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FOLIAR APPLICATION EFFECTS OF ZINC OXIDE NANOPARTICLES ON GROWTH, YIELD AND DROUGHT TOLERANCE OF SOYBEAN

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Abstract

In modern agriculture, the application of nano fertilizers is increasing the productivity and stability of different crops by reducing the destructive effects of abiotic stresses. The nano particles are significant for their particle shape, potential reactivity, tunable pore size and high surface area. A pot experiment was conducted at the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during *Rabi* season (December 2020 to March 2021) to evaluate the effects of foliar spray of nano zinc (ZnO) in varying doses (0, 100 and 200 ppm) on growth, dry matter accumulation and yield of soybean under drought (40% of field capacity, FC) and control (80% of FC) conditions. The trial was conducted in a completely randomized design (CRD) with three replications. Plant height; fresh and dry weight of leaf, stem and whole plant; number of pods per plant; number of seeds pod^{-1}; 100-seed weight and seed yield of soybean were suppressed due to drought stress. Under both drought and control conditions, the foliar application of nano zinc substantially improved the growth, dry matter accumulation and yield of soybean. In drought condition, the foliar spray of 200 ppm nano zinc solution increased plant height, total fresh and dry weight by 21.69, 34.23 and 76.03%, respectively. In drought conditions, nano Zn particles at 100 and 200 ppm increased seed yield by 26.79 and 63.50%, and in control conditions by 13.07 and 23.56%, respectively. As such the results indicated that foliar application of nano zinc oxide improved growth, yield and drought tolerance of soybean.

Introduction

Soybean (*Glycine max)* is an annual self-pollinated diploid legume under the family Fabaceae. Soybean is recognized as an oil seed containing several useful nutrients including protein, carbohydrate, vitamins, and minerals. According to Index Mundi (2021), the soybean production in 2020 was 156,000 MT and also it has been increased about 44% for last decade in Bangladesh. The annual soybean production and imports are increasing gradually to meet the feed requirements for the livestock, poultry and fisheries sectors, and soybean oil for human consumption (USDA, 2019).

Among the abiotic factors, the drought is considered the most devastating, affecting all plants growth and development stages causing huge losses in soybean yield (Engels *et al.*, 2017). Drought stress is a multidimensional stress and causes changes in the physiological, morphological, biochemical, and molecular traits in plants which ultimately affects negatively on yield of plants (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). Since drought tolerance is a quantitative trait and many genes are involved, it is difficult to create a molecular marker for a plant's response to a water deficit (Sinclair, 2011). Furthermore, the timing, intensity, and duration of a drought varies (Mir *et al.*, 2012), creating a hurdle for properly testing varieties. Therefore, plant breeders typically combine data from multi-location trials to test new varieties, determine how genotypes respond across environments, and minimize effects of genotype by environment interactions (Crossa, 1990).

The use of nano fertilizers has led to the increased productivity, stability due to reduction of biotic and abiotic stresses as well as reduced production costs in the last decade (Kashyap *et al.*, 2016, 2017). According to Davar *et al.* (2014), different trace elements and their oxides of nano particles were used for enhancing drought stress resistance in different plants. Additionally, Sharifi and Khoramdel (2016) was found that foliar application of Zn nano particles combined with the inoculum of *Bradyrhizobium japonicum* improved yield of soybean. Sheykhbaglou *et al.* (2010) observed that foliar application of nano-iron oxide at the concentration of 750 ppm increased leaf and pod dry weight over the control.

Nano particles are characterized by its particle shape, tunable pore size, potential reactivity and high surface area (Seleiman *et al.*, 2021). In plants, the cellular organelles are targeted, and certain contents are released through the nano particle target (Cunningham *et al.*, 2018). In agriculture, a wide scope of nano technology is still unexplored. Above circumstances, the present research was conducted to study the effect of the foliar application of nano ZnO fertilizer on growth, dry matter accumulation and yield of soybean plant under drought condition.

Materials and Methods

Location

The pot experiment was conducted under semi-controlled environment in poly house condition in the department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh during 2020-2021. The experimental site was situated in a sub- tropical climatic zone of Madhupur tract (AEZ 28) (24.09° N latitude and 90.26° E longitude) above 8.4 m above the mean sea level and about 40 km north of Dhaka. Plastic pots (30 cm length and 24 cm diameter) were used in the experiment which was filled with soil, holds about 28% moisture at field capacity (FC). Soil used in the plastic pots was clay loam with 40.51% sand, 28.78% silt and 30.71% clay having pH of 6.93. The soil organic carbon, available P, Exchangeable K were 0.61%, 0.8 mg $100g^{-1}$ soil and 0.79 $cmol_c$ kg^{-1} dry soil, respectively. The soil was mixed with cowdung at 1:0.25 ratio and dried well. Then each pot was filled with 11 kg of that soil mixture.

Plant materials and preparation of nano solution

Soybean variety BU soybean-1 was used in this experiment. This high yielding variety was released by the Bangabandhu Sheikh Mujibur Rahman Agricultural University. For the preparation of nano zinc solution, zinc nano powder of having 50-100 nm particle size and 15-25 m^2/g have been used and zinc was 79.1-81.5% (complexometric titration) (Sigma-Aldrich, 2016).

To prepare 100 ppm nano ZnO solution, 100 mg of this powder was added to 1 L of distilled water and similarly, 200 ppm nano ZnO solution was prepared by adding 200 mg of that ZnO powder into 1 L of distilled water. Both of the solutions were heated at 60°C temperature for 16 hours on magnetic stirrer with hot plate. After that, sonication bath was given to both solutions with vibration to mix all the particles into the water homogenously, so that the solution can penetrate through the plant leaves effortlessly during the application (Sandhya *et al.*, 2021). Then those solutions were stored in a plastic bottle at room temperature. The required amount of solution was inserted into the hand sprayer during the application of the solution into the plant.

Treatments and intercultural operation

Ten healthy seeds were sown maintaining uniform spacing in each pot. After sowing, minimal irrigation was applied by using a beaker to maintain uniform germination. Thinning was done during the appearance of first two leaf stage and kept three uniform and apparently healthy plants in each pot. Uniform application of 0.32 g urea, 0.933 g TSP and 0.64 g MoP was given into each pot corresponding to 80-205-128 kg urea, triple super phosphate and muriate of potash ha^{-1}, respectively (FRG, 2018). After trifoliate stage the pots were investigated regularly to identify the moisture level of soil by using portable digital moisture meter (POGO Soil Sensor II, Stevens, USA) and 1 L of water was added on a regular basis to maintain the field capacity to 80% in nine pots of well-watered (Control) plants and 500 mL was added to the rest of the each pot which were kept in water stressed condition by having 40% field capacity (Drought) throughout the growing season. The experiment consisted of two factors. Factor A: i. well water (Control), ii. Drought, Factor B: 3 doses of nano ZnO. The control and drought treated plants leaves were sprayed with water (0 ppm nano ZnO), 100 ppm nano ZnO solution and 200 ppm nano ZnO solutions through a hand sprayer after 15 days beginning of drought stress. The experiment was laid out in Completely Randomized Design (CRD) with three replications.

Growth and yield parameters

Morphological growth related parameters, such as height of plants, leaf, stem fresh weight and total dry weight were observed after 15 days of spraying (at flowering stage). At the physiological maturity stage, seed yield plant^{-1}, number of pods plant^{-1}, number of seed pod^{-1}, and 100-seed weight were also recorded.

Statistical analysis

The Crop Stat statistical software version 7.2 has been used to analyze the recorded data. Mean values of all treatments were compared using Least Significance Difference (LSD) test at 5% level of significance

Results and discussion

Plant height

Plant height is one of the most important morphological traits to determine the growth and development of the plant. The scarcity of water affected the increment of plant height remarkably (Figure 1). The height of soybean plants had been decreased by 15.85% under drought relative to the control condition.

In control condition, plant height had reached maximum to 32.07 cm after the application of 200 ppm of nano ZnO fertilizer and the lowest was 26.95 cm in untreated plants. In drought condition the highest height was found into the plants which were treated with 200 ppm nano fertilizer. Among all of the treatments, the untreated plants remained to the rock-bottom level with only 22.68 cm height under drought. More specifically, at 15 days after the application of 100 and 200 ppm of nano ZnO fertilizers, the height of the BU soybean-1 had been increased by 17.17 and 18.1%, respectively in control condition compared to the untreated plants. Simultaneously, in drought condition, plant height had been increased significantly by 12.87 and

21.7%, due to the application of 100 and 200 ppm of nano ZnO fertilizers, respectively. Similar result was reported by Gholinezhad (2017), that application of nano fertilizer increased plant height by 20% in comparing to the control (no fertilizer) condition. According to Dola *et al.* (2022), application of 200 ppm nano iron fertilizer enhanced the plant height by 21.24% in drought condition compared to the untreated plants. Due to having small and high solubility compound, nano fertilizer absorbed quickly by plants and solved food shortages plants and increased plant growth (Rasht, 2013). According to Linh *et al.* (2020), height of nano particle treated plants reached approximately 50 cm, while 40 cm in the untreated control plants under drought conditions.

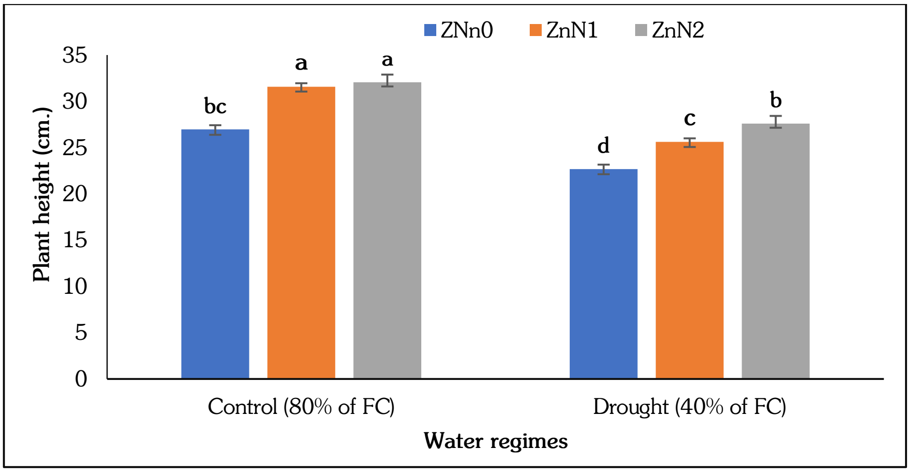

Fig. 1. Effect of zinc fertilizer on plant height of soybean after 15 days of spraying. Bars indicate (±SE). ZnN0, ZnN1, ZnN2 = 0, 100 and 200 ppm nano fertilizer, respectively.

Fresh weight

Drought is one of the most alarming abiotic stress for soybean plants which affects almost all of the morphological traits of the plants severely. The water stressed condition affects fresh weight of leaf, stem and finally the total fresh weight of soybean plants adversely. Due to drought condition, fresh weight of soybean leaves had been attenuated drastically by 28.36% compared to the control condition (Table 1). But after the application of nano fertilizers, the amount of loss had been minimized. In control condition, the maximum leaf fresh weight was recorded 14.28 g after 200 ppm nano zinc solution spray, whereas, 11.32 g was the lowest. Considering the water deficit condition, maximum fresh weight of leaves was 9.92 g found into 200 ppm nano zinc solution treated plants. Among all of the experimental plants, untreated drought stressed plants were bearing the lowest sized leaves which were only 8.11 g. In drought stressed condition, fresh weight of soybean leaves increased by 13.07 and 22.32% at 15 days later of the foliar application of 100 and 200 ppm nano ZnO solution, respectively relative to untreated plants. In control condition, 100 and 200 ppm nano zinc fertilizers incremented the leaf fresh weight by 19.43 and 26.15%, respectively compared to the untreated plants. Stem weight is also an impactful parameter which influences the total weight of a plant. The drought condition in vegetative stage of soybean plants deteriorated the fresh weight of stem by 37.24% compared to the control condition (Table 1). The highest stem fresh weight of soybean was encountered as 6.08 g into the plants treated with 200 ppm nano zinc fertilizer and the minimum of 4.35 g into the untreated plants in control condition. In drought condition, the maximum fresh weight of soybean stem was 4.63 g achieved by the application of 200 ppm nano fertilizer and the lowest stem fresh weight only 2.73 g into the untreated plants. In control condition, stem fresh weight improved by 17.47 and 39.77% at 15 days after 100 and 200 ppm nano ZnO fertilizers treatments application, respectively related to untreated plants. Moreover, in drought condition, 100 and 200 ppm nano zinc solution accelerate the fresh weight of soybean plants exponentially by 28.94 and 69.6%, respectively compared to the plants remained untreated.

Evaluating all of the data mentioned above, it could be manifested that the solution of nano zinc particles also had a great impact on the total fresh weight of soybean plants. Certainly, the water deficit condition into the soybean plants curtailed the total fresh weight by 30.82% compared to the plants grown in control condition (Table 1). In control condition, the highest total fresh weight of soybean was found 20.36 g after the exogenous use of 200 ppm nano ZnO fertilizer and the minimum value was 15.67 g observed in totally untreated plants. In drought condition, the untreated plants had the lowest total fresh weight of 10.84 g only. But 15 days after the application of 200 ppm nano zinc particles in drought condition increased the total fresh weight of soybean to 14.55 g. In control condition, 100 and 200 ppm solution of nano ZnO improved the total fresh weight of soybean plants by 18.89 and 29.93%, respectively compared to the untreated plants. Furthermore, the acceleration of the total fresh weight of soybean plants was 17.01 and 34.23% with the foliar application of 100 ppm and 200 ppm nano zinc fertilizer, respectively in drought condition relative to the untreated plants. This phenomenon should be considered as a noticeable increment in morphological growth of soybean plants. Similar result was reported in green gram (*Vigna radiata* L.) by Raju *et al.* (2016). According to Gülser *et al.* (2019), the highest stem fresh weight was found after the application of 30 mg kg^{-1} iron. Linh *et al.* (2020) also found that treatment of nano particles improved the shoot development in both well-watered and drought-stressed plants at the vegetative stage. Liu and Lal (2014) have also claimed the similar result that using nano solutions increased the growth rate of soybeans by 33%. According to Askary *et al.* (2017), the application of Fe_2O_3 nanoparticles had significantly increased plant growth than the untreated plants.

Table 1. Effect of zinc nano fertilizer on leaf fresh weight, stem fresh weight and total fresh weight of soybean after 15 days of spraying

Nano zinc (ppm)	Leaf fresh weight (g $plant^{-1}$)		Stem fresh weight (g $plant^{-1}$)		Total fresh weight (g $plant^{-1}$)	
	Control	Drought	Control	Drought	Control	Drought
0	11.32b	8.11c	4.35ab	2.73b	15.67b	10.84c
100	13.52ab	9.17bc	5.11ab	3.52b	18.63ab	12.69bc
200	14.28a	9.92bc	6.08a	4.63ab	20.36a	14.55b
$LSD_{(0.05)}$	1.72		2.28		1.81	
CV (%)	11.6		23.2		11.5	

Means followed by diverse letters differ significantly by LSD at $p < 0.05$

Dry weight

The harmful effect of water stressed condition in soybean plants declined the dry weight of different plant parts in vegetative stage remarkably. Due to the drought condition, dry weight of soybean leaves had been decreased by 55.37% in vegetative stage of plants compared to the control condition (Table 2). At 15 days after the foliar application of 200 ppm nano zinc fertilizer in control condition, the highest dry weight of soybean leaves was estimated as 2.48 g, whereas in this similar condition and stage, the untreated plants provided only 1.77 g dry weight of leaves in soybean plants. In drought condition, maximum 1.47 g of leaf dry weight was observed due to the foliar application of 200 ppm nano zinc solution. Evidently, among all of the treatments and conditions, the lowest leaf dry weight (0.79 g) was found into the untreated drought affected plants. After the application of 100 and 200 ppm nano ZnO solution in control condition, dry weight of soybean leaves increased by 18.64 and 40.11%, respectively compared to the untreated plants. In drought condition, a significant increment of leaf dry weight by 37.97 and 86.08%, had been observed after the application of 100 and 200 ppm solution of nano zinc particles, respectively relative to the untreated plants.

Stem dry weight of soybean plants at vegetative stage had been deteriorated by 70.83% due to the hazardous effect of drought condition compared to the control condition (Table 2). The highest amount of stem dry weight of soybean was 2.12 g, achieved by the foliar application of 200 ppm nano zinc solution in control condition. But due to avoiding all of the doses of nano zinc solution, stem dry weight of soybean plants was measured as only 1.44 g in control condition. Conversely, the lowest stem dry weight of soybean plants was only 0.42 g found into the untreated plants grown in drought condition. In vegetative stage of soybean plants, 100 and 200 ppm nano zinc solution enhanced stem dry weight by 29.86 and 45.83%, respectively in control condition relative to the untreated plants. In drought condition, application of 100 and 200 ppm of nano ZnO fertilizer incremented stem dry weight by 35.71 and 59.52%, respectively compared to the untreated plants.

Table 2. Effect of zinc nano fertilizer on leaf dry weight, stem dry weight and total dry weight of soybean after 15 days of spraying

Nano zinc (ppm)	**Leaf dry weight ($g\ plant^{-1}$)**		**Stem dry weight ($g\ plant^{-1}$)**		**Total dry weight ($g\ plant^{-1}$)**	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**
0	1.77b	0.79c	1.44b	0.42c	3.22c	1.21f
100	2.1ab	1.1c	1.87a	0.57c	3.97b	1.66e
200	2.48a	1.47bc	2.12a	0.67c	4.60a	2.13d
$LSD_{(0.05)}$	0.41		0.27		0.40	
CV (%)	14.1		13.0		8.1	

Means followed by diverse letters differ significantly by LSD at $p < 0.05$.

After the analysis of the above mentioned data, it was evident that the nano zinc particles could outperform to improve the dry weight of soybean plants in vegetative stage. Due to the harmful effect of drought, the total dry weight of soybean plants had dropped down by 62.42% relative to the control condition (Table 2). The maximum total dry weight of soybean had been observed as 4.60 g into the plants treated with 200 ppm nano ZnO fertilizer in control condition. In control condition, only 3.22 g of total dry weight had been also noticed into the untreated plants. In drought condition, the highest amount of total dry weight of soybean plants was 2.13 g, attained after the application of 200 ppm nano zinc whereas, the lowest amount was only 1.21 g, obtained from the untreated plants suffering from drought condition. After the foliar application of 100 and 200 ppm of nano ZnO fertilizer in control condition, the total dry weight of soybean plants increased by 23.29 and 42.86%, respectively compared to untreated plants. In drought condition, the total dry weight had been accelerated sharply by 37.19 and 76.03% with the application of 100 and 200 ppm of nano ZnO fertilizer, respectively. Chakralhoseini *et al.* (2002) found that iron at low concentration of 2.5 mg kg^{-1} in soil increased dry matter weight of soybean. Sheykhbaglou *et al.* (2010) reported that application of nano-iron oxide at the concentration of 750 ppm caused an increase in dry pod weight and dry weight of leaf plus pod in soybean. According to Vaghar *et al.* (2020), in early grain filling stage, the combined Fe and Zn foliar application increased the total dry matter content by 20.9%.

Yield and yield contributing characters

The number of pods is considered as one of most important yield components of soybean plants. Due to the drought condition, the number of pods of soybean plants decreased by 25.36% compared to the control condition (Table 3). The highest number of pods plant^{-1} was 61.86 found into 200 ppm nano ZnO fertilizer treated plants in control condition. In control condition, 54.11 pods plant^{-1} on average also found due to the lack of nano iron solution. The lowest number of pods were counted as 40.39 pods plant^{-1} into drought condition when none of the treatments were applied. In drought condition, about 52.94 pods plant^{-1} was also found after the application of 200 ppm of nano ZnO fertilizer. This foliar application of 100 and 200 ppm nano ZnO fertilizer in control condition increased the number of pods plant^{-1} by 8.43 and 14.32%, respectively in relation to the untreated plants. In drought condition, the application of 100 and 200 ppm nano ZnO fertilizer raised the average number of pods plant^{-1} by 47.78 and 52.94%, respectively compared to the untreated plants.

The number of seeds pod^{-1} is directly related with the final yield content of soybean plants. About 27.62% less number of seeds pod^{-1} was found into a drought affected soybean plants compared to the control condition (Table 3). The highest average number of seeds pod^{-1} (2.66) was recorded into 200 ppm nano ZnO fertilizer treated plants in control condition. In control condition, an average number of seeds pod^{-1} (2.10) when the plants remained untreated. The lowest number of seeds pod^{-1} was only 1.82 encountered into the untreated plants in drought condition. The foliar application of 200 ppm of nano iron solution increased seeds pod^{-1} to 1.86 seeds in drought condition. The application of 100 and 200 nano ZnO fertilizer increased the number of seeds pod^{-1} by 17.62 and 26.66%, respectively compared to the untreated plant. Also in drought condition, the application of 100 and 200 ppm nano ZnO fertilizer improved the average number of seeds pod^{-1} in soybean plants by 13.16 and 19.74%, respectively relative to the untreated plants.

Table 3. Effect of zinc nano fertilizer on number of pods plant^{-1}, number of seeds pod^{-1}, 100-seeds weight and seed yield of soybean

Nano zinc (ppm)	**Number of pods plant^{-1}**		**Number of seeds pod^{-1}**		**100- seeds weight (g)**		**Seed yield (g plant^{-1})**	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**
0	54.11b	40.39d	2.1b	1.52c	9.94ab	6.41c	11.63c	4.74f
100	58.67ab	47.78c	2.47ab	1.72bc	10.1a	7.52bc	13.15b	6.09e
200	61.86a	52.94bc	2.66a	1.82bc	10.49a	8.76b	14.37a	7.75d
$LSD_{(0.05)}$	5.89		0.50		1.26		1.18	
CV (%)	6.3		13.8		8.0		6.9	

Means followed by diverse letters differ significantly by LSD at $p < 0.05$.

The obvious negative effect of drought condition also affects the 100-seeds weight noticeably. Due to the drought condition in soybean plants, the 100-seeds weight of soybean had been declined by 35.51% compared to control condition (Table 3). The maximum 100-seeds weight of soybean plants (10.49 g) was estimated into the 200 ppm of nano ZnO fertilizer treated plants in control condition. Moreover, into this control level, 9.94 g of 100-seeds weight was also measured when the plants remained totally untreated. The lowest value of 100-seeds weight of soybean plant was only 6.41 g found into the untreated plants suffering from drought. Whereas, the 100-seeds weight of 8.76 g was also observed in drought condition when the plants treated with 200 ppm nano ZnO fertilizer. The application of 100 and 200 ppm nano ZnO fertilizer incremented the 100-seeds weight of soybean plants in control condition by 1.51 and 5.53%, respectively compared to the untreated plants. The soybean under drought condition also manifested an increase of 100-seeds weight by 17.32 and 36.66%, due to the application of 100 and 200 ppm nano ZnO fertilizer, respectively compared to the untreated plants.

The yield is one of the most vital components of any crop which defines the productivity of that crop. The average yield of soybean had been declined due to the adverse effect of drought condition by 59.24% compared to the control condition (Table 3). The highest seed yield was 14.37 g plant^{-1} in 200 ppm of nano ZnO fertilizer treated plants in control condition. In control condition, only 11.63 g plant^{-1} yield was also calculated into the untreated plants. The lowest amount of yield was only 4.74 g plant^{-1} estimated into the drought affected plants which remained untreated. An increased yield of 7.75 g plant^{-1} was also found in drought condition, after the application of 200 ppm nano ZnO fertilizer. The application of 100 and 200 ppm nano ZnO fertilizer in control condition increased the yield by 13.07 and 23.56%, respectively compared to the untreated plants. In drought condition, the soybean plants treated with 100 and 200 ppm nano ZnO fertilizer depicted a raise by 26.79 and 63.50% of yield, respectively compared to the untreated plants. Similar result was also found by Vaghar *et al.* (2020) that under stressed conditions in the flowering, pod formation and seed filling stage, Fe and Zn nano-chelates increased the number of grains plant^{-1}, average seed yield of soybean by 43.8% and, reached the production rate of 2901.93 kg ha^{-1} more than other treatments.

Zn and Mn foliar application increased the number of pods plant^{-1} by 23.2% compared with control (Vaghar *et al.*, 2020). In irrigation withheld conditions in pod formation, and seed filling stages, the treatment of Zn and Mn increased the number of pods plant^{-1} by 25.2%, and 20.6%, respectively compared to the control treatment. Zn and Mn treatment raised the 100-seed weight, where this increase was 16.5% higher than in the control. Under stress conditions, it was 22% (Vaghar *et al.*, 2020).

Conclusion

The growth, dry matter accumulation, and yield of soybean have been negatively impacted by the water deficiency condition. However, the negative effects of drought stress reduced when ZnO nanoparticles were applied as foliar to plants. The foliar application of 200 ppm ZnO nano particles performed the best to rectify the negative effect of drought and increases the plant characters (plant height, fresh and dry weight of leaf, stem and whole plant) and yield contributing traits (seed yield, 100-seed weight, number of seeds pod^{-1}, number of pods plant^{-1}) of soybean. According to these findings, it can be suggested that the foliar application of ZnO can amend the adverse effect of drought condition and uphold the yield of soybean.

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