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Determination of Chlorophyll Content, Carbonic Anhydrase Activity, Bio-Productivity and Composition of Groundnuts under five Zinc Oxide (ZnO) Applications.

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Abstract

The low level of carbonic anhydrase activity in groundnut grown on soil with limited zinc (Zn) content is one of the factors responsible for low yield in groundnuts in Nigeria. Hence, this study was carried out to further strengthen the significance of Zn in enhancing photosynthetic processes through carbonic anhydrase activity. It also determines how Zn application affects biochemical components, growth, yield and composition of *Arachis hypogaea* cv SAMNUT 22. In this study, a potexperiment was performed to investigate the effects of five concentrations of Zn applied in form of zinc oxide (0.00, 1.00, 2.00, 3.00 and 4.00 gL^{-1}ZnO) on the chlorophyll, carbonic anhydrase activity, bio-productivity and proximate composition of groundnut seeds. Data on the aforementioned parameters were subjected to One-way Analysis of Variance and means separated using Duncan Multiple Range Test at $p<0.05$. The results showed that total chlorophyll in leaves increased with increasing concentration of ZnO and hence the lowest and highest chlorophyll were recorded in 1.00 gL^{-1} and 4.00 gL^{-1}respectively. Carbonic anhydrase, after flowering showed a marked improvement with increases in ZnO application compared to the control. The bio-productivity in terms of growth components and yield attributes were respectively enhanced when the concentration of the applied ZnO is increased. Carbohydrate, protein and ash content of harvested seeds were significantly higher ($p<0.05$) in ZnO treated plants when compared to the control. The results indicated that application of ZnO at 4.00 gL^{-1} is considered optimum for enhancement of biochemical, growth and yield attributes of the studied groundnut. Also, the reported concentrations of ZnO showed positive influences only on carbohydrate, protein and ash contents of the harvested seeds.

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1. Introduction

Groundnut (*Arachis hypogaea* L.) also known as peanuts, earthnuts, gobbers, pinders, Manila nuts is an important food and cash crop across West Africa (Beghin, *et al.,* 2003). It is native to South America, Mexico and Central America (Seijo*et al*., 2003). It belongs to genus *Arachis* in subtribe Stylosanthinae of tribe Aeschynomenea of family Fabaceae. It is a self-pollinated, tropical, annual legume (Ntare*et al*., 2008). Groundnut is an important oil and food crop as it is the third major oil seed globally next to soybean and cotton, while it also serves as a dietary protein source for a large segment of low income populations (Aletor and Ojelabi, 2003) The uses of groundnut make it an excellent cash crop for domestic markets and for foreign trade in several developing and developed countries(Food Agricultural Organization, 2006). Low-yield constitutes a constraint to groundnut production often caused by impaired

photosynthetic assimilate partitioning on account of decreased photosynthetic activity (Fang *et al*., 2008). Carbonic anhydrase (CA; EC, 4.2.1.1) has been identified as an important enzyme that is closely associated with photosynthetic activity of plants. It is a Zn-containing metalloenzyme, which catalyzes the reversible hydration of CO_2 to HCO_3^- and H^+. This biochemical reaction plays a k ey physiological role in diverse biological systems (Xin *et al*., 2001; Kahan and Javid, 2004). However, light, CO_2 and Zn concentrations are essential for its catalytic activity (Silverman, and McKenna, 2007; Rupak*et al*., 2014).Albeit more prominent with carbonic anhydrase, the role of Zn in catalysis is also not negligible in more than 300 other enzymes, cutting the six classes of enzymes (McCall *et al*. 2000) Superoxide dismutase (EC 1.15.1.1), RNA polymerase (EC 2.7.7.6) and alcohol dehydrogenase (EC 1.1.1.1) are some other notable enzymes in plants where Zn plays essential roles (Castillo-González *et al*. 2018) Unlike light and CO_2 which are relatively available, Zn is a microelement that is only available for plant absorption in the form of Zn^{2+}. In addition, Zn^{2+} is required in critical concentrations for normal functioning of several key plant physiological pathways including photosynthesis, protein synthesis, growth regulation, fertility and seed productionin several oil seed crops (Pandey *et al*., 2006). Hence, the main objective of the present study was to evaluate effects of ZnO application on biochemical components, yieldand composition of seeds of *A. hypogaea* (cv SAMNUT 22).

2. Materials and Methods

2.1 Description of study site.

A pot experiment was conducted at the University of Ilorin Botanical Garden located in the Southern Guinea Savanna ecological zone of Nigeria (Latitude 8^0, 29'N and longitude 4^0, 35'E) between the months of May and August in 201 8. The area experiences an annual rainfall of 1000-1240 mm. The textural class of the experimental soil was loamy-sand with a pH of 6.5. The soil organic carbon was 0.24% while the total nitrogen, available phosphorus, potassium and magnesium were 0.14%, 4.23 $mgkg^{-1}$, 0.45 $mgkg^{-1}$ and 1.42 mg/kg respectively. The soil Zn content and the Cation Exchangeable Capacity were 17.8 mg/ kg and 7.43 $cmolkg^{-1}$ respectively.

2.1.1 Experimental layout and treatment details

The experimental layout was a co mpletely randomized design (CRD) with five treatments and five replications comprising twenty five polythene bags each having a dimension 0.4 m high and 0.3 m wide. The bags were filled with 4 kg of the loamy-sand soil. The soil was saturated to field capacity with water and thereafter perforated at the bottom to facilitate drainage. T he treatment details were 0.00 (control), 1.00 2.00, 3.00, and 4.00 $gL^{-1}$of ZnO which were applied by soil drenching technique.

2.1.2 Planting and crop management

Viable seeds of Samnut 22 groundnut obtained from the Institute of Agricultural Research, (IAR) Zaria, Nigeria were sown at the rate of two seeds per bag after pre-treating with seed dresser containing 20% imidacloprid, 20% metalaxyl- M and 2% Tebuconazole. The plants were thinned to one plant per polythene bag at 2 weeks after planting following seedling establishment. Weeding was done at intervals of two weeks throughout the experiment and 500 ml of water was used to irrigate the soil at intervals of two days to maintain normal crop growth.

2.1.3 Data collection

The total chlorophyll content was determined by the summation of chlorophyll a and chlorophyll b. Chlorophyll a and b content were determined by homogenizing 0.1 g leaf in 5 mL of 80% acetone using pestle and mortar, and allowed to stand overnight at room temperature. The absorbance of the supernatant was recorded at 645 and 663 nm with a UV/Visible light spectrophotometer (JENWAY 6300). The chlorophyll concentrations were calculated according to the following equation (Lichtenthaler and Wellburn, 1983).

$$\frac{\text{Mg chlorophyll a}}{\text{g tissue}} = \frac{12.7\ (A_{663}) - 2.69\ (A_{645})\ \text{x V}}{1000\ \text{x W}}$$

$$\frac{\text{Mg chlorophyll b}}{\text{g tissue}} = \frac{22.9\ (A_{645}) - 4.68\ (A_{663})\ \text{x V}}{1000\ \text{x W}}$$

Where;
A: Absorbance at specific wavelength
V: Final volume of chlorophyll extracted in 80% acetone
W: Fresh weight of tissue extracted
The carbonic anhydrase (CA) activities were assayed before and two weeks after anthesis on groundnut leaves. The leaves were homogenized in 0.1M potassium phosphate buffer solution at pH 8.3, 50 µl of the homogenate was immediately transferred in to an Eppendorf tube containing 500 µl of Tris-HCl buffer (pH 7.6) and 500 µl of an already standardized carbonated water. A stop watch was used to monitor the time for the required

colour change from blue to yellow in the solution. Thereafter, CA was calculated as df (Tb/Tc − 1) and activity was expressed as units /ml of enzyme, where;
df: dilution factor,
Tb: Time required for change in colour of blank,
Tc: Time required for change in colour of sample.

Tris-HCl buffer (pH 7.6) was prepared by dissolving 12.14 g of Trizma base in 50 m l of distilled water, and then the pH was adjusted to 7.6 with 4.2 ml of concentrated hydrochloric acid. Carbonated water was prepared by bubbling pure CO_2 through distilled water. The CO_2 concentration in the gas-saturated water was measured by back titration against 0.1 N $C_2H_2O_4$ solution after the addition of 0.1 N NaOH to the carbonic acid solution or a CO_2-free distilled water control. The titration end point was detected with bromothymol blue as the indicator. Based on the titration values, the time required for gas saturation was calculated and adopted for the experiments (Warrier*et al*., 2014).

Morphological growth parameters such as plant height, number of leaves, primary branches, stem girth and leaf areas (m^2) were measured at harvest. Reproductive attributes such as number of days at 50% flowering were also determined. Component yield such as numbers of matured pods per plant, pod weight per plant, number of seeds per plant and seed weight per plant (g) were determined at harvest. The proximate composition of the harvested seed samples such as moisture, ash, fibre, lipid, crude protein and carbohydrate were determined following the standard methods of Association of Official Analytical Chemists (AOAC, 2005).

The Zn uptake in seeds was determined by weighing 0.5 g of air-dried seeds into a digestion glass tube and mixture of concentrated HNO_3 and HCO_4 (10:4) was added. The digestion vessel was closed and heated in the microwave oven. The obtained solutions were allowed to cool at room temperature, and then were filtered by Whatman No.1 filter paper into a 25 mL volumetric flask. The filtrate was used for determination of Z n with the use of Atomic Absorption Spectrophotometer (AAS) (Shimadzu, Japan AA-6200) following the method of Jajda *et al.* (2013)

2.1.4 Data analysis

Collected data were subjected to One Way Analysis of Variance (ANOVA) using Statistical Package of Social Science (SPSS version 22.0). Means were separated using Duncan Multiple Range test at $p<0.05$ level of probability.

3. Results

The results showed that the chlorophyll content was significantly higher in the groundnut that received the highest concentration of ZnO (4.00 g/l) with a value of 2.18 ± 0.15 mg/gf.w This was closely followed by those of 3.00, 2.00 and 1.00 g/l of ZnO with respective values of 1.64 ± 0.23 1.17 ± 0.12, and 0.99 ± 0.03 mg/gf.w (Table 1). Before and after anthesis, carbonic anhydrase followed the same pattern as recorded for chlorophyll content. However, carbonic anhydrase activity was generally low before anthesis when compared to post-anthesis irrespective of the treatments (Table 1).

Groundnut grown under 4.00 $gL^{-1}$of ZnO showed significantly higher ($p<0.05$) morphological growth parameters such as plant height, number of leaves, number of primary branches and stem girth. This was followed in decreasing order of magnitude by those grown under 3.00, 2.00 and 1.0 gL^{-1} of ZnO. The control had the lowest values of all the mentioned growth attributes (Table 2). The results of the leaf area growth followed similar trends as recorded for growth attributes (Table 2).

The application of ZnO had no significant effect ($p< 0.05$) on the time taken for 50% of the plants to flower as shown in Table 3. Nonetheless, groundnut receiving zero application of ZnO and those treated with lower concentrations (1.00, 2.00 gL^{-1}) of ZnO reached their 50% flowering 1-2 days earlier compared to those treated with higher concentration (3.00,4.00 gL^{-1} of ZnO). Days to 50% flowering increased with higher treatments of ZnO (Table 3). Data pertaining to yield components such as number of pods, weight of pods, number of seeds and weight of seeds increased with higher concentration of ZnO. In such parameters, the control recorded the lowest values of yield components (Table 3). Groundnut plant receiving 4.00 gL^{-1} of ZnO had significantly higher pod weight per plant with respect to control and followed in decreasing order of magnitude by 3.00, 2.00 and 1.00 $gL^{-1}$of ZnO (Table 3).

Table1: Chlorophyll content and carbonic anhydrase activity of *Arachis hypogaea*(SAMNUTT22) as influenced by different concentration of ZnO.

Treatments (gL^{-1})	Total Chlorophyll (mg/gf.w)	Carbonic anhydrase activity (EUg^{-1})	
		Before flowering	After flowering
0.00	0.84 ± 0.09^{d}	1.16 ± 0.57^{a}	1.45 ± 0.57^{c}
1.00	$0.99 \pm 0.03c^{d}$	1.10 ± 0.62^{a}	5.38 ± 1.33^{b}
2.00	1.17 ± 0.12^{c}	1.15 ± 0.44^{a}	6.30 ± 0.71^{ab}
3.00	1.64 ± 0.23^{b}	1.31 ± 0.29^{a}	6.97 ± 0.79^{a}
4.00	2.18 ± 0.15^{a}	1.33 ± 0.67^{a}	7.18 ± 1.33^{a}
p-value	0.02	0.42	0.01

Values are Mean ± SEM, n=5: Values with same superscript (s) across the column are not significant at $p<0.05$

Table 2: Growth parameters of *Arachis hypogaea* (Samnut 22) at harvest as influenced by different concentrations of ZnO

Treatments (gL^{-1})	Plant height (cm)	Number of primary branches	Stem girth (cm)	Leaf area (m^2)
0.00	41 ± 0.50^{d}	23 ± 0.50^{e}	0.61 ± 0.50^{d}	4.20 ± 0.50^{d}
1.00	48 ± 0.50^{d}	24 ± 0.50^{d}	0.71 ± 0.50^{c}	5.10 ± 0.50^{c}
2.00	52 ± 0.50^{c}	28 ± 0.50^{c}	0.75 ± 0.50^{b}	6.80 ± 0.50^{bc}
3.00	58 ± 0.50^{b}	32 ± 0.50^{b}	0.80 ± 0.50^{a}	7.60 ± 0.50^{b}
4.00	61 ± 0.50^{a}	34 ± 0.50^{a}	0.81 ± 0.50^{a}	8.50 ± 0.50^{a}
p-value	0.01	<0.001	0.02	0.04

Values are Mean ± SEM, n=5: Values with same superscript(s) along the column are not significant at $p<0.05$;

Table 3: Days to 50% flowering and yield components in *Arachis hypogaea* (Samnut 22) as influenced by different concentration of ZnO

Treatments (gL^{-1})	50% flowering	Number of pods per plant	Pod weight per plant (g)	Number of seeds per plant	Seed weight per plant (g)
0.00	47.40 ± 3.89^{a}	4.30 ± 1.49^{a}	2.21 ± 0.82^{b}	6.50 ± 2.37^{a}	1.43 ± 0.24^{a}
1.00	47.60 ± 4.33^{a}	4.50 ± 1.27^{a}	2.24 ± 0.50^{ab}	6.60 ± 2.07^{a}	1.50 ± 0.25^{a}
2.00	48.20 ± 4.42^{a}	4.70 ± 2.31^{a}	2.33 ± 0.43^{ab}	6.90 ± 1.91^{a}	1.52 ± 0.26^{a}
3.00	49.10 ± 4.38^{a}	5.10 ± 1.52^{a}	2.58 ± 0.51^{ab}	7.50 ± 2.01^{a}	1.52 ± 0.26^{a}
4.00	50.60 ± 2.84^{a}	5.80 ± 2.94^{a}	2.78 ± 0.46^{a}	7.80 ± 1.99^{a}	1.61 ± 0.20^{a}
p-value	0.39	0.40	0.01	0.3	0.20

Values are Mean ± SEM, n=5: Values with same superscript along the column are not significant at $p<0.05$; T_0 = control, T_1=1 g/dm^3 T_2 =2 g/dm^3, T_3=3 g/dm^3, T_4 =4 g/dm^3

Percentage moisture, lipid, ash, protein, fibre and carbohydrate ranged between 3.99-4.79%, 38.11-40.00%, 2.02-2.96%, 37.83-38.89%, 2.40-2.97%, 12.38-14.20% respectively (Table 4). Application of ZnO influenced all the proximate compositions significantly except moisture (Table 4). ZnO application showed a pronounced effect on percentage ash, protein and carbohydrate as compared to the control (Table 4). The aforementioned parameters were higher in those groundnut grown under different concentrations of ZnO when compared to those in the control. Statistical differences were not recorded for ash and protein values in groundnut receiving 2.00 gL^{-1} and 3.00 gL^{-1} ZnO. The recorded values for protein and ash from the aforementioned treatments were however significantly higher in groundnut receiving the lowest treatment (1.00 gL^{-1}) of ZnO and highest concentration of ZnO (4.00 gL^{-1}) as shown in Table 4. The lipid content was higher in seeds harvested from the control compared to groundnut grown under ZnO application. Application of ZnO reduced the fibre content of the seeds when compared with the control (Table 4).

Results of Zn uptake in seeds as influenced by different concentration of ZnO showed that there was a significant difference between the treatments and the control. Zn uptake was generally low in the control and groundnut receiving low concentrations of ZnO as depicted in Table 5.

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Table 4: Proximate composition of seeds of groundnut as influenced by different concentration of ZnO

Treatments (gL^{-1})	Moisture	Lipid	Ash	Protein	Fibre	Carbohydrate
			(%)			
0.00	4.79 ± 0.01^{a}	40.01 ± 0.01^{a}	2.02 ± 0.03^{c}	37.83 ± 0.06^{c}	2.97 ± 0.04^{a}	12.38 ± 0.06^{c}
1.00	4.61 ± 0.21^{a}	38.11 ± 0.15^{d}	2.39 ± 0.01^{bc}	38.18 ± 0.06^{b}	2.40 ± 0.08^{c}	14.20 ± 0.10^{a}
2.00	4.25 ± 0.22^{a}	39.01 ± 0.01^{bc}	2.96 ± 0.56^{a}	38.78 ± 0.15^{a}	2.63 ± 0.07^{b}	12.49 ± 0.67^{c}
3.00	3.99 ± 0.87^{a}	38.76 ± 0.28^{c}	2.57 ± 0.05^{a}	38.89 ± 0.01^{a}	2.70 ± 0.01^{b}	12.79 ± 0.53^{bc}
4.00	4.60 ± 0.20^{a}	39.09 ± 0.06^{b}	2.09 ± 0.07^{bc}	38.18 ± 0.16^{b}	2.71 ± 0.01^{b}	13.38 ± 0.06^{b}
p-value	0.21	<0.001	0.007	<0.001	<0.001	0.001

Values are Mean ± SEM, n=5: Values with same superscript (s) along the column are not significant at $p<0.05$.

Table 5: Zn uptake in the harvested seeds of *Arachis hypogaea* (Samnutt22) as influenced by different concentration of ZnO

Treatment (gL^{-1})	Zn uptake(mgg^{-1})
0.00	0.13 ± 0.03^{d}
1.00	0.15 ± 0.02^{c}
2.00	0.19 ± 0.03^{b}
3.00	0.21 ± 0.01^{a}
4.00	0.19 ± 0.01^{b}
p-value	0.001

Values are Mean ± SEM, n=5: values with same superscript (s) along the column are not significant at $p<0.05$

4. Discussion

Biochemical attributes such as chlorophyll content of leaves and carbonic anhydrase activity were enhanced in groundnut receiving Zn applications compared to the control. The enhanced biochemical attributes could be due to the role of Zn as the co-factor for carbonic anhydrase enzyme, which is intricately linked with photosynthesis. Evidence in support of this was reflected in the enhancement of growth attributes in groundnut plants receiving various concentrations of Zn in the form of its oxide. Similar findings, namely increase in chlorophyll content of leaves upon application of Zn oxide in groundnut had been reported by Prasad *et al.* (2012). Amin *et al.* (2014) reported increase in carbonic anhydrase activity with higher Zn supply in sweet corn. The enhancement of growth attributes were concentration-dependent upon Zn oxide application and were comparable with studies from the past (Sharma *et al*., 2010; Fathi*et al*., 2012; Sonkar *et al*., 2012; Yasari and Vahedi, 2012).

A reproductive character such as time taken for 50% of flowering in plants was not significantly influenced by rates of ZnO application compared to the control. In terms of yield components only pod weight per plant was statistically higher in plants receiving ZnO application than the control. However, all the other yield components were found to be greater in ZnO applied plants when compared to the control. The slight increase in yield components with increasing levels of ZnO could be due ability of Zn to assist in formation of stamen and pollen which ultimately translated to yield. Slaton *et al*. (2001) also reported that treating rice with increasing concentration of Zn significantly increased grain yield.

The moisture values recorded in this work were low and in consonance with the results obtained from other groundnut cultivars which ranged from 6.6-8.9% (Musa *et al*., 2010). The difference could depend on the number of days used to dry the seeds (Olayinka and Etejere, 2013). In this study, application of ZnO significantly enhanced ash, protein and carbohydrate of the harvested seeds compared to the control. Zn is known to be an essential micronutrient for growth, development and health of plants (Fageria*et al*., 2002). It also enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen that is responsible for higher protein content as well as ash (Fagaria, *et al*., 2002). Zn plays a vital role in carbohydrate and proteins metabolism as it f orms an essential component of dehydrogenase, protease, and peptidase enzymes needed for these processes (Fagaria, *et al*., 2002). Similarly, Fang *et al*. (2008) had shown that Zn is an essential micronutrient for higher plants, especially oil crops where it is required for the activity of various types of enzymes (dehydrogenases, RNA and DNA polymerases), carbohydrate metabolism and protein synthesis. Zn has been known not to play a significant role in lipid metabolism. Therefore, the decrease in lipid content of seeds when ZnO was

applied is not unusual. Similarly, ZnO could not promote the fibre contents in the harvested seeds.
The Zn uptake in the seeds could be low when compared to the values reported for twenty varieties of groundnut grown in Ghana with mean values of 5.2 g/100g. However, application of ZnO at higher concentration tends to favour greater uptake of Zn. The amount of Zn present in the seeds at different concentrations is nutritionally significant and low. This is due to the small quantities that are needed by the human body (Asibuo, *et al*., 2008).

5. Conclusions

The study had shown the role of Zn in promoting growth and activity of carbonic anhydrase enzyme in groundnut. It also indicated that the applied concentrations of this trace element promoted an increase in ash, protein and carbohydrate contents but limited the fibre and lipid contents of the harvested seeds.

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