Salicylic acid and boric acid improve flower growth, yield, and cold tolerance in French marigold (*Tagetes patula*)

Toktam Moradian[1], Rahele Ghanbari Moheb Seraj[2] and Ahmad Asgharzade[1]*

1. Department of Horticultural Sciences, Islamic Azad University, Shirvan Branch, Shirvan, Iran
2. Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

Abstract

A decrease in air temperature harms many ornamental plants, especially flowers grown in green space. To prevent cold stress, various elicitors are used that reduce the harmful effects of low temperature. Therefore, in this study, we investigated the impact of two elicitors, viz. salicylic acid (SA) and boric acid (BA), on the growth and yield parameters of French marigold (*Tagetes patula*). Plants were exposed to cold stress, and their physiological and morphological traits were studied. Results showed that the application of elicitors, especially at higher concentrations, had a positive effect on morphological and physiological traits. Interaction of SA×BA with a concentration of 2 mM, increased flower diameter (20%), stem height (19%), stem and root fresh weights (27% and 35% respectively), stem and root dry weight (31% and 39% respectively), and flower quality (26%). Also, the interaction of these two elicitors led to a decrease in ion leakage (22%), malondialdehyde (28%), and proline (24%) content, and an increase in chlorophyll fluorescence (28%), catalase (20%), and ascorbate peroxidase (33%) activity. In general, combined treatment with2 mM salicylic acid and boric acid improved growth and yield characteristics as well as cold stress tolerance in French marigold.

Keywords: antioxidant enzymes, boric acid, cold stress, French marigold, proline, salicylic acid

Moradian, T., R. Ghanbari Moheb Seraj, A. Asgharzade. 2023. 'Salicylic acid and boric acid improve flower growth, yield, and cold tolerance in French marigold (*Tagetes patula*)'*. Iranian Journal of Plant Physiology 13* (4), 4741-4751.

Introduction

Plants experience a variety of abiotic stresses including high and low temperatures, drought, salinity, and heavy metals (Verma et al., 2013). Cold stress causes many physiological changes in plants, including variations in growth characteristics, relative electrolyte leakage (RLR), chlorophyll fluorescence, proline content, soluble sugars, malondialdehyde (MDA), and antioxidant enzymes (CAT, APX, SOD), etc. (Liu et al., 2018).

Application of plant growth regulators (PGR) plays a significant role in the plants facing various abiotic stresses. Among different plant growth regulators, salicylic acid (SA) plays a diverse role in improving several morphological, cytological, physiological, and biochemical attributes in plants facing biotic and abiotic stresses (Saleem et al., 2021). Accumulation of SA in chilling stress has

---

* *Corresponding Author*
E-mail Address: asgharzadeh.a@iau-shirvan.ac.ir
Received: March, 2022
Accepted: March, 2023

been revealed in numerous plant species (Kosová et al., 2012; Wan et al., 2009). SA application has improved tolerance to cold stress in several plant species. It was first demonstrated in maize (Janda et al., 1999), followed by various other species such as potato, rice, etc. (Koyro et al., 2012). In addition to hormones, macro and micro-nutrients also affect the yield and quality of crops and also stress tolerance (Aftab et al., 2015). Like other mineral nutrients, an optimal boron concentration (B) is required for plant growth. Boron deficiency is the second essential micronutrient limiting plant growth after zinc (Ahmad et al., 2012).

Application of boron increases stress tolerance in plants by regulating the activity of antioxidant enzymes, proline, the content of secondary metabolites, stabilizing the integrity of proteins, and reducing reactive oxygen species (Riaz et al., 2018). Boron deficiency in plants reduces biomass performance and disrupts the metabolism of phenols and carbohydrates (Dugger, 1983). In many plants, including cereals, boron deficiency causes financial losses due to a significant reduction in flowering and fruit development (Misra and Patil, 1987). In addition, boron participates in osmotic adjustment, phenolic metabolism, oxidative stress, and sugars translocation (Marschner, 2011). Low B nutrients caused damage in cell membranes through an enhanced accumulation of phenolics and production of free oxides that ultimately diminished plant growth (Naeem et al., 2018). However, sufficient B supply corrected B nutrient deficiency and caused substantial improvement in the growth of *Brassica rapa* (Hajiboland and Bastani, 2012) and *Zea mays* (Lordkaew et al., 2011). B application as a foliar spray improved the photosynthetic rate (Pn) by improving stomatal conductance (Naeem et al., 2018). This argument is further supported by the fact that B plays a vital role in maintaining chloroplast structure, regulating Calvin Cycle enzymes, and photochemical efficiency of PSII (Han et al., 2008; Lu et al., 2014).

French marigold (*Tagetes patula* L.) belongs to the family Asteraceae and a genus of 52 species of annual and perennial herbaceous plants. They are native to the area stretching from the southwestern United States to Mexico and South America (Edward, 1999). It has pretty flowers that are gaining commercial status because of a wide range of adaptation and increasing demand in a wide area of the world (Asif, 2008). French marigold is also one of the most important herbaceous ornamental plants with an aromatic scent in flowerbeds for mass display, pots, borders, and with a capability of being used effectively in the window and porch boxes (Vasudevan et al., 1997). The flowers are used in medicines and pharmaceuticals, processed foods, confectionery, and poultry industry. Furthermore, its essential oil has applications as antiparasitic, antispasmodic, antibiotic, antimicrobial, and antiseptic agent (Chowdhury et al., 2009).

Many ornamental plants, especially those cultured in urban green spaces, are constantly exposed to low temperatures, and many of them are destroyed. In this study, we investigated the effect of salicylic acid and boric acid and their interaction on morphological and physiological parameters of French marigolds (*Tagetes patula*) under cold stress in

Materials and Methods

Experimental site and design

This experiment was performed in the Research Greenhouse of Islamic Azad University, Shirvan Branch in Northern Khorasan, Iran, and physiological parameters were measured in the Plant Physiology Research Laboratory. This experiment was performed in a completely randomized design with four concentrations of salicylic acid and boric acid in three replications.

Cold stress and elicitor treatments

French marigold seeds French obtained from Pakanbazr, Isfahan, were planted in pots with a certain soil mixture, including ordinary soil + manure + sand. The pots were then named according to the type of treatments. Then, the flowers were irrigated every two days during the growth stages. To apply treatments, the plants were sprayed with salicylic acid and boric acid solutions twice a week. The concentrations used for each elicitor included 0, 0.5, 1 and 2 mM. Then, the effect of foliar application of salicylic acid and boric acid was evaluated on morphological traits at the end of the growth stage. Next, the pots were placed in a cold room to apply cold stress (research freezer, cold stress test, and Grock engineering design). The room temperature started at 25 ℃ and dropped by one degree every half hour. After reaching -4 ℃, the plants were exposed to cold for one hour, and then their physiological parameters were calculated.

Morphological parameters

Fresh flower diameter and stem height were measured using a caliper with an accuracy of 1 mm. Flower quality was graded from zero to 100 according to the appearance and freshness of the flower. For dry weight of roots and shoots, the plants were placed in an oven at 70 ℃ for 24 hours, and then their weight was measured using a digital scale with an accuracy of 0.01 g. The fresh weight of roots and shoots was also measured immediately after sampling using a digital scale with an accuracy of 0.01 g.

Physiological parameters

Fluorescence chlorophyll content

A fluorometer was used to measure chlorophyll fluorescence. For this purpose, the clamp of the device was attached to the leaves, and darkness was induced in the desired part of the leaf. After half an hour, the diode was connected to the clamp and Fv/Fm index of plants was read (Saadati et al., 2019).

Ion leakage percentage

Two discs with a diameter of 1 cm were prepared from each leaf and placed in an Erlenmeyer flask containing 10 ml of double-distilled water at room temperature. After 24 hours, the electrical conductivity of the water inside the Erlenmeyer was measured by an EC meter. Then, the Erlenmeyer containing the sample was autoclaved for one hour at 120 ℃ (HIRAYAMA brand HG-80), and its electrical conductivity was determined again. Finally, the percentage of ion leakage was calculated from the following formula (Afrousheh et al., 2018).

$$Ion\ leakage\ (\%) = \frac{initial\ leakage}{final\ leakage} \times 100$$

Proline content

Proline content was determined by the ninhydrin method (Bates et al., 1973). The absorbance of the solution was measured at 520 nm using a spectrophotometer. A calibration curve was created with proline as a standard.

Malondialdehyde (MDA)

Lipid peroxidation was specified by the method described in Stewart and Bewley (1980). The samples' absorption was read at 532 nm, and the quantity of nonspecific absorption was read at 600 nm and subtracted from 532 nm absorption. The malondialdehyde content was determined by the extinction coefficient of 155 $cm^{-1}mmol^{-1}$.

Antioxidant enzymes

Activity of catalase enzyme was determined following Bergmeyer method (Aebi, 1974). The absorbance was recorded at 240 nm for 3 minutes, and then the catalase activity was calculated.

The ascorbate peroxidase enzyme activity was measured by the method of Nakano and Asada (Nakano and Asada, 1987). The mixture absorption was read at 290 nm for 3 minutes, and then the enzyme activity was calculated. A reaction mixture without enzymatic extracts was used as spectrophotometer control.

Statistical Analysis

Statistical analysis for all morpho-physiological data was performed using R version 3.5.3 (Team, 2017) and RStudio version 1.1.463 (Allaire, 2012). Data were analyzed by ANOVA for completely randomized blocks design with treatments as fixed effects and replications as random effect. Mean values were compared using Duncan test function provided in the Agricolae Package (De Mendiburu, 2014) at 5% significance level of probability. The Pearson correlation coefficient was also calculated for all parameters using R, Corrplot Package. All figures and tables were provided with the Word and Excel 2013 software.

Table 1
ANOVA analysis of interaction of salicylic acid and boric acid on some morphological parameters of French marigold

Sources of variation	df	Means of Square						
		Flower diameter	Plant height	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Flower quality
SA	3	0.812^{**}	1.559^{**}	3.836^{**}	9.499^{**}	0.024^{**}	0.044^{**}	570.6^{**}
BA	3	0.601^{**}	1.278^{*}	1.369^{**}	4.618^{**}	0.009^{**}	0.127^{**}	603.5^{**}
SA×BA	9	0.818^{**}	2.559^{**}	4.515^{**}	4.734^{**}	0.024^{**}	0.043^{**}	281.9^{**}
Error	30	0.106	0.314	0.029	0.186	0.001	0.001	6.372
CV (%)	-	8.76	5.67	7.50	15.09	11.54	14.31	3.27

*, **, and ns: significance at 5 and 1 of probability levels and non-significance, respectively

Table 2
ANOVA analysis of interaction of salicylic acid and boric acid on some physiological parameters of French marigold under cold stress

Sources of variation	df	Means of Square					
		Ion leakage	Chlorophyll fluorescence	Proline	Malondialdehyde (MDA)	Catalase (CAT)	Ascorbate peroxidase (APX)
SA	3	5107.5^{*}	0.004^{ns}	0.758^{*}	434^{**}	91.29^{**}	1.611^{**}
BA	3	2138.5^{ns}	0.014^{ns}	1.343^{**}	721^{**}	107.73^{**}	6.238^{**}
SA×BA	9	2277.4^{*}	0.024^{*}	0.170^{*}	463^{**}	92.07^{**}	0.696^{**}
error	30	1245.6	0.010	0.178	14	25.37	0.005
CV (%)	-	32.48	17.33	38.65	20.25	18.15	14.12

*, **, and ns: significance at 5 and 1 of probability levels and non-significance, respectively

Results

The results of ANOVA analysis of morphological parameters showed that the simple effects of SA and the interaction of SA×BA in all traits of flower diameter, plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and flower quality were significant at P≤0.01, but the simple effect of BA, except for the plant height which was significant at 0.05, in other traits was significant at 0.01 (Table 1).

Variance analysis of physiological traits also showed that the simple effect of SA on MDA and enzyme activity of CAT and APX was significant at P≤0.01, but on ion leakage and proline content the effect was significant at P≤0.01. The simple effect of SA was not significantly different on chlorophyll fluorescence. The simple effect of BA on proline, MDA, CAT, and APX enzymes was significant at P≤0.01 while chlorophyll fluorescence and ion leakage were not significantly different. SA×BA interactions on MDA, CAT, and, APX enzymes were also significant at 0.01 probability level, but on ion leakage and chlorophyll fluorescence the interaction effects were significant at P≤0.01 (Table 2).

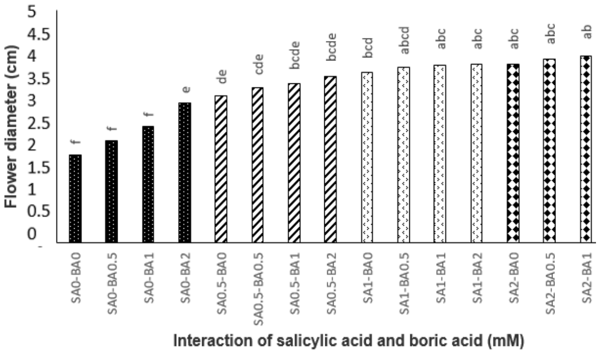

Fig. I. Interaction of different concentrations of salicylic acid and boric acid on the flower diameter of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

Interaction of salicylic acid and boric acid on morphological parameters of French marigold

Simultaneous application of salicylic acid and boric acid, especially at higher concentrations, significantly increased flower diameter (Fig. I). Foliar application of both elicitors with a concentration of 2 mM resulted in the highest flower diameter (4.33 cm) while control plants showed the lowest flower diameter (1.9 mm).

According to the mean comparison results, the combined application of salicylic acid and boric

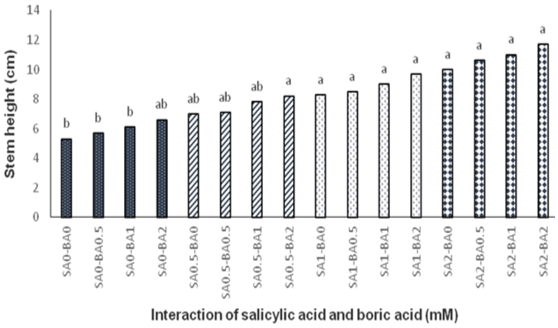

Fig. II. Interaction of different concentrations of salicylic acid and boric acid on stem height of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

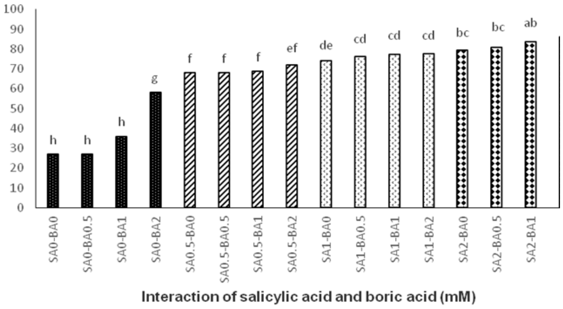

Fig. III. Interaction of different concentrations of salicylic acid and boric acid on flower quality of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

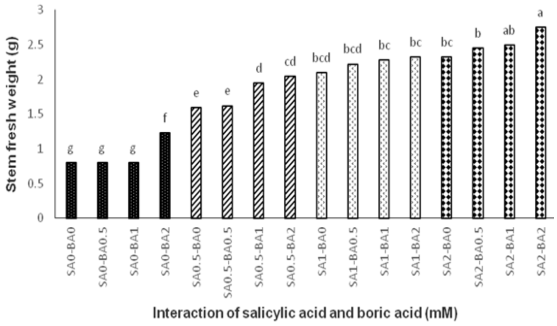

Fig. IV. Interaction of different concentrations of salicylic acid and boric acid on fresh stem weight of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

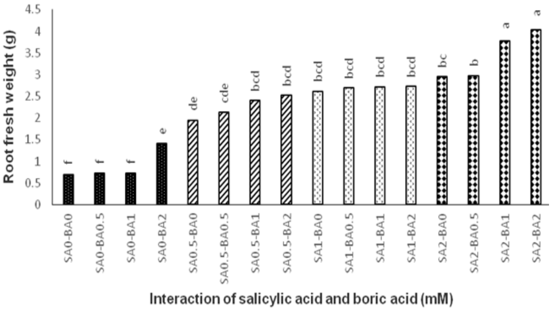

Fig. V. Interaction of different concentrations of salicylic acid and boric acid on fresh root weight of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

acid elicitors led to a significant increase in stem height (Fig. II). The highest stem height (11.7 cm) was observed in the combined treatment of salicylic acid (2 mM) and boric acid (2 mM). In comparison, the lowest height of the stem (5.3 cm) was observed in control plants (without foliar application of both elicitors). The analysis of variance also showed that 0.1 boric acid treatment significantly reduced plant height.

The quality of flowers also increased with increasing the concentration of elicitors in the interaction treatment of two elicitors (SA×BA) (Fig. III). The highest flower quality (86.08%) was observed in SA (2 mM) × BA (2 mM) treatment and the lowest was observed in treatments with SA (0 mM) × BA (0 mM), SA (0 mM) × BA (0.5 mM), and SA (0 mM) × BA (1 mM) with flower quality percentage of 27, 27, and 36%, respectively. There was no significant difference between different concentrations of BA, but the application of SA had a significant effect in increasing flower quality (Fig. VIII).

According ANOVA results, the interaction of salicylic acid and boric acid elicitors significantly increased the fresh weight of stem and root (Figs. IV & V). The use of lower concentrations of these elicitors had more negligible effect than higher concentrations. The highest fresh weights of the stems (2.75 g) and roots (4.03 g) were observed in the combined treatment with salicylic acid and boric acid (2 mM), and the lowest fresh weights of stems (0.8 g) and roots (0.7 g) were recorded in the control treatment, the interaction of salicylic acid (0 mM) and boric acid (0.5 mM), and the interaction of salicylic acid (0 mM) and boric acid (1 mM) concentration (Figs. IV & V).

Similar to fresh weight, the interaction of the two elicitors led to an increase in dry weights of stems and roots. Also, with increasing concentration of elicitors, dry weight increased. The highest dry weights of stems (0.29 g) and roots (0.45 g) were observed in SA×BA treatment (2 mM) while the lowest dry weights of stems and roots were observed in control plants (0.068-0.058 g), the interaction of salicylic acid (0 mM) and boric acid (0.5 mM) (0.077-0.07 g), and the interaction of salicylic acid (0 mM) and boric acid (1 mM) (0.077-0.07 gr), in that order. SA had a more significant effect on stem and root dry weighs than BA (Fig. VI and VII).

Interaction of salicylic acid and boric acid on physiological parameters of French marigold under cold stress

Ion leakage percentage: comparison of mean ion leakage showed that cold stress generally increased ion leakage in plants. Application of elicitors with higher concentrations reduced ion leakage. Ion leakage had the lowest value (60, 60, and 53%) in SA (2 mM) × BA (0.5 mM), SA (2 mM) × BA (1mM), and SA (2 mM) × BA (2 mM) treatments, and in other treatments, ion leakage had a high value, showing no significant difference (Fig VIII).

Chlorophyll fluorescence: Under cold stress, chlorophyll fluorescence decreased. The use of elicitors (SA and BA) increased chlorophyll fluorescence in a dose dependent manner (Fig. IX). The highest range of chlorophyll fluorescence was observed in the interaction of SA×BA (2 mM) (0.71 Fv/Fm) while the lowest value (0.2 Fv/Fm) was recorded with the interaction of SA (0 mM) × BA (0 mM), SA (0 mM) × BA (0.5 mM), and SA (0 mM) × BA (1 mM). There was no significant difference between other treatments (Fig. IX).

Proline content: Under cold stress, the proline content increased. Foliar spraying of elicitors, especially SA, reduced the proline content. Treatments with SA (2 mM) × BA (0.5 mM), SA (2 mM) × BA (1 mM), and SA (2 mM) × BA (2 mM) resulted in the lowest proline content (0.62, 0.62, and 0.55 mmol/FW, respectively). Other treatments resulted in high proline content and no significant difference was observed between them (Fig. X).

MDA content: Cold stress led to an increase in MDA (Fig. XI). The use of SA and BA elicitors resulted in a reduction in the lipid peroxidation in cells and thus, a decrease in MDA. The application of a higher concentration of elicitors reduced the amount of MDA. The highest amount of MDA was

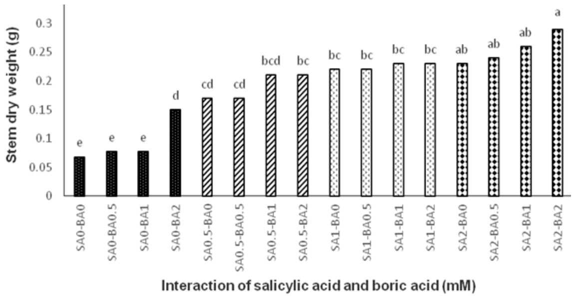

Fig. VI. Interaction of different concentrations of salicylic acid and boric acid on dry stem weight of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

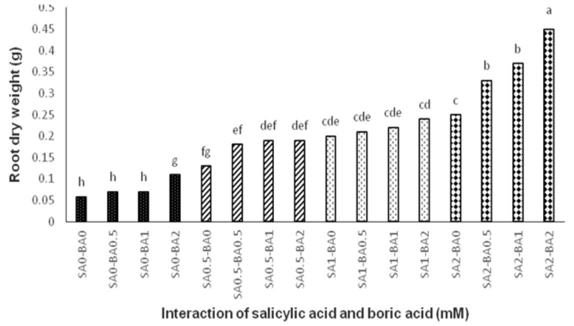

Fig. VII. Interaction of different concentrations of salicylic acid and boric acid on dry root weight of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

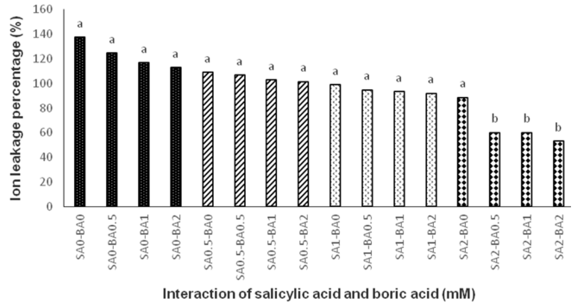

Fig. viii. Interaction of different concentrations of salicylic acid and boric acid on the percentage of ion leakage of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

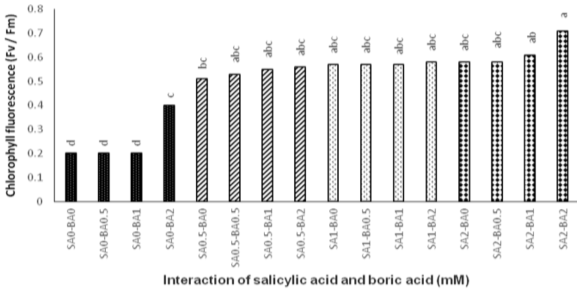

Fig. IX. Interaction of different concentrations of salicylic acid and boric acid on chlorophyll fluorescence of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

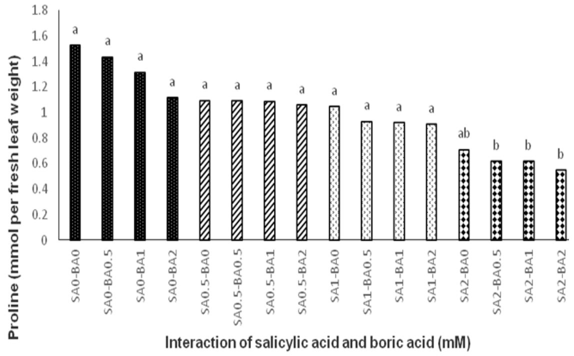

Fig. X. Interaction of different concentrations of salicylic acid and boric acid on proline content of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

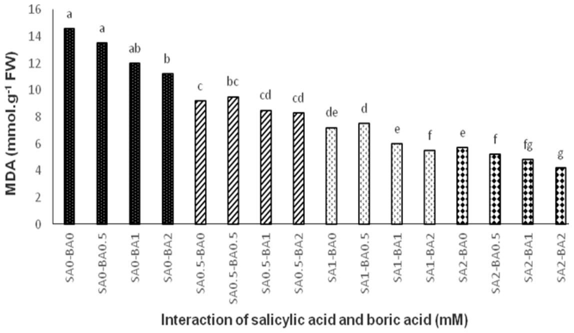

Fig. XI. Interaction of different concentrations of salicylic acid and boric acid on MDA content of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

observed in control (14.6 m mol g^{-1} FW) and BA 0.5 mM (13.5 m mol g^{-1} FW) while the lowest MDA concentration was recorded in the treatment with SA×BA (2 mM). Therefore, 2 mM of both elicitors responded better to cold stress and prevented lipid peroxidation.

Antioxidant enzymes activity: the activity of antioxidant enzymes (CAT and APX) increased under cold stress, but the use of elicitors increased enzymes even more (Figs. XII & XIII). Higher concentrations of these elicitors, especially SA, caused more increase in enzymes activity. The highest levels of CAT and APX activity were observed in the treatment with SA (2 mM) × BA (1 mM) (15.7-121.5 $U.min^{-1}g^{-1}FW$) and SA (2 mM) × BA (2 mM) (16.3-123.6 U $min^{-1}g^{-1}FW$), respectively while the lowest levels were recorded with the control (7.1-25.3 U $min^{-1}g^{-1}FW$) and SA (0 mM) × BA (0 mM) (7.5-28.8 $U.min^{-1}g^{-1}FW$), respectively (Fig XII and XIII).

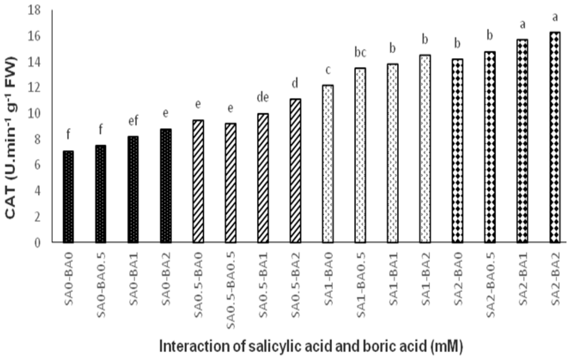

Fig. XII - Interaction of different concentrations of salicylic acid and boric acid on CAT activity of French marigold; different letters indicate statistically significant differences (Dunn's test, p<0.05).

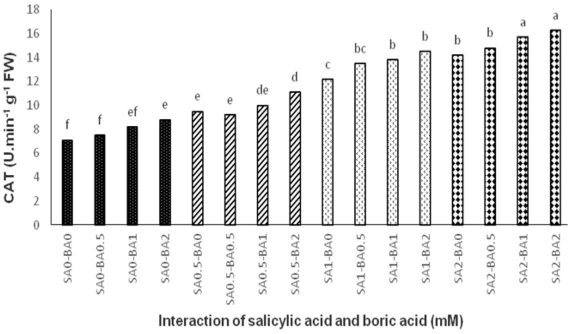

Fig. XIII. Interaction of different concentrations of salicylic acid and boric acid on APX activity of French marigold. different letters indicate statistically significant differences (Dunn's test, p<0.05).

Discussion

French marigold is used as a container and bedding plant in the landscape and urban green spaces, respectively (Sun et al., 2018). Cultivation of this plant in urban green spaces exposes it to temperature changes, including air cooling. Cold stress affects plant growth and development, causing physiological, biochemical, and molecular processes (Yadav, 2010). In the first stage, it affects the permeability of the cell membrane (Yadav, 2010). Next, it disrupts the stability of proteins and reduces the activity of ROS inhibitory enzymes. These processes then lead to disruption of photosynthesis and damage to the membrane (Ruelland et al., 2009; Siddiqui and Cavicchioli, 2006).

The results of this experiment showed that application of salicylic acid improved the morphological and physiological parameters of French marigold. Moreover, plants receiving higher concentrations of SA responded better to cold stress tolerance, showing improved growth characteristics. Molaei et al. (2014) (Mansouri, 2012) reported that foliar application of salicylic acid increased the heights of the rose gerbera stems, respectively. Salicylic acid increases growth by increasing cell division in the terminal meristem and elongation of the cell (Sauter and Kende, 1992).

Saleem et al. (2021) reported a comprehensive study on the role of salicylic acid in the improvement of cold stress in plants. Chilling stress promoted the accumulation of endogenous free SA and glucosyl SA in plants (Kosová et al., 2012; Scott et al., 2004; Wan et al., 2009), suggesting that SA is involved in regulating cold responses. Mild cold stress and SA application through influencing the quality of protein translation, priming of seed metabolism, synthesis of antioxidant enzymes, and mobilization of the seed storage proteins could alter the morphological and physiological traits of plants and seed vigor (Horváth et al., 2007; Rajjou et al., 2006). Khandan-Mirkohi et al. (2021) investigating the effect of mild cold stress and salicylic acid in two methods of seed priming and foliar spraying on growth and physiological traits of periwinkle (*Catharanthus roseus* Don.) showed that mild cold stress with salicylic acid (0.5 mM) in both methods was the best treatment to improve the growth parameters and activity of antioxidant enzymes. According to their reports, mild cold stress increased the positive effect of salicylic acid on plants' adaptation to cold, especially in the spraying method on the plant. Other researchers have also reported the effect of salicylic acid in reducing the harmful effects of cold stress in various plants, including *Phaseolus vulgaris* (Soliman et al., 2018), *Brassica napus* L. (KESHAVARZ et al., 2016), *Hordeum vulgare* L. (Mutlu et al., 2016).

Our results showed that application of boric acid improved the morphological and physiological parameters of French marigold, although its effects were less than those of salicylic acid. There is evidence indicating that the use of boric acid in cold conditions leads to increased plant growth (Bellaloui et al., 2013; Sindoni et al., 1994) and tolerance to cold stress by increasing proline and chlorophyll content, increasing leaf photosynthesis, maintaining osmotic pressure, and reducing MDA (Movahhedi Dehnavi et al., 2017; Nasef et al., 2006). (Dezhabad and Haghighi, 2020) investigated the effect of feeding tomato seedlings with boric acid on some characteristics of tomato plants under cold stress. Boron is involved in many processes, including sugar transport, cell wall synthesis and maintenance, membrane integrity, RNA, indole acetic acid (IAA), and phenol metabolism (Dordas and Brown, 2000; Matoh, 1997). The precise role of this micronutrient has not been elucidated. It was recently shown that boron is essential in pollen germination and tube formation. It has a vital function in fertilization of flowering crops as well (BIGDELI et al., 2016). There is limited evidence that cold in the root zone limits the ability of boron absorption, but the nature of this interaction depends on the cold tolerance of plant species (Huang et al., 2005). In our experiment, we overcame the absorption problem by spraying this element.

According to our results, the simultaneous use of SA and BA had an increasing effect on growth, yield, and cold stress tolerance in French marigold. Foliar application of both elicitors with a concentration of 2 mM resulted in the highest flower diameter (4.33 cm) while control plants showed the lowest flower diameter (1.9 mm). Flower diameter has an essential role in the marketing of cut flowers (Babarabie et al., 2018). Mortazavi et al. (2015) reported that salicylic acid increased the diameter of Lilium florets (Mortazavi et al., 2015). They attributed the increase in flower diameter to the hormone-like activity of salicylic acid (Fig. II). Application of salicylic acid was also reported to improve the toxic effects of higher concentrations of boron in plants (Nawaz et al., 2020). Therefore, in our experiment, the harmful effects of boric acid may be mitigated by salicylic acid. Based on these findings, more studies are recommended on simultaneous application of these two elicitors with other ornamental plants.

Conclusion

French marigold is one of the flowers with a high color variation and is used to beautify green spaces. Since this flower is grown in open air, it is exposed to cold weather. Salicylic acid and boric acid are two compounds whose effects on cold conditions have been confirmed by various researchers. Therefore, in this experiment, we used these two elicitors simultaneously to investigate their combined impact on French marigold. According to our results, the combined application of these two compounds improved the growth and yield parameters of plants. In addition, under cold stress, chlorophyll fluorescence, osmolytes content, and antioxidant enzyme activity increased while lipid peroxidation and electrolyte leakage decreased. Therefore, simultaneous application of these compounds is recommended to improve cold stress tolerance in French marigold.

Acknowledgments

We would like to thank the Islamic Azad University, Shirvan Branch for providing us with financial means to complete this project.

References

Aebi, H. 1974. In HU Bergmeyer (ed.). Methods of Enzymatic Analysis. Academic Press, New York

Afrousheh, M., H. Hokmabadi, H. Arab and A. Tajabadipour. 2018. Evaluation of Freezing Damage in some Pistachio Seedling Rootstocks. *Journal of Nuts,* 9, (1) 77-83.

Aftab, M., M. Anwar-Ul-Haq, J. Akhtar and E. A. Waraich. 2015. Salinity and boron tolerance in cotton (*Gossypium hirsutum*) varieties: A short-term hydroponic study. *International Journal of Agriculture and Biology,* 17, (4)

Ahmad, W., M. H. Zia, S. S. Malhi, A. Niaz and S. Ullah. 2012. Boron Deficiency in soils and crops: a review. *Crop plant,* 2012, 65-97.

Allaire, J. 2012. RStudio: integrated development environment for R. *Boston, MA,* 770, (394) 165-171.

Asif, M. 2008. Effect of various NPK levels on growth, yield and xanthophyll contents of Marigold. *MSc Th esis Inst of Hort Sci, Univ of Agric, Faisalabad, Pakistan,* 95.

Babarabie, M., H. Zarei and S. Badeli. 2018. Morphological, physiological and biochemical response of *Chrysanthemum* to thiamine and salicylic acid. *Journal of Plant Physiology and Breeding,* 8, (2) 109-120.

Bates, L. S., R. P. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil,* 39, (1) 205-207.

Bellaloui, N., Y. Hu, A. Mengistu, M. A. Kassem and C. A. Abel. 2013. Effects of foliar boron application on seed composition, cell wall boron, and seed δ15N and δ13C isotopes in water-stressed soybean plants. *Frontiers in Plant Science,* 4, 270.

Bigdeli, M. M., A. Imani and M. Shamili. 2016. Effects of boron and cold stress on germination of almond pollen in vitro culture.

Chowdhury, M. S. H., M. Koike, N. Muhammed, M. A. Halim, N. Saha and H. Kobayashi. 2009. Use of plants in healthcare: a traditional ethno-medicinal practice in rural areas of southeastern Bangladesh. *International Journal of Biodiversity Science & Management,* 5, (1) 41-51.

De Mendiburu, F. 2014. Agricolae: statistical procedures for agricultural research. *R package version,* 1, (1)

Dezhabad, F. and M. Haghighi. 2020. Bottom-cold stress was less harmful than cold-air stress on tomato seedling production treated with boric acid. *Acta Physiologiae Plantarum,* 42, (4) 1-13.

Dordas, C. and P. Brown. 2000. Permeability of boric acid across lipid bilayers and factors affecting it. *The Journal of membrane biology,* 175, (2) 95-105.

Dugger, W. 1983. Boron in plant metabolism. In: Encyclopedia of plant physiology (Eds.: A. Pirson and MH Zimmermann). Vol. 158 (New Series). Springer-Verlag, Berlin

Edward, F. 1999. Fact Sheet FPS-569. *Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida,*

Hajiboland, R. and S. Bastani. 2012. Tolerance to water stress in boron-deficient tea (*Camellia sinensis*) plants. *Folia Horticulturae,* 24, (1) 41.

Han, S., L.-S. Chen, H.-X. Jiang, B. R. Smith, L.-T. Yang and C.-Y. Xie. 2008. Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. *Journal of plant physiology,* 165, (13) 1331-1341.

Horváth, E., G. Szalai and T. Janda. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. *Journal of Plant Growth Regulation,* 26, (3) 290-300.

Huang, L., Z. Ye, R. W. Bell and B. Dell. 2005. Boron nutrition and chilling tolerance of warm climate crop species. *Annals of botany,* 96, (5) 755-767.

Janda, T., G. Szalai, I. Tari and E. Paldi. 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. *Planta,* 208, (2) 175-180.

Keshavarz, H., S. a. M. M. Sanavy and R. S. G. Moghadam. 2016. Impact of foliar application with salicylic acid on biochemical characters of canola plants under cold stress condition. *Notulae Scientia Biologicae,* 8, (1) 98-105.

Kosová, K., I. T. Prášil, P. Vítámvás, P. Dobrev, V. Motyka, K. Floková, O. Novák, V. Turečková, J. Rolčik and B. Pešek. 2012. Complex phytohormone responses during the cold acclimation of two wheat cultivars differing in cold tolerance, winter Samanta and spring Sandra. *Journal of plant physiology,* 169, (6) 567-576.

Koyro, H.-W., P. Ahmad and N. Geissler. 2012. Abiotic stress responses in plants: an overview. *Environmental adaptations and stress tolerance of plants in the era of climate change,* 1-28.

Liu, X., Y. Zhou, J. Xiao and F. Bao. 2018. Effects of chilling on the structure, function and development of chloroplasts. *Frontiers in plant science,* 9, 1715.

Lordkaew, S., B. Dell, S. Jamjod and B. Rerkasem. 2011. Boron deficiency in maize. *Plant and Soil,* 342, (1) 207-220.

Lu, Y.-B., L.-T. Yang, Y. Li, J. Xu, T.-T. Liao, Y.-B. Chen and L.-S. Chen. 2014. Effects of boron deficiency on major metabolites, key enzymes and gas exchange in leaves and roots of *Citrus sinensis* seedlings. *Tree physiology,* 34, (6) 608-618.

Mansouri, H. 2012. Salicylic acid and sodium nitroprusside improve postharvest life of chrysanthemums. *Scientia Horticulturae,* 145, 29-33.

Marschner, H. 2011. *Marschner's mineral nutrition of higher plants*. Academic press

Matoh, T. 1997. Boron in plant cell walls. *Plant and Soil,* 193, (1) 59-70.

Misra, S. and B. Patil. 1987. Effect of boron on seed yield in Lucerne (*Medicago sativa* L.). *Journal of Agronomy and Crop Science,* 158, (1) 34-37.

Mortazavi, S., V. Karimi and M. Azimi. 2015. Pre-harvest foliar application of humic acid, salicylic acid and calcium chloride to increase quantitative and qualitative traits of Lilium longiflorum cut flowers. *Journal of Science and Technology of Greenhouse Culture,* 6, (23)

Movahhedi Dehnavi, M., A. Yadavi and M. Merajipoor. 2017. Physiological responses of sesame (*Sesamum indicum* L.) to foliar application of boron and zinc under drought stress conditions. *Journal of Plant Process and Function,* 6, (20) 27-36.

Mutlu, S., Ö. Atıcı, B. Nalbantoğlu and E. Mete. 2016. Exogenous salicylic acid alleviates cold damage by regulating antioxidative system in two barley (*Hordeum vulgare* L.) cultivars. *Frontiers in Life Science,* 9, (2) 99-109.

Naeem, M., M. S. Naeem, R. Ahmad, R. Ahmad, M. Y. Ashraf, M. Z. Ihsan, F. Nawaz, H.-U.-R. Athar, M. Ashraf and H. T. Abbas. 2018. Improving drought tolerance in maize by foliar application of boron: water status, antioxidative defense and photosynthetic capacity. *Archives of Agronomy and Soil Science,* 64, (5) 626-639.

Nakano, Y. and K. Asada. 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant and cell physiology,* 28, (1) 131-140.

Nasef, M., N. Badran and A. Abd El-Hamide. 2006. Response of peanut to foliar spray with boron and/or rhizobium inoculation. *Journal of Applied Sciences Research,* 2, (12) 1330-1337.

Nawaz, M., S. Ishaq, H. Ishaq, N. Khan, N. Iqbal, S. Ali, M. Rizwan, A. A. Alsahli and M. N. Alyemeni. 2020. Salicylic Acid Improves Boron Toxicity Tolerance by Modulating the Physio-Biochemical Characteristics of Maize (*Zea mays* L.) at an Early Growth Stage. *Agronomy,* 10, (12) 2013.

Rajjou, L., M. Belghazi, R. Huguet, C. Robin, A. Moreau, C. Job and D. Job. 2006. Proteomic investigation of the effect of salicylic acid on *Arabidopsis* seed germination and establishment of early defense mechanisms. *Plant physiology,* 141, (3) 910-923.

Riaz, M., L. Yan, X. Wu, S. Hussain, O. Aziz, Y. Wang, M. Imran and C. Jiang. 2018. Boron alleviates the aluminum toxicity in trifoliate orange by regulating antioxidant defense system and reducing root cell injury. *Journal of environmental management,* 208, 149-158.

Ruelland, E., M.-N. Vaultier, A. Zachowski and V. Hurry. 2009. Cold signalling and cold acclimation in plants. *Advances in botanical research,* 49, 35-150.

Saadati, S., B. Baninasab, M. Mobli and M. Gholami. 2019. Measurements of freezing tolerance and their relationship with some biochemical and physiological parameters in seven olive cultivars. *Acta Physiologiae Plantarum,* 41, (4) 1-11.

Saleem, M., Q. Fariduddin and T. Janda. 2021. Multifaceted role of salicylic acid in combating cold stress in plants: a review. *Journal of Plant Growth Regulation,* 40, 464-485.

Sauter, M. and H. Kende. 1992. Gibberellin-induced growth and regulation of the cell division cycle in deepwater rice. *Planta,* 188, (3) 362-368.

Scott, I. M., S. M. Clarke, J. E. Wood and L. A. Mur. 2004. Salicylate accumulation inhibits growth at chilling temperature in Arabidopsis. *Plant Physiology,* 135, (2) 1040-1049.

Siddiqui, K. S. and R. Cavicchioli. 2006. Cold-adapted enzymes. *Annu Rev Biochem,* 75, 403-433.

Sindoni, M., J. Zamora and R. Ramirez. 1994. Symptoms of boron deficiency and dry matter production in sesame. *Agronomic Tropical (Maracay),* 44, (1) 135-150.

Soliman, M. H., A. A. Alayafi, A. A. El Kelish and A. M. Abu-Elsaoud. 2018. Acetylsalicylic acid enhance tolerance of Phaseolus vulgaris L. to chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. *Botanical studies,* 59, (1) 1-17.

Sun, Y., G. Niu, C. Perez, H. B. Pemberton and J. Altland. 2018. Responses of marigold cultivars to saline water irrigation. *HortTechnology,* 28, (2) 166-171.

Team, R. C. 2017. R Foundation for Statistical Computing; Vienna, Austria: 2018. R: A language and environment for statistical computing.

Vasudevan, P., S. Kashyap and S. Sharma. 1997. Tagetes: a multipurpose plant. *Bioresource Technology,* 62, (1-2) 29-35.

Verma, S., S. Nizam and P. K. Verma. 2013. Biotic and abiotic stress signaling in plants. In *Stress Signaling in Plants: Genomics and Proteomics Perspective, Volume 1*:25-49: Springer. Number of 25-49 pp.

Wan, S.-B., L. Tian, R.-R. Tian, Q.-H. Pan, J.-C. Zhan, P.-F. Wen, J.-Y. Chen, P. Zhang, W. Wang and W.-D. Huang. 2009. Involvement of phospholipase D in the low temperature acclimation-induced thermotolerance in grape berry. *Plant Physiology and Biochemistry,* 47, (6) 504-510.

Yadav, S. K. 2010. Cold stress tolerance mechanisms in plants. A review. *Agronomy for sustainable development,* 30, (3) 515-527.