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Vitamin and Mineral Level Analysis in Fluted Pumpkin (*Telfairia occidentalis* Hook.F) Leaves As Influenced by Physical Mutagens

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Abstract:
The nutritious and economic value of fluted pumpkin (Telfairia occidentalis Hook. F) Leaf cannot be overemphasized. Countless queries have stemmed this research study which was carried out at the National Soil Science Laboratory Research Farm of Federal Ministry of Agriculture and Rural Development Umuahia, Abia State to investigate the influence of physical mutagens, (x-ray irradiation) on the vitamin and mineral levels in fluted pumpkin leaf in M_1 and M_2 generations. The treatments include 0.00 mGy, 6.75 mGy, 10.08 mGy, 14.08 mGy and 18.75mGy. The experiment was set up in a randomized complete block design (RCBD) with five replications. Treated seeds were planted, data obtained in both generations revealed that the level of minerals such as calcium, phosphorus and nitrogen decreased significantly ($P<0.05$) as the x-ray treatment dose increased. The level of iron however increased significantly ($P<0.05$) at 18.75mGy treatment in the M_1 generation. Vitamins such as niacin and ascorbic acid reduced significantly ($P<0.05$) in level as the treatment dose increased in both generations. At 18.75mGy treatment, an increase above all treatments was obtained in thiamin, though not significant ($P>0.05$). A significant increase ($P<0.05$) in riboflavin level was recorded in M_2 generation at 10.08mGy. The variations in mineral and vitamin contents of the treated pumpkin leaves are reliable indication of the effects of physical mutagens.
Keywords— *Mutation: Mineral: Telfairia occidentalis: Vitamins: X-ray irradiation*

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I. Introduction

The remodelling of the DNA component of an organism using physical mutagens like x-ray irradiation have proved useful in the creation of desirable mutants with profiting traits after vigorous selection and breeding to homozygosity.

X-rays have been used to induce desirable agronomic traits such as nutritional attributes, disease resistance, yields, and height (Iwo *et al.*, 2013) in plants. It allows for the induction of the desired characteristics that are either not exhibited naturally or lost during the evolutionary passage. Akpaniwo *et al*. (2015) reported that the seed of fluted pumpkin when exposed to high doses of X-rays reduced germination and survival rates of pumpkin. Also, (Al-Enezi *et al.* 2012) revealed significant retardation in seed germination and survival rate in date palm. Hameed *et al*. (2008) and Al-Salhi *et al*. (2004) has found that high exposure to gamma rays disrupt protein synthesis, leaf gas exchange, hormonal balance, and activities of enzymes in seeds. The process of Induced mutation has long been used to improve cultivars, and it has proven to be a powerful tool for expanding the resources in genetics, particularly for odd plants, and for selecting mutants with favorable agronomic features (Taheri *et al*., 2014). Induced mutation of about 170 different plant species have given rise to above 2700 improved varieties (Luo *et al.*, 2013).

Fluted pumpkin (*T. occidentalis*), a perennial plant with coiled tendrils and bifids that enables it in climbing belongs to the Cucurbitaceae family. In different countries and languages, *T. occidentalis* is known as follows: Telfairia nut, fluted gourd, fluted pumpkin, oil nut, and oyster nut, (English); Oroko, pondokoko and Gonugbe (Sierra Leone); Krobonko (Ghana); Costillada (Spanish); Ugwu (Igbo-Nigeria), Ikong (Efik/Ibibio-Nigeria) and Aworoko, Eweroko (Yoruba-Nigeria) (Gbile, 1984).

T. occidentalis is a vegetable crop consumed mainly for its high nutritional and medicinal value mainly: High in oil content (30%) (Akoroda, 1990). The Shoots of *T. occidentalis* contain a high amount of iron and potassium, while the seeds are composed of 27% and 53% crude proteins and fats respectively (Aiyelaagbe *et al*., 2002). There is high concentration of antioxidants on the leaves (Nwanna *et al*., 2008). The seeds are recorded to contain high level of fat and protein. The leaves possess high level of carbohydrates, iron, copper, potassium, and manganese (Idris, 2012). Its medicinal benefits include: anti-microbial functions according to Oyewole *et al*., (2012), anti-diabetic functions (Stanaway *et al.*, 2000), treatment of infertility (Akang *et al.*, 2010), hematological and anti-malaria functions (Okokon *et al*., 2009).

All these have led to its increase in demand with less variability, availability and supply.

To close this gap, mutation breading which is a source of increasing variability and improvement without significantly altering acceptable phenotype is needed to supplement conventional breeding (Ojomo *et.al.,* 1979).

In this research, five different x-ray doses (0.00 mGy, 6.75 mGy, 10.08 mGy, 14.08 mGy and 18.75mGy) were used to treat five different sets of fluted pumpkin by exposure to x-ray irradiation.

The objective of this research was to establish the influence of x-ray irradiation on the level of vitamin and mineral components of fluted pumpkin leaves and also to determine the x-ray dose that can induce an increase in the level of vitamins and minerals components.

II. Material And Methods

The research was carried out at the National Soil Science Laboratory Research Farm of Federal Ministry of Agriculture and Rural Development Umuahia, Abia State Nigeria. The site is located within longitude 07°33E and latitude 05°29N with altitude of 122m above sea level. Umuahia is a humid tropic with total average rainfall of about 286mm/annum and classified as sandy loam ultisol (Agboola, 1979).

Source of planting material

The fluted pumpkin pods were obtained from the National Agricultural Seed Council of National Root Crop Research Institute Umudike. The pods were cut open and the fluted pumpkin seeds extracted.

Treatments

Treatment of the fluted pumpkin seeds with different doses of X-ray irradiation were carried out at Mecure Diagnostic Center of Abia State Specialist Hospital, Umuahia. Five samples of twenty seeds each were treated with different doses of X-ray irradiation (0.00 mGy, 6.75 mGy, 10.80 mGy, 14.80 mGy and 18.75 mGy) respectively using a therapeutic medical X-rays device (Clinac 23EX Linear Accelerator, Varian Medical Systems, USA).

Agronomic practices

The field for planting was properly cleared, ploughed and harrowed to fine tilt, beds were made and planting was done on March 20^{th} 2018 to raise the M_1 generation and March 1^{st} 2019 to raise the M_2 generation. The experiment was designed in a randomized complete block design with five replications. The plot size was 2m x 2 m with 0.5 m apart. The planting distance was 120 cm x 60 cm with 2 seeds sown per hole and later thinned to one. All cultural practices such as staking, weeding and fertilizer application were carried out two weeks and four weeks after planting respectively. Six (6) weeks after planting, some portion of the fresh leaves were harvested from all treatment and analyzed for mineral and vitamin levels while some were left to grow to full maturity and seeds were harvested and used to raise the M_2 generation.

Mineral Analysis

This was carried out at O-J Laboratories Umudike, Abia state. The mineral content of the tests samples was determined by the dry ash extraction method following each specific mineral element. A 2.0g of the sample was burnt to ashes in a muffle (as in ash determination). The resulting ash was diluted to 100ml in a volumetric flask using distilled water. The digest so obtained was used for the various analysis below.

- Calcium determination

Calcium was determined by complexiometric titration according to the versanate EDTA titrimetric method of AOAC (2005). From the titre values obtained, the Ca^{2+} content were calculated as shown below:

$$Ca\ \ mg = \underline{100} \times T - B\ (N \times Ca) \times \frac{\underline{Vt}}{WVa}$$

Where W= weight of sample
T=Titre value of sample
B= Titre value of Blank
Ca= Calcium equivalence
N=Normality of titrant (0.02N EDTA)
Va= Volume of extract analyzer
Vt= Total extract volume

- Phosphorus Determination

Phosphorus in the test sample was determined by the Vanadomobydate spectrophotometery method (James 1995).
The phosphorus content was calculated using the formular below:

$$P\,(mg/100g) = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where:
W=weight of sample ash
Au= absorbance of test sample
As= absorbance of standard phosphorus solution
C= concentration of standard phosphorus solution
Vt= total extract volume
Va= volume of extract analyzer.

- Determination of Total Nitrogen (Kjeldahl method)

% Nitrogen was calculated as follows:
(ml std A x NA)-(ml blank x NB)]-(ml std B x NB)x100
1.4007 Weight of sample in grams
Where:
NA=normality of acid
NB= normalty of base
A= acid
B= base
A= Acid

- Iron Determination

This was done following the Orthophenantroline method.

Vitamin Analysis
This was carried out at O-J Laboratories Umudike, Abia state.
Data were generated for vitamin analysis on the different treatment based on the following:

I**.** Thiamin (Vit. B_1)
This was calculated according to Okwu and Ndu (2006).

$$\text{Thiamine (mg/100g)} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where: *W*=weight of sample ash
Au= absorbance of test sample
As= absorbance of standard thiamine solution
C= concentration of standard thiamine solution
Vt= total extract volume
Va= volume of extract analyzer.

III. Riboflavin (Vit B_2).
This was calculated according to Okwu and Ndu (2006).

$$\text{Riboflavin mg/100g} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where:
W=weight of sample ash
Au= absorbance of test sample
As= absorbance of standard phosphorus solution
C= concentration of standard phosphorus solution
Vt= total extract volume
Va= volume of extract analyzer

III. Niacin (Vit B_3).
This was calculated according to Okwu and Ndu (2006).

$$\text{Niacin (mg/100g)} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where:
W=weight of sample ash
Au= absorbance of test sample
As= absorbance of standard phosphorus solution
C= concentration of standard phosphorus solution
Vt= total extract volume
Va= volume of extract analyzer.

IV. Ascorbic acid (Vit.C)
This was calculated according to Okwu and Ndu (2006).
Vitamin C content was calculated as

$$\text{Mg/100g sample} = \frac{100}{WVa} \times Vf \times 0.88T$$

Where:

W = weight of sample

Vf = total volume of extract

Va = volume of sample extract.

III. Result

Fig.1. Effect of different x-ray doses on the mineral levels of fluted pumpkin

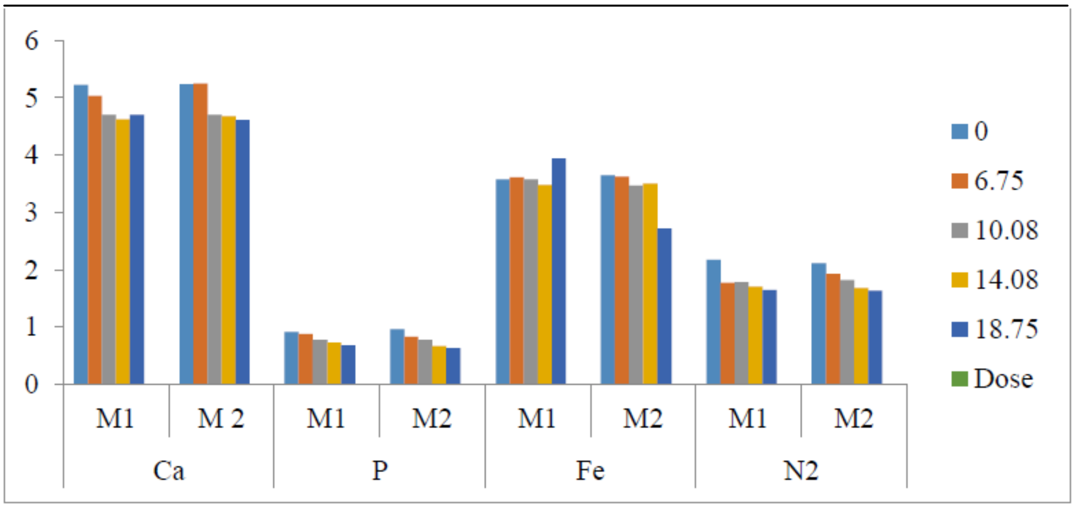

Fig 1. Is a bar chart representation of the effects of different doses of x-ray treatment on Calcium, Phosphorus, Iron and Nitrogen level of fluted pumpkin leaves during M_1 and M_2 generations.

- ***Calcium level***

In the M_1 generation, it was observed that as the treatment concentration doses were increased, the level of calcium decreased. The calcium content during M_1 generation was significantly affected by the mutagen treatments when compared to the control plants. A gradual decrease in calcium content was observed with increase in the x-ray doses. Treatment 6.75mGy recorded 5.03mmol/L whereas 18.75mGy recorded 4.70mmol/L. The untreated plants however recorded the highest (5.23mmol/L) calcium content.
In M_2 generation, the highest level (5.25mmol/L) was recorded on 6.75mGy. This was followed by the control treatment (5.24mmol/L) which was higher that other treatment doses.
Analysis of variance showed a very significant difference ($P<0.05$) on the effect of the x-ray dose treatments.

- ***Phosphorus level***

In both M_1 and M_2 generations, there was a highly significant difference among the treatment doses due to mutagenic effects of the x-ray.
Increase in treatment doses led to a decrease in the level of phosphorus which was highly significant ($P<0.05$).
0.91mmol/L (0.00mGy) and 0.96mmol/L(0.00mGy) were the highest levels of phosphorus as recorded in M_1 and M_2 generations respectively while 0.68mmol/L (18.75mGy) and 0.63mmol/L (18.75mGy) were the lowest levels recorded in M_1 and M_2 generations respectively.

III. *Iron*

In M_1 generation, the highest level of iron (3.94mcg/dL) was recorded on 18.75mGy dose treatment. This was followed by 6.75mGy (3.61mcg/dL). The control (0.00mGy) gave 3.58mcg/d L). Analysis of variance shows that there was a very significant difference on the effect of the treatment doses.
In M_2 generation, the result obtained also showed a very significant difference ($P<0.05$) when compared to the control plants. The result showed a decrease in the iron content with increase in the x-ray doses.

IV *Nitrogen*

In both M_1 and M_2 generations, there was a highly significant difference ($P<0.05$) on the effect of the different treatment doses when compared to the control as shown by analysis of variance.
A trendy decrease in the level of nitrogen was recorded as the treatment concentrations increased. The control plants recorded 2.17mmol/L of which were the highest level as compared to all other treatment levels in M_1 generations.
In M_2 generation, the result showed a significant difference ($P<0.05$) on the nitrogen content when compared to the control. The control (0.00mGy) treatment gave the highest value (2.11mmol/L) which started decreasing as the treatment concentration increased.

Fig.2. Effect of different x-ray doses on the vitamin levels of fluted pumpkin

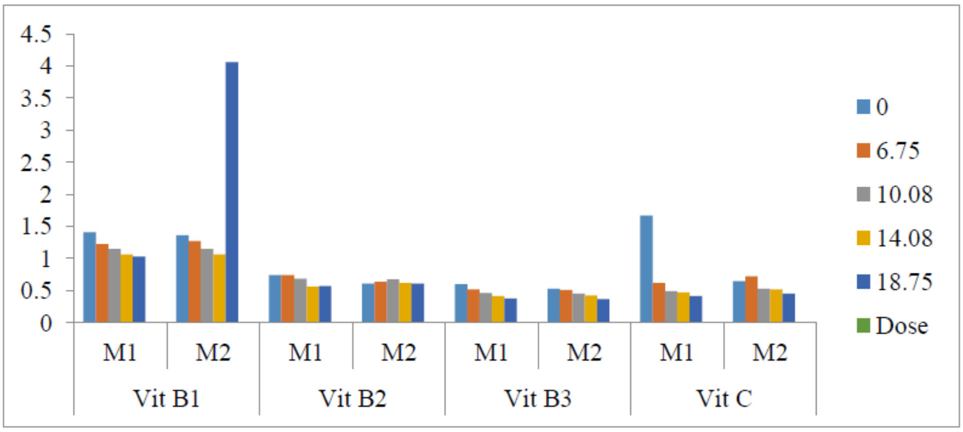

Fig 2 is a bar chart which shows the effects of different doses of x-ray treatment on the vitamin level of fluted pumpkin. These vitamins include: Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin (Vitamin B3) and Ascorbic acid (Vitamin C).

I. Thiamin (Vit B1)

In M_1 generation, a decrease in vitamin B1 level in fluted pumpkin leaves were recorded as the treatment dose increased in concentration. The control treatment (0.00mGy) gave the highest level (1.41) of vitamin B1, followed by 6.75mGy treatment dose (1.22) and continued with the decrease till the least which gave 1.03 at 18.75mGy treatment dose.
The result of the analysis of variance showed that the effect of the x-ray doses of the vitamin B1 content of the fluted pumpkin was highly significant ($P<0.05$).
In M_2 generation, the highest level of vitamin B1 (4.06mGy) was gotten at 18.75mGy dose of x-treatment. This was followed by the 0.00mGy treatment dose(1.36) and a decrease in vitamin B1 level followed as the concentration increased up till 14.08mGy(1.06).
However, analysis of variance shows that there was no significant difference ($P>0.05$) on the effect of the treatment on the different samples.

II***. Riboflavin (Vitamin B2)***
The bar chat (Fig 2) shows that as the concentration of the x-ray treatment dose was increasing, the level of vitamin B2 was decreasing in the M_1 generation.
Analysis of variance showed a significant difference ($P<0.05$) on the effect of treatments.
However, in the M_2 generation, the highest level of vitamin B2 (0.67) was gotten at 10.08mGy x-ray treatment dose, followed by 6.75mGy treatment dose which gave 0.63 and then a decrease.
Analysis of variance showed a significant difference ($P<0.05$) on the effects of the different treatment doses on the samples.

III. Niacin (Vitamin B3)
Fig 2 shows that in both generations of M_1 and M_2, there were trendy decrease in the level of vitamin B3 in fluted pumpkin as the concentration of treatment doses increased. The highest levels of vitamin B3 were gotten from the control (0.00mGy) treatment doses in both generations.
Analysis of variance shows that there was a highly significant difference ($P<0.05$) in the effect of different doses of the x-ray on the different samples in both generations.

IV. Ascorbic acid (Vitamin C)
The result obtained in the vitamin C content during M_1 generation showed a highly significant effect of the mutagen. There was a decrease in the vitamin C content with increase in the x-ray doses. Amongst the treatment levels, 6.75mGy recorded the highest vitamin C content (0.62) followed by 10.08mGy (0.49) whereas 18.75mGy recorded 0.41 in vitamin C content. The control plants however recorded 1.67 in vitamin C content which was the highest.
Also, in M_2 generation, the result of the analysis of variance showed that the effect of the x-ray doses on the vitamin C content was highly significant ($P<0.05$) when compared to the control plants. Plants treated with the lower x-ray doses 6.75mGy recorded the highest vitamin C content (0.72).

IV. Discussion

The mineral make-up of fluted pumpkin leaves whose seeds were treated with different doses of x-ray showed a decreasing trend in the calcium, phosphorus and nitrogen contents with increased treatment doses in M_1 and M_2 generations while the iron content was significantly enhanced by 18.75mGy in M_1 generation.

Vitamin B1 level increased at 18.75mGy treatment, B2 at 10.08mGy and vitamin C at 6.75mGy all in M2 generation while vitamin B3 recorded a decrease with increasing concentration.

The variability in biochemical content of the treated pumpkin seeds is a reliable indication of the effects of physical mutagens. Previous study had shown that gamma irradiation had a significant effect on morphology and yield traits of *Cajanus cajan* (Udensi and Ntui; 2013). As reported by Udensi and Ntui 2013, frequently observed symptoms in the low-or high dose-irradiated plants are enhancement or inhibition of biological responses. Abu *et.al* 2019 recorded an increase in vitamin C with high doses of x-ray and gamma ray in two varieties of red pepper (Shombo and Tatashi) and this agrees with our findings in this research. It was also recorded that iron and calcium levels in shombo and Tatashi varieties of pepper were increased with high concentrations of physical mutagens (Abu et.al 2019). This agrees with our result in iron level but contrary to that of calcium.

The possible increase in phyto-minerals can be attributed to physical mutagen effects which induces oxidative stress and ultimately affects structural and functional molecules of a plant by causing a disturbance in normal metabolic pathways (Ashraf *et.al* 2003).

V. Conclusion

This study reveals that the mineral and vitamin levels of fluted pumpkin are very sensitive to physical mutagen effects in diverse ways.
At 18.75mGy dose of x-ray irradiation, high iron level can be induced in fluted pumpkin leaves.
High level of vitamin B1, B2, and vitamin C can be induced with 18.75mGy, 10.08mGy, and 6.75mGy x-ray doses respectively in second mutant generation.
Iron, vitamin B1, B2 and C are high value nutritional supplements which without doubt remains traits of interest to not only farmers but also nutritionists.

References

[1]. Abu, N.E; Omeka, J.O and Ojua, E.O. (2019). Effect of Different Mutagens on Some Mineral, Phytochemical and Proximate Composition of Two Red Pepper Varieties. Annual research and review in biology 33(2), 1-13; 2019. Article no: ARRB 51702. ISSN-2347-565X. NLM ID: 101632869
[2]. Agboola, S. A. (1979). "**Agricultural Atlas of Nigeria**," OUP Catalogue, Oxford University Press, number 9780195754087.
[3]. A.O.A.C. (2005). Methods of analysis of the association of official analytical chemist, 18th ed. Washington, D.C. USA**.**

[4]. Aiyelaagbe, I. O. O. and Kintomo, A. A. (2002)."Nitrogen response of fluted pumpkin (Telfairia occidentalis Hook. F) grown sole or intercropped with banana." Nutrient Cycling in Agroecosystems, 64: 231-35.

[5]. Akang, E.N., Oreemosu, A.A., Dosumu, O.O., Noronha, C.C. and Okanlawon, A.O. (2010).The effects of fluted pumpkin (Telfairia occidentalis) seed oil (FPSO) on testis and semen parameters, Agriculture and Biology Journal of North America, 1(4): 697-700.

[6]. Akoroda, M. (1990). Ethnobotany of Telfairia occidentalis (Cucurbitaceae) among Igbos of Nigeria. Economic Botany, 44(1): 29-39.

[7]. Akpaniwo, E. G., Ittah, M. A. and Iwo, G. A. (2015). Field performance and morphological variations of X-ray induced M_2 mutant lines of fluted pumpkin (Telfairia occidentalis Hook. F.). International Journal of Research in Agriculture and Forestry 2(I0): 12-16.

[8]. Akpaniwo, E. G., Ittah, M. A. and Iwo, G. A. (2015).Field performance and morphological variations of x-ray induced m2 mutant lines of fluted pumpkin (Telfairia occidentalis, Hook. F.) International Journal of Research in Agriculture and Forestry, 2: I10- 134.

[9]. Al-Enezi, N. A. and Al-Khayri, J.M. (2012). Alterations of DNA, ions and photosynthetic pigments content in date palm seedlings induced by X-irradiation. International Journal of Agricultural Biology, 14: 329–336.

[10]. Al-Salhi, M., Ghannam, M. M., Al-Ayed, M.S., El-Kameesy, S. U. and Roshdy, S. (2004). Effect of gamma-irradiation on the biophysical and morphological properties of corn. Nahrung., 48: 95-98.

[11]. Ashraf M, Cheema AA, Rsahid M, Qamar Z. Effect of gamma rays on M1 generation in basmati rice. Paskistan J Bot. 2003;35 (5):791-795.

[12]. Gehan G. Mostafa, (2015). Effect of some Chemical Mutagens on the Growth, Phytochemical Composition and Induction of Mutations in Khaya senegalensis. International Journal of Plant Breeding and Genetics,9:57-67. **DOI:** 10.3923/ijpbg.2015.57.67**URL:** https://scialert.net/abstract/?doi=ijpbg.2015.57.67

[13]. Hameed, A., Mahmud, T. S., Atta, B. M., Haq, M. A., Sayed, H. (2008). Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. Pakistan Journal Botany. 40, 1033–1041 (2008).

[14]. Hammed, A., Shah, T.M., Atta, B. M., Ahsanul, H. M. and Sayed, H. (2008). Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. Pakistan Journal of Botany, 40:1033-1041

[15]. Idris, S. (2012). Compositional Studies of Telfairia occidentalis Leaves. American Journal of Chemistry, 1(2): 56-59

[16]. Iwo, G. A., Amadi, C. O., Eleazu, C. O. and Ukpabi, J. U. (2013). Induced mutagenesis on ginger for improved yield components and oleoresin content. Canadian Journal of Plant Breeding, 1(3): 90 -96.

[17]. James, C. S. (1995). Experimental methods in analytical chemistry of foods. New York: Chapman and Hall.

[18]. Luo, X. M., Tinker, N. A., Jiang, Y., Xuan, P., Zhang, H. Q. and Zhou, Y. P. (2013). "Suitable dose of ^{60}Co γ-ray for mutation in Roegneria seeds," Journal of Radioanalytical and Nuclear Chemistry, 295(2): 1129–1134, 2013.

[19]. Nwanna, E. E. and Oboh, G. (2008). Antioxidant and hepatoprotective properties of polyphenol extracts from Telfairia occidentalis (fluted pumpkin) leaves on acetaminophen induced liver damage. Pakistan Journal of Biological Sciences, 10: 2682-2687.

[20]. Ojomo, A.O; Omueti, O; Raji, J.A and Omueti, O. (1979) Studies in induced mutation in cowpea, 5. The variation in protein content following ionizing radiation, Nig. J. Appl. Sci. 21 61-64

[21]. Okokon, J. E., Ekpo, A. J., Eseyin, O. A. (2009). Evaluation of in vivo antimalarial activities of ethanolic leaf and seed extracts of Telfairia occidentalis, Journal of Med Food, 12:1-9.

[22]. Okwu, D. E and Ndu, C. E. (2006). Evaluation of phytonutrients, minerals and vitamin content of some varieties of yam. International Journal of Molecular and Advanced Sciences, 2(2):199-203.

[23]. Oyewole, O. and Abalaka, M. (2012). Antimicrobial Activities of Telfairia occidentalis (fluted pumpkins) Leaf extract against selected intestinal pathogens. Journal of Health Science, 2(2): 1-2.

[24]. Stanaway, S. and Gill, G. (2000). Protein glycosylation in diabetes mellitus: biochemical and clinical considerations. Pract Diabetes Int, 17(1): 21-23.

[25]. Taheri, S., Abdullah, T. L., Ahmad, Z. and Abdullah, N. A. P. (2014). "Effect of acute gamma irradiation on Curcuma alismatifolia varieties and detection of DNA polymorphism through SSR marker," BioMed Research International, pp. 245–256

[26]. Udensi O, Ntui VO. Evaluating the performance of amiprophos methyl and γirradiated seeds on growth and yield traits of pigeon pea. Int J Agric Biol. 2013; 15:885 – 890

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