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TiO₂ nanoparticles can enhance germination and seedling growth of mung bean (*Vigna radiata* L.)

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Abstract

The present experimental investigation demonstrated the effect of TiO₂ nanoparticle on germination and seedling growth parameters of mung bean. The experiment was carried out with six treatments using 0, 10, 20, 30, 40, 50 ppm of TiO₂ nanoparticles including control with sterilized water under factor CRD manner following 3 replications. Application of TiO₂ nanoparticle significantly stimulate germination and seedling growth with most of the treatment as compared with control. Lower concentration of nano TiO₂ treatment in mung bean was found effective to enhance almost all the seedling characters like germination percentage, root length, shoot length, root fresh weight, shoot fresh weight, total fresh weight, root dry weight, shoot dry weight, total dry weight, root/shoot ratio, vigor index except root/shoot ratio of Sonali which showed no positive influence. Most effective treatment showing best performance considering all the varieties was found in lower concentration for all the seedling characters. Panna for germination percentage, IPM 23 for root length, shoot fresh weight, total fresh weight, shoot dry weight, total dry weight, vigor index and Sonali for shoot length, root fresh weight, root dry weight, root/shoot ratio were found most responsive with the treatment of TiO₂ nanoparticle. The increase in seed germination along with seedling growth in specific concentration of TiO₂ nanoparticle suggested optimum dose limit on growth of seedlings. Interaction of treatments with varieties was evident in most cases which could be considered as variable response on different varieties for which standardization of concentration of nanoparticle should be done to obtain most effective result.

Keywords: TiO₂ nanoparticle, mung bean, germination, growth parameters

Introduction

India is the largest producer and consumer of pulses in the world accounting for 33% of world's area and 22% of world's production of pulses. It is cultivated across the country through out the year with an area and production of 2.53 m ha and 1.93 mt, respectively (Annual Report, 2014-2015) [1]. The genus *vigna* includes a number of grain legume crops of high economic importance in the world. This includes mung bean [*Vigna radiata* (L.) Wilczek]; urd bean [*Vigna mungo* (L.) Hepper]; cowpea [*Vigna unguiculata* (L.) Walp.]; rice bean (*Vigna umbellata* Thunb.); moth bean (*Vigna aconitifolius*); bambara groundnut (*Vigna subterranea*); adzuki bean [*Vigna angularis* (Willd.) Ohwi & H. Ohashi]; Sarawak bean [*Vigna hosei* (W.G.Craib) Backer]; beach bean (*Vigna marina*), etc. Out of these mung bean is extensively cultivated in West Bengal as well as India. Mung bean is one of the thirteen food legumes grown in India and third most important pulse crop of India after chickpea and pigeon pea. The average yield of mung bean is low due to its indeterminate growth habit, photoperiod sensitivity, late and non-synchronous maturity, susceptibility to lodging, pod shattering, and losses due to pests and diseases (Fernandez and Shanmugasundaram, 1988) [7].

Increasing production and productivity of crops through crop improvement, crop management and crop protection from pest and disease have been in vogue from time immemorial. Conventional and improved technologies have their own limitations. Technologies available are unable to break through some of the bottlenecks. Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties encountered in agriculture (Chinnamuthu and Boopathi, 2009) [3]. Agriculture is the backbone of most developing countries, with more than 60% of the population reliant on it for their livelihood (Brock *et al.*, 2011) [2]. Nanotechnology can improve our understanding of the biology of different crops potentially enhancing yields or nutritional values by developing improved systems for monitoring environmental conditions and delivering nutrients or pesticides as appropriate (Welch and Graham, 1999) [18].

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In plants, titanium stimulates production of more carbohydrate, encourage growth and photosynthesis rate (Choi *et al.*, 2005; Owolade *et al.*, 2008) [4, 14]. The most important effects of Titanium compounds on plants are enhancement of the yield of various crops (about 10–20%); an improvement of some essential element contents in plant tissues; an increase in the peroxidase, catalase, and nitrate reductase activities in plant tissues; and an enhancement of the chlorophyll content in paprika (*Capsicum anuum* L.) and green alga (*Chlorella pyrenoidosa*) (Hruby *et al.*, 2002) [11]. Plants interact with their atmospheric and edaphic environments strongly and are expected to be affected by exposure to engineered nanoparticles (Ruffini Castiglione and Cremonini, 2009) [16]. Hong *et al.* (2005 a & b) [9, 10]; Yang *et al.* (2006) [19] reported that a proper concentration of nano-TiO₂ was found to improve the growth and development of spinach by promoting aged seeds vigor and chlorophyll formation and stimulates Ribulose 1, 5-bisphosphate carboxylase (Rubisco) activity and increases photosynthesis. TiO₂ NPs increases light absorbance, hasten the transport and conversion of the light energy, protect chloroplasts from aging, and prolong the photosynthetic time of the chloroplasts. It may be due to TiO₂ NPs protects the chloroplast from excessive light by augmenting the activity of antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase (Hong *et al.*, 2005a) [9]. Tarafdar *et al.*, (2013) [17] revealed that the leaf chlorophyll content (56–287%) was significantly enhanced due to field application of TiO₂ nanoparticles on 14 days old arid crops (pearl millet, cluster bean, moth bean and mung bean). A significant improvement was also observed in shoot length (17.02%), root length (49.6%), root area (43%), root nodule (67.5%), chlorophyll content (46.4%) and total soluble leaf protein (94%) as a result of TiO₂ NPs application by Raliya *et al.*, (2015) [15]. The present project, therefore, aims at evaluation on the impact of TiO₂ nano particle on germination and seedling characters in 3 varieties of mung bean.

Material and Methods

To evaluate the effect of TiO₂ nanoparticle on germination and seedling growth in mung bean, the present experiment was conducted in the laboratory of Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal in the year 2013 using three varieties of mung bean viz. Panna, Sonali and IPM 23 collected from Department following factorial CRD manner with 3 replications. There are six treatments using different concentration of TiO₂ (0, 10, 20, 30, 40, 50 ppm) including control with sterilized water in the experiment. Uniform and healthy seeds were selected and sterilized with 0.1% Mercuric Chloride solution for 1 min then vigorously rinsed with sterilized distilled water. Ten seeds for each concentrations of nano TiO₂ were treated for 48 hours. After treatment seeds were implanted on glassplates at room temperature for further observations. The important characters considered in the present experiment were germination %, root length, shoot length, root fresh weight, shoot fresh weight, total fresh weight, root dry weight, shoot dry weight, total dry weight, root shoot ratio and vigor index. Observations were recorded from 3 week old seedling. TiO₂ (Anatase) nano particle was purchased from Sigma-Aldrich Company, St.Louis, MO, USA with a purity of 99.5%, particle size of (<100 nm). Data were subjected to analysis of variance and mean separation was assessed by critical difference (CD) at 5%

probability. Data was analyzed using OPSTAT software.

Results and Discussion

Effect of TiO₂ nanoparticles on germination and seedling characters of mung bean

The analysis of variance revealed significant difference within varieties, treatments with different concentrations of TiO₂ nanoparticle and interaction between varieties and treatments for all the seedling characteristics except germination percentage. Results registered by the different mung bean varieties at various levels of concentrations of TiO₂ nanoparticle are presented in the table 1 and in the figures 1 & 2.

Germination percentage (%)

In control condition, highest germination percentage was recorded in Sonali (83.33%) and lowest in Panna (80.00%) whereas IPM 23 recorded 81.67% germination. Statistically significant increment in germination percentage was observed across all concentrations of TiO₂ nanoparticle over control. 10 ppm was found as best concentration among all concentration of TiO₂ nanoparticle. Panna recorded 96.67% of germination, Sonali recorded 93.33% of germination and IPM 23 recorded 95.00% of germination at 10 ppm and there was no significant difference with 20 ppm (95.00%, 90.00% & 93.33% respectively).

Root length (cm)

Maximum root length was observed in Sonali (12.71 cm) and minimum in Panna (11.67 cm) followed by IPM 23 (11.83 cm) as control. Statistically significant promotional impact on root length was found with application of all the concentrations as compared to respective control. The most favorable effect on root length was obtained with 10 ppm of TiO₂ nanoparticle in all varieties. At this concentration of 10 ppm, Panna recorded 15.92 cm of root length, Sonali recorded 16.68 cm length of root and IPM 23 recorded 16.57 cm of root length, all followed by 20 ppm correspondingly (13.45 cm, 13.95 cm & 15.76 cm).

Shoot length (cm)

There was no significant difference across varieties for shoot length. Sonali was found to have longest shoot (13.99 cm) and exhibited no significant difference with Panna (13.96 cm) whereas IPM 23 recorded 13.60 cm of shoot length. A significant improvement of shoot length was reflected across the concentration levels in TiO₂ over control. 10 ppm of TiO₂ nanoparticle was revealed as best concentration for this character as all varieties performed their best at this concentration, Panna displayed most favorable shoot length with 18.51 cm, Sonali recorded 19.59 cm length of shoot and IPM 23 was found to had 19.30 cm of shoot length; the performance superseded the corresponding shoot lengths obtained by 20 ppm level respectively with 17.02 cm, 17.47 cm & 18.44 cm.

Root fresh weight (g)

In control condition, not much variation was noticed for root fresh weight by all varieties. In such case, IPM 23 was found to have maximum fresh weight of root (0.055 g) and displayed no significant difference with Panna (0.054 g) whereas Sonali recorded 0.052 g fresh weight of root under control condition. A significant gain in root fresh weight was recognized with all concentrations of TiO₂ nanoparticle over

respective control. 10 ppm of TiO₂ nanoparticle was reported as best concentration for this character as all varieties given their best result at the same concentration; Panna recorded 0.080 g fresh weight of root, Sonali recorded 0.100 g of root fresh weight and IPM 23 was also found to had 0.100 g of fresh weight of root followed by 20 ppm (0.077 g, 0.096 g & 0.093 g respectively).

Shoot fresh weight (g)

IPM 23 was found to have maximum shoot fresh weight (0.107 g) and Sonali recorded minimum fresh weight of shoot (0.092 g) at par with Panna (0.094 g) in control condition. Statistically significant increase in shoot fresh weight was observed with TiO₂ nanoparticle treatment. 10 ppm was revealed as best concentration for this character as all varieties performed best at this concentration, Panna recorded 0.161 g of shoot fresh weight, Sonali recorded 0.130 g of shoot fresh weight and IPM 23 recorded 0.185 g of fresh weight of shoot followed by 20 ppm correspondingly with the following values 0.153 g, 0.111 g & 0.179 g accordingly.

Total fresh weight (g)

IPM 23 was found to have maximum total fresh weight of seedling (0.164 g) and Sonali recorded minimum total fresh weight (0.147 g) at par with Panna (0.149 g) under control. Statistically significant gain in total fresh weight was observed with different concentrations of TiO₂ nanoparticle as compared to respective control. 10 ppm of TiO₂ nanoparticle was revealed as best concentration contributing to this character in all the varieties (Fig. 1), Panna recorded 0.241 g of total fresh weight, Sonali recorded 0.230 g of total fresh weight and IPM 23 recorded 0.288 g of total fresh weight of seedling followed by 20 ppm (0.231 g, 0.220 g & 0.275 g accordingly).

Root dry weight (g)

All varieties (Panna, Sonali and IPM 23) recorded the same dry weight of root i.e. 0.005 g, no significant differences among them were observed as control. A significant increment in root dry weight was augmented with application of all concentrations of TiO₂ nanoparticle over control. Panna recorded similar increments in root dry weight with 10 ppm, 20 ppm and 30 ppm (0.007 g) followed by 40 ppm (0.006 g) while Sonali and IPM 23 found to had similar dry weight of root (0.010 g) at 10 ppm both followed by 20 ppm having akin result (0.009 g) for TiO₂ nanoparticle.

Shoot dry weight (g)

In control condition, no such differences were found between varieties for shoot dry weight. In that case, IPM 23 was found to have maximum dry weight of shoot (0.010 g) and Panna and Sonali recorded similar dry weight of shoot (0.009 g). A significant gain in shoot dry weight was observed with nanoparticle treatment over control. 10 ppm of TiO₂ nanoparticle was reported as best concentration for this character as all varieties given their best result at this concentration, Panna recorded 0.016 g dry weight of shoot and exhibited no significant difference with 20 ppm, 30 ppm & 40 ppm which were found with similar dry weight of shoot (0.015 g); Sonali recorded 0.013 g of shoot dry weight at 10 ppm followed by 20 ppm (0.011 g) and IPM 23 was found to had 0.018 g dry weight of shoot at 10 ppm & 20 ppm at par with 30 ppm & 40 ppm which exhibited similar shoot dry weight (0.017 g).

Total dry weight (g)

In control condition, total dry weight of seedling was identical for all varieties; no significant variations were noticed. In such case, IPM 23 recorded 0.015 g of total dry weight; Sonali recorded 0.014 g total dry weight and Panna recorded 0.013 g of total dry weight. A significant enhancement in total dry weight was observed with TiO₂ nanoparticle. Panna recorded 0.023 g of total dry weight at 10 ppm & 20 ppm; Sonali and IPM 23 also recorded 0.023 g & 0.028 g of total dry weight at 10 ppm followed by 20 ppm (0.020 g & 0.027 g) (Fig. 1).

Root/Shoot Ratio

Highest root/shoot ratio was highlighted in Panna (0.541) and lowest in IPM 23 (0.502) on par with Sonali (0.506) under control condition. Statistically significant increment in root/shoot ratio was achieved by all varieties with treatment of TiO₂ nanoparticle over control. Sonali was observed with preeminent root/shoot ratio at 30 ppm (0.839) which exhibited no significant difference with 20 ppm (0.836) and IPM 23 was found to have paramount root/shoot at 10 ppm (0.527) at par with 20 ppm (0.510) of TiO₂ nanoparticle.

Vigor index

Highest vigor index was accounted by Sonali (22,241.33) and lowest by Panna (20,504.00) which displayed no significant difference with IPM 23 (20,772.67) as control. Significant enhanced vigor index was highlighted with application of TiO₂ nanoparticle in comparison with control (Fig. 2). Appreciated vigor index was recorded at 10 ppm of TiO₂ nanoparticle; Panna estimated vigor index value (33,272.00), Sonali figured the value of vigor index was 33,853.33 and IPM 23 accounted 34,087.33 vigor index value at the same concentration followed by 20 ppm (28,937.17; 28,281.00 & 31,928.00 respectively).

Lower concentration of nano TiO₂ treatment in mung bean was found effective to enhance almost all the seedling characters like germination percentage, root length, shoot length, root fresh weight, shoot fresh weight, total fresh weight, root dry weight, shoot dry weight, total dry weight, root/shoot ratio, vigor index except root/shoot ratio of Sonali which showed no positive influence. Most effective treatment showing best performance considering all the varieties was found in lower concentration for all the seedling characters. Panna for germination percentage, IPM 23 for root length, shoot fresh weight, total fresh weight, shoot dry weight, total dry weight, vigor index and Sonali for shoot length, root fresh weight, root dry weight, root/shoot ratio were found most responsive with the treatment of TiO₂ nanoparticle.

Seed germination could be considered as index of plant growth, development and yield and as a beginning of physiological process, seed germination required adequate water absorption. The effect of nano particles on germination could be related to water imbibition that directed enhanced germination. Seed coat played protective role in preventing penetration of nanoparticles to embryo and keep the embryo unharmed from many external factors but after germination, radicles penetrating the seed could come in direct contact with nanoparticles. Recently demonstrated that multiwalled nanotubes (MWCNTs) at relatively low doses (10-40 µg/mL) can penetrate thick seed coats, stimulate germination, and activate enhanced growth in tomato plants due to promotion in water uptake (Khodakovskaya *et al.*, 2009 and 2011)^[13, 12]. Water uptake in seed germination is important because mature

seeds are fairly dry and need a large amount of water to start cellular metabolism and growth. Penetration of nanoparticle might be through stomata of plant leaves. The uptake rate depends on the size and the surface properties of the nanoparticles. Nanoparticles could enter the xylem via the cortex and the central cylinder and may accumulate in the vacuole. Gao *et al.* (2006) [8]; Hong *et al.* (2005b) [10]; Zheng *et al.* (2005) [20] found significant positive effect on growth following nano sized TiO₂ treatments. Higher shoot growth could normally be attributed to higher photosynthesis as also observed after TiO₂ application on spinach. High photosynthesis would require steady supply of water and nutrients that is primarily through roots. Thus, a better root system would be logically expected to support the higher photosynthesis.

The increase in seed germination along with seedling growth in specific concentration of TiO₂ nanoparticle suggested optimum dose limit on growth of seedlings. Interaction of treatments with varieties was evident in most cases which

could be considered as variable response on different varieties for which standardization of concentration of nanoparticle should be done to obtain most effective result. Similar results were obtained by several researchers which supports the result obtained in this study. Feizi *et al.*, (2012) [6] too revealed that employing nano sized TiO₂ in suitable concentration could promote the seed germination of wheat and also enhance shoot length, seedling length and root dry matters, significantly. Das *et al.*, (2015) [5] found a significant positive effect on seed germination and seedling growth characteristics with application of nano ZnO, P and TiO₂ in different concentrations nanoparticle which could be due to highly positive responses of nanoparticles on various seedling growth characteristics in tomato. Again a similar report of significant improvement in mung bean for shoot length (17.02%), root length (49.6%), root area (43%) and root nodule (67.5%) was reported by Raliya *et al.*, (2015) [15] as a result of TiO₂ NPs application.

Table 1: Effect of Nano TiO₂ on germination & seedling characters of mung bean

Source of Variation	DF	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Total fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Total dry weight (g)	Root/Shoot Ratio	Vigor index	Anova
Mean squares													
Factor(A)	2	22.685	12.768**	3.941**	0.001**	0.014**	0.012**	0.000**	0.000**	0.000**	0.439**	19,722,011.310**	
Factor(B)	5	249.630**	26.531**	37.767**	0.002**	0.004**	0.012**	0.000**	0.000**	0.000**	0.025**	212,933,930.841**	
Factor(A X B)	10	8.796	1.408**	1.554**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.024**	3,554,125.338	
Error	34	22.222	0.032	0.202	0.000	0.000	0.000	0.000	0.000	0.000	0.004	2,392,826.130	

Two Way Mean

	Germination percentage (%)							Root length (cm)						
	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A
Panna	80.00	96.67	95.00	93.33	90.00	83.33	89.72	11.67	15.92	13.45	12.31	11.95	10.96	12.71
Sonali	83.33	93.33	90.00	88.33	86.67	83.33	87.50	12.71	16.68	13.95	13.63	12.80	10.80	13.43
IPM23	81.67	95.00	93.33	90.00	86.67	83.33	88.33	11.83	16.57	15.76	14.58	14.33	13.26	14.39
Mean B	81.67	95.00	92.78	90.56	87.78	83.33		12.07	16.39	14.39	13.51	13.03	11.67	
Factors	C.D. at 5%		SE(d)			SE(m) ±		C.D. at 5%		SE(d)			SE(m) ±	
Factor(A)	N/A		1.571			1.111		0.121		0.060			0.042	
Factor(B)	4.525		2.222			1.571		0.171		0.084			0.060	
Factor(A X B)	N/A		3.849			2.722		0.297		0.146			0.103	

	Shoot length (cm)							Root fresh weight (g)						
	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A
Panna	13.96	18.51	17.02	15.88	14.99	14.66	15.84	0.054	0.080	0.077	0.074	0.062	0.057	0.067
Sonali	13.99	19.59	17.47	17.12	15.31	12.67	16.03	0.052	0.100	0.096	0.086	0.078	0.066	0.080
IPM23	13.60	19.30	18.44	17.26	16.60	15.15	16.73	0.055	0.100	0.093	0.078	0.074	0.055	0.076
Mean B	13.85	19.13	17.64	16.75	15.64	14.16		0.054	0.093	0.088	0.079	0.072	0.059	
Factors	C.D. at 5%		SE(d)			SE(m) ±		C.D. at 5%		SE(d)			SE(m) ±	
Factor(A)	0.305		0.150			0.106		0.002		0.001			0.001	
Factor(B)	0.431		0.212			0.150		0.003		0.001			0.001	
Factor(A X B)	0.747		0.367			0.259		0.005		0.003			0.002	

	Shoot fresh weight (g)							Root dry weight (g)						
	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A
Panna	0.094	0.161	0.153	0.150	0.149	0.138	0.141	0.005	0.007	0.007	0.007	0.006	0.005	0.006
Sonali	0.092	0.130	0.111	0.107	0.103	0.100	0.107	0.005	0.010	0.009	0.008	0.008	0.006	0.008
IPM23	0.107	0.185	0.179	0.177	0.171	0.160	0.163	0.005	0.010	0.009	0.008	0.007	0.005	0.007
Mean B	0.097	0.158	0.147	0.144	0.141	0.133		0.005	0.009	0.008	0.008	0.007	0.006	
Factors	C.D. at 5%		SE(d)			SE(m) ±		C.D. at 5%		SE(d)			SE(m) ±	
Factor(A)	0.002		0.001			0.001		0.000		0.000			0.000	

Factor(B)	0.003	0.001	0.001	0.001	0.000	0.000
Factor(A X B)	0.005	0.002	0.002	0.001	0.000	0.000

	Shoot dry weight (g)							Root/Shoot Ratio						
	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A	Control 0 (ppm)	TiO ₂ 10 (ppm)	TiO ₂ 20 (ppm)	TiO ₂ 30 (ppm)	TiO ₂ 40 (ppm)	TiO ₂ 50 (ppm)	Mean A
Panna	0.009	0.016	0.015	0.015	0.015	0.014	0.014	0.541	0.463	0.468	0.454	0.421	0.392	0.457
Sonali	0.009	0.013	0.011	0.010	0.010	0.009	0.010	0.506	0.748	0.836	0.839	0.796	0.645	0.728
IPM23	0.010	0.018	0.018	0.017	0.017	0.016	0.016	0.502	0.527	0.510	0.442	0.431	0.342	0.459
Mean B	0.009	0.016	0.015	0.014	0.014	0.013		0.516	0.580	0.605	0.578	0.549	0.460	
Factors	C.D. at 5%		SE(d)			SE(m) ±		C.D. at 5%		SE(d)			SE(m) ±	
Factor(A)	0.000		0.000			0.000		0.041		0.020			0.014	
Factor(B)	0.001		0.000			0.000		0.058		0.028			0.020	
Factor(A X B)	0.001		0.000			0.000		0.100		0.049			0.035	

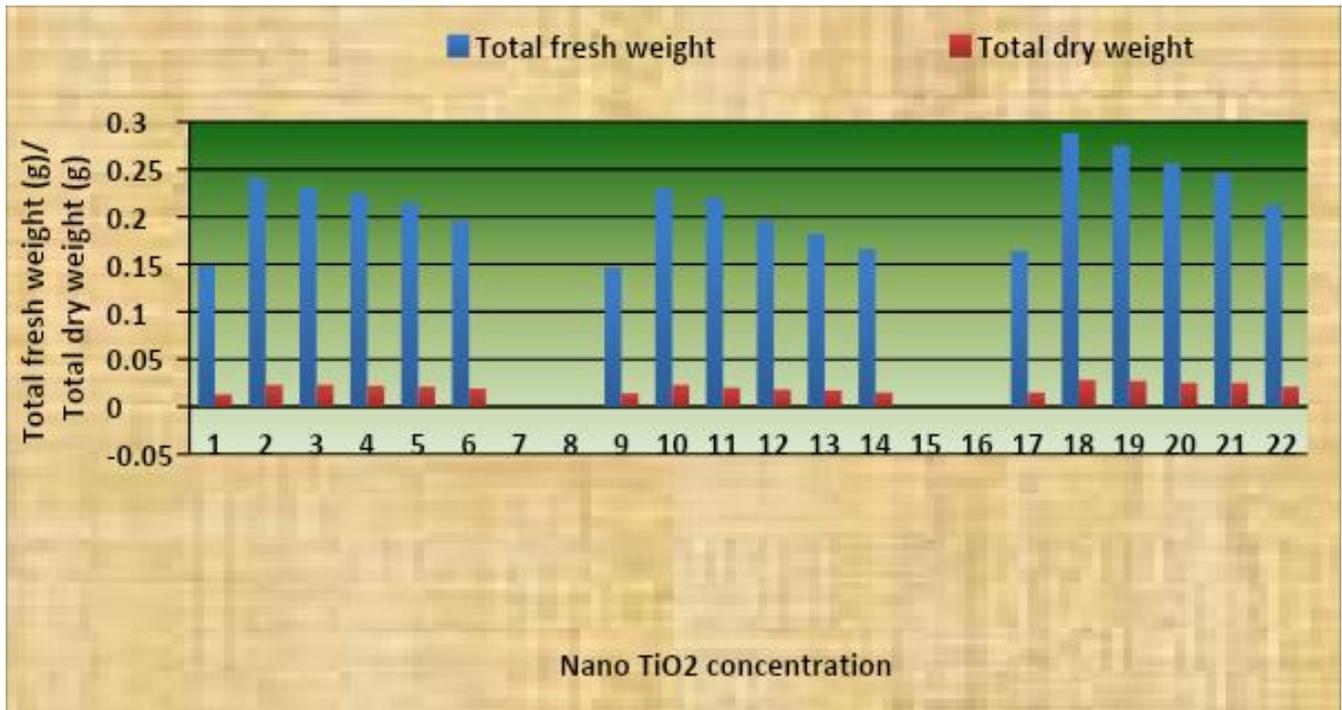


Fig 1: Effect of nano TiO₂ on total fresh and dry weight of mung seedling

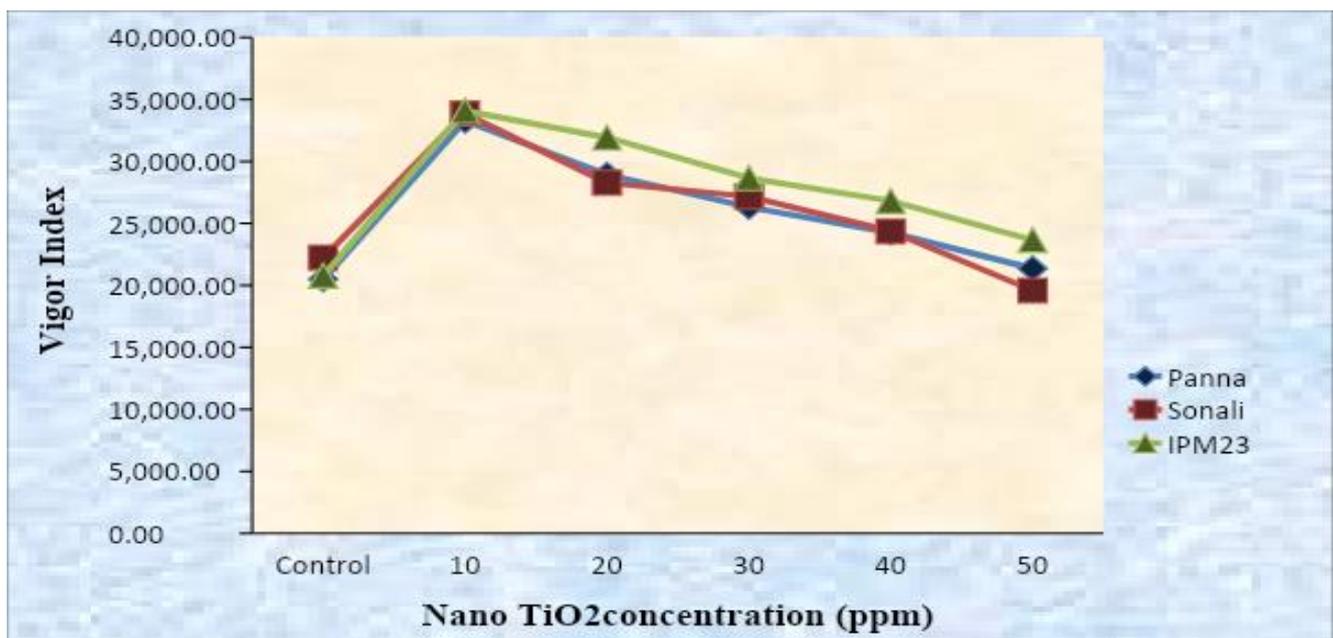


Fig 2: Dose response curve of different conc. of TiO₂ nanoparticle on vigor index of mung seedling

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