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Full Length Article

Exploring the impact of titanium dioxide nanoparticles ($nTiO_2$) at varied concentrations in combination with *Azospirillum brasilense* on wheat growth and physiology

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ABSTRACT

Background: Nanoparticles (NPs), as a novel source of Nano fertilizers in crop production. Titanium dioxide nanoparticles ($nTiO_2$) also have the potential to improve plant growth, but their effect on the wheat crop is not studied enough. Nothing is known about that how $nTiO_2$ specifically effect on wheat crops especially with soil microbes and how much its dose is effective. *Azospirillum brasilense* is a promising bio-fertilizer that can be applied in combination with nano-fertilizers to increase crop productivity and can enhance the efficiency of other fertilizers.
Methods: The present study was planned to investigate the role of different doses of titanium dioxide nanoparticles ($nTiO_2$) with *Azospirillum brasilense* on the growth and physiology of wheat, having three replications. Eleven treatments were planned in the field condition (T_0 = Control (No *A. brasilense* and No $nTiO_2$), T_1 = $nTiO_2$ @20 mg/L, T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*, T_3 = $nTiO_2$ @30 mg/L, T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*, T_5 = $nTiO_2$ @40 mg/L, T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*, T_7 = $nTiO_2$ @50 mg/L, T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*, T_9 = $nTiO_2$ @60 mg/L, T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*) having randomized complete block design (RCBD).
Results: Results revealed that the individual application of *A. brasilense* showed significantly higher results in all treatments, but $nTiO_2$ application shows a positive impact on wheat growth, yield, and physiological parameters when used in a lower concentration. $nTiO_2$ @30 mg/L with *A. brasilense* gives the highest results as compared to all other treatments with the production of higher antioxidant enzymes, nutrient uptake, higher leaf area index, and photosynthesis. Use of $nTiO_2$ @40 mg/L or with the higher dose negatively affects wheat crops, but with the *A. brasilense* application its negative effect control up to a certain level.
Conclusion: This study highlights the potential advantages of combining *A. brasilense* with $nTiO_2$, for the growth of wheat crops. While *A. brasilense* alone consistently produced favorable results, lesser doses of $nTiO_2$ also showed promising results but with the combination of $nTiO_2$ and *A. brasilense* @ 30 mg/L producing the greatest outcomes. The detrimental effects of excessive nanoscale titanium dioxide on plant growth and development,

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which may result in stress and physiological abnormalities in the plants, are probably the cause of the decrease in crop output at greater $nTiO_2$ concentrations. However, care should be used while utilizing larger $nTiO_2$ concentrations since they could harm wheat crop growth and yield.

1. Introduction

Wheat (*Triticum aestivum*) is the main food crop for the one-third population of the world (FAO, 2023). The fertile land of Iraq, Syria, Jordan, Palestine, and Lebanon is the origin of the wheat crop. The wheat crop can be cultivated in a wide range of environmental conditions from arid, semi-arid, and dry regions of different countries, event it can also be a well-grown crop in the cool and wet regions of North America and Europe (Mujeeb-Kazi et al., 1996). Wheat crop is playing significant role to control the global food security and also provide the sustainability in agriculture production. It is reported by the FAO that only wheat crop is providing the 60 % of daily calories and protein needs for the people of word (FAO, 2017). But with the increasing global population, is a need for time to enhance wheat production events under the current challenges of climatic change, poor soil fertility, drought stress, and event under disease outbreaks (Hussai et al., 2021; Luo et al., 2022).

Nanoparticles are particles with a size of 1–100 nm having unique characteristics. Uses of nanoparticles in agriculture have been explored to enhance crop yield and overcome the different problems of farming. Foliar application of Nano fertilizers can improve plant physiology, can reduce the use of agrochemicals, and can save the plant from different kinds of adverse environmental conditions (Yu et al., 2024). The application of nanoparticles in agriculture is a promising development for contemporary farming methods, with numerous potential advantages (Muhammad et al., 2019). By utilizing their special physicochemical qualities, nanoparticles have the potential to greatly boost agriculture outputs. nTiO2 can act as nanocarriers for compounds that are necessary for growth and development of the plants (Khodakovskaya et al., 2012). Increased agricultural productivity is the outcome of precision delivery system, which makes sure that plants get the resources they require at the correct moment and location. Through their ability to catalyze biological processes, nanoparticles can have a significant impact on photosynthesis, respiration, and the metabolism of nutrients in plants (Ibrahim et al., 2020).

Nanoparticles such as zinc oxide, silver, and copper can improve the plant growth and physiological properties of wheat crops, but the use of the proper dose of titanium dioxide nanoparticles ($nTiO_2$) does not study enough. Different researchers reported the positive effect of $nTiO_2$ on the wheat crop (Khodakovskaya et al., 2012) and many are against it (Ibrahim et al., 2020; Muhammad et al., 2019). Some mentioned the suitable dose and some reported using its little quantity to improve particular growth traits (Ibrahim et al., 2020; Muhammad et al., 2019; Khodakovskaya et al., 2012). A titanium dioxide nanoparticle ($nTiO_2$) is becoming the most effective nanoparticle to improve and boost the different chemical reactions in plants. $nTiO_2$ is highly reactive and has the most effective catalytic properties. $nTiO_2$ can improve photosynthesis, chlorophyll content, nutrient uptake, and efficiency in plants. $nTiO_2$ has a positive effect on different traits, but it also harms human health and the environment (Khodakovskaya et al., 2012).

Different organic soil ammendments have the postive impact on plant growth and development (Jiang et al., 2023; Zhang et al., 2020). Some microbes have the positive effect on crop growth and some have the negative effect (Wang et al., 2023). *Azospirillum brasilense* is a nitrogen-fixing, gram-negative bacterium that were isolated from the maize plant roots in 1979 brazil (Baldani et al., 1983) since it is reported to be the growth-promoting bacteria in different crops such as wheat, rice, maize, etc. *A. brasilense* can produce different kinds of the growth hormones such as cytokinin and auxins that can enhance the cell division and different physiological processes of the wheat plant that result to enhance crop yield. *A. brasilense* can also improve the soil structure and fertility in the soil and can also break the chelate molecules from the soil to make available for plant uptake. Several studies reported that *A. brasilense* can enhance wheat crop growth under adverse environmental conditions and can also control the negative effect of different chemicals in the soil. *A. brasilense* has the great potential to improve soil fertility and plant growth, so it can use as a bio-fertilizer for sustainable crop production (Baldani et al., 1983).

Azospirillum brasilense can also control the negative effect of different nanoparticles in the plant by enhancing cell division with the production of cytokinins in the plant. Some nanoparticles harm wheat crops when used in higher amounts, so the use of *A. brasilense* in crop production can alter the plant morphology and can alleviate the negative effect (Singh et al., 2018). A different study was reported to control the toxicity of silver nanoparticles with *A. brasilense*. *A. brasilense* with the wheat seed inoculation is recommended by many researchers to enhance growth and yield under adverse environmental conditions (Baldani et al., 1983) but little study available with the $nTiO_2$. So, the present study was planned to understand the role of different doses of titanium dioxide nanoparticles ($nTiO_2$) with the seed inoculation of *Azospirillum brasilense*, on the growth and physiology of wheat crops.

We hypothesized that the *Azospirillum brasilense* can control the negative effect of $nTiO_2$ on wheat growth and yield. Our current study will also provide a suitable dose of $nTiO_2$ that can enhance yield and can also provide directions to understand the combined effect of *Azospirillum brasilense* and $nTiO_2$ on the growth and physiology of wheat crops.

2. Materials and methods

2.1. Experimental site and design

A well-planned experiment having three replications was conducted with randomized complete block design (RCBD) in the field conditions to investigate the role of different doses of titanium dioxide nanoparticles ($nTiO_2$) with *A. brasilense* on the growth and physiology of wheat crop at agricultural research area, the Khwaja Fareed University of Engineering and Information Technology (KFUEIT), Rahim Yar Khan, Pakistan during 2021–22. "Galaxy-2013" approved wheat variety by Punjab seed corporation was obtained from the Regional Agriculture Research Institute (RARI), Bahawalpur. Experiment was consisted of 11 treatments (T_0 = Control (No *A. brasilense* and No $nTiO_2$), T_1 = $nTiO_2$ @20 mg/L, T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*, T_3 = $nTiO_2$ @30 mg/L, T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*, T_5 = $nTiO_2$ @40 mg/L, T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*, T_7 = $nTiO_2$ @50 mg/L, T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*, T_9 = $nTiO_2$ @60 mg/L, T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*. Titanium dioxide nanoparticles ($nTiO_2$) were obtained from the institute of physics, KFUEIT, and applied on the tillering stage of the wheat crop (After 20 days of germination). *Azospirillum brasilense* bacterial strains were obtained from the Government College (GC), University of Lahore, and inoculated with wheat seeds before the sowing of the crop. Wheat seed was inoculated with the bacterial strain by following the procedure described by Fukami et al. (2016) but before the seed inoculation, the wheat seed was sterilized with 70 % ethanol and sodium hypochlorite. The average humidity and temperature during this experiment at the experimental site is given in Table 1. Soil analysis was done before the sowing of wheat crops in the research area, and the experimental soil properties are shown in Table 2. The sowing date was 15th November 2022. Recommended four irrigations were applied, 1st at tillering stage, 2nd at booting, 3rd at the anthesis stage, and 4th was at the grain filling. Fertilizers were also applied according to the

Table 1
Average humidity and temperature of the experimental site (2021–22).

Month	Humidity (%)	Temperature (°C)
November	62	21
December	72	15
January	74	14
February	69	19
March	63	24

Table 2
Properties of soil in experimental area.

Parameters	2021–2022
Organic matter (%)	0.75
Ph	7.51
EC (μS/cm)	225
T.S.S. (%)	0.53
Available-P (ppm)	5.13
Available-K (ppm)	114
Saturation percentage	32
Soil separates	
Sand (%)	37
Silt (%)	39
Clay (%)	24
Textural	Loam Soil

recommendations of the Agriculture Department, Punjab (120–80-60 NPK.

2.2. Measured parameters

Crop growth and yield-related parameters (Plant height, spike length, spikelets per spike, grains per spike, 1000-grain weight, and grain yield) were recorded after harvesting the crop by following the standard procedure. All other physiological and biochemical analysis was done by taking the samples at the anthesis stage of the wheat crop. An infrared gas analyzer (Cl-340) was used to measure the photosynthetic rate and stomatal conductance of wheat crop and chlorophyll contents were observed with the use of a chlorophyll meter (CL-1). LAI and CGR were noticed according to the procedure described by Gardner et al. (1985). Macronutrient (NPK) uptake was noticed in the anthesis stage of wheat crop by following the procedure described by Bar-Tal et al. (2004). Proline contents wan noticed in μg/g by following the Spectrophotometry and ninhydrin method reported by Bates et al. (1973). The leaf sample (0.5 g) was kept at 4 °C in distilled water for 24 h in the room. Turgid weight was recorded and put the samples in the oven to dry them noticed dry weight, for 48 h at 65 °C. RWC was noticed by using the following formula described by Barr and Weatherley (1962),

$$RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$$

Electrolyte leakage (EL) was noticed by using the procedure described by Zaheer et al. (2019) and with the use of Sullivan and Ross's (1979) equation given below,

EL ¼ Initial electrical conductivity = Final electrical conductivity

Enzymatic activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were noticed by using the standard procedure reported by Nakano and Asada (1981), Vanacker et al. (2000) and Beyer and Fridovich (1987), respectively.

2.3. Statistical analysis

Data from the three replications was analyzed and LSD (least significant difference) obtains by using the computer statistical software statistix 8.1 at 5 % probability level.

3. Results

Data regarding the growth and yield of wheat (Table 3) shows that the titanium dioxide nanoparticles ($nTiO_2$) and *Azospirillum brasilense* significantly effected on wheat plant. Highest plant height (99.35 cm), spike length (13.27 cm) and number of spikelets per spike (26.23) were noticed in T_4 when $nTiO_2$ applied @30 mg/L with the seed inoculation of wheat with *A. brasilense* followed (Plant height: 98.32 cm, spike length: 12.97 cm, number of spikelets per spike: 25.12) by T_3 when applied $nTiO_2$ @30 mg/L only and lowest results were seen in T_9 (Plant height: 89.23 cm, spike length: 07.12 cm, number of spikelets per spike: 16.42) when $nTiO_2$ applied @60 mg/L without *A. brasilense*. Number of grains per spike, 1000-grain weight and grain yield were also significantly affected by the $nTiO_2$ and *Azospirillum brasilense*. $nTiO_2$ application improve the plant growth but when applied on lower concentration till 30 mg/L after that it adversely effected on growth and yield related parameters. Highest grain per spike (49), 1000-grain weight (38.23 g) and grain yield (5191 kg/ha) were noticed in T_4 followed by T_3 (Grains per spike: 47, 1000-grain weight: 36.34 g, grain yield: 5045 kg/ha) and lowest were noticed in T_9 (Grains per spike: 29, 1000-grain weight: 18.35 g, grain yield: 4162 kg/ha).

Photosynthetic rate, leaf area index (LAI), and stomatal conductance were positively affected with the application of $nTiO_2$ till 30 mg/L and with the higher concentration of $nTiO_2$ all mentioned parameters negatively affected but *A. brasilense* seed inoculation overcome the negative effect and improved the Photosynthetic rate, leaf area index and stomatal conductance (Table 4). Highest photosynthetic rate (9.32 μmol m^{-2} s^{-1}), LAI (3.91), and stomatal conductance (262 $mmolm^{-2}$ s^{-1}) was noticed in T_4 followed by T_3 (photosynthetic rate: 9.19 μmol m^{-2} s^{-1}, LAI: 3.78, stomatal conductance: 256 $mmolm^{-2}$ s^{-1}) and lowest were observed in T_9 (photosynthetic rate: 7.33 μmol m^{-2} s^{-1}, LAI: 2.03, stomatal conductance: 168 $mmolm^{-2}$ s^{-1}). Electrolyte leakage linked with the physical damage of cell membrane. Highest electrolyte leakage was noticed in T_9 (68 %) Followed by T_{10} (65 %) and lowest were noticed in T_4 (21 %).

Chlorophyll content, crop growth rate (CGR), relative growth rate (RGR) and proline contents of wheat significantly affected by all treatments (Table 5). $nTiO_2$ application with lower concentration is helpful to improve mentioned parameters and with *A. brasilense* seed inoculation, it is more beneficial. Highest chlorophyll content (84.92 %), CGR (10.33 $gm^{-2}day^{-1}$), RGR (89 %) and proline contents (48.32 μg/g) were noticed in T_4 followed by T_3 (chlorophyll content: 83.33 %, CGR: 10.01 $gm^{-2}day^{-1}$, RGR: 87 %, proline contents: 47.34 μg/g) and lowest results were observed in T_9 (chlorophyll content: 74.34 %, CGR: 07.34 $gm^{-2}day^{-1}$, RGR: 65 %, proline contents: 38.53 μg/g). Data regarding N-P-K uptake by wheat plant shows that nutrients uptake also significantly affected with all studied treatments. Highest N-P-K uptake (0.064–1.92–6.19 mg/g) was noticed in T_4 followed by T_3 (0.050–1.83–6.00 mg/g) and lowest (0.018–0.93–5.72 mg/g) was noticed in T_9. $nTiO_2$ application above of 30 mg/L negatively affected on nutrients uptake but *A. brasilense* seed inoculation also helpful to overcome this damage (Table 6).

Antioxidant enzymes protect the plants from oxidative stress and detoxify reactive oxygen species (ROS). Ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (POD), Superoxide dismutases (SOD) activities increase with the application of *A. brasilense* in all treatments but with the application of $nTiO_2$ their activities increased till the application of 30 mg/L but after that their activities decreased (Figs. 1–4). Highest APX (3.29 Umg^{-1} Protein), CAT (7.32 Umg^{-1} Protein), POD (7.43 Umg^{-1} Protein) and SOD activities (289 Umg^{-1} Protein) were noticed in T_4 when $nTiO_2$ applied @30 mg/L with *A. brasilense*, followed by T_3 (APX: 3.16 Umg-1Protein, CAT: 7.03 Umg^{-1} Protein, POD: 7.05 Umg-1Protein, SOD: 281 Umg^{-1} Protein) when only $nTiO_2$ applied @30 mg/L without *A. brasilense* application. Lowest enzymatic activities (APX: 2.26 Umg^{-1} Protein, CAT: 3.76 Umg^{-1} Protein, POD: 3.81 Umg^{-1} Protein, SOD: 227 Umg^{-1} Protein) were seen in

Table 3
Effect of $nTiO_2$ with *A. brasilense* on the growth and yield of wheat.

Treatments	Plant Height (cm)	Spike Length (cm)	Number of Spikelets per Spike	Number of Grains per Spike	1000-Grain Weight (g)	Grain yield (kg/ha)
T_0 = Control (No *A. brasilense* and No $nTiO_2$)	95.32 e	11.12 d	22.35 e	41 e	30.21 e	4756 e
T_1 = $nTiO_2$ @20 mg/L	96.34 d	11.89c	23.34 d	43 d	32.25 d	4862 d
T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*	97.54c	12.23b	24.54c	45c	34.25c	4954c
T_3 = $nTiO_2$ @30 mg/L	98.32b	12.97 a	25.12b	47b	36.34b	5045b
T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*	99.35 a	13.27 a	26.23 a	49 a	38.23 a	5191 a
T_5 = $nTiO_2$ @40 mg/L	93.22 g	10.26f	20.33 g	37 g	26.12 g	4546 g
T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*	94.34f	10.89 e	21.35f	39f	28.26f	4638f
T_7 = $nTiO_2$ @50 mg/L	91.21 i	09.32 h	18.21 i	33 i	22.54 i	4328 i
T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*	92.31 h	09.98 g	19.32 h	35 h	24.28 h	4461 h
T_9 = $nTiO_2$ @60 mg/L	89.23 k	07.12 j	16.42 k	29 k	18.35 k	4162 k
T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*	90.12 j	08.93 i	17.28 j	31 j	20.17 j	4211 j

Table 4
Effect of $nTiO_2$ with *A. brasilense* on photosynthetic rate, leaf area index, Stomatal conductance and electrolyte leakage of wheat crop.

Treatments	Photosynthetic rate (Pn = μmol m^{-2} s^{-1})	Leaf area index (LAI)	Stomatal conductance (mmolm^{-2} s^{-1})	Electrolyte leakage (%)
T_0 = Control (No *A. brasilense* and No $nTiO_2$)	8.52 e	3.17 e	228 e	41 g
T_1 = $nTiO_2$ @20 mg/L	8.76 d	3.33 d	234 d	36 h
T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*	8.94 c	3.56 c	248 c	31 i
T_3 = $nTiO_2$ @30 mg/L	9.19 b	3.78 b	256 b	26 j
T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*	9.32 a	3.91 a	262 a	21 k
T_5 = $nTiO_2$ @40 mg/L	8.16 g	2.77 g	207 g	51 e
T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*	8.36f	2.98 f	216 f	46 f
T_7 = $nTiO_2$ @50 mg/L	7.77 i	2.34 i	186 i	61 c
T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*	7.96 h	2.57 h	198 h	56 d
T_9 = $nTiO_2$ @60 mg/L	7.33 k	2.03 k	168 k	69 a
T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*	7.53 j	2.23 j	176 j	65 b

T_9 when $nTiO_2$ applied @60 mg/L without *A. brasilense*.

4. Discussion

Titanium dioxide nanoparticles ($nTiO_2$) foliar application significantly affected the growth, yield, and physiology of wheat crops, but up to a certain concentration. Our results follow the results of many researchers such as Zhang et al. (2017), Saber and Abdeen (2021) reported the positive effect of $nTiO_2$ on plant growth but with the lower concentration. Different researchers reported different concentrations, but our study conducted in the hot region of the desert shows that the $nTiO_2$ application up to 30 mg/L positively affected wheat growth and yield, but after that with the increase of the concentration it negatively affected. A 10.14 % increase in wheat grain yield was noticed with the application of $nTiO_2$ @30 mg/L but when we increase the $nTiO_2$ concentration @40 mg/L grain yield decreased by 3.93 % and this percentage increased with increasing the $nTiO_2$. Zhang et al. (2017) also reported that the higher concentration of $nTiO_2$ negatively affected plant growth. Several researchers (Wang et al., 2017) also reported that the $nTiO_2$ application on plants in high-temperature areas negatively affects growth and yield, so there might be the cause of our results that the $nTiO_2$ application up to 30 mg/L is beneficial and after that, it adversely affects. $nTiO_2$ application with lower concentration can improve the chlorophyll contents in the leaf, so it is very beneficial to

Table 5
Effect of $nTiO_2$ with *A. brasilense* on chlorophyll content, crop growth rate (CGR), relative growth rate (RGR) and proline contents of wheat.

Treatments	Chlorophyll Content (%)	CGR (g m^{-2} day^{-1})	RWC (%)	Proline Content (μg/g)
T_0 = Control (No *A. brasilense* and No $nTiO_2$)	80.31 e	09.12 e	82 e	44.33 e
T_1 = $nTiO_2$ @20 mg/L	81.45 d	09.44 d	83 d	45.62 d
T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*	82.65c	09.74c	85c	46.23c
T_3 = $nTiO_2$ @30 mg/L	83.33b	10.01b	87b	47.34b
T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*	84.92 a	10.33 a	89 a	48.32 a
T_5 = $nTiO_2$ @40 mg/L	78.33 g	08.67 g	78 g	42.45 g
T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*	79.34f	08.89f	81f	43.34f
T_7 = $nTiO_2$ @50 mg/L	76.34 i	08.01 i	73 i	40.45 i
T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*	77.33 h	08.45 h	75 h	41.36 h
T_9 = $nTiO_2$ @60 mg/L	74.34 k	07.34 k	65 k	38.53 k
T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*	75.45 j	07.78 j	69 j	39.46 j

Table 6
Effect of $nTiO_2$ with *A. brasilense* on NPK-Uptake of wheat plant.

Treatments	N-Uptake (mg/g)	P-Uptake (mg/g)	K-Uptake (mg/g)
T_0 = Control (No *A. brasilense* and No $nTiO_2$)	0.042 e	1.56 e	5.94 e
T_1 = $nTiO_2$ @20 mg/L	0.046 d	1.63 d	5.97 d
T_2 = $nTiO_2$ @20 mg/L + *A. brasilense*	0.050 c	1.75 c	6.00 c
T_3 = $nTiO_2$ @30 mg/L	0.064 b	1.83 b	6.13 b
T_4 = $nTiO_2$ @30 mg/L + *A. brasilense*	0.068 a	1.92 a	6.19 a
T_5 = $nTiO_2$ @40 mg/L	0.034 g	1.36 g	5.88 g
T_6 = $nTiO_2$ @40 mg/L + *A. brasilense*	0.038f	1.43f	5.91f
T_7 = $nTiO_2$ @50 mg/L	0.026 i	1.15 i	5.81 i
T_8 = $nTiO_2$ @50 mg/L + *A. brasilense*	0.030 h	1.26 h	5.84 h
T_9 = $nTiO_2$ @60 mg/L	0.018 k	0.93 k	5.72 k
T_{10} = $nTiO_2$ @60 mg/L + *A. brasilense*	0.022 j	1.09 j	5.77 j

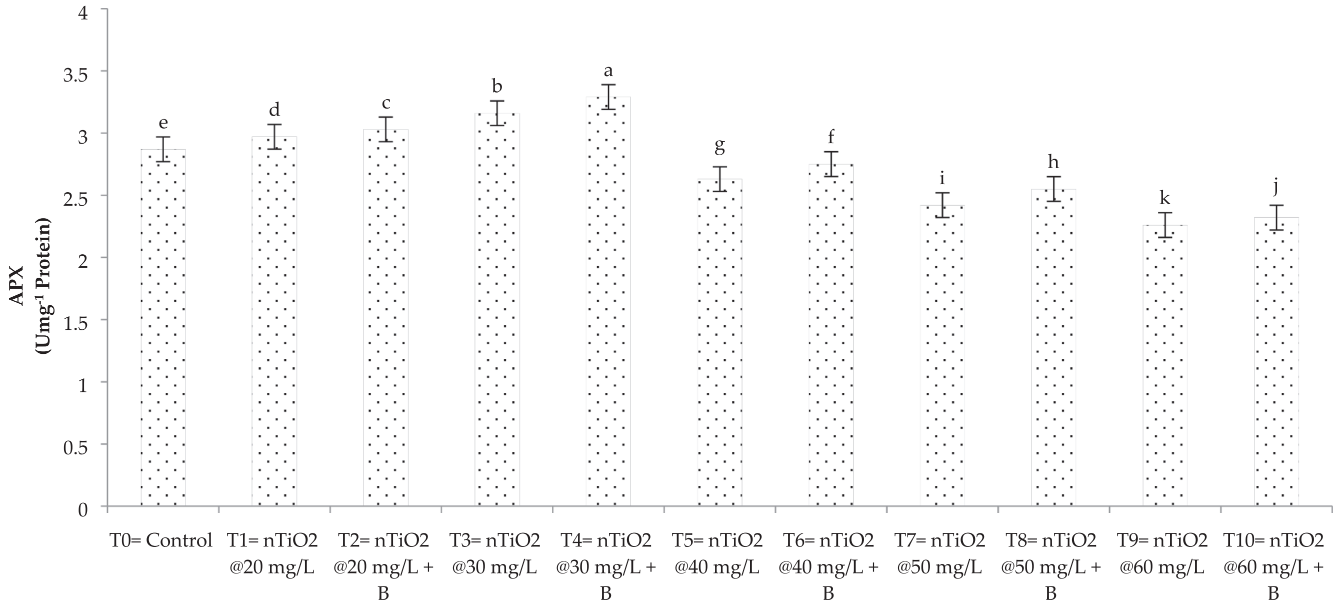

Fig. 1. Effect of $nTiO_2$ with *A. brasilense* on APX (ascorbate peroxidase) of wheat plant. B (*A. brasilense* bacterial strain), $nTiO_2$ (titanium dioxide nanoparticles).

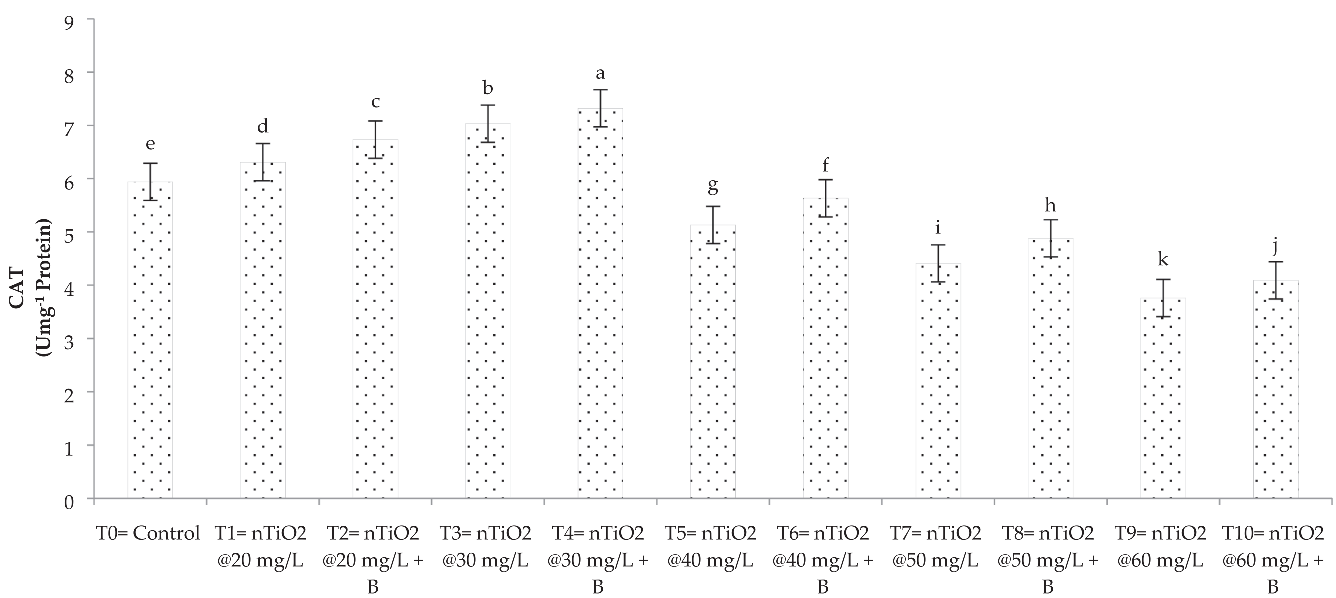

Fig. 2. Effect of $nTiO_2$ with *A. brasilense* on CAT (catalase) of wheat plant. B (*A. brasilense* bacterial strain), $nTiO_2$ (titanium dioxide nanoparticles).

improve the growth and yield-related parameters such as plant height, spikelets per spike, and grain weight. Nour et al. (2019) also reported that the foliar application of $nTiO_2$ increases the root-shoot ratio in the plant, which can enhance the plant growth rate and yield. Seed inoculation of agricultural crops with soil bacteria showed the positive effect on growth and yield (Qin et al., 2022). Zaheer et al. (2019) reported that the *A. brasilense* inoculation enhances the nutrient uptake from the soil and enhances the growth hormones produced in the plant, helpful to increase wheat growth and yield. Combining the application of *A. brasilense* with $nTiO_2$ @30 mg/L is more beneficial as compared to the other treatments. It was also observed that the *A. brasilense* controls the negative effect of the higher concentration of $nTiO_2$.

A. brasilense inoculation with the wheat seed also enhances the leaf area index and chlorophyll contents in the leaf by the production of more phytohormones such as cytokinin, gibberellins, and Indole-3-acetic acid (Lu et al., 2022). Combining the application of $nTiO_2$ @30 mg/L with seed inoculation is more beneficial to increase the chlorophyll contents and leaf area index. This increase in the LAI and chlorophyll content also enhances the photosynthetic rate and stomatal conductance. Increasing the photosynthetic rate by up to 23 % was noticed with *A. brasilense* seed inoculation. Wang et al. (2017) reported that the nanoparticle foliar application enhances and facilitates the plant for more nutrient's uptake from the soil and on the other hand availability of the *A. brasilense* in the root zone enhances nutrients available for plant uptake (Zaheer et al., 2021) so their combine application enhance the growth hormones and physiological processes in the wheat crop that enhance the LAI and Chlorophyll contents. More LAI and chlorophyll contents lead to a higher photosynthetic rate and stomatal conductance. *A. brasilense* and $nTiO_2$ application enhance the cytokinin production in the plant that enhances the cell division, leaves expansion, and chlorophyll accumulation, all these things directly enhance the photosynthetic rate, LAI, stomatal conductance, and chlorophyll contents (Zaheer et al., 2019; Nour et al., 2019; Saber and Abdeen 2021).

Electrolyte leakage (EL) is used to determine the cell membrane

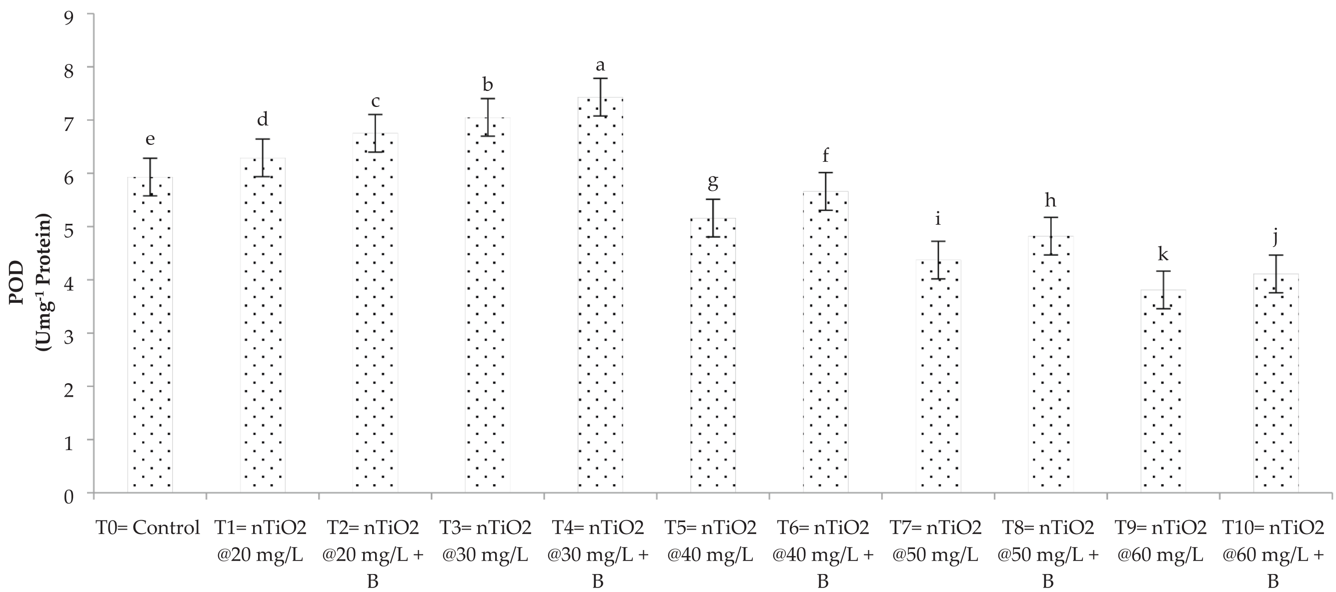

Fig. 3. Effect of $nTiO_2$ with *A. brasilense* on POD (guaiacol peroxidase) of wheat plant. B (*A. brasilense* bacterial strain), $nTiO_2$ (titanium dioxide nanoparticles).

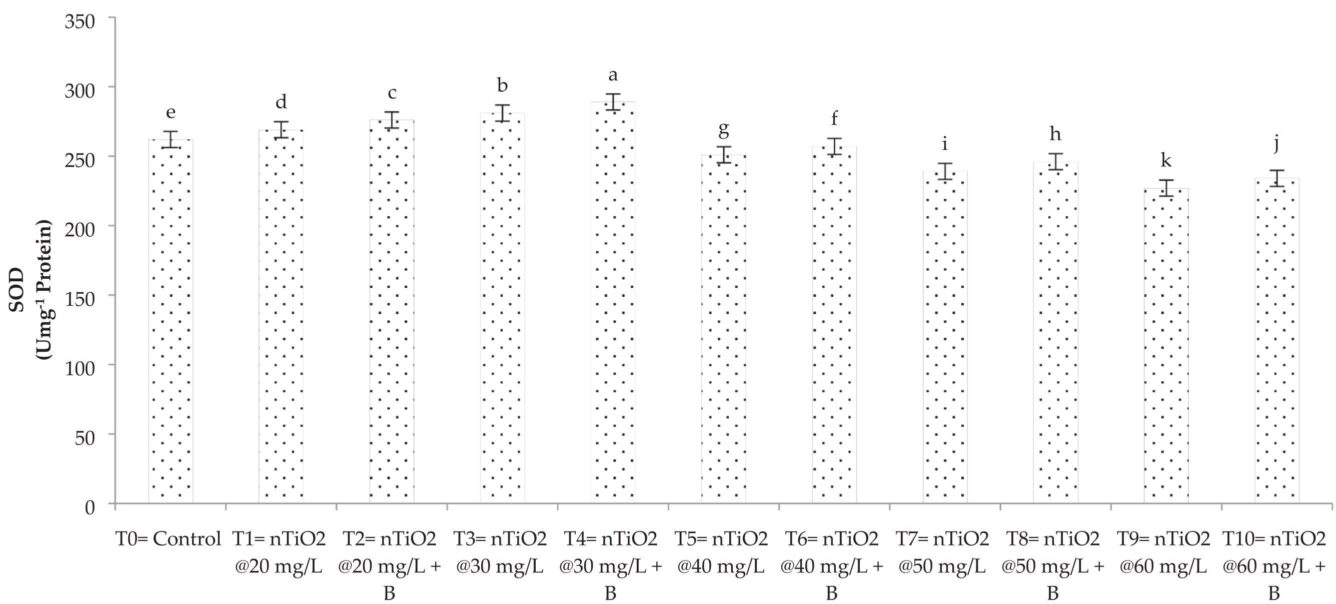

Fig. 4. Effect of $nTiO_2$ with *A. brasilense* on SOD (Superoxide dismutases) of wheat plant. B (*A. brasilense* bacterial strain), $nTiO_2$ (titanium dioxide nanoparticles).

damage due to any stress condition. Higher EL decreases crop yield and growth-related parameters (Zhang et al., 2019). It was observed that $nTiO_2$ application higher than @30 mg/L leads towards higher EL in wheat plant leaf, which means higher EL level converting the higher $nTiO_2$ application towards the stress condition. 68.29 % increase in EL was noticed with the application of $nTiO_2$ @60 mg/L but the application of *A. brasilense* with the same $nTiO_2$ dose decrease this level up to 57.32 %. *A. brasilense* application with $nTiO_2$ @30 mg/L showed the best results. Zaheer et al. (2022) reported that *A. brasilense* decreases the EL by enhancing the antioxidant enzyme production in wheat plants with higher growth hormone production. Pii et al. (2019) also reported that the *A. brasilense* decrees EL in the wheat plant by increasing cell growth with the production of cytokinin which is essential for cell division. *A. brasilense* control the negative effect of the higher concentration of $nTiO_2$ with the production of cytokinin, Zaheer et al. (2022) and Zaheer et al. (2019) reported that the *A. brasilense* stimulate the production of enzymes and allow more cell division and cell expansion.

Many researchers reported the positive effect of $nTiO_2$ and *A. brasilense* on the nutrient's uptake by the plant (Wang et al., 2017; Singh et al., 2015) but no one explains the proper concentration of $nTiO_2$. Our results show the positive effect of $nTiO_2$ on the macronutrient (N-P-K) uptake by the wheat plant but till the 30 mg/L concentration, with the higher concentration adverse effect was noticed. *A. brasilense* application increases the nutrient's uptake by the wheat by improving the soil fertility status and availability of nutrients and improves the root development that is also very beneficial for the NPK Uptake with the $nTiO_2$ (Lin et al., 2021; Singh et al., 2015). *A. brasilense* seed inoculation with 30 mg/L $nTiO_2$ is very effective for nutrient uptake and improving wheat crop growth.

Leaf relative water contents and Proline content is significantly affected by the application of $nTiO_2$ and *A. brasilense*. Combining the application of $nTiO_2$ @30 mg/L with *A. brasilense* is more effective as compared to the other treatments. Different researchers show the different effects of $nTiO_2$ on RWC, Wang et al. (2017) reported the negative effect of $nTiO_2$ on leaf RWC due to the damage to the cell membrane and disturb the plant water mechanism but on the other hand, $nTiO_2$ have the positive effect on RWC but when to use in lower concentration, with increasing the higher concentration RWC becomes decreased. Lower concentrations of $nTiO_2$ improve the water use efficiency in the wheat plant that helpful in main the leaf turgor pressure and RWC (Ma et al., 2019). *A. brasilense* enhance the water and nutrients uptake from the soil which is helpful to enhance root growth, which enhances the water availability for wheat growth and maintains higher leaf RWC under stress condition (Zaheer et al., 2019). *A. brasilense* improves the RWC to improve the stomatal conductance and reduce the transpiration loss. Pereyra et al. (2012) reported the production of osmo protectants with the seed inoculation of *A. brasilense* that can enhance leaf turgor pressure and RWC.

$nTiO_2$ enhance the proline content in wheat crop due to an increase in the root shoot ratio, physiological process, and plant growth. Wang et al. (2017) reported higher proline accumulation with the application of $nTiO_2$ which saves the plant from oxidative damage. Our results also supported the same thing but till the application of $nTiO_2$ @30 mg/L on higher concentrations it effects negatively on proline content. Seed inoculation with *A. brasilense* increases the proline contents and antioxidant enzyme activity (APX, CAT, POD, and SOD) in wheat crops (Zaheer et al., 2021; Zaheer et al., 2022). Ghosh et al. (2016) observed higher SOD and CAT activities with $nTiO_2$ application, but POD activity was unaffected. Wang et al. (2017) also reported higher SOD, CAT, POD, and APX activities with $nTiO_2$ application in maize. Cytokinin production with the *A. brasilense* maintaining physiological functions under stress conditions and reduce the production of reactive oxygen species (ROS), Lower the ROS and availability of nutrients and favorable growth conditions enhance the antioxidant enzyme activity (Zaheer et al., 2021). Synthesis of the P5CS gene can increase the proline formation with increasing the cytokinin production in the plant cell. More microbial activities and organic matter in the soil leads towards positive impact on the plant growth (Zhang et al., 2021). Zaheer et al., (2021,2022) reported the higher cytokinin formation with the seed inoculation of *A. brasilense* that can leads towards the higher proline production.

5. Conclusions

Titanium dioxide nanoparticles ($nTiO_2$) are effective for wheat growth, yield, and physiological parameters, but their concentration more than of 30 mg/L adversely affected wheat growth and yield-related parameters. Combining application of $nTiO_2$ @30 mg/L with *A. brasilense* is very effective for the wheat crop. It is recommended to not use the higher concentration of $nTiO_2$ for crop production and to use *A. brasilense* for sustainable agriculture.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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