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Regulation of cold stress and biochemical responses in marigold var. Pusa Narangi Gainda during the winter season

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ABSTRACT

This study aimed to compare various methods to alleviate cold stress and investigate biochemical responses in marigold var. Pusa Narangi Gainda. This study was conducted over two years (2021-2022 and 2022-2023) at the Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute, New Delhi involving the sowing of marigold variety Pusa Narangi Gainda on three different dates. The seedlings were then cultivated under two different growing conditions, using various treatments and their respective combinations. The biochemical responses were assessed and analysed. Results revealed that plants grown inside a low plastic tunnel showed the lowest activity of anti-oxidant enzymes, proline content, malondialdehyde content, total phenol content, total sugars and soluble protein content significantly compared to those grown in open field conditions. Additionally, the plants from the sowing date of October 15th recorded the lowest values compared to the other two sowing dates (November 1st and November 15th). Through the examination of different treatments, it was observed that the combination of Chito Oligosaccharide at a concentration of 200 ppm with *Arbuscular mycorrhiza* recorded the increased anti-oxidant enzyme activity, proline content, total phenol content, TSS, soluble protein content and reduced malondialdehyde content comparing to the control and other treatments. This study showcases the beneficial effects of using low plastic tunnels in different sowing dates and treatments to increase plant resilience to cold stress.

Key words: *Tagetes erecta* L., arbuscular mycorrhiza, cold stress, chito oligosaccharide, salicylic acid,

INTRODUCTION

The Marigold (*Tagetes erecta* L.) flower, belonging to the Asteraceae family, is highly valued in India for its commercial potential. It is one of the most often used flowers for economic purposes, primarily for its ornamental flowers and as a pigment source for poultry feed. Fresh flowers are sold individually or in garlands at retail establishments. The marigold plant is grown for its visual appeal, especially in the beautification of landscapes, due to its wide array of colours and different heights. Marigold thrives in moderate climates and can be grown three times a year, throughout the rainy, winter, and summer seasons. However, the cultivation of marigold in northern India presents farmers with a substantial obstacle due to the detrimental effects of exceedingly low temperatures leading to delayed flowering which in turn delays commercial yield. This causes an increase in production expenses, which leads to a loss of profitability for farmers. Cold stress, or low-temperature stress, is a key environmental factor that has harmful consequences on higher plants (Zhou *et al.*, 20). Plants under cold stress display several phenotypic symptoms, including diminished leaf growth, drooping, yellowing, and even tissue death (necrosis). It interferes with specific physiological and biochemical processes and significantly impairs reproductive growth and flower quality. The intricate nature of plant stress tolerance mechanisms and reactions requires the investigation of novel and efficient approaches to improve stress tolerance. The utilization of bioagents and the implementation of biotic or abiotic elicitors have become prominent methods for improving plant resilience to various abiotic stresses. For instance, chito oligosaccharide has been employed to alleviate cold stress in tea, rice, and wheat. Salicylic acid (SA) has been utilized in cucumber and wheat, while Arbuscular mycorrhiza has been applied in tomato and cucumber. The main aim of this study was to investigate the biochemical responses of marigold plants to cold stress and various methods considering their economic importance and ornamental value.

MATERIALS AND METHODS

The investigation was conducted at the Centre for Protected Cultivation Technology (CPCT) in New Delhi for two consecutive years (from 2021 - 2022

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and 2022 - 2023). The marigold seeds of the Pusa Narangi Gainda variety were procured from the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi and were sown on raised nursery beds on three different dates such as October 15th (SD_1), November 1st (SD_2), and November 15th (SD_3), with a spacing of 6-8 cm and a depth of 2 cm. After 30 days from seeding, robust and disease-free seedlings were meticulously chosen and then transplanted in the main field (CPCT, New Delhi) with a spacing of 45 × 35 cm. As treatment sprays, SA and Chito Oligosaccharide (COS) were applied at doses of 100, 200, and 300 parts per million (ppm) on 20, 30, and 40 days after sowing whereas Arbuscular mycorrhiza (AM) was applied during the sowing process at the rate of 500 g per square meter. The treatment details consist of the following: 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 in the main field were cultivated under two different conditions such as open field (E_1) and protected conditions (E_2) using a Factorial Randomized Block Design (FRBD) with three replications. To maintain optimal environmental conditions, when the temperature dropped below the threshold, the plants were covered by a low plastic tunnel with a thickness of 20 microns and upon the attainment of an ideal temperature conducive to plant growth, the plastic tunnel cover was removed. The weather data was collected daily to measure the cold stress conditions. The data relating to biochemical responses was estimated using a UV-VIS spectrophotometer by following protocols of Castillo *et al.* (4) (GPX), Aebi (1) (CAT), Dhindsa *et al.* (6) (SOD), Heath and Packer (8) malondialdehyde (MDA), Bates *et al.* (2) (proline), Singleton and Rosie (15) (TPC), Homme *et al.* (9) (TSS), Bradford (3) (SPC) and pooled over two years then data was subjected to statistical analysis using ANOVA. The significance of the results was analysed using the F test, and the critical difference (CD) was calculated using R software with a significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

The data on biochemical responses of marigold var. Pusa Narangi Gainda presented in Table 1 and Table 2 demonstrates the influence of cold stress, elicitors (COS, SA, and AM), sowing dates, and low plastic tunnels in enhancing plant tolerance. Significantly lowest activity of anti-oxidant enzymes like guaiacol peroxidase (3.69 milli mol/mg protein/min.), superoxide dismutase (9.52 milli mol/mg protein/min.), catalase (5.28 milli mol/mg protein/min.), proline content (2.87 µmol/g FW), malondialdehyde content (94.25 µg/g FW), total phenol content (94.98 µg/g FW), TSS (119.49 mg/g FW) and soluble protein content (4.81 mg/g FW) was recorded in the plants cultivated under low plastic tunnel conditions (E_2) compared to those grown in open field conditions (E_1). Similarly, the plants from the sowing date of October 15th (SD_1) recorded the lowest values in guaiacol peroxidase (6.20 milli mol/mg protein/min.), superoxide dismutase (13.87 milli mol/mg protein/min.), catalase (9.14 milli mol/mg protein/min.), proline content (4.00 µmol/g FW), malondialdehyde content (182.53 µg/g FW), total phenol content (115.61 µg/g FW), TSS (161.03 mg/g FW) and soluble protein content (6.68 mg/g FW) compared to those from the other two sowing dates. Among different treatments evaluated, the treatment of Chito Oligosaccharide at a concentration of 200 ppm in combination with Arbuscular mycorrhiza (T_{12}) recorded the highest values in the case of guaiacol peroxidase (9.34 milli mol/mg protein/min.), superoxide dismutase (19.83 milli mol/mg protein/min.), catalase (16.60 milli mol/mg protein/min.), proline content (6.69 µmol/g FW), total phenol content (152.84 µg/g FW), TSS (274.04 mg/g FW) and soluble protein content (9.90 mg/g FW) while it recorded reduced content of malondialdehyde (181.63 µg/g FW) followed by the treatment of salicylic acid at a concentration of 300 ppm in combination with Arbuscular mycorrhiza (T_{10}) with the values of guaiacol peroxidase (9.05 milli mol/mg protein/min.), superoxide dismutase (19.43 milli mol/mg protein/min.), catalase (15.91 milli mol/mg protein/min.), proline content (6.20 µmol/g FW), total phenol content (147.30 µg/g FW), TSS (269.23 mg/g FW) and soluble protein content (9.52 mg/g FW) compared to other treatments.

The plants from the sowing date of October 15th and cultivated under low plastic tunnels displayed favourable responses likely because they were well established by the onset of cold stress. Further, low plastic tunnels provide a favourable microclimate for plant growth even under stress conditions. Under ideal growing conditions, the levels of reactive oxygen species (ROS) are kept low by enzymatic (e.g., superoxide dismutase, catalase, peroxidase) and nonenzymatic antioxidant molecules (e.g., phenols, proline, sugars, and proteins) (Kusvuran *et al.*, 10). Under cold stress conditions, ROS accumulation exceeds the plant's tolerance level due to the reduced activity of antioxidant enzymes responsible for the detoxification of ROS leading to oxidative stress, characterised by the peroxidation of membrane lipids and damage to proteins, carbohydrates, and DNA

Table 1. Effect of elicitors and bioagents on antioxidants enzyme responses of marigold (var. Pusa Narangi Gainda) under cold stress across different sowing dates (Pooled data of two years 2021-22 and 2022-23)

Factor	GPX (milli mol/ mg protein/min.)	SOD (milli mol/ mg protein/min.)	CAT (milli mol/ mg protein/min.)	Proline Content (μmol/g FW)
Growing condition				
Open (E_1)	10.64	24.16	20.10	5.56
Low plastic tunnel (E_2)	3.69	9.52	5.28	2.87
SEm (±)	0.01	0.03	0.03	0.01
CD (*p <0.05*)	0.04	0.08	0.07	0.02
Sowing Date				
October 15th (SD_1)	6.20	13.87	9.14	4.00
November 1st (SD_2)	7.12	15.53	12.25	4.11
November 15th (SD_3)	8.17	21.13	16.70	4.53
SEm (±)	0.02	0.04	0.03	0.01
CD (*p <0.05*)	0.05	0.10	0.09	0.02
Treatments				
Control (T_0)	4.64	13.80	9.34	1.79
SA @ 100 ppm (T_1)	5.10	14.11	9.56	2.33
SA @ 200 ppm (T_2)	5.85	15.15	10.60	3.06
SA @ 300 ppm (T_3)	6.75	16.13	11.53	2.89
COS @ 100 ppm (T_4)	5.85	14.70	9.82	3.17
COS @ 200 ppm (T_5)	7.06	16.36	12.00	3.45
COS @ 300 ppm (T_6)	6.44	15.84	10.96	3.87
AM (T_7)	7.13	16.73	13.00	4.47
SA @ 100 ppm + AM (T_8)	7.76	18.13	13.62	4.82
SA @ 200 ppm + AM (T_9)	8.29	18.30	14.89	5.37
SA @ 300 ppm + AM (T_{10})	9.05	19.43	15.91	6.20
COS @ 100 ppm + AM (T_{11})	8.18	18.07	14.12	5.19
COS @ 200 ppm + AM (T_{12})	9.34	19.83	16.60	6.69
COS @ 300 ppm + AM (T_{13})	8.88	19.20	15.74	5.67
SEm (±)	0.04	0.08	0.07	0.02
CD (*p <0.05*)	0.10	0.22	0.19	0.05

GPX: Guaiacol peroxidase, SOD: Superoxide dismutase, CAT: Catalase

(Gill, 7). The observed reduction in MDA content in plants subjected to low plastic tunnel conditions and those treated with chito oligosaccharides (COS) and AM fungi in open field conditions may be due to COS modulating antioxidant enzyme activities, and regulating the expression of antioxidant enzyme genes under cold stress (Zou *et al.*, 21). The antioxidant properties of chitosan and chito oligosaccharides are attributed to their amino groups and abundant active hydroxyl groups, which can react with ROS to form relatively non-toxic macromolecular radicals (Xie *et al.*, 19). Under cold stress, the activities of non-enzymatic antioxidants were notably higher in plants cultivated in open field conditions and plants treated with Chito oligosaccharides. This suggests that these treatments effectively enhance the activities of ROS-scavenging enzymes, fortifying the plants to withstand cold stress conditions better. Similar results are documented by Zou *et al.* (21) in wheat; Li *et al.* (11) in tea; Wang *et al.* (17) in banana; Shi *et al.* (14) in peanut; and Tan *et al.* (16) in cucumber. AM symbiosis-induced low hydrogen peroxide (H_2O_2) levels may serve as a signalling molecule in defence and adaptive responses (Chen *et al.*, 5). Numerous

Table 2. Effect of elicitors and bioagents on non-enzymatic molecules responses of marigold (var. Pusa Narangi Gainda) under cold stress across different sowing dates (Pooled data of two years 2021-22 and 2022-23)

Factor	MDA (μg/g FW)	TPC (μg/g FW)	TS (mg/g FW)	SPC (mg/g FW)
Growing condition				
Open (E_1)	344.49	153.94	306.68	10.34
Low plastic tunnel (E_2)	94.25	94.98	119.49	4.81
SEm (±)	0.39	0.18	0.44	0.01
CD ($p < 0.05$)	1.08	0.51	1.22	0.04
Sowing Date				
October 15th (SD_1)	182.53	115.61	161.03	6.68
November 1st (SD_2)	216.07	126.74	211.88	7.74
November 15th (SD_3)	259.50	131.04	266.34	8.31
SEm (±)	0.47	0.22	0.54	0.02
CD ($p < 0.05$)	1.32	0.62	1.50	0.05
Treatments				
Control (T_0)	253.22	94.99	151.59	5.20
SA @ 100 ppm (T_1)	245.55	99.88	159.23	5.55
SA @ 200 ppm (T_2)	240.17	107.73	175.31	6.27
SA @ 300 ppm (T_3)	223.66	120.79	194.32	7.13
COS @ 100 ppm (T_4)	243.38	102.50	169.00	5.81
COS @ 200 ppm (T_5)	223.80	122.03	211.80	7.69
COS @ 300 ppm (T_6)	232.30	113.74	182.07	6.70
AM (T_7)	213.23	128.84	224.86	7.92
SA @ 100 ppm + AM (T_8)	214.16	134.15	225.87	8.17
SA @ 200 ppm + AM (T_9)	207.09	139.54	244.15	8.77
SA @ 300 ppm + AM (T_{10})	186.34	147.30	269.23	9.52
COS @ 100 ppm + AM (T_{11})	204.66	135.88	239.62	8.44
COS @ 200 ppm + AM (T_{12})	181.63	152.84	274.04	9.90
COS @ 300 ppm + AM (T_{13})	201.97	142.30	262.11	8.96
SEm (±)	1.02	0.48	1.16	0.04
CD ($p < 0.05$)	2.85	1.34	3.23	0.10

MDA: Malondialdehyde content, TPC: Total phenol content, TS: Total sugars, SPC: Soluble protein content.

studies have revealed that AM fungi could assist host plants in alleviating temperature stress by increasing the activities of antioxidant enzymes and reducing membrane electrolyte permeability. Additionally, AM symbiosis can accumulate nonenzymatic antioxidant components like phenols, proline, sugars, and proteins to scavenge ROS under low-temperature stress. The findings align with those reported by Wu and Zou (18) in citrus, Liu *et al.* (12) in tomato, Chen *et al.* (5) in cucumber, and Pasbani *et al.* (13) in eggplant.

The present study examined the interaction effect of different factors (Fig. 1) and the results revealed that the interaction of plants sown on November 15th, treated with COS at 200 ppm with AM under open field conditions (E_1 SD_3 T_{12}) recorded the highest guaiacol peroxidase (15.25 milli mol/mg protein/min.), catalase (31.42 milli mol/mg protein/min.), proline content (9.55 μmol/g FW), total phenol content (205.70 μg/g FW) and soluble protein content (15.22 mg/g FW) while the highest superoxide dismutase (38.11 milli mol/mg protein/min.) was recorded by the interaction of plants sown on November 15th, treated with COS at 300 ppm with AM under open field conditions (E_1 SD_3 T_{13}) and the highest TSS (476.51 mg/g FW) by the interaction of plants sown on November 15th, treated with SA at 300 ppm in with AM under open field conditions (E_1 SD_3 T_{10}). However, the reduced malondialdehyde content (79.35 μg/g

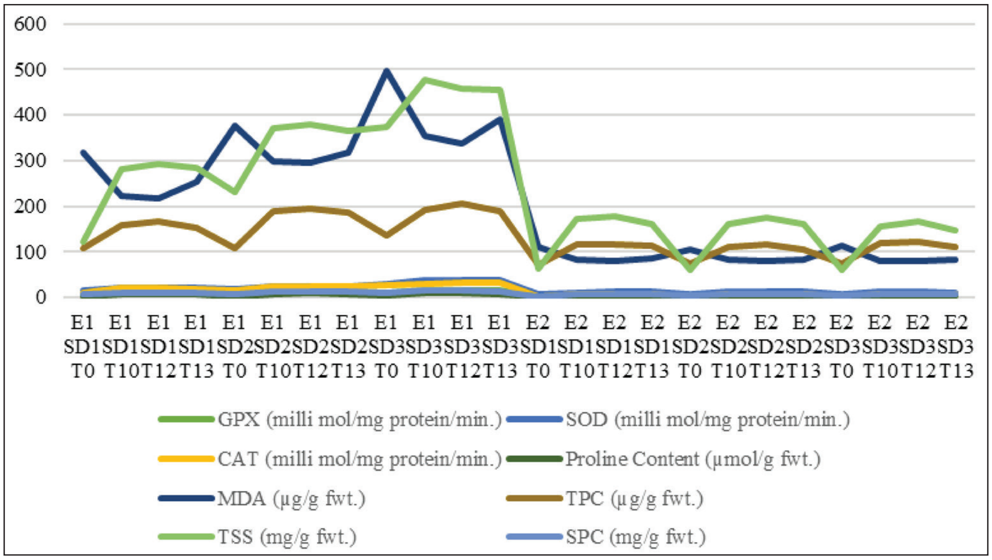

Fig. 1. Interaction effect of elicitors and bioagents on biochemical responses of marigold (var. Pusa Narangi Gainda) under cold stress across different sowing dates (Pooled data of two years 2021-22 and 2022-23)

GPX: Guaiacol peroxidase, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde content, TPC: Total phenol content, TS: Total sugars, SPC: Soluble protein content

FW) was observed in the interaction of plants sown on November 1st, treated with COS at 200 ppm with AM under low plastic tunnel conditions (E_2 SD_2 T_{12}).

In conclusion, the results of the experiment present a favourable outlook on the feasibility of low-plastic tunnels and their usage for protecting plants under cold temperatures during the winter season crop of marigold. Further, the results also show the beneficial effects of applying exogenous chemicals and bioagents in enhancing plants' resilience to cold stress.

AUTHORS' CONTRIBUTION

The Conceptualization of research (KPS, PK, MCS, SP, RJ); Designing of the experiments (KPS, MCS); Contribution of experimental materials (KPS, MCS, SP, RJ); Execution of the experiments and data collection (RT, TBK); Analysis of data and interpretation (RT, VKLN); Preparation of the manuscript (RT, PK).

DECLARATION

The authors declare that they do not have any conflict of interest.

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REFERENCES

1. Aebi, H. 1984. Catalase in vitro. *Methods Enzymol*. **105**:121-26.
2. Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* **39**: 205–07.
3. Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **72**: 248-54.
4. Castillo, F.J., Penel, C. and Greppin, H. 1984. Peroxidase release induced by ozone in *sedum album* leaves: involvement of Ca. *Plant Physiol*. **74**: 846-51.
5. Chen, S., Jin, W., Liu, A., Zhang, S., Liu, D., Wang, F., Lin, X. and He, C. 2013. Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low-temperature stress. *Sci. Hortic*. **160**: 222–29.
6. Dhindsa, R.S., Dhindsa, P.P. and Thorpe, T.A. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*. **32**: 93-101.

7. Gill, T. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**: 909-30.

8. Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*. **125**: 189-98.

9. Homme, P.M., Gonzalez, B. and Billard, J. 1992. Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenne* L.) as affected by source/link modification after cutting. *J. Plant Physiol.* **140**: 282-91.

10. Kusvuran, S., Kiran, S. and Ellialtioglu, S. S. 2016. Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. in abiotic and biotic stress in plants *R. Adv. F. Persp.* 490-92.

11. Li, Y., Zhang, Q., Ou, L., Ji, D., Liu, T., Lan, R., Li, X. and Jin, L. 2020. Response to the cold stress signaling of the tea plant (*Camellia sinensis*) elicited by chitosan oligosaccharide. *Agron*. **10**: 915.

12. Liu, A., Chen, S., Wang, M., Liu, D., Chang, R., Wang, Z., Lin, X., Bai, B. and Ahammed, G. J. 2015. Arbuscular mycorrhizal fungus alleviates chilling stress by boosting redox poise and antioxidant potential of tomato seedlings. *J. Plant Growth Regul.* **35**: 109-20.

13. Pasbani, B., Salimi, A., Aliasgharzad, N. and Hajiboland, R. 2020. Colonization with arbuscular mycorrhizal fungi mitigates cold stress through improvement of antioxidant defence and accumulation of protecting molecules in eggplants. *Sci. Hortic.* **272**: 109575.

14. Shi, X., Sun, Z., Xue, X., Xu, H., Wu, Y., Zhang, Y., Yang, Y., Han, S., Zhao, R., Zhang, M. and Zhang, X. 2023. Amelioration of hypothermia-induced damage on peanut by exogenous application of chito oligosaccharide. *Agric.* **13**: 217.

15. Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **16**: 144-58.

16. Tan, C., Li, N., Wang, Y., Yu, X., Yang, L., Cao, R. and Ye, X. 2023. Integrated physiological and transcriptomic analyses revealed improved cold tolerance in cucumber (*Cucumis sativus* L.) by exogenous chitosan oligosaccharide. *Int. J. Mol. Sci*. **24**: 6202.

17. Wang, A., Li, J., AL-Huqail, A.A., AL-Harbi, M.S., Ali, E.F., Wang, J., Ding, Z., Rekaby, S.A., Ghoneim, A.M. and Eissa, M.A. 2021. Mechanisms of chitosan nanoparticles in the regulation of cold stress resistance in banana plants. *Nanomater*. **11**: 2670.

18. Wu, Q.S. and Zou, Y.N. 2010. Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Sci. Hortic*. **125**: 289-93.

19. Xie, W., Xu, P. and Liu, Q. 2001. Antioxidant activity of water-soluble chitosan derivatives. *Bioorg. Med. Chem. Lett.* **11**: 1699–701.

20. Zhou, M., Chen, H., Wei, D., Ma, H. and Lin, J. 2017. Arabidopsis I3 and DELLAs positively regulate each other in response to low temperature. *Sci. Rep*. **7**: 1–13.

21. Zou, P., Tian, X., Dong, B. and Zhang, C. 2017. Size effects of chitooligomers with certain degrees of polymerization on the chilling tolerance of wheat seedlings. *Carbohydr. Polym*. **160**: 194-202.

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