International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 12(2): 121-127, June 2019
DOI: 10.30954/0974-1712.06.2019.7

SOIL SCIENCE

Effect of Nano-Titanium Dioxide Polymorphs Priming on Seed Germination and Seedling Growth of French Bean (*Phaseolus vulgaris* L.)

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Paper No. 774 Received: 10-02-2019 Revised: 22-05-2019 Accepted: 26-05-2019

ABSTRACT

A laboratory experiment was performed to examine the effect of nano-TiO_2 polymorphs priming on seed germination and seedling growth of french bean (*Phaseolus vulgaris* L.). Nano-TiO_2 polymorphs i.e. anatase and rutile, were synthesized by the sol-gel method using titanium tetraisopropoxide as Ti-precursor and 2-propanol as solvent. Seeds of french bean were treated with nine different concentrations (in water) of each nano-TiO_2 polymorphs (0, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50 %) by soaking in different concentrations suspension (prepared by ultrasonication method) for 24 hours. At the end of the experiment, the percentage of seed germination, seed germination index, seedling vigour index, root length, shoot length, number of lateral roots, seedling fresh weight, seedling dry weight were evaluated at 15 days following paper towel method. The results showed that increase in concentration of each nano-TiO_2 polymorphs caused a significant increase in each study parameters. The best concentration of nano-TiO_2 polymorphs was 2.50 %. The findings illustrated the beneficial effect of nano-TiO_2 polymorphs priming on seed germination and plant growth of french bean.

Highlights

- Nano-TiO_2 polymorphs improved seed germination and seedling growth of french bean.
- Performance of smaller polymorph, anatase (14 nm) was higher than rutile (52 nm).

Keywords: nano-TiO_2 polymorph, sol-gel, french bean

Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles (NPs) have unique properties, i.e., high surface area, high reactivity, tunable pore size and particle morphology. Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Roco, 2003), that can drastically modify their physical–chemical properties compared to the bulk material (Nel *et al*. 2006; Feizi *et al*. 2012).

Titania (TiO_2) exists in three main polymorphic crystallographic structures, namely, rutile, anatase and brookite. Titania-based nanocatalysts are being increasingly used in photocatalysis. Sol-gel is the most simple and sophisticated method (Byun *et al.* 2002) among the various methods, in which various material parameters such as the powder morphology, surface area, average crystallite size and phase structure are controlled in determining photocatalytic activity of TiO_2.

French bean (*Phaseolus vulgaris* L.) is an herbaceous annual plant that is distributed worldwide for its edible fruit. Beans are an important food among the fabaceae family for people of all income categories as a source of dietary protein, vitamins, fibre, complex carbohydrates (Kutos *et al*. 2003) and bioactive compounds with antioxidant capacities (Granito *et al.* 2008).

Till date few studies have focused on the effect of TiO_2-NPs on seed germination of wheat, maize, rapeseed, radish, tomato, onion, parsley and fennel (Haghighi *et al.* 2014; Song *et al.* 2013; Feizi *et al.* 2012; Feizi *et al.* 2013; Dehkourdi *et al.* 2013; Song *et al*. 2013; Kim *et al*. 2015). Haghighi and Dasilva (2014) reported that there was 100% seed germination of tomato and onion with 100 mg L^{-1} of TiO_2-NPs and for radish, 100% germination was observed with 400 mg L^{-1}, respectively.

Keeping these points in mind this work, we decided to find out the effect of various types polymorphs of Titania (TiO_2) nano particles on seed germination and seedling growth of french bean (*Phaseolus vulgaris* L.).

MATERIALS AND METHODS

Synthesis of nano-titanium dioxide polymorphs

10 mL of Titanium tetraisopropoxide (TTIP) as precursor molecule was added drop wise to 30 mL of 2-propanol and the mixture was stirred for 15 minutes on magnetic stirrer. The resulting solution was then added drop wise to another mixture which was prepared by mixing 10 ml 2-propanol with 10 ml distilled water and stirred on magnetic stirrer for 15 minutes. 5 drops of concentrated HCl was added to the final mixed solution to bring the solution pH to 3.0 after which obtained sol converted into a gel. The obtained gel was then dehydrated in an air circulating oven at 100°C for 12 hours. The dehydrated material was then calcined in an induction furnace at 500°C and 850°C for 5 hours for obtaining anatase and rutile TiO_2 nanoparticles, respectively. White powder obtained after calcinations was cooled, ground and weighed to estimate the yield of the final product. The size of the anatase and rutile was determined in transmission electron microscopy (TEM) and the values are 14 nm and 52 nm, respectively.

Seed Priming by nano-TiO_2 & germination test of french bean seeds

Germination tests performed using paper towel method. 50-100 seeds of french bean (*var*. Kashi Rajhansh, ICAR-IIVR) of similar size were randomly selected and surface-sterilized using 10% sodium hypochlorite solution. Seeds were washed in deionized water, soaked in distilled water (control samples) and in TiO_2 nanoparticle suspension (treatment) at eight different concentrations (0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 & 2.50%) of nano-TiO_2 two polymorphs, viz. rutile & anatase each for 24 hours. All the samples were transferred to germination paper folded with butter paper to germinate until 3 & 5 days in controlled environmental conditions (temp: 25°C & RH: 65%). Three replicates of each concentration were prepared. Seed was considered as germinated when their radicle showed at least 2 mm length. At the end of the germination period, observation of the various germination test parameters and seedling growth parameters were taken.

(i) Germination percentage =

$$\frac{\text{Total number of seeds germinated}}{\text{Total number of seeds in all replicates}} \times 100$$

(ii) Germination Index =

$$\frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \frac{\text{Number of germinated seeds}}{\text{Days of second count}} + \ldots\ldots\ldots\ldots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

(iii) Seedling Vigor Index = (Shoot + Root length in cm) × Germination %

(iv) Mean germination time = $\frac{\sum F.X}{\sum F}$

F = Number of seeds newly germinated at the time X &

X = Number of days from soaked with water

Statistical analysis

Experimental data generated during the seed germination and seedling growth test were analyzed statistically for their test of significance as per completely randomized design, at 5% level of significance.

RESULTS AND DISCUSSION

Impact of nano-TiO_2 particles on seed germination of french bean

The seed priming with two polymorphs of nano-TiO_2 particles, viz. anatase (14 nm) and rutile (52 nm) in different concentration showed significantly improvement in the percentage of germination as well as kinetic of germination i.e. mean seed germination time. The significant improvement of germination by seed priming (Table 1 & 2 and Fig. 1 & 2) with both the polymorphs was noticed in the concentration of suspension from 1.0 to 2.5% and maximum percentage was observed in 2.5% suspension of both the anatase and rutile. The percentage enhancement of germination at 2.5 % concentration was 40.0 and 46.6 in the suspension of anatase and rutile, respectively. The germination index was also significantly improved by priming the seed with nano-TiO_2; maximum germination index was noticed in both the polymorphs at 2.5% concentration. It was noticed from the data on mean germination time that the kinetics of biochemical processes of germination was significantly increased in respect of control on seed priming with lower concentration of anatase (0.75%) and higher concentration of rutile (1.50 to 2.50%).

The reason possibly that nano-TiO_2 can penetrate through hard seed coat of french bean and may be activate the biochemical processes in embryo. Both the nano-TiO_2 polymorphs of nano-TiO_2 particle, viz. anatase (14 nm) and rutile (52 nm) activated photogeneration of active oxygen like superoxide and hydroxide anions that resulting enhanced

Table 1: Effect of anatase nanoparticle (TiO_2) on seed germination of french bean

Sl. No.	Treatment	Number of seed germination		Percentage of seed germination		Germination index	Mean seed germination time (days)
		Days		Days			
		3	5	3	5		
1	T_0 (0 %)	3.33	5.33	33.33	53.33	2.17	3.57
2	T_1 (0.10 %)	4.00	6.00	40.00	60.00	2.53	3.64
3	T_2 (0.25 %)	6.67	8.67	66.67	86.67	3.97	3.46
4	T_3 (0.50 %)	6.00	7.33	60.00	73.33	3.39	3.30
5	T_4 (0.75 %)	7.67	7.67	76.67	76.67	4.18	3.00
6	T_5 (1.00 %)	7.67	8.33	76.67	83.33	4.22	3.17
7	T_6 (1.50 %)	8.00	8.33	80.00	83.33	4.34	3.07
8	T_7 (2.00 %)	7.33	8.00	73.33	80.00	4.05	3.17
9	T_8 (2.50 %)	8.67	9.33	86.67	93.33	4.75	3.15
	SE(±)	0.79	0.78	7.94	7.78	0.38	0.17
	CD (P = 0.05)	2.38	2.33	23.58	23.11	1.13	0.51

Table 2: Effect of rutile nanoparticle on seed germination of french bean

Sl. No.	Treatment	Number of seed germination		Percentage of seed germination		Germination index	Mean seed germination time (days)
		Days		Days			
		3	5	3	5		
1	T_0 (0 %)	2.33	4.33	23.33	46.67	1.64	3.84
2	T_1 (0.10 %)	3.00	5.00	30.00	50.00	2.02	3.81
3	T_2 (0.25 %)	3.67	5.67	36.67	53.33	2.37	3.74
4	T_3 (0.50 %)	5.00	7.00	50.00	70.00	3.06	3.56
5	T_4 (0.75 %)	5.67	6.67	56.67	66.67	3.22	3.28
6	T_5 (1.00 %)	4.33	5.67	43.33	56.67	2.57	3.74
7	T_6 (1.50 %)	7.33	8.00	73.33	80.00	4.04	3.16
8	T_7 (2.00 %)	7.33	8.33	73.33	83.33	4.11	3.29
9	T_8 (2.50 %)	8.00	9.33	80.00	93.33	4.53	3.27
	SE(±)	0.92	0.92	9.23	9.43	0.46	0.18
	CD (P = 0.05)	2.74	2.74	27.42	28.01	1.36	0.52

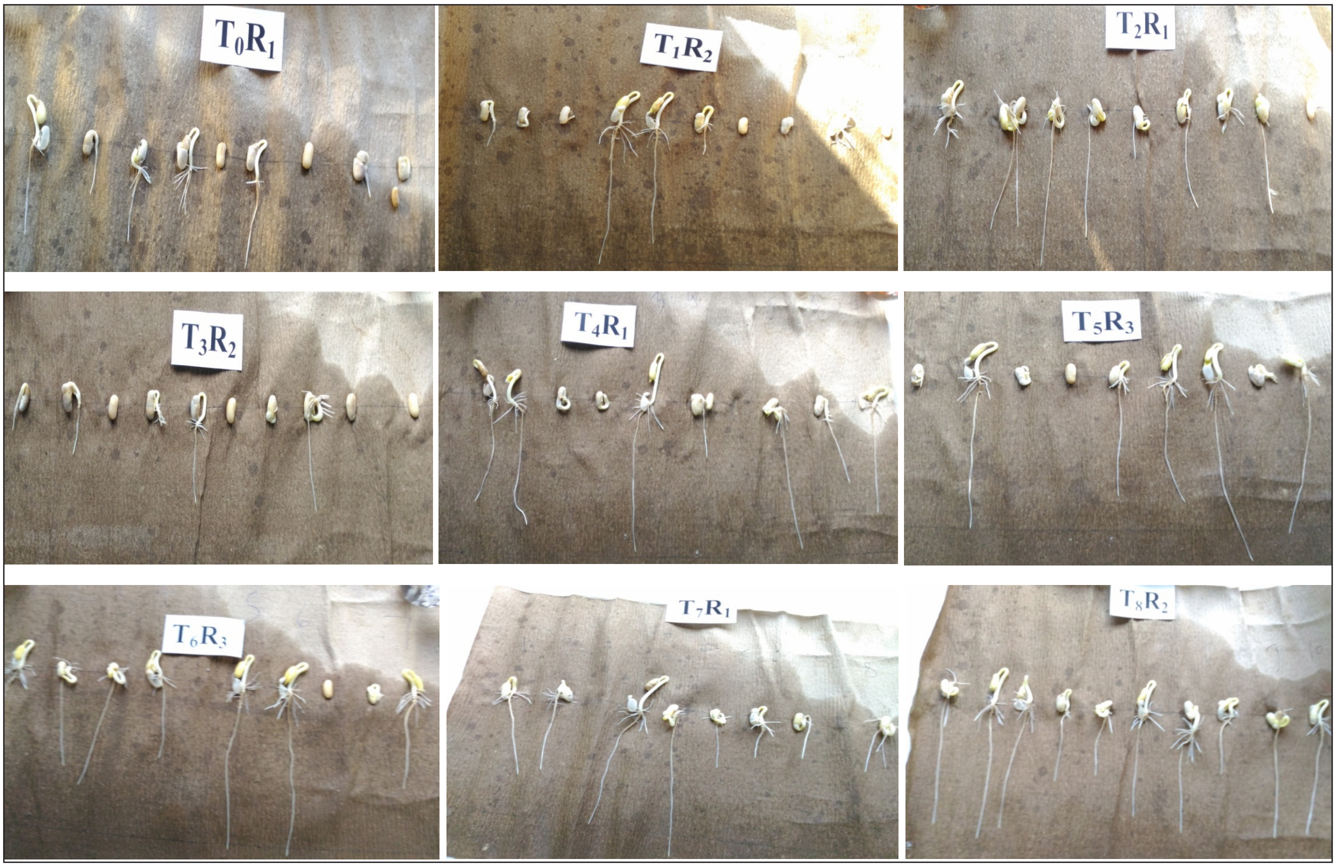

Fig. 1: Effect of anatase nanoparticle (TiO_2) on seed germination of french bean

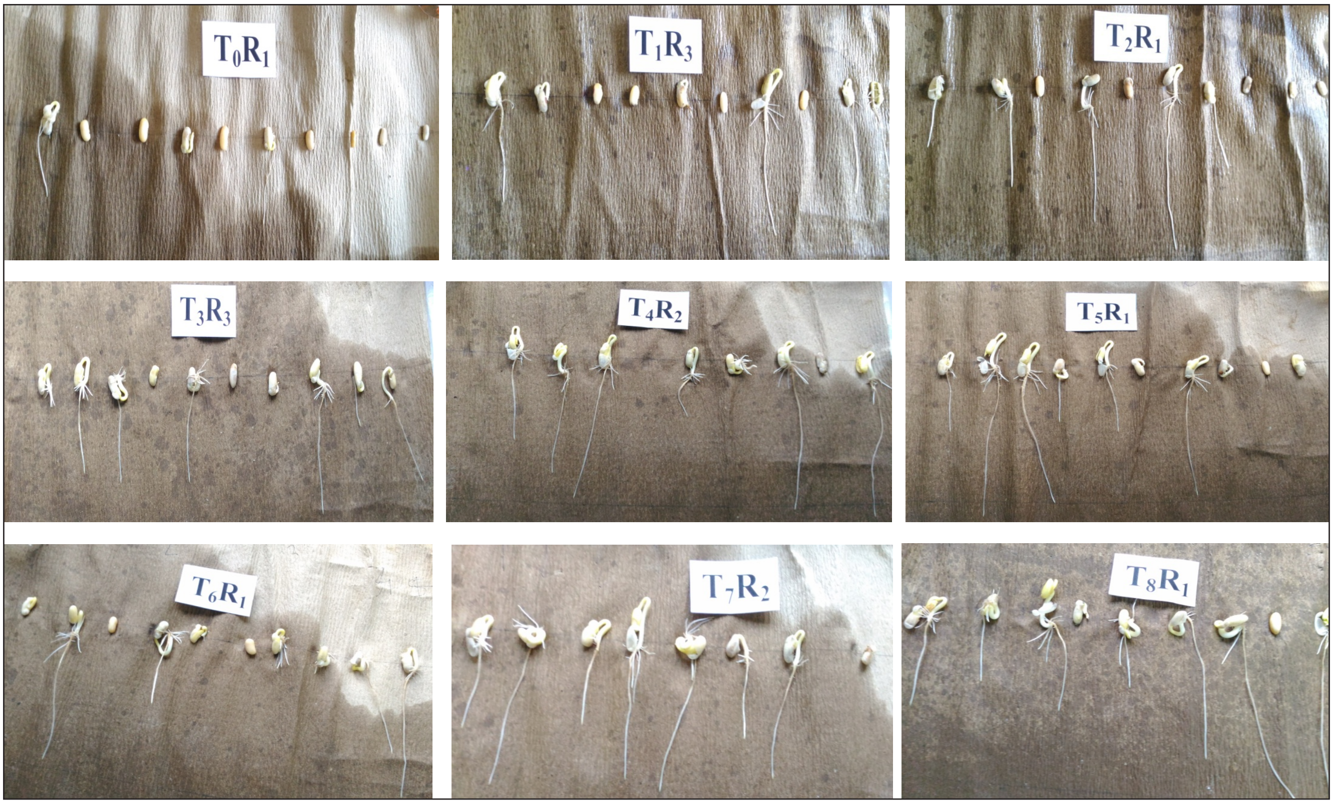

Fig. 2: Effect of rutile nanoparticle (TiO_2) on seed germination of french bean

penetrability of the seed capsule, facilitating the admission of water and dioxygen into the cells and thus improving the biochemical processes of metabolism and germination of seeds. In addition to that mechanism, penetration of nano-TiO_2 into embryo would induce redox reactions via the superoxide ion radical during germination in the dark, resulting in the quenching of free radicals in the germinating seeds. Oxygen generated in such process could be used for respiration, which would further promote rate of respiration and promote germination kinetics. Zheng *et al*. (2005) also observed that when 2.5% nano-TiO_2 was used on spinach seed, it could accelerate metabolism, photosynthesis and enzyme activity, as well as germination characteristics such as germination rate and vigor index.

Impact of nano-TiO_2 particles on seedling growth of french bean

The shoot and root length, fresh and dry weight, vigour index and the number of lateral roots of seedling were significantly increased (Table 3 and 4) due to seed priming with both the nano-TiO_2 particles in different concentration i.e. 0.1 to 2.5% of anatase and rutile. The fresh and dry weights of seedling in 7 and 21 days were significantly higher than those of the untreated control. The increase of dry weight was more significant than that of fresh weight. The maximum increment of the root and shoot length, vigour index as well as fresh and dry weight were found at 2.5% rutile and 2.0 to 2.5% of anatase (T_7 and T_8 was significantly at par). As size of nano-TiO_2 polymorph, anatase (14 nm) particle

Table 3: Effect of anatase nanoparticle on seedling growth of french bean

Sl. No.	Treatment	Seedling Vigour Index		Root length (cm)		Shoot length (cm)		Number of lateral roots	Fresh weight of seedling (g)	Dry weight of seedling (g)
		Days		Days		Days				
		3	5	7	21	7	21			
1	T_0 (0 %)	7.23	8.20	3.64	14.89	7.09	9.57	41.33	1.70	9.47
2.	T_1 (0.10 %)	12.11	17.76	8.47	19.50	13.57	13.80	63.67	3.33	19.33
3	T_2 (0.25 %)	22.17	28.41	8.88	23.50	11.60	13.00	116.00	2.90	15.80
4	T_3 (0.50 %)	18.67	20.92	5.96	19.83	10.80	12.29	59.00	2.27	14.30
5	T_4 (0.75 %)	24.83	26.33	7.61	20.75	13.42	17.00	88.67	3.20	16.30
6	T_5 (1.00 %)	27.30	29.85	8.69	25.33	14.03	18.50	132.00	3.60	16.43
7	T_6 (1.50 %)	27.93	29.07	8.71	21.25	13.74	16.25	124.33	3.47	16.53
8	T_7 (2.00 %)	30.70	33.44	8.73	25.58	19.30	22.58	135.00	3.80	18.93
9	T_8 (2.50 %)	35.47	37.92	9.28	24.00	16.34	16.75	98.00	2.77	13.70
	SE(±)	3.42	2.78	0.93	1.83	1.21	1.71	11.22	0.39	0.02
	CD (P = 0.05)	10.16	8.24	2.76	5.44	3.58	5.08	33.34	1.15	0.06

Table 4: Effect of rutile nanoparticle on seedling growth of french bean

Sl. No.	Treatment	Seedling Vigour Index		Root length (cm)		Shoot length (cm)		Number of lateral roots	Fresh weight of seedling (g)	Dry weight of seedling (g)
		Days		Days		Days		—	—	—
		3	5	7	21	7	21	—	—	—
1	T_0 (0 %)	7.36	12.81	4.44	9.22	10.31	9.42	57.69	2.23	10.37
2	T_1 (0.10 %)	8.89	12.75	6.23	15.92	11.83	16.08	77.17	2.40	12.17
3	T_2 (0.25 %)	11.53	18.66	8.21	21.83	15.80	16.58	106.83	3.07	14.53
4	T_3 (0.50 %)	14.57	20.36	5.14	15.83	17.88	18.00	82.50	2.63	12.37
5	T_4 (0.75 %)	16.32	19.35	6.64	14.58	14.35	15.17	36.57	2.71	12.61
6	T_5 (1.00 %)	12.84	17.22	6.25	16.23	17.21	17.00	86.50	2.97	12.63
7	T_6 (1.50 %)	22.85	24.72	6.94	18.12	19.22	19.45	81.00	2.60	11.60
8	T_7 (2.00 %)	26.00	28.83	5.25	15.42	17.17	17.83	73.50	3.50	15.00
9	T_8 (2.50 %)	28.24	33.33	6.38	18.92	20.30	27.08	93.17	3.17	15.37
	SE(±)	3.43	3.45	0.59	2.06	1.79	2.31	16.28	0.35	0.02
	CD (P = 0.05)	10.18	10.25	1.76	6.11	5.31	6.86	48.39	1.04	0.05

is smaller than those of rutile (52 nm), the effect of enhancement of plant growth due to priming was comparatively higher in anatase (Table 3) than rutile (Table 4). Further, the number of lateral roots in seedling of 21 days old were remarkablely improved in seed treated with both the nano-TiO_2 particles and the highest number were noticed in T_2 (at par with T_7 of anatase), i.e. 0.25% anatase and 0.25 % of rutile. As a consequence these lateral roots system, water and nutrient absorption likely to obviously increase in comparison to untreated nano-TiO_2 particles. It was observed that seed priming with 0.25% anatase and rutile enhanced 2.8 times and 1.8 times lateral root growth of seedling. Thus, smaller size of nanoparticles (anatase : 14 nm & rutile : 52 nm) was observed major factor for biochemical activities in seed germination and seedling growth.

Increased water absorption (Zheng *et al*. 2005), photosynthetic processes (Jiang *et al.* 2017) for nitrogen metabolisms and enzymatic activities particularly nitrate reductase (NiR), glutamate dehydrogenase, glutamine synthase & glutamine –pyruvic transaminase (Yang *et al.* 2006) play critical roles of plant seed germination and seedling growth of french bean by nano-TiO_2 particles priming. Active oxygen radicals, may be activated by NP-TiO_2 (Khot *et al.* 2012) and increase the penetrability of the water and oxygen through seed capsule and thus enhanced the growth of seedling. Thus, smaller size of nano-TiO_2 polymorph, viz. anatase showed better performance in both seed germination and seedling growth.

CONCLUSION

Seed priming with nano-TiO_2 of different size, i.e. anatase (14 nm) and rutile (52 nm) particularly at 2.5 % proved remarkable improvement in seed germination, germination kinetics, seedling shoot and root growth as well as fresh and dry weight after 21 days. Performance of anatase was observed comparatively better than that of rutile particle due to better penetration in seed coat and involvement in different biochemical activities during germination and seedling growth through photocatalytic activities of nano-TiO_2.

ACKNOWLEDGEMENTS

First author is thankful to UGC for the National Fellowship for OBC (F/2017-18/NFO-2017-18-OBC-ASS-61264) during her course of investigation. Authors are also thankful to Prof. A.K. Ghosh, Nano-technology Laboratory in the Department of Physics, Banaras Hindu University, Varanasi for infrastructural support to synthesis of nano-TiO_2 particle.

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