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Study of agronomic traits of pinto bean (Phaseolus vulgaris L.) under nano TiO_2 spraying at various growth stages

Ahmad Ebrahimi[1,2*], Mohammad Galavi[3], Mahmood Ramroudi[3] and Payam Moaveni[4]

1.Department of Agronomy, Faculty of Agriculture, Zabol University, PO. Box: 98615-538, Zabol, Iran
****Corresponding Author**: ae.iranshahr@gmail.com*
2. Agriculture Department, Islamic Azad University, Iranshahr Branch, Iranshahr, Iran
[3]Department of Agronomy, Faculty of Agriculture, Zabol University, PO. Box: 98615-538, Zabol, Iran
[4]Agriculture Department, Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran

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ABSTRACT

Experiments were done on the effects of treatments of titanium dioxide spray on Pinto bean (Phaseolus vulgaris L. c.v 'c.o.s.16'). The study was conducted as a factorial experiment in a randomized complete block design with four replications. Treatments consisted of two factors; the first factor was stage of plant growth that spraying was applied (rapid vegetative growth, flowering and pod fill); and the second factor was that of different concentrations of titanium dioxide nanoparticles (TiO_2) that consisted of spray with water (control), nano titanium dioxide at concentrations of 0.01%, 0.02%, 0.03% and 0.05%. Results showed that effect of nano TiO_2 was significant on chlorophyll content a, seed protein content, grain yield, biological yield and grain weight. The maximum amount of chlorophyll a, seed protein, biological yield, grain weight and grain yield were recorded from the treatment of nano TiO_2 spray at the rapid vegetative growth by Nano particles 0.02 percentage, from the treatment of nano TiO_2 spray at the rapid vegetative growth by Nano particles 0.05 percentage, from the treatment of nano TiO_2 spray at the pod fill growth by Nano particles 0.05 percentage, from the treatment of nano TiO_2 spray at pod fill growth by Nano particles 0.02 percentage and from the treatment of nano TiO_2 spray at the flowering by Nano particles 0.03 percentage in comparison with other treatment. So, utilization of nanoparticles (nanao TiO_2) can facilitate an increase in crop yield, particularly Pinto bean yield and we hope that the results of this research could provide nanoparticles as nanofertilizers to attain to stable performance in sustainable agriculture.
Keywords: *Agronomic traits, pinto bean (Phaseolus vulgaris L.), various growth stages, Nano TiO_2*

INTRODUCTION

Common beans (*Phaseolus vulgaris* L.), have been characterized as a nearly perfect food because of their high protein, fiber, prebiotic, vitamin B, and chemically diverse micronutrient composition (1,2). Dry beans can also be grown in a variety of eco-agricultural regions and distributed in multiple forms, such as whole unprocessed seeds, as part of mixes, canned products, or as a gluten free wheat flour substitute. As a result, dry beans are used throughout the world representing 50% of the grain legumes consumed as a human food source (3). Pinto beans account for the largest market class that, are grown in most of the dry bean growing regions in Iran. Nanotechnology is a novel, innovative, interdisciplinary scientific approach that involves designing, development and application of materials & devices at molecular level in nanometre scale i.e. at least one dimension ranges in size from 1 to 100 nanometres, a billionth of meter (4). The use of nanotechnology in agriculture is getting importance because it is possible advantages vary from enhanced food values, reduced agricultural inputs, improved nutrient contents and longer shelf life (5). Nanotechnology has been found to solve many of the agriculture-related problems with tremendous improvement, as compared to conventional agriculture systems. The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice (40). During the last decade, an array of exploratory experiments has been conducted to gauge the potential impact of nanotechnology on crop improvement. Two comprehensive reviews have presented evaluation of a variety of nanomaterials (NMs), mostly metal-based (MBNMs) and carbon-based (CBNMs), for their absorption, translocation, accumulation, and importantly, effects on growth and development in an array of crop plants (6 ,7). Metal oxide nanoparticles (MONPs, such as *n*CeO2, *n*CuO, *n*TiO2, and *n*ZnO) are increasingly incorporated into agricultural products, such as fertilizers (65). Some of these studies have showed positive effects on plant growth and development upon nanomaterial exposure. The positive morphological effects included enhanced germination percentage and rate; length of root and shoot, and their ratio; and vegetative biomass of seedlings in many crop plants including corn, wheat, ryegrass, alfalfa, soybean, rape, tomato, radish, lettuce, spinach, onion, pumpkin and cucumber. Enhancement of many physiological parameters related to plant growth and development were also reported by MBNMs in plants and crops including barley (8,9), corn (10,11),canola (12), Common bean (11), Wheat (13,26,27), soybean (14,15), *Cicer arietinum* (51), spinach (16,17), calendula(18), *Ulmus elongata* (19), *Mentha piperita* (20), bitter melon (21), peanut (22), mung (23), *Boswellia ovalifoliolata* (24), pepper (27), borage (28), tomato (29,30), *Lemna minor* (31), *Alyssum homolocarpum*, *Sinapis alba*, *Carum copticum*, *Nigella sativa* (32), cowpea (33) and *Arabidopsis thaliana* (34). Briefly, physiological and biochemical effects of MONPs on higher terrestrial plants has been shown in Figure 1 (66). Of course, physiological effects, depending on the nanomaterial type, particle size, concentration , and plant species (55) For example, it is reported that TiO_2 nanoparticles in higher concentration had pronounced effects on photosynthetic pigments while lower concentration of NP-TiO_2 had significantly increased root length (20) In another experiment, it was reported that nano scale TiO_2 at 100 mgL^{-1} proved to be effective in improving both shoot and root length. At higher concentration of nano scale TiO_2 (more than 100 mgL^{-1}), shoot and root length decreased (13). Also, it was pronounced that small concentrations of silver nanoparticles had a stimulating effect on the growth of the plantlets, while the enhanced concentrations induced an inhibitory effect (11). Interactions of plants with dosage and size of TiO_2 were studied by measuring the germination indices and vigor index of wheat seeds. The results indicated that the nanosized TiO_2 treatments in proper concentration accelerates the germination of the wheat seeds and increases its vigor. Nano TiO_2 improves the mean germination time and growth of wheat seedling in comparison to bulk TiO_2 and untreated control (26). Only recently, the genetic implications of such nanoparticle-induced positive changes have been validated through investigations on enhanced mRNA expression and protein level in spinach (35) by nano-TiO_2, generational transmission of fullerol through seeds in rice (36), changes in gene expression at plant and cellular level in tomato and tobacco (37,38) and *Allium cepa* (52) by (multi-walled carbon nanotubes) MWCNTs. Nanomaterials can thus cause a variety of adversities and affect the expression of photosynthesis-related genes (53). These effects are closely related to the surface chemical properties of the nanomaterials (54). Despite such promise towards enhanced plant growth and development, there is some reports on the improvement of agronomic traits that documented increased leaf and pod dry weight and grain yield of soybean by exposure to nano-iron oxide (39), increasing almost all agronomic traits including gluten and starch content by exposur to titanium dioxide nanoparticles in wheat under water deficit stress condions (27) nano-anatase (TiO_2), caused to a significant increase in the percentage of germination, germination rate index, radicle and plumule length, fresh weight and vigor index of seedlings of pepper (*Capsicum annum* L.) (25), TiO_2 NPs caused stability of chlorophyll and carotenoid contents during cold stress. Results suggest that TiO_2 NPs confer an increased tolerance of chickpea plants to cold stress, decreasing the level of injuries and increasing the capacity of defense systems (64). Increasing of photosynthetic pigment contents, carotenoids and anthocyanins of maize under spray treatment of nano TiO_2 (10), Supplying of chlorophyll contents in the sunflower seedlings with magnetic nanoparticles (60) and nano ZnO on growth of Vigna radiata and Cicer arietinum seedlings using plant agar method (41) and Peanut (42). The mechanisms of nanomaterials on living organisms have aroused considerable scientific interest (50) why so, nanomaterials with different modifications have different biological effects (51). Application of Nano SiO_2 significantly enhanced the characteristics of seed germination. Among the treatments, 8gL^{-1} of nSiO_2 improved percent seed germination, mean germination time, seed germination index, seed vigour index, seedling fresh weight and dry weight. Therefore, it is very clear that nSiO_2 has a significant impact on the seed germination potential (30). Nano-TiO_2 treatment, in proper concentration, accelerates the germination of the aged seeds of wheat (48) in comparison to bulk TiO_2. Similarly, canola Seeds treated with 2000 mg l-1 nanoscale TiO_2 recorded significant germination percentage (75%), germination rate and seedling vigor (12). In another study, The nanoparticle suspensions of ZnO, FeO and ZnFeCu-oxide were able to affect the development and growth processes of the mung (*Vigna radiata*) plant by foliar spray. Pronounced effect on increasing in root and shoot length as well as accumulation of biomass was recorded for nanoparticle treated plant as compared to the reference. Among the different nanoparticle suspensions, the maximum effect was found at 50 ppm ZnFeCu-oxide followed by 50 ppm FeO and least for 20 ppm ZnO depending on their chemical composition, size and surface energy (23). It was found that aluminum nanoparticles can promote the growth of *Lemna minor*, particularly the root elongation. The authors believed that this metal nanoparticle can promote the conversion of light energy as well as the photosystem II (PSII) quantum yield (49). These findings show that the use of nanoparticle suspensions by foliar spray for directed delivery of substances into plant cells is a feasible application (23). The role of titanium (Ti) in plant metabolism is not so far fully clear. Many positive beneficial effects as well as a few adverse effects of Ti application are described in literature (45),It can stimulate total chlorophyll content in wheat leaves (44) , enzyme activities and uptake of major and minor nutrients (43). Titanium dioxide (titania, TiO_2) is chemically inert, semiconducting material that also exhibits photocatalytic activity in the presence of light with an energy equal to or higher than its band-gap energy. These characteristics offer a wide range of applications. For these reasons, and because of the relatively low price of the raw material and its processing, titania has gained widespread attention over recent decades (46). Numerous technological improvements, based on nano-sized TiO_2, have been introduced that enable its use for antifogging and self-cleaning coatings on glass, for building facades, in confectionary, in the plastics industry, and so on. Furthermore, TiO_2 is accepted as a food and pharmaceutical additive (47). Nano-titanium dioxide (TiO_2) has excellent optical and biological properties and has recently caught the attention of plant Physiologists (19). Nano-TiO_2 can significantly promote the genetic expression of Arabidopsis thaliana light-harvesting complex II b (56). Nanoanatase TiO_2 can significantly promote the activity of nitrate reductase to accelerate the conversion of inorganic nitrogen (NO_3- N and NH_4-N) to organic nitrogen (protein and chlorophyll) (57). Spinach treated with nano-anatase TiO_2 can even directly absorb N_2 or reduce N_2 to NH_3 in nitrogen-poor nutrient solutions under sunlight, thereby significantly increasing plant nitrogen content (58). Nano-anatase TiO_2 can affect the spinach microenvironment of PSII and increase the visible-light absorption of leaves, thereby improving the energy transport capacity (59). It was also found that TiO_2 nanoparticles could dramatically increase callugenesis and the size of calli in Barley (*Hordeum vulgare* L.) Tissue Culture . As well, TiO_2 nanoparticles are effective bactericides with an aseptic effect, causing no negative change in the quality of the callus (8). It is reported that the different concentrations of NP-TiO_2 and TiO_2 had a negative significant effect on germination percentage and shoot length. However, root length was significantly influenced by 100mg L^{-1} concentration of NP-TiO_2 rather than nonNP-TiO_2 concentrations. Pronounced effect on photosynthetic pigments (chlorophyll a and b and carotenoids) was found in 200mg L^{-1} concentration of TiO_2 and 100mg L^{-1} concentration of NP-TiO_2 (20). The results showed that the effect of TiO_2 NPs on plant growth was more obvious than bulk TiO_2. Titanium dioxide NPs stimulated plant growth in low concentrations, but inhibited plant growth at high concentrations in duckweed (*Lemna minor*) (31). Also, It was concluded that effects of nano Tio_2 spray on evaluations for traits of seed number per pod, 1000 seed weight, grain yield, leaf area, pod number per plant and pod length of Vina unguiculata, were significant and evaluations of these traits significantly increased compared to the control (61), Or in another experiment it has been report that, ultra-small anatase TiO_2 nanoparticles enter plant cells, conjugate enediol and catechol group-rich flavonoids in situ, and exit plant cells as flavonoid-nanoparticle conjugates. The source plant tissues remain viable after treatment. As predicted by the surface chemistry of anatase TiO_2 nanoparticles, quercetin-based flavonoids were enriched amongst the nanoharvested flavonoid species. Nanoharvesting eliminates the use of organic solvents, allows spectral identification of the isolated compounds, and opens new avenues for use of nanomaterials for coupled isolation and testing of bioactive properties of plant-synthesized compounds (62) Or, in another experiment, it was declared that both TiO_2-NP (14 nm or 25 nm anatase TiO_2-NP) are accumulated in these plantlets upon root exposure and that Ti content is higher in rapeseed than wheat. Ti distribution in root cross sections depended on NP agglomeration state. NP are also accumulated in plantlets upon leaf exposure. Finally, it was found that TiO_2-NP exposure induced increased root elongation but did not affect germination, evapotranspiration, and plant biomass. Taken together, these results confirm that TiO_2-NP may be accumulated in plant crops but may only moderately impact plant development (63). However, all previously described plants are edible ones (55) and were studied mainly in a laboratory environment, and the pathway of the interactions between nanoparticles and plants is unclear. Therefore, mechanism by which nano-TiO_2 affects plant growth requires further investigation, examination and elucidation. So, this study was done to investigate changes of physiological aspects in Pinto bean (*Phaseolus vulgaris* L.) sprayed with different concentrations of nano TiO_2 at various stages of growth and development.

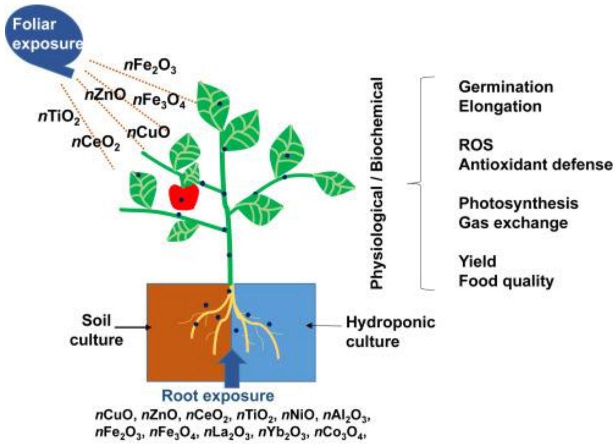

Figure 1. Schematic diagram of physiological and biochemical effects of MONPs on higher terrestrial plants (Wenchao et al., 2016)

Materials and Methods

This survey was done as a factorial experiment in a complete randomized block design. Treatments consisted of two factors. The first factor tested spraying application at various stages of plant development (rapid vegetative growth, flowering and pod fill) and the second factor tested different concentrations of titanium dioxide nanoparticles (control or sprayed with water, and nano titanium dioxide with the concentrations of 0.01%, 0.02%, 0.03% and 0.05%. Pinto bean seeds were planted in May 2015. Fertilization and plant feeding was done according to recommendations from results of a soil test. Spraying treatment was based on growth stages and concentrations of nanoTio_2. Plants were treated with 240 ml titanium solution per square meter. Control plants were treated with distilled water. Evaluations were made for the characters of chlorophyll a content, seed protein content, grain weight , grain yield, biological yield.

Leaf chlorophyll a content. The amount of chlorophyll a was measured in accordance with the method cited in Arnon (1967). Finally, chlorophyll a content was determined by the following formula;

Chlorophyll a = (19.3 × A663 - 0.86 × A645) V/100W

Seed protein content. The content of bean seed protein was measured by near-infrared reflectance (NIR) spectrometer (DA7200 model; Perten company, Sweden). Approximately 20 g of pinto bean flour was placed into a small sample ring cup (diameter 50 mm, height 75 mm). Samples were irradiated using nearinfrared monochromatic light and spectra were collected using a lead sulfide detector in the wave number range of 4,000-12,000 1/cm. Each sample was scanned twice and the two spectra were recorded and averaged automatically.

Grain weight. From each sample 100 seeds were counted and weighed on a laboratory balance (model GF6000, Japan).

Grain yield & Biological yield. In order to measuring grain yield, plants per In each experimental plot calculated. To calculating biological yield, all plant weight measured after harvesting plants per plot and then biological yield determined.

Characterization analysis of TiO_2 NPs

The anatase TiO_2 NPs was purchased from Nano Pars Lima Company. The TiO_2 NPs had a purity of greater than 99.5%, average of particle diameter of 21 nm, and a surface area of 60 m^2/g. Then, in order to prepare concentrations of nano Tio_2, 20 g nano TiO_2 was dissolved into water and then 0.01 ml of solution was filled up to 1000 ml. Thus, different concentrations of titanium dioxide (0.01%, 0.02%, 0.03% and 0.05%) were prepared. An ultrasound instrument was used to homogenize the solution. Titanium dioxide nanoparticles were sprayed on plants using a calibrated pressurized backpack sprayer (capacity 20 l).

Statistical Calculations

The obtained data was studied based on a factorial experiment in randomized complete block design. Data were subjected to analysis of variance (ANOVA) using software Statistical Analysis System 9.0 (SAS Institute 1988) and followed by Duncan's multiple range tests. Terms were considered significant at $P \leq 0.01$. Also, to determine the correlation between the traits were used by Pearson's correlation coefficients, significant at 5 and 1 percent levels and for drawing of charts was used with software Excel.

Table 1 Results of variance analysis of the *Phaseolus vulgaris* treated with nano Tio_2 at vegetative and reproductive stages

Means square						
Biological yield	**Grain yield**	**Grain weight**	**Seed protein**	**Chlorophyll a**	**df**	**Sources of variation**
16.01 $^{n.s}$	1.36 $^{n.s}$	1.76 $^{n.s}$	89.56 $^{n.s}$	0.21 $^{n.s}$	3	Replication
7.27 $^{n.s}$	4.65 $^{n.s}$	1.12*	25.01*	1.16 $^{n.s}$	4	(a) concentrations of nano Tio_2
6.30 $^{n.s}$	16.44*	0.03 $^{n.s}$	30.22*	3.24*	2	(b)Times of spraying
14.97 $^{n.s}$	1.88*	1.10*	13.56*	0.62*	8	b × a
13.78	4.22	0.93	600.41	5.96	42	Error

Note: Ns, Non Significant, * and **, Significant at 5% and 1% levels respectively.

Results

Results of evaluations of plant characters showed that The effect of different amounts of titanium dioxide nanoparticles (TiO_2) was significant on seed protein content and 100 grain weight traits ($P \leq 0.01$, Table 1). The effect of spraying times was significant ($P \leq 0.01$, Table 1) on chlorophyll a amount, seed protein content and grain yield traits. The effect of different amounts and times of titanium dioxide nanoparticles (TiO_2) applications, was significant, on all traits with the exception of biological yield ($P \leq 0.01$, Table 1). According to Tables 2, 3 and 4, the highest amount of chlorophyll a was obtained by spraying of nano titanium dioxide at the concentration of 0.02% at the stage of the flowering and the lowest evaluation for this trait was achieved by spraying of nano titanium dioxide at the concentration of 0.01% at the stage of the rapid vegetative growth. The highest amount of seed protein content was obtained by spraying of nano titanium dioxide at the concentration of 0.02% at the stage of the rapid vegetative growth and the lowest evaluation for this trait was achieved by spraying of nano titanium dioxide at the concentration of 0.05% at the stage of the flowering. Also, The highest amount of 100 grain weight was obtained by spraying of nano titanium dioxide at the concentration of 0.05% at the stage of the pod filling and the lowest evaluation for this trait was achieved by spraying of nano titanium dioxide at the concentration of 0.03%

at the stage of the pod filling. The highest amount of grain yield was obtained by the use of nano titanium dioxide at the flowering stage. But effects of nano TiO_2 spraying at different doses were not significant on grain yield trait. The lowest evaluation for this trait was achieved by spraying of nano titanium dioxide at the concentration of 0.02% at the stage of the rapid vegetative growth. Effects of TiO_2 NPs usage at different concentrations were not significant on biological yield trait. Moreover, Effects of nano titanium dioxide spraying times were not significant on this trait. The highest amount of biological yield was obtained by spraying of nano titanium dioxide at the concentration of 0.03% at the stage of the pod filling and the lowest evaluation for this trait was achieved by spraying of nano titanium dioxide at the concentration of 0.01% at the stage of the rapid vegetative growth.

Discussion

This research results illustrate that TiO_2 NPs to be effective on increasing of all traits with the exception of biological yield and higher amounts of all traits obtained with spraying of nano TiO_2 different concentrations at reproductive Stages of Pinto bean (flowering and pod filling) with the exception of seed protein content trait that Increased at the stage of the rapid vegetative growth that previous studies demonstrated that the response of plants to nanoparticles varied with the growth stages of plants (67,68). Grain yield can not to be correlate with biological yield at the stages of flowering and pod set but Shoot biomass correspond to grain yield at pod filling stage. During pod filling photoassimilate, nitrogen and other nutrients such as phosphorus accumulate in shoots and because of, remobilization function from leaves to grains, which resulted in higher grain yield. Therefore, application of titanium dioxide nanoparticles at reproductive stages of Phaseolus vulgaris, increased amounts of 100 grain weight, grain yield and biological yield triats. increasing of fresh weight of shoots by TiO_2 NPs concentrations was in accordance with the report on wheat by Mahmoodzadeh et al. (2013). The root length increased with the bulk TiO_2 concentration perhaps because bulk TiO_2 has the capacity to adsorb nutrition in the culture media. When a nutrient (such as phosphorus) is lacking in culture media, the root length of L. minor increases to absorb more nutrition. The fresh weight of Lemna minor increased when the TiO_2 NP concentration was lower than 500 mg/L, but the fresh weight decreased when the TiO2 NP concentration was higher than 500 mg/L. The fresh weight increased with the bulk TiO_2 concentration increase in this test concentration. The fresh weight changes showed the same trend as root length changes of Lemna minor with an increase in TiO_2 NPs or bulk TiO_2: this is explained by the fact that the plant is composed of fronds and root, and the root is the main part of the plant body (31). Based on the results of an investigation that was expressed nano-anatase with the increase penetration power of the seed, causes facilitate the entry of water and oxygen into the cell that with increasing absorption of nutrients in the seed, processes related to germination resonant and occur ultimately stimulate germination. Germination indices in spinach more increases by nano titanium compared to bulk titanium. During the growth period of spinach, is rise dry weight, chlorophyll content and photosynthetic rates finally (Zheng et al., 2007), can be concluded that titanium dioxide nanoparticles increase penetration power of the root cell, causes facilitate the entry of water, oxygen and nutrients specially nitrogen into the cell that with increasing absorption of nutrients in the root, nitrogen uptake efficiency goes up and inasmuch as, nitrogen is a key and main essential element in crops and too, it is a basic and cardinal material of protein. Plants produe different types of amino acids, required for the specific protein. So, foliar application of TiO_2 NPs at vegetative growth stage increased seed protein content in Pinto bean. The same results were obtained by Hediat and Salama (2012). Our results show that foliar application of nano titanium dioxide at flowering stage increased chlorophyll a content in Phaseolus vulgaris. Concentrations of nitrogen effect on green leaves stability containing of chlorophyll and gas exchange, especially at flowering stage of crops. So, foliar application of TiO_2 NPs at this stage cause increasing of nitrogen uptake efficiency, As a result, the amount of leaves chlorophyll rises, That this process leads to increasing of rate of photosynthesis in plants, following that the biological yield, economic yeild and grain weight increases, that associated with this process, was reported that, the nano TiO_2, attributed to their photocatalytic property and thermal conductivity, enhanced water absorption, improving light absorption in chlorophyll a, and inducing oxygen evolution rate, consequently showing beneficial effects on photosynthesis and production (69,70) as well as, In another studies were reporetd that, nano TiO_2 effect on transformation from light energy to electronic energy, water photolysis, oxygen evolution and increasing of total chlorophyll content at 750 mg/kg (71), Not only electron under visible light but also energy enriched electron from TiO_2 NPs, which entered chloroplast under ultraviolet light, were transferred in photosynthetic electron transport chain and made NADP+ reduced into NADPH, and coupled to photophosphorylation and made electron energy be transformed to ATP. Moreover, TiO_2 NPs h+, which photogenerated electron holes, captured an electron from water, which accelerated water photolysis and oxygen evolution (72), also, the protein expression of Rubisco from the nano-anatase-treated spinach was increased by 40% compared with the control. Further analysis indicated that the activity of Rubisco in the nano-anatase-treated spinach was significantly higher than the control, by up to 2.33 times, whereas bulk TiO_2 treatment had no such significant effects. The researchers concluded as follows: One of the molecular mechanisms of carbon reaction promoted by nano-anatase is that nano-anatase treatment results in the enhancement of Rubisco mRNA amounts, protein levels, and activity of Rubisco, thereby leading to improved Rubisco carboxylation and a high rate of photosynthetic carbon reaction (73). Thus, according to the results of this study and studies of other researchers, consumption of of nano titanium dioxide would be applied to increase crops yield efficiency and also, pinto bean production because of increase of some of agronomic traits amounts.

Table 2 Means comparsion of nano TiO_2 concentrations on traits of *Phaseolus vulgaris*

Biological yield (Kg/ha)	**Grain yield (Kg/ha)**	**Grain weight (g)**	**Seed protein (%)**	**chlorophyll a ($mg.g^{-1}.fw$)**	**concentrations of nano Tio_2**
352.45a	109.97a	14.667ab	15.792b	35.817a	(Distilled water) Control
288.27a	87.90a	13.225ab	19.425a	32.663a	(0.01%) Nano Tio_2
319.53a	85.23a	12.975ab	17.958ab	43.401a	(0.02%) Nano Tio_2
349.58a	110.08a	10.917b	18.933a	38.525a	(0.03%) Nano Tio_2
336.93a	99.80a	17.358a	17.192ab	33.992a	(0.05%) Nano Tio_2

Note: Means in the same columns and rows, followed by the same letter are not significantly difference ($P < 0.01$).

Table 3 Means comparsion of nano TiO_2 spraying times on traits of *Phaseolus vulgaris*

Biological yield (Kg/ha)	**Grain yield (Kg/ha)**	**Grain weight (g)**	**Seed protein (%)**	**chlorophyll a ($mg.g^{-1}.fw$)**	**Times of spraying**
321.61a	85.97b	13.690a	19.175a	37.150ab	Rapid vegetative growth
350.13a	118.72a	13.840a	16.740b	41.665a	Flowering

316.32a	91.11b	13.955a	17.665ab	31.824b	Pod fill

Note: Means in the same columns and rows, followed by the same letter are not significantly difference ($P < 0.01$).

Table 4 Means comparison of spraying of times and concentrations of nano TiO_2 on traits of *Phaseolus vulgaris*

Biological yield (Kg/ha)	**Grain yield (Kg/ha)**	**Grain weight (g)**	**Seed protein (%)**	**chlorophyll a ($mg.g^{-1}.fw$)**	**Times of spraying**	**concentrations of nano Tio_2**
402.63a	110.35 ab	12.575 abc	15.875 b	39.25ab	Rapid vegetative growth	(Distilled water) Control
251.03a	79.05b	15.550 abc	19.475 ab	28.43b	Rapid vegetative growth	(0.01%) Nano Tio_2
294.10a	64.53b	13.600 abc	22.500 a	46.73ab	Rapid vegetative growth	(0.02%) Nano Tio_2
294.18a	85.10b	12.675 bc	20.350 ab	40.53ab	Rapid vegetative growth	(0.03%) Nano Tio_2
366.10a	90.80b	14.050 bc	17.675 ab	30.83b	Rapid vegetative growth	(0.05%) Nano Tio_2
385.75a	112.80 ab	16.375 ab	15.650 b	35.68ab	Flowering	(Distilled water) Control
266.28a	108.35 ab	12.975 bc	18.775 ab	38.50ab	Flowering	(0.01%) Nano Tio_2
362.43a	99.65ab	12.300 bc	15.800 b	53.55a	Flowering	(0.02%) Nano Tio_2
349.95a	152.10 a	13.450 abc	18.400 ab	42.00ab	Flowering	(0.03%) Nano Tio_2
386.23a	120.68 ab	14.100 abc	15.075 b	38.60ab	Flowering	(0.05%) Nano Tio_2
268.98a	106.75 ab	15.050 abc	15.850 b	32.53ab	Pod fill	(Distilled water) Control
347.50a	76.30b	11.150 bc	20.025 ab	31.07b	Pod fill	(0.01%) Nano Tio_2
302.08a	91.50b	13.025 bc	15.575 b	29.93b	Pod fill	(0.02%) Nano Tio_2
404.60a	93.05b	6.625c	18.050	33.05ab	Pod fill	(0.03%) Nano Tio_2

			ab			
258.45a	87.93b	23.925 a	18.825 ab	32.55ab	Pod fill	(0.05%) Nano Tio_2

Note: Means in the same columns and rows, followed by the same letter are not significantly difference ($P < 0.01$).

Conclusion

In conclusion, these results of the current study reveal that the foliar application of nano titanium dioxide at the growth stages of Pinto bean significantly enhanced chlorophyll a content, percent seed protein, grain weight, grain yield and biological yield. However, the present experiment invites dear scholars to realize the interplay mechanism between nano titanium dioxide and crops which establishes that $nTiO_2$ could be applied as a nutrient for the crop improvement.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EM was responsible for writing paper and literature review and contributed to pay cost of design, MG and MR contributed to the study of design and helped conduct literature review and writing paper and PM helped to the statistical analyses and Laboratory work and responsible for the farm working. All authors read and approved the final manuscript.

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