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Influence of Npk Inorganic Fertilizer Treatment on the Proximate Composition of the Leaves of *Ocimum gratissimum* (L.) and *Gongronema latifolium* (Benth)

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Abstract: The influence of NPK inorganic fertilizer treatment on the proximate composition of the leaves of *Ocimum gratissimum* (L.) and *Gongronema latifolium* (Benth) was investigated. Cultivated *O. gratissimum* and *G. latifolium* were treated with NPK (15:15:15) fertilizer at 100, 200, 300, 400 and 500 kg h^{-1} treatment levels in planting buckets derived using the furrow slice method two months after seedling emergence. No fertilizer treatment served as control. The leaves of the plants were harvested for analysis one month after treatment. The leaf was used for the analysis because it the most eaten part. Fertilizer treatment significantly ($p<0.05$) increased the dry matter, moisture content, ash, crude protein, crude fibre, crude fat contents of the leaves of both plants. On the other hand, fertilizer treatment significantly, ($p<0.05$) decreased the carbohydrate and the calorific value of the leaves of the plants. The increase in the concentrations of these substances as a result of fertilizer of fertilizer treatment might be due to the role of fertilizer in chlorophyll content of plant's leaves, which in turn enhanced the process of photosynthesis leading to increased synthesis of these substances. The decrease in the carbohydrate content might be due to its conversion to other materials in the plants. The results obtained were discussed in line with current literatures.

Key words: Proximate composition, fertilizer treatment, *Ocimum gratissimum*, *Gongronema latifolium*

INTRODUCTION

Plants have been known to play important role as source of food and maintenance of good health since ages in the world. Research reports indicate that plants play vital roles in the maintenance of good health (Burkill, 1995, 1997; Moerman, 1996). In Nigeria and most other African Countries, indigenous people traditionally use a wide range of plants as food and medicine. These plants constitute great reservoir of a wide variety of compounds which exhibit some medicinal and nutritive properties, thus are used as spices, food or medicine (Edeoga *et al.*, 2003; Osuagwu and Nwosu, 2006; Osuagwu *et al.*, 2007). Investigations have been made on the proximate, vitamin and mineral content of some Nigerian vegetables and have been found to rich sources of carbohydrates, proteins, ash, crude fibre, crude fats, food energy, vitamins and minerals (Aletor and Adeogun, 1995; Aletor *et al.*, 2002; Matasyoh *et al.*, 2007; Osuagwu, 2008).

Carbohydrate provide source of energy for the cells and tissues, thus is the major source of fuel for the body (Insel *et al.*, 2007; Ward-Law and Kessel, 2002). Fats also form major source of energy for the body, provide structure for body cells, transport fat soluble vitamins and provide starting materials such as cholesterol for making hormones in the (Whitney and Roifes, 2005; Insel *et al.*, 2007). Proteins build and maintain the body, form structure of the body, regulate body process and used as source of energy (Ward-Law and Kessel, 2002, Insel *et al.*, 2007). Water is used to control the temperature of the body, lubricates joints and transport nutrients and wastes (Whitney and Roifes, 2005; Insel *et al.*, 2007). Fibers lowers the total and LDL cholesterol levels in the body, thereby reducing the risk of heart failure, it also regulates blood sugar, thus reduce the symptoms of metabolic syndromes and diabetes (Salvin, 2008; Spiller *et al.*, 2001). Fibres also speed the passage of food through the digestive system, hence facilitates regular defecation. Furthermore, they add bulk to the stool thereby alleviating constipation and they balance intestinal pH and stimulate intestinal fermentation, thus reducing the risks of colorectal cancer (Drummond and Brefere, 2007; Fotiadis *et al.*, 2008).

The concentration of chemical constituents of plants is actually determined by the nature and amount of nutrients available in the soil in which the plants are growing, thus fertilizer treatment affects the proximate content of plants.

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Increased proximate content of plants has been reported by researchers: Carbohydrates (Khafaga *et al*., 2000; Manyawu *et al*., 2003), moisture content (Elsheikh and Elzidany, 1997; Myers, 1998), dry matter (Bolovic *et al*., 2005) ash (Iqbal *et al*., 1998; Tolera *et al*., 2006), crude proteins (Khan *et al*., 1999; Jacobs *et al*., 2002; Springer *et al*., 2005) crude fibre (Iqbal *et al*., 1998; Akanbi *et al*., 2007), crude fat (Elsheikh and Elzidany, 1997; Khafaga *et al*., 2000; Akanbi *et al*., 2007). However, on the other hand, fertilizer treatment decreased the proximate content of plant parts: Carbohydrates (Fife and Nambiar, 1997; Jacobs *et al*., 2002; Hendawy, 2008), dry matter (Khan *et al*., 1999), crude fibre (Eppendorfer and Eggum, 1996; Akanbi *et al*., 2007), crude fat (Peoples *et al*., 1994).

Ocimum gratissimum a dicotyledonous shrub plant belongs to lamiaceae family. It is popularly used in herbal medicine for the treatment of diseases such as upper respiratory tract infection, diarrhea, pile, cough, fever, pneumonia, surface wound, gonorrhea, worm infection and stomach ache (Burkill, 1995; Iwalokun *et al*., 2003; Makker *et al*., 2007). The leaves are used to reduce blood glucose levels (Owoyele *et al*., 2005; Mohammed *et al*., 2007). It plays important role in blood coagulation and renal function (Edemeka and Ogwu, 2001; Anigbogu and Uzoaga, 2006). Its leaves are used to prepare soups and porridge for women after delivery among the Igbo's of Nigeria (Ijeh *et al*., 2004). The leaves are also used as spices for preparation of food (Burkill, 1995; Ijeh *et al*., 2004).

Gongronema latifolium belongs to the Asclepiadaceae family. It is a slender climber often 3-4 m long. Leaves are simple with blade that is ovate with cordate base and acuminate apex. The margin of the leaf is entire with petiole that is up to 4 cm long (Grubben and Denton, 2004). Its use in the treatment and cure of cough, loss of appetite, diabetes, malaria, improved liver function and asthma have been reported (Burkill, 1985; Morebise *et al*., 2002; Nwanjo and Alumanah, 2006; Nwosu and Malize, 2006; Okeke and Elekwa, 2006; Oshinubi *et al*., 2006; Ugochukwu *et al*., 2003). The leaves and seeds are used as spices or condiments in the diet of nursing mothers and they are also used raw as salad and to flavour meat preparations zand fresh fish pepper soup (Nwosu and Malize, 2006).

This research work investigated the influence of inorganic fertilizer (NPK 15:15:15) on the proximate content of the leaves of *O. gratissimum* and *G. latifolium* and to determine if fertilizer treatment will increase or decrease the levels of the proximate compounds in the leaves of the plants in view of their nutritive and health values.

MATERIALS AND METHODS

Plant samples: The seeds of *Ocimum gratissimum* were collected from a home stead garden in Amaogwu village, Bende town, Bende Local Government Area of Abia State. The fresh and succulent stem cuttings of *G. latifolium* were obtained from the forest strip of the Forestry Department, College of Natural and Environmental Management, Michael Okpara University of Agriculture, Umudike Umuahia Abia State, Nigeria. Both plant materials were identified by the taxonomic unit of the Botany section of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike Umuahia, Abia State, Nigeria. The seeds of *O. gratissimum* were raised into seedlings in nursery boxes before they were transplanted into planting buckets. Stem cuttings of *G. latifolium* were planted directly into the planting buckets.

Cultivation of the plants was carried out using 48 plastics planting buckets containing 8 kg of sterilized soil.

The soil used for the study was earlier on analysed to determine its physiochemical properties (Table 1). Treatments were carried out in four replicates of each treatment. The inorganic fertilizer (NPK 15:15:15) used for the store of the Abia State Ministry of Agriculture Umuahia, Abia State. Five levels of fertilizer treatments 100, 200, 300, 400 and 500 kg h^{-1} derived using the furrow slice method (Brady and Weil, 1999) in four replicates used. No fertilizer treatment served as control. Treatment occurred two months after seedling emergence. Harvesting of plant leaves for proximate analysis occurred one month after treatment. The research was carried out in the Green House of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike Umuahia, Abia State, Nigeria.

Table 1: Physical properties of soil

Particle size distribution	Values
Sand (%)	70.90
Silt (%)	15.40
Clay (%)	13.70
Texture (L)	5.00
pH (H_2O)	5.01
Organic carbon (%)	0.75
Organic matter (%)	1.29
Available phosphorus (mg kg^{-1})	46.00
Total nitrogen (%)	0.08
Exchangeable bases (mg 100 g^{-1})	
Ca^{++}	2.40
Mg^{++}	2.00
K^{+}	0.07
Na^{+}	0.23
Exchangeable acidity (Me/100 g)	1.20
Effective cation exchange capacity (Me/100 g)	5.90

Preparation of leaf sample for analysis: The harvested leaves were oven dried using the Selecta Model 150-900 L ovens at 65°C for 24 h and ground into powder using Thomas Willey Milling machine. The leaves were used for analysis because they are the part normally used in preparing meals. Powdered samples were stored in sample bottles and kept in a dry place to be used for analysis.

Determination of carbohydrate content: The percentage carbohydrate was determined using Muller and Tobin (1980).

The total carbohydrate was estimated as the balance after accounting for ash, crude fiber, protein and fats.

Carbohydrate (%) = 100-(% crude protein + % crude fibre+% ash+% crude fat).

Determination of moisture and dry matter content: The percentage moisture and dry matter content were determined using the method of AOAC (1990).

An empty dried moisture can was weighed using a sensitive balance. Two grams of the test sample was placed in the moisture can and dried in the oven for 8 h. The sample was then placed in desiccator to cool and weighed with a sensitive balance. The sample was then returned to the oven for some time. The process of drying, cooling and weighing continued till a constant weight was obtained. The moisture content was calculated and expressed as percentage moisture content:

$$\text{Percentage moisture content} = \frac{\text{Weight of moisture}}{\text{Weight of sample 1}} \times 100$$

where, the percentage dry matter content was determined by subtracting the percentage moisture content from that of the sample.

Determination of crude fibre and ash content: The percentage crude fibre and ash content was determined using AOAC (1990) method.

Two grams of the sample was defatted using petroleum ether and transferred into a beaker. Hundred mL of 1.25% sulphuric acid was added and hydrolyzed for about 30 min, filtered and washed with 100 mL of boiled water. The sample was transferred and 100 mL of 1.25% sodium hydroxide was added with boiled distilled water. The residue again was recovered into a crucible, dried in the oven and weighed. This was transferred into a muffle furnace and ignited at 500°C to ash. The weight of the fibre was determined by the difference between the weight of the hydrolyzed sample and the weight of the ash. The weight of the ash was determined by subtracting the weight of the crucible from the weight of the crucible and the ash. The weight obtained was expressed in percentage.

Determination of crude fat content: The crude fat content of the leaves was determined using the ether extraction method by the Reflux Soxhlet extraction method described by AOAC (1990).

Two grams of each sample was weighed and placed in the thimble of the soxhlet apparatus. A 500 mL round bottom flask was weighed while empty and filled with about 300 mL of petroleum ether and fixed in the reflux set up and placed on a heating mantle for the extraction of fat. The extraction continued until all the fats in the sample were washed away into the solution. The thimble was removed from the apparatus and the ether recovered from the solution. The fat was finally dried up in the oven at 80°C for 20 min and placed in the dessicator to cool. The weight of the fat and the flask were taken. The difference between the weight of the flask and that of the empty flask is the weight of the fat. The result obtained was expressed in percentage:

$$\text{Crude} = \frac{\text{Weight of fat}}{\text{Weight of sample1}} \times 100$$

Determination of crude protein: The crude protein content of the leaves was determined using the Kjedahl method described by AOAC (1990).

A 0.2 g of the sample was digested and made up to 50 mL of flask. Ten milli liter of the digested sampled was distilled and titrated with 0.2 m sulphuric acid for the nitrogen determination:

$$\text{Crude protein (\%)} = \text{Total nitrogen} \times 6.25$$

Determination of food energy value content: The food energy value content of the plants leaves was estimated using the method of Osborne and Voogt (1978).

The food energy value in grams per calories was derived using the equation below:

$$\text{FEV} = (\%\ \text{CP}\times 4) + (\%\ \text{Lipid}\times 9) + (\%\text{CHO}\times 4)$$

Where:

FEV = Food energy value (grams per calories)
CP = Crude protein
CHO = Carbohydrate

Statistical analysis: The design of the research was complete randomized design in four replicates of each treatment. Analysis of variance (ANOVA) was used to analyse the data and LSD at the 0.05 probability level was used to determine the differences among treatment levels.

RESULTS AND DISCUSSION

Fertilizer treatment significantly affected the proximate composition of the leaves of *Ocimum gratissimum* and *Gongronema latifolium* (Table 2, 3).

Treatment with inorganic fertilizer significantly ($p<0.05$) decreased the carbohydrate content of the leaves of *O. gratissimum* and *G. latifolium* (Table 2). Reduction of carbohydrate due to fertilizer treatment has been reported (Jacobs *et al*., 2002; Hendawy, 2008). This observed decrease in the total carbohydrate content is due to its conversion to other substances. Hendawy, 2008, suggested that fertilizer treatment led to the conversion of carbohydrate to mucilage in *Plantago arengna*. On the other hand, research reports showed that fertilizer treatment caused increased carbohydrate content in plants (Khafaga *et al*., 2000; Manyawu *et al*., 2003).

Fertilizer treatment led to significant ($p<0.05$) increase in the levels of the moisture content, dry matter, ash, crude protein, crude fibre and crude fats in the leaves of both plants used for the study (Table 2, 3). This observed increase in the concentration of these substances in the leaves of the plants might be as a result of increased nutrients in the soil due to fertilizer application, which consequently led to increased absorption of nutrients by the plants. The properties of the soil are known to be affected by NPK fertilizer application. Yagodin (1984), reported that NPK fertilizer had predominant effect on the chemical nature and mineral composition of the soil. The hydrogen of the carboxyl groups in the soil are replaced by the various cations to yield salts known as humates which the plants absorb. The humates of univalent cations (Na^+, K^+, NH^+) are water soluble compounds which are made available to the plants (Yagodin 1984; Okwu, 1999). The NPK fertilizer is converted to soluble nitrates, phosphates, sulphates or chloride which are absorbed by plants. Furthermore, fertilizer increased the supply of nitrogen to the plants, which in turn increased the number of leaves and photosynthetic surface, hence increasing photosynthetic activities and enhancing physiological processes leading to production of more assimilates which significantly increased the chemical composition of plants (Alabi, 2006; Amujoyegbe *et al*., 2007; Lawal and Rahman, 2007). Increased concentration of these proximate compounds as a result of fertilizer application was earlier on observed (Myers, 1998; Amujoyegbe *et al*., 2007; Tolera *et al*., 2006; Springer *et al*., 2005; Akanbi *et al*., 2007).

There was a general increase in the concentration of the proximate compounds in the leaves of the plants studied with increase in the level of fertilizer treatment.

The calorific value content of the leaves of the plants studied was affected by fertilizer treatment. Fertilizer treatment significantly at ($p<0.05$), caused reduction in the calorific value of the leaves of *O. gratissimum* and led to significant increase ($p<0.05$)

Table 2: Effect of NPK (15:15:15) fertilizer treatment on the percentage carbohydrate, moisture content, dry matter and ash content of the leaves of *Ocimum gratissimum* and *Gongronema latifolium*

	Ocimum gratissimum				*Gongronema latifolium*			
Treatment	CHO	MC	DM	ASH	CHO	MC	DM	ASH
Control	64.330^{d}	5.925^{c}	94.060^{f}	8.120^{b}	70.090^{b}	6.140^{e}	93.860^{b}	11.440^{c}
100 (kg ha^{-1})	62.380^{f}	6.340^{d}	93.060^{a}	9.400^{e}	69.470^{b}	7.800^{f}	92.200^{a}	10.593^{a}
200 (kg ha^{-1})	61.825^{bc}	5.880^{b}	94.123^{d}	7.200	65.130^{a}	5.406^{b}	94.593^{e}	13.850^{f}
300 (kg ha^{-1})	60.780^{a}	5.900^{bc}	94.100^{f}	10.110^{f}	64.330^{a}	5.200^{a}	94.800^{f}	10.850^{b}
400 (kg ha^{-1})	63.780^{d}	5.840^{a}	94.160^{f}	8.260^{f}	64.160^{a}	6.040^{d}	93.960^{f}	12.600^{d}
500 (kg ha^{-1})	61.140^{ab}	6.360^{d}	93.618^{b}	9.040^{d}	65.010^{a}	5.940^{c}	94.060^{d}	12.850^{e}
LSD (<0.05)	2.072	0.078	0.077	0.264	2.219	0.054	0.090	0.461

CHO: Carbohydrate, MC: Moisture content, DM: Dry matter, Values with different letters significantly different at $p<0.05$

Table 3: Effect of NPK (15:15:15) fertilizer treatment on the percentage crude fibre, crude protein, crude fat content and the calorific value (mg cal^{-1}) of the leaves of *O. gratissimum* and *G.* latifolium.

	Ocimum gratissimum (mg cal^{-1})				*Gongronema latifolium* (mg cal^{-1})			
Treatment	CF	CP	CFA	CV	CF	CP	CFA	CV
Control	7.880^{a}	15.630^{f}	4.040^{f}	356.450^{e}	6.840^{b}	9.190^{a}	2.440^{f}	339.055^{bc}
100 (kg ha^{-1})	13.400^{f}	16.940^{e}	3.880^{b}	312.055^{a}	6.440^{a}	10.500^{b}	2.840^{d}	345.440^{c}
200 (kg ha^{-1})	12.660^{e}	17.500^{f}	3.840^{b}	351.760^{e}	7.080^{c}	10.960^{f}	2.993^{e}	337.330^{bc}
300 (kg ha^{-1})	11.890^{d}	14..000^{a}	3.360^{a}	326.640^{b}	9.420^{e}	11.380^{d}	4.020^{f}	339.045^{bc}
400 (kg ha^{-1})	9.440^{b}	14.440^{b}	3.880^{b}	347.500^{cd}	10.600^{c}	10.500^{b}	2.140^{a}	317.900^{a}
500 (kg ha^{-1})	9.680^{f}	16.060^{d}	4.080^{f}	345.500^{c}	7.640^{d}	12.250^{e}	2.250^{b}	329.570^{b}
LSD (<0.05)	0.225	0.229	0.242	10.676	0.258	0.229	0.195	21.283

CF: Crude fibre, CP: Crude protein, CFA: Crude fat, CV: Calorific value, Values with different letters significantly different at $p<0.05$

in that of the leaves of *G. latifolium* (Table 3). This observed difference in the calorific values of the leaves of the two plants in response to fertilizer treatment can be attributed to differences exhibited by different plant species to the same environmental condition.

It could be concluded from the finding of this research that the application of inorganic fertilizer (NPK 15:15:15) in the appropriate quantity will lead to increase in the concentration of almost all the proximate compounds in the leaves of *O. gratissimum* and *G. latifolium* which in turn enhances their nutritive and health value.

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