

Effects of Fish guano and Abscisic acid on Leaf area index, Stomatal conductance, Photosynthetic active radiation and Relative water content of Grain Amaranth (*Amaranthus cruentus* L.) under moisture stress conditions

Comment [DP1]: Suggested title: Impact of Fish Guano and Abscisic Acid on Stomatal Conductance, Relative Water Content, Leaf area Index and Photosynthetic Efficiency in Grain Amaranth (*Amaranthus cruentus* L.) Under moisture Stress

ABSTRACT: Field trials were conducted during the 2021 and 2022 at the Teaching and Research Farm of the Faculty of Agriculture Bayero University Kano (11° 09' 98.6" N 8° 42' 03.7" E) and Research Farm of Aliko Dangote University of Science and Technology Wudil (11° 25' N and 8° 09' E), to determine the effects of fish guano (FG) and abscisic acid (ABA) on leaf area index (LAI), stomatal conductance (SC), photosynthetic active radiation (PAR) and relative water content (RWC) of grain amaranth under moisture stress conditions. Treatments consisted of moisture stress stages (vegetative, flowering and grain filling), FG (0, 0.1 and 0.2 kg) and ABA concentrations (0, 20 and 50 $\mu\text{mol L}^{-1}$) and laid out in a split-plot design and replicated three times. Stress was placed in the main plots while FG and ABA rates were placed in the subplots. Data was collected on LAI, SC, PAR and RWC and subjected to analysis of variance (ANOVA) using GENSTAT 17th Edition software. Significant treatment means were compared using the Student Newman-Keuls Test (SNK) at 5% probability level. Plants were highly significant ($p < 0.001$) for LAI and RWC at 0.2 kg FG levels and 50 $\mu\text{mol L}^{-1}$ ABA at BUK and Wudil 2021/2022. Plants under moisture stress were significant ($p < 0.05$) at RWC at flowering at BUK in 2021 season. Stomatal conductance at 20 $\mu\text{mol L}^{-1}$ ABA was also significant ($p < 0.05$) at BUK and Wudil in 2021/2022. The PAR was significant with control having significant differences in BUK 2021/2022 seasons. This study indicated the potential of FG and ABA as valuable tools for enhancing the resilience and performance of grain amaranth crops in under moisture stress.

1 Introduction

Grain amaranth is a pseudocereal that has been widely studied and associated with functional properties and attractive medical benefits (Nataly *et al.*, 2023). Amaranth seeds contain more protein (17.6%) per 100g than other crops like corn and rice and contain several essential amino acids (Soriano *et al.*, 2018). It is high in fibre and low in saturated fat (DPP 2010). Greer and Driver (2000) reported that some aquatic organisms such as algae and fish waste had nutritional content similar to mineral fertilizer exploited in greenhouse cultivation. ABA, an isoprenoid phytohormone, regulates various physiological processes, including stomatal opening and protein storage, and provides adaptation to many plants' stresses (Sah *et al.*, 2016). There is little information on the role of increased fertilization rate and ABA concentration in mediating plant response to water stress, even though ABA is known to be a general growth inhibitor (Milborrow, 1974). Experiments on the effects of water stress and ABA treatment on

leaf morphology and floral development in spinach were undertaken to investigate ABA's role in water stress's developmental consequences and this suggests that ABA may have an essential role in determining the pattern of plant development, at least in the presence of water stress. The application of fish guano can provide readily available nutrients to support the plant's physiological processes under moisture stress conditions, when the uptake of nutrients can be challenging due to reduced root activity. Exogenously applying ABA can enhance the plant's ability to cope with moisture stress by triggering various physiological and biochemical changes which can ultimately improve the plant's resilience and growth under water-limited conditions. This study was therefore carried out to examine the effects of fish guano and ABA on LAI, SC, PAR and RWC of grain amaranth under moisture stress conditions.

2 Materials and Methods

2.1 Experimental sites

The experiment was conducted during the 2021 and 2022 dry seasons at Teaching and Research Farm Faculty of Agriculture Bayero University Kano and Aliko Dangote University of Science and Technology Farm Wudil, Kano State.

2.2 Treatments and Experimental design

Treatments consisted of induced moisture stress at (vegetative, flowering and grain filling), FG (0, 0.1 and 0.2 kg) and abscisic acid concentration (0, 20 and 50 $\mu\text{mol L}^{-1}$) and laid out in a split plot design with three replications. Induced moisture stress was assigned to the main plot while FG and ABA were assigned to the sub plot.

2.3 Preparation and application of materials

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

2.4 Cultural practices

2.4.1 Land preparation: The experimental sites were cleared manually, ploughed and harrowed to obtain a fine tilth soil. The site was divided into gross plots size of 3 x 4.5 m (13.5 m²) made up of 6 rows each and net plot area of 3.0 m². A spacing of 1.5m between replications and 1.0 m between blocks was left as an alley. Discard between sub-plots was 0.5m.

2.4.2 Sowing: Seeds were sown in mid-January 2021 on a prepared nursery bed for four weeks by drilling method. Seeds were covered with soil and mulched to speed up the germination process.

2.4.3 Transplanting: Seedlings were transplanted to the field manually, 10 seedlings were transplanted on each of the 6 rows at a depth of 2.0 cm with a spacing of 75 cm x 30 cm between stands and rows, respectively.

2.4.4 Weeding: Weeding was done manually using a hoe

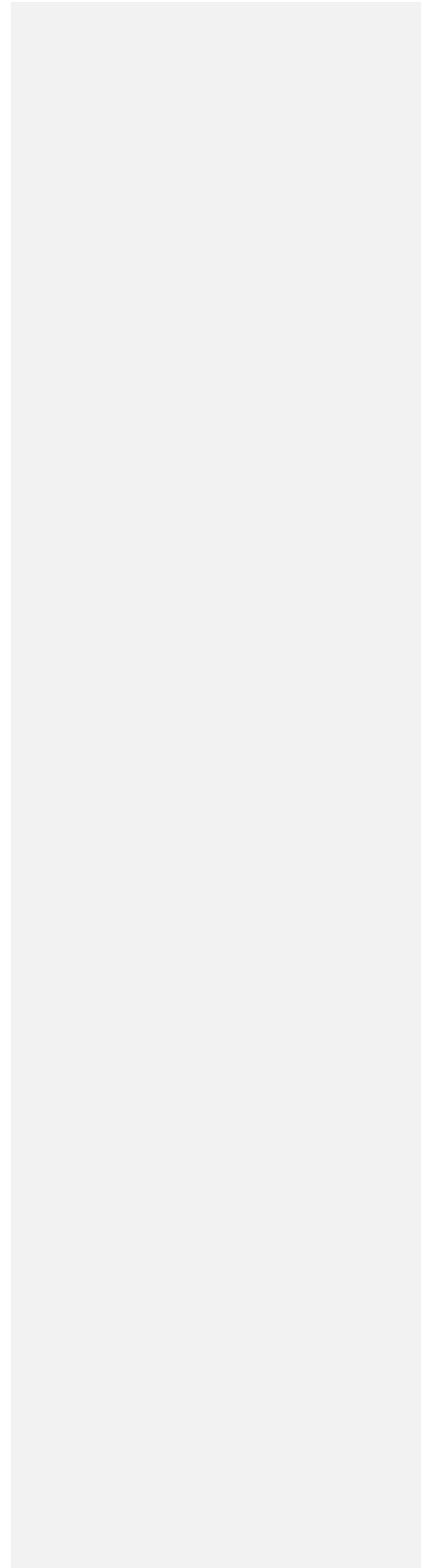
2.4.5 Pesticides application: Garlic extracts were applied thrice to mitigate the infection of leaf miner *Liriomyza huidobrensis*.

2.4.6 Harvesting: The Amaranth grains were harvested from the net plot area

Comment [DP2]: Soil moisture stress should be measured by Soil Water Tension. What is the extent of induced moisture stress that should be incorporated in the treatment details?

byuprootingusingahoeat12WAT.Threshing was done manually.

UNDER PEER REVIEW



2.5 Data Collection

Data were collected at the vegetative stage, flowering stage and grain filling which coincide with 4, 8 and 12 weeks after transplanting (WAT) on 5 tagged plants from the two inner rows (net plot). Data was collected using standard agronomic procedures on:

2.5.1 Leaf area index: The LAI was determined using the AccuPAR model LP-80PAR/LAI Ceptometer (Decagon Devices, INC. Pullman, USA).

2.5.2 Photosynthetically active radiation: This was determined by using ECA-PB0402 Photosynthetic apparatus Beijing Yikangnong science and technology development CO. LTD.

2.5.3 Stomatal conductance: This was measured to know the functions of a leaf using a leaf Porometer AP4Delta devices-Cambridge- U.K.

2.5.4 Relative water content (%): The RWC was measured as described by Hayat *et al.* (2007). Five fresh leaves from tagged plants (two cm diameter, excluding midrib) were taken, weighed and immediately floated on deionized water in Petri dishes. Each Petri dish was supplied with 100 ml deionized water to saturate the leaf disc for 24h, under dark conditions. At 24h, the leaf discs were removed from the water and blotted with tissue paper to remove the adhering water and the turgor mass was recorded. The leaf discs were then dried at 70°C for 48h. The RWC was calculated as follows:

Where, FW = fresh leaf weight/mass; DW = dry leaf weight and TW = turgor weight of leaf

2.6 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using Genstat 17th edition. Significant treatment means were separated using the Student Newman-Keuls test at 5% level of probability.

3 Results

3.1 Leaf Area Index

The effect of fish guano and ABA on LAI of grain amaranth under stress condition at BUK and Wudil in 2021 and 2022 dry season is presented in Table 1. There were no significant ($p > 0.05$) differences in vegetative, flowering and grain filled at BUK and Wudil in 2021 and 2022. No significant ($p > 0.05$) differences was recorded for LAI of amaranth for FG at BUK in 2021 and 2022. However, there was significant difference ($p < 0.05$) in LAI at 4 and 8 WAT at Wudil in 2022. At 4 WAT, the highest (113.8) LAI was obtained at 0.1 kg FG, followed by 0 kg (90.5) while least was recorded at 0.1 kg FG (87.2). Similar trend was also observed at 8 WAT (Table 1). The result of ABA concentration on LAI of grain amaranth showed significant difference at BUK in 2021. In 2022, at BUK, the LAI result indicated that there was no significant ($p > 0.05$) increases at 4 WAT and 12 WAT while at 8 WAT there was significant ($p < 0.05$) increases with increase in the level of ABA with the highest LAI of 166.3 at 50 μ m ABA. In 2021, at Wudil, there was significant ($p < 0.05$) increase with increase in the dosage of ABA at 4, 8 and 12 WAT. The highest LAI recorded for 50 μ m ABA were 81.5, 117.3 and 157.8 at 4, 8 and 12 WAT respectively. The result further showed that there were significant ($p < 0.05$) increase of LAI in 2022 at Wudil at 4, 8 and 12 WAT. The highest (227.2) LAI was obtained with 50 μ m ABA at 12 WAT while the least (83.9) was recorded at 0 μ m ABA at 4 WAT at Wudil in 2022. There was significant interaction of stress x ABA at 8 and 12 WAT and fish guano x ABA at 4, 8 and 12 WAT in 2022 at Wudil (Table 1). At 12 WAT, there was significant ($p < 0.05$) interaction on LAI at

Comment [DP3]: Two additional observations are required to clarify the results and discussion more accurately:

- 1. Leaf Proline Content:** Although the author has not conducted any antioxidative study in this paper, the measurement of proline content would justify the RLWC (Relative Leaf Water Content) and endogenous ABA levels at different stages of observation.
- 2. Weather Data:** As this is a field experiment, the author should incorporate the weather data for the time period to provide a more comprehensive analysis.

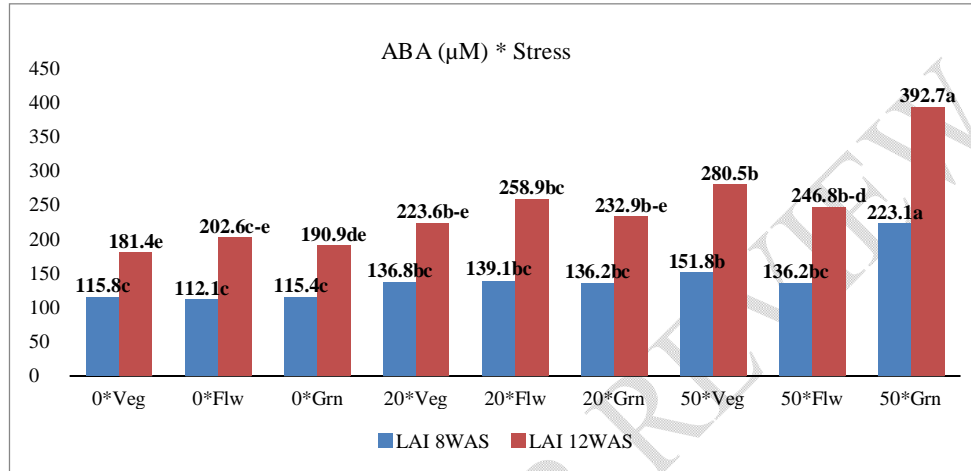
both vegetative to grain filled stage where the highest (275.5) LAI interaction was equally recorded at 50µm ABA at vegetative stage while least (139.4) was recorded at vegetative stage at 0µm ABA in 12WAT Wudil 2022 dryseason.

Table 1: Effect of Fish Guano and Abscisic Acid Concentrations on Leaf Area Index per plant of `grain Amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

Treatments	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
	4	8	12	4	8	12	4	8	12	4	8	12
<u>Stress (S)</u>												
Vegetative	76.2	94.0	114.1	154.0	153.3	218.0	64.7	100.0	130.1	96.7	133.5	178.0
Flowering	78.1	102.0	118.8	204.0	143.8	245.0	68.6	99.0	129.8	81.4	132.7	170.9
Grain Filling	84.1	136.0	116.1	104.0	126.7	195.0	67.9	101.0	129.7	113.4	156.0	196.8
p-value	0.351	0.545	0.869	0.438	0.539	0.568	0.826	0.954	0.999	0.070	0.225	0.593
SE±	3.55	26.5	6.25	49.3	15.82	30.9	4.60	4.48	6.82	6.79	8.92	17.32
<u>Fish Guano (F) (kg)</u>												
0	75.8	100.0	114.9	237.0	142.0	191.0	64.2	100.5	130.3	90.5 ^b	134.8 ^b	169.3
0.1	80.4	100.0	116.7	109.0	141.9	221.0	65.6	97.8	125.4	87.2 ^b	129.1 ^b	174.9
0.2	82.2	132.0	117.4	115.0	140.0	246.0	71.5	101.7	134.0	113.8 ^a	158.3 ^a	201.6
p-value	0.280	0.465	0.874	0.235	0.989	0.343	0.271	0.672	0.250	0.008	0.011	0.053
SE±	2.86	20.6	3.49	59.0	10.63	26.1	3.33	3.15	3.61	6.27	6.96	9.77
<u>ABA (A) (µM)</u>												
0	64.9 ^a	78.0 ^b	90.8 ^c	237.0	107.4 ^b	186.0	50.4 ^a	77.3 ^a	98.8 ^a	83.9 ^b	114.4 ^a	141.9 ^a
20	80.1 ^b	100.8 ^{ab}	118.6 ^b	109.0	150.2 ^a	221.0	69.4 ^b	105.5 ^b	133.0 ^b	94.1 ^b	137.4 ^b	176.6 ^b
50	93.5 ^a	153.1 ^a	139.5 ^a	115.0	166.3 ^a	253.0	81.5 ^a	117.3 ^a	157.8 ^a	113.6 ^a	170.4 ^a	227.2 ^a
p-value	<.001	0.039	<.001	0.394	<.001	0.203	<.001	<.001	<.001	0.006	<.001	<.001
SE±	2.86	20.6	3.49	59.0	10.63	26.1	3.33	3.15	3.61	6.27	6.96	9.77
<u>Interaction</u>												
S*F	0.340	0.408	0.638	0.747	0.923	0.459	0.784	0.700	0.631	0.865	0.393	0.184
S*A	0.292	0.481	0.105	0.275	0.817	0.665	0.777	0.265	0.726	0.175	0.007	0.048
F*A	0.574	0.303	0.234	0.726	0.711	0.286	0.244	0.142	0.163	0.039	0.002	0.004
S*F*A	0.364	0.633	0.297	0.318	0.196	0.479	0.976	0.924	0.858	0.973	0.710	0.707

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

Figure 1: Interaction of Stress x Abscisic Acid on Leaf Area Index of grain Amaranth at 8 and 12 WAT at Wudil during 2022 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

3.2 Photosynthetic active radiation (PAR)

The result of FG and ABA concentrations on PAR of grain amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry season are presented in Table 2. The stressed group revealed that at BUK in 2021 season, there were no significant ($p > 0.05$) differences of PAR at 4, 8 and 12 WAT while in 2022 season, the result showed significant ($p < 0.05$) difference at 4 and 12 WAT on PAR of grain amaranth. It was recorded that 637.1 was the highest PAR obtained at grain filling condition at 4 WAT with 444.9 as the lowest PAR recorded at flowering condition. At 12 WAT, in 2021 season at BUK, 44.50 was the highest PAR value while 6.93 was the lowest value recorded. At Wudil, the result showed clearly that there were no significant ($p > 0.05$) differences at 12 WAT across the 2021 and 2022 seasons. The FG group indicated that from 4 to 12 WAT at BUK 2021 season, there were no significant ($p > 0.05$) differences. Similar, with the exception ($p < 0.05$) of 8 WAT, there were no statistical ($p > 0.05$) differences. At 8 WAT, grain amaranth give highest PAR value at 0 kg fish guano (35.95) followed by 0.1 kg (34.67) while lowest PAR value were recorded at 0.2 kg fish guano concentration level. The effect of fish guano on PAR at Wudil in both 2021 and 2022 seasons revealed that there were no significant ($p > 0.05$) differences in grain amaranth PAR across 4, 8 and 12 WAT. In this study it was clearly shown that ABA

concentration level had no significant ($p > 0.05$) responses on grain amaranth PAR at BUK and Wudil in both 2021 and 2022 dry season despite all numerical variations.

Table 2: Effect of Fish Guano and Abscisic Acid Concentration on Photosynthetic Active Radiation ($\mu\text{mol s}^{-1}$) per plant of grain amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

Treatments	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	0.59 6	0.458 2	0.61 2	575.7 ^a	36.09	44.50 ^b	495.0	467. 0	539.0	324.0	562. 0	525.0
Flowering	0.47 8	0.305 7	0.46 7	444.9 ^b	34.31	6.93 ^b	714.0	502. 0	521.0	232.0	522. 0	535.0
Grain Filling	0.64 9	0.405 0	0.49 0	637.1 ^a	33.75	43.61 ^a	578.0	553. 0	556.0	328.0	529. 0	483.0
p-value	0.32 7	0.560 2	0.11 2	0.008	0.327	<.001	0.304	0.64 2	0.729	0.591	0.70 2	0.554
SE \pm	71.7	94.8	39.2	21.90	0.996	1.806	86.4	61.5	29.3	69.7	34.4	33.4
Fish Guano (F) (kg)												
0	0.55 1	0.354 8	0.48 8	569.1	35.95 ^a	32.26	519.0	515. 0	452.0	246.0	537. 0	517.0
0.1	0.66 3	0.348 6	0.53 6	527.7	34.67 ^{ab}	31.81	544.0	401. 0	608.0	312.0	566. 0	540.0
0.2	0.50 9	0.465 4	0.54 4	560.8	33.53 ^b	30.97	724.0	606. 0	556.0	326.0	511. 0	486.0
p-value	0.22 2	0.340 1	0.74 1	0.144	0.032	0.710	0.103	0.09 9	0.280	0.569	0.24 1	0.138
SE \pm	64.0	62.9	55.2	15.41	0.628	1.112	72.4	66.0	69.2	56.1	22.8	19.2
ABA (A) (μM)												
0	0.59 9	0.369 8	0.48 8	556.6	34.53	31.97	574.0	484. 0	570.0	313.0	553. 0	520.0
20	0.58 1	0.448 2	0.56 2	525.8	35.18	31.46	558.0	541. 0	583.0	321.0	522. 0	509.0
50	0.54 3	0.351 8	0.51 8	575.2	34.44	31.61	655.0	497. 0	463.0	250.0	539. 0	514.0
p-value	0.82 4	0.518 2	0.64 2	0.083	0.661	0.945	0.597	0.81 4	0.411	0.624	0.62 9	0.923
SE \pm	64.0	62.9	55.2	15.41	0.628	1.112	72.4	66.0	69.2	56.1	22.8	19.2
Interaction												
S*F	0.22 4	0.716 8	0.08 8	0.155	0.994	0.738	0.033	0.25 8	0.547	0.353	0.78 6	0.474
S*A	0.12 7	0.991 5	0.12 5	0.091	0.983	0.858	0.610	0.71 4	0.823	0.398	0.96 4	0.505
F*A	0.79 8	0.456 0	0.50 0	<.001	0.418	0.537	0.834	0.32 7	0.650	0.240	0.94 8	0.771
S*F*A	0.17 4	0.369 9	0.54 9	0.254	0.840	0.065	0.326	0.76 8	0.570	0.607	0.96 7	0.415

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

3.3 Relative Water Content (RWC)

The result on the effect of FG and ABA concentration on RWC of grain amaranth under moisture stress conditions at BUK and Wudil are presented in Table 3. The result on the effect of stress on RWC of grain amaranth indicated that there was significant ($p < 0.05$) differences at 4 and 12 WAT. At 12 WAT highest RWC was obtained at flowering stage with 71.7%. There were no significant ($p > 0.05$) differences of RWC at BUK in 2022 season. Moreover, there were no significant ($p > 0.05$) difference of RWC of grain amaranth at Wudil in 2021 and 2022 under different stress condition. The result on RWC of grain amaranth in FG group revealed significant ($p < 0.05$) differences at 4, 8 and 12 WAT at BUK in 2021. At 4 WAT, 48.29% was the highest RWC at 0.2 kg fish guano while 40.09% was the lowest at 0 kg FG concentration level. There were linear increases of RWC with increase concentration levels of fish guano at 8 and 12 WAT where 58.92 and 71.2% were the highest RWC obtained at 0.2 kg FG for 8 and 12 WAT respectively. In 2022 at BUK, the result revealed significant ($p < 0.05$) difference at 12 WAT with the highest RWC of 50.2% at 0.2 kg fish guano concentration level. At Wudil, the result showed that fish guano has no significant ($p > 0.05$) effect on RWC of grain amaranth at 2021 and 2022. The result on the effect of ABA concentration on RWC of amaranth showed that with the exception of 12 WAT ($p < 0.05$) there were no significant ($p > 0.05$) difference at both BUK and Wudil in 2021 and 2022 season respectively. At 12 WAT, at BUK in 2022, the result indicated the highest (70.5) RWC of grain amaranth. The result further revealed significant ($p < 0.05$) interactions between FG and ABA at 8 WAT at BUK in 2021 season. The effect of FG and ABA interaction on RWC at 8 WAT at BUK in 2021 dry season is presented in Figure 2. The result indicated clearly that highest RWC (66.45) was recorded at 0.2 kg FG x 50 μ M ABA followed by 57.00 at 0.2 kg FG x 20 μ M ABA while least (47.39) was recorded at 0 kg FG x 0 μ M ABA.

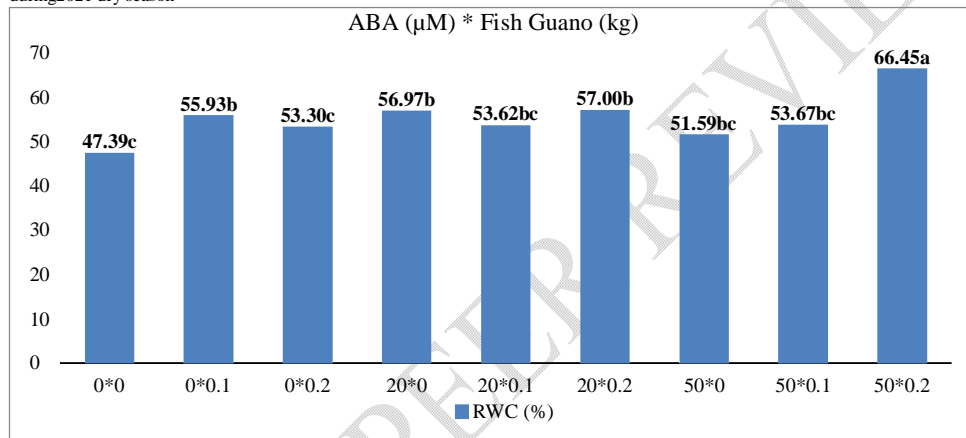
Table 3: Effect of Fish Guano and Abscisic Acid Concentrations on the Relative Water Content (%) per plant of grain amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

Treatments	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	44.50 ^a	52.90	65.7 ^b	29.8	25.13	41.4	47.5	26.0	38.4	47.5	26.0	38.4
Flowering	47.15 ^a	57.99	71.7 ^a	33.3	27.40	47.6	57.0	24.5	34.9	57.0	24.5	34.9
Grain Filling	40.73 ^b	54.41	64.4 ^b	33.3	28.96	47.4	50.2	33.6	37.6	50.2	32.5	37.6
p-value	0.018	0.085	0.035	0.69	0.762	0.480	0.504	0.50	0.771	0.504	0.60	0.771
SE \pm	0.896	1.184	1.33	3.17	3.566	3.74	5.44	5.39	3.49	5.44	5.64	3.49
Fish Guano (F) (kg)												
0	40.09 ^a	51.98 ^b	61.9 ^b	30.5	26.24	38.0 ^b	50.8	25.6	35.5	50.8	25.6	35.5
0.1	44.00 ^b	54.40 ^b	68.7 ^a	34.1	28.06	48.2 ^a	50.5	29.7	39.1	50.5	28.6	39.1
0.2	48.29 ^a	58.92 ^a	71.2 ^a	31.9	27.18	50.2 ^a	53.3	28.7	36.3	53.3	28.7	36.3
p-value	<.001	0.008	0.002	0.39	0.733	0.039	0.814	0.54	0.459	0.814	0.67	0.459
SE \pm	1.362	1.511	1.76	1.85	1.630	3.50	3.38	2.68	2.10	3.38	2.78	2.10
ABA (A) (μM)												
0	41.89	52.20	64.2 ^b	30.9	24.85	39.9	49.9	29.2	34.5	49.9	29.2	34.5
20	44.26	55.86	67.0 ^b	30.5	29.25	48.7	53.3	31.8	38.4	53.3	30.7	38.4
50	46.23	57.24	70.5 ^a	35.1	27.38	48.0	51.5	23.0	38.0	51.5	23.0	38.0

p-value	0.089	0.061	0.049	0.161	0.170	0.159	0.767	0.068	0.364	0.767	0.129	0.364
SE±	1.362	1.511	1.76	1.85	1.630	3.50	3.38	2.68	2.10	3.38	2.78	2.10
Interaction												
S*F	0.881	0.718	0.948	0.225	0.810	0.748	0.949	0.149	0.430	0.949	0.268	0.430
S*A	0.131	0.147	0.188	0.093	0.773	0.568	0.898	0.594	0.672	0.898	0.794	0.672
F*A	0.226	0.011	0.145	0.779	0.083	0.673	0.614	0.436	0.945	0.614	0.405	0.945
S*F*A	0.916	0.975	0.500	0.590	0.861	0.995	0.590	0.906	0.307	0.590	0.925	0.307

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

Figure 2: Fish Guano x Abscisic Acid interaction on Relative Water Content (%) of grain Amaranth at 8WAT at BUK during 2021 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

3.4 Stomatal Conductance

The result on the effect of FG and ABA concentrations on stomatal conductance of grain amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry season is presented in Table 4. The stress group indicated that there were significant ($p < 0.05$) difference on stomatal conductance of grain amaranth at BUK 2021 season at 4WAT and 12WAT. At 4WAT the result showed a linear increase of stomatal conductance with advancing of stress condition from vegetative to grain filled stage. At 2022 season in BUK the result however showed significant ($p < 0.05$) differences at 12WAT where grain filling had a very high (637.1) stomatal conductance followed by vegetative condition (575.7). At Wudil, the result showed that at 4WAT and 12WAT, there were significant ($p < 0.05$) differences in which the highest (44.67) stomatal conductance was found in grain filling while lowest stomatal conductance (5.51) was recorded at vegetative condition in 4WAT. At 12WAT highest stomatal conductance were found at vegetative stage (61.8) with the least (7.5) at grain filling at 2021 season.

Table 4: Effect of Fish Guano and Abscisic Acid Concentrations on Stomatal Conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) per plant of grain amaranth under moisture stress conditions at BUK and Wudil during 2021 and 2022 dry seasons.

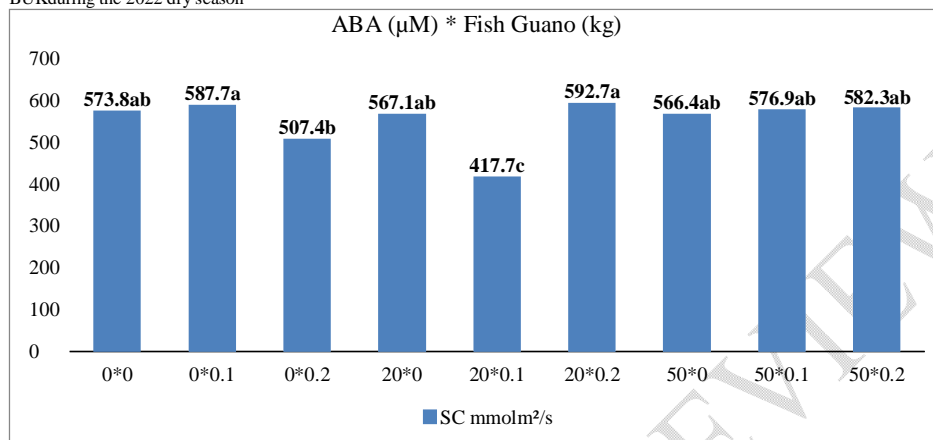
BUK Wudil

Treatments	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	5.83 ^b	37.0	63.8 ^a	74.7	30.47	575.7 ^a	5.51 ^b	37.9	61.8 ^a	10.8	37.7	61.4 ^a
Flowering	46.90 ^b	17.9	50.7 ^b	74.4	30.63	444.9 ^b	42.12 ^a	44.0	49.5 ^a	14.1	44.1	50.0 ^b
Grain Filling	47.64 ^a	51.0	8.1 ^c	74.0	31.08	637.1 ^a	44.67 ^a	23.8	7.5 ^c	6.9	23.8	7.50 ^b
p-value	<.001	0.336	<.001	0.99	0.727	0.008	0.001	0.70	<.001	0.697	0.71	<.001
SE±	2.336	13.81	3.21	3.88	0.537	21.90	2.954	16.7	3.58	5.73	16.7	3.59
Fish Guano (F) (kg)												
0	33.37	33.8	41.9	76.6	30.53	569.1	30.44	35.4	41.0	15.0	35.4	41.1
0.1	33.13	37.0	40.4	74.8	30.36	527.7	32.13	35.5	38.3	9.8	35.5	38.4
0.2	33.88	35.1	40.2	71.6	31.29	560.8	29.74	34.8	39.5	7.1	34.8	39.5
p-value	0.935	0.512	0.812	0.32	0.232	0.144	0.534	0.96	0.658	0.201	0.96	0.660
SE±	1.483	1.94	2.01	2.34	0.405	15.41	1.540	1.99	1.95	3.15	1.98	1.95
ABA (A) (µM)												
0	33.53 ^{ab}	34.6	41.3	73.7	30.50	556.6	28.79	35.6	40.0	8.9	35.6	40.0
20	36.27 ^a	35.2	41.6	73.5	30.86	525.8	30.41	34.4	40.2	12.0	34.5	40.2
50	30.57 ^b	36.1	39.6	75.9	30.81	575.2	33.11	35.7	38.7	11.0	35.7	38.7
p-value	0.032	0.846	0.759	0.73	0.797	0.083	0.146	0.87	0.831	0.774	0.87	0.831
SE±	1.483	1.94	2.01	2.34	0.405	15.41	1.540	1.98	1.95	3.15	1.98	1.95
Interaction												
S*F	0.686	0.306	0.873	0.05	0.765	0.155	0.218	0.63	0.727	0.786	0.63	0.727
S*A	0.608	0.439	0.933	0.34	0.214	0.091	0.532	0.07	0.754	0.512	0.07	0.754
F*A	0.908	0.929	0.368	0.52	0.157	<.001	0.545	0.73	0.321	0.252	0.73	0.321
S*F*A	0.117	0.861	0.143	0.73	0.313	0.254	0.215	0.56	0.080	0.423	0.56	0.080

Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

At Wudil, in 2022, the result revealed that there were no significant ($p>0.05$) differences on stomatal conductance at 4 and 8WAT while significant ($p<0.05$) difference was recorded at 12WAT in which vegetative state had the highest (61.8) followed by 49.5 while the least was recorded at grain filled condition with 7.50 stomatal conductance in 2022. The result on the effect of FG on stomatal conductance of grain amaranth revealed no significant ($p>0.05$) differences at BUK and Wudil at 2021 and 2022 seasons. In responses to the effect of ABA the result showed that there were only significant ($p<0.05$) differences at 4WAT at BUK in 2021 while others no significant ($p>0.05$) differences on stomatal conductance were observed in both BUK 2022 and Wudil 2021 and 2022 seasons. The interaction result indicates significant ($p<0.05$) interactions of fish guano and ABA at 12WAT at BUK in 2022 season. The effect of fish guano x ABA interaction on stomatal conductance at 12 WAT at BUK during 2022 dry season is presented in Table 9. The result revealed that there were higher ($p<0.05$) stomatal conductance interaction between 0.2kg FG and 20µM ABA (592.7) followed 0.1kg FG concentration and 0µM ABA (587.7) while lowest stomatal conductance interactions were recorded at 0.1kg FG x 20µM ABA concentration level.

Figure 3: Fish Guano x Abscisic Acid interaction on stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) of grain Amaranth at 12WAT at BUK during the 2022 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

4 Discussion

Stress had significant effect on the SC of grain amaranth at BUK and Wudil in 2021 and 2022. The significant increases may be attributed to external influences of environmental factors that are responsible for certain stress like air temperature rises will increase SC, independent of plant water status and photosynthesis (Jeangueninet *et al.*, 2016). Light may be the primary external factor that determines SC as stomata are activated and open during daylight; maybe due to humidity since stomata will open in high humidity even if the leaf water content is less or may be due to soil water and nutrient status which will also impact stomatal conductance, which will be high when soil moisture is high and decreases when soil moisture is less (Buckley, 2019). The PAR was significantly affected by stress at BUK in 2022. Photosynthesis in plants is inhibited due to a loss of balance between the production of reactive

oxygen species and antioxidant defense (Fu and Huang 2001; Reddy *et al.*, 2004). Due to O₂ plant organelles and cytoplasmic activities take place at about a normal rate which helps plants to perform well in terms of growth, photosynthesis and assimilate partitioning to grain filling (Ludlow and Muchow 1990; Subbarao *et al.* 2000).

The capacity of plants to utilize the light absorbed by them declines significantly when they are exposed to environmental stresses such as drought, salinity, low temperature and high light intensity (Dubey 1997; He *et al.*, 1995). Stress showed a significant effect on the RWC of grain amaranth at BUK. During stomatal closure, decreased gas exchange results in the reduction of photosynthate production while decreased transpiration can reduce water loss from leaves (Suzuki *et al.* 2013; Mittler and Blumwald, 2015). In drought conditions, ABA alteration of guard cell ion transport promotes stomatal closure and prevents stomatal opening, reducing water loss (Kim *et al.*, 2010). In sunflower water shortage at the flowering stage reduced yield by 29 % (Velue and Palanisami, 2001).

Leaves are the major eco-physiological parts of a plant that interact with the atmosphere (Addai and Alimiya, 2015) and FG had a significant effect on the LAI of grain amaranth at Wudil 2022. These significant differences

Comment [DP4]: In this experiment, the extent of induced moisture stress is unclear, which is why the leaf ABA content prior to exogenous ABA application is not well-defined. It is essential to determine the ABA content in the leaf at the time of moisture stress to interpret the results accurately

observed from the LAI due to the foliar application of FG tea fertilizer to the leaves were in agreement with the assertions of Davies (2009) who stated that FG is a high-quality organic fertilizer that contains natural ingredients that can promote vigorous growth for outdoor vegetables, flowers, fruit, trees, ornamental plants, and indoor house plants. The PAR and RWC were significantly affected by FG at BUK. Fish guano deposition and absorption by the leaves may have altered some cellular functions of the plant which causes the stomata to close, as in the water deficit case (Lassouane *et al.*, 2013).

The concentration of ABA significantly affects the SC and the RWC of grain amaranth at BUK and the LAI of grain amaranth at both locations. Similar results have been reported by Goreta *et al.* (2007) reported that reductions in SC by ABA enabled the maintenance of leaf water potential and prevented increases in electrolyte leakage and leaf abscission. It is well documented that ABA acts as a stress signal, which triggers adaptive changes in the physiology and morphology of plants (Taiz and Zeiger, 2002). These findings may be linked to the concentration of the ABA in the xylem sap (Carminati and Javaux, 2020) or may be further attributed to the internal influences (plant-level factors) such as signals from the guard cell and stomatal density, changes in leaf water potential, need to take CO₂ for photosynthesis or association with arbuscular mycorrhizal fungi which form symbiotic associations with 80% of plant species, can change SC (Kirkham, 2014).

In drought conditions, ABA alteration of guard cell ion transport promotes stomatal closure and prevents stomatal opening, reducing water loss (Kim *et al.*, 2010). Berkowitz and Rabin (1988) found that bell pepper seedlings dipped entirely in 1-mM ABA solution had higher stomatal resistance and leaf water potential than untreated seedlings after transplanting. It is also known that ABA is involved in the inhibition of leaf growth (Van Volkenburgh, 1999). Several studies reported that restricted leaf expansion was correlated with ABA increases in xylem sap (Ismail *et al.*, 2002) or leaves (Alves and Setter 2000). A reduction in the leaf area of sunflowers has been observed under a continuous 30-day drought period, ending at anthesis rather than full irrigation (Hammadeh *et al.*, 2005). In recent results on spinach production, a foliar application using S-ABA ConTego effectively held leaf size at its marketable value even before the harvesting period (Racsko *et al.*, 2014).

The exogenous application of ABA affected water use efficiency more in the droughty plants than well-watered plants of jewelweed (Heschel and Hausmann 2001). Similar results have been reported by Goreta *et al.* (2007). The relative water content of grain amaranth obtained in this study was similar to those reported by Cechiet *al.* (2022) that foliar sprays of ABA applied before withholding water to muskmelon seedlings improved maintenance of leaf water potential and RWC thus minimizing dehydration-induced damage to membranes. In addition to stomatal closure, ABA is involved in the inhibition of leaf expansion which can reduce plant water use by limiting increases in transpiration area (Alves and Setter, 2000; Bacon *et al.*, 1998; He and Cramer, 1996).

5 Conclusion

The use of Abscisic acid at concentrations of 50 μM and 0.2 kg Fish Guano plays a vital role in regulating plant growth and development, especially in conditions of water scarcity. The combined application of fish guano and ABA demonstrated noteworthy effects on water relations grain amaranth plant exposed to stress conditions at different stages, including the vegetative, flowering, and grain-filling phases. These findings highlight the potential

Comment [DP5]: Subscript it

Comment [DP6]: Add comma

of fish guano and ABA as valuable tools for enhancing the resilience and performance of grain amaranth crops in water-limited environments.

Comment [DP7]: The conclusion section should be written in a more scientific manner

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