Comprehensive assessment of ZnO, P and TiO_2 nanoparticles sustaining environment in response to seed germination, antioxidants activity, nutritional quality and yield of Spinach Beet (*Beta vulgaris* var. bengalensis)

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Abstract

A comprehensive investigation was carried out under laboratory and field by ZnO, P and TiO_2 NPs on seedling growth and antioxidant activity, nutrient accumulation, yield traits and biochemical response of spinach beet. Spinach beet variety All Green were treated with sixteen different concentrations nanoscale ZnO (5, 10, 15, 20, 25 ppm), P (10, 20, 30, 40, 50 ppm) and TiO_2 (10, 20, 30, 40, 50 ppm) including untreated control and each treatment has three replicates. TiO_2 NPs treatments found drastic positive impact on seed germination, root and shoot length, seed vigour index and antioxidant enzyme activity (CAT, PAD, SOD and GR). Application of ZnO, P and TiO_2 NPs improve morphological parameter and that of increase seed yield (gm plant^{-1}) with 12.48% 13.01% and 10.93% respectively, compared to control. Study also revealed that zinc and iron content in leaf tissues increases by 50.76%, 7.71%, and 6.41% and 12.69%, 18.49% and 14.17%, respectively of ZnO, P and TiO_2 NPs NPs. Among the biochemical parameter maximum chlorophyll content obtained from 10 ppm TiO_2, leaf protein and carotene from 50 ppm P, ascorbic acid from 40 ppm TiO_2, crude fibre from 20 ppm ZnO and total phenol from 30 ppm TiO_2. Our result clearly demonstrates that application of ZnO, P and TiO_2 NPs improve the antioxidant enzyme activity, yield and nutritional quality of spinach beet and these nanoparticles can be utilized with their effective doses which may reduce fertilizer cost and environmental pollution.

1. Introduction

To meet demand of food supply over increasing population, recently nanotechnology will occupied a prominent position in transforming agricultural sector and environment sustainability. The expansion of nano devices (<100nm size) could be use wide range of extensive applications for increasing seed germination and seedling growth, plant morho-physiological development and alleviation of stress environments. The unusual idiomatic properties of nanoparticles with high surface to volume ratio, better catalytic surface, high reactivity capacity, rapid chemical reaction and adsorb abundant water may result in different environmental behaviors than the bulk materials. The metal based NPs (ZnO, TiO_2, Fe_2O_3, CuO) were capable to penetrate into the plant cell causing damage by interacting with sulfur and phosphorus containing compounds such as DNA and protein (Amarendra and Krishna, 2010) and also optimizing plant metabolic processes at the early growth stages (Taran et al. 2014). The interaction between NPs and plant cell results changes of gene expression and connected biological pathways which finally changes the plant characteristics. The impact of NPs positive effect were documented in array of crop plants (Monica and Cremonini 2009) and their response dependent type of NPs use, concentration and species (Gao et al. 2006; Hong et al. 2005; Ma et al. 2010). Application of MWCNT (multiwalled carbon nanotubes) increases seed germination and seedling growth of different crop plants related to tomato (Khodakovskaya et al. 2010), soybean, corn, barley (Lahiani et al. 2013), maize, peanut and wheat (Srivastava et al. 2014; Joshi et al. 2018). Use of metal-based ZnO, TiO_2 and P NPs largely utilized in seed sciences and crop improvement, which exploit contradictory results on seed germination and seedling growth in tomato (Das et al. 2015). NPs application also acts as antioxidant enzyme activity in plants that protect from oxidative stress damage (Gupta et al. 2018; Jadczak et al. 2020). In addition to germination and seedling growth the nanoscale ZnO and TiO_2 NPs were reported to positive influences of crop growth and development with nutritional quality enhancement in many crop species including peanut (Prasad et al. 2012), soybean (Zhang et al. 2021), rice (Debnath et al. 2020), spinach (Yang et al. 2006) and carrot (Gholami et al. 2020). Application of TiO_2 NPs in spinach produced significant effects on growth, increases photosynthesis and nitrogen metabolism (Gao et al. 2006; Hong et al. 2005; Zheng et al. 2005) and induce redox reactions, chlorophyll formation and Rubisco activity (Siddiqui et al. 2015; Hegde et al. 2016). NPs may enhanced nutrient use efficiency (Zulfiqar et al. 2019), nutrient content (Sabir et al. 2014) and improve yield and also decrease its accumulation in soil. Albeit reports of improvement on seedling growth, physiological attributes, yield and quality, information on the genetic mechanism underline these changes is meager. In viewpoint of the above facts the present study delineates ZnO, P and TiO_2 NPs laboratory observation of seed germination, seedling growth and antioxidant activity and field study of yield attributing and nutritional quality responsive traits in spinach beet (Figure 1).

2. Materials And Methods

2.1. Material used

Titanium dioxide (TiO_2) NPs were purchased from Sigma-Aldrich Company, St. Louis, MO, USA with a purity of 99.5%, particle size of (<100 nm). Biosynthesized Zinc Oxide (ZnO) and Phosphorus (P) NPs were collected from Central Arid zone Research Institute (CAZRI), Jodhpur, Rajastan. Characterization of particles in stipulations of size (<100 nm), shape, structure and elemental proportion presented our previous report (Das et. al. 2015).

2.2. Seed treatment with ZnO, P and TiO_2 NPs

The present study was conducted at research laboratory and Instructional farm of College of Agriculture (Extended Campus), Uttar Banga Krishi Viswavidyalaya, Majhian, West Bengal, India during 2018 and 2019. Healthy and uniform seeds of spinach beet variety All Green were selected and sterilized with 0.1% Mercuric Chloride solution for 1 min and rinsed properly with sterilized distilled water two to three times. Then seeds were treated with different concentrations of ZnO (0, 5, 10, 15, 20, 25 ppm), P (10, 20, 30, 40, 50 ppm) and TiO_2 (10, 20, 30, 40, 50 ppm) NPs in aerated solutions for 24 hrs at 25 ± 2^{o}C and these concentration were chosen based on our previous studies (Das et al. 2015). The untreated seeds with sterilized water considered as control.

2.3. Germination test and seedling growth

Germination percentage of spinach beet seeds was estimated based on AOSA method (AOSA, 1990). In an incubator, three replicates of 50 seeds of each treatment were placed in 12 cm diameter petri plates, between the layers of moist Whatman-42 filter papers at 27°C and arranged in a complete randomized design (CRD). Germination percentage was counted based on emergence of seedling from embryo. The average root length, shoot length and their fresh seedlings weight recorded from 10 seedlings of each treatment at one week of age. Vigour Index (VI) was estimated as per following equation (Abdul Baki and Anderson, 1973):

Vigour Index = (Shoot length average + Root length average)*Germination

2.4. Extraction and Antioxidant enzyme activity

Catalase enzyme (CAT) activity was measured following the procedure of Aebi (1984) with some minor modifications. All steps in preparation of the enzyme extracts were carried out at 0 to 4°C. The ultra violet (UV) light absorbance of hydrogen peroxide solution can be measured between 230 and 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. Fresh leaves (0.1 g) were homogenized in 5 mL of cooled phosphate buffer (50 mM pH 7.8) with a pinch of Poly Vinyl Pyrrolidone (PVP).The extracted leaves were centrifuged with 15000 rpm for 10 minutes and supernatant collected for enzyme estimation. Three ml of analysis mixture contained 50 μl distilled water whereas, control contained devoid of H_2O_2. The catalase activity was measured by reduce of H_2O_2 absorbance on 240 nm and expressed as μmol H_2O_2 decomposing/min/g.

Peroxidase enzyme (POD) was estimated following the procedure of Castillo et al. (1994) at absorbance of 470 nm using a reaction mixture containing 12 mM hydrogen peroxide and 96 mM guaiacol in phosphate buffer (pH 7.0). The optical density increases due to oxidation which produce tetra-guaiacol and POD expressed as μmoles/cm/min/g fresh weigh. The Superoxide dismutase (SOD) was estimated following the procedure by Gong et al. (2005) by nitroblue tetrazolium (NBT) with an absorbance at 560 nm. Glutathione reductase (GR) was estimated following the procedure describe by Shanker et al. (2004).

2.5. Field study of NPs treated seeds and recording of yield attributing traits

Beside seedling experiments All Green seeds with above sixteen different concentrations of NPs treatment including control also sown in field based on randomized block design (RBD) by maintaining spacing of 40cm x 20cm. The fertilizer applied in soil following recommended dose of NPK (75:40:40) and necessary cultural practices maintained for plant growth. The initial soil fertility status of experimental field was presented in Table 1. One foliar exposure of above NPs treatment in each case was done at 30 days of sowing. During the entire growth period (150 days) of spinach beet plants morpho-phenotypic and yield attributes such as plant height (60 DAS), leaf number, leaf length (cm), leaf width (cm), stem thickness (mm), leaf area (cm^2), fresh leaf weight (gm) and seed yield plant^{-1} (gm) were recorded by selecting average of five plant in each treatment. The fresh weight (gm) of the leaves at was recorded after 50 days sowing.

Table 1
Soil fertility status under field condition during crop growth stage

Particular	Values	Status
Texture class		
Sand	58%	-
Silt	27	-
Clay	15	Sandy loam
pH	5.54	Acidic
Soil nutrients		
Organic carbon (%)	0.41	Medium
Available N (Kg $ha^{-1)}$	185.36	Medium
Available P_2O_5 (Kg $ha^{-1)}$	12.07	Low
Available K_2O (Kg $ha^{-1)}$	296.78	High
Source: Regional Research Station (Old Alluvial Zone), UBKV, Majhian		

2.6. Dry matter and Elemental Analysis

For estimation of dry matter content each treated plants were taken out during maturity stage (60DAS), dried under shade and then placed in a hot air oven at 70 ± 5^{o}C for 48 hours to a constant weight, and weighed to record dry matter content (gm). The dried leaf were finely ground and digested for estimation of total NPK content. Nitrogen content from dried leaves was estimated by the micro-Kjeldahl method (Subbiah et al. 1956). For phosphorus and potassium content tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4) was used and determined by flame photometer (Jackson 1973). Iron and zinc contents each treated leaves extract digested in triple acid was estimated by employing the method given by Jackson (1973) by using atomic absorption spectroscopy (PinAAcle 990F; PerkinElmer, Singapore).

2.7. Estimation of Biochemical parameters

Biochemical properties like total chlorophyll content from young leaf of spinach beet was estimated by spectrophotometric method (Davies 1976). Carotene content of spinach leaf was estimated by the standard procedure of AOAC (1984) using 80% of acetone. Protein content from the fresh leaves was estimated following the method of Lowry, et al., 1951. Ascorbic acid content was determined by using 2-6, Dichlorophenyl Indophenol dye solution (Ranga 1979) and expressed as mg/100g of fresh weight. Total polyphenol concentrations of leaf material were determined using the Folin-Ciocalteu the method by Waterman and Mole (1994) as described by Mudau et al. (2006). The finely ground leaves samples were used crude fibre content by AOAC method (1970).

2.5 Statistical Analysis

Each treatment was conducted with three replicates and the results were presented as mean±SE (standard error of the mean) and CD value. All the data were statistically analyzed by Fisher's technique and Duncan's Multiple Range Test ($p \leq 0.05$). The analysis was carried out using the SPSS software 21 version and graphical representation done by OriginPro 21 software.

3. Result

3.1. Effect of ZnO, P and TiO_2 NPs for germination and seeding growth

The use of ZnO, P and TiO_2 NPs treatments significantly affected germination and seedling growth of spinach beet. Improvement in germination percentage was found from all the ZnO, P and TiO_2 NPs treatments (Figure 2A). Enhanced germination percentage over control was highlighted for almost all the treatments except 5 ppm of ZnO. Highest germination percentage was found in treatment with 30 ppm of TiO_2, followed by 15 ppm of ZnO. The increment in germination percentage was observed to the tune of 10.92%, 13.66% and 17.01% in ZnO, P and TiO_2 respectively, as compare to control. The data regarding length of root and shoot at one week of age has been presented in the Figure 2B. Most favourable effect on root and shoot length was highlighted by respective treatments of 40 ppm of TiO_2. The data also depicts that the TiO_2 NPs provides maximum response of root and shoot length than other treatment which an increase of 28.14% and 22.34% respectively, over control. Similar to the data on fresh seedling weight the maximum was recorded with 50 ppm TiO_2 which was 36.46% higher than control, whereas, the least fresh seedling weight of shoot was associated with 20 ppm of P (Figure 2C). Enhancement of vigor index was maximum by TiO_2 NPs at 40 ppm and 30 ppm. Over all vigor index increase with 25.16%, 26.92% and 30.39% by ZnO, P and TiO_2 NPs, respectively from the control.

3.2 Effect of ZnO, P and TiO_2 Nanoparticles for antioxidant enzymatic activity

NPs treatments significantly influence the antioxidant enzymatic response during seedling growth of spinach beet. The data indicate highly significant ($p \leq 0.05$) differences of antioxidant enzyme activity (CAT, POD, SOD and GR) under seedling stage of spinach beet (1 week old seedling) in response to ZnO, P and TiO_2 NPs application (Figure 3A, 3B). The catalase (CAT) activity increased statistically ($p \leq 0.05$) with the treatments (Figure 3A). The maximum expression of catalase activity was recorded when treated with TiO_2 at 40 ppm followed by 20 ppm and 30 ppm. The average cumulative effect of catalase activity increased from 21.79%, 8.08% and 43.78% respectively, by ZnO, P and TiO_2 NPs respectively, over the control. The maximum peroxidase enzyme activity was recorded in 20 ppm TiO_2 and an average increment by ZnO, P and TiO_2 NPs treatments found with 17.31%, 6.15% and 34.05% respectively, as compared to control plants (Figure 3A). During seedling growth, the SOD activity tended to produce considerably at different NPs treatment (Figure 3B). The SOD enhancement occurred by ZnO, P and TiO_2 NPs treatments was 22.59%, 22.02% and 45.22% respectively, from control. On the other hand the expression of GR activity in spinach beet leaves was found maximum with TiO_2 NPs at 30 ppm (49.22%). Overall enhancement of the GR activity was noticed 18.41%, 10.68% and 44.50% by ZnO, P and TiO_2 NPs treatments (Figure 3B).

3.3 Effect of ZnO, P and TiO_2 NPs on morphological and yield traits of spinach beet

Application of ZnO, P and TiO_2 NPs treatments of spinach beet had profound effect on plant morphological traits (Figure 4). At maturity stage of spinach beet (80 DAS), the maximum plant height was found in TiO_2 NPs treatments and increased by 35.27% at 30 ppm (Table 2). Shortest plants were observed under untreated control condition. The positive effect of plant height with statistically at per treatments were 40 ppm and 50 ppm TiO_2 NPs, 20 ppm and 30 ppm P NPs, 10 ppm and 15 ppm ZnO NPs. Other characteristics noticed maximum response with the application of TiO_2 NPs at 30 ppm of leaf number; at 40 ppm of leaf length, leaf width and leaf area; at 10 ppm of stem thickness. For ZnO NPs treatments the promising effect found at higher concentration of 25 ppm for plant height, number of leaf $plant^{-1}$ and leaf area whereas lower concentration beneficial for stem thickness. In case of P NPs treatment the higher concentration found satisfactory results almost all the morphological characteristics. In situation to yield characteristics of spinach beet leaf weight and seed yield showed significant ($p < 0.05$) impact with by application of ZnO, P and TiO_2 NPs treatments (Table 2). Significantly enhancement of fresh leaf weight (gm $plant^{-1}$) exhibited with 25 ppm of ZnO, 40 ppm P and 20 ppm of TiO_2 with an increase of 17.16%, 18.33% and 16.41% respectively, in assessment to control. Whereas, seed yield (gm $plant^{-1}$) an average increase by ZnO, P and TiO_2 NPs occurred with 12.48% 13.01% and 10.93% respectively, compared to control (Table 2).

Table 2
Effect of ZnO, P and TiO_2 NPs plant morphological and yield traits of spinach beet

Treatment (ppm)	Plant height (cm)	Number of leaf $plant^{-1}$	Leaf length (cm)	Leaf width (cm)	Stem thickness (mm)	Leaf area (cm^2)	Fresh leaf weight (gm)	Seed yield $plant^{-1}$ (gm)
Control	79.81 ±1.77h	13.81 ±1.01g	14.57±1.17e	10.35±0.52f	11.07 ± 0.28g	193.12 ±3.62k	71.45 ±0.75k	12.45 ±0.30h
ZnO-5	80.35 ±0.10h	14.72 ±1.05efg	17.43 ±0.43abcd	12.46 ±0.28def	12.04 ±0.41fg	198.44 ±2.95jk	74.89 ±0.35j	14.46 ±0.26bcde
ZnO-10	85.40 ±2.84fgh	17.67 ±1.12bcd	19.16 ±0.59a	12.05 ±0.91ef	15.93 ±0.87ab	212.31 ±3.24hi	78.36 ±0.27hi	13.13±0.12fgh
ZnO-15	84.80 ±2.78fgh	16.07 ±0.80defg	17.15 ±0.65abcd	11.53 ±1.24f	14.09 ±0.51cd	227.86 ±5.73efg	75.99 ±0.75 ij	15.35 ±0.37ab
ZnO-20	89.43 ±2.38ef	16.48 ±0.85def	17.75 ±1.12abcd	11.87 ±0.91ef	12.52±0.40efg	225.90 ±5.13fg	84.80 ±0.33bcd	13.89 ±0.32def
ZnO-25	92.88 ±1.61de	20.87 ±1.51a	17.75 ±0.49abcd	11.95 ±0.77ef	15.72 ±0.50ab	239.81 ±2.70cd	86.24 ±1.09 ab	16.08 ±0.34a
Avg. of ZnO	86.57	17.16	17.85	11.97	14.06	220.69	80.06	86.57
P-10	82.50 ±1.81gh	15.67 ±0.54defg	18.29 ±1.05ab	15.67 ±1.27abc	13.61 ±0.67cde	219.47 ±3.34gh	80.13 ±1.18 gh	14.59 ±0.43bcd
P-20	98.15 ±1.21d	17.98 ±0.67bcd	18.31 ±0.67ab	17.98 ±0.30a	12.79 ±0.61def	207.12 ±2.98ij	83.98 ±0.94bcde	14.42 ±0.34bcde
P-30	96.17 ±1.57d	14.73 ±0.55fg	15.61 ±0.93de	14.74 ±0.79bcd	12.77 ±0.05def	229.80 ±3.20ef	87.49 ±0.38a	14.37 ±0.25bcde
P-40	93.05 ±2.55de	16.82 ±0.75de	17.62 ±0.70abc	16.82 ±1.01ab	12.92 ±0.63def	248.70 ±3.50bc	77.75 ±2.22hi	12.86 ±0.31gh
P-50	87.55 ±1.17efg	14.10 ±0.94fg	18.91 ±0.53a	14.11 ±0.74cde	13.63 ±0.57cde	259.21 ±1.79a	83.46 ±0.38cdef	15.32 ±0.77ab
Avg. of P	91.49	15.86	17.75	15.86	13.14	231.80	82.56	91.49
TiO_2-10	117.07 ±1.76b	17.09 ±0.66cde	19.11 ±1.33a	11.70 ±0.66f	16.67 ±0.21a	241.88 ±3.10cd	81.15 ±0.59fg	13.07 ±0.35fgh
TiO_2-20	120.84 ±1.67ab	19.33 ±0.58abc	15.47 ±0.60de	10.72 ±1.01f	13.44 ±0.48cdef	236.86 ±2.58de	82.29 ±0.23efg	13.91 ±0.20def
TiO_2-30	123.29 ±1.30a	19.77 ±0.73ab	15.89 ±1.04cde	10.79 ±0.70f	13.09 ±0.38def	254.77 ±2.89ab	82.54 ±0.25defg	13.56 ±0.26efg
TiO_2-40	111.00 ±1.12c	17.38 ±0.49cde	18.48 ±1.18ab	12.43 ±0.41f	13.92 ±0.83cde	234.24 ±2.75def	85.48 ±0.36abc	14.25 ±0.18cde
TiO_2-50	110.18 ±2.62c	19.29 ±0.76abc	16.26 ±0.60bcde	11.29 ±0.53f	14.71 ±0.28bc	258.60 ±2.62a	84.39 ±0.20bcde	15.10 ±0.12abc
Avg. of TiO_2	116.48	18.57	17.04	11.39	14.37	244.66	83.17	116.48

*Mean value± SE. Each value represents the mean of three replication of each treatment. Different letter of mean value are significantly different at P<0.05 according to the Duncan's multiple range test (DMRT) and the same letters indicate no significant difference P<0.05.

3.2.2 Effect of ZnO, P and TiO_2 NPs on dry matter production and nutrients accumulation in spinach beet

Effect of NPs on dry matter and nutrients accumulation were presented in Table 3. Our study revealed that maximum dry matter was observed by ZnO NPs at 25 ppm (36.55 g), P NPs at 20 ppm (35.66 g) and TiO_2 NPs at 40 ppm (34.35g) that which 22.74%, 20.81% and 17.76% enhancement monitored as compared to control. The nutrient uptake in spinach beet leaves exposed by NPs, indicating their differential response with the treatments. Among the analyzed elements, significant difference in total N, P, K, Zn and Fe contents was found in leaves exposed to NPs. Our results depicted that improvement of major and minor elements in the leaves with an average increase 15.60%, 9.39% and 12.01% of N, 21.62%, 52.44% and 16.00% of P, 10.13%, 14.11%, and 11.75% of K, 50.76%, 7.71%, and 6.41% of Zn and 12.69%, 18.49% and 14.17% of Fe by ZnO, P, and TiO_2 NPs respectively, over the control plants (Table 3).

Table 3
Effect of ZnO, P and TiO_2 NPs on dry matter production and nutrients accumulation in spinach beet

Treatment (ppm)	**Dry matter (gm plant^{-1})**	**N (%)**	**P (%)**	**K (%)**	**Zn (ppm)**	**Iron (ppm)**
Control (0 ppm)	28.24 ± 1.14f	0.608 ± 0.02e	0.775 ± 0.05d	3.56 ± 0. 29e	40.26 ± 1.64e	212.36 ± 2.40g
ZnO-5	29.26 ± 2.60ef	0.636 ± 0.02e	0.955 ± 0.05cd	3.69 ± 0.25de	63.56 ± 2.22c	232.55 ± 2.20f
ZnO-10	30.24 ± 1.12cdef	0.672 ± 0.06cde	0.966 ± 0.05cd	3.88 ± 0.05bcde	70.69 ± 0.99b	236.56 ± 5.17ef
ZnO-15	29.65 ± 2.35def	0.756 ± 0.02abc	0.942 ± 0.05cd	4.12 ± 0.12abcd	88.66 ± 1.76a	254.36 ± 4.86bc
ZnO-20	33.56 ± 0.91abc	0.779 ± 0.02a	1.020 ± 0.09c	4.13 ± 0.13abcd	92.21 ± 3.00a	241.37 ± 4.26def
ZnO-25	36.56 ± 1.24a	0.759 ± 0.05ab	1.061 ±0.02c	3.99 ± 0.02bcde	93.66 ± 1.28a	251.33 ± 2.70cd
Avg. of ZnO	31.86	0.72	0.99	3.96	81.76	243.23
P-10	34.66 ± 0.92bcde	0.656 ± 0.03de	1.454 ± 0.11b	3.89 ± 0.04bcde	42.34 ± 1.75de	244.12 ± 2.20de
P-20	35.66 ± 1.11ab	0.689 ± 0.03bcde	1.560 ± 0.18ab	3.97 ± 0.10bcde	44.22 ±1.19de	245.36 ± 3.02cde
P-30	28.66 ± 0.52f	0.671 ± 0.03cde	1.661 ± 0.09ab	4.23 ± 0.12ab	41.66 ± 2.17de	262.56 ± 2.49b
P-40	34.66 ± 0.92ab	0.685 ± 0.03bcde	1.713 ± 0.12a	4.12 ± 0.16abcd	46.25 ± 1.76d	274.38 ± 4.17a
P-50	33.55 ± 0.94abc	0.654 ± 0.04de	1.760 ± 0.02a	4.53 ± 0.18a	43.66 ± 1.76de	276.22 ± 5.47a
Avg. of P	33.02	0.67	1.63	4.15	43.62	260.53
TiO_2-10	33.25 ± 0.58abcd	0.668 ± 0.03de	0.911 ± 0.05cd	4.12 ± 0.24abcd	42.34 ± 2.18de	232.25 ± 2.83f
TiO_2-20	33.22 ± 0.97abcd	0.724 ± 0.01abcd	0.923 ± 0.06cd	3.87 ± 0.13bcde	41.34 ± 0.35de	233.67 ± 1.21f
TiO_2-30	32.67 ± 0.88bcde	0.723 ± 0.01abcd	0.922 ± 0.02cd	4.16 ± 0.20abc	44.22 ± 2.25de	254.36 ± 2.65bc
TiO_2-40	34.35 ± 1.2ab	0.653 ± 0.02de	0.932 ± 0.01cd	4.33 ± 0.16ab	45.61 ± 2.26de	263.12 ± 2.69b
TiO_2-50	33.46 ± 0.96abc	0.687 ± 0.01bcde	0.925 ± 0.03cd	3.70 ± 0.15cde	41.58 ± 1.71de	254.68 ± 4.56bc
Avg. of TiO_2	33.39	0.69	0.92	4.04	43.02	247.61

*Mean value± SE. Each value represents the mean of three replication of each treatment. Different letter of mean value are significantly different at P<0.05 according to the Duncan's multiple range test (DMRT) and the same letters indicate no significant difference P<0.05.

3.2.2 Effect of ZnO, P and TiO_2 NPs on biochemical responses in spinach beet

The application of different NPs treatments increase total chlorophyll content in leaves where, TiO_2 NPs at 10 ppm proved to be best and increased by 17.18%, over the control (Table 4). All though successful improvement also detected from 10 ppm and 50 ppm of TiO_2 which were statistically at per. A progressive increase of total chlorophyll content by ZnO, P, and TiO_2 treatments with 30.17%, 28.24%, and 40.69% respectively, compared to control.

Table 4
Effect of ZnO, P and TiO_2 NPs on biochemical response in spinach beet

Treatment (ppm)	Total Chlorophyll (mg g-1 FW)	Protein (g/100g)	Carotene (mg/100g)	Ascorbic acid (mg/100g)	Total phenol (mg/100g D.W)	Crude fibre (%)
Control (0 ppm)	2.11 ± 0.11f	3.01 ± 0.22d	9.26 ± 0.61d	93.55 ± 2.54h	608.37 ± 4.51h	7.16 ± 0.15d
ZnO-5	2.52 ± 0.30ef	3.31 ± 0.10cd	10.26 ± 1.11cd	115.26 ± 2.69g	725.34 ± 4.84g	8.66 ± 0.09abc
ZnO-10	3.15 ±0.15bcd	3.57 ± 0.46bcd	12.36 ± 0.59abc	118.78 ± 3.19g	736.56 ± 1.90g	9.55 ± 0.44ab
ZnO-15	3.39 ± 0.10bc	3.49 ± 0.27bcd	11.38 ± 0.91abcd	144.66 ± 3.30e	825.45 ± 9.23cd	9.58 ± 0.47ab
ZnO-20	3.02 ± 0.10cd	4.08 ± 0.05ab	12.33 ± 1.08abc	135.44 ± 2.20f	837.55 ± 2.23ab	9.65 ± 0.83a
ZnO-25	3.03 ± 0.08cd	3.95 ± 0.24abc	10.36 ± 1.04bcd	138.34 ± 1.19ef	822.66 ± 4.07d	8.56 ± 0.31abc
Avg. of ZnO	3.02	3.68	11.34	130.50	789.51	9.20
P-10	3.13 ± 0.09bcd	3.48 ± 0.16bcd	12.56 ± 1.20abc	136.66 ± 2.45f	765.64 ± 10.49e	8.69 ± 0.46abc
P-20	3.24 ± 0.13bc	3.92 ± 0.17abc	12.36 ± 0.98abc	144.68 ± 2.15e	789.56 ± 4.01e	9.12 ± 0.57abc
P-30	2.46 ± 0.14 ef	3.86 ± 0.15abc	12.78 ± 0.58ab	166.57 ± 1.61c	796.22 ± 4.65e	8.68 ± 0.65abc
P-40	3.13 ± 0.17bcd	4.25 ± 0.05a	12.88 ± 0.19a	175.66 ± 5.42b	801.67 ± 3.34e	8.79 ± 0.54abc
P-50	2.74 ± 0.22de	4.25 ± 0.20a	12.36 ± 0.49abc	167.67 ± 1.54c	796.66 ± 2.42e	9.00 ± 0.26abc
Avg. of P	2.94	3.95	12.59	158.25	789.95	8.86
TiO_2-10	4.05 ± 0.13a	3.91 ± 0.34abc	11.66 ± 0.90abcd	156.65 ± 2.79d	842.33 ± 2.89 b	8.22 ± 0.46cd
TiO_2-20	3.08 ± 0.11cd	3.96 ± 0.08ab	12.58 ± 0.72abc	179.66 ± 2.86b	848.66 ± 3.30ab	8.34 ± 0.17bcd
TiO_2-30	3.55 ± 0.19b	4.03 ± 0.31ab	12.67 ± 0.81abc	189.66 ± 3.20a	857.12 ± 4.63a	8.55 ± 0.36abc
TiO_2-40	3.08 ± 0.11cd	4.09 ± 0.11ab	10.26 ± 1.03cd	193.24 ± 2.36a	844.21 ± 3.71ab	8.11 ± 0.41cd
TiO_2-50	4.02 ± 0.13a	4.09 ± 0.21ab	9.22 ± 0.82d	179.23 ± 1.94b	837.67 ± 3.83ab	7.88 ± 0.31cd
Avg. of TiO_2	3.56	4.02	11.28	179.69	846.00	8.22

*Mean value± SE. Each value represents the mean of three replication of each treatment. Different letter of mean value are significantly different at P<0.05 according to the Duncan's multiple range test (DMRT) and the same letters indicate no significant difference P<0.05.

The perusal of data in Table 4 revealed that use of NPs treatment significantly influences the leaf protein content of spinach beet. Protein content recorded maximum 4.25 g that of 40 ppm and 50 ppm concentration of P, where as plants under control had produced

3.15g only. ZnO, P, and TiO_2 NPs treatments protein content increased by 11.57%, 21.21%, and 21.09% respectively, over the control. Carotene content among the treatments varied considerable with the treatment. It was revealed that moderate concentration of NPs was found to be more effective for carotene content in the leaves. The application of NPs at higher concentration shows the decreasing tends of carotene. Total Carotene content of ZnO, P, and TiO_2 NPs treatments increased by 18.30%, 26.43%, and 17.85% respectively, compared to control. On the other hand ascorbic acid content on leaves also significantly influenced by NPs treatment (Table 4). The maximum of ascorbic acid content was recorded from 40 ppm TiO_2, which was also statistically at par with 40 ppm TiO_2. Total ascorbic acid content increased by 28.31%, 40.88%, and 48.03% of ZnO, P, and TiO_2 treatments respectively, as compared to control. The maximum total phenol was recorded from 30 ppm of TiO_2 NPs. An average effect of ZnO, P, and TiO_2 treatments on total phenol content increased by 22.94%, 22.99%, and 28.09% respectively, compared to control. In response to crude fibre content maximum was found when were treated with 20 ppm of ZnO NPs, whereas an enhancement of 22.21%, 19.18%, and 12.92% occurred by ZnO, P, and TiO_2 treatments respectively, compared to control.

4. Discussion

4.1. Germination and seeding development

Seed germination considered as an index of plant growth which required adequate water absorption that responses to yield characteristic. Our study exhibited that seed germination in spinach beet significantly enhanced by TiO_2 NPs than ZnO and P NPs and the result fully agreement with Hajra et al (2017). TiO_2 NPs found better germination percentage over ZnO NPs and P NPs may be due to production of reactive anions which accumulate more water absorption and boost the oxygen rate for uptake during germination (Khot et al. 2012). In viewpoint of Zheng et al. (2007) suggested that due to small particle size of TiO_2 NPs can easily penetrate into the seed coat of spinach and enhanced activity during growth. Zheng et al. (2005) in spinach and Feizi et al. (2012) in wheat observed accelerated germination in proper concentration of TiO_2 treatment. The study revealed that seed priming and presoaking techniques using different NPs can significantly improve spinach beet performance by increasing seedling growth and vigour, although response varied with different NPs and concentrations. In general, treatments with lower to moderate doses of TiO_2 NPs than ZnO and P NPs showed significant positive response in most of the seedling characters. Previously, Haghighi et al. (2014) opined that higher doses of TiO_2 NPs (up to 200 ppm) in tomato and onion be capable of enhanced root and shoot lengths. Seedling growth enhances by TiO_2 NPs treatment possibly due to it regulates the activity of nitrogen-metabolizing enzyme which helps to convert nitrogen for chlorophyll accumulation (Yang et al. 2006; Mishra et al. 2014). The enhancement of seedling weight and vigor index by ZnO, P and TiO_2 NPs also described our earlier report in tomato (Das et al. 2015) and TiO_2 NPs in rice (Debnath et al. 2020).

4.2. Antioxidant enzymatic activity

Spinach beet one of the important vegetables contains high antioxidant capacity and it protects the plant cells from oxidative damage during stress. Our results depicted that the enzymatic activities of CAT, POD, SOD, and GR responses significantly by application of ZnO, P and TiO_2 NPs treatment during seedling stage and varies upon changes of concentration (Figure 3A, 3B). Higher concentration of Phosphorus and ZnO NPs the enzymatic activity were increasing trends which suggested that these elemental particles actively utilized various metabolic processes as well as protein synthesis also decrease H_2O_2 levels and involve oxidative stress response in spinach beet. On the other hand higher concentration of TiO_2 a decreasing trend of CAT and GR activity was noticed, which might affect the failure of plant defense system. The highest activity of peroxidase was noticed by ZnO NPs at 15 ppm and positive effect also agreements with previous study (Faizan et al. 2017). Pretreatments of seeds by ZnO nanoparticles can improve germination and seedlings as well as antioxidant enzymes (García-López et al. 2018). The observed increase in GR activity suggests that glutathione is also involved in spinach plants, either directly to oxidative stress response or indirectly via the production of phytochelatins (Potters et al. 2002). The antioxidant activity effective towards dose-dependent with lower doses of ZnO NPs increases the catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) but decreases at extremely high concentration (200 ppm) in rapeseed (Sohail et al. 2020), in potato (Raigond et al. 2017) and in wheat and maize (Srivastav et al. 2021; Wang et al. (2019). Additionally, Khater (2015) reported that TiO_2 increased the production of antioxidant content in coriander. Additionally, Çatav et al. (2020) showed that higher doses of Cd severely increased GR, POD and SOD in wheat.

4.3. Morphological and yield traits of spinach beet

Our finding clearly depicted positive response plant growth and yield responses in nanoparticles treated plants as compared to control plants. Lower concentration of ZnO showed optimum improvement for the characters plant height, leaf length, leaf width, number of leaves and exposure to extremely high concentration showed optimum improvement for the characters stem thickness and leaf area (Table 2). Similarly the economic product fresh leaf weight showed optimum response at moderately high concentration. With high concentration of P NPs were found to produce effective improvement for majority of the characters except leaf length and number of leaves, where comparatively lower concentration had given the effective result. Extremely higher concentration of TiO_2 NPs treatments produced optimum improvement in economic product fresh leaf weight along with its component like plant height, number of leaves, stem thickness. Our present study both laboratory and field evaluation found a positive impact on spinach beet growth by NPs and the suggested mechanisms explain by increased nutrient adsorption and active transport of nutrients to the roots, activate enzyme for nutrient accumulation in leaf, enhanced synthesis of starch which ultimately increased the carbohydrate production and photosynthesis (Song et al. 2018). These mechanisms could be a contributing factor for increase average leaf yield found in spinach beet. In addition, the small size and high surface ratio with photo catalysis property of NPs which can interact with cellular system that contribute to overall effect of plant phenotypic changes and yield. Interestingly, Elizabath et al. (2017) reported a combination of ZnO and FeO NPs at 50ppm found good response of plant height, leaves plant^{-1}, petiole length, leaf area (cm^2) and yield of carrot. Recently Zhang et al. (2021) reported ZnO NPs application obtained higher yield of rice with more panicle number (4.83–13.14%), spikelets panicle^{-1} (4.81–10.69%), test weight (3.82–6.62%) and filled grain rate (0.28–2.36%). Kheyri et al. (2019) suggest that Zn and Si NPs applications in rice enhance grain yield upto 9.5, 9.2, and 6.9%, respectively, which reduce fertilizer costs as well as environmental pollution. Tarafder et al. (2014) reported grain yield in pear millet at crop maturity was improved by 37.7% due to application of zinc nanofertilizer. On the other hand, Khater, (2015) described TiO_2 with low concentration had enhances the plant height, branches and fruit yield in coriander. Recently, Waani et al. (2021) suggested TiO_2 with dose dependant action both positive and negative effect on plant growth and yield of rice. Earlier report also suggested that CeO and iron oxide NPs increased wheat and soybean yield by 36.6% and 48%, respectively (Yang et al. 2006; Sheykhbaglou et al. 2010).

4.4. Effect of NPs nutrient accumulation of spinach beet

The application of NPs found to be beneficial in increasing dry matter of plant as compared control. Better production of dry matter by NPs treatments suggested that plant accumulate higher nutrient supply in respective plant parts in particularly leaf and produce larger size. Our study revealed that by ZnO NPs with higher concentration maximum accumulation of nitrogen content found in spinach beet leaf and this might have accelerated the activity of several enzymes which in turn results better nitrogen content. On the other hand the phosphorus accumulation in treated leaves significantly increased by P NPs (Table 3). This result also supported by application of TiO_2 NPs that enhanced acid phosphatase activity and increase phosphorus nutrients (Raliya and Tarafdar 2014). In another report, it has been suggested that use of 500 mg kg^{-1} TiO_2 NPs increased the P content in shoots (138.9%) in rice (Zahra et al. 2017). The maximum potassium content of observed in 50 ppm of P NPs. Our investigation also suggested that application of ZnO NPs significantly increased the zinc and iron content in spinach beet. The enrichment of zinc and iron content in spinach beet leaves might be due to availability and increased uptake of ZnO NPs by plants. Du et al. (2019) recommended that to overcome the problem of Zn deficiency in plants Zn NPs will be best responsive slow release fertilizer. Recently, Zhang et al. (2021) reported that ZnO NPs significantly increased Zn content in polished rice which could be remarkable achievement under biofortified nutrition rich rice grain. The application of TiO_2 NPs at higher concentration (500 mg kg^{-1}) significantly many fold changes of Fe, Zn, Cu and P accumulation occurred in rice (Arshad et al. 2021). This contrasts with results by Hruby et al. (2002), who found that *Avena sativa* L. showed increased Mg and Fe uptake with increased Ti exposure at 2–18 mg L^{-1}. Additionally, Li et al. (2021) reported hydroxyapatite NPs were promising beneficial response for nutrient uptake of Cu, Zn, Fe, Mn, and B in soybean tissues. With the application of Fe_3O_4 NPs tremendous nutrient accumulation of P, K, Mn, Ca and Fe content recorded in different tissues of common bean (Souza et al. 2019).

4.5. Biochemical trait responses in spinach beet

The experimental results indicate that TiO_2 and ZnO NPs with moderate to higher concentration could significantly improve spinach beet growth and accelerate maximum chlorophyll synthesis. Surprisingly, Morteza et al. (2013) described utilizing of nano TiO_2 at 0.03% concentration could increase chlorophyll content in corn. In contrast to this, opposite response was found by ZnO NPs plants which accumulate more chlorophyll and carotene content than TiO_2 NPs treated plants (Hajra et al. 2017).

Spinach beet being a leafy vegetable contains good source of vitamin C, protein, fibre, calcium and iron. In the current study, maximum protein content in spinach beet was observed at 50 ppm of P NPs (Table 4) suggesting that acceleration of nutrients particularly nitrogen which enhances protein content in spinach beet leaves. Tarafder et al. (2014) found significant enhancement in chlorophyll content (24.4%), leaf protein (38.7%) and plant dry biomass (12.5%) in *Pennisetum americanum* by use of Zn NPs. Following nano TiO_2 treatments also accumulate more photosynthetic activity, enhanced mRNA expression and protein content in spinach (Gao et al. 2006). Our results showed that chlorophyll and carotene content were increased by increasing the doses of TiO_2 NPs and this reports are agreement with recent study in carrot (Gholami et al. 2020). Moreover, Sohail et al. (2020) reported that application of Zn NPs drastically improved protein content, total soluble sugar, proline, total flavonoid, and phenolic content in rapeseed. Whereas, Ghafari et al. (2013) suggested that nano-iron oxide (2 g L^{-1}) increases chlorophyll, grain protein and iron contents in wheat.

Conclusion

In this study a comprehensive assessment of ZnO, P and TiO_2 NPs was achieved to target for improvement of seed germination antioxidant activity, nutritional quality and yield of spinach beet. NPs treatments showed the capability to increase seedling growth and many fold volume of antioxidant enzyme in spinach beet. Variable response to different concentrations of nanoparticles was noticed changes of plant phenotypic character in spinach beet. Higher concentration of P and moderate to high concentration of ZnO and TiO_2 NPs improve the plant nutrient (Zn and Fe content) content and quality traits as well as economic yield of spinach beet. Development of nanotechnology paved the way or increase productivity of spinach beet but at the same time it has raised concern for its adverse effect on biological system, food safety and environment system. It needs further study to get answer of question as to what extent molecular and genetic factors may mediate plant response to nano exposure with mitigation of adverse effect on plant development and environment.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

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Figures

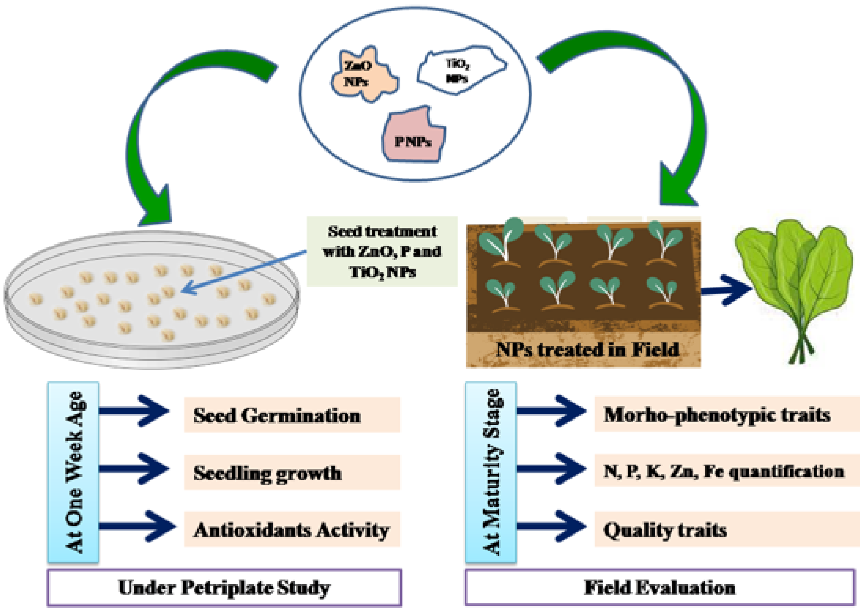

Figure 1

Schematic illustration of ZnO, P and TiO_2 NPs under laboratory and field study of Spinach beet variety All Green

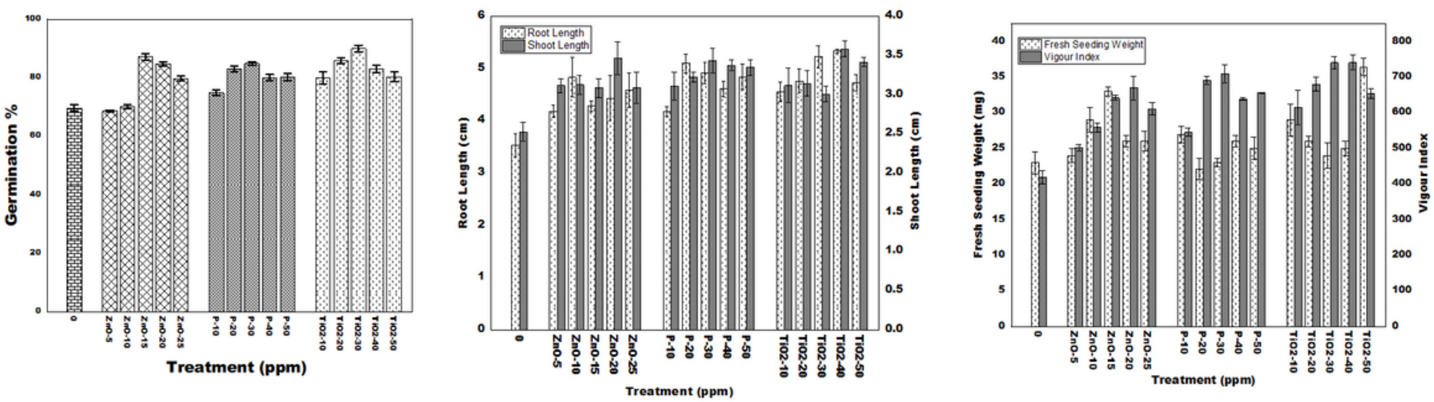

Figure 2

A. Effect of ZnO, P and TiO_2 NPs treatments for germination percentage of Spinach beet

B. Effect of ZnO, P and TiO_2 NPs treatments for root length and shoot length of Spinach beet

C. Effect of ZnO, P and TiO_2 NPs treatments for fresh seedling weight and vigour index of Spinach beet

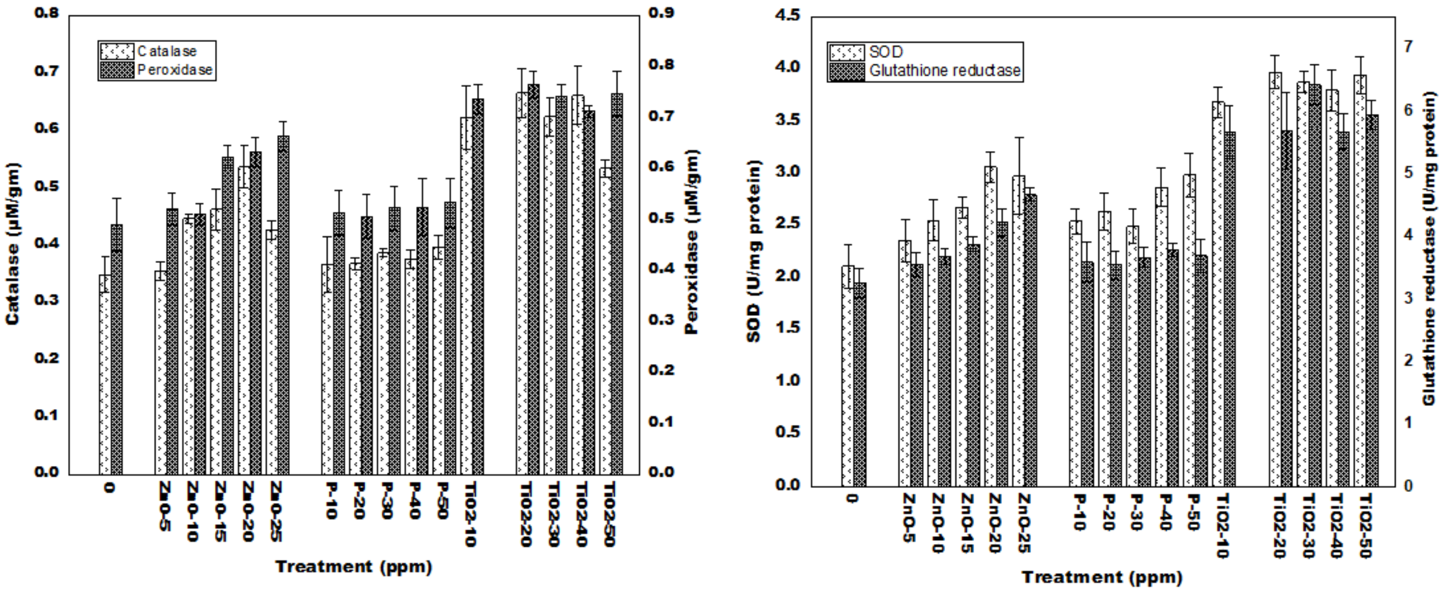

Figure 3

A. Effect of ZnO, P and TiO_2 NPs treatments for Catalase (CAT) and Peroxidase (POD) enzyme activity of Spinach beet at one week of seedling

B. Effect of ZnO, P and TiO_2 NPs treatments for Superoxide dismutase (SOD) and Glutathione reductase (GR) enzyme activity of Spinach beet at one week of seedling

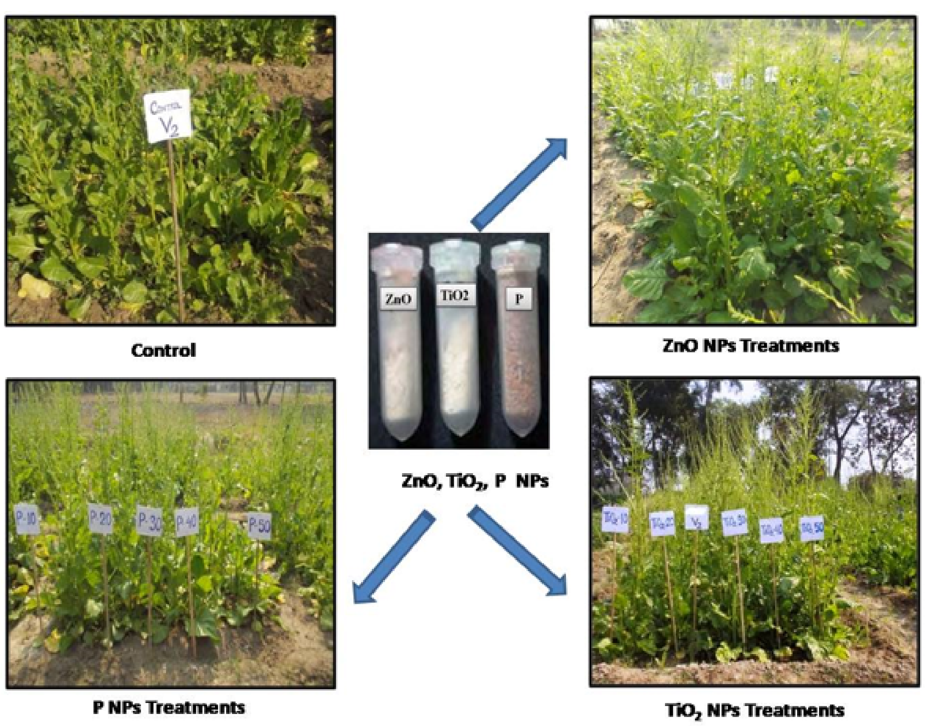

Figure 4

ZnO, P and TiO_2 NPs treatments responses under field evaluation of Spinach beet variety All Green