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Research Article

Germination and Growth Characteristics of Mungbean Seeds (*Vigna radiata L.*) affected by Synthesized Zinc Oxide Nanoparticles

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Abstract

The present work is focused on synthesis and characterization of high quality ZnO NPs were synthesized by wet chemical method. The precursors are zinc acetate, sodium hydroxide, methanol. ZnO has been characterized by XRD, FTIR, TGA/DTA, UV and TEM etc. From the XRD results the crystalline size is calculated using Scherer equation. ZnO nanoparticles application in different fields like seed germination, sensors, biomedical, semiconductor etc. In present study, the different concentration (0,20,40,60 and 100mg) of ZnO NPs are prepared in distilled water and sonicator for 15 minutes are used for the treatment in Mungbean (Vigna radiata L.) seeds to study the effect on bioavailability of seed germination and observed early seedling growth and growth characteristics of Mungbean. The experiment was carried out under greenhouse conditions.

Keywords: *Zinc oxide, Characterization, mungbean, bioavailability, growth characteristics.*

Introduction

Nanomaterials have gained increasing attention because of their novel properties, including a large specific surface area and high reaction activity (K. Suresh babu *et al*, 2013: A Khorsand Zak *et al*, 2011). Nanomaterials have also been used for various fundamental and practical applications (T.V.Kolekar *et al*, 2011). The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice studied the effect of mixtures of nano-SiO_2 and nano-TiO_2 on soybean seed. They found that the mixture of nanoparticles increases nitrate reductase in soybean increasing its germination and growth (K. Prasad *et al*, 2009) . There are reports that nanomaterials on higher plants wherein both positive and negative effects (Laurent S. *et al*, 2008: Kikui S *et al*, 2005).Experts feel that the potential benefits of nanotechnology for agriculture, food, fisheries and aquaculture need to be balanced against concerns for the soil, water, and environment and the occupational health of workers. Raising awareness of nanotechnology in the agri-food sector, including feed and food ingredients, intelligent packaging and quick-detection systems, is one of the keys to influencing consumer acceptance. On the basis of only a handful of toxicological studies, concerns have arisen regarding the safety of nanomaterials, researchers and companies will need to prove that these nanotechnologies do not have more of a negative impact on the environment. Agri-food nanotechnology is multidisciplinary in nature. Nanotechnology application to the agriculture and food sectors is relatively recent compared with its use in drug delivery and pharmaceuticals. Nanotechnology has the potential to protect plants, monitor plant growth, detect plant and animal diseases, increase global food production, enhance food quality, and reduce waste for "sustainable intensification"(Locke J.M. *et al*, 2000) .

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Material and methods

In this experiment 0.1 M zinc acetate was dissolved in methanol stirring on magnetic stirrer with heating at 40^0C and 0.2 M Sodium hydroxide was dissolved in methanol with stirring. Then Sodium hydroxide was added drop wise to the zinc acetate solution with heating. A milky white solution was obtained and stirring continued for 4hrs. The solution was aging for 24hrs then filtered with Whatman filter paper. It washed with methanol several times to remove any impurities in this solution. The sample was placed in oven at 70^0 C for 12hrs and then white powder was collected in ceramic crucible. The crucible placed in the mafule furnace and heat- treated at 400^0C for 2hrs

Seeds

The mungbean seeds were purchased from market these seeds were kept in a dry place in the dark under the room temperature before using.

Seedling Exposure

The seeds were checked for their viability by suspending them in double distilled water. The seeds which are settled to the bottom were selected for further study. The seeds were rinsed in double distilled water thrice and then surface sterilization of seeds was done. The seeds were sonicated for 3hr in prepared nanoparticle suspensions (20, 40, 60, 80 and 100mg) using a sonicator instrument. The soaked seeds were put in prepared pots. Observe of the growth is studied.

Seed germination test

This test was conducted on mungbean seeds. The seed germination rate (RSG) and relative root growth (RRG) were calculated using the equation and germination index (GI).

Relative seed germination Rate = $(S_C \backslash S_S)\times 100$
Relative root growth= $(R_S \backslash R_C) \times 100$
Germination index = (RSG\RRG) ×100
Where S_S is the number of seed germinated in sample, S_C is the number seed germinated in control, R_S is the average root length in sample and R_C is the average root length in control.

Root and shoot length

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transission zone up to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale.

Seedling vigour index

The seedling vigour index was determined by using the formula given by Abdul baki and Anderson (1973). Seedling vigour index= Average root length in cms + Average shoot length in cms ×Germination percentage.

Fresh and Dry weight

The fresh weight of root and shoot of seedlings was determined by weigh of the root and shoot separately on electric balance. After the fresh weight taken then the seedlings was kept in a hot air oven at 60 ° C for 48 hrs then the weight of dry matter was recorded.

Results and discussions

X- Ray diffraction analysis

Figure 1 the X-ray diffraction pattern for sample calcinated at 500°C. Study of standard data JCPDS 76-0704 confirmed that the synthesized material has a hexagonal ZnO phase (wurtzite Structure).The pattern was indexed with hexagonal unit cell and the lattice parameters are given. The peak and relative intensities obtained for the ZnO match with the literature values (Maguire et al, 1982) 8). There were no Characteristic peaks of impurity observed. The peaks at scattering angles of 31.64°, 34.85°, 36.65°, 46.1°, 57.12°,63.2°, 68.9° which corresponds to (100), (002), (101) (102) (110), (103) and (112) crystal plane respectively. X-ray diffraction pattern of the ZnO nanoparticles shown above has the average crystal size 19.23nm which is calculated using Scherrer formula.

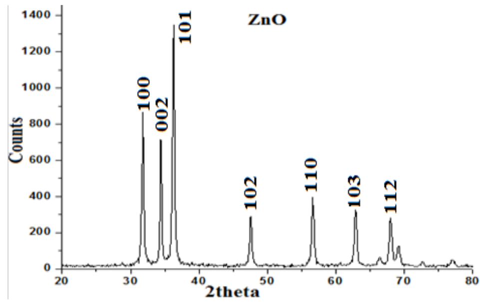

Fig.1 XRD Pattern of ZnO nanoparticles

FTIR Spectroscopy

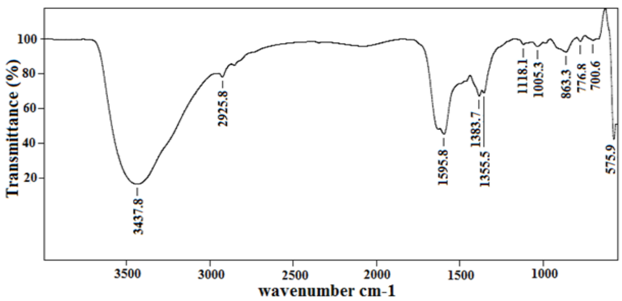

Fig.2 FTIR spectra of ZnO nanoparticles

Synthesized zinc nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with the synthesized nanoparticles. The peaks indicate the characteristics functional group present in the synthesized zinc oxide nanoparticles. It is inferred that the samples have absorption peaks in the range of 3437.8 cm-1, 2925.8 cm^{-1}, 1595.8 cm^{-1}, 1383.7 cm^{-1}, 1355.5 cm^{-1}, 1118.1 cm^{-1}, 1005.3 cm^{-1}, 863.3 cm^{-1}, 776.8 cm^{-1}, 700.6 cm^{-1} and 575.9 cm^{-1}. The absorption peak at 575.9 cm^{-1} corresponds to metal-oxygen (ZnO stretching vibrations) vibration mode. The peak at 1005.3 cm^{-1} is ascribed to the stretching vibration of C-N bond of the primary amine or to the stretching vibration of the C-O bond of the primary alcohol. The peak at 1118.1 cm^{-1}, 1355.5 cm^{-1}, and 1383.7 cm^{-1} are ascribed to primary, secondary alcohol in-plane bend or vibration. The peak at 1595.8 cm^{-1} is ascribed to the vibration modes of aromatic nitro compounds and alkyl. The peaks at 2925.8 cm^{-1} and 3437.8 cm^{-1} are ascribed to the stretching vibration of hydroxyl compounds.

TG/DTA analysis

TG curves of ZnO nanoparticles are as shown in Figure.3. The temperature range is 30°C to 800°C. In the TG analysis, the total weight loss percentage of the sample is 2.3%. The initial weight loss observed below 200°C it corresponds to the liberation of adsorbed moisture and water content on the surface of the sample. At transition range of temperature 400-500°C, it corresponds to the liberation of inorganic compounds in the sample. In the final stage, temperature range from 700-800°C, in this weight loss of the sample is very small. In the DTA analysis there is an exothermic peak it might indicate the existence of organic material in small amount.

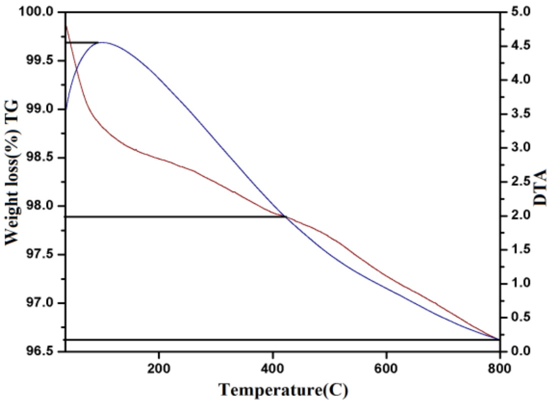

Fig.3 TG\DTA analysis of ZnO nanoparticles

Particle Analysis

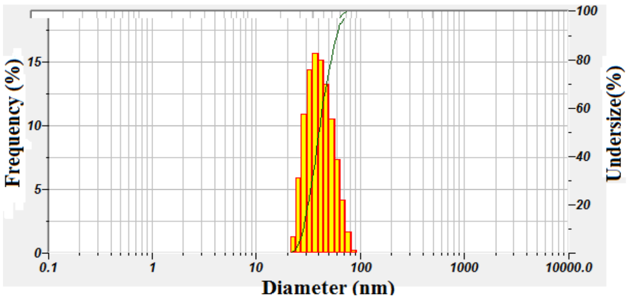

Fig.4 PSA of ZnO nanoparticle

The as-prepared ZnO nano particles were ultra-sonicated and suspended in the distilled water. The sizes of the agglomerated colloids in the suspensions were estimated using particle size analyzer. The histograms of the particle sizes Vs under size percentages are shown in Fig.4. The ZnO nano particles when analyzed by the dynamic light scattering show the mean particle size as 18 nm. It is correlating with the crystallite size 19.23 nm calculated from the XRD pattern.

UV spectrophotometer

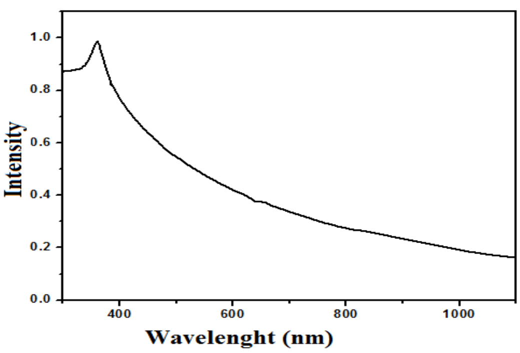

Fig.5 UV – vis of ZnO nanoparticle

Optical properties of ZnO nanoparticles were characterized based on UV absorption spectroscopy. The UV–vis absorption curves of ZnO nanoparticles calcination at 400^{o} C for 2hrs presented and absorption spectra showed a broad band emission and a relatively narrow absorption band at 360.68 nm respectively.

SEM Analysis

SEM images of the ZnO nanoparticle prepared by chemical method. The SEM analysis has showed agglomeration of the particles and in the range of 10 µm. The TEM analysis particles size is 10nm.

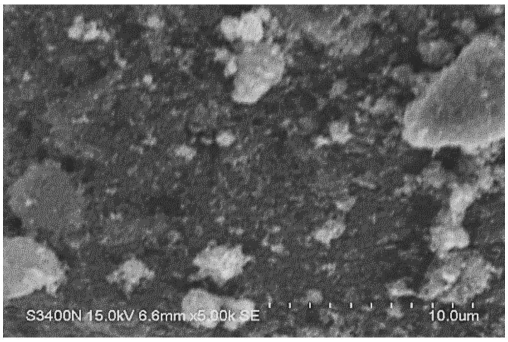

Fig.6 SEM image of ZnO nanoparticles

TEM Analysis

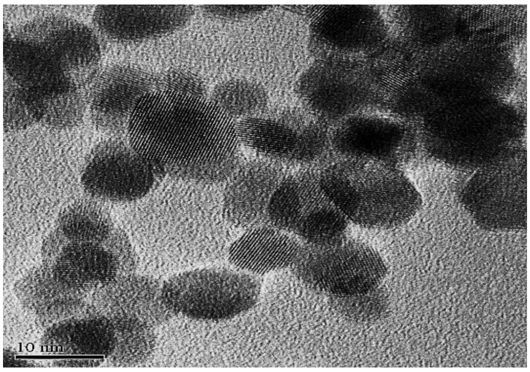

Fig.7 TEM image of ZnO nanoparticle

Table 1 Synthesized ZnO nano suspensions solution

ZnO NPs	RSG	RRG	GI
Control	-	-	-
100mg	88.8	141.6	62.7
60mg	80	158.3	50.5
40mg	80	175	45.7
20mg	80	200	40

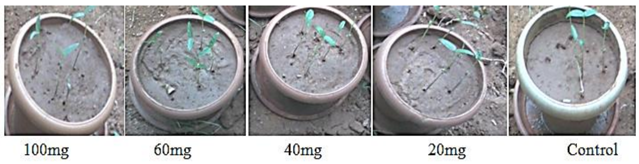

Fig .8 Mungbean seeds treated with synthesized ZnO nano suspensions solution preparation of particle suspensions and seed treatments

Table.2 Measurement growth characteristics of mungbean at different concentration of ZnO NPs (Control, 100mg, 60mg, 40mg, 20mg) within7days)

Treatment	Germination index	Seed germination (%)	Seedling growth(cms)		Fresh weight (gms)		Dry weight (gms)	
			Root length	Shoot length	Root	Shoot	Root	Shoot
Control	568.6	80	1.2	14.2	0.019	0.116	0.0031	0.0072
100mg	671.35	90	1.7	14.9	0.023	0.121	0.0039	0.0121
60mg	755.95	100	1.9	15.1	0.025	0.126	0.0041	0.0125
40mg	781.05	100	2.1	15.6	0.027	0.130	0.0041	0.0127
20mg	786.2	100	2.4	15.9	0.029	0.135	0.0042	0.0129

Table.3 Measurement growth characteristics of mungbean at different concentration of ZnONPs (Control,100mg,60mg, 40mg, 20mg)

Treatment	Germination index	Seed germination (%)	Seedling growth(cms)		Fresh weight (gms)		Dry weight (gms)	
			Root length	Shoot length	Root	shoot	Root	Shoot
Control	624.95	80	1.9	15.6	0.024	0.210	0.0043	0.0081
100mg	712.15	90	2.3	15.8	0.026	0.250	0.0049	0.0133
60mg	796.45	100	2.9	15.9	0.027	0.275	0.0051	0.0135
40mg	804.15	100	3.3	16.05	0.030	0.282	0.0053	0.0136
20mg	806.25	100	3.5	16.09	0.031	0.297	0.0057	0.0139

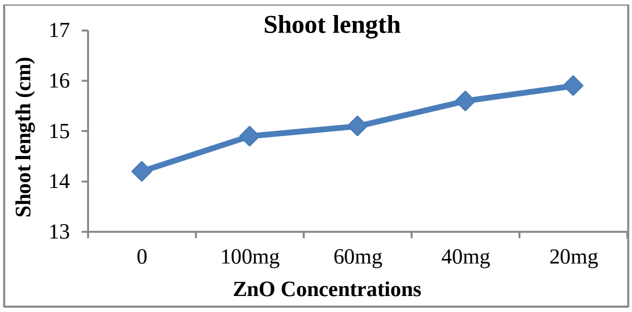

Fig.11 Shoot length of respect treatment within 7 days

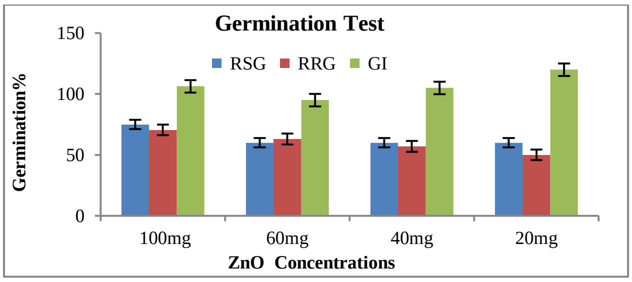

Fig.9 Germination test conducted on Seed germination mungbea seed

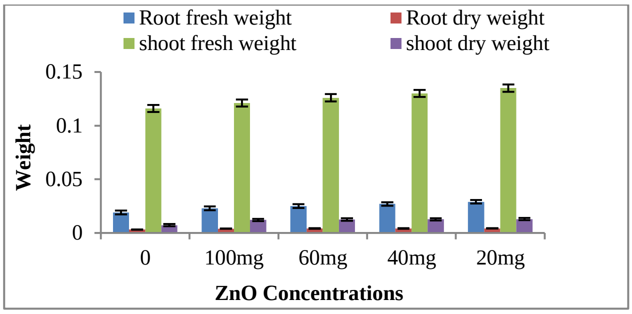

Fig.12 Fresh and dry weight of Root, Shoot respectivetreatmentswithin7days

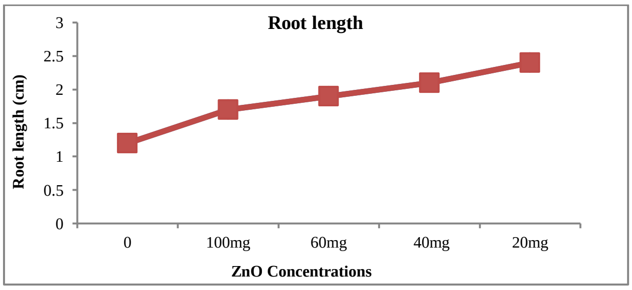

Fig.10 Root length of respect treatment within 7 days

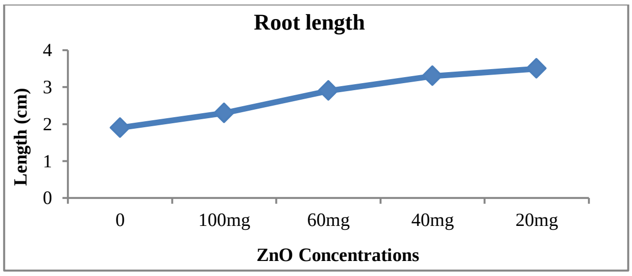

Fig.13 Root length reatment within 15days

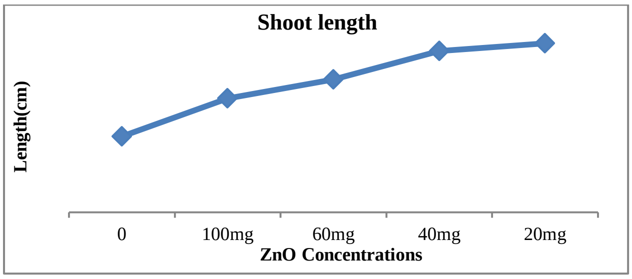

Fig.14. Shoot length treatment within 15days

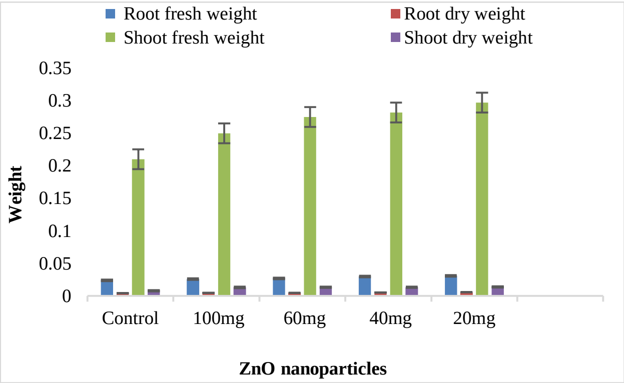

Fig.15 Fresh and dry weight of Root, Shoot of respective treatments within 15days

Evaluation of Germination using Zinc oxide nanoparticles treatments

Percentage of seed germination was significantly affected by the interaction of Zinc oxide nanoparticles. Due to interaction of ZnO NPs the percentage of germination has been increased. In comparison between the control and ZnO NPs treatments there was significant increase in germination. There was an increase in germination significantly when seeds were treated with ZnO NPs for 4hrs at different concentrations of 20, 40, 60, 80 and 100mg. According to the results the control has shown the 80% germination, zinc oxide nano particles treated seeds has shown increase in germination at different concentrations viz., 20mg shown 100%, 40mg-95%, 60mg-90%, 80mg-90% and 100mg of ZnO NPs shown 85% germination in Mungbean seeds. When the concentration of ZnO NPs increased there was decrease in germination of seeds. When the concentration of ZnO NPs decreased there was increase in germination of seeds. Control showed statistically significant difference and could not improve root length and shoot length. This treatment also increases the levels of root length and shoot length significantly.

Effect of zinc oxide nanoparticles on root and shoot of mungbean

In present study the zinc oxide nanoparticles showed increased root and shoot length with decreasing the concentration of the zinc oxide nanoparticles. The length of root were taken in centimeter, roots and shoots were treated by ZnO at different concentrations (20, 40, 60 and 100 mg) (Vashisth A *et al*, 2010) . At low concentrations the ZnO nanoparticles shows good effect on root and shoot was more prominent. The control shown 80% germination, the reduction in root and shoot growth at higher doses may be attributed to toxic level of nanoparticles. The evidence for demonstrating that mungbean seedlings respond to added nanoparticles in a limited range, above a limited range the toxic levels of nano particles reached causing subsequent declines in growth. (Lee WM *et al*, 2008).

Shoot Growth Characteristics

The effect of treatment of ZnO NPs at different concentration was measured (Naderi MR *et al*, 2012) . The results showed that the plant shoot measurements of mungbean as expressed by shoot length, shoot fresh weight and dry weight of shoot were influenced by different concentrations of ZnO NPs. The dry shoot weight was significantly different among the treatments. 20mg of concentration effect on mungbean shoot length compared to other concentration like 40mg, 60mg, 100mg and control. It was observed in the limited concentration of the plant that there was increase in plant shoot growth. It can also effect plant growth hormone enhancement. However, the lowest fresh weight and dry weight was noticed in control, the maximum shoot length was recorded in 20mg compared to other concentrations and control (Zhu H *et al*, 2008).

Root Growth Characteristics

Results showed that the root length, dry root weight, were significantly increased with different concentrations (control, 20mg, 40mg, 60mg, and 100mg) of ZnO nanoparticles treatments. Initially the number of second roots were not significantly different among the treatments. The maximum root length was recorded in 20mg concentration compare to other concentrations and control. 40mg, 60mg, 100mg increases the root length the minimum root length observed in control (Liu XM *et al*, 2005). It might have enhance the plant growth hormones in current plant by the treatment of different concentration of ZnO NPs (M. Koizumi *et al*, 2008).

Conclusion

In current study the synthesized ZnO NPs were characterized by different techniques for calculation of crystalline size, particles size, morphology, chemical compositions, thermal analysis etc. The different concentrations of ZnO nanoparticles effect on mungbean seed germination and the length of root and shoot were studied. Nanoparticles of metal oxide are quickly transported through the plant and included in the metabolic processes. We observed in mungbean seeds germination lowest concentration (20mg) of ZnO suspension solution shown good shoot, root growth results compare other concentrations and control in this experiment.

Acknowledgement

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