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Evaluation Effect of Copper Sulphate and Manganese on Yield and Chemical Markers of Maize under Water Deficit Condition

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Water deficit is considered among the abiotic stresses most impacting agriculture for its ability to interfere with crop development and quality.

OBJECTIVES: Practices and innovations that could contain the deleterious effects of such stress with copper and manganese are importance for maintaining acceptable crop yields. In this study water deficit stress (end of irrigation at initiation of corn formation) on maize seedlings and the effects of Cu^{++} and Mn^{++}, the accumulation of hydrogen peroxide (H_2O_2) and lipid peroxidation products (MDA), total markers compounds (8-oH-dg), were investigated in control samples, in samples treated with $CuSo_4$ alone, and in samples treated with $MnSO_4$.

METHODS: The experiment was conducted to split split plot based on randomized complete block design such as water deficit in main plot (normal and end of irrigation at initiation of corn formation), copper sulphate (0, 15 and 30 $kg.ha^{-1}$) in sub plots and manganese (0, 10, 20 and 30 $kg.ha^{-1}$) which foliar application at 50 and 60 days after sowing with three replicates, respectively.

RESULT: The results showed that Cu^{++} and Mn^{++} significantly mitigated the impact of the water deficit stress on seed yield and ChlA, ChlB and reduced the chemical markers activity. Regarding the oxidative status these elements revealed lower amounts of 8-oH-dg and MDA, while maize seedlings grown with water deficit alone exhibited the highest increases in reactive oxygen species. The explanation for these effects is provided by highlighting the effectiveness of the elements in avoiding to oxidative stress, which resulted in a lower content of H_2O_2, MDA and 8-oH-dg activity.

CONCLUSION: Thus the content of malondialdehyde (MDA) and 8-oH-dg (Di hydroxy guanosine) was significant decreased by Cu (15 $kg.ha^{-1}$) and Mn (20 $kg.ha^{-1}$) in comparison with other treatments.

KEYWORDS: *Chlorophyll, Corn, Foliar application, Hydrogen peroxide, Nutrition*.

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1. BACKGROUND

Water deficit is considered among the abiotic stresses that have the highest detrimental effects on plant growth and development (Khan *et al*, 2017). Furthermore, the increase in soil humidity is closely related to climate change (Del buono, 2021); specifically, climate change has led to rises in temperatures and decreases in precipitations, thus degrading water quality of groundwater (Akbari *et al*., 2020). As for as agriculture is concerned, the water of soil and irrigation water has a heavy impact on crop yield. In particular, As a result, water deficit is taking vast areas away from cultivation (Khan *et al*., 2017); to date, it has been estimated that 500 million arable hectares in iran are affected by this problem (Shrivastava *et al*., 2015). Water deficit impacts crops by negatively affecting plant establishment and causing stunted growth (Del Buono *et al*., 2021). High water deficit exert direct toxicity on plants or lead to nutritional disorders (Elrys *et al*., 2020). The adverse effects of water deficit stress can lead to the destruction of water and ion homeostasis in plants, decreased photosynthetic activity, degradation of biomolecules, or alteration of cellular redox potential. (Li *et al*., 2014). In addition, water deficit may hamper the crop's ability to acquire and distribute nutrients among tissues or even interfere with the activity of some critical markers for plant life (Colantoni *et al*., 2017). Further effects prompted by excessive malondialdehyde and 8-oH-dg concentrations can regard alterations in plant growth, and substantial reductions in biomass production (Semida *et al*., 2019). Another critical pathway in which water deficit demonstrates its detrimental action on plants relates to the onset of oxidative stress, namely the overproduction of reactive oxygen species (ROS). ROS are highly harmful to plant survival for their oxidizing capacity, leading to disturbing essential plant cell functions (Elrys *et al*., 2020). In particular, ROS can degrade, among other things, pigments, proteins, nucleic acids, and membranes, thus determining in the most severe cases plant death by chemical markers (Mimmo *et al*., 2015). However, there is recent and increasing attention in using copper and manganese to improve plant resistance to water deficit stress, even though these substances have been initially employed to increase the production and quality of crop species (Calvo *et al*., 2014). Manganese and copper can be defined as substances that promote beneficial effects in crops by stimulating physiological, morphological, and biochemical processes, mainly related to nutrient assimilation, enhanced plant growth and decrease of markers. In addition, the beneficial effects of these elements can often relate to improving the quality of end-products and increasing the plant's ability with biotic and abiotic stresses (Rouphael and Colla, 2020).

2. OBJECTIVES

In order to collect evidence on the possible beneficial effects of these elements, some physiological and biochemical parameters were investigated in samples subjected to water deficit stress, treated or not with compared to control samples. In particular, biomass production, oxidative stress, and markers activities were investigated in maize plants compared to samples grown in the absence of water deficit stress.

3. MATERIALS AND METHODS

3.1. Lab and Treatments Information

The experiment was conducted to split split plot based on randomized complete block design such as water deficit in main plot (normal and end of irrigation at initiation of corn formation), copper sulphate (0, 15 and 30 $kg.ha^{-1}$) in sub plots and manganese (0, 10, 20 and 30 $kg.ha^{-1}$) which foliar application at 50 and 60 days after sowing with three replicates, respectively.

3.2. Measured Traits

The concentration of chlorophylls (Chlorophyll a, ChlA; Chlorophyll b, ChlB) according to the method of Lichtenthaler (2001) and chemical markers (Malondialdehyde and 8-oH-dg) according to the method of panfili, seed yield were determined in maize plants. Malondialdehyde (MDA) and Di hydroxyl guanosine (8-oH-dg) Contents were determined in maize shoots collected at 20 days after Cu and Mn foliar application. To this end, maize leaves (0.5 g fresh weight) The MDA and 8-oH-dg content was determined in maize shoots which were extracted in a solution containing 10% trichloroacetic acid and 0.25% thiobarbituric. The suspension was then centrifuged for 20 min (Panfili *et al*., 2019). After that, the MDA content was determined spectrophotometrically at 532 and 600 nm and referred to the Methanolic Extract One gram of fresh shoots samples was cut and then extracted twice in 80% aqueous methanol (volume for each extraction) by shaking it at room temperature for 90 min.

3.3. Statistical Analysis

Statistical Analysis Data were analysed by one-way minitab (Ver. 14.1) according to a randomised block design with three replicates. The mean values of the determinations in triplicate ± standard errors are shown. In addition, the means were compared with Fisher's least significant difference (Duncan test) at $p < 0.05$ (Lenth, 2016) was used to perform the analysis.

4. RESULT

Effect of water deficit on Maize yield Content in Control Samples and Treated with Cu and Mn Alone or in Combination with severely significant on seed yield, ChlA, chlB and markers (Table 1) compared to control samples. However, when the samples subjected to water deficit were foliar application with Cu and Mn, increases in seed yield were still recorded (Table 2). On the other hand, yield was affected by water deficit and the samples treated with Cu and Mn show statistically significant differences compared to control samples. (Table 1). Different letters within each column indicate statistically significant differences at $p \leq 0.05$. As far as chlA and chlB are concerned, water deficit significantly reduced seed yield, while chlA and chlB was affected (Table 2).

Table 1. Result of analysis of variance effect of treatments on studied traits

S.O.V	df	Seed yield	ChlA.	ChlB.	MDA	8-oH-dg
Replication (R)	2	0.648^{ns}	0.901^{ns}	0.855^{ns}	0.611^{ns}	0.665^{ns}
Water deficit (A)	1	1745.24**	805.26**	832.55**	1202.61**	1156.48**
Error (I)	2	30.25	25.47	29.81	14.82	16.71
Cu (B)	2	744.62**	699.57**	793.55**	911.68**	918.44**
A×B	2	598.51**	462.65**	492.79**	722.55**	713.49**
Error (II)	8	26.44	21.47	24.55	12.81	14.48
Manganese (C)	3	512.68**	445.91**	482.63**	632.51**	628.69**
A×C	3	450.26**	475.81**	466.52**	749.64**	762.53**
B×C	6	348.29**	402.56**	399.67**	613.29**	602.43**
A×B×C	6	275.48**	347.81**	352.66**	479.51.68**	423.67**
Error (III)	36	22.74	23.18	26.65	12.53	14.05
CV (%)	-	7.42	8.15	7.94	6.49	7.11

$^{ns,\ *\ and\ **}$: non-significant, significant at 5% and 1% of probability level, respectively.

Furthermore, significant differences were recorded between samples grown in water deficit, whether Cu and Mn foliar application or not. Regarding MDA and 8-oH-dg, water deficit enhanced the content (Table 2); on the contrary, when samples were treated with the Cu and Mn, the content of these markers was significantly different from those shown by control samples (Table 2). Regarding chlorophyll b (ChlB), samples grown in water deficit without the Cu and Mn showed a significant decrease. As a consequence of the abovementioned effects, the total chlorophyll and seed yield contents were significantly decreased in samples subjected to water deficit alone (Table 2). However, samples subjected to water deficit with elements did evidence significant difference from the untreated control samples. On the other hand, when samples were foliar application with Cu and Mn, a significant reduction in the amount of MDA and 8-oH-dg contained by leaves was recorded, even though it was significantly higher than those shown by the control samples (Table 2). In addition, the highest Cu^{++}/Mn^{++} ratio was recorded in samples grown in water deficit, regardless of 15 and 30 kg.ha^{-1} application respectively (Table 2).

5. DISCUSSION

Water deficit is among the abiotic stresses that have the highest detrimental effects on agriculture for its ability to interfere with crop development and quality (Khan *et al*., 2017). Furthermore, water deficit stress mainly affects crop production in regions with an arid or semi-arid climate, stress mainly affects crop production in regions with an arid or semi-arid climate, particularly the coastal areas (Del Buono *et al*., 2021). Therefore, implementing practices and innovations that can contain the deleterious effects of such stress is considered essential to maintaining acceptable crop yields. For this reason, this work concerned the study of the effects of Cu and Mn foliar application on maize plants on which severe water deficit stress (15 and 20 kg.ha^{-1}) was imposed.

Table 2. Mean comparison effect of treatments on measured traits

Water deficit	Mn ($kg.ha^{-1}$)	Cu ($kg.ha^{-1}$)	Seed yield ($kg.m^{-2}$)	ChlA. ($mg.g^{-1}$ FW)	ChlB. ($mg.g^{-1}$ FW)	MDA ($nm.mg^{-1}$ Leaf pro)	8-oH-dg ($nm.mg^{-1}$ Leaf pro)
I_1	Mn_0	(C_0)	755.48^{c}	2.26^{b}	1.44^{c}	7.65^{a}	1.36^{a}
	Mn_1		769.36^{b}	2.49^{ab}	1.56^{b}	7.39^{bc}	1.24^{b}
	Mn_2		798.66^{a}	2.58^{a}	1.67^{a}	6.98^{c}	1.1^{c}
	Mn_3		768.91^{b}	2.53^{ab}	1.63^{ab}	7.45^{b}	1.26^{b}
	Mn_0	(C_1)	849.11^{c}	2.74^{b}	1.53^{b}	7.11^{a}	1.16a
	Mn_1		894.55^{b}	2.92^{ab}	1.64^{ab}	6.76^{b}	1.09^{b}
	Mn_2		936.11^{a}	3.01^{a}	1.72^{a}	6.27^{cd}	1.02^{c}
	Mn_3		918.25^{ab}	2.97^{ab}	1.69^{ab}	6.35^{c}	1.07^{b}
	Mn_0	(C_2)	821.44^{d}	2.59^{c}	1.49^{bc}	7.28^{a}	1.27^{a}
	Mn_1		845.29^{c}	2.67^{b}	1.52^{b}	7.19^{ab}	1.22^{ab}
	Mn_2		889.12^{a}	2.79^{a}	1.59^{a}	6.71^{c}	1.08^{bc}
	Mn_3		866.34^{b}	2.72^{ab}	1.56^{ab}	6.8^{bc}	1.11^{b}
I_2	Mn_0	(C_0)	535.16^{c}	1.78^{c}	1.12^{c}	10.87^{a}	1.91^{a}
	Mn_1		561.45^{b}	1.91^{b}	1.2^{b}	10.44^{b}	1.79^{b}
	Mn_2		594.37^{a}	2.02^{a}	1.31^{a}	10.05^{c}	1.64^{cd}
	Mn_3		569.72^{b}	1.96^{ab}	1.26^{ab}	10.26^{bc}	1.68^{c}
	Mn_0	(C_1)	586.22^{c}	1.89^{bc}	1.28^{d}	9.98^{a}	1.74^{a}
	Mn_1		621.45^{b}	1.97^{b}	1.44^{c}	9.43^{b}	1.59^{b}
	Mn_2		679.39^{a}	2.24^{a}	1.57^{a}	9.14^{c}	1.48^{c}
	Mn_3		658.12^{ab}	2.15^{ab}	1.51^{b}	9.31^{bc}	1.52^{bc}
	Mn_0	(C_2)	544.49^{bc}	1.74^{bc}	1.17^{bc}	10.94^{a}	1.97^{a}
	Mn_1		578.22^{b}	1.79^{b}	1.25^{b}	10.57^{b}	1.83^{b}
	Mn_2		619.46^{a}	1.96^{a}	1.43^{a}	9.38^{cd}	1.58^{cd}
	Mn_3		601.25^{ab}	1.88^{ab}	1.38^{ab}	9.71^{c}	1.66^{c}

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).
I_1: Normal and end of irrigation, I_2: End of irrigation at initiation of corn formation.
Mn_0: 0 $kg.ha^{-1}$ Manganese, Mn_1: 10 $kg.ha^{-1}$ Manganese, Mn_2: 20 $kg.ha^{-1}$ Manganese , Mn_3: 30 $kg.ha^{-1}$ Manganese.
C_0: 0 $kg.ha^{-1}$ Copper sulphate, C_1: 15 $kg.ha^{-1}$ Copper sulphate, C_2: 30 $kg.ha^{-1}$ Copper sulphate.

In order to provide evidence of possible positive effects of foliar application on this adversity, specific biochemical parameters, the content of markers as well as certain antioxidant activities were investigated in control samples treated only with elements, or with water deficit in combination with a foliar application. In general, water deficit stress causes physiological and biochemical changes in plants, leading to impaired or stunted growth, yield losses and decreased end-products quality (Colla *et al*., 2012). When maize was grown in water deficit and foliar application with elements, it maintained a content of ChlA and ChlB at levels similar to those shown by control samples (Table 2). Therefore, this result reveals that the foliar application may permit maize, even though grown in water deficit, to show a high chlorophyll content to sustain higher photosynthetic activity than unfoliar samples. It is in line with the increase in seed yield found in foliar application samples (Table 2) which can protect plants from degradation of MDA (Martynenko *et al*., 2016) or stimulate its biosynthesis (Abbas *et al*., 2013). This beneficial effect was attributed to the capacity of Cu and Mn to activate the expression of specific genes involved in stress responses and tolerance, and it allowed plants to recover quicker when the stress was removed (Sonobe *et al*., 2020). Therefore, the results show that Mn and Cu can help the plant to cope with the water deficit stress stimulating the content of chlorophyll a and the activity of markers (Table 2). The importance of this effect prompted by the in biological role of element since they operate as biochemical molecules and exert a protective function (Bartucca et al., 2020). In particular, Cu and Mn remove ROS, leading to the protection of chloroplast from markers, whose overproduction is frequently caused by biotic and abiotic stresses (Bartucca *et al*., 2020). Finally, the interest towards the possibility of increasing seed yield by elements value should be mentioned, given their health-promoting action (Gan *et al*., 2018). Therefore, the effect of Mn and Cu on maize in water deficit stress considered in terms of maintaining a low MDA content has an immediate protective effect against salinity as it is well known that this adversity can give rise to the overproduction of ROS. Our data show that this remained practically the same in control and foliar application maize grown in water deficit, showed a considerable increase in seed yield (Table 2). In particular, the lower values exhibited by no foliar application samples indicated a good relationship between the markers complex and the capacity of yield production (EL Arroussi *et al*., 2018). Several hypotheses have been formulated to explain the mechanisms of tolerance/resistance of crops to water deficit, but, in many cases, they are dependent on the species in question. In general, however, among the most critical pathways adopted by species to counteract the deleterious effects of salinity mechanisms, the controlling of water uptake and ROS production are reputed of particular importance (Acosta-Motos *et al*., 2017). Regarding the latter aspect, a large body of literature documents that salinity causes oxidative stress, and this mainly affects chloroplasts and mitochondria with negative consequences on their functionality (Acosta-Motos *et al*., 2017). Our results indicate that plants exposed to water deficit without the Cu and Mn exhibited a non-selective and deleterious decrease in seed yield, accompanied by an increase MDA and 8-oH-dg. On the contrary, these elements strongly limited the amount of markers accumulated in the leaf protein (Desoky *et al*., 2019). However, limiting the concentration of MDA in the leaf is a crucial mechanism for plant survival in water deficit, and it was prompted by the Mn and Cu investigated in this study. Water deficit stress can give rise to the overproduction of ROS, thus affecting the redox status of cells and generating oxidative stress. Excessive ROS accumulation has a negative impact mainly for its ability to degrade proteins, membranes, and DNA (Nxele *et al*., 2017). H_2O_2 is one of the main oxidizing species and can seriously affect cell functionality if accumulated to excessively high levels (Liang *et al*., 2018). Furthermore, as already stated, the accumulation of H_2O_2 in water deficit could lead to selective Cu^{++} acquisition by plants, as appeared to happen in our experiments. Despite this, Cu and Mn significantly decreased the amount of H_2O_2, as evidenced in Table 2 compared to maize samples grown without these. This last aspect should be discussed with the other parameter investigated in this study, thus the content of malondialdehyde (MDA) and 8-oH-dg (Di hydroxy guanosine).

6. CONCLUSION

Thus the content of malondialdehyde (MDA) and 8-oH-dg (Di hydroxy guanosine) was significant decreased by Cu (15 $kg.ha^{-1}$) and Mn (20 $kg.ha^{-1}$) in comparison with other treatments.

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FOOTNOTES

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