biology and life sciences forum

MDPI

Proceedings

Effects of salinity on edible marigold flowers (*Tagetes patula* L.)†

María Rita Guzman [1] and Isabel Marques [2,*]

[1] Estacion Biológica La Ceiba, Chisec, Guatemala; mrguzman@gmail.com
[2] Forest Research Center (CEF) & Associate Laboratory TERRA, Instituto Superior de Agronomia (ISA), Universidade de Lisboa (UL), Tapada da Ajuda, 1349-017 Lisbon, Portugal; isabelmarques@isa.ulisboa.pt
* Correspondence: isabelmarques@isa.ulisboa.pt
† Presented at The 3rd International Electronic Conference on Agronomy.

Abstract: Salinization is an increasing problem worldwide, limiting crop production. Soil salinity causes ion toxicity, osmotic stress, nutrient deficiency and oxidative stress on plants, leading to the overproduction of reactive oxygen species (ROS). To counterbalance these effects, plants activate a complex detoxification system through the action of antioxidant pigments, carotenoids, phenolics and flavonoids, and the accumulation of minerals, that play an important role in human health against several diseases. In this study, we investigated the impacts of salinity (0, 50, 100, 300 mM NaCl) on the flowers of three *Tagetes patula* cultivars harvested after 14 days, recording total carotenoids, minerals, carotenoids, ascorbic acid, total polyphenol content, and total flavonoid content. Results showed an overall increase in all compounds with the increase in salinity levels, in comparison with control conditions. Nevertheless, salinity (most especially 100 and 300 mM) strongly affected plant size and flower production. Results showed that edible marigold flowers are a promising crop with enriched nutritional contents and antioxidant activity that can be a new source of source of nutraceuticals. However, further tests are needed to evaluate the implications that salinity might have in the viability and yield of flowers.

Keywords: edible flowers; antioxidant; nutraceutical food; salinity; tolerance

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Currently, there is an increasing consumers' demand for functional and healthy foods [1]. In this context, the market niche for edible flowers is very promising and several species have already been used in the human diet since ancient times [2]. Edible flowers add aesthetic value to food and drinks, introducing new colors and flavors in gourmet dishes, providing new opportunities for gastronomic innovation [3]. In addition, many edible flowers contain several antioxidants, vitamins, and mineral compounds that provide a wide range of valuable nutraceuticals eventually beneficial to consumers' health [4]. Edible flowers offer a wide range of phytochemicals related to the prevention of several human diseases [4]. Thus, edible flowers are promising horticultural crops providing new solutions to farmers, helping to diversify agroecosystems and the sustainable use of natural resources. However, not all flowers are edible since several plants have toxic substances and should not be included in the human diet [5]. Thus, it is important to understand the nutritional composition of flowers and the content of functional compounds that might be useful in the human diet.

Marigolds (*Tagetes* L.) are a good source of edible flowers. They have a growing demand in the food, medicinal, and floricultural industries [6]. Marigolds have high levels of antioxidant components including carotenoids, flavonoids, and phenolic acids [7] that play an important role in human health [8]. For instance, flower extracts of *T. erecta* have been reported to have a high neuroprotective potential against neurodegenerative disorders [9]. Short-saline exposure of *T. patula* plants (10 days at 50, and 100 mM NaCl) helps to increase the levels of antioxidants and other protective compounds [6]. This is because salinity causes oxidative stress in plants, leading to the overproduction of reactive oxygen species (ROS) [10]. To counterbalance these effects, plants activate a complex detoxification system through the action of antioxidant pigments, carotenoids, phenolics, and flavonoids, and the accumulation of minerals [11]. The use of saline water or the ability to grow under saline soils is also a useful trait in the floriculture market although studies remain scarce [6]. In this context, we tested the effects of salinity on the nutritional components of *Tagetes patula* flowers, grown under 0, 50, 100, and 300 mM NaCl. Specifically, we quantified the mineral composition, total proteins, fats, and phenolic compounds using three *Tagetes patula* cultivars to determine the potential beneficial effects of salinity to the production of these compounds.

2. Materials and Methods

2.1. Plant material and experimental conditions

Three *Tagetes patula* cultivars (cv. Aurora Orange, Fireball, Safari Scarlet) were grown in 2 L capacity pots, in a controlled environmental chamber under a long-day photoperiod (16 h light), temperature of 23/19 °C (light/dark period), and relative humidity of 72-76%. Plants were watered every two days using Hoagland nutritive solution. One-month plants were exposed to the following treatments: 0, 50, 100, and 300 mM NaCl levels. Each treatment consisted of 10 biological replications, totaling 40 plants per cultivar. Two weeks after treatments, flowers were harvested for the determinations stated below.

2.2. Determination of mineral contents, total fats, and total phenols

Mineral contents were determined by digesting flower samples (0.2–0.3 g) with hydrochloric acid (2 N HCl) (Merck, Darmstadt, Germany) [6]. K and Na were determined using flame photometry (Corning 410, Corning, Halstead, England), while Mg, Ca, Cu, Fe were determined by an atomic absorption spectrophotometer (Model 2280 Perkin Elmer, Spain). Data was expressed in g kg^{-1} and mg kg^{-1} of dry weight (DW) for macro- and micronutrients. The total phenols content (TPC) of flowers (0.5 g) was determined using the Folin-Ciocalteu method with 10 mL of methanol (50% v/v) and measured at 755 nm. Results were expressed as equivalents of gallic acid (Scharlau, Spain) per gram of DW. The crude protein content of samples was estimated by the macro-Kjeldahl method (N × 6.25). The total fat was determined using a Soxhlet procedure.

2.3. Statistical analysis

Mean values (± SE) were calculated from the 10 replicates per cultivar, using IBM SPSS v.22. To analyze the effects of salinity we used a multivariate ANOVA (at the 1% significance level) after checking the homogeneity of variance using Levene's Test for Equality of Variances. Significant differences between means were also followed by Tukey's test for post-hoc comparisons (at the 5% significance level).

3. Results and Discussion

Exposure to salinity significantly increased the levels of N, K, Ca, and Mg in the flowers of *Tagetes patula* cultivars although results depended on the levels of salinity (Table 1). Mineral contents were usually enhanced with the increase in salinity levels especially the levels of K (Table 1). Nevertheless, flowers grown under 300 mM NaCl usually showed lower values of minerals even in comparison with control conditions, except for K values (Table 1). Overall, plants grown under 50 mM NaCl showed the highest values of mineral contents (Table 1). Positive effects of NaCl have also been found in *Zygophyllum xanthoxylum* plants where cultivation under 50 mM NaCl resulted in optimal plant growth and reduced the negative impacts induced by different osmotic stresses [12]. Maximum growth also occurred in *Calligonum caput-medusae* seedlings while higher salinity levels decreased growth, net photosynthetic rate, and stomatal conductance [13].

The three cultivars also showed significant differences in the mineral contents (always $P<0.001$) being lowest in cv. Aurora Orange and highest in cv. Safari Scarlet. Similar values of N and K were reported in other *T. patula* flowers (cultivars not reported) grown during 10 days under 50, and 100 mM NaCl [6]. However, in that study, the levels of Ca and Mg decreased with salinity [6] while here, the response varied between cultivars (Table 1) but overall suggests a high tolerance to salinity. The vast majority of plants are intolerant to salt and unable to grow in saline soils [14]. Some plants cultivated with intermediate levels of salinity usually show high levels of antioxidant compounds although reports are usually related to halophyte species such as *Salicornia ramosissima* [15] or *Crithmum maritimum* [16]. It is thus, interesting to find edible flowers that can grow under, at least, some saline levels as found in this study.

Table 1. Effects of salinity levels (0, 50, 100 and 300 mM NaCl) on the mineral contents (N, K, Ca and Mg) of flowers from three *Tagetes patula* cultivars (cv. Aurora Orange, cv. Fireball, cv. Safari Scarlet). Results are expressed as means ± SE (n=10). Different superscripts in the same row indicate significant differences between salinity levels for the same species (ANOVA followed by a Tukey test at $p<0.001$).

Minerals	Cultivars	0	50	100	300
N	cv. Aurora Orange	11.11 ± 1.22 ^{a}	16.31 ± 1.22 ^{b}	18.25 ± 1.12 ^{c}	19.37 ± 1.56 ^{d}
	cv. Fireball	10.13 ± 1.31 ^{a}	16.44 ± 1.18 ^{b}	18.24 ± 1.35 ^{c}	19.20 ± 1.33 ^{d}
	cv. Safari Scarlet	14.21 ± 1.65 ^{a}	17.52 ± 1.11 ^{b}	19.22 ± 1.56 ^{c}	22.11 ± 1.56 ^{d}
K	cv. Aurora Orange	11.09 ± 1.17 ^{a}	12.22 ± 1.26 ^{b}	13.25 ± 1.56 ^{c}	12.15 ± 1.68 ^{b}
	cv. Fireball	11.11 ± 1.21 ^{b}	11.59 ± 1.12 ^{d}	11.16 ± 1.65 ^{c}	10.23 ± 2.55 ^{a}
	cv. Safari Scarlet	11.01 ± 1.13 ^{c}	11.55 ± 1.10 ^{d}	10.20 ± 1.32 ^{b}	10.11 ± 2.15 ^{a}
Ca	cv. Aurora Orange	3.11 ± 1.12 ^{b}	9.55 ± 1.57 ^{d}	3.21 ± 1.99 ^{c}	3.01 ± 2.95 ^{a}
	cv. Fireball	3.15 ± 1.26 ^{c}	9.50 ± 1.44 ^{d}	3.10 ± 2.60 ^{b}	3.00 ± 2.78 ^{a}
	cv. Safari Scarlet	4.03 ± 1.19 ^{b}	8.52 ± 1.35 ^{d}	4.22 ± 2.24 ^{c}	3.99 ± 2.67 ^{a}
Mg	cv. Aurora Orange	0.18 ± 0.04 ^{b}	1.55 ± 0.57 ^{d}	0.21 ± 0.05 ^{c}	0.11 ± 0.01 ^{a}
	cv. Fireball	0.14 ± 0.05 ^{b}	1.50 ± 0.57 ^{d}	0.10 ± 0.08 ^{a}	0.20 ± 0.02 ^{c}
	cv. Safari Scarlet	0.28 ± 0.04 ^{c}	1.52 ± 0.61 ^{d}	0.22 ± 0.06 ^{b}	0.19 ± 0.03 ^{a}

Tagetes patula flowers are also a promising nutraceutical food as the levels of TPC and proteins were relatively high (Figure 1). TPC showed a significant increase in all cultivars as salinity levels also increased ($F_{3,14}=121.265$; $p<0.001$; Figure 1A). All flowers showed the highest levels of TPC at 300 mM NaCl (Figure 1A). Some differentiation in the levels of TPC was found between cultivars with cv. Safari Scarlet triggering a higher level than the other cultivars but only under control ($t=3.013$; $p<0.001$) and 50 mM NaCl ($t=3.011$; $p<0.001$) conditions. Protein contents also varied significantly with salinity levels ($F_{3,14}=123.887$; $p<0.001$). The highest values of proteins were found under 50 mM and 100 mM NaCl while the lowest values were reported under 300 mM NaCl for all cultivars (Figure 1B), except for cv. Safari Scarlet where no statistical differences were found between salinity levels ($F_{3,14}=2.025$; $p=0.891$).

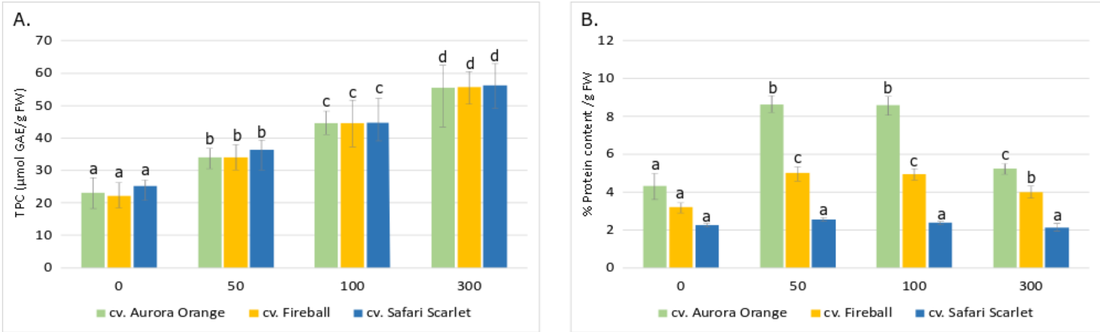

Figure 1. Effects of salinity levels (0, 50, 100 and 300 mM NaCl) on the total phenolic contents (A) and the total protein content (B) of flowers from three *Tagetes patula* cultivars (cv. Aurora Orange, cv. Fireball, cv. Safari Scarlet). Results are expressed as means ± SE (n=10). Different superscripts in the same row indicate significant differences between salinity levels for the same species (ANOVA followed by a Tukey test at $p<0.001$).

Total fat contents remained low in all cultivars despite exposure to salinity (always $P>0.05$) with an average content of 1.56 ± 0.87 in cv. Aurora Orange, 2.25 ± 0.99 in cv. Fireball and 1.02 ± 0.83 in cv. Safari Scarlet. Thus, along with its bioactive potential, the edible flowers of *T. patula* also have a nutritional combination desirable from a health point of view, with good levels of proteins but low in fatty acids. The levels of proteins reported in this study are similar to the ones found in bananas [17], the sunflower [17], or the pot marigold, among other edible flowers [18]. The replacement of high-fat food with other options that are more beneficial to public health is crucial, and edible flowers as the ones studied here are a good option in helping feed the world.

4. Conclusions

Short exposure to salinity increased the levels of compounds useful for the human diet and therefore supports the use of marigold flowers as a source of nutraceutical foods. Considering that salinity increases the levels of antioxidants and minerals in *T. patula*, farmers can consider the use of saline water for short irrigation periods in marigolds. However, future studies should also address the impacts that salinity might have on plant growth and flower production.

Author Contributions: Conceptualization, I.M.; methodology, I.M.; formal analysis, M.G.; investigation, M.G.; data curation, M.G..; writing—original draft preparation, M.G.; I.M; writing—review and editing, M.G.; I.M.; supervision, I.M.; project administration, I.M.; funding acquisition, I.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received national funds through the FCT—Fundação para a Ciência e a Tecnologia, I.P., Portugal through the research unit UIDB/00329/2020 (CE3C), UIDB/00239/2020 (CEF), and under the Scientific Employment Stimulus—Individual Call (CEEC Individual)—2021.01107.CEECIND/CP1689/CT0001 (IM).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript itself.

Acknowledgments: In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Guiné, R.; Florença, S.; Ferrão, A.C.; Correia, P. Study about the use of edible flowers for gastronomic purposes In Portugal. *Int. Conf. Mediterr. Diet Gastron.* **2018**, 41–41.
2. Navarro-González, I.; González-Barrio, R.; García-Valverde, V.; Bautista-Ortín, A.B.; Periago, M.J. Nutritional composition and antioxidant capacity in edible flowers: Characterisation of phenolic compounds by HPLC-DAD-ESI/MSn. *Int. J. Mol. Sci.* **2015**, *16*, 805–822. 10.3390/ijms16010805.
3. Cunningham, E. What Nutritional Contribution Do Edible Flowers Make? *J. Acad. Nutr. Diet.* **2015**, *115*, 856. 10.1016/j.jand.2015.03.002.
4. Benvenuti, S.; Mazzoncini, M. The Biodiversity of Edible Flowers: Discovering New Tastes and New Health Benefits. *Front. Plant Sci.* 2021, *11*, 569499. 10.3389/fpls.2020.569499.
5. Alasalvar, C.; Pelvan, E.; Özdemir, K.S.; Kocadagìli, T.; Mogol, B.A.; Pasli, A.A.; Özcan, N.; Özçelik, B.; Gökmen, V. Compositional, nutritional, and functional characteristics of instant teas produced from low- and high-quality black teas. *J. Agric. Food Chem.* **2013**, *61*, 7529–7536. 10.1021/JF4015137.
6. Chrysargyris, A.; Tzionis, A.; Xylia, P.; Tzortzakis, N. Effects of salinity on tagetes growth, physiology, and shelf life of edible flowers stored in passive modified atmosphere packaging or treated with ethanol. *Front. Plant Sci.* **2018**, *871*. 10.3389/FPLS.2018.01765.
7. Cicevan, R.; Al Hassan, M.; Sestras, A.F.; Prohens, J.; Vicente, O.; Sestras, R.E.; Boscaiu, M. Screening for drought tolerance in cultivars of the ornamental genus Tagetes (Asteraceae). *PeerJ* **2016**, *2016*. 10.7717/PEERJ.2133.
8. Hajhashemi, V.; Vaseghi, G.; Pourfarzam, M.; Abdollahi, A. Are antioxidants helpful for disease prevention? *Res. Pharm. Sci.* 2010, *5*, 5–12.
9. Moliner, C.; Barros, L.; Dias, M.I.; López, V.; Langa, E.; Ferreira, I.C.F.R.; Gómez-Rincón, C. Edible flowers of tagetes erecta l. As functional ingredients: Phenolic composition, antioxidant and protective effects on caenorhabditis elegans. *Nutrients* **2018**, *10*, 2002. 10.3390/nu10122002.
10. Hasanuzzaman, M.; Fujita, M. Plant Responses and Tolerance to Salt Stress: Physiological and Molecular Interventions. *Int. J. Mol. Sci.* 2022, *23*, 4810. 10.3390/ijms23094810.
11. Laamari, I.; Marques, I.; Ribeiro-Barros, A.I.; Zoubeir, B.; Abassi, M. Can saline preconditioning enhance plant survival in degraded soils? Physiological, biochemical, and molecular responses in Casuarina glauca saplings. *Plant Ecol. 2023* **2023**, *1*, 1–15. 10.1007/S11258-023-01346-W.
12. Yue, L.J.; Li, S.X.; Ma, Q.; Zhou, X.R.; Wu, G.Q.; Bao, A.K.; Zhang, J.L.; Wang, S.M. NaCl stimulates growth and alleviates water stress in the xerophyte Zygophyllum xanthoxylum. *J. Arid Environ.* **2012**, *87*, 153–160. 10.1016/j.jaridenv.2012.06.002.
13. Lu, Y.; Lei, J.Q.; Zeng, F.J.; Zhang, B.; Liu, G.J.; Liu, B.; Li, X.Y. Effect of NaCl-induced changes in growth, photosynthetic characteristics, water status and enzymatic antioxidant system of Calligonum caput-medusae seedlings. *Photosynthetica* **2017**, *55*, 96–106. 10.1007/S11099-016-0234-6/METRICS.
14. Green, T.G.A.; Sancho, L.G.; Pintado, A.; Saco, D.; Martín, S.; Arróniz-Crespo, M.; Casermeiro, M.A.; Caravaca, M.T. de L.C.; Cameron, S.; Rozzi, R. Sodium chloride accumulation in glycophyte plants with cyanobacterial symbionts. *AoB Plants* **2017**, *9*. 10.1093/aobpla/plx053.
15. Lima, A.R.; Castañeda-Loaiza, V.; Salazar, M.; Nunes, C.; Quintas, C.; Gama, F.; Pestana, M.; Correia, P.J.; Santos, T.; Varela, J.; et al. Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of Salicornia ramosissima commercially produced in soilless systems. *Food Chem.* **2020**, *333*, 127525. 10.1016/j.foodchem.2020.127525.
16. Ventura, Y.; Myrzabayeva, M.; Alikulov, Z.; Omarov, R.; Khozin-Goldberg, I.; Sagi, M. Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. *AoB Plants* **2014**, *6*. 10.1093/AOBPLA/PLU053.
17. Takahashi, J.A.; Rezende, F.A.G.G.; Moura, M.A.F.; Dominguete, L.C.B.; Sande, D. Edible flowers: Bioactive profile and its potential to be used in food development. *Food Res. Int.* **2020**, *129*, 108868. 10.1016/j.foodres.2019.108868.
18. Jakubczyk, K.; Koprowska, K.; Gottschling, A.; Janda-milczarek, K. Edible Flowers as a Source of Dietary Fibre (Total, Insoluble and Soluble) as a Potential Athlete's Dietary Supplement. *Nutrients* **2022**, *14*. 10.3390/nu14122470.