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Research Article

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Identification of Phytochemicals and Bioactive Compounds in Grain Amaranth (*Amaranthus cruentus*. L) as Affected by Fish Guano and Abscisic Acid under Water Stress Condition

Aisha Muhammad Aliyu[1], Shehu Usman Yahaya[2], Abdurrahman Lado[3], Hauwa Muhammad Aliyu[4], Abdulhakeem B. Yakub[5]

[1]Department of Crop Science Aliko Dangote University of Science and Technology, Wudil, Kano State
[2,3,5]Department of Agronomy, Faculty of Agricultural Technology, Bayero University Kano
[4]Colleges of Nursing and Midwifery, Kano State
[1] aliyuaisha02@gmail.com, [2] suyahaya.agr@buk.edu.ng, [4]Mhauwa87@gmail.com [5]yakabdulhaeembam@gmail.com

Abstract Field trials were conducted during the dry seasons of 2021 and 2022 to identify the phytochemicals and bioactive compounds present in grain amaranth (*Amaranthus cruentus*. L), as affected by fish guano and abscisic acid under moisture stress conditions. The research was conducted at the Teaching and Research Farm of the Faculty of Agriculture Bayero University Kano (110 97′ 98.6″ N 8042′03.7″ 'E) and the Research Farm of Aliko Dangote University of Science and Technology Wudil (11° 25′ N and Long 9° E). The treatments consisted of moisture stress (vegetative, flowering and grain filling), fish guano (FG) (0, 0.1 and 0.2 kg) and abscisic acid (ABA) (0, 20 and 50 µmol/L) treatments. The plants were laid out in a split-plot design and replicated 3 times. Stress occurred in the main plots, and fish guano and abscisic acid rates occurred in the subplots. Phytochemical screenings were carried out for qualitative and quantitative secondary metabolites. Phytochemical screening of the seed extracts revealed the presence of bioactive compounds, which is an indication that the plant possesses some pharmacological activities that will enhance the nutritional and health status of the populace to support food security. The GC–MS chromatogram of the seed extract revealed a high percentage of fatty acids and their derivatives, which are good sources of therapeutic drugs.

Keywords Grain amaranth, Fish guano, Abscisic acid, Secondary metabolites, Bioactive compounds

Introduction

Amaranth grain is a pseudocereal that has been widely studied and is associated with functional properties and attractive medical benefits (Nataly *et al.,* 2023). It is emerging at the forefront among most grains because of its remarkable nutritional value; high contents of minerals (calcium, iron and phosphorous), vitamins, carotenoids, and bioactive components such as phytosterols, squalene, flavonoids, and phenolic acids (Pasko *et al.,* 2009). It also contains primary proteins called albumin and globulins, which are more soluble and digestible than prolamins in wheat. Seeds are also beneficial for sores. Seeds and leaves are used as astringents for preventing bloody diarrhea excrement, hematuria and excessive menstruation (Sumner *et al*., 2003, Weckwerth, 2003; Kopka *et al*., 2014). Amaranth also contains phytochemical compounds such as rutin and nicotiflorin, and peptides can help lower hypertension and the incidence of cancer. It also cures cardiovascular diseases (CVDs) linked to high blood cholesterol (hyperlipidemia), hypertension, obesity, and diabetes; thus, scientists have reported that reducing saturated fat while increasing unsaturated fatty acids can prevent CVD. Amaranth was studied for these findings and was found to be potentially beneficial for CVD patients (Mercola, 2016).

Fish guano is an organic matter recognized for its high nutritional and agronomical value through the maintenance and development of the microfauna and microflora of the soil, which are essential for good exchanges of elements between the earth and plants. In a greenhouse, Greer and Driver (2000) reported that some aquatic organisms, such as algae and fish waste, had nutritional contents similar to those of mineral fertilizers used in greenhouse cultivation. Plant hormones are essential for their primary role in regulating plant growth and development (Peto *et al*., 2011) and their roles in improving plant tolerance to biotic and abiotic stress (Krishnamurthy and Rathinasabapathi, 2013). Exogenous application of abscisic acid (ABA) improves plant tolerance to various stresses (Meng *et al*., 2009). ABA, an isoprenoid phytohormone, regulates various physiological processes, including stomatal opening and protein storage, and promotes adaptation to many plant stresses (Sah *et al*., 2016).

Most indigenous people consider amaranth species available in their vicinity tobe the same (Ogwu *et al*., 2018). They serve multipurpose ethnological roles as medicines, dyes, home decorators, animal feed, human food, and superstitious practices for local gods. The development of sustainable value chains for grain amaranth, from cultivation in the field to the production of different value-added products and an understanding of its numerous health benefits, could enable significant interventions to uplift millions of rural and poor urban households in developing countries where malnutrition is glaring about its attendant health consequences. Thus, the seeds of amaranth appear to be an economically viable underutilized crop with great potential. Fish guano is a natural source of essential nutrients such as nitrogen, phosphorus, and potassium, as well as micronutrients, which are vital for plant growth and development. Under moisture stress conditions, when the uptake of nutrients can be challenging due to reduced root activity, the application of fish guano can provide readily available nutrients to support the plant's physiological processes. Exogenously applied ABA can enhance the plant's ability to cope with moisture stress by triggering various physiological and biochemical changes, such as osmotic adjustment, antioxidant activity, and stomatal regulation. By improving water availability, nutrient availability, and stress tolerance, the combined application of fish guano and exogenous ABA can potentially increase the yield and nutritional content of grain amaranth under moisture stress conditions.

Materials and Methods

Experimental Sites

The experiment was conducted during the 2021 and 2022 dry seasons at the Teaching and Research Farm Faculty of Agriculture Bayero University Kano (11^0 9798.6^{i} N 8^0 42$^{'}$ 03.7^{i} E) and Aliko Dangote University of Science and Technology Farm Wudil (11˚25′ N and Long 9˚E 400-430 m above sea level).

Treatments and experimental design

The treatments consisted of induced moisture stress (vegetative, flowering and grain filling), fish guano (0, 0.1 and 0.2 kg) and abscisic acid (0, 20 and $^{50\ \mu mol/L}$). These plots were laid out in a split plot design with three replications. Induced moisture stress was assigned to the main plot, while fish guano and ABA were assigned to the subplot.

Preparation and application of materials

Fish guano powder (200 g) was suspended in 4 liters of distilled water. It was mixed thoroughly, serially diluted to the prescribed rates and sprayed across seedlings using a handheld watering can (Angibaud, Derome & Specialties, 2016). A stock solution was prepared by mixing 100 mg of ABA powder in 1 ml of ethanol. A weighed quantity of ABA (as per treatment) was added to a graduated cylinder, and a 1 L volume was made in a volumetric flask by adding distilled water. The foliage was immediately applied to the solutions with a hand sprayer (PhytoTechnology, Laboratories, 2016).

Harvesting

All cultural practices were carried out, and the amaranth grains were harvested by uprooting using a hoe at 12WAT, and threshing was performed manually.

Qualitative analysis of phytochemicals

To test for alkaloids, one milliliter of each extract was added to 2 test tubes, and 3 drops of Dragendoff's reagent were added separately. A red/orange precipitate/turbidity was produced immediately, which indicates the presence of alkaloids (Ciulci, 1994).

Test for flavonoids: Two to three drops of dilute sodium hydroxide were added per 1 ml of extract. An intense yellow colour was produced in the plant extract, which later became colourless upon the addition of 2 drops of dilute acid (HCl), which indicates the presence of flavonoids (Trease and Evans, 1989).

Test for saponins: Half a gram (0.5 g) of each extract was placed in a test tube, and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15 min was observed, which indicates the presence of saponins (Trease and Evans, 1989).

Test for tannins: Solutions of the extracts were made with distilled water, and 3 drops of 5% ferric chloride ($FeCl_3$) solution were added. A green–black or blue–black colouration indicates the presence of tannins (Ciulci, 1994).

Test for terpenoids: To 4ml of chloroform, 10 kg of the crude extract was added, followed by the careful addition of 5 ml of concentrated H_2SO_4. The formation of reddish brown colouration at the interface is an indication of a positive result for the presence of terpenoids (Parekh and Chands, 2008).

Test for cardiac glycosides: To 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, 5 ml of the plant extract was added, followed by the addition of 1 ml of concentrated sulfuric acid. A brown ring was formed at the interface, which indicated the presence of the deoxy sugar of the cardenolides. A violet ring may appear below the brown ring, although in the acetic acid layer, a greenish ring may also form just progressively throughout the layer (Parekh and Chands, 2008).

Quantitative Analysis of the Phytochemicals

To 500 ml of beaker, 10 g of the dried amaranth leaves and 400 ml of 10% acetic acid in ethanol were added, and the beaker was then covered and allowed to stand for 4 hours. This mixture was then filtered, extracted and concentrated in a water bath to one-quarter of the original volume. This was preceded by dropwise addition of concentrated ammonium hydroxide to the extract until the precipitation was completed. The whole solution was allowed to settle, and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue was the alkaloid, which was dried and weighed to a constant mass (Trease and Evans, 1989).

$$\text{Formula} = \frac{\text{B} - \text{A}}{\text{S}} \times 100$$

where B = the weight of Whatman filter paper.
A = Weight of Whatman filter paper after drying
S = Sample weight.

Estimation of saponins: Fifty (50) kg of 20%aqueous ethanol was added to 10 g of dry amaranth leaves in a conical flask. At approximately 55°C, for 4 hours with continuous stirring, the mixture was heated using a hot water bath, after which the mixture was filtered, and the residue was re-extracted with a further 100 ml of 20% ethanol. The combined extracts were reduced to 20 ml in a water bath at approximately 90°C. The concentrate was transferred to a 100 ml separatory funnel,10 ml of diethyl ether was added, and the mixture was shaken vigorously. The aqueous layer was recovered, and the ether layer was then discarded. The purification process was repeated three times. Thirty milliliters of n-butanol was added. The combined n-butanol extracts were washed twice with 5 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven to a constant weight; the saponin content was calculated as the percentage of the starting material (Obadoni and Ochuko, 2001).

$$\text{Formula} = \frac{\text{B} - \text{A}}{\text{S}} \times 100$$

where B = the weight of Whatman filter paper.
A = Weight of Whatman filter paper sample
S = Sample weight.

Estimation of phenols: To 0.5 ml of freshly prepared plant extracts in test tubes, 8 ml of distilled water and 0.5 ml of Folin‟s Ciocalteu reagent were added to all the tubes. All the tubes were kept in a biological oxygen demand chamber for 10 minutes at 40°C for incubation. This was followed by the addition of 1 ml of sodium carbonate solution to all the test tubes; subsequently, the tubes were incubated in the dark for an hour. The colour developed was read spectrophotometrically at 660 nm. A standard curve was drawn using tannic acid as a standard. Different concentrations of tannic acid were prepared, and the OD was read at 660 nm in a Shimadzu UV-1650 spectrophotometer. The concentrations of the samples were calculated based on the standard curve (Mallick and Singh, 1980).

Estimation of total flavonoids: A total of 200 ml of 80% aqueous methanol was used for recurrent extraction of 20 g of dry amaranth leaves at room temperature. Whatman filter paper No 42 was then used for filtration of the whole solution. The filtrate was then transferred into a crucible and evaporated to dryness over a water bath; the dry content was weighed to a constant weight (Osuntokun *et al.*, 2014).

Estimation of tannins: Samples of 100 mg of tannic acid were dissolved in 100 ml of distilled water. Five milliliters of stock solution was diluted to 100 ml with distilled water. 1 ml containing 50 µg tannic acid. Extraction of Tannin: 0.5 gm of the powdered material was weighed and transferred to a 250 ml conical flask, and 75 ml of water was added. The flask was heated gently and boiled for 30 min and centrifuged at 2,000 rpm for 20 min, after which the supernatant was collected in a 100 ml volumetric flask. One milliliter of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of water. Then, 5 ml of folin denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml with water. The plate was shaken well. The absorbance was read at 700 nm after 30 min. If the absorbance was greater than 0.7,a 1 + 4 dilution of the sample was made. A blank was prepared with water instead of the sample. A standard graph was prepared by using 100 mg tannic acid. The tannin content of the sample was calculated as tannic acid equivalents fs from the standard graph. The data are expressed as the mean ± standard deviation (SD) of triplicate samples.

Identification of the Active Compounds (GC–MS Analysis) of Aqueous Extracts

The extract was analysed by GC–MS electron impact ionization on a GC–17A gas chromatograph (Shimadzu) coupled to a GC–MS QP 5050A mass spectrometer (Shimadzu); a fused silica capillary column (30 m × 0.25 mm; 0.25 mm film thickness) coated with DB–5 (J&W); a column temperature of 100°C (2 min) to 250°C at a rate of 3°C/min; and a carrier gas, helium, at a constant pressure of 90 kPa. The acquisition parameters were full scanand a scan range of 40-350 amu. Compound identification was performed by comparing the National Institute of Standard Technology (NIST) library data of the peaks with those reported in the literatureand mass spectra of the peaks with literature data. The percentage composition was computed from the GC–MS peak areas on the DB-5 column without applying correction factors.

Results

Phytochemical Constituents of the Plant Extracts

Table 1 shows the results of the preliminary phytochemical screening of grain amaranth at BUK in 2021 and 2022 at vegetative, flowering and grain filling stages. By subjecting fractions of grain amaranth plant extracts to quantitative phytochemical tests, aqueous extracts of grain amaranth plants were found to contain some secondary metabolites, such as alkaloids, saponins, flavonoids and phenols. Table 2 shows the results of the quantitative phytochemical

analysis of different fractions of *Amaranthus cruentus* seed extract from the BUK during the 2021 and 2022 dry seasons. The secondary metabolites detected in the plants were further quantified. Alkaloids at 0.1kg of fish guano/50ABA (41.06 µg/ml ± 2.80[a]) had the most significant effect, whereas the control had the least significant effect (4.78 µg/ml ± 0.25[b]) during the 2021 dry season in the BUK. However, 0.2kg of guano/0ABA (47.89 µg/ml ± 5.85[a]) significantly increased the alkaloid content and decreased the alkaloid content (2.89 µg/ml ± 1.46[b]) at 0kg of guano/50ABA during the 2022 dry season at the BUK compared to the other treatment combinations during the water stress stages.

Flavonoids at BUK 2021 and 2022 were more abundant (10.75 µg/ml ± 1.66[a]) at 0.2kg of fish guano/0ABA and 16.27 µg/ml ± 0.04[a], respectively, while they were less abundant at 0 kg of fish guano/50ABA (6.04 µg/ml ± 0.82b[c]) and 5.43 µg/ml± 0.04[c], respectively, at 0.1kg of fish guano/0ABA. Phenols (60.54 µg/ml ± 1.62[a]) at 0.2 kg of fish guano/20ABA and (434.95 µg/ml ± 66.98[a]) at 0 kg of fish guano/20ABAwere significantly more abundant in the aqueous amaranth seed extract, while the lowest were obtained at 0.1 kg of fish guano/OABA (13.76 µg/ml ± 10.43[b]) and 100FG/50ABA (15.69 µg/ml± 3.23[c]) in both the 2021/2022 dry seasons. In 2021, more saponins were reported to be present in grain amaranth extract (17.87 µg/ml ± 1.04[b]) at 0 kg guano/50ABA and (47.19 µg/ml ± 12.26[c]) at 0.2 kg guano/0ABA, whereas grain extract was found to have the lowest concentration of saponins (1.31 µg/ml ± 0.13[b] and 17.23 µg/ml ± 1.29[b]) at BUK for both the 2021 and 2022 dry seasons for the control treatment (0 kg guano/0ABA).

In Wudil, aqueous extracts of grain amaranth plants contain similar secondary metabolites which were not altered by water stress at vegetative, flowering and grain filling including alkaloids, saponins, flavonoids and phenols (Table 3). Alkaloids at 0kg of fish guano/20ABA (54.95 µg/ml ± 6.98[a]) had the highest value, whereas 0.2kg of fish guano/50ABA had the lowest value (22.44 µg/ml ± 4.09[a]) during the 2021 dry season at the BUK (Table 42). However, 0.2kg of fish guano/0ABA (30.83 µg/ml ± 3.00[a]) had the highest content and 0.2kg of fish guano/50ABAhad the lowest content (19.22 µg/ml ± 0.19[d]) during the 2022 dry season compared to the other treatment combinations. The flavonoid content in Wudil was 49.11 µg/ml ± 0.08[a] at 0kg of fish guano/0ABA in 2021 and 16.27 µg/ml ± 0.04[a] at 0kg of fish guano/20ABA in 2022, while it was lowest at 0kg of fish guano/0ABA (6.15 µg/ml ± 0.04b[c]) and 9.49 µg/ml± 0.07[b] at 0.1kg of fish guano/20ABA.

Phenols (102.47 µg/ml ± 0.19[a]) at 0.1 kg of fish guano/20ABA and (79.49 µg/ml ± 53.25[a]) at 0 kg of fish guano/50ABA were more abundant in the aqueous amaranth seed extract, while the lowest were obtained at 0 kg of fish guano/0ABA (9.31 µg/ml ± 0.17[d]) and 0.2FG/0ABA (20.00 µg/ml± 0.18[c]) in the 2021 and 2022 dry seasons, respectively.

Table 1: Preliminary phytochemical screening of grain amaranth at BUK

	Treatments	**Alkaloids**	**Tannins**	**Saponins**	**Flavonoids**	**Coumarin**	**Phenols**	**Terpenoids**	**Cardiac glycosides**
					2021				
Vegetative	0FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.1FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.1FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.1FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.2FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.2FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.2FG/50ABA	+	-	++	+	++	+	+	-
					2022				

Vegetative	0FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.2FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.2FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.2FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.3FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.3FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.3FG/50ABA	+	-	++	+	++	+	+	-

Key: (+): presence of chemical constituents, (-): absence of chemical constituents

Table 2: Quantitative phytochemical analysis results of different fractions of *Amaranthus cruentus* seed extract from the BUK during the 2021 and 2022 dry seasons.

		2021				2022			
Stress	**Sample ID**	**Flavanoids**	**Phenols**	**Saponins**	**Alkaloids**	**Flavanoids**	**Phenols**	**Saponins**	**Alkaloids**
Vegetative	0FG/0ABA	5.66 ± 0.15^{b}	18.49 ± 4.85^{b}	1.31 ± 0.13^{b}	4.78 ± 0.25^{b}	16.27± 0.04^{a}	304.62 ±13.78^{d}	17.23 ± 1.29^{b}	6.22 ± 1.29^{b}
Flowering	0FG/20ABA	7.26 ± 0.46^{b}	13.33 ± 7.66^{b}	5.67 ± 5.35^{b}	12.11 ± 1.35^{b}	9.42 ± 0.07^{c}	434.95 ±66.98^{a}	45.51 ± 2.73^{c}	4.78 ± 1.78^{b}
Grain filling	0FG/50ABA	6.04 ± 0.82b^{c}	23.44 ± 1.04^{b}	17.87 ± 1.04^{b}	12.36 ± 1.79^{b}	7.39 ± 0.34^{b}	96.34 ± 14.27^{a}	37.60 ± 30.60^{c}	2.89 ± 1.46^{b}
Vegetative	0.1FG/0ABA	6.27 ± 0.64^{b}	13.76 ± 10.43^{b}	6.26 ± 5.23^{b}	13.22 ± 0.51^{b}	5.43 ± 0.04^{c}	27.09 ± 4.88^{c}	20.38 ± 3.09^{b}	19.33 ± 8.53^{b}
Flowering	0.1FG/20ABA	6.34 ± 1.20^{b}	13.68 ± 15.53^{b}	8.09 ± 4.63^{b}	16.17 ± 1.59^{b}	8.45± 0.11^{d}	51.18 ± 3.85b^{c}	20.30 ± 0.83^{b}	19.67 ± 10.69^{b}
Grain filling	0.1FG/50ABA	8.36 ± 1.09^{b}	53.76 ± 21.61^{c}	13.41 ± 2.69^{b}	41.06 ± 2.80^{a}	6.34 ± 0.41^{e}	15.69 ± 3.23^{c}	21.69 ± 4.67^{b}	23.06 ± 23.95^{c}
Vegetative	0.2FG/0ABA	10.75 ± 1.66^{a}	47.53 ± 0.49^{c}	3.86 ± 1.18^{b}	12.29 ± 1.05^{b}	7.26 ± 0.04b^{c}	82.58 ± 1.41b^{c}	47.19 ± 12.26^{c}	47.89 ± 5.85^{a}
Flowering	0.2FG/20ABA	6.45 ± 0.43^{b}	60.54 ± 1.62^{a}	16.85 ± 0.29^{b}	15.16 ± 1.77^{b}	10.49 ± 0.18^{b}	97.42 ±0.56^{b}	75.36 ± 0.5^{a}	39.83 ± 5.34^{a}
Grain filling	0.2FG/50ABA	9.20 ± 0.65^{b}	21.83 ± 3.94^{b}	68.98 ± 6.25^{a}	2.44 ± 0.35^{b}	10.68 ± 0.07^{b}	120.65 ±1.16^{b}	43.22 ± 0.06^{c}	36.94 ± 4.67^{a}

The values are presented as the means ± standard deviations of triplicate readings; n=3. Values within the same column bearing the same superscript letters are significantly different at $p<0.05$.

Table 3: Preliminary phytochemical screening of grain amaranth at Wudil

	Treatments	**Alkaloids**	**Tannins**	**Saponins**	**Flavonoids**	**Coumari**	**Phenols**	**Terpenoids**	**Cardiac glycosides**
		2021							
Vegetative	0FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.1FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.1FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.1FG/50ABA	+	-	++	+	++	+	+	-

Vegetative	0.2FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.2FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.2FG/50ABA	+	-	++	+	++	+	+	-
					2022				
Vegetative	0FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.1FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.1FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.1FG/50ABA	+	-	++	+	++	+	+	-
Vegetative	0.2FG/0ABA	+	-	++	+	++	+	+	-
Flowering	0.2FG/20ABA	+	-	++	+	++	+	+	-
Grain filling	0.2FG/50ABA	+	-	++	+	++	+	+	-

Key: (+): presence of chemical constituents, (-): absence of chemical constituents

Table 4: Quantitative phytochemical analysis results of different fractions of *Amaranthus cruentus* seed extract from Wudil during the 2021 and 2022 dry seasons

		2021				**2022**			
Stress	**Sample ID**	**Flavanoids**	**Phenols**	**Saponins**	**Alkaloids**	**Flavanoids**	**Phenols**	**Saponins**	**Alkaloids**
Vegetative	0FG/0ABA	49.11 ± 0.08a	9.53 ± 0.49d	91.31 ± 0.17b	45.95 ± 4.09a	11.29 ± 0.11b	74.70 ± 0.37a	93.45 ± 1.59b	26.17 ± 3.18b
Flowering	0FG/20ABA	9.35 ± 0.0b	34.30 ± 0.19c	123.48 ± 0.45a	54.95 ± 6.98a	17.63 ± 3.63a	22.62 ± 0.21c	126.52 ± 0.11a	23.83 ± 1.59b
Grain filling	0FG/50ABA	6.38 ± 0.14c	100.27±49.53a	115.22 ± 9.94ab	38.75 ±23.13a	12.26 ± 0.62b	79.49 ± 53.25a	127.08 ± 6.89a	23.25 ± 3.65b
Vegetative	0.1FG/0ABA	6.15 ± 0.04c	74.84 ± 0.32b	101.01 ±0.11b	27.06 ± 2.84a	14.09 ± 0.04b	71.13 ± 8.60a	85.24 ± 0.06b	29.06 ± 0.98c
Flowering	0.1FG/20ABA	16.32 ± 0.07c	102.47 ± 0.19a	98.54 ± 0.27b	25.78 ± 1.36a	9.49 ± 0.07b	76.91 ± 49.06a	91.19 ± 0.23b	27.89 ± 1.51ac
Grain filling	0.1FG/50ABA	6.81 ± 0.07bc	14.41 ± 0.67e	95.24 ± 0.64b	26.17 ± 3.18a	13.31 ± 0.11b	30.06 ± 2.85a	83.75 ± 0.36b	28.11 ± 3.09c
Vegetative	0.2FG/0ABA	10.85 ± 0.04b	13.87 ± 0.32d	93.45 ± 1.59b	23.83 ± 1.59a	15.33 ±0.07ab	20.00 ± 0.18c	105.05 ± 12.09b	30.83 ± 3.00a
Flowering	0.2FG/20ABA	12.09 ± 0.07b	60.54 ±1.63b	126.52 ± 0.11a	24.06 ±3.81a	13.15 ± 0.07b	57.71 ± 4.65b	111.65 ± 11.76ab	25.00 ±5.05a
Grain filling	0.2FG/50ABA	10.80 ± 2.96b	21.83 ± 3.94c	133.37 ± 0.19a	22.44 ± 4.09a	10.68 ± 0.07b	20.43 ± 1.79c	109.55 ± 7.77b	19.22 ±0.19d

The values are presented as the means ± standard deviations of triplicate readings; n=3. Values within the same column bearing the same superscript letters are significantly different at $p<0.05$.

In 2021, the highest quantity of saponins was detected in the grain amaranth extract (133.37 µg/ml ± 0.19^{b}) at 0.2 kg of fish guano/50ABA and 126.52 µg/ml ± 0.11^{a} at 0 kg of fish guano/20ABA,whereas the lowest concentration of saponins was detected in the grain extract (93.45 µg/ml ± 1.59b) for 0.2 kg of fish guano/0ABA and 83.75 µg/ml ± 0.36b for 0.1 kg of fish guano/50ABA in the 2021 and 2022 dry seasons, respectively.

Bioactive Compounds

Table 5 shows the composition of the aqueous extract of *Amaranthus cruentus*. The number of peaks were detected, and the retention time in minutes and retention index of the identified compounds were determined together with the mass spectra (i.e., the base peak and the most abundant peaks). The total ion concentration was also recorded as a percentage. The results of the GC–MS analysis of all the compounds in the aqueous extract of *A. cruentus* revealed various constituents. All the compounds were effectively matched and identified based on their retention indices by computer matching against library spectra built from authentic compounds and mass spectrum data (Koenig, 1998; Moronkola and Kunle, 2014). The major constituents identified included ally cyclohexane, acetoxy-3-methoxystyrene, p-hydroxyamphetamine, hexadecanoic (palmitic acid), acid, trimethylsilyl ester acid, pentamethyldisilyl ester, hexadecanoic acid methyl ester, tetradecanoic acid, methyl ester, methyl (oxopropyl) and hexadecane. Some of these compounds are fatty acids or their derivatives. These include palmitic acid (n-hexadecanoic acid) and oleic acid methyl ester.

Discussion

After the secondary metabolites of both plant extracts were subjected to quantitative and qualitative phytochemical tests, the results showed that the aqueous extracts of amaranth seeds contained some secondary metabolites, such as saponins, alkaloids, flavonoids, tannins, cardiac glycosides and phenols. However, the results of this study revealed that moisture stress had no effect on the phytochemical compounds in the grain of amaranth. Some of these metabolites were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Singh and Bhat, 2003). These findings agree with the findings of Barku and Abban (2013), who reported that *A leiocarpus* extracts contain all the secondary metabolites that were detected in these studies, including tannins, saponins, flavonoids, steroids, amino acids and reducing sugars, even though carbonyls, which were not detected in this study, were present. Fish guano and abscisic acid had no effect on phytochemicals, and antioxidants had no significant effect at maturity when irrigation resumed.

The results obtained from this study are in line with the findings of Tibiri *et al.* (2007) and Philips (2010), who reported that tannins and alkaloids are natural products that have medicinal properties. The activity exhibited may be a result of the presence of phytochemicals. Several components such as tannins, saponins, flavonoids and phenols have been reported to exhibit antibacterial and antioxidant activities (Kunle and Egharevba 2009; Ayoola *et al.,* 2008). However, few studies with conflicting results have been conducted on the nutritional activity of amgarath essential oils.

Table 5: Chemical Composition of Grain Amaranth Seeds at BUK and Wudil using Gas Chromatography (GC–MS) during 2021/2022

Peak No.	MS [Basepeak + most abundant peaks	Identified compd.	RT [mins]	Cal. RI
1	55,70,73,80,90,97,105,110,115,120,130,135,147, 157,207,253,330	1-Ally cyclohexane-1,2-diol($C_9H_{16}O_2$)	3.949	156
2	55,60,70,75,90,95,105,120,135,145,150,156,255, 330,405	4-Acetoxy-3-methoxystyrene ($C_{11}H_{128}O_3$)	4.361	192
3	55,69,73,72,85,91,97,109,129,135,147,163,171, 195,207,253	pHydroxyamphetamine($C_9H_{13}N$)	5.334	151
4	105,77,51,122,227,64,41	Hexadecanoic acid, trimethylsilyl ester ($C_{19}H_{40}O_2Si$)	8.904	328
5	55,69,73,83,98,115,129,149,157,171,183,199,207, 213,227,242,57,281,285,439,4	Hexadecanoic acid, pentamethyldisilyl ester ($C_{12}H_{46}O_2Si_2$)	9.373	386
6	55,89,74,81,97,112,123,129,152,171,183,211.2, 257,264,281,288,433,467	Hexadecanoic acid methyl ester($C_{17}H_{34}O_2$)	10.57	270
7	107,91,135,150,80, 119,67,55,	Tetradecanoic acid,12-methyl-,methyl ester ($C_{16}H_{32}0_2$)	10.77	256
8	52,54,56,58,60,65,67,68,68,69,70,73	3-Methyl-2-(2-oxopropyl)furan ($C_8H_{10}O_2$)	11.02	138

9	52,70,83,90,100,110,122,130,135160,170,185, 211,232,260,290300,310,330,340,370,400,420, 440,470	7-Hexadecana,(z)-l ($C_{16}H_{30}O$)	11.313	238

Studies on grain amaranth aqueous extract fractions are scarce in the literature. Fatty acids are the primary constituents of edible oils and medicinal herbs and are reported to interfere with bacterial growth and survival (Benkendorff *et al.,* 2005). Many fatty acids are known to have antibacterial and antifungal properties (Knapp and Melly, 1986). Many other fatty acids, such as palmitic acid and oleic acid, have been reported to have potential antibacterial and antifungal activities (Agoramoorthy *et al.,* 2007). Indeed, FAs are often identified as the active ingredients in ethnic and herbal medicines (McGaw *et al.,* 2002). Hexadecanoic acid methyl ester is an antioxidant, flavour and hypocholesterolemic (Sermakkani and Thangapandian, 2012). n-Hexadecanoic acid (palmitic acid) is the most common saturated fatty acid that occurs in natureand is an antioxidant, hypocholesterolemic, nematicide and pesticide. Oleic acid is a long-chain monounsaturated acid and a potent antibacterial agent (Dilika *et al.,* 2000; Zheng *et al.,* 2005). In addition, the application of ABA also maintained the activities of antioxidant enzymes at lower levels. ABA reportedly acts in several ways to improve the antioxidant defense system in plant cells (Peleg & Blumwald, 2011).

Conclusion and Recommendations

The combined application of fish guano and ABA had notable effects on the phytochemical and bioactive constituents of grain amaranth. Farmers should adopt growing grain amaranth to enhance the nutritional and healthy living status of the populace for food security, especially in areas with low water availability in Nigeria.

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