Ergin *et al. Journal of Cotton Research* (2024) 7:21
https://doi.org/10.1186/s42397-024-00183-0

Journal of Cotton Research

RESEARCH Open Access

The ability of biostimulants and copper-containing fungicide to protect cotton against chilling stress

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Abstract

Background Cotton (*Gossypium hirsutum* L.), adapted to tropical and subtropical regions of the world, is highly sensitive to low temperatures throughout its life cycle. The objective of this study was to evaluate the mitigating effects of different doses of animal-derived (0.25%, 0.50%, and 1.00% Isabion®), seaweed-based (0.165%, 0.330%, and 0.660% Proton®) biostimulants, as well as a copper (Cu)-containing fungicide application, on cotton cultivar Lazer seedlings at the four true leaves (V4) stage. The plants were exposed to a low temperature of 5 °C for 48 h, and the changes in morphological (seedling fresh and dry weight, plant height, and stem diameter) and physiological parameters (leaf temperature, chlorophyll content, relative water content, electrolyte leakage, and relative injury) were examined.

Results The results revealed that chilling stress reduced plant growth, while biostimulants helped protect the plants and overcome the adverse effects of chilling. Under chilling stress, there was a considerable reduction in seedling fresh weight (SFW), seedling dry weight (SDW), plant height (PH), stem diameter (SD), leaf temperature (LT), and relative water content (RWC). Cotton seedlings treated with the animal-derived biostimulants showed significantly enhanced SFW, SDW, PH, SD, LT, chlorophyll content (Chl), electrolyte leakage (EL), and relative injury (RI), although there were no positive changes in RWC. No significant differences in the morphological traits were observed among the doses of seaweed biostimulants. For SDW, PH, EL, and RI, the best results were obtained with the application of a fungicide containing copper.

Conclusion These results show the efficiency of the biostimulant and fungicide treatments in mitigating low-temperature stress in cotton seedlings. Applying a copper-containing fungicide to cotton seedlings helped to counteract the negative effects of low-temperature stress and to protect the plants from damage by maintaining electrolyte balance. Among the biostimulant applications, all levels of animal-derived biostimulant applications, as well as the 0.660% level of the seaweed-derived biostimulant, led to increased tolerance of cotton plants to chilling stress.

Keywords *Gossypium hirsutum* L., Cold stress, Electrolyte leakage

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Introduction

Low temperatures pose a widespread environmental stress that significantly inhibits agricultural productivity worldwide by impeding plant growth and development (Mishra et al., 2019). Low temperatures can be divided into two categories: chilling, which occurs between 5 and 15 °C, and freezing, which happens below 0 °C (Ruelland et al., 2009; Theocharis et al., 2012; Liu et al., 2018; Hajiboland, 2022).

Low temperatures considerably and adversely alter physiological and biochemical mechanisms (Snider et al., 2022; Fu et al., 2023), leading to a loss in plant height, leaf area, and aboveground biomass (Liang et al., 2023; Wang et al., 2023). Additionally, plant damage to tissues caused by low temperatures can postpone plant growth and development (Bhattacharya, 2022). Under low temperatures, plants can experience structural damage (Moriyama et al., 1989), accumulation of reactive oxygen species (ROS) (Sharma et al., 2012), increased permeability of cell membranes (Kawamura, 2008), changes in membrane lipids (Uemura et al., 1999), protein content and enzyme activities (Ruelland et al., 2010), cellular osmotic concentrations, gene expression (Hiraki et al., 2014), and necrosis or death (Theocharis et al., 2012). In addition, transgenic cotton plants with *AmDUF1517* and *GhDREB1B* have been shown to increase the resilience to cold stress by regulating relative water content, chlorophyll levels, soluble sugar content, and electrolyte leakage (Ahmed et al., 2024).

In recent years, biostimulants derived from seaweed, plants, microorganisms, or animals containing amino acids, vitamins, enzymes, and plant nutrition, have been available for the induction of plants' tolerance to various abiotic stresses (Kauffman et al., 2007). These biostimulants help stressed plants cope better with adverse conditions by benefiting many metabolic pathways. Biostimulants can increase crop yield (Bulgari et al., 2015) by influencing the plant's metabolism (Nephali et al., 2020), improving the growth and development of aboveground and underground parts (Halpern et al., 2015; Rouphael et al., 2020), promoting chlorophyll biosynthesis and enhancing photosynthesis (Rashid et al., 2021), and increasing nutrient use efficiency (Du Jardin, 2015; De Pascale et al., 2017). Due to their beneficial effects, biostimulants have been advised for use on plants damaged by low temperatures (Niu et al., 2022).

Copper (Cu) is an essential element for plant growth and plays a significant role in many physiological processes, including photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, protein metabolism, antioxidant activity, cell wall metabolism, hormone perception, seed germination, drought resistance, and water supply (Yamasaki et al., 2008; Sundin et al., 2016). However, it can also catalyze the formation of harmful free radicals such as hydroxyl, peroxyl, and alkoxyl radicals, resulting in oxidative stress. The activity of one or more antioxidant enzymes generally increases in plants exposed to stress conditions, and this elevated activity correlates with increased stress tolerance (Pilon et al., 2006). Copper is extensively used as a protectant (La Torre et al., 2018), exhibiting distinctive fungistatic and bactericidal activity through the release of copper ions (Cu^{++}) into water (Yruela, 2005). These ions, when dissolved in water layers on plant surfaces, can penetrate the cell protoplasm of oomycetes, fungi, and bacteria.

Cotton (*Gossypium hirsutum* L.) is a unique fiber crop for textiles in Türkiye. It also helps oil production as the seeds contain 17%–21% crude oil. It is mainly grown in southern Anatolia, followed by the Mediterranean and Egea regions, where the climate is characterized by high temperatures in summer and moderate temperatures in winter. For optimal growth, cotton requires a minimal temperature of 12–15 °C and an optimum temperature of 20–30 °C (Reddy et al., 1991; Singh et al., 2018; Li et al., 2019). Because of its long vegetation period, growers are willing to plant early soil and weather temperatures are unfavorable for young cotton plants (Basal et al., 2019). In such cases, cotton is often exposed to low temperatures from the seedling stage up to the four true leaves (V4), which typically occurs in late May and early June. This study investigated how biostimulants derived from animals and seaweed, as well as the traditional application of a copper-containing fungicide for root rot and damping off, might improve the tolerance of cotton seedlings to chilling stress.

Materials and Methods

Treatment preparation

This study was conducted at Seed Science and Technology Laboratory at Eskişehir Osmangazi University, Türkiye, in 2023. Cotton cultivar Lazer and two commercial biostimulants, one animal-derived (Isabion®) and the other seaweed-based (Proton®), were used in the study. In addition, Tricopper Forte®, a commonly used fungicide for root rot and damping off, was applied. Isabion® is composed of 62% (mass fraction, same as below) organic matter, 29% organic carbon, 8% organic nitrogen, and 10% free amino acids, with a pH of 5.5–7.5. Proton® is composed of 45% organic matter, 1.5% alginic acid, and 10% potassium oxide (K_2O), with a pH of 9.0–11.0. Tricopper Forte® fungicide with copper was applied by foliar spraying at a concentration of 0.3%. The active substances of Tricopper Forte® consist of 21% copper salts equivalent to metallic copper (Bordeaux mixture + copper oxychloride + copper carbonate) and 20% mancozeb.

Experimental setup and growth conditions

The seeds were sown in two 70-well vials filled with a mixture of peat, perlite, and vermiculite (in a ratio of 3:1:1) at a depth of 2 cm and irrigated with tap water. Seedlings were grown in a growth chamber (16 h light / 8 h dark at 25 °C and 60% relative humidity) for 15 days and transplanted to Polyvinyl chloride pots (9 cm in diameter, 12 cm in height) filled with the same mixture medium. Nine treatments with seven plants for each treatment were arranged as follows:

T1: control (no stress).
T2: chilling.
T3: 0.25% Isabion® + chilling.
T4: 0.50% Isabion® + chilling.
T5: 1.00% Isabion® + chilling.
T6: 0.165% Proton® + chilling.
T7: 0.330% Proton® + chilling.
T8: 0.660% Proton® + chilling.
T9: 0.300% fungicide with copper + chilling.

The biostimulants were sprayed on the cotton seedlings at the V4 stage at the dose of 50 mL per plant using a hand sprayer. An advised dose of fungicide was applied to protect the plants from diseases. One day after applying biostimulant, the plants were exposed to chilling stress by cycling at 15 °C for 24 h, followed by 5 °C for 48 h.

Growth measurements

Measurements were conducted on three randomly selected seedlings per experimental unit. Seedling fresh weight (SFW), seedling dry weight (SDW), plant height (PH), stem diameter (SD), leaf temperature (LT), chlorophyll content (Chl), relative water content (RWC), electrolyte leakage (EL), and relative injury (RI) were measured after chilling stress. PH was defined by the distance from the soil surface to the highest point of the cotton seedlings. SD was measured with a caliper below the cotyledonary leaves. To determine the seedling dry weight, the plants were cut at the growth medium surface, and SFW and SDW (dried for 48 h at 70 °C) were determined. LT was determined by an infrared thermometer (Trotec BP21, Germany).

Chlorophyll content

The Chl readings of the second fully expanded leaves were performed with a chlorophyll meter (Konica-Minolta SPAD-502, Japan) and expressed as SPAD units.

Relative water content

RWC was calculated using the formula described by Ghoulam et al. (2002): RWC (%) = [(FW−DW) / (TW−DW)] × 100, where FW = fresh weight of leaf, DW = dry weight of leaf, and TW = turgid weight of leaf.

Electrolyte leakage

Four discs with a 10 mm diameter were excised from the second leaf for EL. The leaf samples were washed with deionized water to remove any remaining electrolytes from the leaf surface. These discs were weighed and placed into a glass tube filled with 20 mL of deionized water. After the incubation period of 24 h at 20 °C, the electrical conductance (EC) of the solution (Li) was directly read using an EC meter (WTW 3.15, Germany). Then, they were taken into the water bath at 90 °C for 45 min, and the EC (Lo) was re-recorded. It was calculated by the formula (Ghoulam et al., 2002): EL (%) = (Li / Lo) × 100.

Relative injury

RI was calculated using the formula described by Gulen et al. (2003): RI (%) = (ELs−ELc) / (100−ELc) × 100, where ELs and ELc are EL values belonging to the seedlings under stress or control, respectively.

Data analysis

The experiment was performed using a complete randomized block design, including nine treatments with three replicates per treatment. All data were subjected to analysis of variance (ANOVA). The data were analyzed using the MSTAT-C program (Freed et al., 1991), and the statistical difference between treatments was determined by Duncan's test ($P < 0.05$) (Duncan, 1955). The correlation between morphological parameters and physiological parameters was determined using the correlation coefficients (r) at the 1% and 5% significance levels.

Results

The investigated characteristics of cotton plants treated with biostimulants and copper-containing fungicide against chilling stress were significantly different. Chilling stress caused significant inhibition in the morphological characteristics of cotton seedlings, as shown in Fig. 1.

All biostimulant applications had lower SFW compared to the untreated seedlings. There was an increasing linear effect of animal-derived biostimulant doses for SFW, with the highest SFW (1 217 mg·plant^{-1}) recorded in T4 treatment. Also, SFW for seedlings treated with copper-containing fungicide (1 113 mg·plant^{-1}) was close to that of T4 treatment. Similar trends were observed in SDW, which reached the highest value (191 mg·plant^{-1}) in the- fungicide treatment. The lowest SFW and SDW values were observed at all applied doses of seaweed-based biostimulant. PH and SD were reduced by 15%–20% after chilling stress, while increasing levels of the biostimulants promoted the increase of PH. Except for unstressed seedlings, the maximum PH (5.27 cm) was obtained from the T9 treatment. SD was 2.10 cm in unstressed seedlings and decreased to 1.77 cm in chilled seedlings. In addition, the highest SD after chilling stress was measured with Isabion® applications at T3 and T4 doses.

The LT (26.4 ℃) of cotton seedlings grown under unstressed conditions was higher than all treatments under chilling stress (Fig. 2). Compared with T2 treatment, LT increased at all doses of the animal-derived biostimulant. Similar Chl content were recorded in both control treatments, and generally, biostimulant applications and the increase in dosages enhanced Chl. The

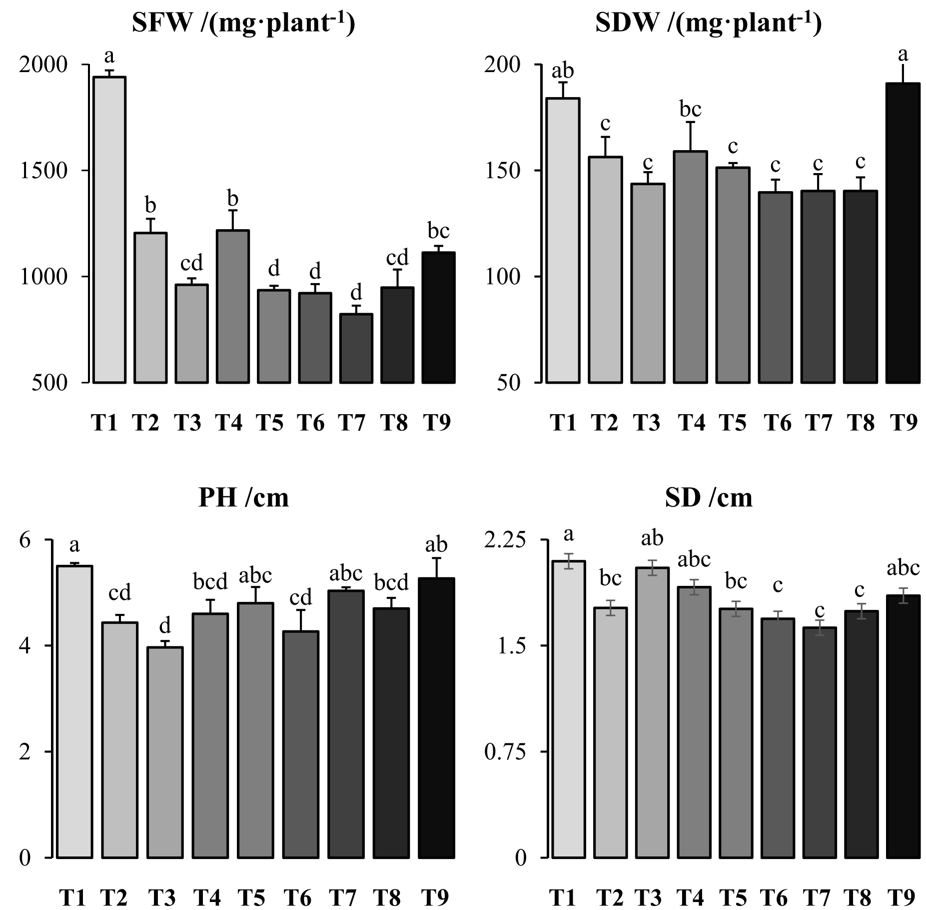

Fig. 1 Changes in SFW (seedling fresh weight), SDW (seedling dry weight), PH (plant height), and SD (stem diameter) of cotton seedlings in different foliar applications under chilling stress (at 5℃ for 48 h). The letter(s) on each column denotes a significance level at $P < 0.05$

peak Chl value (51.6 SPAD) was obtained at the highest dose of Proton® (T8 treatment). Compared with T1 treatment, Chilling stress decreased RWC by 35%. The RWC values of plants receiving T4, T5, T7, and T9 treatment were higher than 50%. Seedlings grown under normal conditions had an EL of 25.0%, whereas under low temperatures it increased to more than 60%. A positive effect of Isabion® on EL was observed in the plant exposed to chilling stress. Among the treatments, fungicide treatment resulted in the minimum EL value (29.9%), followed by T5 (38.2%) and T4 treatment (39.0%).

The highest RI was obtained from T7 treatment , probably due to high electrolyte leakage. RI was lowest in the T9 treatment (copper-containing fungicide), followed by T5 and T4 treatment. Unexpectedly, cotton plants treated with Isabion® (T3, T4, and T5 treatment) exhibited a lower RI than T2 treatment (Fig. 3).

There were significant correlations between the investigated parameters (Table 1). Some physiological traits showed a significant relationship with the morphological characteristics. Specifically, RWC was positively associated with SFW ($r = 0.877^{**}$). However, EL was negatively correlated with SFW ($r = -0.686^{**}$), SDW ($r = -0.658^{**}$), and SD ($r = -0.541^{**}$). Chl was negatively correlated with SFW and RWC.

Discussion

The results show that the chilling stress remarkably inhibited the plant growth of cotton seedlings. Similar results were reported by Singh et al., (2018), who found a reduction in plant height, leaf number, leaf area, leaf weight, stem weight, and total weight in cotton lines subjected to chilling stress. In the present study, a medium dose of animal-derived biostimulant and the spraying of copper-containing fungicide protected the plants and stimulated the growth of cotton after chilling stress. Higher doses of seaweed biostimulant had positive effects on PH and SD. These results were supported by the findings of Polo et al. (2018), who indicated that animal-derived (Pepton®) and seaweed-based (Acadian®) biostimulants improved vegetative parameters, including PH and SD, for cherry tomatoes compared with the control under low-temperature

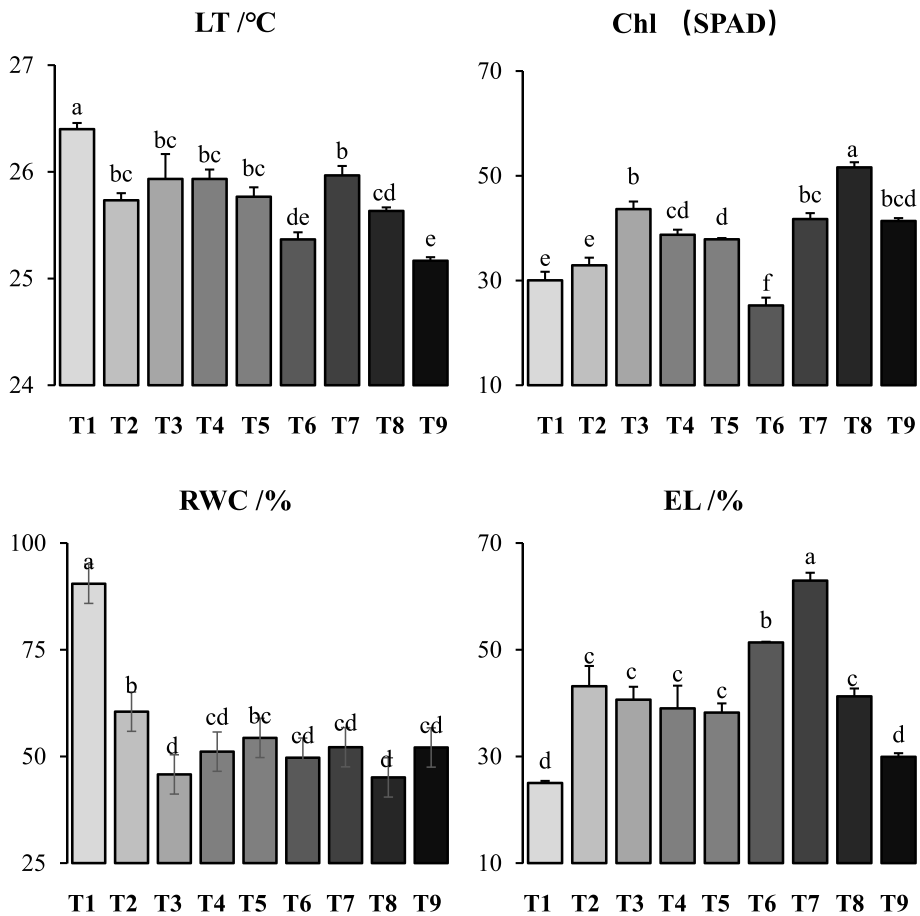

Fig. 2 Changes in LT (leaf temperature), Chl (chlorophyll content), RWC (relative water content), and EL (electrolyte leakage) of cotton seedlings in the different foliar applications under chilling stress (at 5℃ for 48 h). The letter(s) on each column denotes a significance level at $p < 0.05$

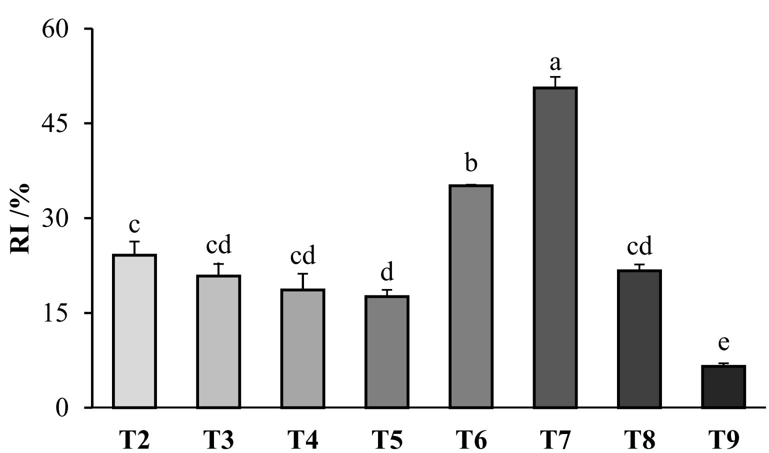

Fig. 3 Changes in RI (relative injury) of cotton seedlings treated with different foliar applications of biostimulants under chilling stress. The letter(s) on each column denotes a significance level at $p < 0.05$

stress. Xu et al. (2017) also confirmed increased root and shoot growth by biostimulants in lettuce. In addition, Rouphael et al. (2023) observed that copper and copper-combined biostimulants applied to basil plants enhanced fresh and dry weights. Similarly, Botta (2013) found that an animal-derived biostimulant led to heavier

Table 1 Correlation coefficients (*r*) between the investigated parameters

	SFW	SDW	PH	SD	LT	Chl	RWC	EL
SFW	-							
SDW	0.664**	-						
PH	0.456**	0.626**	-					
SD	0.542**	0.488**	0.174^{ns}	-				
LT	0.545**	0.065^{ns}	0.163^{ns}	0.428*	-			
Chl	-0.383**	-0.129^{ns}	0.026^{ns}	-0.021^{ns}	-0.026^{ns}	-		
RWC	0.877**	0.539**	0.545**	0.394*	0.560**	-0.497**	-	
EL	-0.686**	-0.658**	-0.335**	-0.541**	-0.125^{ns}	0.057^{ns}	-0.511**	-

SFW seedling fresh weight *SDW* seedling dry weight, *PH* plant height, *SD* stem diameter, *LT* leaf temperature, *Chl* chlorophyll content, *RWC* relative water content, *EL* electrolyte leakage. *, **show significance levels at 5% and 1%, respectively. ns: non-significant

shoot and root fresh weights compared with untreated control lettuce plants. In that study, chilling stress (5°C) led to a significant loss of amino acids and sugars like glucose, fructose, and sucrose from the roots, and the amount of loss increased linearly with increasing duration of chilling temperature. Liu et al. (2013) found that cool temperatures enhanced the activities of Rubisco and cytosolic fructose-1,6-bisphosphatase. Also, dehydration and cellular membrane disintegration in plant tissues commonly occur (Yadav, 2010), resulting in inhibition of root and shoot growth (Hussain et al., 2018). These findings are consistent with the observations of Hund et al. (2007) and Farooq et al. (2009), who found restricted growth of the aboveground part of seedlings.

In the present study, using biostimulants resulted in increased chlorophyll content in the cotton plants compared with the untreated plants. In particular, treatments T3, T7, T8, and T9 exhibited elevated chlorophyll levels compared with other treatments. Both animal- and seaweed-based biostimulants containing bioactive substances such as amino acids, betaines, and minerals have been found to boost chlorophyll levels in plant tissues (Bahmani Jafarlou et al., 2023). These biostimulants regulate osmotic adjustment, nutrient uptake, and water absorption, hence enhancing the tolerance to stress conditions. Niu et al. (2022) found that foliar application of two seaweed extract-based biostimulants (Boosten® and Megafol®) and an animal collagen-derived biostimulant (Isabion®) to tomato seedlings could promote plant biomass accumulation, and that seaweed extract-based biostimulants enhanced chlorophyll synthesis under low-temperature stress. Do Rosário Rosa et al. (2021) showed that the SPAD index of bean plants was higher in all seaweed extract treatments than those in the control under suboptimal temperatures (< 20 °C). However, the findings of the present study indicate a significant correlation between chlorophyll concentration and SFW or RWC. In the present study, chilling stress considerably reduced cell membrane stability, but seaweed-based extract significantly enhanced it. These results are in line with the findings of Bahmani Jafarlou et al. (2023), who determined that a seaweed extract biostimulant triggered cell membrane stability under salinity stress. Electrolyte leakage increases when cotton plants are exposed to stress factors such as drought and high temperatures (Rehman et al., 2021; Majeed et al., 2024). In this study, electrolyte leakage was higher in the plants exposed to chilling stress, and the minimum value for electrolyte leakage was obtained from the plants treated with copper-containing fungicide (T9 treatment).

Conclusion

This study found that low temperatures negatively influenced the morphological and physiological parameters of cotton seedlings at the V4 stage. However, seedlings sprayed with copper-containing fungicide balanced cell membrane stability and improved all morphological parameters following chilling, suggesting that such treatment provides resilience against chilling stress. In addition, all levels of animal-derived biostimulant applications, as well as the 0.660% level of the seaweed-derived biostimulant, led to increased tolerance of cotton plants to chilling. As a result, only copper-containing fungicide treatment may be beneficial for mitigating the damages caused by low temperatures and for protecting cotton seedlings against disease.

Acknowledgements
Not applicable.

Authors' contributions
Ergin N and Kulan EG performed the experiments, analyzed the data, prepared figures and tables, authored and reviewed drafts of the article. Harmanci P assisted in performing the experiments. Kaya MD improved the written language. Kaya MD and Kulan EG conceived and designed the experiments and approved the final draft. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 9 February 2024 Accepted: 20 May 2024
Published online: 01 July 2024

References

Ahmed AI, Khan AI, Negm MAM, et al. Enhancing cotton resilience to challenging climates through genetic modifications. J Cotton Res. 2024;7:10. https://doi.org/10.1186/s42397-024-00171-4.
Bahmani Jafarlou M, Pilehvar B, Modaresi M, et al. Seaweed liquid extract as an alternative biostimulant for the amelioration of salt-stress effects in *Calotropis procera* (Aiton) WT. J Plant Growth Regul. 2023;42(1):449–64. https://doi.org/10.1007/s00344-021-10566-1.
Basal H, Karademir E, Goren HK, et al. Cotton production in Turkey and Europe. In: Jabran K, Chauhan BS, editors. Cotton production. Wiley Online; 2019. p. 297–321. https://doi.org/10.1002/9781119385523.ch14.
Bhattacharya A. Effect of low-temperature stress on germination, growth, and phenology of plants: a review. In: Bhattacharya A, editor. Physiological processes in plants under low temperature stress. Singapore: Springer; 2022. p. 1–106. https://doi.org/10.1007/978-981-16-9037-2_1
Botta A. Enhancing plant tolerance to temperature stress with amino acids: an approach to their mode of action. Acta Hortic. 2013;1009:29–35. https://doi.org/10.17660/ActaHortic.2013.1009.1.
Bulgari R, Cocetta G, Trivellini A, et al. Biostimulants and crop responses: a review. Biol Agric Hortic. 2015;31(1):1–17. https://doi.org/10.1080/01448765.2014.964649.
De Pascale S, Rouphael Y, Colla G. Plant biostimulants: Innovative tool for enhancing plant nutrition in organic farming. Eur J Hortic Sci. 2017;82(6):277–85. https://doi.org/10.17660/eJHS.2017/82.6.2.
Do Rosário Rosa V, dos Farias Santos AL, da Alves Silva A, et al. Enhanced tolerance to cold in common bean treated with biostimulant. Gesunde Pflanz. 2021;73(1):39–50. https://doi.org/10.1007/s10343-020-00526-2.
Du Jardin P. Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic. 2015;196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021.
Duncan DB. Multiple range and multiple *F* tests. Biometrics. 1955;11(1):1–42.
Fareed R, Eisensmith S, Everson E, et al. MSTAT-C: a microcomputer program for the design, management, and analysis of agronomic research experiments. East Lansing, MI, USA: Michigan State University; 1991.
Farooq M, Aziz T, Wahid A, et al. Chilling tolerance in maize: agronomic and physiological approaches. Crop Pasture Sci. 2009;60:501–16. https://doi.org/10.1071/CP08427.
Fu Y, Xin L, Mounkaila Hamani AK, et al. Foliar application of melatonin positively affects the physio-biochemical characteristics of cotton (*Gossypium hirsutum* L.) under the combined effects of low temperature and salinity stress. Plants. 2023;12(21):3730. https://doi.org/10.3390/plants12213730.
Ghoulam C, Foursy A, Fares K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ Exp Bot. 2002;47(1):39–50. https://doi.org/10.1016/S0098-8472(01)00109-5.
Gulen H, Eris A. Some physiological changes in strawberry (*Fragaria* × *ananassa* 'Camarosa') plants under heat stress. J Hortic Sci Biotechnol. 2003;78(6):894–8. https://doi.org/10.1080/14620316.2003.11511715.
Hajiboland R. Silicon-mediated cold stress tolerance in plants. In Etesami H, Al Saeedi AH, El-Ramady H, et al., editors. In silicon and nano-silicon in environmental stress management and crop quality improvement. Salt Lake City, Utah, USA: Academic Press; 2022. p.161–180. https://doi.org/10.1016/B978-0-323-91225-9.00013-3.
Halpern M, Bar-Tal A, Ofek M, et al. The use of biostimulants for enhancing nutrient uptake. Adv Agron. 2015;130:141–74. https://doi.org/10.1016/bs.agron.2014.10.001.
Hiraki H, Uemura M, Kawamura Y. Low-temperature sensing and the dynamics of cytoplasmic calcium in plant cells. Cryobiol Cryotechnol. 2014;60:139–42.
Hund A, Richner W, Soldati A, et al. Root morphology and photosynthetic performance of maize inbred lines at low temperature. Eur J Agron. 2007;27:52–61. https://doi.org/10.1016/j.eja.2007.01.003.
Hussain HA, Hussain S, Khaliq A, et al. Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Front Plant Sci. 2018;9:393. https://doi.org/10.3389/fpls.2018.00393.
Kauffman GL, Kneivel DP, Watschke TL. Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. Crop Sci. 2007;47(1):261–7. https://doi.org/10.2135/cropsci2006.03.0171.
Kawamura Y. Chilling induces a decrease in pyrophosphate-dependent H^+-accumulation associated with a ΔpH_{vac}-stat in mung bean, a chill-sensitive plant. Plant Cell Environ. 2008;31(3):288–300. https://doi.org/10.1111/j.1365-3040.2007.01762.x.
La Torre A, Iovino V, Caradonia F. Copper in plant protection: current situation and prospects. Phytopathol Mediter. 2018;57(2):201–36. https://doi.org/10.14601/Phytopathol_Mediterr-23407.
Liang Y, Liu H, Fu Y, et al. Regulatory effects of silicon nanoparticles on the growth and photosynthesis of cotton seedlings under salt and low-temperature dual stress. BMC Plant Biol. 2023;23(1):504. https://doi.org/10.1186/s12870-023-04509-z.
Li ZB, Zeng XY, Xu JW, et al. Transcriptomic profiling of cotton *Gossypium hirsutum* challenged with low-temperature gradients stress. Sci Data. 2019;6(1):197. https://doi.org/10.1038/s41597-019-0210-7.
Liu J, Ma Y, Lv F, et al. Changes of sucrose metabolism in leaf subtending to cotton boll under cool temperature due to late planting. Field Crop Res. 2013;144:200–11. https://doi.org/10.1016/j.fcr.2013.02.003.
Liu J, Shi Y, Yang S. Insights into the regulation of C-repeat binding factors in plant cold signaling. J Integr Plant Biol. 2018;60(9):780–95. https://doi.org/10.1111/jipb.12657.
Majeed S, Chaudhary MT, Mubarik MS, et al. Genetics of biochemical attributes regulating morpho-physiology of upland cotton under high temperature conditions. J Cotton Res. 2024;7:3. https://doi.org/10.1186/s42397-023-00164-9.
Mishra KB, Mishra A, Kubásek J, et al. Low temperature induced modulation of photosynthetic induction in non-acclimated and cold-acclimated *Arabidopsis thaliana*: chlorophyll a fluorescence and gas-exchange measurements. Photosynth Res. 2019;139:123–43. https://doi.org/10.1007/s11120-018-0588-7.
Moriyama Y, Nelson N. Cold inactivation of vacuolar proton-ATPases. J Biol Chem. 1989;264(6):3577–82. https://doi.org/10.1016/S0021-9258(18)94105-0.
Nephali L, Piater LA, Dubery IA, et al. Biostimulants for plant growth and mitigation of abiotic stresses: a metabolomics perspective. Metabolites. 2020;10(12):505. https://doi.org/10.3390/metabo10120505.
Niu C, Wang G, Sui J, et al. Biostimulants alleviate temperature stress in tomato seedlings. Sci Hortic. 2022;293:110712. https://doi.org/10.1016/j.scienta.2021.110712.
Pilon M, Abdel-Ghany SE, Cohu CM, et al. Copper cofactor delivery in plant cells. Curr Opin Plant Biol. 2006;9(3):256–63. https://doi.org/10.1016/j.pbi.2006.03.007.
Polo J, Mata P. Evaluation of a biostimulant (Pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. Front Plant Sci. 2018;8:2261. https://doi.org/10.3389/fpls.2017.02261.

Rashid N, Khan S, Wahid A, et al. Exogenous application of biostimulants and synthetic growth promoters improved the productivity and grain quality of quinoa linked with enhanced photosynthetic pigments and metabolomics. Agronomy. 2021;11(11):2302. https://doi.org/10.3390/agronomy11112302.
Reddy VR, Baker DN, Hodges HF. Temperature effects on cotton canopy growth, photosynthesis, and respiration. J Agron. 1991;83(4):699–704. https://doi.org/10.2134/agronj1991.00021962008300040010x.
Rehman AU, Rana IA, Majeed S, et al. Intra-plant variability for heat tolerance related attributes in upland cotton. Agronomy. 2021;11:2375. https://doi.org/10.3390/agronomy11122375.
Rouphael Y, Carillo P, Ciriello M, et al. Copper boosts the biostimulant activity of a vegetal-derived protein hydrolysate in basil: morpho-physiological and metabolomics insights. Front Plant Sci. 2023;14:1235686. https://doi.org/10.3389/fpls.2023.1235686.
Rouphael Y, Colla G. Biostimulants in agriculture. Front Plant Sci. 2020;11:40. https://doi.org/10.3389/fpls.2020.00040.
Ruelland E, Vaultier MN, Zachowski A, et al. Cold signalling and cold acclimation in plants. Adv Bot Res. 2009;49:35–150. https://doi.org/10.1016/S0065-2296(08)00602-2.
Ruelland E, Zachowski A. How plants sense temperature. Environ Exp Bot. 2010;69:225–32. https://doi.org/10.1016/j.envexpbot.2010.05.011.
Sharma P, Jha AB, Dubey RS, et al. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. 2012;2012:26. https://doi.org/10.1155/2012/217037.
Singh B, Norvell E, Wijewardana C, et al. Assessing morphological characteristics of elite cotton lines from different breeding programmes for low temperature and drought tolerance. J Agron Crop Sci. 2018;204(5):467–76. https://doi.org/10.1111/jac.12276.
Snider JL, Thangthong N, Rossi C, et al. Root system growth and anatomy of cotton seedlings under suboptimal temperature. J Agron Crop Sci. 2022;208(3):372–83. https://doi.org/10.1111/jac.12591.
Sundin GW, Castiblanco LF, Yuan X, et al. Bacterial disease management: challenges, experience, innovation and future prospects: challenges in bacterial molecular plant pathology. Mol Plant Pathol. 2016;17(9):1506–18. https://doi.org/10.1111/mpp.12436.
Theocharis A, Clément C, Barka EA. Physiological and molecular changes in plants grown at low temperatures. Planta. 2012;235:1091–105. https://doi.org/10.1007/s00425-012-1641-y.
Uemura M, Steponkus PL. Cold acclimation in plants: relationship between the lipid composition and the cryostability of the plasma membrane. J Plant Res. 1999;112:245–54. https://doi.org/10.1007/PL00013882.
Yamasaki H, Pilon M, Shikanai T. How do plants respond to copper deficiency? Plant Signal Behav. 2008;3(4):231–2. https://doi.org/10.4161/psb.3.4.5094.
Yadav SK. Cold stress tolerance mechanisms in plants. A review. Agron Sustain Dev. 2010;30:515–27. https://doi.org/10.1051/agro/2009050.
Yruela I. Copper in plants. Braz J Plant Physiol. 2005;17:145–56. https://doi.org/10.1590/S1677-04202005000100012.
Wang L, Zhao Y, Long X, et al. A review of molecular regulation studies of low temperature stress in cotton. Crop Design. 2023;2(2):100039. https://doi.org/10.1016/j.cropd.2023.100039.
Xu C, Mou B. Drench application of fish-derived protein hydrolysates affects lettuce growth, chlorophyll content, and gas exchange. HortTechnology. 2017;27(4):539–43. https://doi.org/10.21273/HORTTECH03723-17.