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Effect of Coconut Milk and Benzyl Amino Purine on the Vegetative Growth, Nutritional and Chemical Constituent of *Amaranthus Hybridus* Linnaeus seedlings

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Abstract- Studies were carried out to investigate the effects of 10%, 15% coconut milk and 50mg/L, 100mg/L Benzyl amino purine on the vegetative growth, nutritional and photochemical constituents of *Amaranthus hybridus* Linnaeus seedlings. Plants sprayed with water were the controls. All the treatments resulted in significantly increased leaf number, stem girth, plant height, shoot/root ratio, leaf area ratio and chlorophyll content of the vegetable with highest values recorded in treatments with 15% coconut milk at 14 weeks after planting. Treatments with 15% coconut milk also resulted in greater mineral elements contents at 14 weeks after planting of the vegetable. Alkaloids (3.55), saponins (1.66), phenols (0.36) and flavonoids levels (0.85) were higher in 15% coconut milk treated plants, whereas phytic acid (1.26) and hydrocyanic acids levels (15.30) were greater in 100mg/L Benzyl amino purine and 10% coconut milk respectively at 14 weeks after planting.

Keywords: amaranthus hybridus, coconut milk, benzyl amino purine, chlorophyll content, nutritional contents, phytochemical constituents.

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EFFECTOFCOCONUTMILKANDBENZYLAMINOPURINEONTHEVEGETATIVEGROWTHNUTRITIONALANDCHEMICALCONSTITUENTOFAMARANTHUSHYBRIDUSLINNAEUSSEEDLINGS

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Effect of Coconut Milk and Benzyl Amino Purine on the Vegetative Growth, Nutritional and Chemical Constituent of *Amaranthus Hybridus* Linnaeus seedlings

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Abstract- Studies were carried out to investigate the effects of 10%, 15% coconut milk and 50mg/L, 100mg/L Benzyl amino purine on the vegetative growth, nutritional and photochemical constituents of Amaranthus hybridus Linnaeus seedlings. Plants sprayed with water were the controls. All the treatments resulted in significantly increased leaf number, stem girth, plant height, shoot/root ratio, leaf area ratio and chlorophyll content of the vegetable with highest values recorded in treatments with 15% coconut milk at 14 weeks after planting. Treatments with 15% coconut milk also resulted in greater mineral elements contents at 14 weeks after planting of the vegetable. Alkaloids (3.55), saponins (1.66), phenols (0.36) and flavonoids levels (0.85) were higher in 15% coconut milk treated plants, whereas phytic acid (1.26) and hydrocyanic acids levels (15.30) were greater in 100mg/L Benzyl amino purine and 10% coconut milk respectively at 14 weeks after planting. The study shows that 15% coconut milk persistently had the greater potentials to increase vegetative growth, nutritional and phytochemical constituents of Amaranthus hybridus leading to its significance in nutrients requirements of man and usefulness in medicinal industries.

Keywords: amaranthus hybridus, coconut milk, benzyl amino purine, chlorophyll content, nutritional contents, phytochemical constituents.

I. INTRODUCTION

Vegetables are essential in the diet as they provide plant fibre, mineral elements, vitamins, carbohydrates and proteins (Hollingsworth, 1981). In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acid (Okafor, 1983). Many of the local vegetable materials are under-exploited

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because of inadequate scientific knowledge of their nutritional potentials. Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries abound in literature, much still need to be done. Many workers (Lockeett et al., 2000; Akindahunsi and Salawu, 2005; Edeoga et al., 2006; Hassan and Umar, 2006; Ekop, 2007) have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries. In Nigeria, Amaranthus hybridus L, popularly called 'efo tete', 'tete oyinbo' or 'tete-nla' in Yoruba land (Gbile, 2002), is an annual herbaceous plant of 1-6 feet high. The leaves are alternate petioled, 3 – 6 inches long, dull green, and rough, hairy, ovate or rhombic with wavy margins. The flowers are small, with greenish or red terminal panicles. Taproot is long, fleshy red or pink. The seeds are small and lenticellular in shape; with each seed averaging 1 - 1.5 mm in diameter and 1000 seeds weighing 0.6 - 1.2 g. It is rather a common species in waste places, cultivated fields and barnyards. In Nigeria, Amaranthus hybridus leaves combined with condiments are used to prepare soup (Oke, 1983; Mepha et al., 2007). In Congo, their leaves are eaten as spinach or green vegetables (Dhellot et al., 2006). The growth regulators influence growth and development at very plant low concentrations while they inhibit at high concentrations (Jules et al., 1981). Monthly foliar spraying of geranium (Pelergonium graveolens) resulted in increased plant height and herb production (Mohammed et al., 1983). Spraving of datura plant, Datura innoxia planted in different salinity concentrations with chlormequat, ethephon or kinetin was found to enhance plant growth, alkaloidal and soluble sugar contents of leaves and reduce the harmful effect of salinity on the plant (Abdul-Rahman and Abdel-Aziz, 1983). Application of gibberellic acid, 4-chloroindole and 6-benzyl amino purine on to the standard petal and calyx of Vicia faba var. major was found to significantly enhance pod set (Rylott and Smith, 1990). Currently, there is no information on the effects of coconut milk (CM) (a crude

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source of cytokinin) and benzyl amino purine (BAP) on the growth, nutritional and chemical composition of *Amaranthus hybridus*. This study was therefore designed to evaluate the vegetative growth, nutritional and phytochemical chemical composition of *Amaranthus hybridus* in responses to coconut milk and benzyl amino purine treatments and to determine the optimum concentrations of the hormones that can be recommended for spraying on the vegetable for enhanced growth and quality.

II. MATERIALS AND METHODS

a) Seed Collection and Planting

Seeds of Amaranthus hybridus was obtained from the Institute of Agricultural Research and Training (IAR&T) Ibadan, Oyo State, Nigeria ((7° 23'N and 3° 51'E). Seedlings were raised from seeds planted on sandy loam nursery seed bed (5m x 5m) at the forestry nursery unit of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (7° 11'N and 3° 21'E), in the rainforest belt with an annual rainforest of 963.3mm (Fadimu et al., 2012). Five nursery seed beds in three replicates were made. Each nursery seed bed formed a plot. Water was added to the nursery bed when necessary to keep it moist. Seedlings were thinned to 100 seedlings per bed 2 weeks after planting (WAP) putting uniformity of height into consideration and were considered matured enough to be subjected to treatments.

b) Coconut milk (CM) and Benzyl amino purine (BAP) Treatments on the Amaranthus Hybridus Seedlings

The seedlings of Amaranthus hybridus were sprayed foliarly in this experiment with coconut milk (CM) and benzyl amino purine (BAP). Different concentrations of the coconut milk treatments were 10% and 15% while 50mg/L and 100mg/L for benzyl amino purine. 500ml of the various concentrations of the coconut milk and benzyl amino purine were applied on the seedlings of the Amaranthus hybridus by foliar spraying until the leaf surfaces were properly wet and dripping at 3 weeks intervals, starting from 2 weeks after planting (WAP) while seedlings of Amaranthus hybridus sprayed with 500ml distilled served as the control. The treated and control vegetables were harvested for all the analyses at 14 weeks after planting (WAP). The method of Akubugwo et al. (2007) was used for processing the leaves of Amaranthus hybridus in preparation for various analyses after harvesting. The collected sample was thoroughly mixed, had their stalks removed, rinsed with de-ionized water and the residual moisture evaporated at room temperature before sun-drying for 2 - 3 days on a clean paper with constant turning over to avert fungal growth. The sun-dried sample was ground into fine powder using pestle and mortar, and sieved through a 2.0 mm mesh sieve to obtain a dried powdered sample that was used for all the analyses.

c) Vegetative Growth, Nutritional Chemical Constituent of the seedlings of Amaranthus Hybridus

Treated and control seedlings of *Amaranthus hybridus* were sampled at 14 weeks after planting. The vegetative growth (leaf number, plant height, stem girth, shoot-root ratio and leaf area ratio) were measured according to the method of **Mukhtar (2008)** while the chlorophyll contents of the leaves were determined using the method of **Witham** *et al.* (1971).

The mineral elements, comprising sodium, calcium, potassium, magnesium, iron, zinc and phosphorus were determined according to the method of Shahidi et al. (1999) and Nahapetian and Bassiri (1975) with some modifications. Exactly 2.0 g of each of the processed samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5.0 ml of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5.0 ml of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through whatman No.42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm long cell was used and concentration of each element in the sample was calculated on percentage (%) of dry matter that is mg/100 g sample. Phosphorus content of the digest was determined calorimetrically according to the method described by Nahapetian and Bassiri (1975).

Flavonoids were estimated by the method of Bohm and Kocipal (1974). Alkaloids, phenols and saponins were determined by the method of Harborne (1973) as detailed by Obadoni and Ochuko (2001). Tannin determination was determined according to Van-Burden and Robinson (1981) and determination of Hydrocyanic acid was quantified by the method of Bradbury *et al.* (1991) while Determination of Phytic acid was by the method of Wheeler and Ferrell (1971).

d) Statistical Analysis

The experimental layout was a randomized complete block design containing three replications (Steel and Torrie, 1982). Each replication contained five treatments and every treatments consisted of 100 seedling plants (5 treatments \times 3 replications \times 100 seedling plants = 1500 seedling plants). Data were subjected to analysis of variance (ANOVA) using Duncan Multiple Range Test (DMRT) for mean separation (Sokahl and Rholf, 1969).

III. Results and Discussion

In this study, significant ($P \le 0.05$) increases in all the vegetative growth parameters (leaf number, stem girth, plant height, shoot/root ratio, leaf area ratio) were

observed in the Amaranthus hybridus seedlings treated with different concentration of coconut milk (CM) and benzyl amino purine (BAP) at 14 weeks after planting in comparison with the control. 15% Coconut milk treatment recorded the highest number of leaf number (144cm), stem girth (3.98cm), plant height (167cm), shoot/root ratio (2.14), leaf area ratio (365.5) and chlorophyll content (3.01mg/100g) (Table 1). Ebofin et al. (2004) similarly recorded enhancement in leaf number and plant height in Prosopis africana and Albizia lebbeck. Spraying of kinetin on Datura innoxia plant at 1mg/L, 5mg/L and 10mg/L was found to cause increased vegetative growth (Abdel-Rahman and Abdel-Aziz, 1983). An increase in stem circumference was likewise observed according to Kadiri (1991) personal communication in his studies with Abelmoschus esculentus and Lycopersicum esculentus treated with various concentration of GA₃ and 2, 4-D. Furthermore, similar results were observed by Mukhtar (2008) where chlorophyll content (1.08mg/g) at 9 weeks was obtained in Hibiscus sabdariffa treated with 15% coconut milk, followed by 100ppm GA₃ (0.93mg/g). The production of high shoot-root ratio in Amaranthus hybridus plants raised with hormones in this study suggests that the rate of absorption of available nutrients might have significantly been enhanced. Akhtar et al. (2008) explained that increases in shoot-root ratio by hormone treatments are due to the fact that they enhance the stem elongation plants. In addition, cytokinins such as those contained in coconut water, benzyl amino purine (BAP) also facilitate cell division and sprouting (Pan, 2001). GA₃ and IAA had regulatory effect to enhance the plant height, number of branches, numbers as compared to other plant growth regulators and control (Sarkar et al., 2002). GA3 and IAA treated plants exhibited higher values of dry weight and chlorophylls content than the control (Abdel-Lateef, 2003; Afroz et al., 2005; Abou Al-Hamd, 2007). Enhanced germination and seedling growth by plant growth regulators may be mediated through changes in the activities of carbohydrate metabolism enzymes (Kaur et al., 2000).

Mineral composition (Potassium, sodium, calcium, magnesium, zinc, iron and phosphorus) recorded in mg/100g in all the various treatments of coconut milk (CM) and benzyl amino purine (BAP) applied on Amaranthus hybridus showed significant differences in comparison with the control. Treatment of 15% CM produced the maximum K (53.70), Na (7.21), Ca (43.01), Mg (229.30), Zn (3.12), Fe (13.30) and P (34.22) (Table 2). The ratio of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) were also shown in Table 2. Magnesium content (229.30 mg /100 a) and Iron content (13.30 ma/100a) of the leaves are within the range reported in some green vegetables (Ladan et al., 1996; Ibrahim et al., 2001; Antia et al., 2006; Hassan and Umar, 2006). Magnesium is a component of chlorophyll (Akwaowo et al., 2000) while McDonald et al., (1995) highlighted magnesium for efficient metabolism of carbohydrates and lipids, involved in cellular respiration and general cellular biochemistry and function. It is also an important mineral element in connection with ischemic heart disease and calcium metabolism in bones according to Ishida et al., (2000). Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats (Adeyeye and Otokiti, 1999). The Zinc content (3.12 mg/100 g) compares favourably to most values reported for green leafy vegetables in literatures (Ibrahim et al., 2001; Hassan and Umar, 2006). Zinc is involved in normal function of immune system (Akubugwo et al., 2007). Also, zinc stabilizes the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity (Emebu and Anyika, 2011). The Na/K ratio in the body is of great concern for prevention of high blood pressure (Akubugwo et al., 2007). Na/K ratio less than one is recommended (FND, 2002). Therefore, high blood pressure diseases would be minimized due to constant of Amaranthus hybridus because intake Na/K composition is lower than one. For good Ca to P intestinal absorption, Ca/P ratio should also be close to unity (Gull-Guerrero et al., 1998) and the ratio in this study supports this requirement, hence provides evidence of good Ca to P intestinal absorption.

Table 3 contains full details of the effects of different concentrations of coconut milk (CM) and benzyl amino purine (BAP) at 14 weeks after planting (WAP) on the phytochemical composition of the leaves of Amaranthus hybridus in mg/100g (DW). The result (3.55mg/100g), indicates that alkaloid saponin (1.66mg/100g), phenol (0.36mg/100g) and flavonoids (0.85mg/100g) recorded highest values from 15% Coconut milk treatment at 14 weeks after planting while 10% coconut milk treatment at 14 weeks after planting favoured highest values of tannin (0.45mg/100g) and hydrocyanic acids (15.30mg/100g). 100mg/L benzyl amino purine treatment recorded 1.26mg/100g of phytic acids at 14 weeks after planting. Result also shows that at 14 weeks after planting, all the hormonal treatments showed no significant (P< 0.05) differences with the controls in all the phytochemical compositions except 15% coconut milk (Table 3). This present result of the alkaloid content of 3.55mg/100g by 15% coconut milk treatment disagreed with results obtained by Edeoga et al., (2005); Okwu and Josiah, (2006); Akubugwo et al., (2007) based on the fact that it is higher than the values reported for the leafy vegetables of Aspilia Africana, Brvophyllum pinnatum. Cleome rutidosperma and Emilia coccinea and Amaranthus hybridus consumed in Nigeria. The tannin contents (0.45mg/100g) agrees with those of Edeoga et al., (2005) and Okwu and Josiah (2006). The flavonoid content of the leaves (0.85 mg/100

g) and saponin (1.66 mg/100 g) also agreed with the result obtained for Amaranthus hybridus in Nigeria but was lower than values reported for *O. gratissiumum* and Hsypits sauvelens (Akubugwo et al., 2007). The Hydrocyanic acid content (15.30mg/100 g) and phytic acid content (1.26mg/100 g) of the leaves were lower than the values reported for A. hybridus and I. batatas (Akubugwo et al., 2007; Antia et al., 2006). Phytochemical composition of Amaranthus hybridus shows that it may not only be useful due to its dietetic value but also medicinally and pharmacologically (Akubugwo et al., 2007). Alkaloids are known to play some developmental control in living system, metabolic role and have a protective role in animals (Edeoga et al., 2006; Edeoga and Eriata, 2001). The anti-nutrients, for example hydrocyanic acid have been suggested in cerebral damage and lethargy in man and animals. Tannins are capable of reducing available protein by antagonistic competition and can therefore cause protein deficiency syndrome, kwashiorkor while phytic acid has complex effect in human system including indigestion of food and flatulence (Maynard, 1997). According to Ekop et al. (2004), Eka and Osagie (1998) and Ekop and Eddy (2005) the anti-nutrients present in this plant is within the tolerant levels and can easily be rendered harmless by soaking, boiling or frying. The results from this research work showed that 10%, 15% coconut milk and 100mg/L benzyl amino purine treatments at 14 weeks after planting increases the vegetative growth, nutritional and phytochemical constituents of Amaranthus hybridus. This therefore added to its suitability as edible vegetable, nutritional and therapeutic values. These treatments could therefore be used to promote the growth and quality of Amaranthus hybridus.

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Table 1 : Effect of different concentrations of Coconut milk (CM) and Benzyl amino purine (BAP) at 14 weeks after planting on leaf number, stem girth (cm), plant height (cm), shoot / root ratio, leaf area ratio and chlorophyll content (mg/g) of *Amaranthus hybridus* seedlings.

	Treatments						
Parameters	10% CM	15% CM	BAP (50 mg/L)	BAP (100 mg/L)	Control		
Leaf number	128 ^b	144 ^a	103°	125 ^b	87 ^d		
Stem girth (cm)	3.30 ^b	3.98 ^a	2.87°	3.03 ^{bc}	1.74 ^d		
Plant height (cm)	132 ^b	167 ^a	134 ^b	151 ^a	94°		
Shoot/root ratio	1.78 ^b	2.14 ^a	0.98 ^d	1.32°	0.76 ^e		
Leaf area ratio	283.1 ^{ab}	365.5ª	264.0 ^{bc}	228.4°	171.6 ^d		
Chlorophyll (mg/g)	2.63 ^b	3.01ª	2.60 ^b	2.62 ^b	2.27 ^c		

Values followed by different letters in the same row differ significantly (P=0.05) according to Duncan's multiple comparison.

 Table 2 : Effect of different concentrations of Coconut milk (CM) and Benzyl amino purine (BAP) at 14 weeks after planting (WAP) on the mineral element contents of Amaranthus hybridus seedlings.

	Treatments					
Composition (mg/100g)	10% CM	15% CM	BAP (50 mg/L)	BAP (100 mg/L)	Control	
Potassium (K)	50.30 ^{ab}	53.70 ^a	48.00 ^b	48.80 ^{ab}	42.50 ^c	
Sodium (Na)	7.01 ^{ab}	7.21ª	6.24°	6.33 ^{bc}	5.50 ^d	
Calcium (Ca)	40.30 ^{ab}	43.01 ^a	33.50°	38.70 ^b	28.80 ^d	
Magnesium (Mg)	201.40 ^b	229.30 ^a	173.80 ^c	198.30 ^b	146.40 ^d	
Zinc (Zn)	2.78 ^b	3.12ª	2.31°	2.44 ^c	1.89 ^d	
Iron (Fe)	10.90 ^b	13.30 ^a	6.27 ^d	8.49 ^c	4.90 ^e	
Phosphorus (P)	32.29 ^a	34.22ª	26.80 ^b	31.00 ^a	23.10 ^c	
Na/K	0.14 ^a	0.13ª	0.13 ^a	0.13 ^a	0.13 ^a	
Ca/P	1.25 ^a	1.26ª	1.25 ^a	1.25 ^a	1.25ª	

Values followed by different letters in the same row differ significantly (P=0.05) according to Duncan's multiple comparison.

Table 3 : Effect of different concentrations of Coconut milk (CM) and Benzyl amino purine (BAP) at 14 weeks after planting (WAP) on the constituents of alkaloids, saponin, tannins, phenols, flavonoid, phytic acid and hydrocyanic acids (mg / 100g dry weight) of *Amaranthus hybridus* seedlings.

	Treatments					
Phytochemicals	10% CM	15% CM	BAP (50 mg/L)	BAP (100 mg/L)	Control	
Alkaloids	3.53 ^{ab}	3.55 ^a	3.51 ^b	3.50 ^b	3.50 ^b	
Saponin	1.64 ^a	1.66 ^a	1.64 ^a	1.63 ^a	1.65 ^a	
Tannins	0.45 ^a	0.44 ^a	0.44 ^a	0.44 ^a	0.41 ^a	
Phenols	0.35ª	0.36 ^a	0.34 ^a	0.35ª	0.33 ^a	
Flavonoid	0.84 ^a	0.85 ^a	0.78 ^a	0.82 ^a	0.80 ^a	
Phytic acid	1.24 ^a	1.25 ^ª	1.24 ^a	1.26 ^a	1.13 ^a	
Hydrocyanic acids	15.30 ^a	15.20 ^a	14.40 ^a	15.10 ^a	14.10 ^a	

Values followed by different letters in the same row differ significantly (P=0.05) according to Duncan's multiple comparison.

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