



Research Article

Evaluation Effect of Nano-TiO₂ Foliar Application and Humic Acid on Grain Yield, Its Components and Biochemical Traits of Corn (S.C 704) Affected Water Deficit Stress

Reza Jalakani, Mohammad Reza Dadnia*^{ID}, Alireza Shokuhfar^{ID},
Saeed Zakernejad

Department of Agronomy, Ahv.C., Islamic Azad University, Ahvaz, Iran

*Corresponding author: rezadadnia1352@iau.ac.ir

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Abstract

Background: Drought stress is one of the most important factors limiting the agricultural products. The use of humic acid and Titanium dioxide nanoparticles led to keep crop production under water stress conditions.

Objectives: Current research was conducted to assess effect of Nano titanium dioxide and Humic acid on Corn yield under water deficit stress. Current research was conducted to assess effect of Nano titanium dioxide and Humic acid on Corn yield under water deficit stress.

Methods: This study was done according combined analysis split-split plot experiment based on randomized complete blocks design with four replications along two agronomic years (2021-22 and 2022-23). The main factor included different Irrigation regime (I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence) and sub factors consisted different level of Humic acid foliar spraying (H₁: Control, H₂: 1000 ppm.ha⁻¹, H₃: 2000 ppm.ha⁻¹, H₄: 4000 ppm.ha⁻¹) 60 days after planting. Also different level of Nano titanium dioxide (T₁: Control, T₂: 0.02% ha, T₃: 0.04% ha) belonged to sub-sub plot.

Result: The results of analysis of variance showed that the triple interaction of irrigation regime, humic acid, and titanium dioxide on the number of grains per ear and grain yield was significant at 1% probability level. Also, the interaction of irrigation regime and humic acid on the number of grain per row, 1000-grain weight, carotenoids, leaf relative humidity, Dityrosine and Dihydroxyguanosine was significant. The highest grain yield (9094.1 kg.ha⁻¹) was obtained under normal irrigation conditions with the application of 2000 ppm.ha⁻¹ of humic acid and 0.02% ha of Nano Titanium Dioxide, which showed an increase of about 38.5% compared to the treatment of cut irrigation at ear emergence stage and nonuse humic acid and Nano Titanium Dioxide (5579 kg.ha⁻¹). Under limit irrigation conditions, the concentration of biochemical biomarkers such as Dityrosine and Dihydroxyguanosine increased, but their levels were reduced by the application of humic acid and Nano Titanium Dioxide under stress conditions.

Conclusion: According to the results obtained from this study, use of 2000 ppm.ha⁻¹ humic acid and 0.02% ha Titanium Dioxide Nanoparticles as foliar sprays is recommended under water deficit stress conditions led to produce economic yield and advised to producer in studied region.

Keywords: Carotenoid, Dityrosine, Irrigation, Leaf relative humidity, Maize

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1. Background

Corn has spread rapidly around the world due to its high adaptability to various climatic conditions, so that it has the largest cultivated area after Wheat and Rice (Fareghi et al., 2021). Drought or water shortage is one of the major abiotic stresses in the world, which limits the production of cereals, including Corn. Today, providing the water needed to irrigate crops in the agricultural sector is the main challenges facing humanity, because farmers are one of the largest consumers of water resources worldwide (Bonea, 2020). Water deficit stress by reduction in growth length, disruption in photosynthesis and translocation assimilates led to reducing growth and yield of Corn, also water stress reduces Leaf area index and stem height (Charabeh et al., 2024). Therefore, it is important to use methods that can be effective in increasing plant resistance to drought stress. Nanotechnology, as a new interdisciplinary technology and a pioneer in solving problems and deficiencies in many scientific and industrial fields, has created great hopes for solving deficiencies and developing the agricultural sector (Lei et al., 2008). Titanium dioxide nanoparticles, as one of the metal oxide semiconductor nanocrystals, have found a special place in today's industrial world and have attracted the attention of many scientists in various fields, including agriculture, due to their good electrical, optical, and photocatalytic properties (Khan et al., 2017). Nano titanium dioxide improves light absorption and Rubisco enzyme activity, increases nitrate absorption, and accelerates the conversion of inorganic materials into organic plant materials. Titanium dioxide increases yields by up to 30 percent by increasing photosynthesis and reducing damage from pests and diseases (Gao et al., 2013). Accordingly, Khairy et al. (2013) investigated the effects of Titanium Dioxide Nanoparticles on some biochemical traits of corn crop and stated that different concentrations of Titanium Dioxide Nanoparticles showed a significant effect on traits such as the amount of superoxide dismutase enzyme and the degradation biomarker Dihydroxyguanosine. In general, the use of higher amounts of nanoparticles led to an increase in antioxidant enzymes and a decrease in destructive biomarkers in the plant. The use of natural fertilizers, including humic acid, without harmful environmental

effects, can be effective in increasing yield under water stress conditions. Humic acid, with its natural polymer structure, is a part of organic matter that is generally obtained in soils and running waters as a result of the decomposition of plants and animal remains and is used to increase yields (Fan et al., 2014). Humic acid can directly have positive effects on plant growth. The growth of the aerial part and root of the plant is stimulated by humic acid, but its effect on the root is more pronounced. This combination increases root volume and improves the effectiveness of the root system. The beneficial effects of humic acid directly and indirectly, by penetrating cell membranes, improve protein synthesis, change enzyme activity, micronutrient solubility, improve soil structure, increase cation exchange capacity, and increase microbial population in the soil (Meganid et al., 2015). Researchers stated in their reports that the application of humic acid at the time of germination had a positive effect on ear diameter, 1000-grain weight, leaf area index, grain yield, and harvest index, and increased these traits (Eryigit and Husamalddin, 2023).

2. Objectives

Current research was conducted to assess effect of Nano titanium dioxide and Humic acid on Corn crop production under water stress condition.

3. Materials and methods

3.1. Field and treatments information

This study was done according combined analysis split-split plot experiment based on randomized complete blocks design with four replications along two agronomic years (2021-22 and 2022-23). Place of research was located in Gotvand city at longitude 48°34'E and latitude 32°27'N in Khuzestan province (Southwest of Iran). The main factor included different Irrigation regime (I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence) and sub factors consisted different level of Humic acid foliar spraying (H₁: Control, H₂: 1000 ppm.ha⁻¹, H₃: 2000 ppm.ha⁻¹, H₄: 4000 ppm.ha⁻¹) 60 days after planting. Also different level of Nano titanium dioxide (T₁: Control, T₂: 0.02% ha⁻¹, T₃: 0.04 ha⁻¹) belonged to sub-sub plot. The physical and chemical properties of studied soil was mentioned table 1.

Table 1. The physical and chemical characteristics of studied field (0-30 cm)

Year	Soil texture	pH	Organic matter (%)	Electrical conductivity (ds.m ⁻¹)	Nitrogen (%)	Phosphorus (mg.kg ⁻¹)	Potassium (mg.kg ⁻¹)
2021	Clayloam	7.32	0.89	3.94	0.05	11.35	175
2022	Clayloam	7.20	0.91	3.71	0.04	12.50	181

Soil sampling was done by Cross-over method from four points on the side of the field and four points in the middle of the field from a depth of 0 to 30 cm. The bed preparation operation included plowing with a Moldboard, a disk, and finally leveling with a land leveler. The hybrid cultivated was Single Cross 704, a late-maturing hybrid, and was obtained from the Safi-Abad Agricultural and Natural Resources Research and Education Center in Dezful (North of Khuzestan province, Southwest of Iran). This experiment consisted of 144 plots. Each plot had seven lines by four meters long and 75 cm distance, with a spacing of 17 cm between bushes in a row, with a density of 75,000 plants per hectare. The distance between the main plots was considered to be equal to two non-planting lines (1.5 m), the distance between the subplots was equal one non planting, line and the distance between the sub-subplots was considered to be equal to 0.5 m.

3.2. Farm management

To provide the required nutrients, after preparing the land, the required phosphorus fertilizer was applied pre-planting from a triple superphosphate source based on 100 kg of pure phosphorus and nitrogen fertilizer from a urea source (46%) at a rate of 200 kg per hectare, half of which was spread in the field with a disc and the other half of the nitrogen was applied as foliar spray at the six-leaf stage. The seed sowing operation was carried out manually on middle of furrow at a depth of four centimeters at August 15, 2021 and August 16, 2022. The Humic acid application rates included nonuse or control, one gram per liter (1000 ppm), two grams per liter (2000 ppm), and three grams per liter (3000 ppm), and its source was Humex WSG 95 powder. The amount of Nano Titanium dioxide consisted nonuse or control, 0.02% ha (0.0158 grams per liter of water), and 0.04% ha (0.0316 grams per liter of water), and its source was a 25-gram pack from the Army Nano brand. The first irrigation was done after planting. Irrigation treatments including normal irrigation that continued until harvest (Normal irrigation means irrigation at intervals of 6 to 7 days), cut irrigation at ear emergence and cut irrigation 7 days after ear emergence were carried out in the field. Weed control was carried out by hand weeding during the growth stage. Diazinon was used during the plant growth period to control pests.

3.3. Measured traits

The maturity of the seeds was determined by the formation of a black layer at the base of the seeds, and the final harvest was carried out by removing 50 cm from the beginning and end of the lines from an area equivalent to two square meters. To measure grain yield in each experimental plot, after removing 0.5 meters from both

ends of the lines, all ears in the three middle lines with a length of two meters were manually harvested and after drying in an oven, the grains were manually separated and weighed at a moisture content of 14%. The number of grains per row was determined based on counting and averaging the number of grain from the beginning to the end of the ear in 5 ears. The number of rows in the ears harvested from each plot and the number of grains in the row of the ears harvested from each plot were counted and recorded, and the number of grains per ear was obtained from the multiply of these two components. After removing the leaves around ear harvested from each plot, their grains were carefully separated from cob. Two samples of 500 grains were placed in an oven at 72°C for 48 hours and then weighed. If the difference between two samples was less than five percent, their total weight was recorded as the weight of a thousand grains (Taleb Zadeh and Marashi, 2018). In order to determine the concentration of dityrosine in leaves at the beginning of the emergence of female inflorescences, first half a gram of fresh leaves was completely powdered in a 20% thiochloroacetic acid (TCA) solution containing half a percent Thiobarbituric acid (TBA), and then this mixture was heated for 25 minutes at 95°C in a bain-marie bath. Then, this mixture was cooled in an ice bath and the concentration of dityrosine was measured at a wavelength of 532 nm according to the method of Valentovic et al. (2006). To calculate Dihydroxyguanosine, the leaf tissue sample, it was heated in 1.6 M monosodium phosphate bicarbonate buffer solution (acidity 7.5) and then rapidly homogenized in the presence of ice and cold conditions. Then, the amount of Dihydroxyguanosine was calculated based on the method of Bogdanov et al. (1999). Arnon (1975) method was used to calculate carotenoids at the silking stage. In this method, 0.5 grams of fresh leaf tissue was weighed, chopped into small pieces, and then crushed with some 80% acetone in a porcelain mortar. Then, its volume was brought to 25 ml by adding acetone. Then, the resulting solution was centrifuged for 15 minutes at 5000 rpm. In the next step, the optical density of the leaf extract was read with a spectrophotometer at a wavelength of 470 nm and the concentration of carotenoids was calculated using the following equations in milligrams per gram of fresh weight of the sample. The Relative water content was measured by equation 2 (Schlemmer et al., 2005).

$$\text{Car (mg.gr}^{-1}\text{)} = (1000 \times A_{470} - 1.8 \times \text{Chl.a} - 85.02 \times \text{Chl.b})/198 \quad (1)$$

Car: carotenoid, Chl.a: Chlorophyll A, Chl.b: Chlorophyll B.

$$\text{RWC (\%)} = (W_f - W_d)/(W_t - W_d) \times 100 \quad (2)$$

RWC: Relative water content, W_f is the fresh weight of leaf (g); W_d is the dry weight of leaf (g); W_t is the weight of leaf saturated with water (g).

3.4. Statistical analysis

After use Bartlett test, combined analysis of variance was done via SAS (Ver.8) software. Mean comparison was conducted with Duncan test at 5% probability level.

4. Results and discussion

4.1. Number of grain per row

According result of analysis of variance effect of Irrigation regime, Humic acid, Titanium dioxide, interaction effect of irrigation regime and humic acid, and irrigation regime and Titanium dioxide on number of grain per row was significant at 1% probability level (Table 2). Mean comparison result of different level of Irrigation regime indicated that maximum number of grain per row (35.31) was noted for I₁ and minimum of that (28.17) belonged to I₂ treatment (Table 3). As for Duncan classification made with respect to different level of humic acid maximum and minimum amount of number of grain per row belonged to H₃ (33.24) and H₁ (29.53) (Table 3). Evaluation mean comparison result indicated in different level of Titanium dioxide the maximum number of grain per row (34.61) was noted for T₂ and minimum of that (27.85) belonged to T₁ treatment (Table 3). Evaluation mean comparison interaction effect of irrigation regime and humic acid indicated maximum number of grain per row (37.35) was noted for I₁H₃ and lowest one (24.84) belonged to I₂H₁ (Table 4). It seems in this study, different levels of humic acid reduced the effect of water deficit stress on the number of grain per row. Also assess interaction effect of irrigation regime and Titanium dioxide indicated maximum number of grain per row (36.06) was noted for I₁T₂, and lowest one (26.88) belonged to I₂T₁ treatment (Table 5). Nouri et al. (2017) reported that foliar spraying of Titanium dioxide nanoparticles increased grain yield and its components under drought stress conditions, which is consistent with the results of this study. Eryigit and Husamaldin (2023) reported humic acid application acted as a supplement in water deficit conditions by keeping the rhizosphere moist and providing sufficient nutrients to the crop, it led to an increase in the number of grains per row. Also Anwar et al. (2016) stated that the highest number of grains per spike was obtained by applying humic acid at a rate of 15 kg.ha⁻¹. Khater (2015) reported the use of Nano-Titanium dioxide under water deficit stress conditions has increased the number of grains in the ear row by affecting plant photosynthesis ability and keep produce assimilate. Some researchers believe that application Nano-Titanium dioxide increases the absorption of nutrients, especially Nitrogen, and by accelerating the activity of the Nitrate reductase enzyme, it led to increases the synthesis of amino acids, proteins, and photosynthesis in crop (Khalilvand Behrouzfar et al., 2019).

4.2. Number of grain per ear

Result of analysis of variance revealed effect of irrigation regime, humic acid, Titanium dioxide, interaction effect of irrigation regime, Humic acid and Titanium dioxide on number of grain per ear was significant (Table 2). According result of mean comparison of different level of irrigation regime, maximum number of grain per ear (492.75) was obtained for I₁ and minimum of that (380.23) was for I₂ treatment (Table 3). Evaluation mean comparison result indicated in different level of Humic acid the maximum number of grain per ear (450.31) was noted for H₃ and minimum of that (384.55) belonged to H₁ treatment (Table 3). Assessment mean comparison result indicated in different level of Titanium dioxide the maximum number of grain per ear (455.66) was noted for T₂ and minimum of that (394.83) belonged to T₁ treatment (Table 3). Assess mean comparison interaction effect of irrigation regime, humic acid and Titanium dioxide indicated maximum number of grain per ear (512.34) was noted for I₁H₃T₂ and lowest one (339.57) belonged to I₂H₁T₁ (Table 6). In the treatment of cut irrigation at the ear emergence, the number of grain per ear decreased significantly due to poor formation of reproductive organs and abnormal pollination (Meseka et al., 2018). Under water deficit stress conditions, the application of humic acid as a foliar spray with irrigation water has increased cell division and consequently the number of grains in the ear due to its presence of growth hormones such as Cytokinin and Auxin (Ludwig-Muller, 2000; Huang et al., 2023). On the other hand, the use of Titanium Nanoparticles increases light absorption by chloroplasts, thereby increasing photosynthetic capacity (Ze et al., 2011). Under drought stress conditions, Nano-Titanium dioxide increases Rubisco enzyme activity and improving the content of some essential elements in plant tissues led to increase crop yield and its components (Khater, 2015). According to the results of Shahim Germi et al. (2021), foliar application of 0.02% Nano titanium dioxide resulted in the highest number of grains per ear, while no foliar application of titanium dioxide resulted in the lowest one. On the other hand, Esmaeili et al. (2014) reported that deficit irrigation treatment reduced growth, grain number per ear, and yield of corn in compared to the control sample, but the use of Titanium Nanoparticles had a positive effect on increasing the growth of photosynthetic pigments and crop production (under deficit irrigation conditions), the mentioned result which was consistent with the results of this study.

4.3. 1000-grain weight

According result of analysis of variance effect of year, irrigation regime, humic acid, Titanium dioxide,

interaction effect of irrigation regime and humic acid, and irrigation regime and Titanium dioxide on 1000-grain weight was significant (Table 2). The results showed that the 1000-grain weight in 2021 was 8 percent higher than in 2022 (Table 3). Mean comparison result of different level of irrigation regime indicated that maximum 1000-grain weight (225.96 gr) was noted for I₁ and minimum of that (219.68 gr) belonged to I₂ treatment (Table 3). As for Duncan classification made with respect to different level of humic acid maximum and minimum amount of 1000-grain weight belonged to H₃ (238.27 gr) and H₁ (224.08 gr) (Table 3). Evaluation mean comparison result indicated in different level of Titanium dioxide the maximum 1000-grain weight (237.01 gr) was noted for T₂ and minimum of that (229.34 gr) belonged to T₁ treatment (Table 3). Evaluation mean comparison interaction effect of irrigation regime and humic acid indicated maximum 1000-grain weight (260.76 gr) was noted for I₁H₃ and lowest one (212.44 gr) belonged to I₂H₁ (Table 4). Also assess interaction effect of irrigation regime and Titanium dioxide indicated maximum 1000-grain weight (259.16 gr) was noted for I₁T₂, and lowest one (217.44 gr) belonged to I₂T₁ treatment (Table 5). It can be stated that different levels of humic acid application led to a reduction in the negative effects of water deficit stress on thousand-grain weight. The application of humic acid increased water transfer from roots to shoots and reduced the severity of water deficit stress (Tsanaktsidis et al., 2013). In this regard, Zhou et al. (2019) stated that the increase in grain weight with the application of humic acid is due to the increase in the number of endosperm and amyloplast cells and photosynthetic materials (assimilates), so, the grain weight has increased, probably due to the effect of growth hormones on cell division. It seems that in this study, Nano Titanium dioxide increased the thousand-grain weight under water deficit stress conditions by affecting plant photosynthesis. Foliar application of Nano-Titanium dioxide fertilizer increased catalase enzyme activity under water deficit stress and reduced oxygen free radicals (hydrogen peroxide) in Corn. Nano titanium dioxide was able to increase the 1,000-grain weight in all studied treatments by reducing the negative effects of water deficit stress, which indicates the positive effects of this compound on crop (Khalilvand Behrouzayar et al., 2019). It should be noted that the results of other research also indicate this issue (Salehi et al., 2023).

4.4. Grain yield

Result of analysis of variance showed effect of year, irrigation regime, humic acid, Titanium dioxide, interaction effect of irrigation regime and humic acid, and irrigation regime and Titanium dioxide, interaction effect

of irrigation regime, humic acid and Titanium dioxide on grain yield was significant at 1% probability level (Table 2). The results showed that the year 2021 had a higher grain yield (due to the high yield components in this year), which increased the grain yield by 6% compared to the year 2022 (Table 3). According result of mean comparison of different level of irrigation regime, maximum grain yield (8895.1 kg.ha⁻¹) was obtained for I₁ and minimum of that (6764.6 kg.ha⁻¹) was for I₂ treatment (Table 3). Evaluation mean comparison result indicated in different level of Humic acid the maximum grain yield (8003.6 kg.ha⁻¹) was noted for H₃ and minimum of that (6747.5 kg.ha⁻¹) belonged to H₁ treatment (Table 3). Assessment mean comparison result indicated in different level of Titanium dioxide the maximum grain yield (7903.3 kg.ha⁻¹) was noted for T₂ and minimum of that (7162.4 kg.ha⁻¹) belonged to T₁ treatment (Table 3). Assess mean comparison interaction effect of irrigation regime, humic acid and Titanium dioxide indicated maximum grain yield (9094.1 kg.ha⁻¹) was noted for I₁H₃T₂ and lowest one (5579.3 kg.ha⁻¹) belonged to I₂H₁T₁ (Table 6). It seems that the higher grain yield under water deficit stress conditions in crop treated with humic acid and Nano Titanium Dioxide led to greater root growth and more water in the leaves, and ultimately an increase in improved water use efficiency through an increase in root activity and a decrease in crop transpiration. In this study, the increase in grain yield is indicative of the ability of humic acid to affect the plant's reproductive process under water deficit stress conditions, such that high grain yield is associated with a higher number of grains per ear and 1,000-grain weight. Humic acid accelerates cell division and increases plant root growth, which can reduce the destructive effects of environmental stresses (Wang et al., 2014). It can be stated that Nano Titanium dioxide reduces the destructive effects of drought stress by reducing oxygen free radicals and malondialdehyde and increasing antioxidant enzymes. This compound also increases crop yield by increasing the photo reduction activity of photosystem II, oxygen release, chloroplast photophosphorylation activity, and improving the content of some essential elements in crop tissues (Khater, 2015). In this regard, Jaberzadeh et al. (2013) reported that Titanium Dioxide Nanoparticles increase crop growth, grain yield and its components under water stress conditions.

4.5. Carotenoid

Result of analysis of variance revealed effect of year, irrigation regime, humic acid and Titanium dioxide on carotenoid was significant at 1% probability level (Table 2). The results showed that the year of 2021 had a higher amount of carotenoids, which increased the amount of

carotenoids by 11.35% compared to the year 2022 (Table 3). Mean comparison result of different level of irrigation regime indicated that maximum carotenoid (3.93 mg.g^{-1}) was noted for I_1 and minimum of that (3.14 mg.g^{-1}) belonged to I_2 treatment (Table 3). As for Duncan classification made with respect to different level of humic acid maximum and minimum amount of carotenoid belonged to H_3 (3.90 mg.g^{-1}) and H_1 (3.03 mg.g^{-1}) (Table 3). Evaluation mean comparison result indicated in different level of Titanium dioxide the maximum carotenoid (3.58 mg.g^{-1}) was noted for T_2 and minimum of that (3.45 mg.g^{-1}) belonged to T_1 treatment also it doesn't have significant difference with T_3 treatment (Table 3). The rate of carotenoid synthesis in leaves increases at the beginning of environmental stresses due to their role in protecting against free radicals, but over time and as the crop adapts to the stress, its rate decreases (Junaid et al., 2023). The decrease in carotenoid content could be due to their oxidation by active oxygen and destruction of their structure, studies also showed that the amount of carotenoids decreased with increasing drought stress (Ben Ahmed et al., 2009). In another study, it was reported that carotenoid content decreased significantly with increasing drought stress compared to the control treatment (Godarzi et al., 2017), Which was consistent with the results of this study. In this study, humic acid increases crop production through positive physiological effects, including effects on crop cell metabolism and increased concentrations of chlorophyll and leaf carotenoids (Abou-Aly and Mady, 2009). On the other hand, Ferrara et al. (2008) stated that humic acid increases root growth, chlorophyll and photosynthetic pigments such as carotenoids in leaves, which was consistent with the results of this study. The mean comparison showed effect of Titanium Nanoparticles on photosynthetic pigments indicated that with increasing Titanium Nanoparticle concentration, the amount of carotenoids showed an increasing trend. Nano Titanium Dioxide can improve the efficiency of the photosynthetic system and increase the crop ability to absorb sunlight, which affects the production and conversion of light energy into active electrons and chemical activities, and increases photosynthetic pigments such as chlorophylls and carotenoids in the crop (Akbari et al., 2015). The favorable effect of using Titanium Dioxide Nanoparticles on carotenoids and photosynthetic pigments in corn leaves has been reported (Morteza et al., 2013), which was consistent with the results of this study.

4.6. Leaf relative humidity

According result of analysis of variance effect of year, irrigation regime, humic acid, Titanium dioxide, interaction effect of irrigation regime and humic acid, and

irrigation regime and Titanium dioxide on leaf relative humidity was significant (Table 2). The results showed that the year 2021 had a higher leaf relative humidity content with an average of 84.1%, which increased the relative leaf water content by 3% compared to the year 2022 (Table 3). According result of mean comparison of different level of irrigation regime, maximum leaf relative humidity (88.41%) was obtained for I_1 and minimum of that (79.67%) was for I_2 , also it doesn't have significant difference with I_3 treatment (Table 3). Evaluation mean comparison result indicated in different level of Humic acid the maximum leaf relative humidity (84.00%) was noted for H_3 and minimum of that (81.64%) belonged to H_1 treatment (Table 3). Assessment mean comparison result indicated in different level of Titanium dioxide the maximum leaf relative humidity (83.60%) was noted for T_2 and minimum of that (82.39%) belonged to T_1 , also it doesn't have significant difference with T_3 treatment (Table 3). Evaluation mean comparison interaction effect of irrigation regime and humic acid indicated maximum leaf relative humidity (89.46%) was noted for I_1H_3 (also it doesn't have significant difference with I_1H_4) and lowest one (78.33%) belonged to I_2H_1 (Table 4). Also assess interaction effect of irrigation regime and Titanium dioxide indicated maximum leaf relative humidity (89.1%) was noted for I_1T_2 , and lowest one (79.31%) belonged to I_2T_1 treatment (Table 5). The results of the present study showed that the effects of drought stress are reduced by applying humic acid, therefore, by increasing the relative content of leaves, intracellular pressure is provided for cell growth and allows cell wall expansion, and ultimately, it increases the flexibility of the cell membrane to provide situation for cell growth. Therefore, it seems that by using biofertilizers and improving the physical conditions of the soil, including the water capacity of the soil, the crop is less exposed to drought conditions and shows less inclination to activity in increasing membrane production, Also, by improving soil structure, it stimulates crop growth (Nassotti Mianadab et al., 2000). In this regard, Mozafari et al. (2017) also obtained similar results indicating an increase in the relative amount of leaf water in the presence of humic acid. On the other hand, the use of titanium has disrupted the process of deterioration and reduction in vegetative and reproductive growth of plants under water shortage conditions and has provided better yield by increasing the relative water content (Berahmand et al., 2012). An increase in relative leaf water content with the application of Nano-Titanium dioxide under water deficit stress has also been reported by Kiapour et al. (2015).

4.7. Dityrosine

Result of analysis of variance showed effect of year,

irrigation regime, humic acid, Titanium dioxide, interaction effect of irrigation regime and humic acid, and irrigation regime and Titanium dioxide on Dityrosine was significant (Table 2). The results showed that dityrosine was 4% higher in 2021 than to 2022 (Table 3). Mean comparison result of different level of irrigation regime indicated that maximum Dityrosine (1.87 nmol.mg leaf protein) was noted for I₂ and minimum of that (1.41 nmol.mg leaf protein) belonged to I₁ treatment (Table 3). As for Duncan classification made with respect to different level of humic acid maximum and minimum amount of Dityrosine belonged to H₁ (2.02 nmol.mg leaf protein) and H₃ (1.31 nmol.mg leaf protein) (Table 3). Evaluation mean comparison result indicated in different level of Titanium dioxide the maximum Dityrosine (1.70 nmol.mg leaf protein) was noted for T₁ and minimum of that (1.53 nmol.mg leaf protein) belonged to T₂ treatment also it doesn't have significant difference with T₃ treatment (Table 3). Evaluation mean comparison interaction effect of irrigation regime and humic acid indicated maximum Dityrosine (2.38 nmol.mg leaf protein) was noted for I₂H₁ and lowest one (1.25 nmol.mg leaf protein) belonged to I₁H₂ (also it doesn't have significant difference with I₁H₃ and I₃H₃) (Table 4). Also assess interaction effect of irrigation regime and Titanium dioxide indicated maximum Dityrosine (2.00 nmol.mg leaf protein) was noted for I₂T₁, and lowest one (1.30 nmol.mg leaf protein) belonged to I₁T₂ treatment (Table 5). Under the conditions of this study, the application of humic acid and Nano Titanium dioxide at different concentrations reduced the content of Dityrosine in the crop. Under the conditions of this study, the application of humic acid and Nano Titanium dioxide at different concentrations reduced the content of Dityrosine in the crop. This result probably indicates that humic acid and Nano Titanium dioxide reduce the effect of drought stress through another defense mechanism, which has prevented the increase in the volume of Dityrosine and the activity of antioxidant enzymes (Aktas et al., 2007). In this regard, Khairy et al. (2013) stated that different concentrations of Titanium Dioxide Nanoparticles showed a significant effect on traits such as the amount of degradation biomarkers Dityrosine and Dihydroxyguanosine at the 0.01 level. In general, the use of higher amounts of nanoparticles led to an increase in antioxidant enzymes and a decrease in destructive biomarkers in the crop. This increase in the amount of enzymes at higher levels of nanoparticles is one of the desirable results of foliar spraying in plants, which was consistent with the results of this study.

4.8. Dihydroxyguanosine

According result of analysis of variance effect of year, irrigation regime, humic acid, Titanium dioxide,

interaction effect of irrigation regime and humic acid, and irrigation regime and Titanium dioxide on Dihydroxyguanosine was significant at 1% probability level (Table 2). The results showed that Dihydroxyguanosine was 18 percent higher in 2021 than in 2022 (Table 3). According result of mean comparison of different level of irrigation regime, maximum Dihydroxyguanosine (1.97 nmol.mg leaf protein) was obtained for I₂ and minimum of that (1.73 nmol.mg leaf protein) was for I₁ treatment (Table 3). Evaluation mean comparison result indicated in different level of Humic acid the maximum Dihydroxyguanosine (2.26 nmol.mg leaf protein) was noted for H₁ and minimum of that (1.54 nmol.mg leaf protein) belonged to H₃ treatment (Table 3). Assessment mean comparison result indicated in different level of Titanium dioxide the maximum Dihydroxyguanosine (2.00 nmol.mg leaf protein) was noted for T₁ and minimum of that (1.69 nmol.mg leaf protein) belonged to T₂ treatment (Table 3). Evaluation mean comparison interaction effect of irrigation regime and humic acid indicated maximum Dihydroxyguanosine (2.59 nmol.mg leaf protein) was noted for I₂H₁ and lowest one (1.47 nmol.mg leaf protein) belonged to I₁H₂ (also it doesn't have significant difference with I₁H₃ and I₃H₃) (Table 4). Also assess interaction effect of irrigation regime and Titanium dioxide indicated maximum Dihydroxyguanosine (2.24 nmol.mg leaf protein) was noted for I₂T₁, and lowest one (1.52 nmol.mg leaf protein) belonged to I₁T₂ treatment (Table 5). It can be stated that the increase in the amount of Dihydroxyguanosine is due to the increased production of superoxide free radicals as a result of oxidative stress in the crop. An increase in Dihydroxyguanosine under drought stress conditions indicates damage to the plant's genetic structures and the destruction of nucleic acids. When oxidative stress occurs, peroxidation of unsaturated fatty acids in lipids increases. As a result of free radical attack on lipids, aldehydes, various aldehydes, including malondialdehyde, are formed (Wenho and Russel, 2000). In this regard, Pourabtehaj et al. (2012) showed that the highest production of Dihydroxyguanosine with 15.9 $\mu\text{mol.g}^{-1}$ protein was obtained from drought stress treatment. The changes in Dihydroxyguanosine under drought conditions and its role in creating drought stress resistance in plants are not yet known. It seems that humic acid and Nano Titanium Dioxide treatments reduce stomatal resistance in crop and, as a result, increase water use efficiency and relative water content of leaves, leading to a decrease in the amount of Dihydroxyguanosine under drought stress conditions (Anjum, 2015), these reports confirmed the results of this study. Therefore, the approach of using fertilizers of organic and natural origin, such as humic acid, along with the use of Nano Titanium Dioxide, while maintaining crop production, prevents environmental pollution.

Table 2. Result analysis of variance of measured traits

S.O.V	df	No. Grain per row	No. Grain per ear	1000-grain weight	Grain yield
Year (Y)	1	50.24**	105.1*	39.57*	101.5**
Year × Replication	6	0.006	9.44	6.12	0.60
Irrigation (I)	2	736.89**	197446.6**	10896.3**	1010840.5**
Y × I	2	0.0003 ^{ns}	0.001 ^{ns}	0.15 ^{ns}	0.015 ^{ns}
Error I	12	13.66	3168.5	377.4	19258
Humic acid (H)	3	459.33**	234200.1**	12761.7**	111923.5**
Y × H	3	0.002 ^{ns}	0.0006 ^{ns}	0.48 ^{ns}	0.105 ^{ns}
I × H	6	688.05**	0.056 ^{ns}	9033.71**	135232.6**
Y × I × H	6	0.001 ^{ns}	0.07 ^{ns}	5.16 ^{ns}	0.06 ^{ns}
Error II	54	10.74	2730.3	315.1	15237.5
Titanium dioxide (T)	2	361.8**	15308.85*	4257.6**	821490.2**
Y × T	2	0.04 ^{ns}	0.001 ^{ns}	20.31 ^{ns}	0.002 ^{ns}
I × T	4	543.1**	0.014 ^{ns}	7449.65**	253361.07**
Y × I × T	4	0.007 ^{ns}	0.02 ^{ns}	6.11 ^{ns}	0.11 ^{ns}
H × T	6	0.001 ^{ns}	0.0003 ^{ns}	2.77 ^{ns}	0.005 ^{ns}
Y × H × T	6	0.006 ^{ns}	0.023 ^{ns}	1.19 ^{ns}	0.017 ^{ns}
I × H × T	12	0.013 ^{ns}	136547.1**	7.23 ^{ns}	350291.5**
T × H × I × Y	12	0.007 ^{ns}	0.009 ^{ns}	5.12 ^{ns}	0.06 ^{ns}
Error III	144	8.95	2010.5	277.02	13005.1
CV (%)	-	9.48	10.59	7.14	15.09

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively

Continue of **table 2.**

S.O.V	df	Carotenoid	Leaf relative humidity	Dityrosine	Dihydroxyguanosine
Year (Y)	1	0.1**	7.28*	0.014**	0.013**
Year × Replication	6	0.003	1.15	0.0006 ^{ns}	0.00012
Irrigation (I)	2	27.8**	22245.6**	5.24*	6.66*
Y × I	2	0.0003 ^{ns}	0.0001 ^{ns}	0.0001 ^{ns}	0.00005 ^{ns}
Error I	12	0.44	51.32	0.77	0.84
Humic acid (H)	3	15.21**	617.7**	6.377**	11.83**
Y × H	3	0.0003 ^{ns}	0.007 ^{ns}	0.00003 ^{ns}	0.00004 ^{ns}
I × H	6	0.05 ^{ns}	499.11**	13.511**	7.281**
Y × I × H	6	0.006 ^{ns}	0.0002 ^{ns}	0.0005 ^{ns}	0.0003 ^{ns}
Error II	54	0.32	40.41	0.16	0.35
Titanium dioxide (T)	2	40.55**	1137.18**	8.371**	4.167**

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively

Continue of **table 2.**

S.O.V	df	Carotenoid	Leaf relative humidity	Dityrosine	Dihydroxyguanosine
Y × T	2	0.004 ^{ns}	0.011 ^{ns}	0.00005 ^{ns}	0.00007 ^{ns}
I × T	4	0.071 ^{ns}	866.38 ^{**}	6.77 ^{**}	5.03 ^{**}
Y × I × T	4	0.031 ^{ns}	0.001 ^{ns}	0.0001 ^{ns}	0.0002 ^{ns}
H × T	6	0.0001 ^{ns}	0.003 ^{ns}	0.00017 ^{ns}	0.0001 ^{ns}
Y × H × T	6	0.0002 ^{ns}	0.007 ^{ns}	0.0001 ^{ns}	0.0003 ^{ns}
I × H × T	12	0.0002 ^{ns}	0.004 ^{ns}	0.0031 ^{ns}	0.0008 ^{ns}
T × H × I × Y	12	0.0003 ^{ns}	0.077 ^{ns}	0.0002 ^{ns}	0.0003 ^{ns}
Error III	144	0.27	25.71	0.09	0.06
CV (%)	-	14.88	6.12	18.63	15.21

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively**Table 3.** Mean comparison effect of Irrigation regime, Humic acid and Titanium dioxide on measured traits of Corn along two agronomic year

Treatment	No. Grain per row	No. Grain per ear	1000-grain weight (gr)	Grain yield (kg.ha ⁻¹)
Year				
Y ₁	33.06a	429.1a	242.7a	7802.00a
Y ₂	30.00b	417.6b	223.3b	7313.2b
Irrigation regime				
I ₁	35.31a	492.75a	255.96a	8895.1a
I ₂	28.17b	380.23c	219.68b	6764.6c
I ₃	29.94ab	396.71b	223.37ab	6954.00b
Humic acid				
H ₁	29.53c	384.55c	224.08c	6747.5c
H ₂	30.12bc	421.14b	234.07b	7675.7b
H ₃	33.24a	450.31a	238.27a	8003.6a
H ₄	31.67b	439.08ab	235.58b	7724.8ab
Titanium dioxide				
T ₁	27.85c	394.83c	229.34c	7162.4c
T ₂	34.61a	455.66a	237.01a	7903.3a
T ₃	30.96b	420.09b	232.65b	7548.00b

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

Y₁: 2021, Y₂: 2022I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergenceH₁: Control, H₂: 1000 ppm.ha⁻¹ Humic acid, H₃: 2000 ppm.ha⁻¹ Humic acid, H₄: 4000 ppm.ha⁻¹ Humic acidT₁: Control, T₂: 0.02% ha⁻¹ Titanium dioxide, T₃: 0.04 ha⁻¹ Titanium dioxide

Continue of table 3.

Treatment	Carotenoid (mg.g ⁻¹)	Leaf relative humidity (%)	Dityrosine (nmol.mg leaf protein)	Dihydroxyguanosine (nmol.mg leaf protein)
Year				
Y ₁	3.70a	84.1a	1.68a	1.77a
Y ₂	3.28b	81.5b	1.54b	1.45b
Irrigation regime				
I ₁	3.93a	88.41a	1.41c	1.73c
I ₂	3.14c	79.67b	1.87a	1.97a
I ₃	3.41b	80.44b	1.57b	1.85b
Humic acid				
H ₁	3.03bc	81.64b	2.02a	2.26a
H ₂	3.39b	82.83ab	1.64b	1.88b
H ₃	3.90a	84.00a	1.31d	1.54c
H ₄	3.65ab	82.90ab	1.51c	1.73b
Titanium dioxide				
T ₁	3.46b	82.39b	1.70a	2.00a
T ₂	3.58a	83.60a	1.53c	1.69c
T ₃	3.45b	82.53b	1.63b	1.86b

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

Y₁: 2021, Y₂: 2022

I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence

H₁: Control, H₂: 1000 ppm.ha⁻¹ Humic acid, H₃: 2000 ppm.ha⁻¹ Humic acid, H₄: 4000 ppm.ha⁻¹ Humic acid

T₁: Control, T₂: 0.02% ha⁻¹ Titanium dioxide, T₃: 0.04 ha⁻¹ Titanium dioxide

Table 4. Mean comparison interaction effect of Irrigation regime and Humic acid on measured traits

Irrigation regime	Humic acid	No. Grain per row	1000-grain weight (gr)	Leaf relative humidity (%)	Dityrosine (nmol.mg leaf protein)	Dihydroxyguanosine (nmol.mg leaf protein)
I ₁	H ₁	32.63c	244.47c	87.28b	1.72c	1.99bc
	H ₂	34.11b	257.45b	87.85b	1.25de	1.47e
	H ₃	37.35a	260.76a	89.46a	1.25de	1.43e
	H ₄	35.14b	258.17ab	89.05a	1.42cd	1.64d
I ₂	H ₁	24.84f	212.44f	78.33f	2.38a	2.59a
	H ₂	29.31de	218.9e	79.33de	1.99b	2.25b
	H ₃	30.43d	225.36d	80.69cd	1.47cd	1.70d
	H ₄	30.12d	222.75de	80.01d	1.65c	1.89c
I ₃	H ₁	27.92e	217.33e	79.30e	1.94b	2.20b
	H ₂	30.13d	222.87de	80.00d	1.69c	1.95bc
	H ₃	31.17cd	228.65d	81.69c	1.21de	1.44e
	H ₄	30.53d	225.83d	80.78cd	1.45cd	1.67d

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence

H₁: Control, H₂: 1000 ppm.ha⁻¹ Humic acid, H₃: 2000 ppm.ha⁻¹ Humic acid, H₄: 4000 ppm.ha⁻¹ Humic acid

Table 5. Mean comparison interaction effect of irrigation regime and Titanium dioxide on measured traits

Irrigation regime	Titanium dioxide	No. Grain per row	1000-grain weight (gr)	Leaf relative humidity (%)
I ₁	T ₁	33.84b	253.22b	87.90b
	T ₂	36.06a	259.16a	89.1a
	T ₃	34.03b	255.52b	88.23b
I ₂	T ₁	26.88e	217.44d	79.31d
	T ₂	30.58cd	221.71cd	80.00cd
	T ₃	28.06de	223.59c	79.81cd
I ₃	T ₁	29.15cd	218.35d	80.19cd
	T ₂	31.86c	224.17c	80.67c
	T ₃	29.80d	223.49c	80.47c

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence

T₁: Control, T₂: 0.02% ha⁻¹ Titanium dioxide, T₃: 0.04 ha⁻¹ Titanium dioxide

Continue of **table 5.**

Irrigation regime	Titanium dioxide	Dityrosine (nmol.mg leaf protein)	Dihydroxyguanosine (nmol.mg leaf protein)
I ₁	T ₁	1.49d	1.70cd
	T ₂	1.30e	1.52d
	T ₃	1.45d	1.66cd
I ₂	T ₁	2.00a	2.24a
	T ₂	1.78bc	2.02b
	T ₃	1.84b	2.08b
I ₃	T ₁	1.62c	1.88c
	T ₂	1.49d	1.72cd
	T ₃	1.60c	1.83c

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence

T₁: Control, T₂: 0.02% ha⁻¹ Titanium dioxide, T₃: 0.04 ha⁻¹ Titanium dioxide

Table 6. Mean comparison interaction effect of Irrigation regime, Humic acid and Titanium dioxide on measured traits

Irrigation regime	Humic acid	Titanium dioxide	No. Grain per ear	Grain yield (kg.ha ⁻¹)
I ₁	H ₁	T ₁	468.32cd	7659.6e
		T ₂	479.66c	8010.8d
		T ₃	478.37c	7930.7d
	H ₂	T ₁	488.29bc	8853.3c
		T ₂	498.71b	8905.7bc
		T ₃	497.43b	8873.3c
	H ₃	T ₁	490.52bc	8986.7b
		T ₂	512.34a	9094.1a
		T ₃	495.86b	8986.2b
	H ₄	T ₁	491.37bc	8958.4b
		T ₂	508.43a	9060.8a
		T ₃	494.34b	8962.4b

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test

I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence

H₁: Control, H₂: 1000 ppm.ha⁻¹ Humic acid, H₃: 2000 ppm.ha⁻¹ Humic acid, H₄: 4000 ppm.ha⁻¹ Humic acid

Continue of table 6.

Irrigation regime	Humic acid	Titanium dioxide	No. Grain per ear	Grain yield (kg.ha ⁻¹)
I ₂	H ₁	T ₁	339.57h	5579.3j
		T ₂	404.48ef	6916.4h
		T ₃	363.34g	5925.7i
	H ₂	T ₁	411.47e	6958.4gh
		T ₂	414.9e	6972.7gh
		T ₃	412.51e	6975.4gh
	H ₃	T ₁	430.46de	7141.1fg
		T ₂	432.34de	7155.6fg
		T ₃	431.00de	7145.5fg
	H ₄	T ₁	413.5e	7001.3gh
		T ₂	419.61e	7078.1g
		T ₃	418.57e	7045.8g
I ₃	H ₁	T ₁	411.62e	6953.2gh
		T ₂	405.42ef	6856h
		T ₃	404.16ef	6887h
	H ₂	T ₁	417.27e	7003g
		T ₂	419.78e	7078.3g
		T ₃	418.45e	7047.5g
	H ₃	T ₁	436.64de	7232.8f
		T ₂	444.47d	7316.4f
		T ₃	438.39d	7262.1f
	H ₄	T ₁	427.14e	7154.4fg
		T ₂	435.91de	7175.3fg
		T ₃	431.52de	7162fg

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test
 I₁: Control or normal irrigation, I₂: Cut irrigation at ear emergence, I₃: Cut irrigation 7 days after ear emergence
 H₁: Control, H₂: 1000 ppm.ha⁻¹ Humic acid, H₃: 2000 ppm.ha⁻¹ Humic acid, H₄: 4000 ppm.ha⁻¹ Humic acid

5. Conclusion

In general, the findings of the present study showed that the application of humic acid and Titanium Dioxide Nanoparticles had positive effects on Corn crop, and by increasing the crop resistance to stress conditions, it contributes significantly to its better growth and higher yield. Under water deficit stress conditions, reduced absorption and increased wastage of humic acid fertilizer due to water deficit in the soil, reduced the positive effect of increasing humic acid on increasing grain yield. According to the results obtained from this study, use of 2000 ppm.ha⁻¹ humic acid and 0.02% ha Titanium Dioxide Nanoparticles as foliar sprays is recommended under water deficit stress conditions led to produce economic yield and advised to producer in studied region.

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Authors Contribution

All authors are equally involved.

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Conflict of interests

Authors declared no conflict of interest.

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