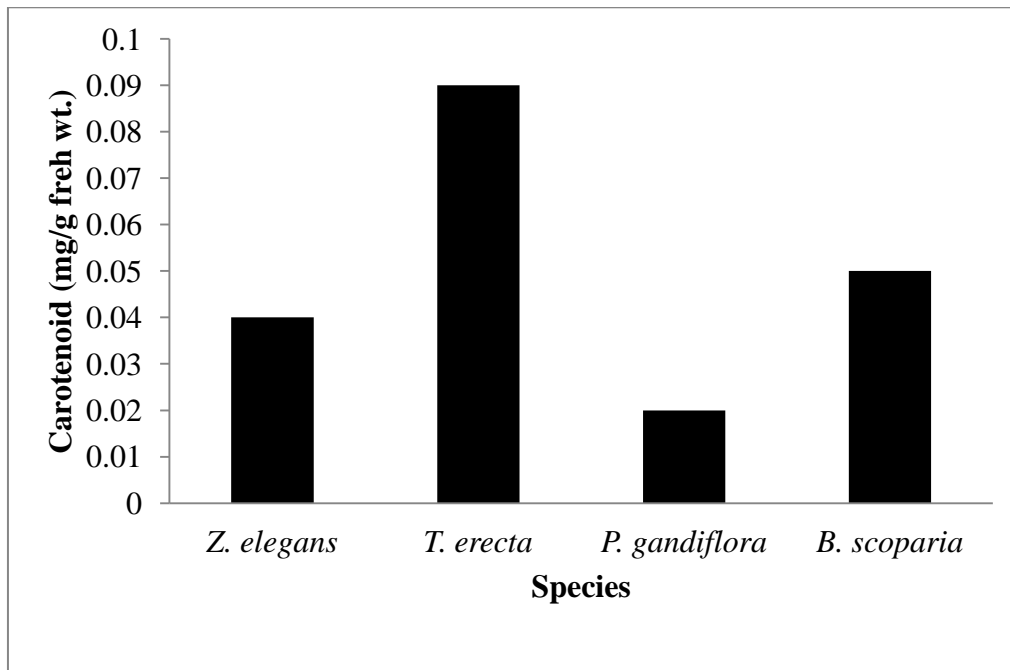


Fig. 2. Effect of NaCl on mean carotenoid content (mg/g fresh weight) of four ornamental annual species

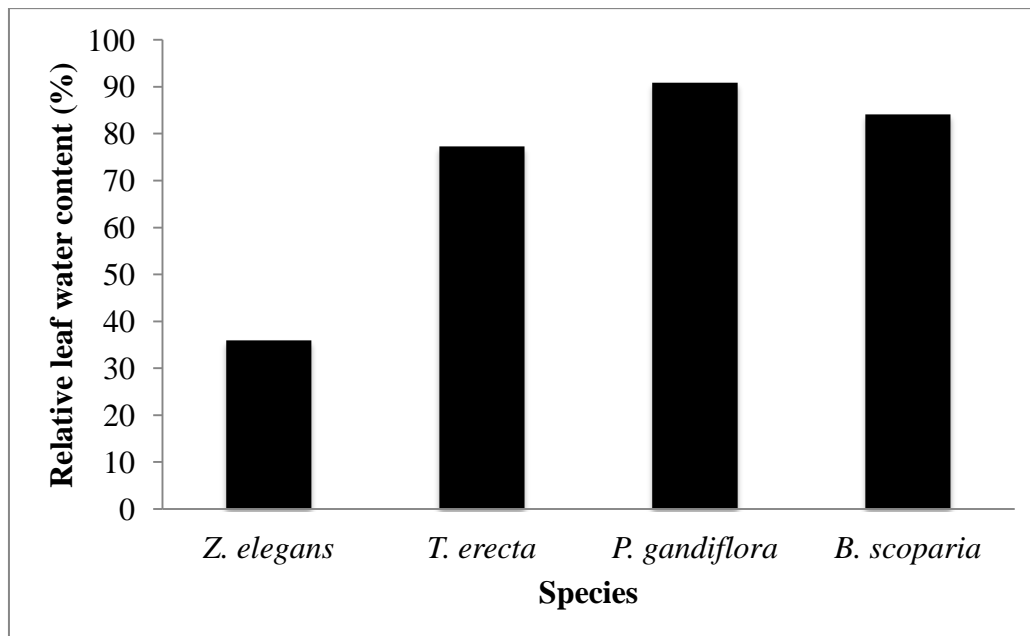


Fig. 3. Effect of NaCl on mean relative leaf water content (%) of four ornamental annual species

### 3.4 Proline (mg g<sup>-1</sup> 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*. The solute such as proline help the plants to cope with salt stress by decreasing 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 low proline 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 (30 mM) and T3 (60 mM) and in *B. scoparia*, it was more observed under T5 (120 mM) i.e. higher dose of NaCl in the experiment as shown in Table 5. Similar results were observed by Mazhar et al. [23] that proline content in *Chrysanthemum indicum* increased as a result of increasing salinity. Thus, *P. grandiflora* and *B. scoparia* had

highest salt tolerance index as compared to other two species. The results of current investigation agreed with Chen et al. [24] and Rahdari et al. [25] where they observed that Proline accumulated significantly with consequent increase in level of salinity. Don et al. [26] 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.

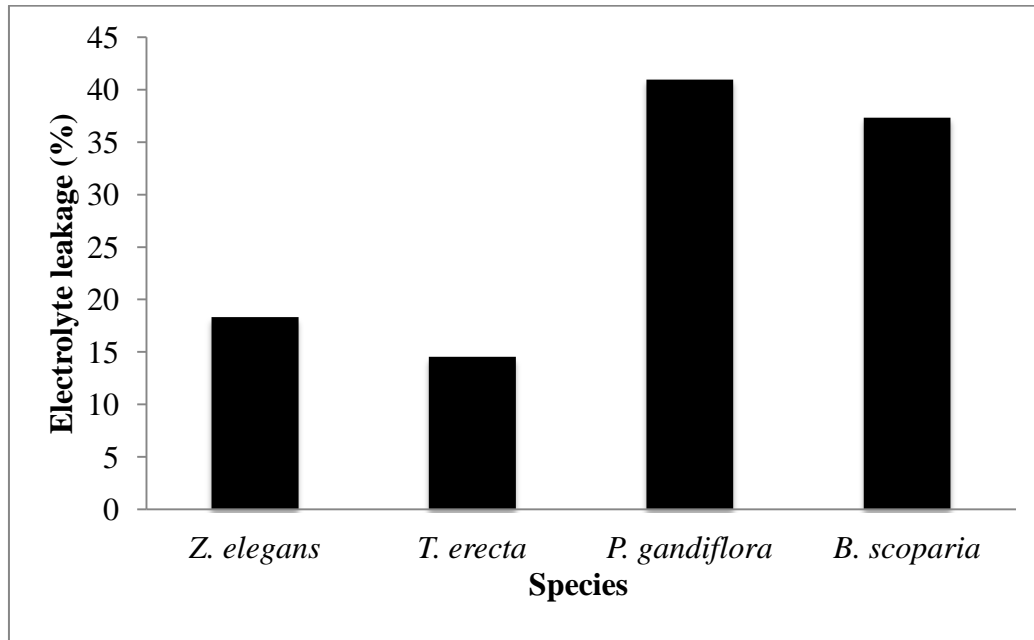


Fig. 4. Effect of NaCl on mean electrolyte leakage (%) of four ornamental annual species

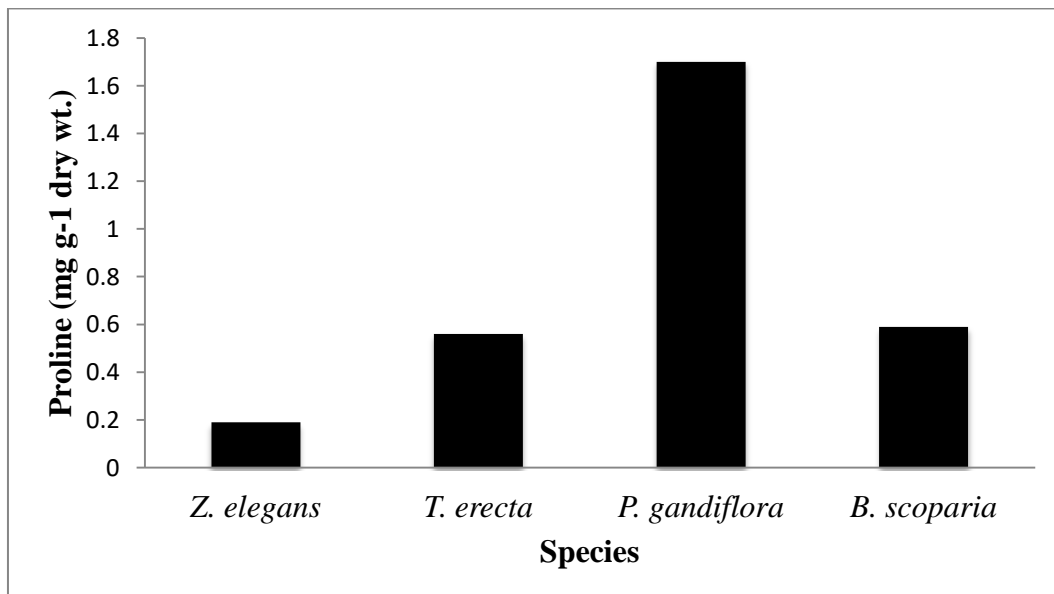


Fig. 5. Effect of NaCl on mean proline content (mg g<sup>-1</sup> dry wt.) of four ornamental annual species

#### 4. CONCLUSION

The whole experiment was conducted to evaluate the usage of saline water to irrigation ornamental plant species in pots under sub-tropical condition of Punjab for summer season. The results of the experiment depicted that the increased salinity concentration remarkably lowers 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 maintained with increase in salinity level during the experiment. The electrolyte leakage increased with the high 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 NaCl and minimum salt tolerance was observed in *Z. elegans* followed by *T. erecta*.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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