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## Synthesis and Characterization as Zinc Oxide Nanoparticles as a Source of Zinc micronutrient in Organic Fertilizer

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Deficiency of micronutrients in plant will result to a reduced yield and in severe cases it may cause plant death. Among these micronutrient deficiencies, Zn deficiency is the most damaging to crop yield. Zinc is a co-factor that is involved in enzyme systems and metabolic reactions in plants. In this study, aloe vera leaf extract was utilized to synthesize ZnO nanoparticles. The synthesized ZnO nanoparticles were dispersed in organic fertilizer (fish amino acid + chitosan) which can be a potential source of zinc for plants. Zinc oxide nanoparticles were synthesized using aloe vera leaf extract as stabilizing agent. The synthesized nanoparticles were characterized using UV-Vis Spectrophotometer, Fourier Transform Infrared Spectrophotometer (FTIR) and Scanning Electron Microscopy (SEM). The zinc oxide nanoparticles were dispersed in organic fertilizer and was applied to tomato plant to determine its effect on the growth performance of the plant. The synthesized ZnO nanoparticles exhibited SPR peak at 290 nm and a vibrational frequency peak at 478 cm<sup>-1</sup> on its UV-Vis and FTIR spectra. Analysis of the SEM micrograph shows average particle size of 50.67 ± 10.22 nm. Application of the ZnO nanoparticles dispersed in organic fertilizer to tomato plant for seven weeks in pot experiment shows significant increase in height for treatment 1 (15 ppm ZnO NPs) and treatment 2 (30 ppm ZnO NPs) by 17.36% and 22.64% respectively, when compared to the control (0 ppm ZnO NPs). Similarly, the total mass of fruits yield in treatment 1 and treatment 2 was significantly increased by 121.50% and 127.23% relative to the control. Application of ZnO NPs dispersed in organic fertilizer to tomato plant can significantly enhance its growth performance.

**Keywords:** zinc oxide nanoparticles, zinc deficiency, micronutrient, growth performance, nanotechnology

### Introduction

Micronutrients such as Zn, Fe, Mg, and Cu are needed by plants in small amounts but play an important role in many processes controlling plant growth.

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The amount of these micronutrients in grains and leaves determine the quality of food consumed by human and animals. Deficiency of micronutrient in plants will result to a reduced yield and in severe cases it may cause plant death. Among these micronutrient deficiencies, Zn deficiency is the most damaging to crop yield. Zinc is a co-factor that is involved in enzyme systems and metabolic reactions in plants. It is needed in the production of chlorophyll and carbohydrates. The lack of Zn in plants may result discoloration of the foliage and growth abnormalities (Monreal, DeRosa, Mallubhotla, Bindraban, and Dimkpa, 2015).

Today, nanotechnology gained great attention due to its potential applications. Nanomaterial (having size ranging from 1 – 100 nm) properties became different and enhanced compared to that of bulk material (Paul, Syed, Vyawahare, Dakle, and Ghuge, 2016). There are numerous developed methods for synthesizing nanoparticles which were categorized as physical, chemical, and biological (Paul *et al.*, 2016). These physical and chemical methods include wet chemical method carried out in water, organic solvents, ionic water, or microemulsions (Zelechowska, 2014). Gas condensation, vacuum deposition and vaporization, mechanical attrition, hydrolysis, condensation, growth and agglomeration, and electrodeposition were the other methods that were used to synthesis nanoparticles (Rajput, 2015). Most of these methods requires hazardous chemicals, stabilizing agents and capping agents (Paul *et al.*, 2016) that can cause adverse effect in medical application (Sangeeta, Rajeshwari, and Venckatesh, 2011). This concern has led the development of eco-friendly method of synthesizing nanoparticles (Sangeeta *et al.*, 2011) which offers a lot of benefits for medical and pharmaceutical application (Devasenan, Beevi, and Jayanthi, 2016).

This eco-friendly method of synthesizing nanoparticles which they call biological method utilizes plant extracts and microorganisms in producing such nanoparticles (Ayeshamariam *et al.*, 2014). Aloe vera extract was used to synthesize zinc oxide nanoparticles (Sangeeta *et al.*, 2011), copper oxide nanoparticle (Kumar, Shameen, Kollu, Kilyani, and Pammik, 2015), silver nanoparticle and gold nanotriangle (Chandran, Chaudhary, Pasricha, Ahmad, & Sastry, 2006). Phytochemical screening of aloe vera shows the presence of alkaloids, tannins, flavonoids, and terpenoids (Raphael, 2012) which were mainly responsible for the formation of metallic nanopartiles (Kuppusamy, Yussof, and Govindan, 2014).

Zinc oxide is a multifunctional material with its unique chemical and physical properties like high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability (Kołodziejczak-Radzimska and Jesionowski, 2014). It's nanoscale form was

reported to be non-toxic to human cells which make it biocompatible to human cells (Sirelkhatim *et al.*, 2015). This metal oxide was also used as source of zinc in fertilizers but it will be less efficient if used in granular form because its solubility is low. To increase the solubility of this metal oxide it must be finely ground (Sutradhar, Kaiser, Rosen, and Lamb, 2016). This condition best describes the nanoscale size of the zinc oxide which means that it can be a potential source of zinc if incorporated in fertilizers.

In this study, the zinc oxide nanoparticles will be synthesized using aloe vera leaf extract as stabilizing agent. The efficacy of zinc oxide nanoparticles incorporated to the organic fertilizer on the growth of tomato will also be examined.

## **Materials and methods**

### ***Chemicals and Reagents***

Zinc sulphate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), nitric acid ( $\text{HNO}_3$ ), and sodium hydroxide ( $\text{NaOH}$ ) were purchased from Sigma – Aldrich Corporation. Analytical grade chemicals were used in this study.

### ***Preparation of Aloe Vera Leaf Extract***

One hundred twenty five grams (125 g) of aloe vera leaf were thoroughly washed, dried, and finely chopped. The leaves were heated in 500 ml of deionized water for 5 minutes at 80 °C and then cooled to room temperature. The resulting solution was filtered to remove any residue. The filtrate was used for the synthesis of the ZnO nanoparticles (Kumar *et al.*, 2015).

### ***Synthesis of ZnO Nanoparticles***

The aloe vera leaf extract was mixed in 0.1 molar zinc sulphate heptahydrate solution at 1:4 ratio. The solution was constantly stirred with a magnetic stirrer at 60 °C for 3 hours (Devasenan *et al.*, 2016) after the pH was adjusted to 8 by dropwise addition of 0.1 molar NaOH solution. The resulting solution was centrifuged and the supernatant was discarded. The collected ZnO nanoparticles was oven dried at 80 °C for 7 hours after thorough washing with 5% nitric acid (Sangeeta *et al.*, 2011).

### ***Characterization of ZnO Nanoparticles***

The nanoparticles were dispersed in an aqueous solution for optical property analysis using UV-Vis. The chemical composition was determined

using FTIR. The shape, size, and nanostructure of the nano metal oxides was characterized using Scanning Electron Microscope (SEM) (Sangeeta *et al.*, 2011)

### ***Preparation of Organic fertilizer***

One gram of chitosan were dissolved in 200 ml of 1% acetic acid solution. One hundred milliliters (100 ml) of this chitosan solution was added to 400 ml of fish amino acid to form an organic fertilizer made from fish amino acid and chitosan (Han, Nyein, and Nwe, 2008).

### ***Preparation of Organic fertilizer with ZnO Nanoparticles***

Fifty milliliters (50 ml) of the organic fertilizer were diluted to 1 liter using distilled water prior to its application to the tomato plant. The ZnO nanoparticles were dissolved in the prepared organic fertilizer at concentrations of 0, 15, and 30 ppm (Aduloju and Abdulmalik, 2013).

### ***Efficacy Test***

The prepared organic fertilizer with zinc oxide nanoparticle with concentrations 0, 15, and 30 ppm were applied in tomato planted in a pot as foliar spray every 7 days for 7 weeks. The efficacy of the treatment to the plant was evaluated by measuring its height every 7 days, the number of days required to produce flower and fruit, and the total mass of the fruits harvested after 7 weeks. The treatments were done in triplicates and the data were evaluated using one way ANOVA. (Sibounnavong, Utthajadee, Makhonpas, and Soyong, 2012).

## **Results**

### ***Synthesis of ZnO Nanoparticles***

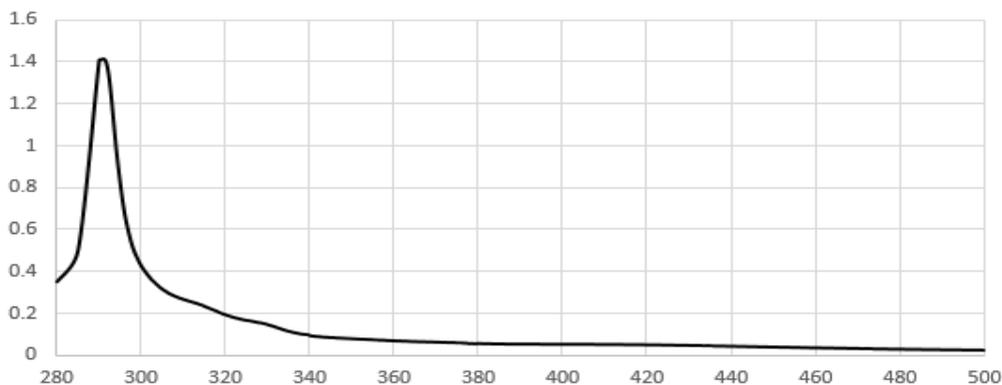
Aloe vera leaf extract and 0.1 molar solution of zinc sulphate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) was mixed in 1:4 ratio and the pH of the solution was adjusted to pH 8 by dropwise addition of 0.1 molar sodium hydroxide (NaOH) solution. The formation of pale yellow precipitate indicates the formation of zinc oxide nanoparticles (Narendhran and Sivaraj, 2015). The precipitate was collected and dried for further characterizations.

### UV – Vis Analysis

As the light hits a metallic nanoparticle, its oscillating electromagnetic field induces a collective oscillation on the free electrons (conduction band electrons) of the metal which creates charge separation with respect to the ionic lattice. The amplitude of the oscillation will have its maximum at a specific frequency which was known as surface plasmon resonance (SPR) (Huang and El-Sayed, 2010).

Analysis of the synthesized ZnO nanoparticles using UV-Vis spectrophotometer shows surface plasmon resonance (SPR) absorption peak at 290 nm which was the characteristic peak of ZnO nanoparticles (Zhou *et al.*, 2016). The absorption peak of the synthesized nanoparticles was far blue shifted from the absorption peak of the bulk size ZnO oxide (385 nm) (Senthilkumar and Sivakumar, 2014) which indicated smaller size of particle produced (Sangeeta *et al.*, 2011).

A hypsochromic shift on the SPR wavelength can be related to the optical band gap using the formula  $E_g = hc/\lambda$  (Yuliah, Bahtiar, Fitrilawati, and E.Siregar, 2016). The optical band gap of the bulk and nanosized ZnO were calculated to be 3.26 eV and 4.28 eV respectively. The increase in the optical band gap was due to the quantum confinement effect (Lin, Cheng, Hsu, Lin, and Hsieh, 2005). Quantum confinement effect was the trapping of electrons and holes in a tiny region as particle size decreases which results in increased transition energy between the valence band and the conduction band also known as the interband transition energy (Atkins *et al.*, 2010). The relationship between the optical band gap and the particle size was observed to be inversely proportional (Lin *et al.*, 2005). Therefore, the increase in the band gap energy indicates smaller particle size of the synthesized ZnO NPs.



**Figure 3.1.** UV-Vis spectra of the synthesized ZnO nanoparticles.

### FTIR Analysis

Fourier Transform Infrared (FTIR) spectra of the synthesized ZnO nanoparticles shows characteristic peak at  $478\text{ cm}^{-1}$  (Sangeeta *et al.*, 2011) which was due to Zn=O stretching (Khan, Khan, Zulfequar, and Khan, 2011). Broad peak appearing at  $3200\text{ cm}^{-1}$  to  $3600\text{ cm}^{-1}$  attributes the presence of O – H stretch. The peaks at  $1560\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$  were due to C=O and C – O stretch. The observed peaks of the organic functional groups on the FTIR spectra possibly indicates the presence of biological molecules on the surface of ZnO nanoparticles which probably came from the aloe vera leaf extract used as stabilizing agent in the synthesis. These organic molecules on the surface of the nanoparticle were responsible for the stabilization of the nanoparticles as it prevents the particles from aggregating and forming a larger particle (Sangeeta *et al.*, 2011).



**Figure 3.2.** FTIR spectra of the synthesized ZnO nanoparticles.

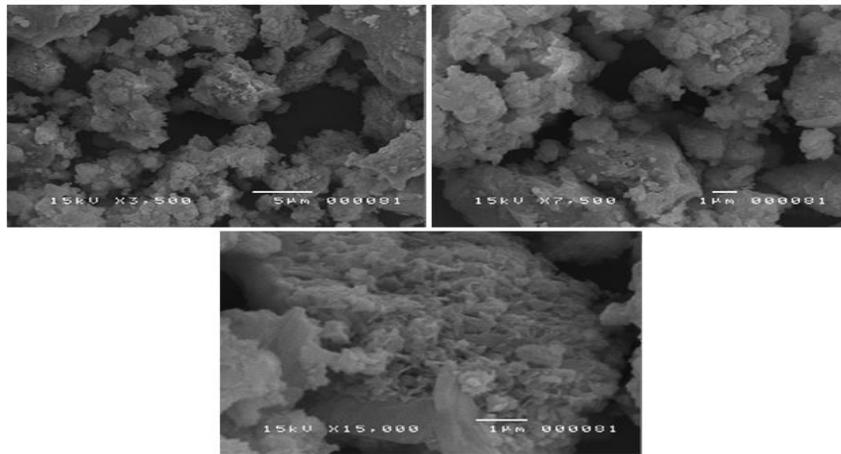
**Table 3.1.** FTIR spectra vibrational frequency peak assignments.

Frequency	Assignment
$478\text{ cm}^{-1}$	Zn=O stretch
$1400\text{ cm}^{-1}$	C – O stretch
$1560\text{ cm}^{-1}$	C=O stretch
$3200\text{ cm}^{-1} - 3600\text{ cm}^{-1}$	O – H stretch

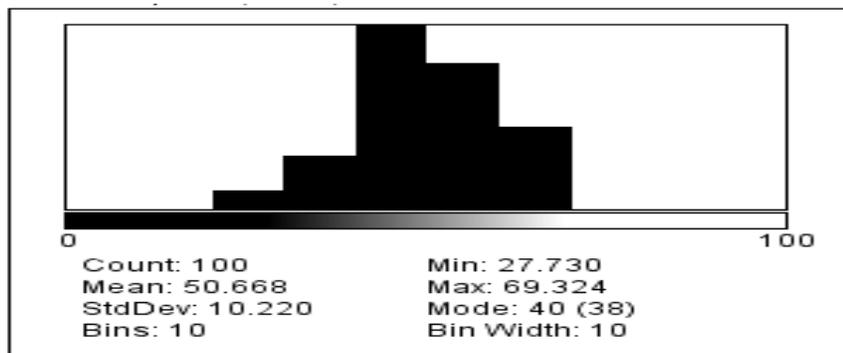
### SEM Analysis

The Scanning Electron Microscopy (SEM) micrograph presented below (Figure 7), shows that nanoparticles produced has a diameter size less than 100 nm with spherical shape. Figure 8 revealed the particle size distribution of the synthesized nanoparticles having an average size of  $50.67 \pm 10.22\text{ nm}$  with a minimum and maximum size of 27.73 nm and 69.32 nm respectively. The ZnO nanoparticles depicted below (Figure 7) showed an image that looks bigger in

size due to agglomeration of the nanoparticles. During the drying process, agglomeration of nanoparticles would takes place (B. Wang *et al.*, 2007). The nanoparticles were attracted with each other via weak forces (adhesion) leading to a formation of larger cluster of particles (Gosens *et al.*, 2010). This process of agglomeration was reversible which means that the agglomerates or cluster of particles could also be separated into its individual particles by disturbing the equilibrium reaction (Sokolov, Tschulik, Batchelor-McAuley, Jurkschat, and Compton, 2015). Reversing agglomeration employed the application of ultrasonic treatment to disperse the nanoparticles in aqueous medium (Walter, 2013).



**Figure 3.3.** SEM micrograph of the synthesized ZnO nanoparticles. Magnifications: a) x3500 b) x7500 c) x15000.



**Figure 3.4.** Particle size (in nanometer) distribution of ZnO nanoparticles.

### *Efficacy Test*

A mixture of Fish Amino Acid (FAA) and chitosan served as organic fertilizer that was used in this study (Han *et al.*, 2008). Zinc oxide nanoparticles were added to the organic fertilizer at concentrations 0, 15, and 30 ppm respectively. The organic fertilizer with 15 and 30 ppm ZnO NPs served as the treatments 1 and 2 and the organic fertilizer with 0 ppm ZnO NPs served as the control.

The fertilizers were applied as foliar spray on tomato for seven weeks. The height of the plants were measured weekly as shown on Table 2. There was no significant increase in height of the treatments 1 and 2 when compared to the control during the first five weeks. During the sixth week, treatment 2 showed significant increase in height by 19.78%. At the end of the seventh week, both treatments 1 and 2 showed significant increase in height by 17.36% and 22.64% when compared to the control. Increase in plant height was directly related to cell elongation (Nick, 2013) which was promoted by zinc micronutrient (Prasad *et al.*, 2012). Application of ZnO NPs also showed significant increase in height of maize (Subbaiah *et al.*, 2016) and peanut (Prasad *et al.*, 2012).

**Table 3.2.** Height of tomato plant from week 1 to week 7.

	<b>Control (cm)</b>	<b>T1 (cm)</b>	<b>T2 (cm)</b>
<b>Start</b>	19.0 ± 1.73	19.3 ± 0.58	19.3 ± 1.15
<b>Week 1</b>	39.0 ± 2.66 <sup>a</sup>	38.0 ± 1.00 <sup>a</sup>	39.7 ± 0.58 <sup>a</sup>
<b>Week 2</b>	61.7 ± 1.53 <sup>a</sup>	62.3 ± 2.08 <sup>a</sup>	66.3 ± 2.52 <sup>a</sup>
<b>Week 3</b>	81.3 ± 8.50 <sup>a</sup>	89.0 ± 1.00 <sup>a</sup>	91.7 ± 2.52 <sup>a</sup>
<b>Week 4</b>	106.7 ± 7.64 <sup>a</sup>	111.7 ± 2.89 <sup>a</sup>	110.3 ± 0.58 <sup>a</sup>
<b>Week 5</b>	111.7 ± 7.64 <sup>a</sup>	121.0 ± 5.29 <sup>a</sup>	123.0 ± 6.08 <sup>a</sup>
<b>Week 6</b>	118.3 ± 7.64 <sup>b</sup>	136.0 ± 1.73 <sup>a,b</sup>	141.7 ± 11.55 <sup>a</sup>
<b>Week 7</b>	125.0 ± 10.00 <sup>b</sup>	146.7 ± 2.89 <sup>a</sup>	153.3 ± 10.40 <sup>a</sup>

Average of 3 replicates. Means within a row followed by the same letter are not significantly different at p = 0.05

The tomato plant from all the treatments was observed to produce flower and fruit at the same number of days (21 days and 28 days). Studies on mustard revealed that application of zinc has negligible effect on flower initiation (Sahito, Solangi, Lanjar, Solangi, and Khuhro, 2014) and was mainly affected by phosphorus (Silva and Uchida, 2000). Deficiency in micronutrients like iron and copper were reported to delay flower initiation (Padney, 2010). The average mass of fruits harvested were shown in Table 3. Statistical analysis revealed that treatments 1 and 2 have significantly increased the average mass of fruit yield by 121.50% and 127.23% when compared to control. Zinc as co-factor enzyme positively affects the sucrose forming enzymes (aldolase), the

starch synthesizing enzyme (starch synthetase) and the number of starch grains and therefore increasing the yield of the plant (Masroor *et al.*, 2016). This study demonstrated the positive effect of ZnO NPs on the growth of tomato which was also observed on peanut, soybean, wheat, onion (Siddiqui, Al-Whaibi, Firoz, and Al-Khaishany, 2015) and maize (Subbaiah *et al.*, 2016).

**Table 3.3** Mass of fruits harvested from tomato plant.

	Control (grams)	T1 (grams)	T2 (grams)
<b>Total mass</b>	223.90	495.95	508.78
<b>Ave. mass</b>	74.63 ± 18.95 <sup>b</sup>	165.31 ± 30.50 <sup>a</sup>	169.59 ± 27.78 <sup>a</sup>

Average of 3 replicates. Means of the same letter are not significantly different at  $p = 0.05$

Foliar application of nanoparticles enters the plant through the stomatal pathway (Chichiriccò and Poma, 2015). However, particles must have a size not exceeding 100 nm in order to penetrate the stomata successfully (W.-N. Wang, Tarafdar, and Biswas, 2013). Thus, the synthesized ZnO NPs having an average size of  $50.67 \pm 10.22$  nm can effectively enter through the stomata. Plants absorb zinc via ion exchange or ion diffusion (Haslett, Reid, and Rengel, 2001) which increases absorption with increased surface area of nanoparticles (Prasad *et al.*, 2012). The low water solubility of ZnO NPs increases its bioavailability on the plant because it inhibits rapid falling off compared to more water soluble supplements (Laware and Raskar, 2014). The sufficient supply of zinc can be used by plant in growth regulation, enzyme activation, gene expression and regulation, phytohormone activity, protein synthesis, photosynthesis, carbohydrate production, seed production and defense against disease (Sadeghzadeh, 2013). All of these factors may be responsible for the increased growth performance of the plant (Prasad *et al.*, 2012).

## Conclusion

Zinc oxide nanoparticles, having an average size of  $50.67 \pm 10.22$  nm were successfully synthesized using aloe vera leaf extract as stabilizing agent. Application of these metal oxide nanoparticles, dispersed in organic fertilizer (fish amino acid + chitosan), to the tomato plant shows a significant positive effect on the growth performance of the plant. The plants in treatments 1 and 2 showed an increase in height by 17.36% and 22.64%. Likewise, the total mass of the fruit yielded was significantly increased in treatment 1 and 2 by 121.50% and 127.23% when compared to the control.

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