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# Effect of biosynthesized zinc oxide nanoparticles on the vegetative growth of *Amaranthus cruentus* plants

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## Abstract

ZnO nanoparticles synthesis using *Ocimum gratissimum* and *Vernonia amygdalina* plant leaf extracts, their characterisation and use as nanofertilizer for growing black-seeded (BS) and pale-seeded (PS)Amaranthus cruentus plants is here presented. The nanoparticles (made possible by the flavones, phenols and flavonoids in the leaf extracts used) were of good crystalline structure, spherical in shape and in clusters. Their UV-vis peak absorbance occurred at 355 nm and 360 nm and their PL spectra showed a UV emission peak and a green emission peak. SEM images show nanoparticles sizes (which depended on the pH level of their synthesis solution and the type of plant leaf extract used) in the range 38 nm to 63 nm. When used as nanofertilizer for the *Amaranthus cruentus* growth, it was discovered that smaller nanoparticles produced taller plants and that both plant varieties tolerate nanofertilizer concentrations as high as 500 $mg/l$ though nanofertilizer concentrations higher than this was detrimental to the plant growth. While the BS plants showed better shoot growth with broad leaves of area 60 $cm^2$, the PS plant variety had narrow and scanty leaves with leaf area as low as 4 $cm^2$. The PS plants produced the highest yield of 60 % when treated with pH 10 500 $mg/l$ Og-ZnO nanofertilizer but the lowest yield of 16 % when treated with pH 12 Og-ZnO nanofertilizer of concentration 500 $mg/l$. This work shows that these ZnO nanofertilizer enhanced the growth of the *Amaranthus cruentus* provided high concentrations of the fertilizers which could be toxic, are avoided.

**Keywords:** Zinc oxide nanoparticles, Characterization, *Amaranthus cruentus* plants, nanofertilizer, vegetative growth, yield percent.

## INTRODUCTION

Population explosion demands an increase in food production and due to poor soil quality, deficit of labour and arable land with an attendant drop in crop yield, farmers have turned their searchlight to chemical fertilizers to boost their harvest and achieve food security[1] Farmers have moved from using organic fertilizers (which sometimes are unhealthy, as some pathogens from them cling to the planted crops ) to bulk chemical fertilizer[1] which are becoming more expensive and not so affordable, and now to nanofertilizers [2].

Nanoparticles when used in Agriculture do not only increase crop yield by 15-30 % with reduced soil toxicity, they protect them against pests and microbial infection [3]. Nanoparticles can even stimulate early flowering in the plants and enhance their nutritional value [4, 5]. Furthermore, nanoparticles are laced with nanomaterials which slowly release nutrients to plants to take it up throughout its growth period .and during adverse weather [6,7]. Furthermore, only small quantities of Nano fertilizers are required to grow plants and plants do not lose their natural minerals while growing.

Zinc oxide(ZnO) is an essential ingredients in chemical fertilizers and is used in place of zinc sulphate. ZnO has also been applied directly to soils to grow crops and fruit trees. Zinc nanoparticles on the other hand are becoming popular and have been reported to possess exceptional optical, physical and antimicrobial properties, giving them a prominent place in agriculture [8].

**Literature review**

Zinc oxide nanoparticles of size less than 100 nm and ionic form of zinc sulphate salt of various concentrations have been applied to maize plant [9,10]. The plant height, root length and dry matter were reported to have grown reasonably and the maize plants roots possess a special mechanism of absorbing the Nano-zinc for its growth.

Peanuts treated with varying concentrations of Nano- zinc oxide (ZnO NPs) and chelated bulk zinc sulphate (Zn$SO_4$) are reported to have flowered early and produced a yield of 34 % when compared with the effect of the chelated bulk Zn$SO_4$ [11]though concentrations as high as 2000ppm produced inhibitory effect. Bulk zinc and nano Zinc oxide and Titanium dioxide ($TiO_2$) have been used to grow wheat [12] . Though the bulk zinc had no significant effect on the wheat, the nano zinc raised the chlorophyll level of the shoots and increased the protein content of its seeds.

Three different concentrations of colloidal ZnO nanoparticles solution synthesized with two different plant leaf extracts (Ocimum gratissimum (Og) and Vernonia amygdalina (Va)) at three different pH levels(pH 8, pH 10 and pH 12) have been used for planting pale-seeded (PS) and black-seeded (BS) *Amaranthus cruentus* [13]. The effect of these ZnO nanoparticles (Og-ZnO and Va-ZnO) on the seedling characteristics (Percent emergence (% E), emergence index (E I) and emergence rate index (ERI)) of this crop after one week was studied [13]. It was discovered that a high concentration of Og ZnO nanoparticles of all 3 pH levels raised the percent emergence of the black-seeded variety but raised the emergence index (E I) of the two seed varieties though with poor growth uniformity. Conversely, high concentrations of pH 12 Va ZnO nanoparticles produced low percent emergence of both seed varieties. Lower concentrations of Og ZnO nanoparticles of all pH levels resulted in low ERI of the pale-seeded variety and all the viable seeds of PS variety germinated in four days while those of the BS variety germinated after 10 days.

From the above review, it is obvious that there is a dearth of information and data on the effect of different concentrations of zinc oxide nanoparticles as described above on the vegetative growth of *Amaranthus cruentus* plants and that is the focus of this work. Amaranthus cruentus is a pseudo-cereal in Central America and eaten in tropical Africa. Its leaves are rich in protein and its extract can give some relief from constipation.

## MATERIALS AND METHODS

(i) (a) Black-seeded (BS) and (b) Pale-seeded (PS) *Amaranthus cruentus*

**a** **b**

(ii) leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* plants
(iii) Zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) of analytical grade, molecular weight 219.50, Purity $> 99$ purchased from Sigma Aldrich
(iv) Sodium hydroxide pellets (NaOH) of Molecular weight 40g from Sigma Aldrich.
(v) Milli-Q water used for preparing all the solutions.
(vi) pH level meter (high precision)

**Preparation of Ocimum gratissimum and Vernonia amygdalina plant leaf extracts** [14]

To prepare 100 ml of Og or Va leaf extract, 130 ml of water was added to 15 grams of each of the grounded shade-dried Ocimum gratissimum or Vernonia amygdalina leaves and the mixture was boiled for one hour and 100 ml of the extract was sieved and stored.

**Zinc oxide (ZnO) nanoparticles synthesis** [13]

Zinc oxide nanoparticles was biosynthesized using Ocimum gratissimum and Vernonia amygdalina plant leaf extracts [13] and labelled as Og-ZnO and Va-ZnO nanoparticles. 70 ml of 0.2 M zinc acetate dihydrate solution was mixed with 70 ml of 0.2 M sodium hydroxide in a 250 ml beaker and 30 ml of either the Og or Va leaf extracts was stirred.

After about 30 mins of continuous stirring, 2 M NaOH solution was added dropwise to adjust the pH of the mixture (monitored using a pH meter) to pH 8, 10 or 12 as required. This mixture was stirred continuously for 2hrs to produce a cloudy zinc hydroxide ($Zn(OH)_2$) precipitate which was centrifuged at 5000 rpm for 60 minutes to give sediment which was dried in an oven set to 70 ℃ to produce the Og- and Va-zinc oxide nanoparticles [14-16]. The pH 8, pH 10 and pH 12 Og- and Va- ZnO nanoparticles were labelled as P1, P2 and P3. The choice of the range of pH of synthesis of the zinc oxide nanoparticles is because high pH of nanoparticles synthesis can shorten the precipitation time but raise the purity level of the nanoparticles [17].

**Characterisation of ZnO Nano powder**

The UV-Vis scan of the synthesized ZnO nanoparticles was done using Shimadzu UV-2600 spectrophotometer and scans were in the range 220 nm – 1400 nm. The X-ray diffraction (XRD) scan of the sample was done using Bruker D8 Advance powder diffractometer with a Cu K α source and λ = 1.54 Å while the FTIR characterisation of the ZnO nanoparticles was done using the Perkin Elmer 100 FTIR spectrometer and sample scan was in the range 600 – 4000 $cm^{-1}$.

The Photoluminescence (PL) study was done using a machine that uses the 325 nm line of He-Cd laser with Renishaw RM 220 spectrometer and Peltier CCD array while the Scanning Electron Microscopy(SEM) studies was done using JOEL JSM 6330 F field emission scanning electron microscope.

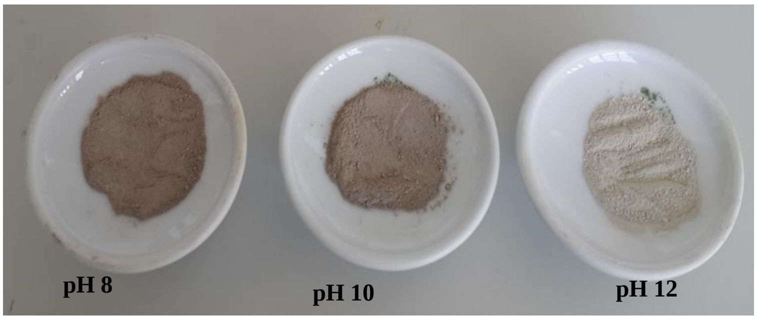


**Figure 1:** Biosynthesized zinc oxide nanoparticles (ZnO Nano powder)

## Preparation of ZnO nanofertilizer

Six concentrations of colloidal solutions of the ZnO nanoparticles in milliQ water namely 10 $mg/l$, 100 $mg/l$, 500 $mg/l$, 1000 $mg/l$, 1500 $mg/l$ and 2000 $mg/l$, were made and labelled as N1, N2, N3, N4, N5, and N6 respectively. These were the nanofertilizer used for the study. The blank control pots which contained untreated soil with *Amaranthus cruentus* plant was labelled as P0N0, while the 10 $mg/l$ of pH 8 ZnO nanoparticles was labelled PIN1 and the 500 $mg/l$ of pH 12 ZnO nanoparticles was labelled as P3N3 etc.

## Planting of *Amaranthus cruentus* [13]

The *Amaranthus cruentus* seeds used are of good quality and the soil pH is 6.00 and made up of 79.5 % of sand, 10.5 % clay and 10.0 % silt. Organic carbon was 1.50 %, and its zinc content was 0.24 mg $kg^{-1}$ [13]. Pots used for the planting each contained $8 \times 10^{-3}\ m^3$ of soil treated with 50 ml of each type of the liquid ZnO Nano fertilizer. The pots were housed in a screen house (Figure 2) to protect the growing plants from all other factors, except the effects of the applied nanofertilizer and seeds were planted in the soil at a depth of 0.7 cm and with three replications of each soil treatment (Figure 3). For this phase of the study, the ZnO Nano fertilizer was applied weekly to the plant. The data from the interactive effect of the pH and concentration of the applied nanofertilizer on the *Amaranthus cruentus* plants was subjected to the analysis of variance (ANOVA) and the tests were done at 95 % confidence Interval using GenStat release 10.3 DE [13].

**Figure 2:** Screen house to shield the plants from factors other than those investigated.


**Figure 3: (a)** Layout of pots containing plants with three replications of each treatment**(b)** BS plants showing chlorosis **(c)** PS plants showing long stem and scanty leaves

This study was for five weeks during which the effect of the ZnO nanofertilizer on the plants height, leaf area and yield % was studied. At the end of the five weeks, the fresh plants were harvested, and their fresh weight was determined. The plants were dried in an oven set to 70 ℃ for 72 hours and their dry weight in each case determined. The yield % of the plants was determined using equation (1)

$$Yield\ \% = \frac{Dry\ weight\ of\ plants}{Fresh\ weight\ of\ plants} \times 100 \qquad (1)$$

The yield percent was calculated to see how much dry biomass resulted from the fresh plants harvested and to identify the particular nanoparticle concentration which produced the best yield % and the concentration beyond which there was a sharp decrease in the yield of the *Amaranthus cruentus* plants termed the critical point was also determined. To achieve this, the plants were treated with higher concentrations of the ZnO Nano fertilizer (1000 $mg/l$ , 1500 $mg/l$ and 2000 $mg/l$) and obtained results were discussed.

The effect of the ZnO nanofertilizer on the height is shown in Figure 7a, 7b, that on the leaf Area is shown in Figure 11 using bar charts while and yield % are as shown in Tables 2 and 3 and Figures 12-14. To add credibility to the bar chart results, error bars were added and by drawing lines across, from the bottom of the error bar with the highest dependent or from the top of the error bar with the lowest dependent variable [18] it was possible to tell which differences were significant and which ones were not.

## RESULTS AND DISCUSSIONS

The UV-vis absorbance spectrum (Figure 4) established the ZnO nanoparticles identity and the peak absorbance was within the range specified for ZnO nanoparticles in available literature. For the Og ZnO nanoparticles, the absorption occurred at 360 nm while that of the Va ZnO nanoparticle occurred at 355 nm. These values suggest that these nanoparticles do not absorb light in the visible part of the spectrum. The optical energy bandgap values $E_g$ of the Og- zinc oxide nanoparticles increased with pH of its synthesis and was in the range 3.22-3.24 eV while the reverse was the case for the Va- zinc oxide nanoparticles whose $E_g$ decreased with nanoparticles synthesis pH and in the range 3.29-3.32 eV[14].

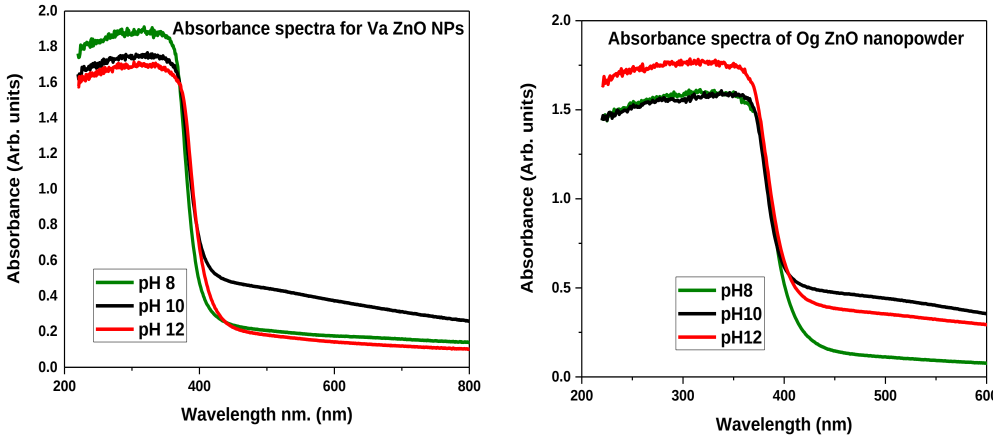


**Figure 4:** UV-vis spectra of the synthesized ZnO nanoparticles

The XRD scan (Figure 5) showed that the nanoparticles were of a good crystalline structure. Ten peaks were identified, with the strongest reflection coming from plane 101. The pattern confirmed that the nanoparticles has the hexagonal Wurtzite structure of ZnO according to JCPDS (2000)36-1451,.

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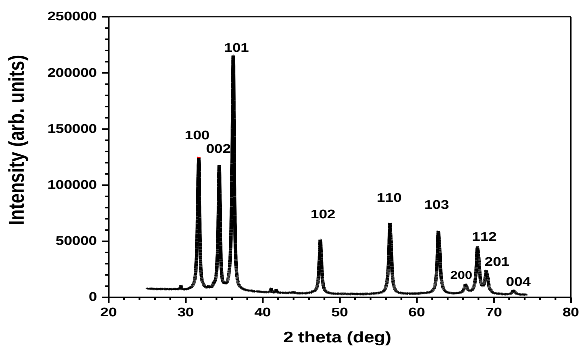


**Figure 5:** XRD pattern of the zinc Nano powder

The Photoluminescence study was for characterising the optical and electronic properties of the ZnO nanoparticles, The PL spectra (Figure 6) showed two emission peaks namely the peak at 2.2-2.3 eV which is the band gap excitonic emission and the peak at 3.2 eV due to singly ionized oxygen vacancies.

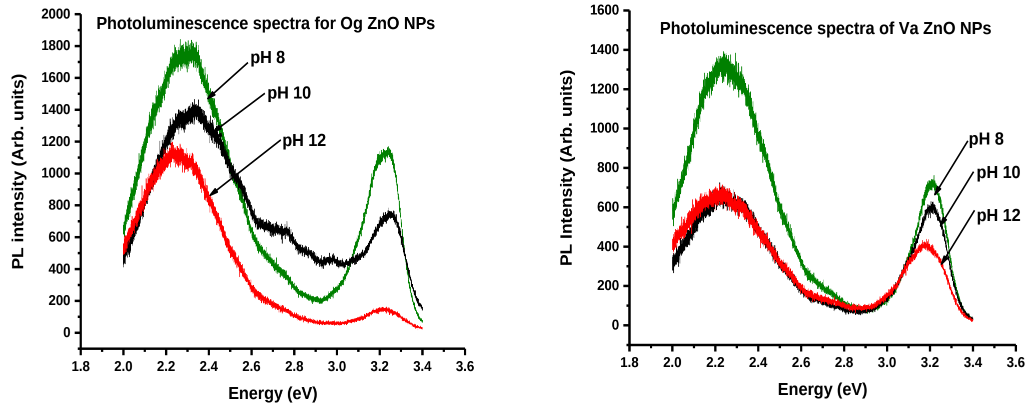


**Figure 6:** Photoluminescence spectra of the ZnO Nano powder.

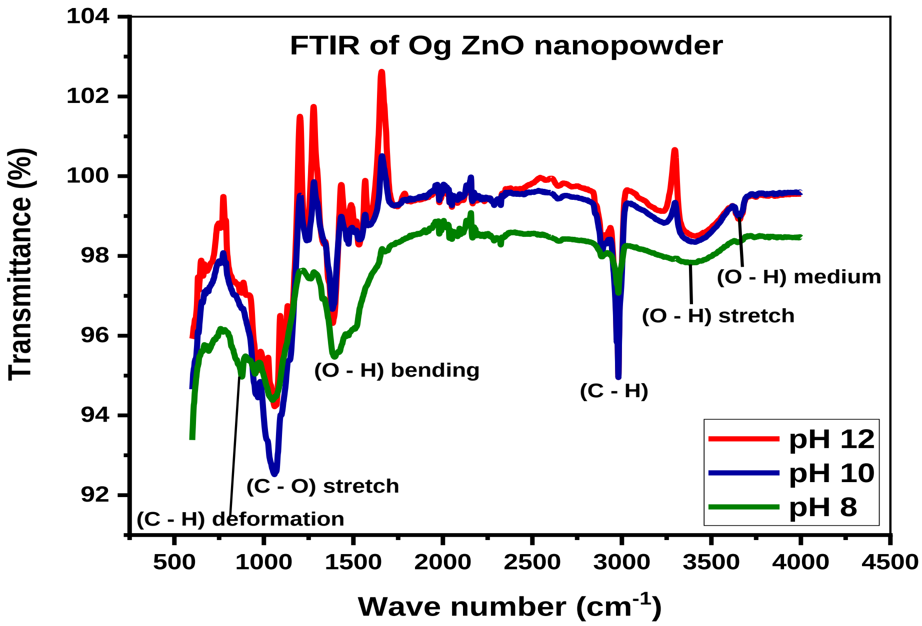


**Figure 7a:** FTIR spectra of the Og-ZnO Nano powder.

The FTIR spectra (Figure 7a) shows (O-H) stretch at 3377 $cm^{-1}$ which indicate the presence of phenols and flavonoids and (O-H) medium represents the presence of phenols and alcohols while the (O-H) bending at 1392 $cm^{-1}$ denote phenols. The (C-H) deformation at $868cm^{-1}$ indicate phenolic compounds while (C-O) stretch at $1065cm^{-1}$ and (C-H) at $2986cm^{-1}$ represent primary alcohol and Alkane respectively.

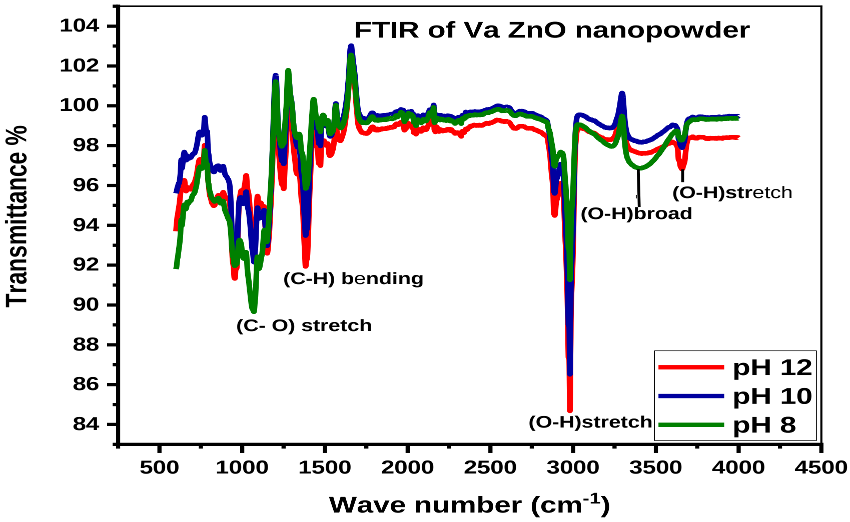


**Figure 7b:** FTIR spectra of the Va-ZnO Nano powder

Figure 7b shows the (C-O) stretch at 1077 $cm^{-1}$ which represents alcohols and cellulose and the (C-H) bending at 1387 $cm^{-1}$ for aldehydes and flavones. Other functional groups identified were the (O-H) stretching at 3661$cm^{-1}$ due to alcohols and phenols, the (O-H) stretch at 2982$cm^{-1}$ and centred at 3000$cm^{-1}$, which represents carboxylic acids. The (O-H) broad at 3382$cm^{-1}$ is due to alcohols and phenols. These FTIR results indicate that flavonoids, phenols and flavones inherent in the two plant leaf extracts, aided in the nanoparticles synthesis.

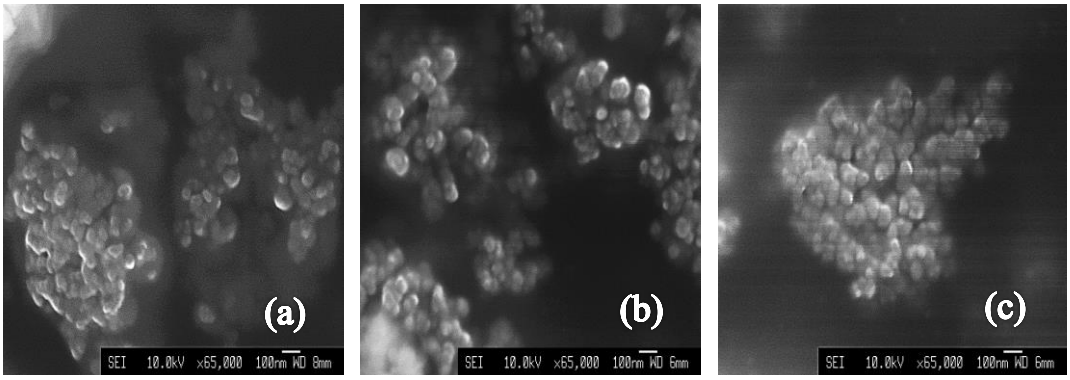


**Figure 8a:** SEM images of (a) pH 8 (b) pH 10, (c) pH 12 Og zinc oxide nanoparticles

The SEM images (Figures 8a and 8b) show spherical nanoparticles which are in clusters, an indication that they are pure [17] with sizes which were pH (of synthesis) and plant leaf extract dependent as shown in Table 1.

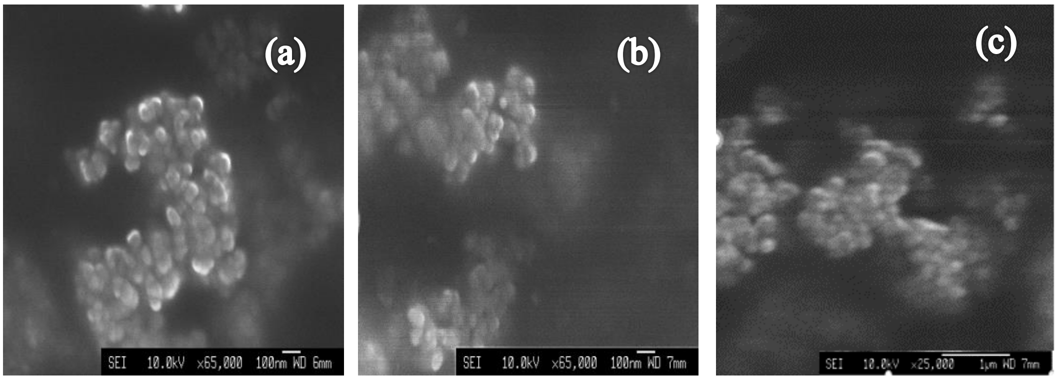


**Figure 8b:** SEM images of (a) pH 8 (b) pH 10, (c) pH 12 Va zinc oxide nanoparticles

**Table 1:** Showing the sizes of ZnO nanoparticles synthesized at various pH levels

| pH of ZnO nanoparticle synthesis | Size of Og-ZnO nanoparticles (nm) | Size of Va-ZnO nanoparticles(nm) |
|---|---|---|
| 8 | 38 ±0.4 | 44 ±0.8 |
| 10 | 43 ± 0.6 | 57±0.5 |
| 12 | 63±0.8 | 62±0.6 |

## Plant height

The plants reached their maximum heights after 5 weeks because no significant increase was observed after that. The plants growth was compared with the control plant (P0N0) In all cases, the treated plants grew taller than the control pot plant (P0N0) which came out the shortest (Figures 9 and 10).

With the Og-zinc oxide nanofertilizer, the BS plant variety was the tallest with treatment P2N3 while the PS plant variety was tallest with P2N2 treatment. Conversely and with the Va-zinc oxide nanofertilizer, the P1N3 treatment made the BS plant taller and the P1N1 treatment produced the tallest PS plant (Figure 9 and 10). From these analyses it can be inferred that low pH synthesized ZnO nanofertilizer or smaller nanoparticles, produced taller plants. It was also observed that the pale-seeded (PS) plants had thicker stems when compared with the BS plants.

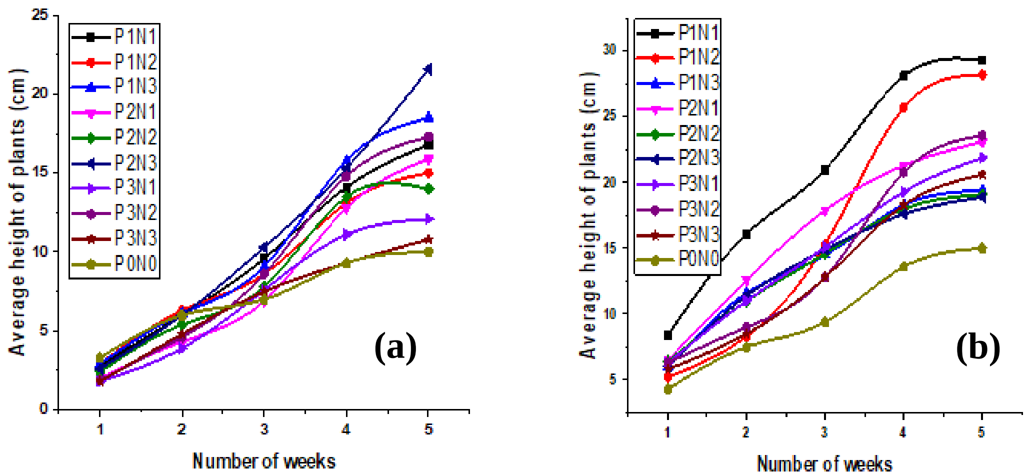


**Figure 9:** Variation of plant height with weeks for Og- (a) black-seeded and (b) pale-seeded variety plants.

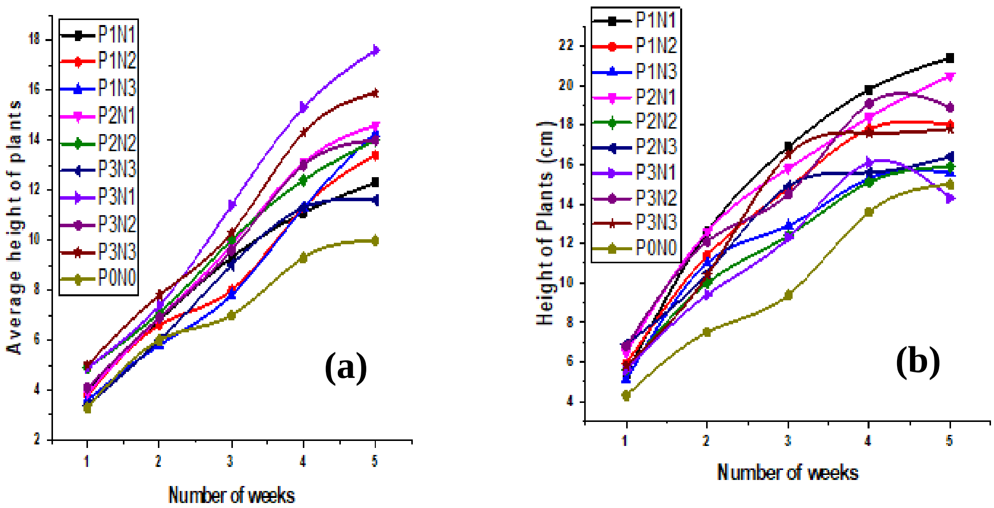


**Figure 10:** Variation of plant height with weeks for Va-(a) black-seeded and (b) pale-seeded variety plants.

## Leaf area

Treatments with the Og-zinc oxide nanofertilizer produced BS plants with better shoot. The largest leaf area of 63 $cm^2$ was for the BS plants and for treatment P2N3. This leaf area was significantly different from the outcome of other treatments (Figure 11). For the PS plants, coincidentally, the P2N3 treatment produced the worst leaf area of 4 $cm^2$which was significantly different from the outcome of other treatments. While the BS plants had broad leaves, the PS plants had scanty and narrow leaves. With the Va-zinc oxide nanofertilizer, the largest leaf area of 53 $cm^2$was produced in the PS plants given the P1N3 treatment and the worst outcome of 5 $cm^2$ for the PS plants which though significant for the BS plants was not significant for the PS plants with other treatments.

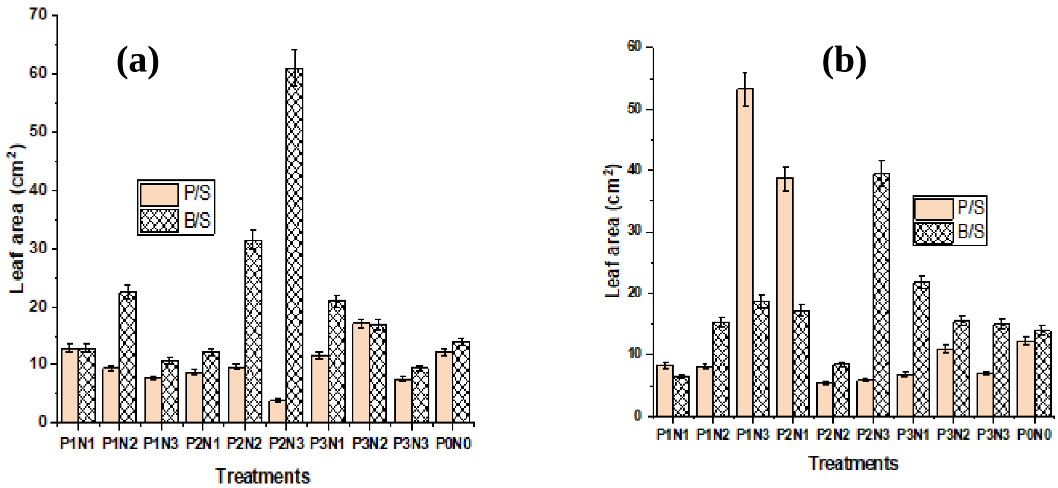


**Figure 11:** Effect of pH and concentration of (a)Og – and (b) Va-ZnO nanofertilizer on leaf area of plants.

## Yield percent of plants

Tables 2 and 3. give a summary of the yield % for different Og- or Va- ZnO nanofertilizer treatments given to the pale-seeded (PS) or black-seeded plants.

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**Table 2:** Effect of Og-ZnO nanofertilizer on treated plants yield %

| ZnO synthesis pH level | Treatment | Plant variety/Yield % | Plant variety/Max. Yield % |
|---|---|---|---|
| 8 | P1N2 | BS (19 %) | |
| | P1N1 | PS (23 %) | |
| | P1N3 | PS (21 %) | PS (23 %) |
| 10 | P2N3 | BS (25 %) | |
| | P2N3 | PS (60 %) | PS (60 %) |
| 12 | P3N1 | BS (35 %) | |
| | P3N3 | PS (16 %) | |
| | P3N3 | PS (18 %) | BS (35 %) |

**Table 3:** Effect of Va-ZnO nanofertilizer on treated plants yield %

| ZnO synthesis pH level | Treatment | Plant variety/Yield % | Plant variety/Max. Yield % |
|---|---|---|---|
| 8 | P1N1 | BS (23 %) | |
| | P1N3 | PS (29 %) | PS (29 %) |
| 10 | P2N1 | BS (27%) | |
| | P2N3 | PS (23 %) | BS (27 %) |
| 12 | P3N3 | BS (22 %) | |
| | P3N3 | PS (25 %) | PS (25 %) |

## Critical points

**G**enerally and for all the *Amaranthus cruentus* plants which were treated with ZnO Nano fertilizer of concentrations 1000 $mg/l$ and 2000 $mg/l$ , there was a sharp decrease in the yield and while some of the plants died out rightly, some were affected by chlorosis. For pH 8 Og-ZnO nanofertilizer, the critical point for BS plants was 100 $mg/l$ and for PS plants it was 500$mg/l$. For pH 8 Va-ZnO nanofertilizer, the critical point for BS plant was 10 $mg/l$ and for PS plants it was 500 $mg/l$ (Figure 12).

The critical point for pH 10 Og-ZnO nanofertilizer for both the PS and BS plants was 500 $mg/l$ and for BS plants treated with Va-ZnO nanofertilizer it was 100 $mg/l$ but 500 $mg/l$ for the PS plants (Figure 13). For the pH 12 Og-ZnO nanofertilizer, the critical points for the BS plants and PS plants were 500 $mg/l$ and 100 $mg/l$ respectively while for the pH 12 Va-ZnO nanofertilizer, the critical point for both the BS and PS plants was 500 $mg/l$ (Figure 14). These results show that both plant varieties tolerate at most 500 $mg/l$ fertilizer of any of the two types of Nano fertilizers and concentrations higher than this were toxic to the *Amaranthus cruentus* plant thwarting their growth and causing chlorosis (Figure 3 b and 3c) .

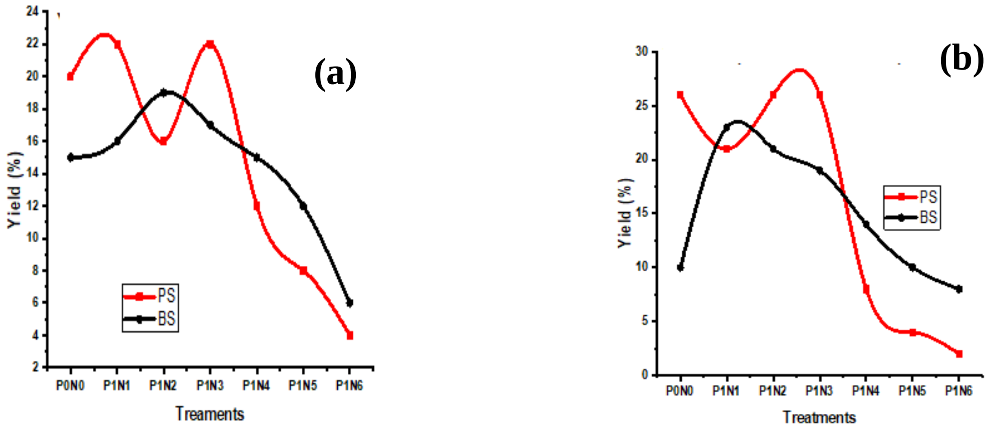


**Figure 12:** Yield % and critical points for pH 8 (a) Og-ZnO and (b) Va-ZnO nanofertilizer treated plants.

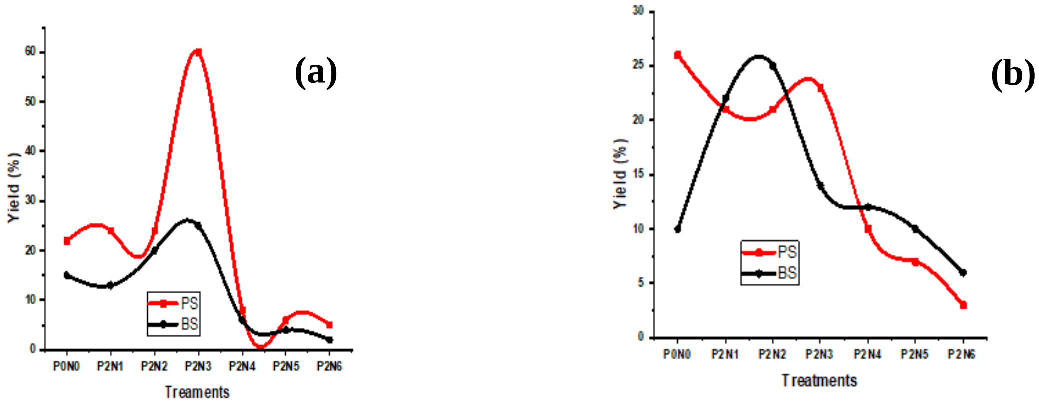


**Figure 13: Yi**eld % and critical points for pH 10 (a) Og-ZnO and (b) Va-ZnO nanofertilizer treated plants.

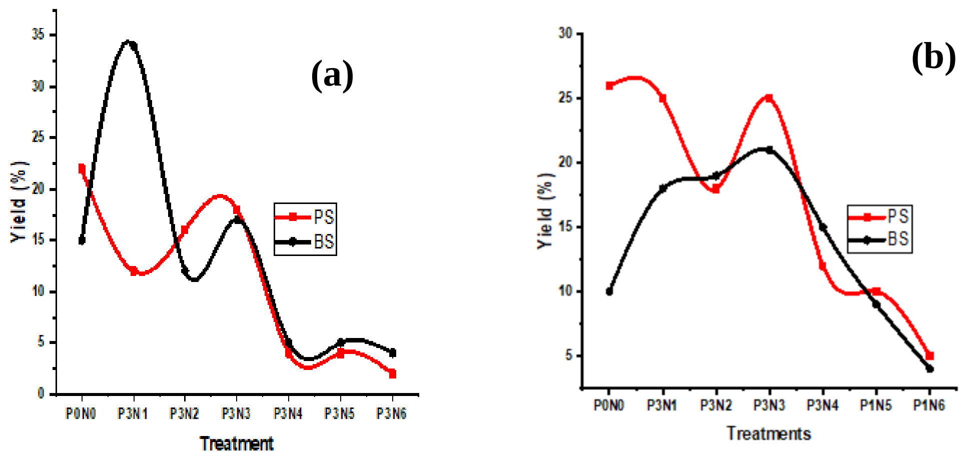


**Figure 14: Yi**eld % and critical points for pH 12 (a) Og-ZnO and (b) Va-ZnO nanofertilizer treated plants.

## CONCLUSION

Zinc oxide nanoparticles were biosynthesized using *Ocimum gratissimum* (Og) and *Vernonia amygdalina* (Va) plant leaf extracts. The nanoparticles were characterised and found to be of good crystalline structure, spherical in shape and in clusters with sizes and optical energy band gap values which depended on their synthesis pH level as well as the type of plant leaf extract used. Different concentrations of the colloidal solutions of these ZnO nanoparticles were used as nanofertilizer to grow *Amaranthus cruentus* plants and were found to enhance the growth of these plants. ZnO nanoparticles synthesized at low pH levels were smaller but produced taller plants while the reverse was the case for larger nanoparticles produced at high synthesis pH. The black-seeded (BS) plants had better shoot with broad leaves and produced the largest leaf area of 63 $cm^2$ while the worst leaf area of 4$cm^2$ was from a pale-seeded (PS) plant. The highest yield of 60 % was obtained from the PS plants treated with (P2N3) ( pH 10, 500 $mg/l$) Og-ZnO nanofertilizer and the worst yield of 16 % was obtained for PS plants given P3N3 treatment (pH 12, 500 $mg/l$).

The critical point study showed that both plant varieties tolerate at most 500 $mg/l$ concentration of any of the two types of the nanofertilizer and that concentrations higher than this were toxic to the plants and either thwarted their growth or killed them. Consequently there is need to carry out a study to ascertain the toxicity levels of the ZnO nanoparticles in the harvested plants in order to know how safe they are for human consumption.

This work may not have exposed all we sought to know about the efficacy of these ZnO nanoparticles for agricultural purposes but it certainly brings us closer to our objective of increasing food production to make it more affordable for sustainable food security to cater for the envisaged population explosion worldwide.

## POTENTIAL CONFLICT OF INTEREST S

The authors declare that there is no conflict of interest in this article

## ACKNOWLEDGMENT

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