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EFFECT OF METAL BASED NANOPARTICLES (ZNO AND TIO_2) ON GERMINATION AND GROWTH OF COWPEA SEEDLING

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ABSTRACT

The present study demonstrated the effect of ZnO and TiO_2 nanoparticles on germination and growth parameters of cowpea seedling. Cowpea seeds were treated with different concentrations of nanoscale ZnO (0, 2,4,8,10,15 ppm), and TiO_2 (0, 10, 20, 30, 40, 50 ppm) including control with sterilized water were used in this study.The experiment was carried out in complete randomized design with 3 replications. Application of ZnO and TiO_2 nanoparticles significantly stimulate germination and seedling growth with most of the treatment as compared with control.The promontory impact was observed in germination (22.91%), root length (65.96% and 104.49%), shoot length (61.82% and 64.56%), total fresh weight (110.7% and 134.8%), total dry weight (118.6% and 148.8%) and vigor index (100% and 116.45%) at 2 ppm ZnO and 20 ppm TiO_2 nanoparticles respectively. The increase in seed germination along with seedling growth in specific concentration of different nano particles suggested optimum dose limit for growth of cowpea seedlings and consequence decrease in highest concentration could be due to toxic effect of nano particles and that reduction in lower concentration may be suggested as least sensitiveness on such treatment or detrimental interaction effect of nano particles and seedling growth.

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INTRODUCTION

Nanoparticles became of interest relatively recently dynamically, mostly because of their possible use in diverse technologies. They can be defined as objects ranging in size from 1-100 nm that due to their size may differ in the properties from the bulk material. This can result from the high surface to volume ratio that increases their reactivity, the ability to penetrate cell membranes and possible biochemical activity (Dubchak *et al.,*2010).

Depending on the origin, three types of nanoparticles: natural, incidental and engineered are found. Engineered nanoparticles are defined simply as any intentionally produced particle that (i) has a characteristic dimension between 1 and 100 nm and (ii) possesses properties that are not shared by non nanoscale particles with the same chemical composition (USNTC, 2004). The second part of this definition recognizes that as a result of their small size, nanomaterials possess unique properties. This is particularly true for nanoparticles $<$20 to 30 nm in size, which are generally characterized as having an excess of energy at the particle surface that makes them highly reactive and thermodynamically unstable (Auffan*et al.,* 2009). Engineered nanoparticles allow truly novel chemical reactions that are previously unknown in nature to occur (Owen and Handy 2007). Engineered nanoparticles including carbon based materials, metal based materials, dendrimersand composites (Lin and Xing 2007). Zinc oxide (nano-ZnO) and Titanium dioxide (nano-TiO_2)are commonly used metal oxide engineered nanoparticles (ENPs). Zinc is one of the essential micro nutrients required for plant growth. It is an important component of various enzymes that are responsible for driving metabolic reactions in all crops (Raliya and Tarafdar 2013). In plants, titanium has been reported to stimulate production of more carbohydrates, encouraging growth and photosynthesis rate (Owolade *et al.,* 2008; Chen *et al.,* 2014; Khodakovskaya and Lahiani2014). Recent publications on zinc oxide (ZnO) nanoparticles reported both positive and negative effects (Lin and Xing 2008; Adhikari *et al.,* 2010; Priester *et al.,* 2012; Prasad *et al.* 2012). Lopez-Moreno *et al.* (2010) investigated the uptake and accumulation of ZnO NPs (8 nm) by soybean (*Glycine max*) seedlings. The application of TiO_2 on food crops has been reported to promote plant growth by increasing the rate of photosynthesis and reducing the incidence of disease resulted enhance yield (Bowen *et al.,* 1992; Maness *et al.* 1999; Frazer, 2001). Hong *et al.* (2005 a & b) and Yang *et al.* (2006) reported that a proper concentration of nano-TiO_2 was found to improve the growth of spinach by promoting photosynthesis and nitrogen metabolism.

Cowpea (*Vigna unguiculata*L. Walp.)is used as experimental material in present investigation because it is an important food and fodder legume cultivated in the tropics and sub-tropics (Singh *et al.,* 1997). Cowpea is of economic and nutritional importance as its high protein content 24.8% (Dominic *et al.,* 2005) makes it extremely valuable in Tropical Africa and as a replacement for meat and fish. Limitedresearch work have been done related to the effect of nanoparticles on cowpea growth, accomplished that TiO_2 nanoparticle significantly increase the growth and yield in cowpea plants by increasing photosynthetic rate and reducing the severity of foliar and pod disease (Owolade *et al.,* 2008) and Afshar *et al.* (2012) revealed that the effect of Nano Fe treatment on leaf Fe content was significant at 1% probability level but these treatments did not affect leaf phosphorus rate and seed Fe content significantly. Mean comparison of the spraying times indicated that the highest values of spike weight (614.88 g), 1000 grain weight (36.10 g), biologic yield (8830 kg/ha), grain yield (3639.5 kg/ha) and protein content (16.01%) were achieved in the first spraying of Fe nanoparticle. Therefore it leaves scope of exploring the possibility of employing different nanoparticles in cowpea.

The present research work, therefore, aims at evaluation on the impacts of ZnO and TiO_2 nanoparticles on germination and seedling growth in cowpea (*Vigna unguiculata*L. Walp.).

MATERIALS AND METHODS

Chemicals

ZnO Nanoparticlewas synthesized and characterized by CAZRI, Jodhpur (Raliya and Tarafdar 2013). TiO_2 (Anatase) nano particle was purchased from Sigma-Aldrich Company, St.Louis, MO, USA with a purity of 99.5%, particle size of (<100 nm).

Characterization of ZnO nanoparticle

DLS analysis

The particle size distribution and zeta potential of ZnO nanoparticles was monitored usingdynamic light scattering (DLS) measurements which determines particle size by measuring the rate of fluctuations in the laser light intensity scattered by particles as they diffuses through solvent. Particle size analyzer (Beckman Delsa Nano C, USA) was used for size measurement and confirmation of nanoparticles size distribution.

TEM and HR-TEM analysis

For the confirmation of size and shape, transmission electron microscope (TEM) measurements were carried out using drop coating method in which a drop of solution containing nanoparticles was placed on the carbon coated copper grids and kept under vacuum desiccation for overnight before loading them onto a specimen holder. TEM and High-resolution transmission electron microscope (HR-TEM) micrographs of the sample were taken using the JEM-2100F TEM instrument. The instrument was operated at an accelerating voltage of 200 kV.

SEM analysis

Scanning electron microscopy (SEM) was extremely useful for the determination of topology and observations of surfaces as they offer better resolution and depth of field than optical microscope. Micrographs of the biologically synthesized samples were taken using the Hitchi-S-3400 N SEM instrument. The instrument was operated at an accelerating voltage of 30 kV.

XRD analysis

The crystal structure and size confirmation were examined by X-ray Diffraction (XRD) analysis. XRD analyses of ZnO nanoparticle were carried out by thin film mode of X-ray Diffraction using X'PERT PRO MRD model of PANalytical system operated at 20 kV voltages and a current of 15mA with CuKá radiations.

Seed treatment and germination of seedling

To evaluate the impact of ZnO and TiO_2 nanoparticles on germination and seedling growth in cowpea, the present experiment was conducted in the laboratory of Department of genetics and Plant Breeding, Bidhan Chandra Krishi Viswa vidyalaya, West Bengal during 2012-2013 using cowpea variety Arka Garima following completely randomized design with 3 replications. There are six treatments using different concentration of each nanopartices ZnO (0, 2, 4, 8, 10, 15 ppm) and TiO_2 (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 ZnO and TiO_2 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, leaf fresh weight, total fresh weight, root dry weight, shoot dry weight, leaf dry weight, total dry weight, root shoot ratio and vigor index. Observations were recorded from 3 week old seedling.

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

Characterization of ZnO nanoparticle

Particle size of biotransformed ZnO nanoparticles was analyzed by DLS using particle size analyzer (Fig. 1). Histogram shows particle size ranges from 1.2 to 6.8 nm and possess an average size of 3.8 nm. The Polydispersity index (PDI) was 0.335 shows high monodispersity of the particle. The size was further confirmed by TEM and SEM analysis. TEM measurements were used to determine the size, shape and morphological study of ZnO nanoparticles. A TEM micrograph (Fig. 2a) showed well distribution of spherical ZnO nanoparticle, which was encapsulated by thin layer of protein

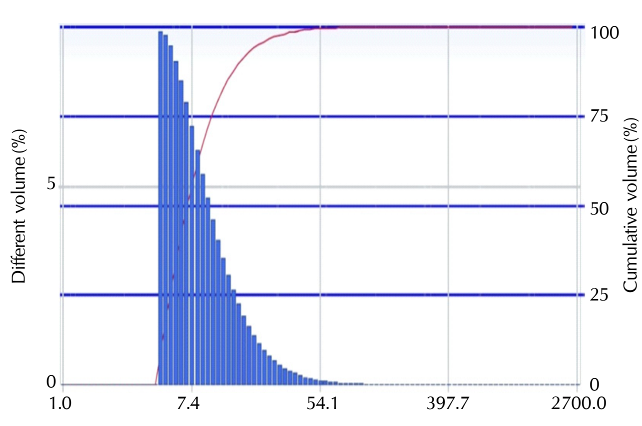

Figure 1: DLS histogram of ZnO nanoparticles for particle size analysis

Table 1: Effect of different concentrations of ZnO nanoparticle on seedling characters

Treatment (ppm)	Germination (%)	Root length(cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight(g)	Leaf fresh weight(g)	Root dry weight (g)	Shoot dry weight(g)	Leaf dry weight (g)	R/S Ratio (%)
Control	80.00	5.423	13.430	0.050	0.195	0.190	0.005	0.018	0.019	0.134
ZnO (2)	98.33	9.000	21.733	0.275	0.335	0.334	0.028	0.034	0.032	0.424
ZnO (4)	86.67	8.473	12.467	0.210	0.245	0.363	0.021	0.027	0.036	0.334
ZnO (8)	88.33	8.770	18.423	0.273	0.285	0.345	0.026	0.030	0.039	0.377
ZnO (10)	85.00	8.267	17.593	0.278	0.275	0.322	0.026	0.029	0.033	0.420
ZnO (15)	76.67	7.937	12.680	0.127	0.268	0.310	0.013	0.026	0.032	0.224
C.D.	10.599	0.192	0.175	0.021	0.008	0.022	0.005	0.003	0.003	0.187
SE(m)	3.402	0.062	0.056	0.007	0.003	0.007	0.001	0.001	0.001	0.060
SE(d)	4.811	0.087	0.079	0.010	0.004	0.010	0.002	0.001	0.001	0.085
C.V.	6.865	1.337	0.605	5.889	1.756	3.882	12.982	6.199	4.410	14.98

Table 2: Effect of different concentrations of TiO_2 nanoparticle on seedling characters

Treatment (ppm)	Germination (%)	Root length (cm)	Shoot length (cm)	Root fresh weight(g)	Shoot fresh weight(g)	Leaf fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Leaf dry weight (g)	R/S Ratio(%)
Control	80.00	5.423	13.430	0.050	0.195	0.190	0.005	0.018	0.019	0.134
TiO_2 (10)	88.33	7.920	18.270	0.270	0.195	0.390	0.029	0.020	0.040	0.483
TiO_2 (20)	98.33	11.090	22.100	0.347	0.280	0.435	0.036	0.028	0.042	0.509
TiO_2 (30)	90.00	8.733	19.700	0.177	0.197	0.210	0.020	0.024	0.021	0.456
TiO_2 (40)	93.33	9.557	20.563	0.350	0.233	0.307	0.036	0.024	0.031	0.662
TiO_2 (50)	85.00	7.547	18.130	0.127	0.198	0.230	0.013	0.019	0.022	0.315
C.D.	10.385	0.152	0.141	0.024	0.017	0.020	0.004	0.006	0.003	0.242
SE(m)	3.333	0.049	0.045	0.008	0.005	0.006	0.001	0.002	0.001	0.078
SE(d)	4.714	0.069	0.064	0.011	0.008	0.009	0.002	0.003	0.001	0.110
C.V.	6.475	1.011	0.418	6.158	4.323	3.829	9.137	13.807	5.760	13.38

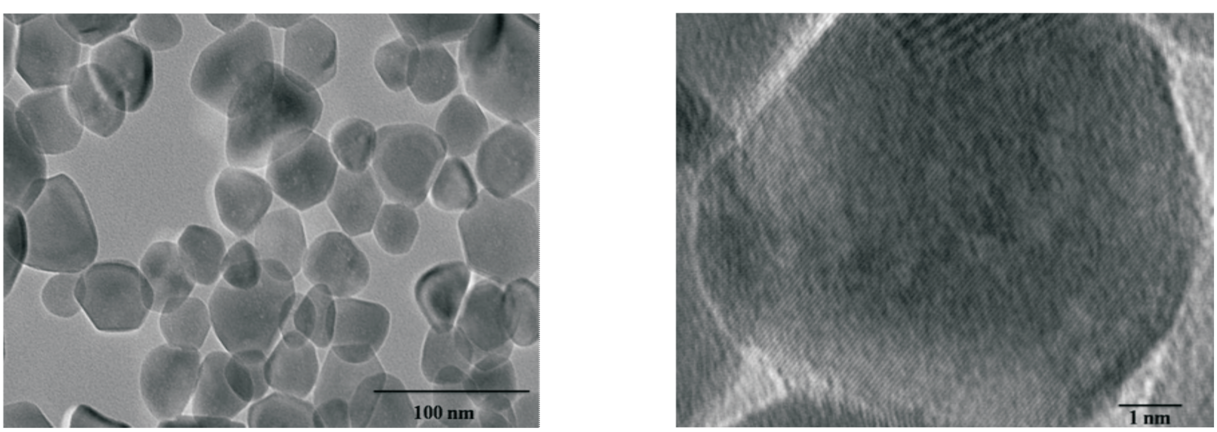

Figure 2(a): TEM micrograph of biologically synthesized ZnO nanoparticle (b) HR-TEMmicrograph of single ZnO

at the measurement scale bar of 100 nm with 200 kV applied voltage. The nanoparticles were oblate spherical with clear edge of crystal and lattice structure observed in HR-TEM micrograph (Fig. 2b), which supports the crystalline nature of ZnO nanoparticle. SEM micrograph revealed the structural and topology of ZnO nanoparticles (Figure 3). Nanoparticles were of oblate spherical and hexagonal in structure which was uniformly distributed (monodispersed) without any significant agglomeration. Analysis of XRD spectra showed well defined peaks at 2è values, which correspond to hexagonal phase of ZnO (Fig. 4).

Effect of ZnO and TiO_2 nanoparticles on cowpea

The results demonstrated significant enhancement in germination and growth parameters of cowpea seedling due to nanoparticles ZnO and TiO_2 at 3 weeks of crop age. The effect of ZnO and TiO_2 nanoparticles on cowpea seedling are shown in Table 1 and 2. All the treatments of ZnO and TiO_2 nanoparticles were found to stimulate germination over control except ZnO (15 ppm), where it was diminished to 76.67 percent. Highest germination was found at TiO_2 (20 ppm) followed by TiO_2 (40 ppm) and ZnO (2 ppm) followed by ZnO (8 ppm). Application of ZnO and TiO_2 nanoparticles in cowpea

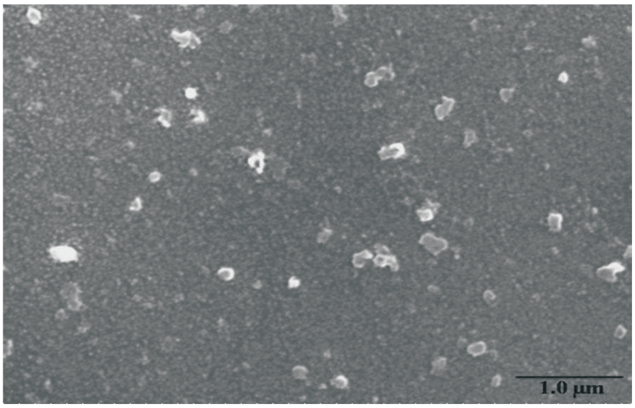

Figure 3: SEM micrograph of monodisperse ZnO nanoparticle

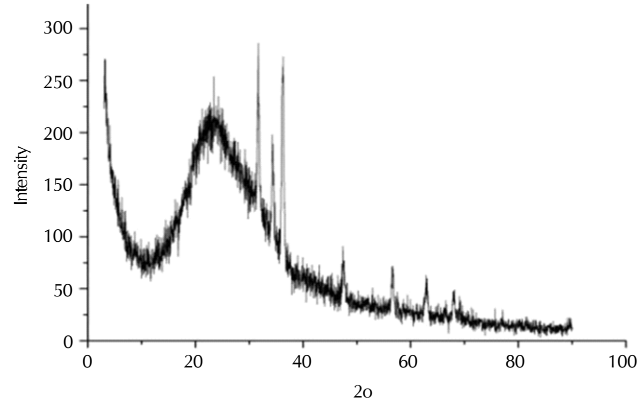

Figure 4: XRD spectra of ZnO nanoparticle

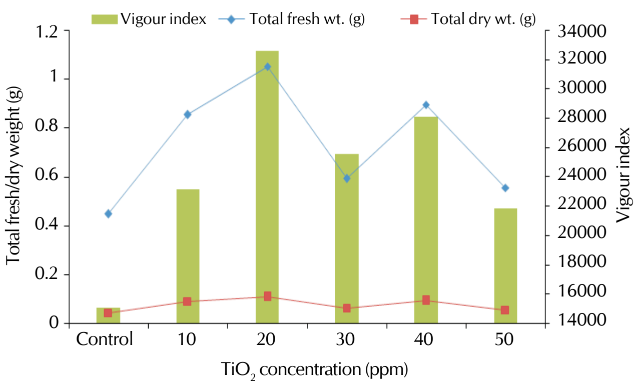

Figure 5: Influence of nano TiO_2 on total fresh weight, dry weight and vigor index of cowpea

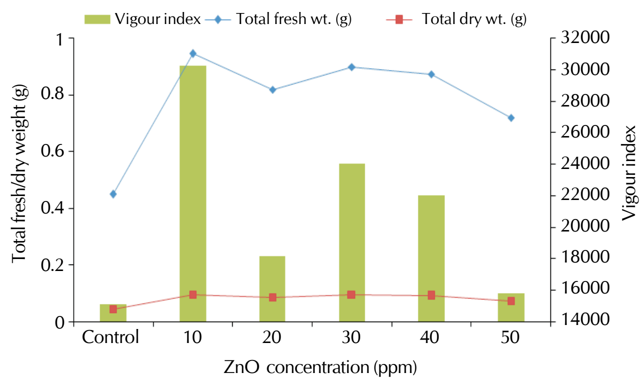

Figure 6: Influence of nano ZnO on total fresh weight, dry weight and vigor index of cowpea

resulted as increase in germination percentage upto 22.91%. Accelerated germination with proper concentration of TiO_2 was also reported by Feiziet *al.*, 2012 in wheat. Zhenget *al.*, (2005) demonstrated that nanosized TiO_2 helped the water absorption by the spinach seeds, and as result of it, the germination of the seeds accelerated. Seed germination could be considered as index of plant growth, development and yield and as beginning of physiological process it require adequate water absorption (Wierzbicka and Obidzinska 1998). Germination percent improved in all concentrations of TiO_2 and ZnO except for extreme concentration in ZnO. Reports indicate that ZnO NPs adversely affect plant growth of green pea, corn, cucumber, rye, zucchini, soybean, and wheat, in a dose dependent manner (Lin and Xing 2007 and 2008; Dimkpa *et al.*, 2012; Bandyopadhyay *et al.*, 2012a and b; Mukherjee *et al.*, 2014a and b).

At low concentration, ZnO and TiO_2 nanoparticles showed good effect on root and shoot length. Most promontory effect on root and shoot length were highlighted with the treatments 20 ppm followed by 40 ppm $TiO_2$and 2 ppm followed by 8 ppm in case of ZnO over control. Appreciating impact on root and shoot length were depicted by treatments TiO_2 (10, 30 and 50 ppm) and ZnO (4, 10 and 15 ppm) for root length. ZnO treated plants recorded increase upto 65.96% in root length and 61.82% in shoot length over control and further rise was observed when the plants were treated with TiO_2 nanoparticle as it increases upto 104.49% root length and 64.56% shoot length. Raliya and Tarafdar (2013) also reported a significant improvement in plant biomass (27.1 %), shoot length (31.5 %), root length (66.3 %), root area (73.5 %) and chlorophyll content (276.2 %). On the contrary ZnO 4 and 15 ppm showed adverse effect on shoot length which indicated that either had least positive effect or detrimental effect with increased dose. Mahajanet *al.* (2011) noted retardation in early growth of chickpea seedlings at high dose of nano-ZnO further strengthening our observation that low dose of nano-ZnO is sufficient to achieve positive response.

Maximum fresh weight of shoot and leaf were found again at 20 ppm followed by 40 ppm TiO_2. However reverse trend was observed for root fresh weight wherein more accumulation was found with 40 ppm rather than 20 ppm TiO_2. In case of ZnO, fresh weight of root was paramount at 10 ppm while shoot and leaf fresh weight at lower concentrations *viz*. 2 and 4 ppm respectively. Dry weight of root, shoot and leaf were found to be in accordance with fresh weight of root, shoot and leaf for corresponding TiO_2 nanoparticles treatment. Nonetheless contradictory trends were depicted for root and leaf dry weight except shoot dry weight with ZnO treatment. The fresh and dry weights were both significantly higher than those of the untreated plants (controls). None of the treatments highlighted with any adverse effect except highest and lowest concentrations.This result is confirmed byBurman *et al.*, (2013b) who demonstrated maximum promontory response with respect to root and shoot dry weight at low concentration (1.5 ppm) of ZnO nanoparticles while at high doses it exerted adverse effects in mung seedling.

Root shoot ratio is the amount of plant tissues that have supportive functions to the amount of those that have growth functions. Root shoot ratio is highest at ZnO 2 ppm (0.424) significantly at par with ZnO 10 ppm (0.420). This ratio is not equal to 1 that means shoot proportion is higher than root. Also in case of TiO_2 treatment similar type of result was found as highest root shoot ratio was found at 40 ppm (0.662) significantly at par with 20 ppm (0.509) which also exhibited more proportion of shoot. All treatment showed the ratio less than 1 which exhibited more proportion of shoot. Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy. Large proportions of shoot production are characteristic of vegetation in early successional phases, while high proportions of root production are characteristic of climax vegetational phases (Allaby, 2015).

Significant increment had been found in total fresh weight and dry weight of cowpea seedling for all the concentrations of ZnO and TiO_2 nanoparticles over control. Highest promotional effect for total fresh and dry weight were observed at 20 ppm of TiO_2 nanoparticle followed by 40 and 10 ppm but extent of improvement was found to be least at 30 and 50 ppm (Fig. 5). With satisfactory enhancement at all doses over control the effect of ZnO nanoparticle on total fresh weight was recorded maximum at 2 ppm (0.944) while dry weight at 8 ppm (0.095) shown in figure 6. For total fresh weight and dry weight per plant were higher than those of the control by 110.7% and 118.6% with ZnO nanoparticle while 134.8% and 148.8% due to TiO_2 nanoparticles respectively.

Seed vigor is animportant quality parameter which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Highest vigor index was highlighted at 2 ppm ZnO and 20 ppm TiO_2 nanoparticles over control followed by 40 ppm TiO_2, 30 ppm TiO_2, 8 ppm ZnO and 10 ppm ZnO nanoparticles (Fig. 5 and 6).The seed lot showing the higher seed vigor index is considered to be more vigorous (Abdul-Baki and Anderson 1973). Increases of upto 100% and 116.45% were recorded for vigor index by the treatment of ZnO and TiO_2 nanoparticles.

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