

Efficiency of Nanoparticles (Titanium Dioxide and Zinc Oxide) in Stimulating the Growth of Different Cultivars of Wheat Plants *Triticum aestivum* **L**

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Doi:10.29072/basjs.2021210

Abstract

The wheat plant, *Triticum aestivum* L., is one of top cereal crops in the world, followed by the rice and corn crops; and it is one of the most important food crops for more than a third of the world's population. in recent times, several studies focused on finding ways to increase and encourage growth and production of wheat, and among these methods is the use of nanomaterials. The present study has been conducted to evaluate the nanomaterials titanium dioxide $(TiO₂)$ and zinc oxide (ZnO) efficacy in the growth promoting of three cultivars of wheat plant (Ibaa 99, Abu Ghraib and Bhooth 22). Laboratory experiments proved the non-toxicity of $TiO₂$ and ZnO on the germination of wheat seeds. All treatments were 100% germinated, whereas the results showed the superiority of $TiO₂$ in stimulating all examined parameters. The examined parameters, whether in Petri plates or pots, including the length of the radicle and hypocotyl, compared to a control treatment. The results of the pots experiment showed that the concentration of 100 ppm significantly exceeded the rest of the tested concentrations (0, 25 and 50 ppm), and the Abu Ghraib cultivar was the most responsive to nanomaterials. It is worth noting that the nanomaterials $TiO₂$ and ZnO at a concentration of 100 ppm led to an increase in some biochemical indicators, including photosynthesis pigments, carbohydrate content and proline, compared to the control treatment in the cultivar Abu Ghraib .The current study recommends that TiO² and ZnO should be used in the treatment of wheat plants, with more studies being *Received:*

conducted on other economic plants.

Article inf.

15/5/2021 Accepted: 29/7/2021 Published: 31/8/2021

keywords:

Biochemical indicators, *in vitro*, poaceae, SEM, wheat

1. Introduction:

The wheat *Triticum aestivum* L., belongs to the Poaceae family and occupies the top position in the list of cereal crops in the world, followed by the rice and corn crops [1], and it is one of the most important food crops for more than a third of the world's population as it contains proteins and calories more than any other crop, it contains 7-22% of protein and 55% of carbohydrates, and 20% of the calories. Wheat is grown over large areas in the world, with a total production up to 734.74 million tons in the agricultural season 2018-2017 [2], while global production of wheat reached about 761.5 million tons worldwide in the year 2020 [3]. In Iraq, the total production of wheat crop reached 4,343 million tons for the year 2019, while Basrah governorate recorded a total production of 29021 tons [4]. As a consequence of high increase in the population, Iraq imported more than two-thirds of its need of wheat [5]. The cultivation as well as production of wheat suffers from a lack of production for many reasons, including a lack of key nutrients, diseases and insects.

 Nanotechnology has emerged using nanomaterials in order to improve growth and production in economic plants, as demonstrated by many applied studies [6, 7, 8]. Nanotechnology has also been used to enhance plant defense responses and in genetic manipulation of plant cells [9], as well as used in modifying the forms of chemical pesticides to make them more effective and less harmful. Nanoparticles were also used in the manufacture of nanopesticides because of their high efficiency in killing pathogens even though at the lowest concentrations. These nanoparticles are used as fungicide carriers and encapsulation of active ingredients in nanocapsules [10]. Global production of nanoparticles is expected to reach 10,000 tons, and total sales are expected to reach 50 billion dollars in 2026 [11]. Rico *et al.* [12] indicated that after nanoparticles entered the plant cell, they can be transported in the form of symplasm or apoplasm and can be transferred through the plasma membrane from one cell to another, the nanoparticles, and once the nanoparticles become in touch with the plant cell surface, they entered through stomatal openings and move towards different tissues. It has also been pointed out that the absorption, transfer or transformation, and accumulation of nanoparticles in plants is a very important state related to the plant species, the stage of growth and the growth environment, functions, biochemistry, and the method of delivery [13-15]. The present study aims to prepare two types of nanomaterials, namely titanium dioxide and zinc

oxide at the laboratory, and to determine their stimulation effects for the growth of different wheat cultivars, based on phenotypic and biochemical characteristics.

2. Materials and Methods

2.1 Preparation of nanomaterials

A 1 g of pure zinc oxide (ZnO) and titanium dioxide $TiO₂$ were taken, and add into 50 ml of distilled water, and transferred to the Sonication device to prepare the ZnO and $TiO₂$ particles nanostructures, the sample time for ultrasound was fixed to 2 seconds and the extinguishing time was 1 second and the total preparation time was 15 minutes. A sample was taken from the prepared material and deposited on crystalline silicon slides for the purpose of SEM scanning electron microscopy [16].

2.2 Phytotoxicity of nanomaterials on the wheat plant

- *On the seeds of different cultivars of wheat in the petri dishes*

Petri dishes with a diameter of 9 cm were used, and 10 seeds were placed in each plate for three cultivars of wheat which were Abu Ghraib, Ibaa 99 and Bhooth 22, with triplicates each, and the concentrations were used 0, 25, 50, 75 and 100 ppm of each substance (Zno and $TiO₂$) [17]. A 5 ml of each treatment was added, and then the treated dishes were placed, as well as the control treatment, in the growth room (incubator) at a temperature of 25 \degree C. Then the germination rate of wheat seeds was calculated after seven days of treatment. The radicle has been separated from the hypocotyl for the growing seedlings and their lengths were measured, then they were dried in an electric oven at 70 \degree C for 72 hours, and then the dry weights were measured [18].

- *On plants of different cultivars of wheat in pots*

Plastic pots of 1 kg, 10 cm high and 15 cm wide, were used as contain of sterile peatmoss, in this experiment three cultivars of wheat were used, which were Abu Ghraib, Bhooth 22 and Ibaa 99, and were irrigated with nanomaterials at a concentration of 0, 25, 50, 75 and 100 ppm and an amount of 250 ml 24 hours before planting the seeds, then 10 seeds were planted in each pot, with three replications for each concentration, and the plants were then watered with water

whenever needed, and a second spray was used with nanomaterials 10 days after planting, a third spray after 20 days , and after 30 days from planting. Several phenotypic characteristics of the plant were measures as:

1. Germination percentage $(GP\%) =$ Number of germinated seeds Total number of seeds X100

Average plant height (cm): The height of the plant is measured by a tape measure from the soil surface to the top of the plant.

4. Fresh and dry weight of the shoot and root (g): The fresh weight was calculated by taking three plants for each treatment by cutting the plants at their contact area with the soil then the plants were dried using an electric oven at a degree of 60 ° C for a period of 72 hours, then the dry weight was calculated using the sensitive scale.

 Some biochemical characteristics were estimated at 100 ppm in the nanomaterials treatments under study with the most responsive cultivar Abu Ghraib:

Photosynthetic pigment:

The pigments chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanins were estimated, and these pigments were extracted according to the method of [19] by grinding 200 mg of leaves into 8 ml of acetone (80%) using chilled ceramic mortar and separating the filtrate using a centrifuge (3000 rpm). Eppendorf model (R5804) German-made. Wavelengths 470, 534, 663 and 645 nm were measured in the filtrate and acetone was used as a control sample for the purpose of calibrating the device using a UV-1100D spectrophotometer manufactured by EMclAB GmbH of Germany with four cells and the values of plant pigments were estimated depending on the following equations according to [20] and expressed in units (mg / g):

Chlorophyll $-a = 12.7 (0D663) - 2.69 (0D645)$ x Vol./Wt) $Chlorophyll - b = 22.9 (OD645) - 4.68 (OD663) x Vol./Wt)$ Total chlorophyll = 20.2 (OD 645) + 8.02 (OD 663) x Vol./ Wt.) Anthocyanins = 0.0821 x A534 – 0.0439 A643 − 0.002423 x A661 Carotenoids = A 740-17.1 x (Chl–a+ Chl-b)-9.479 x anthocyanins)/119.26

Total carbohydrates

Total carbohydrates were estimated by using the modification of phenol-sulphuric acid colorimetric method [21] and expressed in mg /g dry weight unit. Absorbance was measured at a wavelength of 490 nm with a spectrophotometer. Total dissolved carbohydrates were estimated using Glucose standard curve.

Proline content

The proline content in the leaves tissues samples was estimated according to the protocol [22], by mixing 0.5 g of tissues in 10 ml of Sulphosalicylic aqueous solution 3% and the filtrate was separated using a centrifuge at a speed of 6000 rpm, then the filtrate was transferred into new test tubes and add to it 2 ml of glacial acetic acid and 2 ml of acid ninhyderine solution prepared by dissolving 1.25 g of nanhydrin in 50 ml of a mixture of 30 ml of acetic acid and 20 ml of phosphoric acid. Subsequently, the test tubes were heated in a water bath at a temperature of 100 ° C for one hour, then the reaction was stop by placing the tubes in an ice bath, then transferred in to a separating funnel and add 4 ml of toluene with shaking for 20-30 seconds and then leave it at a room temperature, until they are two separate layers, then the transparent (lower) layer is discarded. Estimate the proline content in the (upper) coloured layer by reading the absorbance at a wavelength of 520 nm using a spectrophotometer, and toluene as a control sample, the proline content was estimated using standard proline curve according to the following equation and expressed in units (m mole**/**g fresh weight):

Proline = (micrograms proline x volume of toluene (ml) / 115.5) x (5 / sample weight g) where 115.5 is the molecular weight of proline.

2.3 Statistical analysis

 All laboratory experiments were carried out using complete randomised design, and all results were compared by calculating the least significant difference (LSD). The experiments were carried out with three replications for each treatment. Data percentage was arcsine transformed, and the results were analysed with GenStat Discovery Edition software with probability levels of 0.01 in laboratory experiments and 0.05 in field experiments.

3. Results and discussion

3.1 Surface morphology of prepared nanoparticles

Figure 1 shows the nanoparticle shape of the $TiO₂$ and it was clear that the compound may form in the form of mostly spheres of different sizes, with different grain diameters dominating the range 200-250 nm, with the presence of granules at larger diameters in this range and lesser ones. It is also noticed that granules with diameters less than 100 nanometers appeared in small numbers, and tend to cluster together to form a larger granule. The second figure represents a scanning electron microscope image of the compound ZnO and shows the presence of smallsized granules within the nanoscale. The image showed that the granules were with diameters less than 100 nm, and the predominant range was 30-40 nm, with the appearance of larger masses of these grains due to their clustering together. The microscope image did not show a distinct geometrical shape for the ZnO nanoparticles as in the case of the $TiO₂$ compound in which the grains were formed as spheres.

Figure 1:FESEM images of (A) ZnO and (B) $TiO₂$ nanoparticles

3.2 The effect of nanomaterials (titanium dioxide and zinc oxide) on the seeds of different cultivars of wheat in dishes

3.2.1 Effect of nanomaterials on germination percentage of wheat

The results of the statistical analysis,indicated that there were no significant differences between the treatments, as the germination percentage reached 100%, this result proved that the two nanomaterials had nontoxic effect on wheat seeds. This result is in agreement with the results of several studies, including [23], in the ability of nanoparticles of zinc oxide in improving all indicators of wheat plant growth (germination percentage, root length, fresh and dry weight of the plant and chlorophyll content); and increasing the germination percent of onion plants [24].

3.2.2.Effect of nanamaterials on hypocotyl length (cm)

The results of the statistical analysis as shown in Table (1) proved significant differences between the cultivars in the extent of their response to the treatment with nanomaterials, as the cultivar Abu Ghraib was the most responsive to the nanomaterials reaching the highest hypocotyl length (7.07 cm), followed by bhooth cv. (6.47 cm). The results of the analysis indicated that titanium dioxide surpassed zinc oxide and recorded the highest average of hypocotyl length of 8.07 cm compared with zinc oxide, which recorded an average of 7.770 cm. The concentration of 100 ppm exceeded all examined concentrations in all treatments, regardless of the cultivar and nanomaterial, as the average of hypocotyl length increased from 5.43 cm in the control treatment to 8.68 cm, compared to the concentration of 75 ppm, which recorded a rate of 7.58 cm. The results of the statistical analysis also indicated that the treatment of the cultivar Abu Ghraib with titanium dioxide showed the best response, as it increased the length of the hypocotyl by an average of 7.15 cm, while the treatment of the cultivar with the zinc oxide showed the lowest average (5.91cm).

Table 1: The effect of different concentrations of nanomaterials (zinc oxide and titanium dioxide) on the length of hypocotyl of different wheat cultivar

3.2.3 Effect of nanomaterials on radicle length (cm)

 The results of the statistical analysis indicated that the concentration of 100 ppm exceeded all the concentrations used, as it increased the average of radicle length from 5.31 cm in the control treatment to 7.09 cm, followed by the concentration of 75 ppm, which recorded a rate of 6.94 cm. The results also showed that there were significant differences between the cultivar as Abu Ghraib cv. had a higher response than the other two cultivars, followed by a Bhooth cultivar with a length of 6.00 cm (Table 2). The results also showed that titanium dioxide recorded the highest average of length radicle which was 6.14 cm, compared to the zinc oxide, which recorded 6.04 cm. This result is consistent with what [25] and [7] found, who confirmed the role of titanium dioxide in increasing plant growth characteristics due the role of this substance in increasing the ability of plants to absorb light, which is well reflected in the photosynthesis process. It was also found that zinc oxide enhances the cation exchange capacity of the roots, because of their small size, which absorbed by the roots, and improves the ability of the roots to absorb water and dissolved nutrients, especially the nitrogen [6].

Table 2: The effect of different concentrations titanium dioxide and zinc oxide on the radicle length of wheat cultivars

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3.3 The effect of nanomaterials on wheat plant in pots

3.3.1 The effect of different concentrations of nanomaterials (titanium dioxide and zinc oxide) on the percentage of germination of wheat plant

The results of this experiment also agreed with the experiment of phtotoxicity of nanomaterials in petri dishes, where the results showed that all treatments gave a percentage of 100% germination, and confirmed that both nanomaterial were non-toxic. This result was in agreement with the results of many studies, including [26] who showed that the use of these nanoparticles leads to an increase in the productivity of the coriander plant without any toxic effects mentioned, as well as with [27] mentioned that the use of such materials, leads to an increase in all growth parameters (seed germination, an increase in the fresh and dry weight; and the chlorophyll content) in sunflower plant. It also agreed with findings of [28] that titanium had an important role in fixing nitrogen and converting inorganic nitrogen into organic nitrogen. It is known that nitrogen has an important role in building proteins and new plant tissues, which leads to improved seed germination and an increase in the fresh and dry weight of the plant. [29] mentioned in the fact that the small size of nanoparticles of zinc oxide and titanium oxide can be

easily absorbed by the plant through the stomata.) [30, 31, 32] also pointed out that due to their small size, they were able to easily penetrate the pores of the roots and thus improve the ability of the roots to absorb water and dissolved nutrients. This result also agreed with [33] that the use of zinc oxide nanoparticles leads to increased germination and productivity of peanuts, and with what [34, 35] have shown that Zinc Oxide Nanoparticles increases enzyme activity, as it is a cofactor for more than 300 enzymes inside the cell and has an important role in gene expression and protein synthesis.

3.3.2 The effect of different concentrations of nanomaterials (titanium dioxide and zinc oxide) on plant height (cm)

 The results of the statistical analysis as shown in Table (3) indicated the superiority of the Abu Ghraib cultivar from the other two cultivars, as it recorded the highest average plant height of 25.11 cm, followed by the Bhooth cultivar, which recorded an average of 23.48 cm and did not differ significantly from the Ibaa99 cultivar. The results also showed the superiority of titanium dioxide compared to zinc oxide, where an average plant height was recorded as 24.63 cm, while zinc oxide recorded an average of 23.01 cm. The results of the statistical analysis also showed the significant differences between the concentrations used .

Table 3: The effect of different concentrations of titanium dioxide and zinc oxide on the height of the wheat plant

3.3.3 The effect of different concentrations of nanomaterials on the fresh weight of the shoots (gm).

The results showed that there were no significant differences between the cultivars in the degree of their response to the treatment with nanomaterials, and the results of the statistical analysis indicated the superiority of titanium dioxide, as it recorded the highest rate of fresh weight of 0.335 g, while the average of zinc oxide was 0.014 g as shown in Table (4). Results of the statistical analysis showed that the concentration of100 had the highest average for fresh weight which was 0.370 g, while the control treatment recorded the lowest percentage of 0.273 g. The results also showed that there were no significant differences between the interaction factors of the nanomaterials and the concentration, as well as the treatment of the cultivar and the concentration.

Table 4: The effect of different concentrations of nanomaterials (titanium dioxide zinc oxide nanoparticles) on the fresh weight of the root total of wheat plant cultivars

3.3.4 The effect of different concentrations of nanomaterials on the dry weight of the shoots (g).

 The results of the statistical analysis shown in Table (5) indicated that there were significant differences between the cultivars, as the cultivar Abu Ghraib had the highest shoot dry weight of 0.034 g. While the 100 ppm concentration showed the highest dry weight (0.040 g), compared to the control which was 0.025 g.

The results of the statistical analysis also showed that there were no significant differences between the interaction treatments of the cultivar and the nanomaterial, as well as between the cultivar, concentration and nonomaterials.

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3.3.5 The effect of different concentrations of nanomaterials on the fresh and dry weight of the root system (g).

The results of the statistical analysis shown in Table (6) and (7) showed that there were no significant differences between all examined treatments in their effect on the fresh and dry weight of wheat in its different cultivars.

Table 6: The effect of different concentrations of nanomaterials on the fresh weight of the root system (g)

Table 7: The effect of different concentrations of nanomaterials on the dry weight of the root system (g)

3.4 Efficiency of the nanomaterials TiO² and ZnO (100 ppm) in growth promoting of Abu Gharib cv.

 The results that obtained from biochemical analysis indicated that the treatment of the nanomaterial titanium dioxide led to increase all examined biochemical parameters compared to control treatment and zinc oxide (Table 8). The total chlorophyll increased from 2.88 mg /g in the control treatment to 3.99 mg /g in its treatment, additionally $TiO₂$ treatment showed a significant accumulation of carotenoids and anthocyanin which reached 1.02 and 0.98 mg/g, compared to the control treatment(0.79 and 0.76 mg/g), respectively. The results of the carbohydrate content and proline were identical with the above-mentioned results in terms of stimulating titanium dioxide treatment, as they reached 2.81 and 7.69 mg/g in the control treatment, reaching 3.56 and 8.25 mg/g in $TiO₂$ at a concentration of 100 ppm, respectively. The results of increasing the stability index of plant pigments under study due to nanomaterials are in agreement with many studies that have shown that nanomaterials of titanium oxide increase the ability of plants to absorb light and thus this is reflected in the efficiency of the

photosynthesis process in the plant, including what [36] found in terms of increasing the rate of chlorophyll, carotenoids, and anthocyanin in cucumber plants. Our results were in agreement with [37] who confirmed the increase in the chlorophyll content in mint when treated with titanium oxide nanoparticles. The increase of carbohydrates content as a response to ZnO treatment is in accordance with [38] who showed an increase in the accumulation of carbohydrates in the wheat plant when treated with zinc oxide. As for the significant increase in the content of the amino acid proline in the leaves, this result also agreed with [37] in the ability of titanium oxide nanoparticles to improve growth and productivity as well as increase the proline content in the plant, as well as consistent with what [39] found.

Treatment	Abu Gharib cultivar (mg/g)						
	Chlor. a	Chlor.b	Total chlor.	Caroten.	Antho.	Carbohydrates	Pro.
Control	2.93	0.95	2.88	0.79	0.76	2.81	7.69
TiO ₂	2.20	0.82	3.99	1.02	0.98	3.56	8.25
ZnO	2.23	0.89	3.88	1.00	0.95	2.74	7.99
LSD	0.01	0.01	0.02	0.02	0.05	0.06	1.03
(0.05)							

Table 8: Efficiency of the nanomaterials $TiO₂$ and $ZnO(100$ ppm) in stimulating some biochemical parameters of the wheat plant cultivar Abu Ghraib

4. Conclusions

The results of the current study proved the efficiency of the laboratory-prepared nanomaterils titanium dioxide and zinc oxide in supporting the growth of three different cultivars of wheat plant, which were the Ibaa99 and Abu Ghraib and Bhooth 22. Results proved the non-toxicity of the examined concentrations (0, 25, 50, 75 and 100 ppm) of each nanomaterials on germinated seeds. A promoting effect in both petri plates and pots experiments on wheat cultivars were observed in $TiO₂$ treatment; $TiO₂$ treatments showed the highest level of responses in all examined wheat cultivars. Additionally; the 100 ppm concentration was the best one in wheat growth support compared to all tested concentrations. It is worth noting that the Abu Ghraib cultivar was the most responsive to all the concentrations of the two nanomaterials. Here, the current study recommends the use of $TiO₂$ and ZnO in wheat open fields; further studies are required with more economic plants.

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كفاءة بعض المواد النانوية)ثنائي أوكسيد التيتانيوم وأوكسيد الزنك(في تشجيع نمو اصناف مختلفة من *Triticum aestivum* **L. الحنطة نبات**

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المستخلص

يعد نبات الحنطة .*Triticum aestivum* L من أهم محاصيل الحبوب في العالم، متبوعاً بالرز والذرة، كونه يمثل الغذاء الرئيس لأكثر من ثلثي سكان العالم. وشهدت السنوات الأخيرة توجه الدراسات العلمية الى تشجيع نمو نبات الحنطة وزيادة أنتاجه، ومن بين التوجهات الحديثة استخدام المواد النانوية. نفذت الدراسة الحالية بهدف تقييم كفاءة بعض المواد النانوية)ثنائي أوكسيد التيتانيوم 2TiO وأوكسيد الزنك ZnO)في تشجيع نمو اصناف مختلفة من نبات الحنطة (أباء 99 وأبو غريب وبحوث 22).أثبتت التجارب المختبرية عدم سمية المادتين النانويتين في انبات بذور نبات الحنطة قيد البحث إذ كانت نسبة اإلنبات %100 في جميع المعاملات المختبرة، مع تفوق معاملة $T_{\rm iO_2}$ في تشجيع مؤشرات النمو المدروسة، حيث سجلت تجارب ~ 100 االطباق البترية والسنادين تفوق هذه المادة النانوية كما في مؤشر طول الرويشة والجذير مقارنة بمعاملة السيطرة. كما بينت النتائج تفوق معاملة التركيز 100 جزء بالمليون للمادتين النانويتين في تحسين مواصفات النمو المدروسة مقارنة بالتراكيز 0 و 25 و 50 جزء بالمليون، مع تفوق معاملات الصنف أبو غريب في الاستجابة للمواد النانوية مقارنة بالأصناف الاخرى. واشارت نتائج االختبارات البايوكيميائية إلى أن معامالت 2TiO و ZnO في التركيز 100 جزء بالمليون أديا زيادة معدالت التركيب الضوئي وتراكم الكربوهيدرات والحامض الأميني البرولين قياسأ بمعاملة المقارنة. لذا توصي الدراسة الحالية بضرورة اإلفادة من المواد النانوية)ثنائي أوكسيد التيتانيوم 2TiO وأوكسيد الزنك ZnO)لمعاملة نبات الحنطة للكفاءة العالية التي اظهرتها معامالتها في تشجيع النمو.

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