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Exogenous application of proline on growth, physiological adaptability and flower quality of *Tagetes erecta* L. (Marigold) grown under sea water irrigation

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Abstract

Current study was aimed to investigate the role of exogenous application of proline on physiological adaptability and flower quality of common marigold (*Tagetes erecta* L) cultivated using sea water. Salinity beyond 25 dsm^{-1} severely hindered the growth of marigold and with increasing sea water salinity from 6 to 12 dsm^{-1}, all of growth parameter, phytochemicals were reduced. But, the exogenous addition of proline sustained the growth of all the plants with increasing salinity up to 9 dsm^{-1} beyond that the effects were insignificant. The salinity tolerance index (STI) increased with increasing salinity up to 6 dsm^{-1} in T2 (74.7%) with the application of 10 mg L^{-1} proline. Total protein content in leaves varied significantly among the different treatments applied. The lowest and highest value was observed in T2 (19.3 mg g^{-1}), T3 (17.6 mg g^{-1}) and control (17.3 mg g^{-1}) at 90 DAT. *T. erecta* under the treatment of 6 dsm^{-1} sea water and 10 mg L^{-1} proline showed highest level of alkaloid content (1.356 mg g^{-1}) when compared to control (1.034 mg g^{-1}). Highest marketable flower yield was recorded in control (148.7 g/plant), which was significantly superior to all other treatments. Under sea water irrigation, the highest marketable flower yield was recorded in T2 (123.3 g/plant) followed by T3 (114.3 g/plant). From the results of this investigation, it can be concluded that, the seedling treatments of proline at lower dose (10 mg L^{-1}) can be used as a good tool in the enhancement of biomass yield and alkaloid contents in floriculture crops such as marigold under saline irrigation.

1. Introduction

Curtailing crop losses due to various environmental stressors is a major area of concern to cope with the increasing food requirements (Abbas *et al*., 2019). According to statistics from FAO (2008), more than 50 % of the world's irrigated soils are affected by secondary salinization and/or alkalization and each year, about 10 million hectares of land are abandoned, mainly due to secondary salinization. Biosaline agriculture is an approach to utilize the saline barren lands for cultivating plants that could either produce oil seeds, forage, or food. The success of using saline water for economic crop production can be achieved using best management practices to reduce the negative effects of salinity on crop productivity. Ghadiri *et al*. (2006) explored that the barren lands can be judiciously utilized for raising forestry, forages, agricultural and horticultural crops, and non-conventional crops of high economic value using sea water for irrigation selecting suitable salt-tolerant species.

Salinity causes many adverse effects on plant developmental stages which are due to many factors such as low water potential of soil solution, nutritional imbalance, ions toxicity (salt stress) and hormonal imbalance (Abd El Gayed, 2020). Application of osmoprotectants as seed priming or as a foliar spray can be an economically viable strategy to enhance stress tolerance under adverse environmental conditions (Ali and Ashraf, 2011; Sako *et al*.,

2020). Ali *et al*. (2008) reported that the exogenous application of proline enhanced the nutrient uptake in roots and shoots under water deficit conditions and correlated the results with an enhanced plant transpiration rate. Exogenous application of proline is known to induce abiotic stress tolerance in various plant species (Abdelhamid *et al*., 2013; Soroori *et al*., 2021).

Marigold is an important ornamental plant from the family Compositae which is currently in environmental management and also evaluated as a flower cut. *Tagetes* species are reported to have antimicrobial (Gakuubi *et al*., 2016), insecticidal (Fabrick *et al*., 2020), larvicidal (Giarratana *et al*., 2017) and antioxidant (Sadique *et al*., 2021) properties and are used as traditional medicines in different countries as a treatment for colic, diarrhea, vomiting, fever, skin diseases and liver disorders (Jain *et al*., 2012). Among the ornamental flowering plants, marigold is reported to grow well in saline conditions. A number of marigold cultivars used as flowering cuttings or as ornamental bedding plants in landscaping may be grown with maintained plant quality under salt stress with an ECw ≤8 dS -1 (Valdez-Aguilar *et al*., 2009).

Normally, osmoprotectants protect plants from different abiotic stresses in several ways, including their role in adjusting cellular osmosis, scavenging reactive oxygen species (ROS), protecting cellular membranes, and stabilising proteins/enzymes and enzyme activities (Gill and Tuteja, 2010). In contrast, the effect of proline is dependent on its concentration, as mentioned by Ashraf and Foolad (2007), because an excessive amount of free proline has negative or side effects on cell growth or on protein functions. Hence, it is also necessary to determine the optimal concentrations of exogenously applied proline that can provide beneficial effects in economically important crop plants, such as sunflower when exposed to abiotic stress. Thus the aim of present study was to investigate the role of exogenous application of proline on physiological adaptability and flower quality of common marigold cultivated using sea water.

2. Materials and Method

African marigold var. giant orange (*Tagetes erecta* L.) was chosen for the present study. All experiments were performed in greenhouse cabins at 30°C and 14 h light and at 25°C in the dark for 10 h/day. The experiment was conducted in split plot design comprising of 3 levels of sea water (6.0, 9.0 and 12.0 ds/m) in main plot and 2 levels of proline (10 mg and 20 mg L^{-1}) in sub plots (Kahlaoui et al., 2013). Sea water dilutions for irrigation were prepared by mixing fresh water (1.2 dsm^{-1}) with sea water (51.2 dsm^{-1}) to achieve salinity levels of 6.0, 9.0 and 12.0 dsm^{-1} and applied 2 weeks after sowing. Proline (L – Pro, NICE/AR) was applied as foliar spray at the rate of 10 lit ha^{-1} with the interval of 7 days. Treatments comprised of T0 (Control), T1 (6 dsm^{-1}), T2 (6 dsm^{-1} + 10mg), T3 (6 dsm^{-1} + 20mg), T4 (9 dsm^{-1}), T5 (9 dsm^{-1} + 10mg), T6 (9 dsm^{-1} + 20mg), T7 (12 dsm^{-1}), T8 (12 dsm^{-1} + 10mg) and T9 (12 dsm^{-1} + 20mg).

2.1 Morphological studies

The data on morphological traits including; plant height (PH), number of leaves on main stem (NL), root length (RL), fresh and dry shoot weight (SFW, SDW), and fresh and dry root weight, (RFW, RDW) after drying at 68°C for 48 h were measured. The leaf area, relative growth rate (RGR) and the salt tolerance index (STI) were also calculated (Baci *et al*., 2003).

2.2 Biochemical analysis:

Physiologically active leaf (3rd and 4th from the top) samples from 3 different plants of each plot were plucked randomly for determination of different biochemical parameters such as chlorophyll content (Arnon, 1949), protein (Lowry *et al*., 1948), reducing sugar (Moore and Stein, 1948),alkaloids (Sreevidya and Mehrotra ,2003) and total phenols (Lin and Tang, 2007).

2.3 Flower quality

Flower size, yield, colour, fragrance and marketable quality will be estimated using standard procedures (Augustine *et al*., 2016). The results were analyzed by using analysis of variance (ANOVA) and the group means were compared with the Duncan's Multiple Range Test (DMRT) (Duncan, 1957). Values were considered statistically significant when $P < 0.05$.

3. Results and Discussion

Numerous research results have indicated that exogenous application of osmoprotectants, plant hormones, antioxidants, signaling molecules, polyamines and trace elements provided significant protection against salt-induced damages in plants. These protectants enhanced salt stress tolerance by enhancing their germination, growth, development, photosynthesis, antioxidative capacities and yield. In recent decades exogenous protectant such as osmoprotectants (proline, glycinebetaine, trehalose, etc.), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signaling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, etc.) have been found effective in mitigating the salt induced damage in plant (Yusuf *et al*. 2012). During the current experiment, the higher sea water concentration beyond 25 ds/m severely hindered the growth of plants used.

3.1 Growth Analysis

Highest Stem length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and biomass were achieved under the control and with increasing in sea water salinity from 6 to 12 dsm^{-1}, all of growth parameter, phytochemicals were reduced (Table 1). The plant height increased continuously from 30 DAT to 90 DAT in all the treatments with proline application. A close similarity was observed in plant height with the control and proline treated plants up to 60mM NaCl (6 dsm^{-1}). For *Tagetes erecta* the maximum mean plant height was observed at 90 DAT in Control (T0) (26.7 cm) followed by T2 (21.8 cm) and T3 (19.7 cm) beyond that the effects were insignificant. Though the NaCl salinity hindered the growth of marigold, to certain extent the application of proline sustained the growth. The highest shoot (18.8 cm) and root length (7.2 cm) was observed in T0 (control) followed by T2, whereas, the lower plant heights were statistically insignificant in higher salinity even with increasing proline application. Similarly the leaf area showed (Table 2) a maximum value (38.3 cm^2) by the control

Table 1. Exogenous application of proline on shoot, root length (cm) and plant height (cm) of *Tagetes erecta* under sea water irrigation

Treatments	Shoot length (cm)			Root length (cm)			Plant Height (cm)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	6.8^{a}	12.8^{a}	18.8^{a}	3.8^{a}	4.3^{a}	7.2^{a}	11.8^{a}	16.8^{a}	26.7^{a}
T1	4.8^{c}	5.9^{c}	7.8^{e}	2.7^{b}	2.9^{d}	4.2^{d}	7.7^{d}	9.2^{c}	13.3^{d}
T2	5.4^{b}	7.4^{b}	12.6^{b}	3.3^{a}	3.4^{c}	5.8^{c}	9.1^{b}	10.8^{b}	21.8^{b}
T3	5.0^{b}	7.0^{b}	10.7^{c}	3.1^{a}	3.7^{b}	6.0^{b}	8.7^{c}	10.2^{b}	19.7^{c}
T4	3.2^{e}	5.2^{d}	7.2^{e}	2.2^{c}	2.2^{e}	3.6^{e}	5.2^{f}	7.2^{e}	10.8^{f}
T5	3.8^{d}	5.8^{c}	8.8^{d}	2.8^{b}	3.8^{b}	3.8^{d}	6.3^{e}	9.3^{c}	12.4^{e}
T6	3.8^{d}	5.8^{c}	7.4^{e}	2.8^{b}	3.4^{c}	4.2^{d}	6.2^{e}	9.2^{c}	11.8_{f}
T7	3.1^{e}	5.1^{d}	6.5^{f}	2.1^{c}	2.7^{d}	3.3^{f}	5.1^{f}	7.4^{e}	9.7^{g}
T8	3.3^{e}	5.3^{d}	7.3^{e}	2.3^{c}	3.3^{c}	3.7^{e}	5.6^{f}	8.3^{d}	10.8^{f}
T9	3.2^{e}	4.7^{e}	6.6^{f}	2.2^{c}	3.1^{d}	3.2^{f}	5.2^{f}	7.5^{e}	10.2^{f}

Values followed by the same letter within treatment are not significantly different at p = 0.05

Table 2. Exogenous application of proline on Leaf Area (cm^2), RGR ($g\ g^{-1}dw^{-1}$) and STI (%) of *Tagetes erecta* under sea water irrigation

Treatments	Leaf Area (cm^2)			RGR ($g\ g^{-1}dw^{-1}$)			STI (%)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	16.8^{a}	21.3^{a}	38.3^{a}	0.08^{a}	0.13^{a}	0.16^{a}	-	-	-
T1	8.2^{c}	10.9^{d}	14.7^{d}	0.04^{c}	0.09^{b}	0.11^{b}	42.9^{c}	31.9^{c}	37.9^{c}
T2	9.4^{b}	17.1^{b}	26.6^{b}	0.07^{b}	0.09^{b}	0.13^{b}	61.9^{a}	56.5^{a}	74.7^{a}
T3	9.2^{b}	15.4^{c}	21.3^{c}	0.06^{b}	0.07^{c}	0.12^{b}	54.8^{b}	46.4^{b}	70.1^{b}
T4	6.0^{e}	10.7^{d}	11.4^{f}	0.04^{c}	0.05^{d}	0.09^{c}	28.6^{f}	26.1^{d}	26.4^{e}
T5	7.2^{d}	11.3^{d}	12.8^{e}	0.03^{d}	0.05^{d}	0.07^{d}	38.1^{d}	29.0^{c}	36.8^{c}
T6	6.7^{e}	10.9^{d}	12.2^{e}	0.04^{c}	0.06^{c}	0.06^{d}	35.7^{d}	26.1^{d}	32.2^{d}
T7	6.0^{e}	8.2^{e}	10.0^{f}	0.02^{d}	0.04^{e}	0.05^{e}	26.2^{f}	23.2^{e}	24.1^{e}
T8	6.3^{e}	8.9^{e}	11.3^{f}	0.04^{c}	0.04^{e}	0.06^{d}	31.0^{e}	27.5^{d}	29.9^{d}
T9	6.2e	8.6^{e}	10.4^{f}	0.03^{d}	0.04^{e}	0.05^{e}	28.6^{f}	26.1^{d}	27.6^{e}

Values followed by the same letter within treatment are not significantly different at p = 0.05

plants followed by the plants treated with 10 mg L^{-1} proline at 50 mM (26.6 cm^2) and 100mM NaCl (12.8 cm^2). The data observed on plant height clearly showed that the proline application could possibly alleviate the salinity stress up to 6ds/m beyond that the effects were insignificant. The plant height under 6 ds/m treatment with 10 mg L^{-1} indicated that the application of proline favour the uptake of nutrient at the root zone level that causes the increase in the plant height (Abou El-Ftouh *et al*., 2018). The results on the effect of exogenous application of proline on the dry matter are presented in Table.3. The dry matter decreased with increasing salinity upto 12 ds/m when compared to the control (0.87 g g^{-1}). An increased amount of dry weight was observed in *T. erecta* (0.65 g g^{-1}) with the application of 10 mg L^{-1} proline at the optimal salinity level (6 ds/m). The highest dry matter (0.87 g) was recorded at the control T0 followed by T2 on 90th day samples.

The results on the effect of exogenous application of proline on the fresh and dry weight are presented in Table.2. The fresh weight of shoot and root increased with increasing days of treatment whereas the FW increased with the proline treatment upto 6 ds/m sea water salinity and there after it had decreased. An increased amount of dry weight was observed in *T. erecta* with the application of 10 mg L^{-1} proline up to the optimal salinity level (100 mM). The highest increase in fresh (16.7 g) was recorded in control followed by T2 (12.2g). Similarly the relative growth rate was high in control (0.16 g g^{-1}DW) where as proline application at the rate of 10 mg L-1 showed RGR close to the control (0.13 g g^{-1}DW) at 50 mM salinity (6 ds/m). Banu *et al*., (2009) stated that proline might have protected cell membranes against ion toxicity and salt-induced oxidative stress, increased cellular growth, and thus increased the growth of plants. The enhanced growth characteristics due to exogenous application of proline under low salinity levels might be attributed to its role in osmoregulation, and maintenance of the tertiary structure of proteins and enzymes, to help growing plants tolerate drought stress (Abdelaal *et al*., 2020; El-Nashaar *et al*., 2020).

The results on the effect of exogenous application of proline on salinity tolerance of *T. erecta* are presented in Table.2. The STI increased with increasing salinity upto 6 ds/m and there after it had decreased. An increased STI was observed in T2 (74.7%) with the application of 10 mg L^{-1} proline at salinity level (60 mM). The lowest STI was recorded at 12 ds/m salinity on 90th day samples. Similar to previous reports (Al-Khazrji *et al*., 2020), in *Tagetes erecta* the foliar application of proline enhanced the plant water status and the results also suggested that the exogenous application of proline enhances membrane stability, thus preventing electrolyte leakage, as previously documented by Hayat *et al*. (2012).

3.2 Photosynthetic Pigments

Similar to other crops, deleterious effects of salinity stress on leaf chlorophyll contents have been reported in our present study on *Tagetes erecta*. (Abd El-Samad *et al*., 2011; Abdelhamid *et al*., 2013; Taie *et al*., 2013). In contrast, total chlorophyll content in proline treated plants was sustained to a greater degree than in plants grown without proline. The chlorophyll *a* content in

Table 3. Exogenous application of proline on shoot, root fresh weight (g) and dry matter (g g^{-1}FW) of *T. erecta* under sea water irrigation

Treatments	Shoot FW			Root FW			Dry matter		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	10.3a	12.5a	16.7a	3.8a	4.0a	4.3a	0.42a	0.69a	0.87a
T1	6.51d	7.78d	7.91d	2.4c	2.7c	3.1c	0.18c	0.22d	0.33c
T2	8.86b	9.31b	12.2b	3.4b	3.7b	3.9b	0.26b	0.39b	0.65b
T3	8.14c	8.33c	8.67c	3.4b	3.5b	3.7b	0.23b	0.32c	0.61b
T4	5.51e	7.78d	7.91d	2.2d	2.9c	3.1c	0.12d	0.18e	0.23e
T5	5.62e	7.81d	8.02c	2.8c	3.2b	3.3c	0.16c	0.20d	0.32c
T6	5.43e	7.53d	7.87d	2.6c	3.0b	3.3c	0.15d	0.18e	0.28d
T7	5.12e	5.93e	6.12e	2.2d	2.6c	2.7d	0.11e	0.16e	0.21e
T8	5.32e	5.76e	6.02e	2.4d	2.8c	2.7d	0.13e	0.19e	0.26d
T9	5.22e	5.56e	5.78e	2.3d	2.6c	2.7d	0.12e	0.18e	0.24e

Values followed by the same letter within treatment are not significantly different at p = 0.05

Table 4. Effect of exogenous application of proline on photosynthetic pigments (mg g^{-1}) of *T. erecta* under sea water irrigation

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Carotenoids		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	8.86a	9.03a	9.26a	4.46a	4.87a	5.06a	0.091a	0.108a	0.122a
T1	6.21d	6.37e	6.54e	3.66e	3.82c	4.08c	0.046d	0.051d	0.057d
T2	7.22b	7.73b	8.07b	4.22b	4.37b	4.86b	0.066b	0.074b	0.083b
T3	6.43d	6.57d	6.97d	3.81d	4.07c	4.26c	0.056c	0.061c	0.067c
T4	5.82c	6.06c	6.24c	3.13b	3.36b	3.54b	0.041c	0.053c	0.066b
T5	6.21d	6.37e	6.54e	3.66e	3.82c	4.08c	0.046d	0.051d	0.057d
T6	6.23d	6.31d	6.43e	3.64c	3.77c	4.06c	0.042d	0.047d	0.054d
T7	5.12c	5.36c	5.44c	3.03b	3.16b	3.24b	0.040c	0.052c	0.056b
T8	5.22c	5.66c	5.74c	3.03b	3.36b	3.44b	0.041c	0.055c	0.062b
T9	5.22c	5.46c	5.54c	3.03b	3.26b	3.24b	0.041c	0.053c	0.062b

Values followed by the same letter within treatment are not significantly different at p = 0.05

the leaf tissue of all the plants grown in salt affected soil decreased, by 8.2, 22.3, 30.2 and 35% compared to control. The total chlorophyll content in marigold at T2 and T4 were (8.07 mg g -1) & (6.97 mg g -1) with the application of 10 mg L^{-1} proline (Table 4). These increases in chlorophyll concentrations could be attributed to the more efficient scavenging of ROS by proline and other antioxidant compounds (Abdelhamid *et al*., 2013). Our results are in accordance with findings reported by Jaleel *et al*. (2007) in *Catharantus roseus* and in Maize (Ali *et al*., 2007). Salinity stress increases the generation of reactive oxygen species (ROS), resulting in oxidative damage to plants and degradation of chlorophyll pigments (Hasanuzzaman *et al*., 2020; Mansoor *et al*., 2022). But, as per the previous reports (Altuntaş *et al*., 2020; Semida *et al*., 2020; Tariq *et al*., 2021; Abdou *et al*., 2022) the exogenous application of proline might be responsible for scavenging ROS and other free radicals, enhancing antioxidant enzyme activity, thus increasing stomatal conductance, facilitating higher CO_2 diffusion through leaves and hence, enhance photosynthetic rates. Similar to our results, Islam *et al*. (2021) found that glycine betaine exhibited significant potential to improve photosynthetic pigment biosynthesis under salinity stress in mustard. Alasvandyaria and Mahdavia (2018) documented beneficial effects of exogenous application of glycine betaine on net photosynthesis, stomatal conductance and the Photosystem II (PSII) efficiency in salt-stressed safflower plants.

3.3 Proximate analysis

The effect of exogenous application of proline on the reducing sugar content is shown in Table 5. The reducing sugar increased up to 9 ds/m and then declined thereafter with the application of 10 mg L^{-1}. The highest sugar content was observed in 6 ds/m sea water salinity with proline 20 mg L^{-1} in *T. erecta* (32.3 mg g $^{-1}$) and with 10 mg L^{-1} (30.7 mg g^{-1}). Proline application in the present study showed sustained sugar level in the leaves of *T. erecta*. These findings are in consistent with the findings of Ghadiri *et al*. (2006) who reported enhancement in sugar contents of grapes and *Thuja orientalis*. Application of proline significantly promoted the level of total protein content in leaves of plants treated with sea water when compared to control initially (Table 5). Total protein content in leaves varied significantly among the different treatments applied. The lowest and highest value was observed in T2 (19.3 mg g^{-1}), T3 (17.6 mg g^{-1}) and control (17.3 mg g^{-1}) at 90 DAT Proline has been shown to protect protein integrity and enhance the activities of different enzymes (Abdelhamid *et al*., 2013). Under salinity stress, proline has been reported to enhance growth, physiological, biochemical and anatomical characteristics, and improve antioxidant system defense of plants (Dawood *et al*., 2014)..

In the present study, the TP content of marigold under sea water irrigation was investigated. The results are given in Table 6. The TP content varied in plants treated with proline and ranged from 8.9 to 17.3 mg GAE g–1 DW. The the highest TP content was in T2 (17.3 mg GAE g–1 DW) for and in T3 (15.2 mg GAE g^{-1} DW) indicating that T2 might have the most outstanding antioxidant activity. The TP contents were in the following order: T2 > T3 > T0 > T4 > T5 > T6 for 10 mg L^{-1} proilne. In this regard, phenolic contents protect cells from potential oxidative damage, increase the stability of cell membranes and mitigate salinity stress injuries (Khattab, 2007). At higher concentrations of T7 and T8 the TP contents were insignificant. Table 5 shows that total phenolic concentrations were significantly increased under seawater salinity stress compared with their corresponding controls (T0). In contrast, Khattab (2007) observed decreased

Table 5. Effect of exogenous application of proline on reducing sugar, protein and proline content (mg g^{-1}) of *T. erecta* under sea water irrigation

Treatments	Reducing sugar			Protein			Proline		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	18.3e	20.9c	23.8c	16.4a	16.8b	17.3b	0.52d	0.62c	0.67c
T1	17.6d	18.3b	17.8b	14.0b	15.7c	16.2a	0.82f	0.94e	1.23d
T2	25.6b	27.6a	30.7a	14.7b	15.9c	19.1c	1.74b	1.82b	1.99b
T3	28.5a	29.6a	32.6a	13.6c	15.7d	17.6d	1.82a	1.94a	2.02a
T4	20.2d	22.4b	24.6b	14.6a	15.0a	15.9a	1.46e	1.52d	1.61c
T5	22.6c	23.1b	25.6b	15.2b	16.2b	16.7a	1.66c	1.82b	1.91b
T6	24.2e	20.2c	20.9d	15.6b	16.3c	16.3c	1.56d	1.66c	1.71c
T7	15.6d	18.1b	18.8b	14.0b	15.7c	15.2a	0.82f	0.84e	1.13d
T8	16.6d	18.3b	18.2b	15.0b	15.4c	16.2a	0.92f	0.94e	1.33d
T9	16.4d	17.3b	18.0b	15.3b	15.4c	15.5a	0.92f	0.92e	1.31d

Values followed by the same letter within treatment are not significantly different at p = 0.05

Table 6. Effect of exogenous application of proline on total phenol (mg GAE g^{-1} DW), flavonoids and alkaloids content (mg g^{-1}) of *T. erecta* under sea water irrigation

Treatments	Total Phenol			Flavanoids			Alkaloids		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T0	8.9e	10.2e	11.2g	0.156c	0.168c	0.187c	1.411a	1.465a	1.523a
T1	10.8d	11.2d	12.7f	0.143d	0.158d	0.179d	1.023d	1.038e	1.042e
T2	14.0a	15.1a	17.3a	0.201a	0.217a	0.223a	1.287b	1.309b	1.356b
T3	13.2b	14.8b	16.2b	0.171b	0.183b	0.201b	1.087d	1.165d	1.223c
T4	10.4d	10.8d	11.9f	0.132e	0.156d	0.171d	1.014d	1.023e	1.034e
T5	11.8c	13.5c	15.2c	0.171b	0.186b	0.187c	1.132c	1.212c	1.346b
T6	11.2c	12.8c	14.3d	0.152c	0.163c	0.185c	1.131c	1.231c	1.332b
T7	9.2e	11.8d	12.3f	0.142d	0.146e	0.153e	1.031d	1.110d	1.112d
T8	11.2c	11.8d	13.6e	0.151c	0.153d	0.156e	1.130c	1.131d	1.132d
T9	10.2d	10.8d	13.3e	0.148d	0.148e	0.154e	1.127c	1.131d	1.124d

Values followed by the same letter within treatment are not significantly different at p = 0.05

total phenolic concentration in Faba bean plants treated with an exogenous application of proline under seawater salinity. Consistent with the findings of the present study, Tokarz *et al*. (2020) suggested that the phenol accumulation may protect membranes against excessive lipid peroxidation under stress.

Flavonoids in leaves of marigold under sea water irrigation showed significant level of variation among the treatments (Table 6). The proline treated plants at lower dose showed highest level of flavonoids content (0.223 mg g^{-1}) when compared to control (0.152 mg g^{-1}). Application of proline significantly increased the flavonoids content up to 6 ds/m salinity but the differences were statistically not significant with increasing level of sea water. The application of proline may have stimulated the biosynthesis of sucrose and other sugars which is essential for rapid biosynthesis of flavonoids and thus resulted into enhanced level particularly in treated plants. (Augustine *et al*., 2016). The proline application increased the synthesis of alkaloids also (Table 6). *T. erecta* under the treatment of 6 ds/m sea water and 10 mg L^{-1} proline showed highest level of alkaloid content (1.356 mg g^{-1}) when compared to control plants (1.034 mg g^{-1}). Application of proline significantly increased the alkaloids content up to 9 ds/m in but the differences were statistically not significant with increasing level of salinity.

3.4 Ameliorative effect of proline on flower quality

3.4.1 Number of flowers per plant

Growing conditions had significant influence on the number of flowers per plant, since the interaction effect was significant for all treatment (Table 7). In the present study, control recorded the highest number of flowers per plant (12.7) and T2 recorded the number of flowers per plant (8.08) which was on par with the control. Regardless of the treatments, significantly higher number of flowers per plant was observed in plants grown under sea water irrigation with the application of 10 mg L^{-1}. The number of flowers was significantly influenced by the number of branches per plant. At higher sea water salinity (T7), there was no flowering.

3.4.2 Flower length

It was found that, the interaction effect of growing conditions with exogenous application of proline was not significant with regard to the flower length (Table 7). The mean flower length regardless of treatments was also found not significant. Highest flower length was recorded in control (7.32 cm).This was followed by T2 (6.22 cm) and T3 (5.94 cm) which were on par with control.

3.4.3 Pedicel length

The interaction effect of growing conditions with regard to pedicel length was found not significant during the growing period (Table 7). The plants recorded better pedicel length in the open field condition than under control condition, even though there was no significant difference between sea water salinity. Irrespective of growing conditions, the highest pedicel length was observed in control (5.96 cm).

Table 7. Effect of exogenous application of proline on flower number, flower & pedicel length (cm) and marketable yield (g/plant) of *T. erecta* under sea water irrigation

Treatments	Flower No.	Flower L	Pedicel L	Marketable Yield
T0	12.7a	7.32a	5.96a	148.7a
T1	3.21f	3.32d	2.17c	89.2d
T2	8.08b	6.22b	3.82b	123.3b
T3	7.24c	5.94b	3.71b	104.3c
T4	3.14d	3.08d	2.29c	61.3f
T5	4.88d	3.85c	3.11c	83.2d
T6	4.02e	3.18d	3.11c	71.4e

Values followed by the same letter within treatment are not significantly different at p = 0.05

3.4.4 Marketable flower yield

The marketable flower yield of marigold was significantly influenced by the growing conditions (Table 7). Highest marketable flower yield was recorded in control (148.7 g/plant), which was significantly superior to all other treatments. Under sea water irrigation, the highest marketable flower yield was recorded in T2 (123.3 g/plant) followed by T3 (104.3 g/plant). The reduction in marketable flower yield obtained might be due to the exposure of the crop to the external fluctuating salinity. Previous reports are consistent with the current study's findings that foliar spraying with proline, methionine, and melatonin increased the yield of potato, corn, and tomato plants under drought conditions (Ibrahim *et al*., 2020; El-Yazied *et al*., 2022). Growing conditions had significant influence on the number of flowers per plant, since the interaction effect was significant for all the cultivars. The number of flowers was significantly influenced by the number of branches per plant (Pramila *et al*., 2011). Higher number of flowers under rain shelter condition might be due to the more number of branches recorded under rain shelter condition compared to open field condition. Similar results were also reported in chrysanthemum (Gantait and Pal, 2011). It was found that, the interaction effect of growing conditions on cultivars was not significant with regard to the flower length. The variation in pedicel length might be attributed to the variation in flower length. Gantait and Pal (2011) reported higher flower length in *Chrysanthemum* plants under polyhouse condition compared to open field condition. The interaction effect of growing conditions on cultivars with regard to pedicel length was found not significant during the growing period. The marketable flower yield of the cultivars was significantly influenced by the growing conditions. The reduction in marketable flower yield obtained from open field condition might be due to the exposure of the crop to the external fluctuating climatic conditions, while a controlled atmosphere is created inside the rain shelter. Bibliography (Andersen *et al*., 2011) reported that gerbera plants grown under open field condition were highly sensitive to the external climatic conditions.

The fresh weight of flower was maximum in the control followed by 10 mg L-1 proline treated plants. Similar results were observed by Abou El-Ftouh *et al*., (2018) and Patil *et al.* (2011) in marigold. Flower yield per hectare was maximum in the control and the T2 has a similar results. Similar results were observed by Abou El-Ftouh *et al*., (2018), and Raghuvanshi and Sharma (2011) in marigold. Deivanai *et al*. (2011) demonstrated that rice seeds pretreated with Pro (1, 5 and 10 mM) and grown at different NaCl concentrations counteracted the adverse effect of salt. Yan *et al*. (2011) found that application of exogenous 0.2 mM Pro to salinized nutrient solution alleviated the decrease in fresh and dry weights of *Cucumis melo* seedlings.

3.5 Conclusion

Earlier studies clearly indicated that development of stable formulations of osmoprotectants is of great importance and is a promising approach to a sustainable alternative agriculture. The current results have indicated that all measured traits were significantly affected by using sea water salinity. But the interactions with proline showed significant increase in growth, dry matter production and certain physiologically adaptive chemicals in all the plants. From the results of this investigation, it can be concluded that, the seedling treatments of proline at lower dose (10 mg L^{-1}) can be used as a good tool in the enhancement of biomass yield and alkaloid contents in floriculture crops such as marigold, as it provides an eco-friendly approach and can be used as an agent in saline stress amelioration.

3.6 References

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