Educational Administration: Theory and Practice
2024, 30(6), 1566-1573
ISSN: 2148-2403
https://kuey.net/

Research Article

Response Of Wheat To Various Treatments Of Nanoparticles Under Moisture Stress

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Citation: Anand Kumar Pandey, (2024) Response Of Wheat To Various Treatments Of Nanoparticles Under Moisture Stress *Educational Administration: Theory And Practice*, *30*(6) 1566-1573
Doi: 10.53555/kuey.v30i6.5539

ARTICLEINO	ABSTRACT

The study titled "Physio-biochemical response of wheat to different nanoparticle treatments under moisture stress (drought) conditions" was carried out during the Rabi season of 2022-23 and 2023-24 at the student instructional farm (SIF) of Acharya Narendra Deva University of Agriculture and Technology, located in Kumarganj, Ayodhya- 224229 (U.P.). The experiment was carried out using a randomized block design, with three replications and ten treatments, using the wheat variety HD 2967. The treatments consisted of the following: T1: control (foliar spray of distilled water), T2: Ca_3PO_4 (40ppm), T3: TiO_2 (40ppm), T4: TiO_2 (40ppm) + Ca_3PO_4 (40ppm), T5: TiO_2 (50ppm). T6: TiO_2 (50ppm) + Ca_3PO_4 (40ppm), T7: ZnO (40ppm), T8: ZnO (40ppm) + Ca_3PO_4 (40ppm), T9: ZnO (50ppm), T10: ZnO (50ppm) + Ca_3PO_4 (40ppm). The application of foliar spray occurred 45 days after sowing (DAS). The physio-biochemical parameters were measured at 60, 75, and 90 days after sowing (DAS), whereas the yield data was recorded at harvest time. The application of foliar spray containing both ZnO and TiO_2 nanoparticles, as well as Ca_3PO_4, was found to be highly successful in reducing moisture stress compared to the control group. The addition of Nano ZnO at a concentration of 50ppm, combined with Ca_3PO_4 at a concentration of 40ppm (T10), was shown to be the most efficient in increasing both the total chlorophyll content and total soluble sugar content under conditions of moisture stress. This treatment was followed by treatment T9. However, when considering additional characteristics such as membrane stability index (MSI), relative water content (RWC), grain yield, and biological yield, treatment T9 showed significantly greater values than the control treatment for the crop. Treatment T4 also showed higher values, although to a lesser extent. The current study concludes that nanoparticles effectively reduce moisture stress in wheat. Specifically, ZnO (50ppm) followed by TiO_2 (40ppm) in combination with Ca3PO4 (40ppm) are recommended treatments to alleviate the negative impacts of moisture stress on wheat crops.

Keywords: wheat, nanoparticles, moisture stress, zinc oxide, titanium dioxide.

Introduction

Agriculture is key to economic, commercial, and social progress, particularly in developing nations. The sector makes for roughly 25% of the Gross Domestic Product (GDP) and employs around 42% of the labour force (Mumtaz *et al*., 2019). Wheat is the most economically important crop, contributing to almost 30% of global cereal production. It is grown on around 218 million hectares and yields an average of 771 million tons. Wheat satisfies the dietary needs of about 21% of the world's population (Ahmadian *et al*., 2021). Wheat (Triticum aestivum L.) is a vital staple worldwide, cultivated on all habitable continents (Ahmad *et al*., 2018; Aurangzaib *et al*., 2022). Wheat is third among the major world crops, followed by rice and corn. It is a significant source of carbs and proteins, providing sustenance to many individuals (Daryanto *et al*., 2016; Ahmadian *et al*., 2021). Immobile organisms like plants are consistently subjected to a variety of non-living factors. Environmental fluctuations, such as drought, salinity, alkalinity, flooding, and mineral toxicity/deficiencies, can induce stress in crops, leading to significant decreases in output. While certain plants possess inherent resilience to endure stress, this characteristic is not widespread among most plants (Hayat *et al*., 2023; Luz *et al*., 2023). Water is essential for the plant life cycle as it plays a crucial role in the transportation of nutrients. Water deficit stress, resulting from the physical absence of water (drought) and the physiological inability to acquire water, is prevalent in arid and semiarid areas (Luz *et al*., 2023). Water scarcity is a major concern for sustainable agriculture globally, especially in Asian countries where irrigation-based farming consumes 90% of the available water (Hamdy *et al*., 2003). Studies have shown that wheat yields in areas with limited rainfall and no irrigation can be reduced by 50-90% compared to their potential with irrigation. This water deficit has significant implications for the future world food supply (Reynolds *et al*., 2000). Around 37% of the total geographical area within developing nations is classified as semiarid, meaning it has limited access to water resources. This scarcity of water is particularly challenging for the cultivation of wheat crops. The absence of water inhibits the growth of plants by decelerating cellular processes and metabolic activities, decreasing turgidity and ultimately leading to cell death (Dar *et al*., 2020). Due to drought stress, plants experience impaired photosynthesis, nitrogen uptake, osmotic function, and antioxidant activities. During drought, photorespiration in plants can cause excessive production of reactive oxygen species (ROS). This can lead to the denaturation of proteins, DNA damage, and lipid peroxidation. These processes hinder cell growth and elongation, resulting in poor plant growth and productivity (Waqas Mazhar *et al*., 2022; Hayat *et al*., 2023). In their study, Shahzad *et al.* (2021) identified moisture stress as the primary environmental stressor that impacts all aspects of ensuring the security of cereal food, from production to post-harvest consumption. The primary reason for low wheat production is moisture stress, which has detrimental impacts on crucial growth phases (Rashid *et al*., 2022; Sallam *et al*., 2019). Gull *et al*., 2019 described drought's aggressive effects on several growth stages in wheat. The presence of this phenomenon during the tillering stage might lead to reduced plant height and total tillers per unit area, resulting in decreased biomass, more spiked tillers, reduced grains per spike, and, ultimately, lower grain weight during the grain-filling stage. These effects can potentially cause a 50% decrease in yield (Kulkarni *et al*., 2017). In order to tackle these difficulties, the employment of nanotechnology in the creation of nanoparticles (NPs) shows promise and optimistic prospects for improving plant development and yield in challenging environments (Briefs, 2018). Furthermore, using nanoparticles (NPs) to enhance wheat output by mitigating the harmful effects of drought could substantially impact the address of food security issues (Zhao *et al*., 2016). Recent research has emphasized the contribution of metal-based nanoparticles (NPs) in reducing the negative effects of drought stress by promoting tolerance (Linh *et al*., 2020; Shekhawat *et al*., 2021). Metallic nanoparticles (NPs), including ZnO and TiO_2 NPs, have been extensively employed to alleviate drought stress by enhancing water and nutrient absorption through stress tolerance mechanisms and the activation of genes associated with cellular growth (Linh *et al*., 2020; Shekhawat *et al*., 2021). These nanoparticles have been documented to increase plant growth, including germination parameters, growth rate, biomass, and yield. They also can regulate stomatal conductance and transpiration rate and enhance photosynthetic parameters. In addition, they decrease the leakage of ions across the membrane and improve the absorption of carbon dioxide in leaves (Borišev *et al*., 2016; Semida *et al*., 2021). Zinc, as one of the eight necessary micronutrients, plays a crucial role as a fundamental component of numerous enzymes and proteins. Similarly, titanium has been recorded to enhance crop performance by increasing chlorophyll content and photosynthesis and stimulating specific enzymes. While the application of nanomaterials in agriculture is relatively new compared to other industries, they are a crucial tool for improving stress tolerance and increasing crop output (Dogaroglu & Koleli, 2017). This study aims to investigate the impact of ZnO and TiO_2 nanoparticles, both individually and in combination with Ca_3PO_4, on alleviating moisture stress in wheat crops. The study aims to enhance physiological and biochemical properties and increase crop yield.

Material and methods:

The field study was conducted at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh during the Rabi season of 2022-23 and 2023-24. Geographically the experimental site is situated 42km away from Ayodhya on Ayodhya-Raibarielly Road between latitude of 26.47° North and longitude of 81.12° East on an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh. The weather conditions in terms of minimum and maximum temperature, relative humidity (RH), rainfall (mm) and sunshine hours were recorded during the crop season *i.e*., from November to April during 2022-23 and 2023-24. The data were collected from metrological observatory situated in the main campus of university, Kumarganj, Ayodhya. The experiment was conducted in randomized block design (RBD) with three replications. Popular wheat variety HD 2967 was used for the research experiments. Sowing was done in November and moisture stress conditions were maintained in the field by abstaining irrigation after the CRI (Crown Root Initiation) stage. Full dose of fertilizer was applied as basal @ 120:60:40 $N:P_2O_5:K_2O$ respectively. Zinc oxide nanoparticles (ZnO), titanium dioxide nanoparticles (TiO_2) and calcium phosphate (Ca_3PO_4) were used in different combinations to alleviate moisture stress experienced by the crop (Table.1). Zinc oxide nanoparticles and titanium dioxide nanoparticles were purchased from SRL (Sisco Research Laboratory), Maharashtra, India. Zinc and Titanium nanoparticle solutions were prepared by sonicating the solution at 40 KHz for 20 min and 20 KHz for 15 min respectively. The spray of nanoparticles was done 45 days after sowing (DAS). The various biochemical and physiological parameters were recorded at four different stages i.e. 45, 60, 75 and 90 DAS. Yield related data were taken at the time of harvest. The statistical analysis was carried out for each parameter studied based on the randomized block design following the method of Gomez & Gomez (1984). Associations between parameters were studied using correlation analysis. Means were compared by least significant difference (LSD) at 5% level of significance.

Table1: Different treatment combinations used to mitigate moisture stress in wheat

S. No.	Treatment	Description
1	T1	Control (foliar spray of distilled water 45 DAS)
2	T2	Ca_3PO_4 (40ppm)
3	T3	TiO_2 (40ppm)
4	T4	TiO_2 (40ppm) + Ca_3PO_4 (40ppm)
5	T5	TiO_2 (50ppm)
6	T6	TiO_2 (50ppm) + Ca_3PO_4 (40ppm)
7	T7	ZnO (40ppm)
8	T8	ZnO (40ppm) + Ca_3PO_4 (40ppm)
9	T9	ZnO (50ppm)
10	T10	ZnO (50ppm) + Ca_3PO_4 (40ppm)

Result & Discussion:

Effect of nanoparticles on total chlorophyll content (mg g^{-1} fresh weight) of wheat under moisture stress condition:

The results show that moisture stress reduces the total chlorophyll content of wheat; however, foliar spray with ZnO and TiO_2 nanoparticles (NPs) along with Ca_3PO_4 significantly enhances the total chlorophyll content compared with untreated plot (Table 2). Among the various combinations of TiO_2 applies it was observed that treatment T6 i.e. TiO_2 (50ppm) along with Ca_3PO_4 (40ppm) was most effective also for ZnO among the various combinations applied treatment T10 i.e. ZnO (50ppm) along with Ca_3PO_4 (40ppm) was most effective against moisture stress. Overall it was observed that among all the treatments maximum total chlorophyll content are measured for T_{10} when the ZnO NPs (50 ppm) with 40 ppm Ca_3PO_4 is applied under moisture stress conditions. Similarly, at 75 DAS and 90 DAS maximum chlorophyll content was recorded in treatment T_{10} (7.75) and (3.84) followed by T_9 (50 ppm of ZnO NPs) (7.48) and (3.67) respectively. ZnO NPs with Ca_3PO_4 is more responsive as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum total chlorophyll content at all the crop stages (Table 2). These results are in accordance with El-Bassiouny *et al*, 2022. They observed that the impact of TiO_2 or ZnO NPs (5 and 10mg/L) increased significantly the photosynthetic pigments on wheat plants under various water levels when compared to the corresponding untreated plants. Among the two varieties used the highest values in total photosynthetic pigments were recorded through the application with 10 mgL^{-1} nano-TiO_2 (55.8% and 55.3%) in response to the Gimeza 12 cultivar and using 10 mg/L nano ZnO (52.43 and 49.41) in the Sids 13 at well watered and water stressed conditions, respectively.

Table 2: Effect of nanoparticles on total chlorophyll content (mg g^{-1} fresh weight) of wheat under moisture stress condition

Treatments	Total Chlorophyll Content (mg g^{-1} fresh weight)		
	60DAS	**75DAS**	**90DAS**
T_1	4.21	5.79	2.04
T_2	5.10	7.15	3.21
T_3	5.26	6.81	2.81
T_4	5.52	7.34	3.40
T_5	5.43	7.19	3.41
T_6	5.60	7.41	3.55
T_7	5.94	6.78	3.42
T_8	5.69	7.25	3.07
T_9	5.44	7.48	3.67
T_{10}	6.20	7.75	3.84
S.Em±	0.18	0.18	0.15
C.D. 5%	0.52	0.53	0.44

Effect of nanoparticles on total soluble sugar content (mg g^{-1} dry weight) of wheat under moisture stress condition:

Data pertaining to total soluble sugar content of wheat revealed that under moisture stress condition foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly enhances the total soluble sugar content compared with untreated plot (Table 3). Among the various combinations of TiO_2 applies it was observed that treatment T4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) was most effective. Similarly in case of ZnO among the various combinations applied treatment T10 i.e. ZnO (50ppm) along with Ca_3PO_4 (40ppm) was most effective against moisture stress. Overall it was observed that among all the treatments maximum total soluble sugar content are measured for T_{10} when the ZnO NPs (50 ppm) with 40 ppm Ca_3PO_4 is applied under moisture stress conditions. Similarly, at 75 DAS and 90 DAS total soluble sugar content was recorded in treatment T_{10} (63.50) and (91.02) followed by T_9 (50 ppm of ZnO NPs) (62.57) and (89.56) respectively. ZnO NPs with Ca_3PO_4 is more responsive as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum total soluble sugar content at all the crop stages (Table 3). Amira *et al.* 2015 established that ZnO NPs treatment raised the Total Soluble Sugar (TSS) concentrations which are associated with the salt tolerance in maize. Similarly Abdel Latef *et al.* 2018 and El-Bassiouny *et al.* 2020 stated that zinc oxide nanoparticle treatment augmented the contents of compatible solutes i.e. TSS in lupine and wheat plants under salinity stress.

Table 3: Effect of nanoparticles on total soluble sugar content (mg g^{-1} dry weight) of wheat under moisture stress condition

Treatments	Total Soluble Sugar Content (mg g^{-1} dry weight)		
	60DAS	75DAS	90DAS
T1	44.18	56.36	82.04
T2	46.59	56.85	82.64
T3	46.14	60.13	86.37
T4	47.23	61.32	88.02
T5	44.84	57.67	84.62
T6	46.92	59.55	86.66
T7	47.39	61.40	87.90
T8	47.36	59.99	85.99
T9	47.57	62.57	89.56
T10	48.67	63.50	91.02
S.Em±	0.85	0.98	0.98
C.D. 5%	2.51	2.90	3.86

Effect of nanoparticles on membrane stability index (MSI) (%) of wheat under moisture stress condition:

Data pertaining to the results revealed that moisture stress causes a significant damage to the membranes of the various plant organelles leading to a lower membrane stability index in wheat. However, foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly increases the membrane stability index compared with untreated plot (Table 4). Among the various combinations of TiO_2 applied it was observed that treatment T4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) was most effective also for ZnO among the various combinations applied treatment T9 ZnO (50ppm) was most effective against moisture stress. Overall it was observed that among the various treatment combinations applied maximum membrane stability index was measured for T_9 when the ZnO NPs (50 ppm) is applied under moisture stress conditions. Similarly at 75 DAS and 90 DAS maximum membrane stability index was recorded in treatment T_9 (60.63) and (47.15) followed by T_4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) (60.06) and (46.93) respectively. ZnO NPs were found to be more effective as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum membrane stability index at all the crop stages (Table 4). Similar results were obtained by Pandya *et al.* 2023, who observed that under drought stress, the MSI decreased as a result of increased ion leakage and a reduction in cell turgor. ZnO nanopriming application helped to improve membrane stability and the water content of the cell in a water-stressed condition. The ZnO-treated plants increased the MSI of shoot and root by 41%, and 76% in drought index conditions, respectively, over their control plants.

Table 4: Effect of nanoparticles on membrane stability index (%) of wheat under moisture stress condition

Treatments	Membrane Stability Index (%)		
	60DAS	75DAS	90DAS
T1	64.81	52.34	37.02
T2	69.07	54.73	41.21
T3	68.16	53.51	42.50
T4	70.53	60.06	46.93
T5	67.07	56.72	43.68
T6	68.07	55.61	44.93

T7	70.23	59.94	44.69
T8	69.31	58.38	44.71
T9	70.80	60.63	47.15
T10	69.72	58.12	45.57
S.Em±	0.74	1.59	0.11
C.D. 5%	2.1	4.73	0.31

Effect of nanoparticles on relative water content (%) of wheat under moisture stress condition

The results related to relative water content revealed that moisture stress reduces the relative water content of wheat; however, foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly enhances the relative water content compared with untreated plot (Table 5). Among the various combinations of TiO_2 applied it was observed that TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) was most effective also for ZnO among the various combinations applied ZnO (50ppm) was most effective against moisture stress. Overall it was observed that at 60 DAS among all the treatments maximum relative water content was measured for T_9 when the ZnO NPs (50 ppm) is applied under moisture stress conditions. Similarly, at 75 DAS and 90 DAS maximum relative water content was recorded in treatment T_9 (82.08) and (74.21) followed by T_4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) (74.75) and (71.87) respectively. ZnO NPs is more responsive as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum relative water content at all the crop stages (Table 5). Similar to our findings, Zn NPs improved RWC by 8–10% in two different wheat varieties when exposed to drought stress (Taran *et al.* 2017). Under drought stress, plants treated with ZnO NPs have a higher water retention capacity than non- ZnO-treated plants, which is the most appropriate measurement for plant water status in the case of a cellular water deficit (Linh *et al.* 2020).

Table 5: Effect of nanoparticles on relative water content (%) of wheat under moisture stress condition

Treatments	**Relative Water Content (%)**		
	60DAS	**75DAS**	**90DAS**
T1	82.52	68.63	61.96
T2	84.20	71.13	64.47
T3	87.86	70.02	69.46
T4	89.01	74.75	71.87
T5	84.69	71.83	67.67
T6	86.46	73.78	69.09
T7	88.16	74.72	71.25
T8	87.14	69.73	72.42
T9	90.77	82.08	74.21
T10	84.24	74.38	71.16
S.Em±	0.85	0.42	2.07
C.D. 5%	2.52	1.25	6.16

Effect of nanoparticles on grain yield and biological yield (kg plot^{-1}) of wheat under moisture stress condition:

The perusal of data revealed that moisture stress significantly affected the yield of the crop causing a reduction in the biological yield as well as the grain yield of the wheat variety however, foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly enhances the biological yield as well as the grain yield compared with untreated plot (Table 6). Among the different combinations of TiO_2 applied it was observed that TiO_2 (50ppm) along with Ca_3PO_4 (40ppm) was most effective also for ZnO among the various combinations applied ZnO (50ppm) along with Ca_3PO_4 (40ppm) was most effective against moisture stress. Overall it was observed that among the various combinations applied in the study maximum biological yield and grain yield are obtained for T_9 when the ZnO NPs (50 ppm) was applied under moisture stress conditions i.e. (15.14 kg/plot) and (6.58 kg/plot) respectively followed by T_4 [TiO_2 (40ppm) along with Ca_3PO_4 (40ppm)] having biological yield (13.38 kg/plot) and grain yield (5.72 kg/plot). ZnO NPs is more responsive as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum biological yield and grain yield at all the crop stages (Table 6). These results are in agreement with the results of Adil *et al.*, 2022 who reported that with the addition and increase in ZnO NPs concentration, yield per plant increased. Grain yield per plant varied noticeably among different ZnO NPs concentrations under saline condition. The highest yield per plant (45.33 g) was obtained under the highest ZnO NPs concentration (T_7) i.e. Zinc Nano (0.12 g) + NPK

(150:100:60 kg ha^{-1}) + Salinity (10 dS m^{-1}) compared to the control treatment (36.7g). Similar results were also obtained by Lohani *et al.*, 2023 in case of rice.

Table 6: Effect of nanoparticles on grain yield and biological yield (kg $plot^{-1}$) of wheat under moisture stress condition

Treatments	Grain yield (kg $plot^{-1}$)	Biological yield (kg $plot^{-1}$)
T1	3.62	8.22
T2	5.47	11.14
T3	5.26	10.29
T4	5.72	13.38
T5	5.16	12.37
T6	5.56	12.84
T7	5.17	11.98
T8	4.72	10.78
T9	6.58	15.14
T10	5.46	13.62
S.Em±	0.12	0.04
C.D. 5%	0.35	0.10

Conclusion

Water deficiency conditions significantly impact biochemical and physiological processes, including photosynthesis, nutrition and hormone metabolism, absorption of carbohydrates, and a significant decrease in crop growth and yield. The present study aimed to examine the effects of ZnO nanoparticles (NPs) and TiO_2 NPs, both alone and in combination with Ca_3PO_4, on mitigating moisture stress in wheat. The study focused on evaluating the impact of these NPs on physiological and biochemical features, as well as the yield of wheat. Our study showed that combining TiO_2 nanoparticles at a concentration of 40 ppm with Ca_3PO_4 nanoparticles at 40 ppm was the most successful in reducing the negative impact of moisture stress. Similarly, among the several combinations of ZnO nanoparticles tested, ZnO at a concentration of 50 ppm was shown to be the most efficient in easing the harmful effects of moisture stress. Among all the treatments employed, ZnO (50ppm) effectively mitigated the adverse effects of drought stress and boosted the activity of photosynthetic total pigments, soluble sugars, membrane stability index, and relative water content. In addition, the ZnO NP-treated plants exhibited enhanced economic production and biological yield of the wheat crop. However, additional investigations are required to correctly establish these nanoparticles as a viable method to improve moisture stress in wheat.

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