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Foxtail Millet Responses to Bulk and Nano Zinc Oxcide Particles in Water Stress Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Author ND was corresponding author. Author MJS was supervisor. Authors SGM and AAN were advisers of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims*:* This study was done to investigate the effects of zinc oxide (ZnO) nanoparticles application on foxtail millet in water stress conditions.

Place and Duration of Study: The experiment was conducted at the Agricultural Research Center of Islamic Azad University, Birjand branch, Iran in 2011.

Methodology: Experimental design was split plot based on randomized complete blocks with three replications. Two irrigation treatments (control and water stress) and seven ZnO fertilizer (control without ZnO, three levels of bulk ZnO (3000, 6000 and 12000 ppm) and three levels of ZnO nanoparticles (250, 500 and 1000 ppm) were as main plots and sub plots, respectively.

Result: The results showed that water stress declined peduncle length, stomatal conductivity, germination percentage and grain yield. Seed protein content increased under water stress but ZnO fertilizer treatments had not any significant effect on the mentioned traits. Effect of ZnO application on Relative Water Content (RWC) at the pre-anthesis stage was significant. The highest RWC before flowering were recorded in the ZnO nanoparticles treatment (250 ppm).

Conclusion: The insignificant effect of bulk and ZnO nanoparticles treatments can be attributed to a low level of requirement of this element in millet. Probably, nutrient

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imbalance in the soil may also have contributed to the prevention of any positive effects of zinc consumption on plant growth parameters.

Keywords: Millet; morphology; nano zinc; physiology; protein; water stress; yield.

1. INTRODUCTION

Iran, with average annual precipitation of 240 mm is classified as a dry region. An understanding of the factors affecting growth and yield in plants under water stress can be applied to enhance irrigation efficiency and facilitate plant improvement in terms of its ability for more efficient water use, and to develop methods of dry farming [1].

Millet genotypes have a short growing season. In suitable environmental conditions they are able to produce higher yields with less water [2]. Millet is a forage crop with C4 photosynthetic pathway and is mainly cultivated as forage. Its nutritional value is about 83% that of maize [3]. References [1,4,5] have reported that water stress decreased millet growth and had an adverse effect on plant height.

In most plants, the first response to water stress is the stomata closure to prevent water loss as transpiration, which can be the result of decreasing turgor or leaf water potential. This can be attributed to a decrease in the flow of $CO₂$ through the leaves, which causes more electrons to become active. These electrons combine with other compounds, oxidize them and then separate more easily (active oxygen). However, by decreasing transpiration, leaf temperature increases [6]. The leaf chlorophyll content is a parameter that can be influenced by water stress. Research by Reference [7] maintains that chlorophyll is an important indicator of environmental stress and the report shows that the amount of chlorophyll decreases in plants under stress contributing to less light absorption by a plant. The result of reference [8] stated that decrease in chlorophyll under water stress was caused by the destruction of pigments or a decrease in their ability to synthesize; another contributing factor may be the disruption of the enzyme activity responsible for photosynthesis.

One effect of water stress is the disruption of a plant's nutrient equilibrium. However this problem can be addressed with foliar application of microelements which improve plant growth in water stress conditions [2].

Zinc (Zn) is an important microelement for plants' metabolic activities. Although plants generally require only small amounts of zinc, if it is not available, the plant will suffer physiological stress from dysfunctional enzyme activity and other metabolic processes [9]. This element has an important role in regulating stomata due to its role in retaining the potassium content of protective cells. Zn shortage can cause imbalance in plant nutrients and consequently decrease a crop's quality and yield [10]. Zinc has an important role in protecting plant cells against reactive oxygen species [11].

Most soil in Iran, suffers from an intense shortage of micro nutrients, particularly that of zinc. So, foliar application of zinc (Zn) can be used to improve plant growth in stress conditions [12]. Reference [13] reported that Zn application increased the growth of maize. In an experiment on pearl millet [2] it has been concluded that non stress treatment accompanied by foliar applications of zinc and manganese increased percentages of ash, raw protein and nitrogen of forage.

Although fertilizer consumption is recommended to improve yield in conditions with a shortage of nutrients, chemical fertilizers have harmful effects on the environment and the quality of the produced food. Nowadays nano technology has expanded horizons in all fields of science. Nano technology can be used in crop production to increase yield [14]. Substituting traditional methods of fertilizer application with nano fertilizers is a way to release nutrients in to the soil gradually and in a controlled way, thus preventing autrification and pollution of water resources [15]. Transforming materials to a nano scale changes their physical, chemical and biological characteristics as well as affecting catalytic properties. The chemical and biological activities of most substances increase at nano scale [16].

Reference [17] illustrated that $TiO₂$ nano particles had a significant effect on characteristics of maize. Treatment with $TiO₂$ nano particles on maize had a considerable effect on growth, whereas the effect of TiO₂ bulk treatment was negligible. Titanium nano particles increased light absorption and photo energy transmission. In another experiment, a compound of $SiO₂$ and TiO₂ nano particles increased the activity of nitrate reductase in soybean, and intensified plant absorption capacity, making its use of water and fertilizer more efficient [18]. The result of reference [19] demonstrated that treatment of nano silver (50 mgL $^{-1}$) on wheat increased the percentage of germination, plumule and radical length and improved stabilization. Employing nano sized TiO₂ in suitable concentration could promote the seed germination of wheat in comparison to bulk TiO2 but in high concentrations it had inhibitory effect on wheat [20].

This experiment was conducted to investigate the effects of ZnO (bulk and nano) on morphological, physiological and qualitative characteristics of foxtail millet under water stress.

2. MATERIALS AND METHODS

This experiment was conducted at agricultural research center of Islamic Azad University, Birjand branch, Iran (Latitude of 32^0 53 N, longitude of 59^0 13 E, and altitude of 149 m). Soil characteristics at the site of the experiment are presented in Table 1.

Table 1. The results of soil analysis

The experiment was carried out in randomized complete blocks design with three replications. Each replication included two main plots as irrigation treatments consisting of the following; 1. Control treatment with adequate irrigation according to water requirements determined for plants in the Birjand region. 2. Under irrigation in which 50% of water requirement was applied. Water requirements were determined by the FAO method using evaporation statistics of a class pan and by considering 80%water spreading efficiency on plot surface [21]:

Amounts of consumptive water (lit.m⁻²) in each irrigation treatment are presented in Table 2. Stress treatment was applied after complete plant establishment.

Table 2. Irrigation volume in different treatments

** The amount of water consumed is Regardless of the initial irrigation*

In this experiment each main plot consisted of 7 subplots for foliar application as follows:

- 1. Control (ZnO)
- 2. ZnO spraying with 3000 ppm concentration(Zn_1)
- 3. ZnO spraying with 6000 ppm concentration $(Zn₂)$
- 4. ZnO spraying with 12000 ppm concentration (Zn_3)
- 5. ZnO nanoparticles spraying with 250 ppm concentration (NZn₁)
- 6. Nono ZnO spraying with 500 ppm concentration $(NZn₂)$
- 7. ZnO nanoparticles spraying with 1000 ppm concentration (NZn_3)

Fig. 1. show the size of ZnO nanoparticles by electron microscope.

Fig. 1. The size of ZnO nanoparticles by electron microscope (TEM)

Dimensions of each subplot were 1.80 \times 4.45 m² including 6 planting rows with 50 cm spacing. Cultivation was carried out on both sides of ridges on 30 May 2011. The Bastan cultivar of foxtail millet was used as seed for tests. During the growing season weed control was done by hand. Urea fertilizer was applied in all treatments (150 kg.ha⁻¹). To determine grain yield, harvesting was done when panicles turned completely yellow. The two lateral rows and 0.5m of the first and last rows were crossed out to prevent side effects. SC-1 model prometer measured stomata conductivity. A chlorophyll meter (SPAD) Minolta model was used for measuring chlorophyll indexes in two stages (at the emergence of inflorescence and at the start of the grain filling stage).

Leaf relative water content calculated using the formula [22].
\n
$$
RWC(\%)=[(W1-W3)\times(W2-W3)^{-1}]\times100
$$
\n(3)

Leaf fresh weight: W1 Leaf turgored weight: W2 Leaf dry weight: W3

This parameter was calculated at three stages (before flowering, during flowering and after flowering).

At the final stages of growth, 5 plants from each plot were selected for measuring morphological characters including plant height, number of leaves per stem, panicle and peduncle length, and the number of panicles per plant. Protein percentage was determined by the Kejeldal method. The data were statistically analyzed and the significant means were compared by Duncan's multiple range tests at 5% probability level using the software MSTATC and Excel.

3. RESULTS AND DISCUSSION

The effect of irrigation on yield was significant (Table 3).Water stress reduced the grain yield 81.46% as compared to control (Table 4). It seems that water stress, due to its effect on photosynthesis, respiration, and transition, ionic absorption, nutrients and hormones metabolism processes reduced growth and led to a decrease in flowering and grain filling period which was associated with smaller and fewer grains. The decrease of grain filling due to water stress has been reported by many other researchers including [23,24,25,26,1].

Table 3. Sources of variation, df and mean squares for grain yield and quality traits of foxtail millet as affected by drought stress and Zn fertilizer

ns, and ** are non-significant, and significant at the 0.05 and 0.01 level of probability, respectively*

Means with similar letters in each column are not significantly different

ZnO did not have a significant effect on grain yield (Table 3). According to the results of soil analysis, it seems that macro and micronutrients were not available for plants. Thus, it is possible that ZnO was unavailable to the plants. Nevertheless, a positive effect of Zinc on millet yield has been reported elsewhere [2,27]. Some other reports regarding yield increase in various plants have been found [28,17].

The results of analysis of variance demonstrated that water stress decreased peduncle length significantly (Tables 5 and 6). In desirable irrigation treatment, peduncle length was longer in comparison with stress treatment. This is in agreement with the results of other research [23].

The results of data variance analysis illustrate that treatments of water stress and foliar application of ZnO had non-significant effects on ear length, but their mutual effect on this trait was significant at 5% (Tables 5 and 7).

The result of reference [23] reported that the decrease of ear length associated with water stress is related to the number of florets. Stem elongation of foxtail millet begins sooner than it does in other millet cultivars, but its ear appears later and as a result there is more time available for formation of florets. So, in this particular millet cultivar more seeds are produced.

Sov	df	Mean-square			
		Plant height	Number of leaves on the main stem	Peduncle length	Panicle length
replication	2	168.718	3.844	11.795	0.565
Factor A (irrigation)		529.731 $^{\text{ns}}$	1.524 ^{ns}	221.445	0.154 ^{ns}
Error a		113.370	1.387	13.537	0.940
Factor B (fertilizer)	6	13.584 ns	0.957 ^{ns}	3.397^{ns}	0.618 ^{ns}
(irrigation× fertilizer)	6	76.403 ^{ns}	0.682 ^{ns}	4.720 $^{\text{ns}}$	1.396
Error b	24	30.825	0.559	2.660	0.532
CV(%)	$\overline{}$	14.10	6.94	15.48	12.29

Table 5. Sources of variation, df and mean squares for some morphological traits of foxtail millet as affected by drought stress and Zn fertilizer

ns, and ** are non-significant, and significant at the 0.05 and 0.01 level of probability, respectively.*

Means with similar letters in each column are not significantly different.

Means with similar letters in each column are not significantly different.

The results of analysis of variance showed that the effect of irrigation treatment and ZnO on plant height was not significant (Table 8). References [1,23] reported that water stress decreased plant height. Some research such as references [29,13] reported that applying supplementing zinc in conditions shortage increased plant height.

Means with similar letters in each column are not significantly different.

Leaf RWC is a good indicator of a plant's water condition and it serves as an important index in determining drought resistance. Plants that are able to keep more leaf RWC are more resistant to water stress [5]. Evaluations of leaf water relative content provide an indication of the amount of water present in plant tissue relative to its saturated state. So, in comparison with moisture percentage, it provides a more insightful evaluation for water conditions [25]. Based on results of analysis of variance, leaf RWC, water stress and ZnO had non significant effects on leaf RWC during the periods before and after flowering, but the effect of ZnO on this parameter was significant at 5%before flowering (Tables 9,10). The highest leaf RWC was obtained with the application of nano zinc (0.25 per 1000 concentration), by the average 80 before flowering (Table 11).

Fig. 2. Shows leaf RWC trend in various growth stages of millet under different irrigation treatments.

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Fig. 2. Leaf RWC trend in various irrigation treatments

References [25] found that water stress in corn reduced significantly flag leaf RWC during flowering. This decrease in RWC indicates turgor pressure reduction in plant cells due to increasing water stress, which leads to decreased growth. In such conditions the correlation between RWC and plant water potential diminishes, decreasing the correlation between yield and leaf RWC [30]. However, under water stress there may not be a significant difference between RWC and desirable irrigation condition but the energy consumed by a plant for osmotic control results in a considerably decreased yield [31]. References [25] found that water stress in corn reduced significantly flag leaf RWC during
flowering. This decrease in RWC indicates turgor pressure reduction in plant cells due to
increasing water stress, which leads to d

The result of reference [32] reported that in Sorghum the RWC rate was 81% in leaves with higher greenness and 38%in those with lower greenness. Reference [33] found a significant correlation between chlorophyll index and RWC.

Water stress had a significant effect on the stomata conductivity at both stages (at the start of flowering and at the grain filling stage) that led to decreased stomata conductivity (Fig. 3). But this character was not affected by application of ZnO (Table 9). Water stress had a significant effect on the stomata conductivity at both stages (at the start
of flowering and at the grain filling stage) that led to decreased stomata conductivity (Fig. 3).
But this character was not af

Table 9. Sources of variation, df and mean squares for some physiological traits of foxtail millet as affected by drought stress and Zn fertilizer

ns, and ** are non-significant, and significant at the 0.05 and 0.01 level of probability, respectively*

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Table 10. Effect of irrigation levels on physiological characteristics of millet

Means with similar letters in each column are not significantly different

Table 11. Effect of fertilizer levels on physiological characteristics of millet

Means with similar letters in each column are not significantly different

The first reaction to water shortage in most plants is stomata closure to prevent water loss from transpiration, and this response is related to conditions of turgor or decreased leaf water potential. This reduces the flow $CO₂$ through the leaf and resulting in more electrons available to form active electrons. These electrons then compose with other compounds, oxidize them, and separate easily (active oxygen). However, by decreasing transpiration, leaf temperature increases [6].

In conditions of water shortage the arsenic acid level in roots increases and transfers to leaves where it causes stomata closure. When stomata are closed transpiration decreases. Moreover, water shortage leads to ABA increase in leaves, its redistribution from mesophyll to epidermis and stomata closure. Stomata are able to produce their required ABA. Extensive studies have shown that a decrease in leaf stomata conductivity highly depends on improving ABA levels in xylem. So, ABA can regulate stomata conductivity as a sign of water stress [34]. from transpiration, and this response is related to conditions of turgor or decreased leaf
water potential. This reduces the flow CO_2 through the leaf and resulting in more electrons
available to form active electrons.

Results of analysis of variance illustrate that although water stress causes an increase in the chlorophyll index, this enhancement was not significant. It was also not influenced by ZnO application (Table 9). Nevertheless, some results indicate that zinc has positive effects in terms of increasing chlorophyll content in maize [35,36]. levels in xylem. So, ABA can regulate stomata conductivity as a sign of
of variance illustrate that although water stress causes an increase in the
his enhancement was not significant. It was also not influenced by ZnO
9).

The result of reference [37] in wheat indicated that water stress increased the chlorophyll a/h ratio. This effect was associated with increasing levels of chlorophyll and darkness of the b leaves. The result of reference [38,39] achieved similar results in wheat and alfalfa respectively.

Fig. 4. Shows Chlorophyll index changes of millet in irrigation treatments at both stages (at stages (atthe start of flowering and at the grain filling stage).

Fig. 4. Chlorophyll index changes of millet in irrigation treatments index in irrigation

Reference [40] reported that water stress decreased chlorophyll rate in soybean, probably due to the case that water stress diminishes a plant's water potential and closing stomata is a mechanism that controls a plant's relative water content (RWC). As mentioned before, stomata closure produces oxidative stress and reduces chlorophyll content which serves to reduce photosynthesis thereby affecting the availability of assimilates. This phenomenon Reference [40] reported that water stress decreased chlorophyll rate in soybean, probadue to the case that water stress diminishes a plant's water potential and closing stomat a mechanism that controls a plant's relative w

Fig. 5. Shows Millet leaf temperature changes in irrigation treatments at both stages (at the
start of flowering and at the grain filling stage). start of flowering and at the grain filling stage).

Fig. 5. Millet leaf temperature changes in irrigation treatments leaf temperature

Although there was increased leaf temperature recorded in both measured conditions of water stress, it was negligible and insignificant. Furthermore, ZnO had a non- significant effect on leaf temperature (Table 9). re was increased leaf temperature recorded in both measit was negligible and insignificant. Furthermore, ZnO had temperature (Table 9).

According to these results, water stress had a significant effect at 5% probability level on According to these results, water stress had a significant effect at 5% probability level on
grain protein. Grain protein percentage increased (17.93%) under water stress conditions (Tables 3 and 4). An increase in the percentage of grain protein in maize from water stress in maize has been also reported by [41]. Water stress improves protein percentage in legumes. Probably, there is a decrease of water potential in the cell sap that is a response induced by Probably, there is a decrease of water potential in the cell sap that is a response induced by
stress. However, in conditions of water shortage, the formation of more simple molecules increases automatically. So, transformation of photosynthetic substances leads to protein synthesis [42].

The results showed that ZnO had a non-significant effect on protein percentage. According to the results of another study other than improving yield, Zn application increased the concentration of Zn, grain protein and shoots, and led to better quality of the yield [43].

Reference [44] reported that water stress disrupted processes of photosynthesis, enzyme activity and protein synthesis, which contributed to the displacement of metabolites needed by the grain.

Water stress had a significant effect on seed germination capacity (seed viability) and reduced it which is in agreement with the results of research by reference [45, 46] whereas ZnO had a non-significant effect on seed viability (Table 3).

4. CONCLUSION

Totally the results indicated that water stress had a significant effect on yield and some measured morphological characteristics of millet. Non-significant effect of ZnO fertilizer treatments on the most measured traits could be attributed to a low level of requirement of this element in millet. On the other hand, imbalance soil nutrient content may also have contributed to the prevention of any positive effects of zinc consumption on plant parameters evaluated in the study. sults showed that ZnO had a non-significant effect on protein percentage. According
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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