1. Introduction

Nanotechnology is a rapidly growing discipline of science and technology, which has potential to revolutionize the fields of agriculture, economy, society and environment (Kumar et al., 2012; NAAS, 2013). The market of nanotechnology-based products and their synthesis processes is expected to increase by US$ 1 trillion (Roco and Bainbridge, 2001; Maynard et al., 2006; Rejeski and Lekas, 2008; Husen and Siddiqi, 2014). Its wide application in synthesis process, environmental remediation, cosmetic, agriculture sector, medicine, materials synthesis, etc. has made nanotechnology a thrust area of research and development in recent years. However, it generates both positive and negative responses from the governments, scientists and social media throughout the world (Brumfiel, 2003; Service, 2003; Yang et al., 2006).

Nanomaterials are present in both natural and engineered forms. Engineered nanomaterials have different physical and chemical properties as compared to the naturally occurring nanoparticles, and thus have different effect on the environment (Husen and Siddiqi, 2014). USEPA (2010) grouped different natural and engineered nanomaterials into seven major groups such as (1) carbonaceous (natural or engineered, *e.g.*, fullerenes/buckyballs and nanotubes); (2) metal oxides (natural or engineered, *e.g.*, titanium oxide (TiO_2) and cerium oxide (CeO_2)); (3) zero-valent metals (engineered, *e.g.*, nanoscale zero-valent iron (nZVI) and emulsified zero-valent iron (EZVI)); (4) quantum dots (engineered, *e.g.*, quantum dots made from cadmium selenide (CdSe) and cadmium telluride (CdTe)); (5) dendrimers (engineered, *e.g.*, hyperbranched polymers and dendrigraft polymers); (6) composite nanopolymenrs (engineered, *e.g.*, made with two different nanomaterials or resins); and (7) nanosilver (engineered, *e.g.*, colloidal silver and polymeric silver). The effect of these engineered nanomaterials on the environment is limited.

Engineered nanoparticles have various applications in agricultural research, reproductive science, transformation of agricultural and food waste to energy, nano-bioprocessing of enzymes, plant disease prevention and treatments using nanocides (Carmen et al., 2003). For instance, titanium dioxide (TiO_2) is widely used in many process industries (including agrochemicals, cosmetic medicines, textiles, electronics, pharmaceutics, and environmental remediation) due to its abundant availability, low cost, amphoteric nature and catalytic activities (Feizi et al., 2012). The release of such nanoparticles in the environment through various routes including synthesis processes is considered harmful to the environment. Therefore, proper recycling and disposal of nano-based products is imperative. Also, the effect of disposed nanomaterials in the agricultural systems is of vital importance.

Generally, the effect of nanomaterials on plant and soil depends upon their particle size and nature of crystalline structure (Clément et al., 2013; Dehkourdi and Mosavi, 2013; Feizi et al., 2012, 2013; Song et al., 2013b). For example, the effect of nano-sized TiO_2 on seed germination and growth of various plants showed ambiguous results. Both negative and positive effects ranging from strong toxicity to the root-shoot systems to the growth stimulating effects has been observed depending upon the concentration (Song et al., 2013b). Various positive and negative effects of TiO_2 and other nanoparticles associated with the seed germination and plant growth are summarized in Table 6.1 and 6.2. Various researches found the negative effects of TiO_2 nanoparticles on few plant species (García et al., 2011; Jośko and Oleszczuk, 2013; Mushtaq, 2011; Song et al., 2013a). Moreover, various other effects of nanomaterials on the plants and soil ecology interaction are still unknown (Lin and Xing, 2007). Studies have supported the TiO_2 application to soil for enhancement in the chlorophyll content and enzymatic activities such as peroxidase, catalase and nitrate reductase. It, therefore, positively affects the growth by improving essential element content in plant tissue in various crops (Feizi et al., 2012; Hruby et al., 2002). Therefore, the use of TiO_2 nanoparticles as a possible new approach to overcome the problems with seed germination in plants, having lower germination rate and higher dormancy period such as medicinal, could be of significant importance (Feizi et al., 2013).

How the nanoparticles affects seed germination and plant development could have economic significance for agriculture (Jośko and Oleszczuk, 2013; Gong et al., 2011; Gottschalk and Nowack, 2011). The effect depends upon the concentration, nature, and size of nanoparticles. Presently, carbon-based TiO_2 nanomaterials (Mattle and Thampi, 2013), carbon nano-tubes (Ouyang et al., 2013), Fe_2O_3-TiO_2 composite (Ouyang et al., 2013), N-doped TiO_2 (Sun et al., 2008) catalysts are being widely used for the remediation of various environmental contaminations. During this process, some amounts of these nano-composites are released in the environment and reach the agriculture systems. Limited studies have been done to understand the environmental impacts of TiO_2 and other such semiconductors used as catalysts. Therefore, effect of TiO_2 and activated carbon nano-composite (TiO_2/AC) on the seed germination of two important agricultural crops *viz., Solanum lycopersicon* and *Vigna radiata* have been studied in the present study. Following objectives has been put to achieve the aim in this study.

(1). Characterization of unused TiO_2 (TiO_2/AC) nano-composite after photocatalytic degradation of dye (refer chapter 3), and (2). Evaluation of effect of nano-composite on the seed germination, root and shoot length and weight of two crops

Table 6.1: The effect of TiO_2 and related nano-composites on the seed germination of different vegetable crops

S.N.	Materials	Seed	Effect	Reference
1	TiO_2	*Brassica campestris* ssp. *nippo-oleifera* Makina (oilseed rape), *Lactuca sativa* L. (lettuce), and *Phaseolus vulgaris* var. *humilis* (kidney bean)	No effect on seed germination. Positive effects on root elongation	Song et al. 2013
2	Anatase TiO_2	(*Petroselinum crispum*)	Significant increase in the percentage of germination, germination rate index, root and shoot length, fresh weight, vigor index, and chlorophyll content of seedlings	Dehkourd and Mosavi 2013
3	TiO_2	Tomato, Onion, and Radish Seed	Most positive effect on germination	Haghighi and Silva 2014

4	TiO_2	Spinach	During the growth stage, the plant dry weight was increased, as was the chlorophyll formation, the ribulosebisphosphate carboxylase /oxygenase activity, and the photosynthetic rate	Zheng et al. 2004
5	TiO_2	*Vicia narbonensis* L. and *Zea mays* L	Induced genotoxic effect for both species.	Castiglione et al. 2011
6	TiO_2	Wheat Seed	Promote the seed germination of wheat in comparison to bulk TiO_2 but in high concentrations had inhibitory or any effect on wheat	Feizi et al. 2012
7	AC/TiO_2	*Solanum lycopersicum* and *Vigna radiata*	Promote the seed germination in both species	Present study

Table 6.2: Positive and negative effects of nanomateirals at various concentrations on the seed germination behaviour of different vegetable crops (+ shows the positive response and – shows negative response to root shoot ratio)

Seed	Materials	Concentration of materials	Germination rate (%)	Root: shoot
Vigna radiate	TiO_2/AC nanocomposites	100 ppm	90	+
Vigna radiate	TiO_2/AC nanocomposites	500 ppm	95	+
Vigna radiate	GO	100 ppm	25	_
Vigna radiate	GO	500 ppm	0	_
Vigna radiate	TiO_2 -GO	100 ppm	100	+
Vigna radiate	TiO_2 -GO	500 ppm	82	+
Vigna radiate	Ag-TiO_2-GO	100 ppm	90	+
Vigna radiate	Ag-TiO_2-GO	500 ppm	85	+
Solanum lycopersicum	TiO_2/AC nanocomposites	500 ppm	70	+
Solanum lycopersicum	TiO_2 -GO	100 ppm	30	_
		500 ppm	0	_
Solanum lycopersicum	Ag-TiO_2-GO	100 ppm	40	_
		500 ppm	25	_
Solanum melongena	TiO_2 -GO	100 ppm	0	_
		500 ppm	0	_
Cucumis sativus	TiO_2 -GO	100 ppm	50	_
		500 ppm	30	_
Cucumis sativus	Ce-TiO_2	100 ppm	40	_
Cucumis sativus	Si-Ag	100 ppm	80	+
		500 ppm	40	_

6.2 Experimental: (See chapter 3)

6.3 Results and discussion

6.3.1 Scanning Electron Microscopy

Surface morphology of unused composite was characterized by SEM as shown in (Fig. 6.1). The rough porous surface of activated carbon results from the growth of TiO_2 nanoparticles on it. Most of the TiO_2 nanoparticles were entirely filled into interstitial pores of activated carbon particles (Fig. 6.1a, b). Presence of TiO_2 along with activated carbon has been also inferred by EDX analysis results shown in Fig. 6.1c. This revealed that the TiO_2 nanoparticles get adsorbed on the surface of activated carbon to form TiO_2/AC nano-composites.

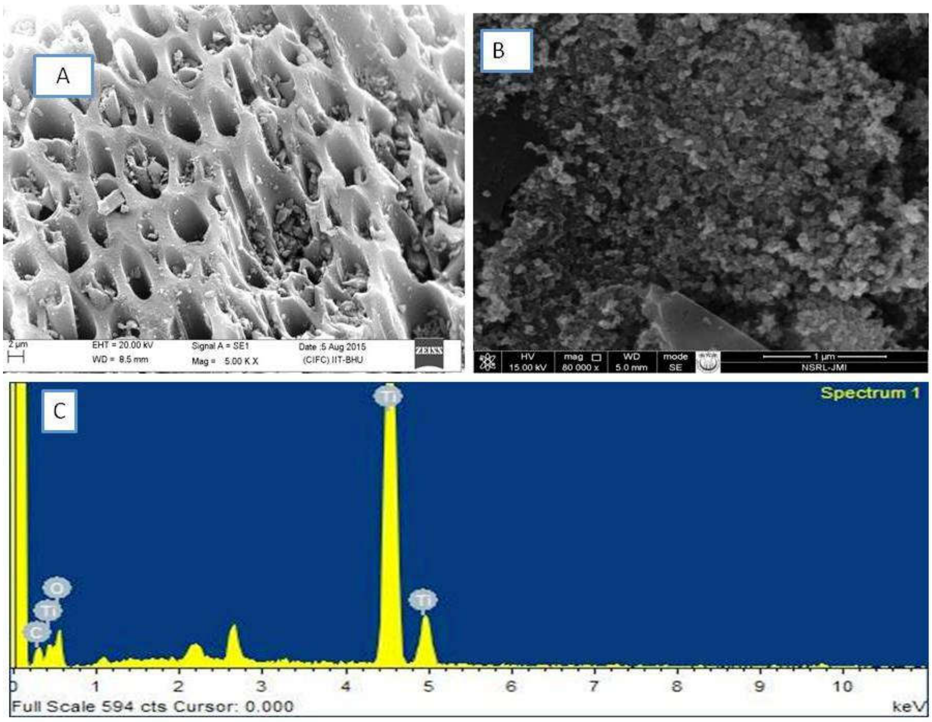

Fig. 6.1: Scanning Electron Microscopy (SEM). (1a) SEM image of Activated carbon (1b) EDX analysis of TiO_2/AC nano-composite, and (1c) EDX peaks of TiO_2/AC nano-composite.

6.3.2 Raman Spectra analysis

The Raman spectra of activated carbon, bare TiO_2 nanoparticles and unused TiO_2 adsorbed on the surface of activated carbon (TiO_2/AC nano-composite) are shown in Fig. 6.2. As per the observation, spectra of activated carbon (Fig. 6.2a) exhibits two well-resolved bands, D (1332 cm^{-1}) and G (1585 cm^{-1}) which clearly indicates vibration in C-C bond in activated carbon (Cuesta et al., 1994). Further, Fig. 6.2b showed the Raman spectra of bare TiO_2 with each unit cell possessing tetragonal structure (Hyun et al., 2005). Raman spectra of single crystal indicates the four modes which appears at 133 cm^{-1}, 190 cm^{-1}, 386 cm^{-1}, 506 cm^{-1}, 630 cm^{-1} (Scepanovic et al., 2009). The first peak appearing at 133 cm^{-1} is slightly broader and shifted than those of a bulk TiO_2 crystal (Zhang et al., 2000). As compared to short-range order of anatase phase in weak broader phase in high frequency region, certain degree of long-range order exists at 133 cm^{-1} peak (Arora et al., 2007). Fig. 6.2c showed the Raman Spectra of TiO_2/AC nano-composites having peaks at same wave numbers as that of spectra of TiO_2. The variation in intensity at given described peaks reflected that activated carbon is successfully exfoliated and incorporated in the nano-composite.

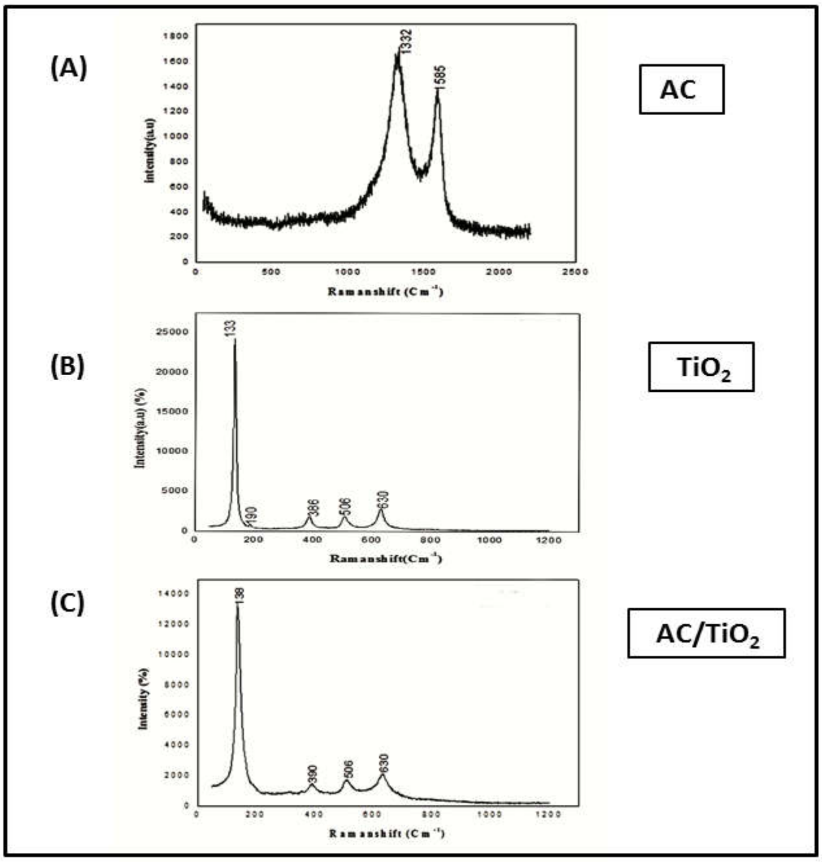

Fig. 6.2: RAMAN spectra. (1a) RAMAN spectra of Activated carbon, (1b) RAMAN spectra of bare TiO_2, and (1c) RAMAN spectra of TiO_2/AC nano-composite.

6.3.3 Fourier Transform-Infrared (FTIR) analysis

FTIR spectra of unspent TiO_2 nanoparticles showed different peaks at different wave numbers revealing various absorption patterns (Fig. 6.3). Absorption peak at 3415 cm^{-1} represents the stretching of hydroxyl (-OH) group in water as moisture (Ba-Abbad et al., 2012), whereas, peak at 1632 cm^{-1} shows the stretching of titanium carboxylate, which was the product of TTIP and ethanol used in sol-gel method. Further, absorption peak at 757 cm^{-1} represents the stretching of Ti-O bond which is the characteristic attribute of the formation of TiO_2 nanoparticles (Hema et al., 2013). FTIR study of TiO_2/AC nano-composite showed the shift in the O-H vibration band towards lower wave number (3400 cm^{-1}). These shifts confirmed the alteration of acid-base characteristics of -OH group in the used samples. The bands near the 600 cm^{-1} is assigned to the stretch vibration of Ti-O bond and proved that the TiO_2 particles are well-distributed on the surface of activated carbon (Zang et al., 2012)

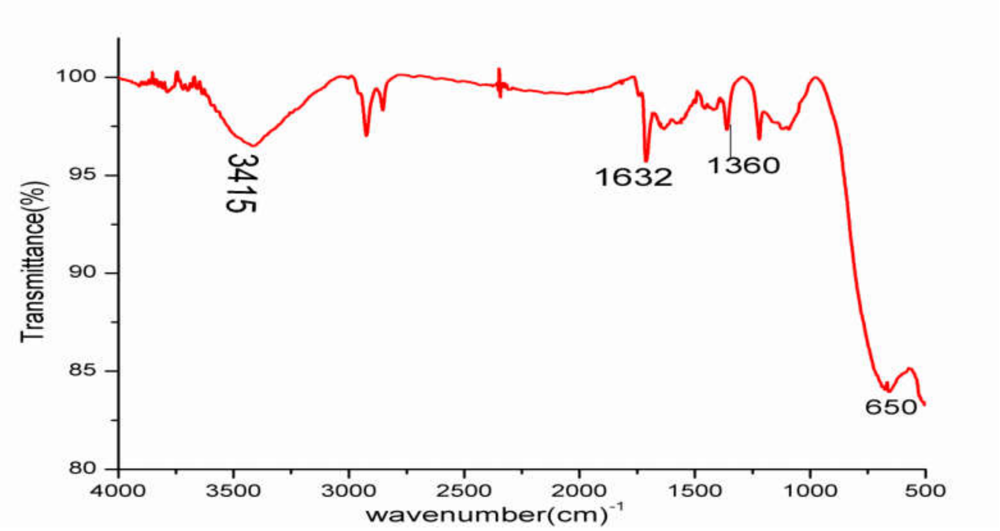

Fig. 6.3: FT-IR analysis of TiO_2/AC nano-composite.

6.3.4 X-Ray Diffraction of TiO_2/AC nano-composite

X-ray diffraction analysis was performed to assess the phase composition, crystalline nature and size of unspent TiO_2/AC nano-composite (Fig. 6.4). Various diffraction peaks (Fig. 6.4) at $2\theta = 25.40°$, 48.02°, 54.19° and 62.72°, were given by TiO_2/AC nano-composite, which were assigned to (101), (200), (105) and (103) reflections of anatase phase and peaks at $2\theta = 27.48°$, 36.07°, 37.80° and 69.00° being assigned to (001), (021), (210) and (220) reflects the rutile phase of TiO_2. The average intensity of rutile phase is considerably less as compared to that of anatase phase. Average crystalline size can be determined using Scherrer's equation (Borchert et al., 2005) as:

$$\mathbf{D} = \frac{K\lambda}{\beta cos\theta} \qquad \text{(Equation. 6.1)}$$

where, K = Scherer constant,

λ = X-ray wavelength,

β = the peak width of half maximum, and

θ = Bragg diffraction angle.

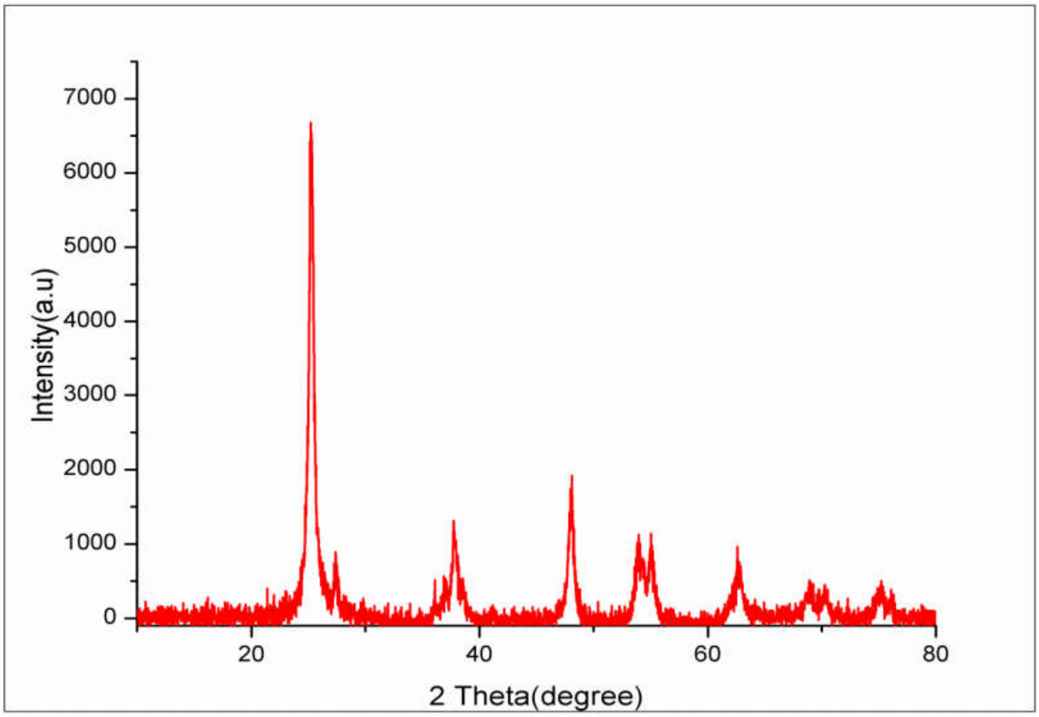

Fig. 6.4: XRD patterns of TiO_2/AC nano-composite (JCPDS 894921).

6.4 Effect of TiO_2/AC nano-composite on seed germination

TiO_2/AC accelerated the process of seed germination after 3 days of incubation and shortened the germination time considerably as compared to the control. The root and shoot lengths in TiO_2/AC treated seed (as shown in Fig. 6.5) was also found much longer than that of the control seeds. Moreover, the root length of *V. radiata* showed higher value at the lower concentration of TiO_2/AC. However, the overall root length showed a decrease with further increase in the concentration.

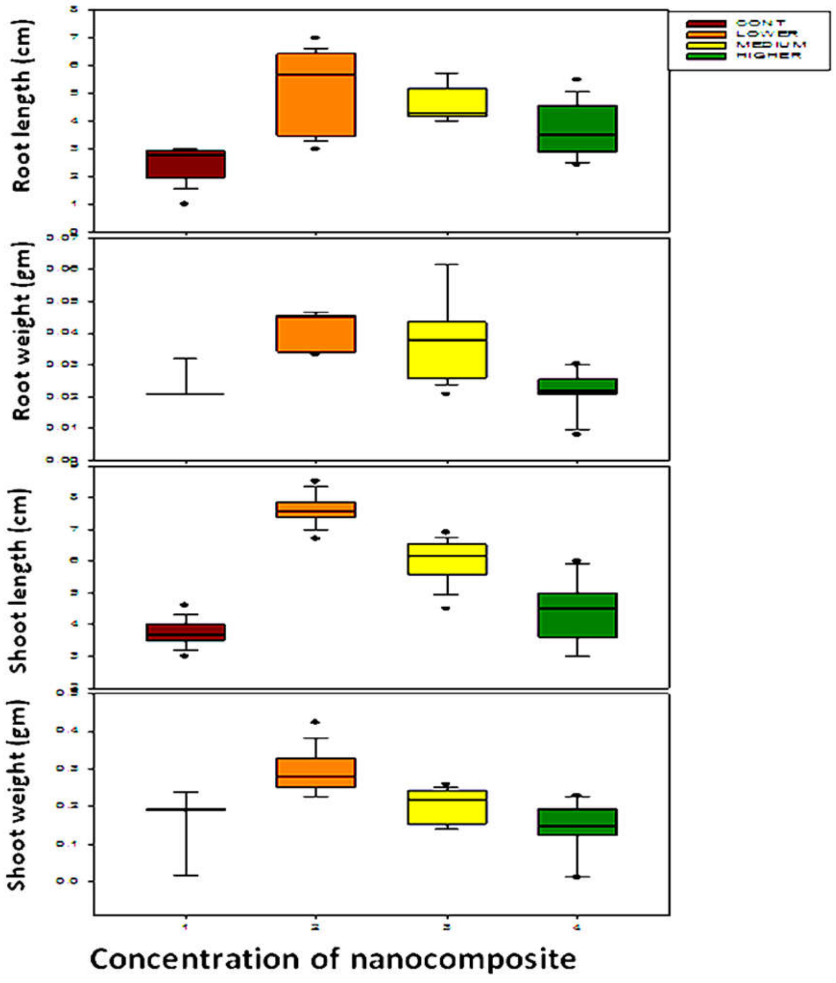

Fig. 6.5: Root and shoot length and weight of *Vigna radiata* growing under different concentration of TiO_2/AC nano-composite.

Fig. 6.6 showed that germination of *S. lycopersicon* seeds at lower concentration was more than the control treatments on the second day. During the next few days, the germination rate was dramatically faster for seeds treated with TiO_2/AC than those of the control seeds. After 4 days of incubation, the germination percentage for the control seeds averaged 80%, while the germination percentage of the TiO_2/AC treated seeds averaged 100% at higher concentration and 90% at lower concentration (Fig. 6.6). It indicates that the accelerated seed germination could be caused by the increasing concentration of nano-composite, which might be due to the penetration of the seed husks by TiO_2. As illustrated in Fig. 6.6, at stage I, the TiO_2/AC was found to densely deposit on the seed surface and penetrate seed husks, which accelerates water uptake inside the seeds. Water uptake is an important process in the seed germination as mature seeds are fairly dry and needs water to initiate the cellular metabolism and growth. In case of *S. lycopersicon*, the germination rate was 0% in 4 days in control, 10% in lower concentration 35% in medium concentration and 80% in higher concentration. After 8 days, the germination rate was increased by 0-50% in control, 50% in lower concentration, 95% in medium concentration and 90% in higher concentration. Zhang et al. (2015) demonstrated that the nano-sized TiO_2 helps the water absorption by the *Spinach* seed, helping in the enhancement of the germination rate. Khodakovskaya et al. (2009) also reported similar finding using carbon nano-tube.

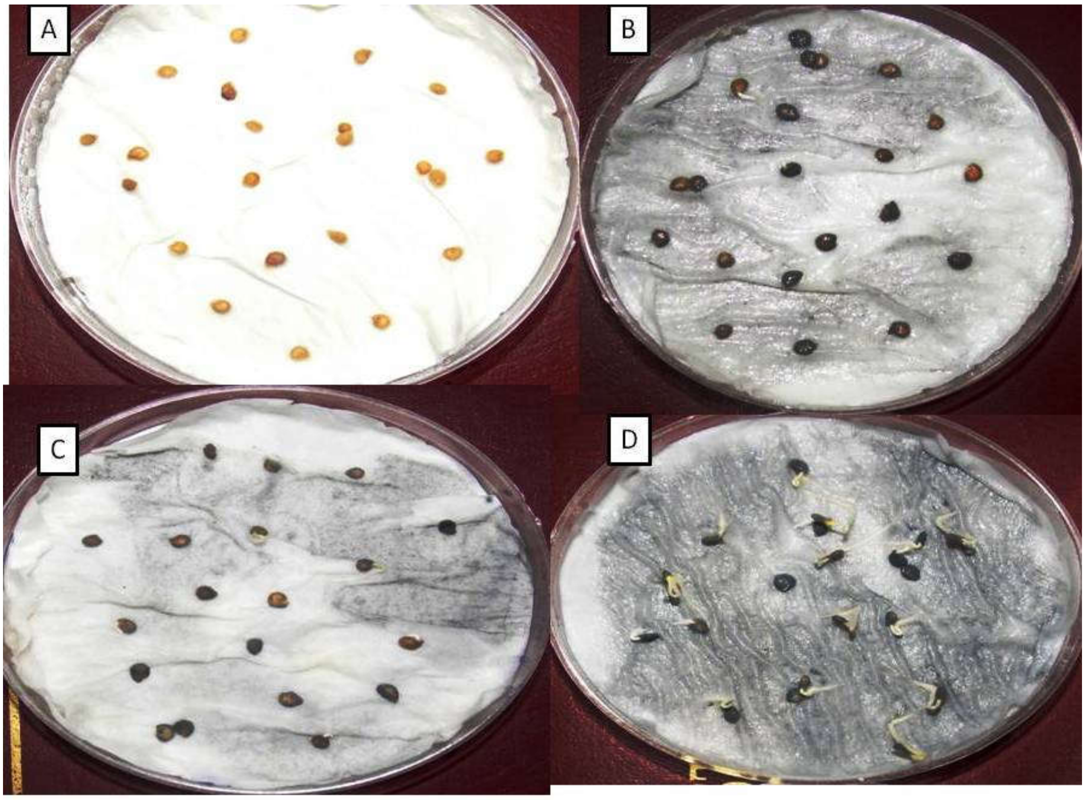

Fig. 6.6: Germination of *Lycopersicum esculentum* at: (A) control, (B) lower concentration, (C) medium concentration, and (D) higher concentration of TiO_2/AC nano-composite.

Mature seeds are relatively dry and need uptake of significant amounts of water to start their cellular metabolism and resume growth. Generally, nanoparticles enhance the seed germination rate in various plant species. Nanoparticles may create new pores in the seed coat, and thereby facilitate the process of water uptake inside the seed embryo, and increase the germination rate. Other studies have reported that TiO_2 nano-particle promotes seed germination, photosynthetic activity and nitrogen metabolisms, which cumulatively promote the growth of plant species at a suitable range of concentrations (Hong et al., 2005; Yang et al., 2007; Zheng et al., 2005). It also increases the activity of several enzymes and promotes the adsorption of nitrate, accelerating the transformation of inorganic-N into organic-N. However, normal-sized TiO_2 does not have these effects (Ma et al., 2010 a, b).

TiO_2/AC nano-composite had positive impacts on the germination of *V. radiata* seeds. It can be attributed to the fact that TiO_2 was able to penetrate the seed husks of these seeds (Fig. 6.7). The penetration might break the husks to facilitate the water uptake, which result in the rapid seed germination and higher percentage of germination rates. Moreover, at the stage of seedling growth, activated carbon may be providing the moisture to the seed. Furthermore, stems and roots of the seedlings with higher concentration of treatment were longer than those of the control. Zhang et al. (2015) found that in the case of graphene, it penetrates the seed husk which might break the husk to facilitate water uptake resulting into faster germination as well as higher germination rate. Carbon nano-materials are known for their light weight and extreme conductivity, which occupy a unique place in agriculture because of their abilities to enhance the seedling growth and development. Effect of nanomaterials on the plant growth also depends upon the type of nanomaterials, size-specific area, functional groups, concentration, plant species, soil type and condition (Josko and Olesozuczhe, 2013; Lin and Xing, 2007; Shouts and Willson, 2011).

Fig. 6.7: Seed germination of *Vigna radiata* after 4 days at: (A) higher concentration, (B) medium concentration (C) lower concentration, and (D) control.

Seed germination was enhanced at lower and medium concentrations of TiO_2/AC nano-composite, whereas root and shoot ratio was found higher in the lower concentration. It showed that the concentration of nano-composite plays a vital role in the plant growth and development. The increase in the seed germination might be due to the photo-generation of active oxygen like superoxide and hydroxide anions which causes re-activation of aged seeds. Activated carbon present in the nano-composite provides large surface areas and moisture for seed germination. It also enhances the penetrability of seed capsule facilitating the admission of water and dioxygen into the cell, resulting in the increased seed germination. TiO_2 also induces oxidation–reduction *via* free superoxide radical during the germination. The oxygen produced in such processes could be used for respiration, which further promotes the seed germination (Zhang et al., 2004). Overall, various plant species show different seed germination and growth behaviour under various concentrations of the nano-materials, which needs to be studied in much details for precise understanding of the impact on nano-materials on the environment.

6.5. Conclusions

The release of nanomaterials into the environment affects various plant growth mechanisms and development from the seed germination to pollination. We observed that the activated carbon based TiO_2 (TiO_2/AC) nano-composite had positive impacts on the germination of *Vigna radiata* and *Solanum lycopersicon* seeds, which can be attributed to the penetration ability of TiO_2 into the seed husks. It envisaged that the increase in root and shoot ratio may be related to the concentration of catalysts; however, elaborative physiological studies are needed for the better understanding of this mechanisms. TiO_2/AC nano-composite enhanced the seed germination in plants and also enhanced the shoot and root ratio depending on concentration. Compared to the control, enhanced germination was found at increased concentration, however, the growth of root and shoot either decreased or remained stable. Overall, these results would help in mechanistic understanding of the interaction of nano-materials with plants species in the environment.

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