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Original Research Paper

Physiological responses of African marigold var. Pusa Narangi Gainda to low-temperature stress, elicitors and bioagents across sowing dates

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ABSTRACT

The Physiological responses of African marigold var. Pusa Narangi Gainda was studied under low-temperature stress conditions using chemical elicitors (Chito Oilgosaccharide and Salicylic acid) and bioagents (Arbuscular mycorrhiza) at different sowing dates for two consecutive years (2021-22 and 2022-23) at the Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute, New Delhi. The findings revealed that marigold plants grown under low plastic tunnels showed superior performance in terms of dry matter accumulation (71.44 g), total chlorophyll content (10.98 mg/g fresh weight) and lowest electrolyte leakage (55.44%) than open field conditions. Among the three sowing dates, October 15th was the most favourable, recording highest dry matter accumulation (69.47 g), total chlorophyll content (10.43 mg/g fresh weight) and lowest electrolyte leakage (61.70%). Regarding treatment efficacy, a combination of 200 ppm Chito Oligosaccharide with Arbuscular Mycorrhiza showed the best results in terms of dry matter accumulation (69.71 g), total chlorophyll content (11.22 mg/g fresh weight) and lowest electrolyte leakage (57.41%), outperforming the control group and other treatments. This study demonstrates potential strategies for reducing the negative impact of low-temperature stress in marigold.

Keywords: Arbuscular mycorrhiza, bioagents, chito oligosaccharide, elicitors, marigold, salicylic acid

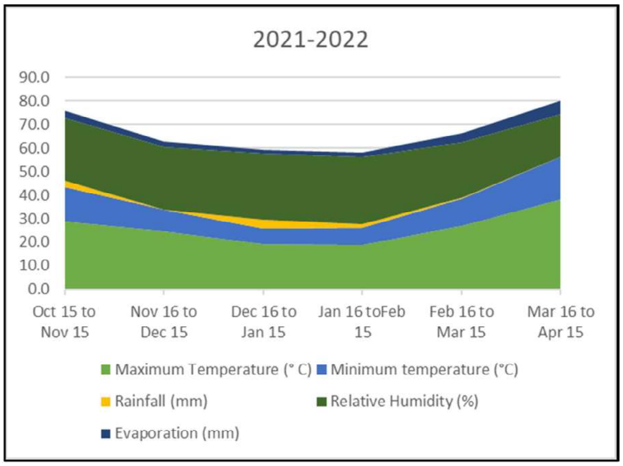
INTRODUCTION

Marigold (*Tagetes* spp.) is one of India's most commercially exploited flowers, belonging to the Asteraceae family. It's a major crop for loose flowers, renowned for its easy cultivation, adaptability, bright colours, various shapes and sizes, and excellent shelf life. These unique features make marigold a highly valuable resource with diverse industrial applications. In India, marigold ranks first among loose flower crops in terms of cultivated area (81,540 hectares) and production (923,430 metric tons), with major growing states including Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, and West Bengal (Anonymous, 2023).

Despite being a major loose flower crop, marigold has a low yield in Northern India during winter due to extreme cold. The harsh winter weather causes damage and blackening of the plants and flowers. Additionally, studies show that marigold plants tend to flower earlier in higher temperatures compared to lower temperatures (Blanchard & Runkle, 2011). To address this issue, the development of low-temperature stress-tolerant varieties could be beneficial, but there's a limited availability of such varieties. This has led to the exploration of innovative approaches to improve stress tolerance in marigold. Among the various strategies, the use of bioagents and elicitors to boost plant resilience to stress has gained prominence in recent years. Given the economic and commercial importance of marigold, a combination of different methods is necessary to ensure high and consistent yields despite adverse weather conditions. With this in mind, the experiment aimed to examine the responses of marigold plants to low-temperature stress and explore ways to mitigate its effects.

MATERIALS AND METHOD

The research was conducted at the Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute, New Delhi, using a factorial randomized block design (FRBD) with three replications over two consecutive years (2021-2022 and 2022-2023). Weather data for the



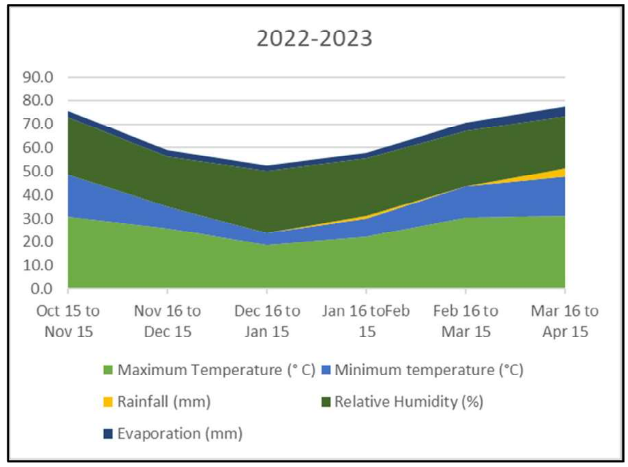


Fig. 1 : Meteorological data during the study period (October, 2021 to April, 2023)

study period are illustrated in Fig. 1. Healthy seeds of the African marigold variety Pusa Narangi Gainda were sourced from the Division of Floriculture and Landscaping at ICAR-Indian Agricultural Research Institute, New Delhi, and sown in raised nursery beds on three different dates namely October 15th (SD_1), November 1st (SD_2), and November 15th (SD_3), with 6-8 cm spacing and 2 cm depth. The most robust and disease-free seedlings were carefully selected and transplanted into the main field at CPCT, with a 45 × 35 cm spacing. Salicylic Acid (SA) and Chito Oligosaccharide (COS) were applied as chemical sprays at concentrations of 100, 200, and 300 ppm at 20, 30, and 40 days after sowing. Arbuscular mycorrhiza (AM) was applied at the time of planting at a rate of 10 grams per plant. The experiment included the following treatment groups: T_0 (control), T_1 (SA 100 ppm), T_2 (SA 200 ppm), T_3 (SA 300 ppm), T_4 (COS 100 ppm), T_5 (COS 200 ppm), T_6 (COS 300 ppm), T_7 (AM alone), T_8 (SA 100 ppm + AM), T_9 (SA 200 ppm + AM), T_{10} (SA 300 ppm + AM), T_{11} (COS 100 ppm + AM), T_{12} (COS 200 ppm + AM), and T_{13} (COS 300 ppm + AM). Plants were grown in two environments *viz.*, open field (E_1) and protected conditions (E_2). To manage low-temperature stress, a plastic tunnel was used to cover plants when temperatures dropped below a specific threshold and removed when temperatures rose to optimal levels. Data was collected on several parameters, including mortality percentage, dry matter accumulation, leaf area (using a LICOR-3000 leaf area meter), total chlorophyll and carotenoid content (measured with a UV-VIS spectrophotometer by DMSO method), relative water content, and electrolyte leakage. Data were compiled for the two years, and significance was tested using the 'F' test, with a critical difference (CD) at P=0.05 for comparing treatment means using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The data in Table 1 and supplementary Table 2 detail the responses of African marigold var. Pusa Narangi Gainda to different elicitors (chito oligosaccharide (COS), and salicylic acid (SA), arbuscular mycorrhiza (AM), various sowing dates, and growing conditions under low-temperature stress. The results showed significant variation across these factors. Plants grown under low plastic tunnels demonstrated higher dry matter accumulation (71.44 g), larger leaf area (27.48 cm^2), higher total chlorophyll content (10.98 mg/g fresh weight), carotenoid content (2.99 mg/g fresh weight), and greater relative water content (91.75%), compared to open field conditions. The plants in the tunnels also had a lower mortality rate (9.44%) and reduced electrolyte leakage (55.44%). Among the different sowing dates, October 15th proved to be the most favourable, with a high dry matter accumulation (69.47 g), leaf area (24.71 cm^2), total chlorophyll content (10.43 mg/g fresh weight), carotenoid content (2.75 mg/g fresh weight), and relative water content (90.36%). This sowing date also had the lowest mortality rate (13.97%) and electrolyte leakage (61.70%). When assessing the effectiveness of different treatments, the combination of 200 ppm chito oligosaccharide with arbuscular mycorrhiza (T_{12}) and 300 ppm salicylic acid with Arbuscular mycorrhiza (T_{10}) had the highest dry matter accumulation (69.71 g and 70.29 g, respectively). For leaf area

Table 1 : Efficacy of elicitors and bioagents on physiological parameters of African marigold var. Pusa Narangi Gainda across different sowing dates under low-temperature stress

Treatment	Mortality (%)	Dry matter accumulation (g)	Leaf area (cm^2)	Total chlorophyll (mg/g fresh weight)	Carotenoid (mg/g fresh weight)	RWC (%)	EL (%)
Growing condition							
Open condition (E_1)	31.50^{a}	63.39^{b}	18.53^{b}	8.72^{b}	2.16^{b}	88.33^{b}	75.84^{a}
Low polytunnel (E_2)	9.44^{b}	71.44^{a}	27.48^{a}	10.98^{a}	2.99^{a}	91.75^{a}	55.44^{b}
SE (m) $\pm$	0.19	0.09	0.04	0.02	0.00	0.13	0.09
CD (P=0.05)	0.52	0.25	0.11	0.04	0.01	0.35	0.26
Sowing date							
October, 15th (SD_1)	13.97^{c}	69.47^{a}	24.71^{a}	10.43^{a}	2.75^{a}	90.36^{a}	61.70^{c}
November, 1st (SD_2)	19.48^{b}	66.20^{c}	22.80^{b}	9.76^{b}	2.59^{b}	90.29^{a}	65.03^{b}
November, 15th (SD_3)	27.96^{a}	66.56^{b}	21.51^{c}	9.37^{c}	2.39^{c}	89.47^{b}	70.18^{a}
SE (m)$\pm$	0.23	0.11	0.05	0.02	0.01	0.15	0.11
CD (P=0.05)	0.64	0.31	0.13	0.05	0.01	0.43	0.32
Treatment							
Control (T_0)	24.89^{a}	65.59^{g}	18.88^{m}	8.36^{l}	1.87^{n}	83.58^{h}	73.56^{a}
SA 100 ppm (T_1)	24.44^{a}	66.45^{f}	19.46^{l}	8.48^{k}	1.97^{m}	85.87^{g}	71.69^{b}
SA 200 ppm (T_2)	22.54^{b}	66.80^{ef}	20.51^{j}	9.05^{i}	2.19^{k}	88.02^{f}	69.91^{c}
SA 300 ppm (T_3)	22.17^{b}	66.25^{fg}	22.18^{h}	9.64^{g}	2.41^{i}	89.20^{e}	68.02^{e}
COS 100 ppm (T_4)	22.47^{b}	66.35^{f}	19.97^{k}	8.71^{j}	2.12^{l}	86.34^{g}	70.33^{c}
COS 200 ppm (T_5)	20.32^{c}	67.32^{cde}	23.27^{g}	9.96^{f}	2.50^{h}	90.78^{d}	66.91^{f}
COS 300 ppm (T_6)	22.25^{b}	66.84^{def}	21.39^{i}	9.43^{h}	2.30^{j}	87.94^{f}	68.83^{d}
AM (T_7)	21.75^{b}	66.65^{ef}	23.81^{f}	10.12^{e}	2.68^{g}	89.78^{e}	65.65^{g}
SA 100 ppm + AM (T_8)	20.10^{cd}	67.54^{cd}	23.71^{f}	10.15^{e}	2.77^{f}	91.43^{d}	64.49^{h}
SA 200 ppm + AM (T_9)	18.76^{d}	67.95^{bc}	24.99^{d}	10.64^{c}	2.98^{d}	92.80^{c}	60.83^{j}
SA 300 ppm + AM (T_{10})	15.50^{f}	70.29^{a}	26.52^{b}	10.96^{b}	3.13^{b}	94.95^{a}	59.21^{l}
COS 100 ppm + AM (T_{11})	19.19^{cd}	67.74^{bc}	24.66^{e}	10.51^{d}	2.86^{e}	91.26^{d}	62.07^{i}
COS 200 ppm + AM (T_{12})	15.17^{f}	69.71^{a}	26.82^{a}	11.22^{a}	3.23^{a}	94.71^{ab}	57.41^{m}
COS 300 ppm + AM (T_{13})	17.03^{e}	68.29^{b}	25.93^{c}	10.71^{c}	3.01^{c}	93.90^{b}	60.01^{k}
SE (m)$\pm$	0.49	0.24	0.10	0.04	0.01	0.33	0.25
CD (P=0.05)	1.38	0.67	0.28	0.11	0.03	0.92	0.68

RWC: relative water content (%); EL: electrolyte leakage (%)

(26.82 cm²), total chlorophyll content (11.22 mg/g fresh weight), and carotenoid content (3.23 mg/g fresh weight), the best results recorded from 200 ppm chito oligosaccharide with arbuscular mycorrhiza (T_{12}). Additionally, the maximum relative water content (94.95%) was recorded for the 300 ppm salicylic acid with arbuscular mycorrhiza (T_{10}). Moreover, the lowest mortality rate (15.17%) and reduced electrolyte leakage (57.41%) were observed in the combination of 200 ppm chito oligosaccharide with arbuscular mycorrhiza (T_{12}). These findings highlight the impact of different elicitors, sowing dates, and growing conditions on marigold under low-temperature stress.

The interaction effect revealed several noteworthy findings. When salicylic acid at a concentration of 300 ppm was combined with arbuscular mycorrhiza fungi and sown on October 15th ($E_2SD_1T_{10}$), the highest dry matter accumulation (76.61 g) and lowest mortality rate (6.83%) were observed. However, the largest leaf area (31.67 cm²) and total chlorophyll content (12.70 mg/g fresh weight) were recorded when chito oligosaccharide at a concentration of 200 ppm was combined with arbuscular mycorrhiza and sown on November 15th ($E_2SD_3T_{12}$). For relative water content, the interaction with the highest value (97.03%) was chito oligosaccharide at 200 ppm with arbuscular mycorrhiza at the sowing date of November 1st ($E_2SD_2T_{12}$). Furthermore, the interaction of chito oligosaccharide at 200 ppm with arbuscular mycorrhiza at the sowing date of October 15th ($E_2SD_1T_{12}$) yielded the highest carotenoid content (3.94 mg/g fresh weight) and the lowest electrolyte leakage (47.44%). These results demonstrate that the interaction between different treatments and sowing dates has a significant impact on the physiological responses of marigold plants under low-temperature stress.

The mortality rate in plants is closely linked to both the duration and timing of exposure to low temperatures. Low-temperature stress adversely affects several physiological processes, including altering membrane fluidity, lowering water potential, changing lipid composition, reducing ATP supply, accumulating toxic substances, disrupting ion balance, and causing solute leakage (Theocharis et al., 2012). These physiological disturbances collectively lead to higher plant mortality rates under low-temperature stress. The increased relative water content (RWC) and decreased electrolyte leakage observed in plants grown under a plastic tunnel, can be attributed to the stable ambient temperatures within the tunnel. The application of chitosan oligosaccharide (COS) and arbuscular mycorrhizal fungi (AM) likely helps maintain membrane integrity, reducing cellular membrane damage. This can occur by enhancing the plant's antioxidant capacity under low-temperature stress, thereby preserving membrane permeability and reducing ion leakage. These results are in line with studies conducted by Zhou et al. (2018) on rice and Zhao et al. (2019) on annual ryegrass. Lower night temperatures might increase the activity of the chlorophyllase enzyme, leading to decreased chlorophyll synthesis in leaves. Treatments with chitosan oligosaccharide (COS) can improve plant photosynthesis by significantly up-regulating genes related to photosystem I (PSI) and photosystem II (PSII), as suggested by Anwar et al. (2018), potentially aiding recovery from low-temperature stress. Carotenoids also helps in stabilizing thylakoid membranes, protecting them from lipid peroxidation and cold-induced damage (Laugier et al., 2010). The higher levels of chlorophyll and carotenoid content observed in plants grown in plastic tunnels and those treated with COS in open field conditions may be due to these mechanisms. These findings are consistent with the results of Li et al. (2020) on tea, Salachna et al. (2021) on ferns, and Tan et al. (2023) on cucumber.

CONCLUSION

This experiment results demonstrated the physiological responses of African marigold var. Pusa Narangi Gainda to low-temperature stress and the influence of different mitigation strategies in enhancing plant tolerance, leading to improved winter crop production even in adverse conditions. The study indicates that growing plants under low plastic tunnels provides favourable outcomes, suggesting that this method is a practical solution for boosting the winter season production of marigold in Northern India. The collective impact of mitigation techniques was found to be most effective when chito oligosaccharide was applied at a concentration of 200 ppm in combination with arbuscular mycorrhiza on plants grown under low plastic tunnel conditions from sowing dates of October 15th and November 15th. This outcome reflects the impact of elicitors, bioagents, and sowing dates.

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REFERENCES

Anonymous. (2023). Area and production of horticulture crops for 2021-22 (Final), Ministry of Agriculture and Farmers' Welfare, Government of India, New Delhi.

Anwar, A., Yan, Y., Liu, Y., Li, Y., & Yu, X. (2018). 5-aminolevulinic acid improves nutrient uptake and endogenous hormone accumulation, enhancing low-temperature stress tolerance in cucumbers. *International Journal of Molecular Sciences*, *19*: 3379.

Blanchard, M., & Runkle, E. S. (2011). The influence of day and night temperature fluctuations on growth and flowering of annual bedding plants and greenhouse heating cost predictions. *HortScience*, *46*: 599-603.

Laugier, E., Tarrago, L., Santos, V. D. C., Eymery, F., Havaux, M., & Rey, P. (2010). *Arabidopsis* *thaliana* plastidial methionine sulfoxide reductases B, MSRBs, account for most leaf peptide MSR activity and are essential for growth under environmental constraints through a role in the preservation of photosystem antennae. *The Plant Journal*, *61*: 271-282.

Li, Y., Zhang, Q., Ou, L., Ji, D., Liu, T., Lan, R., Li, X., & Jin, L. (2020). Response to the cold stress signaling of the tea Plant (*Camellia sinensis*) elicited by chitosan oligosaccharide. *Agronomy*, *10*(6), 915; https://doi.org/10.3390/agronomy10060915.

Salachna, P., Pietrak, A., & Łopusiewicz, Ł. (2021). Effects of chitosan oligosaccharide lactate on growth and overwintering of evergreen fern *Cyrtomium fortunei* var. clivicola. *Biology and Life Sciences Forum*, *1*.

Tan, C., Li, N., Wang, Y., Yu, X., Yang, L., Cao, R., & Ye, X. (2023). Integrated physiological and transcriptomic analyses revealed improved cold tolerance in cucumber (*Cucumis sativus* L.) by exogenous chitosan oligosaccharide. *International Journal of Molecular Sciences*, *24*(7), 6202.

Theocharis, A., Clément, C., & Barka, E. A. (2012). Physiological and molecular changes in plants grown at low temperatures. *Planta, 235*, 1091-1105.

Zhao, J., Pan, L., Zhou, M., Yang, Z., Meng, Y., & Zhang, X. (2019). Comparative physiological and transcriptomic analyses reveal mechanisms of improved osmotic stress tolerance in annual ryegrass by exogenous chitosan. *Genes, 10*(11), 853.

Zhou, J., Chen. Q., Zhang, Y., Fan, L., Qin, Z., Chen, Q., Qiu, Y., Jiang, L., & Zhao, L. (2018). Chitooligosaccharides enhance cold tolerance by repairing photodamaged PS II in rice. *The Journal of Agricultural Science*, *156*, 888–899.

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