Effect of foliar application of Cycocel and micronutrients on the antioxidant enzyme activities of *Triticum aestivum* under drought

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Abstract

This study aimed to investigate the effect of foliar application of Cycocel and s of iron and zinc on wheat yield and physiological traits under drought stress during the crop years of 2017-2018 and 2018-2019. The experiment was performed as a split factorial in a randomized complete block design with three replications. Irrigation at two levels (I_1: regular irrigation and I_2: cut-off irrigation at the beginning of the reproductive stage) was considered as the main factor. Also, Cycocell at two levels (C_1: non-consumption and C_2: Cycocell consumption of 0.5 L ha^{-1}) and foliar application of micronutrients at four levels (F_1: pure water, F_2: foliar application of iron, F_3: foliar application of zinc, and F_4: foliar application of iron + zinc (each in a ratio of 3 per thousand)) were considered as sub-factors. Results showed that under normal irrigation conditions, both application and non-application of Cycocell and also the application of iron + zinc had the lowest amount of ascorbate peroxidase and catalase. Under water stress conditions and non-application of Cycocel, the concentration of ascorbate peroxidase and catalase decreased significantly under micronutrient treatments; however, their concentration increased with the consumption of micronutrients and Cycocel. In general, foliar application of Cycocel and iron + zinc under stress reduced the concentration of antioxidant enzymes.

Keywords: catalase, nutrition, superoxide dismutase, wheat, zinc sulfate

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Introduction

In terms of the area under cultivation and world production, wheat (*Triticum aestivum* L.) ranks the first among other cereals. This strategic crop supplies more than 40% of the world's staple food (Ahangar et al., 2015).

Drought is a global problem that poses major limitations in production of wheat in arid and semi-arid regions of Iran (Amiri et al., 2015). Among various stresses, the most challenging one in terms of measurement is drought stress since different mechanisms lead to resistance. Drought stress is one of the most important factors that may be caused by low rainfall, high heat, and high wind. The life span of a plant depends on the stage of growth at which stress occurs (Naderi et al., 2020).

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Table 1
Maximum and minimum temperature during 2017-2018 and 2018-2019 in Robat Karim, Southern Tehran, Iran

Month	2017-2018		Precipitation (mm)	2018-2019		Precipitation (mm)
	Min °C	Max °C		Min °C	Max °C	
October	11.3	25	1.62	9.5	24	13.49
November	3	18	0.02	4	17.2	28.51
December	-1	15	2.43	0	13	11.28
January	-3	13	62.82	-4.1	11	32.49
February	-1.2	12	19.16	-3	9.8	9.84
March	3.7	15.5	6.05	-1	11.6	58.75
April	8.7	21.8	28.29	3.2	16.1	45.68
May	14.4	29.2	24.28	6.9	22.2	4.08
June	18.5	33.9	9.27	10.7	28.8	0.04

Research has established that drought stress before pollination in wheat can have some negative effects on plant fertility as well as grain filling (Al-Ajlouni et al., 2016). The cell protection enzymes such as superoxide dismutase (SOD), peroxidase, and catalase in leaves play significant physiological roles during the primary growth stage under water stress (Saeidi et al., 2017).

Growth retardants are organic compounds that are used at low concentrations and affect the physiological processes of plant growth and development by changing the amounts of hormones (PirastehAnosheh et al., 2016). Cycocel (CCC) is one of the most widely used plant growth retardants in the world which is widely used today to control plants growth (Moon et al., 2020).

Foliar application of micronutrients is a rapid and helpful strategy for the bio-fortification of wheat with s (Cakmak and Kutman, 2018). Iron (Fe) plays a leading role in photosynthesis in the structure of two major groups of proteins as the electron donor-receptor group in respiratory reactions, in the structure of proteins, such as leghemoglobin (Lb), catalase (CAT), and peroxidase (POD) enzymes, as well as in light-dependent processes (Rout and Sahoo, 2015). The effect of iron alone or in combination with other micronutrients has been reported on chlorophyll content, photosynthesis, and yield factors in wheat (Ramzan et al., 2020). Another is Zinc, and essential trace elements for plants with many physiological roles including protein and carbohydrate synthesis, photosynthetic pigment metabolism, photosynthetic capacity enhancement, cellular metabolic functions, cell membrane protection against ROS, and product generation (KARAMI et al., 2016). Hao et al. (2021) found that foliar application of zinc increased wheat yield compared to the control treatment (Hao et al., 2021). Given the problem of deficiency and the importance of Fe and Zn nutrition in wheat as well as water-deficit stress in Iran, this study aimed to investigate the effects of foliar application Fe and Zn fertilizers and also CCC on some physiological/biochemical traits of wheat under drought stress conditions.

Materials and Methods

This study was carried out during the crop years of 2017-18 and 2018-19 in Robat Karim, Southern Tehran, Iran with a height of 1050 m above sea level, the annual precipitation of less than 272 mm, and maximum and minimum temperatures of 44 ℃ and -15 ℃, respectively. Table 1 shows the meteorological data during the study.

The experiment was carried out using a split-plot in a randomized complete block design (RCBD) with three replications. In this experiment, irrigation at two levels (I_1: normal irrigation and I_2: irrigation cut-off at the onset of reproductive phase) as the main factor, CCC application at two concentrations (C_1: non-use and C_2: 0.5 L ha^{-1}), and foliar application of micronutrients at the ratio of 3 g L^{-1} at four levels (F_1: control, F_2: Fe, F_3: Zn, and F_4: Fe + Zn) were considered as factorials.

To shed light on the physical/chemical properties of the soil in the experiment site, soil samples were collected a depth of 0-30 cm and then transferred to a soil laboratory for the relevant analysis (Table 2).

Table 2
Soil test results in the Robat Karim region

Texture	Sand %	Silt %	Clay %	K (mg kg^{-1})	P (mg kg^{-1})	Na (mgkg^{-1})	C %	N %	Lime %	EC (dSm^{-1})	pH
Loam	45	30	25	197.6	10.2	38.7	0.57	0.04	3.1	0.22	8.5

Land preparation, including autumn tillage, was initially conducted in early October (in both crop years). After plowing, the disc and leveling operations were performed for cultivation. Moreover, 120 kg ha^{-1} of wheat was sown with grain drills in mid-October. Then, foliar Fe and Zn micronutrients from the Fe and Zn sulfate sources were applied based on the recommended ratio (three per thousand) at the designated stage of plant growth (namely, shoot and spike emergence). CCC 500 mg L^{-1} was also used in the tillering stage. Besides, nitrogen fertilizer application was based on the experimental treatments within two phases in the form of top-dressing, before stem elongation from the urea source (50 kg ha^{-1} urea fertilizer).

The wheat cultivar used in this experiment was Pishgam, which was selected from the progeny of a cross between Barakat Iranian bread wheat cultivar and Chinese 90-Zhong 87 winter-habit (Bkt/90-Zhong 87) in 1995. This line is superior to other winter wheat cultivars and is registered for production in cold regions of Iran.

Malondialdehyde (MDA) concentration was measured following Valentovic et al. (2006). Also, hydrogen peroxide (H_2O_2) was determined by the method recommended by Sagisaka (1976). Furthermore, CAT and SOD activities were measured by the approaches developed by Candlee and Scandalios (1984) and Beauchamp and Fridovich (1971), respectively.

Statistical Analyses

Analysis of variance (ANOVA) was performed using the factorial split-plot in the RCBD and mean comparisons via Duncan’s multiple range tests ($p \leq 0.05$), using the Statistical Analysis System (SAS *ver. 9.1.3*) software. Owing to the homogeneity of the data based on Bartlett's test, combined analysis was performed using SAS. All charts were plotted by Microsoft Excel software.

Results

Ascorbate peroxidase (APX)

ANOVA results indicated that the effects of CCC, micronutrients, and interactions of irrigation × CCC, CCC × micronutrients, and irrigation × CCC × micronutrients on the APX concentration were significant at the $p \leq 0.05$ (Table 3). The mean comparisons for the interaction of irrigation × CCC × micronutrients on APX also showed that the foliar application of Fe + Zn resulted in the lowest level of APX under the normal irrigation conditions compared with non-application of CCC (Table 4). The lowest concentration of APX was thus observed in the micronutrients non-application and CCC use under the stress conditions in the reproductive phase. These results suggested that the application of micronutrients under stress conditions could significantly reduce APX in the absence of CCC. However, there was no significant difference in APX concentration under application of micronutrients with and without CCC use (Table 4).

Catalase (CAT)

ANOVA results indicated that the effects of CCC, micronutrients, and interaction of irrigation × CCC, CCC × micronutrients, and irrigation × CCC × micronutrients on the CAT concentration were significant at the 5% level (Table 3). The mean comparisons for the interaction of irrigation × CCC × micronutrients CAT showed that under normal irrigation conditions with and without CCC, foliar applications of both Fe and Zn resulted in the least level of CAT (Table 4). Under the stress conditions during the reproductive phase, with CCC application, the CAT concentration significantly decreased following the application of the micronutrients, but the CAT concentration was augmented as the micronutrients were applied along with CCC (Table 4).

Table 3
ANOVA results for physiological traits of wheat

SOV	D.f	APX	CAT	SOD	H_2O_2	MDA
Year	1	0.0001	0.0001	0.047	0.011	27839.283**
Replication (Year)	4	0.0001	0.014	0.045	0.010	4392.750*
Irrigation	1	0.023	0397	1.534	0.347	12931.141**
Year × Irrigation	1	0.001	0.016	0.040	0.009	438.273
Error	4	0.012	0.179	0.821	0.186	475.584
CCC	1	0.013*	0.342*	0.986*	0.224*	3642.326*
Year × CCC	1	0.001	0.028	0.033	0.008	147.659
Irrigation × CCC	1	0.021*	0.429*	1.542**	0.349**	740.754
Year × Irrigation × CCC	1	0.002	0.010	0.037	0.008	159.187
MN	3	0.012*	0.0191*	0.772*	0.175*	4523.648**
Year × MN	3	0.001	0.004	0.043	0.010	199.868
Irrigation × MN	3	0.002	0.061	0.136	0.031	348.299
Year × Irrigation × MN	3	0.003	0.084	0.241	0.055	252.714
CCC × MN	3	0.009*	0.202*	0.672*	0.152*	2286.054*
Year × CCC × MN	3	0.0001	0.009	0.006	0.001	198.245
Irrigation × CCC × MN	3	0.010*	0.214*	0.719*	0.163*	347.313
Year × Irrigation × CCC × MN	3	0.003	0.062	0.171	0.039	174.874
Error	56	0.003	0.062	0.211	0.048	566.490
CV (%)	-	16.81	17.27	16.61	14.60	12.93

* and ** represent significant at $p \leq 0.01$ and $p \leq 0.05$, respectively,

Table 4
The mean comparison of studied traits

Irrigation	Cycocel	Micronutrient	Ascorbate peroxidase ($mM.g^{-1}$ FW)	Catalase ($mM.g^{-1}$ FW)	Superoxide dismutase ($mM.g^{-1}$ FW)	hydrogen peroxide ($mM.g^{-1}$ FW)
normal	control	Control	0.325 bc	0.014 b-d	2.731 bc	1.300 bc
		Fe	0.293 c	0.013 d	2.462 c	1.173 c
		Zn	0.331 bc	0.015 b-d	2.788 bc	1.328 bc
		Fe+Zn	0.293 c	0.013 d	2.469 c	1.176 c
	Cycocel	Control	0.317 bc	0.013 d	2.670 bc	1.271 bc
		Fe	0.350 bc	0.015 b-d	2.944 bc	1.402 bc
		Zn	0.311 bc	0.014 b-d	2.612 bc	1.244 bc
		Fe+Zn	0.289 c	0.013 d	2.426 c	1.155 c
Cut off irrigation	control	Control	0.441 a	0.019 a	3.710 a	1.767 a
		Fe	0.382 ab	0.017 ab	3.210 ab	1.529 ab
		Zn	0.378 ab	0.017 a-c	3.174 ab	1.511 ab
		Fe+Zn	0.283 c	0.012 d	2.381 c	1.134 c
	Cycocel	Control	0.320 bc	0.014 b-d	2.630 bc	1.252 bc
		Fe	0.316 bc	0.013 d	2.656 bc	1.265 bc
		Zn	0.308 bc	0.014 b-d	2.593 bc	1.235 bc
		Fe+Zn	0.330 bc	0.015 b-d	2.772 bc	1.32 bc

Means not sharing a common letter in a column differ significantly at 0.05 level of probability.

Superoxide dismutase (SOD)

Based on the ANOVA results, the effects of CCC, micronutrients, CCC × micronutrients, irrigation × CCC × micronutrients, and irrigation × CCC interactions on the SOD concentration were significant (Table 3). The mean comparisons for the interaction effects of such treatments on the SOD concentration showed that under the normal irrigation, micronutrients relatively decreased SOD concentration whether CCC was applied or not (Table 4). Under the irrigation cut-off conditions in the reproductive phase without CCC use, utilization of micronutrients decreased SOD concentrations, but the SOD level increased with the application of CCC (Table 4).

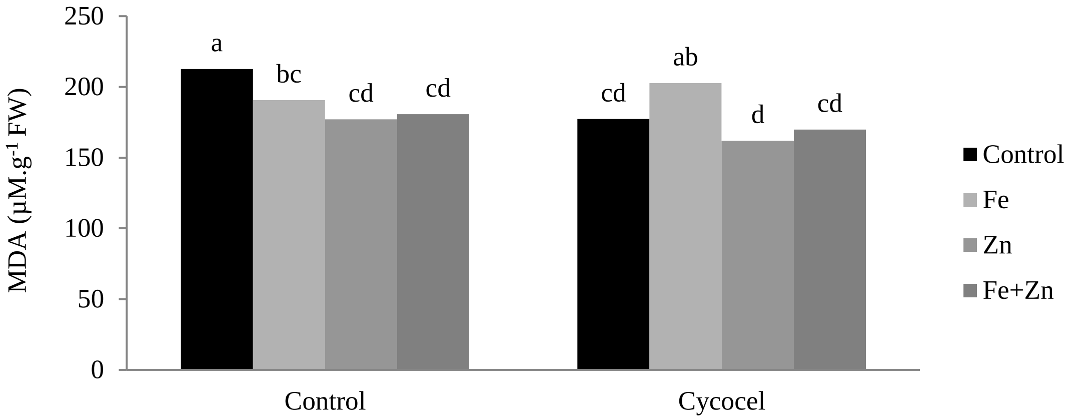

Fig. I. Mean comparisons of the interaction effects of CCC and micronutrient foliar application on MDA concentration

Hydrogen peroxide (H_2O_2)

The ANOVA results showed that the effects of CCC, micronutrients, CCC × micronutrients, irrigation × CCC × micronutrients, and irrigation × CCC interactions, on H_2O_2 concentration were significant at $p \leq 0.05$ (Table 3). The mean comparisons for the interaction effects of the treatments on the H_2O_2 concentration also confirmed that under normal irrigation conditions, applying Fe + Zn reduced the H_2O_2 concentration. Under the CCC use conditions, the H_2O_2 concentration was also diminished by the foliar application of Fe + Zn micronutrients (Table 4). Considering the irrigation cut-off conditions in the reproductive phase, when no CCC was applied, the use of micronutrients reduced H_2O_2, which was more pronounced in the Fe + Zn foliar application. Nevertheless, H_2O_2 concentration was not significantly different in the micronutrients treatments in the presence of CCC (Table 4).

Malondialdehyde (MDA)

The IDA results confirmed that the effects of the year, irrigation, and micronutrients at $p \leq 0.01$ and CCC and interaction of CCC × micronutrients at $p \leq 0{,}05$ were significant on MDA (Table 3). The mean comparisons for the effects of the year on the MDA contents also revealed that the mean MDA content in the first year (198 µM g^{-1} fresh weight) was higher than that in the second year (73 µM g^{-1} fresh weight) due to the lower rainfall. The mean comparisons for the interaction effects of the CCC × micronutrients on the MDA concentration also showed that the application of micronutrients significantly mitigated the MDA concentration in the absence of the CCC use. This decreasing trend also continued under the CCC application conditions. The lowest MDA concentration was thus observed in the Fe + Zn foliar application plus the CCC use (Fig, I).

Discussion

Our results showed that drought stress increased antioxidant activities in wheat. However, foliar application of Cycocel (CCC) and iron and zinc micronutrients moderated the destructive effects of stress. Drought is one of the most important abiotic stresses and the second main factor in reducing the yield of plants after pathogens. This decrease occurs as a result of delay or non-establishment of plants, weakening or destruction of established plants, and physiological and biochemical changes in plant metabolism (Chai et al., 2016).

The results showed that SOD activity significantly increased in plants exposed to water stress. It has been reported that with increasing drought levels, the activity of antioxidant enzymes such as APX, SOD, and guaiacol peroxidase in plant leaf tissues increased significantly (Bahrampour et al., 2019).

Results of the study also showed that foliar application of zinc and iron reduced the harmful effects of drought stress on wheat. Rahimi Jarihani and Abdollahi Mandoulakani (2021) reported that the gene encoding APX increased under zinc deficiency conditions. Increased gene expression can be due to the key role of this enzyme in H_2O_2

scavenging and its important role in the management of reactive oxygen species under stress conditions (Rahimi Jarihani and Abdollahi Mandoulakani, 2021).

In line with Sarafraz Aradakani (2019) drought stress elevated MDA content in the wheat plants under study. On the other hand, application of micronutrients significantly reduced the MDA concentration with or without use of CCC. Application of Zn increments the expression of zinc-finger proteins, improves the activities of antioxidant proteins, and diminishes the MDA aggregation (Wu et al., 2015). The application of Zn under drought stress is likely to increment the activities of Turf, CAT, and APX in cotton and rice, suggesting that application of Zn essentially improves the action of the antioxidant framework, leading to the mitigation of oxidative damage caused by water deficit stress (Wu et al., 2015).

Regarding biochemical indicators, the results generally showed that the use of CCC increased resistance to environmental factors. In other words, it can be argued that the use of CCC preserves the cell in normal conditions. Increasing the concentration of CCC reduced the activity of SOD, CAT, and glutathione peroxidase enzymes. Therefore, the use of CCC growth retardant is recommended to control vegetative growth, reduce the effects of environmental stresses, and consequently, increase crop yield (Afkari and Abbasi, 2018).

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