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Vegetative Growth and Yield Response of *Amaranthus cruenthus* to Arbuscular Mycorrhizal Fungi (AMF), Poultry Manure (PM), Combination of AMF-PM and Inorganic Fertilizer

C. Nwangburuka Cyril[1*], O. Oyekale Kehinde[1], A. Denton Olanrewaju[1], S. Daramola David[1] and Aderemi-Williams Olugbenga[1]

[1]Department of Agronomy and Landscape Design, School of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, PMB, 21244 Ikeja, Lagos, Nigeria.

Authors' contributions

This research was conducted with considerable collaboration among the authors. Authors CNC and AWO were involved in conception, design, formulation of protocol and writing the first draft of the manuscript. Authors OOK, ADO and SDD participated in designing the experiment, statistical analysis and interpretation as well as reading through the manuscript. All authors also read and endorsed the final manuscript.

Original Research Article

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ABSTRACT

The vegetative growth and yield response of five accessions of *Amaranthus cruenthus* to treatments of poultry manure (PM), arbuscular mycorrhizal fungi (AMF) consisting of a mixture of *Glomus* and *Acaulospora* species, combination of AMF-PM, and NPK, was evaluated at the teaching and research farm of School of Agriculture and Industrial Technology, Babcock University, between January and March 2013. The experiment was a randomized complete block design, with three replications. Data was collected on five vegetative and yield related characters. The combined analysis of variance showed significant treatment, accession and accession X treatment interaction effect, on all the characters evaluated at 0.01 and 0.05 probabilities. PM gave significantly highest total leaf weight per plant (2.483g), total root weight per plant (5.68g) and plant weight at six weeks (16.22g), while AMF-PM gave significantly highest plant height at six weeks (29.48cm) and produced leaf size that had no significant difference with NPK,

**Corresponding author: Email: cykem2001@yahoo.com, kenoye3@yahoo.com;*

Suggesting that AMF-PM could be an alternative to NPK. Furthermore accession BUAM 004 performed best in the entire yield characters evaluated and can be considered for yield improvement in *Amaranthus* while accession BUAM 005 was the poorest of the accessions evaluated.

Key word: Leaf size; perennial; Glomus; Acaulospora; plant height; accession.

1. INTRODUCTION

Amaranthus, is a cosmopolitan genus of annual or short-lived perennial plant [1]. Approximately 60 species are recognized, with inflorescences and foliage ranging from purple and red to green or gold. Members of this genus share many characteristics and uses with members of the closely related genus Celosia. Although several species are often considered weeds, people around the world value amaranths as leaf vegetable, cereal, and ornamental. It is also known as "love lies bleeding", In Nigeria it is known as '*tete*'. Amaranthus shows a wide variety of morphological diversity among and even within certain species. Although the family (*Amaranthaceae*) is distinctive, the genus has few distinguishing characters among the species. This complicates the taxonomy of *Amaranthus*, which has generally been considered among systematists as a "difficult" genus [2].

The genus *Amaranthus* has been classified into two subgenera on the basis of their sexual system [3]. These two subgenera are grouped into monoecious and dioecious species. Although this classification was widely accepted, further intrageneric classification was needed to differentiate this widely diverse group. Currently, *Amaranthus* includes three recognized subgenera and 60 species, although species numbers are questionable due to hybridization and species concepts. Intrageneric classification focuses on inflorescence, flower characters and whether a species is monoecious/ dioecious, as in the Sauer [3] suggested classification. A modified intrageneric classification of *Amaranthus* was published by Mosyakin & Robertson [4] and includes three subgenera: *Acnida, Amaranthus,* and *Albersia.* The taxonomy is further differentiated by sections within each of the subgenera Amaranth greens. Cooked amaranth leaves are a good source of vitamin A, vitamin C, and foliate; they are also a complementing source of other vitamins such as thiamine, niacin, and riboflavin, plus some dietary minerals including calcium, iron, potassium, zinc, copper, and manganese [5]. Cooked amaranth grains are a complementing source of thiamine, niacin, riboflavin, foliate, and dietary minerals including calcium, iron, magnesium, phosphorus, zinc, copper, and manganese - comparable to common grains such as oats and others. Amaranth seeds contain lysine, an essential amino acid, limited in other grains or plant sources [6]. Most fruits and vegetables do not contain a complete set of amino acids, and thus different sources of protein must be used.

Vegetable crops production in West Africa, including *Amaranthus* has been plagued with an array of factors such as poor farm input, poor cropping system, incidence of pest and diseases as well as poor soil productivity [7]. However, poor soil productivity status has accounted significantly for high poor performance in crop yield [8]. Donava and Casey [9] reported that constant nutrient replenishment in the soil through the use of fertilizers, or sustainable agronomic practices, soil nutrient decline will be continuous and this critical for *Amaranthus* as a vegetable crop.

Most frequently, inorganic fertilizers have been employed as soil amendment for crop yield increase [9]. Currently there is an increase in the demand for inorganic fertilizer (NPK) to ameliorate the soil nutrient status and enhance crop production [7]. This no doubt has consequently increased the production cost and ultimately will affect the cost of food produced in the market. Furthermore, inorganic fertilizers are also associated with many other adverse effects on the soil and crop such as increase in soil acidity, increase in greenhouse effect [10]. It also induces cytological defects and chromosomal abnormalities in crop cells [11]. Today, there is an advocacy for the use of organic fertilizers in agriculture [7,10].

Organic manure is an important source of nutrient for most plants especially fruits and vegetable. However, organic manure in most cases serves as secondary host for crop pathogens. Furthermore, application of poultry manure a type of organic manure increased soil carbon, organic nitrogen and exchangeable calcium thereby resulting to a significant pH increase, but has slow release of macro nutrients most especially phosphorous [12], Poultry manure is most valued of all the manure produced by livestock it has been historically used as a source of plant nutrient and as soil amendment. On the other hand, arbuscular mycorrhizal fungi is a type of plant-fungus symbiotic association in which the fungus penetrates the cortical cells of the roots of a vascular plant. Arbuscular mycorrhizal fungus (AMF) help plants to capture nutrients such as phosphorus, sulphur, nitrogen and micronutrients from the soil. Recent reports by Olawuyi et al. [13] and Garmendia and Mangas [14] have implicated arbuscular mycorrhizal fungi in the increased yield of cucumber and rose plant respectively. There are other reports of symbiotic microorganisms the Nigerian soil. For instance Daramola et al. [15] reported bacteria of the genus *Bradyrhizobium* are known to infect the root hairs to cause nodulation. The bacteria inhabit the nodules of host plants and are provided with photosynthate by the host plant to enable the bacteria convert elemental N2 into a form usable by host plant.

The effect of crop animal residue as fertilizer has received much attention in recent years. This beneficial effect of organic materials has been studied by researchers. Although poultry manure promotes and enhances the growth and yield of vegetable plants, not all macro nutrients are readily available for plant intake, and this could bring about slow growth and poor yield. The effect of combined arbuscular mycorrhizal fungi and poultry manure (AMF-PM) in different concentrations has been tested in recent times as a new way guaranteeing efficiency in soil productivity. Recent works of Nwangburuka et al*.* [7,16] have demonstrated huge prospects in this direction in enhancing okra and *Corchorus* fruit and leafy vegetable yield and have recommended further investigation using other crops. Similarly, recent reports have indicated that AMF treatment produces significant increase in yield when AMF treatment comprises many species of arbuscular mycorrhizal fungi than only contains one species of mycorrhizal fungus [17].

This work is aimed at evaluating the effect of single application of poultry manure, NPK, AM (composed of *Glomus* and *Acaulospora* species) and combined AMF-PM treatment on the vegetable (leafy) yield and growth of *Amaranthus* species.

2. MATERIALS AND METHODS

This experiment was conducted at Babcock University Teaching and Research farm during the period between late January to early March, 2013. Five accessions of *Amaranthus* collected from the Department of Agronomy and Landscape Design, Babcock University germplasm was used for the study. The accessions were: BUAM 001, BUAM 002, BUAM

003, BUAM 004 and BUAM 005. The Poultry manure used for the study was collected from Babcock University Poultry Farm located at Ilara, Ogun state. The poultry droppings from layer birds were collected wet and sun-dried to reasonable moisture content of 12-15%. Samples of 500g each of poultry manure and soil each were taking from experimental area for routine chemical analysis in the laboratory using standard method described by IITA [18], where micro and macro element of the samples were determined. The soil textural properties were also determined using standard methods as described by IITA [18]. The result of the poultry manure and soil analysis is displayed in (Table 1).

Table 1. Chemical analysis of soil and poultry manure sample

Sample	**N (%)**	**Ca (%)**	**Mg (%)**	**K (%)**	**Na (%)**	**P (pp)**	**Zn (pp)**	**Cu (pp)**	**Mn (pp)**	**Fe (ppm)**
Soil sample	0.215	2.00	0.46	0.15	0.10	7.60	8.61	1.36	73.88	57.15
Poultry manure	3.02	4.98	0.53	1.98	32.3	2.00	44.1	6.48	157.8	194.48

The Arbusclar mycorrhizal fungi treatment consisting of a soil carrier mix *Glomus* (*mosseae, deserticola, intaradices*) and *Acaulospora* (*colossica, scrobicurlata*) was introduced at the time of planting the seeds at a rate as shown below. The experiment was a randomized complete block design with five treatments and three replications; each replication consisting of 5 single row plots

The treatments were:

- A= Arbuscular mycorrhizal fungi (AMF) – (400 kg ha^{-1})
- B= Poultry manure (PM) - (400 kg ha^{-1})
- C= Arbuscular mycorrhizal (AMF) / Poultry manure (PM) - (200 kg ha^{-1}AMF) (200 kg ha^{-1}PM)
- D=$N_{12}P_{12}K_{17}$ fertilizer - (400 kg ha^{-1}NPK)
- E=Control

The treatment was based on the recommendation of the earlier work of Nwangburuka et al. [7,16]. Seed was sown by drilling method on already prepared beds of 1 meter by 1 meter area. Each treatment consisted of 3 rows of 60cm per accession, with spacing of 30cm between rows. Arbuscular mycorrhizal fungi and poultry manure were applied at the day of planting, to ensure early availability of nutrients at the early period of plant life while inorganic fertilizer was applied later; not at the beginning of the experiment. Weeding was done manually by the use of hoe, two weeks after planting.

Data were collected on the following yield characters of *Amaranthus* using destructive sampling at 6 weeks after planting.

- Total leaf weight per plant (fresh)
- Leaf size (length X width) per plant
- Root weight per plant (fresh)
- Plant height at six weeks
- Plant weight at six weeks (fresh)

Data collected were subjected to analysis of variance using Statistical Analysis System (SAS) Microsoft windows 8.0 [19] employing the method outlined by Steel and Torrie [20]. Means were separated using Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

There was significant treatment and accession effect at 0.05 and 0.01 probability in all the traits studied Table 2. The result implies that the treatment was able to influence the expression of the accession in the characters considered; it also suggests variability among the accession, sufficient enough for selection toward crop improvement. The mean performance of *Amaranthus* in five agronomic traits under treatment is shown in Table 3.

Table 2. Means square of variance of response to soil amendment and accession

Source of variance	Degree of frequency	Total leaf weight (g)	Leaf size (cm^2)	Root weight (g)	Plant weight at six weeks(g)	Plant Height t six weeks (cm)
Treatment	4	1795.56**	181.25*	46.36**	191.05**	332.95**
Accession	4	122.39**	3137.78**	25.44**	289.27**	873.41**
Treatment x accession	16	116.61*	1756.13**	21.39**	149.15**	713.31**
Error	50	2.52	61.67	4.32	3.96	10.36
Total	74					

*Key: *Significant at 0.05, **Significant at 0.01,*

Table 3. Means of five soil amendment treatments evaluated for agronomic parameters of *Amaranthus*

Treatments	Total leaf weight(g)	Leaf size (cm^2)	Root weight (g)	Plant weight at six weeks(g)	Plant Height at six weeks(cm)
AMF	0.349b	38.16a	2.84b	8.34d	25.33b
PM	2.483a	30.31c	5.68a	16.22a	22.28c
AMF-PM	0.380b	36.36abc	1.97b	11.98b	29.48a
Inorganic fertilizer	0.363b	36.60ab	1.79b	9.90c	18.98d
Control	0.37b	31.41c	1.24b	7.13d	18.02d

Key: Means with same letter are not significantly different along the column AMF= arbuscular mycorrhizal *fungi, PM= poultry manure,*

Total fresh leaf weight at six weeks (Table 3) ranged from 0.349g to 2.483g, the PM treatment recording the highest significant value of 2.483g, while AMF treatment recording the least value of (0.349g) though not significantly different from the values recorded in the remaining treatments. This result agrees with the report of Nwangburuka et al. [7,16] who reported significantly lower leaf weight in *Corchorus* with AMF treatment, but also reported a significantly higher leaf weight with combined treatment of AMF-PM. The high significant leaf weight recorded in PM treatment, maybe as a result of the high percentage of nitrogen in the PM (Table 1) while the low leaf weight observed in AMF, may be as a result of poor interaction amongst the fungi that contributes to the AMF treatment. This poor interaction could be due to weak or no mutual association between the roots of *Amaranthus* and the AMF species. This could also lead to little or no colonization of the *Amaranthus* root for better absorption of nutrients. This seem to favour the report of Dessai and Rodrigues [21], who suggests that the family *Amaranthaceae* among others does not have mycorrhizal association and therefore does not experience root colonization by AM fungi. Meanwhile, Shwethal et al., [22] had earlier observed variation in AM fungi colonization in *Amaranthus* species. However, Herve Dupre [17] observed that the combinations of more than one AM species produced better effect on crop performance in other crops though not *Amaranthus*.

Similarly, root weight at six weeks (Table 3) followed the same trend as fresh leaf weight ranging from 5.67g to 1.24g. The highest significant root weight value (5.68g) was recorded in PM treatment, followed by AMF treatment (2.84g), however this value is not significantly different from that observed in the control (1.24g) which was the least in this parameter. This unexpected result in the control which had a higher phosphorus level (see Table 1) suggests that the phosphorus in the soil may be in the insoluble form for root absorption and unavailable to the accessions compared to the phosphorus in the PM. Meanwhile, Famuwagun [23], had earlier observed an increase in the root volume of *Cacao* seedling under AMF treatment at 4 weeks. However, the present finding agrees with the report of Bagula et al. [24], who observed that AMF retarded root growth in cassava. Total leaf size (Table 3) ranged between 30.31cm^2 and 38.16cm^2. The recorded leaf size for AMF treatment was (38.16cm^2), which was not significantly different from those obtained, for inorganic fertilizer (36.60cm^2) and the combined treatment of AMF-PM (36.36cm^2), whereas PM treatment recorded the least value of (30.31cm^2). This suggests that AMF treatment and combination of AMF-PM, influences indirectly the photosynthetic capacity of the plant, thereby increasing crop vegetative yield. This agrees with the findings of Nwangburuka et al*.* [25], who observed an increase in leaf size in okra with AMF-PM treatment and AMF sole treatment.

Plant height at six weeks (Table 3) was significantly highest with AMF-PM treatment with mean value of (29.48cm), which was followed by AMF treatment (25.33cm) and PM treatment (22.28cm) whereas the least (18.02cm) was recorded in the control. These values were significantly different from each other. This suggests that combined AMF-PM treatment enhance the height increase in *Amaranthus*, which is positively associated to increased leaf volume and vegetation in *Amaranthus* [26]. This report also agrees with the finding of Nwangburuka et al*.* [7] [16] in their work with okra and *Corchorus*, Famuwagun, [23] in his work with cacao. This result shows that combination of AMF-PM, could adequately replace inorganic fertilizer in *Amaranthus* cultivation. This has already been suggested by Nwangburuka et al. [7].

However, plant weight at 6 weeks (Table 3) ranged from 7.13g to 16.22g. The highest significant value of (16.22g) was recorded for PM tretment, followed by combined AMF-PM treatment with mean value of (11.98g). The least was observed in the control, with mean value of 7.13g. The highest plant height at maturity recorded in PM treatment may be due to high efficiency and economic release of nutrients. This was also enhanced in combined AMF-PM treatment compared to the single treatment of AMF, suggesting a synergy between AMF-PM. This agrees with the finding of Nwangburuka et al. [7]. The combination of AMF-PM seem to eliminate the negative interaction between the arbuscular mycorrhizal species present in the AMF inoculums, leading to a better overall result when compare to AMF sole treatment and inorganic fertilizer treatment.

The mean performance of the five accessions of *Amaranthus* species in five agronomic characters are shown in Table 4. Total leaf weight ranged between 0.19g and 6.99g. Accession BUAM 004 recorded the highest mean leaf weight of 6.99g which was not significantly different from accessions BUAM 001 (6.13g), BUAM 002 (6.78g) and BUAM 003 (6.19g), but significantly different from BUAM 005 with mean value of 0.19g which was least. This suggests that selection for leafy vegetable yield would produce good result when any of the first four accessions is favoured or selected. Accession BUAM 004 recorded the highest mean value in leaf size ($51.88cm^2$), which is significantly higher than the rest. This was followed by accession BUAM 001 with mean value of ($41.07cm^2$), while the lowest leaf size was observed in accession BUAM 005 with the mean value of $13.68cm^2$. This also suggests that accession BUAM 005 may not be a good choice when breeding for leaf yield. Meanwhile root weight per plant (Table 4) ranged from 0.84g to 4.04g. Accession BUAM 002 recorded the highest root weight with a mean value of 4.04g which is not significantly different from that recorded in accessions BUAM 003 (3.69g) and BUAM 004 (2.94g) which were the second and third in the rank, respectively. The least value was observed in accession BUAM 005 (0.84g). This result may suggest that accessions BUAM 002, BUAM 003 and BUAM004 have a high efficiency in water absorption, which will ultimately affect their growth and yield. Plant weight at 6 weeks (Table 4) ranged from 3.34g to 14.30cm, BUAM 004 recorded a significantly higher mean value of 14.30g, which was not significantly different from values obtained in accession BUAM 001(13.79g). However accession BUAM 005 also recorded the least value (3.34g) in the plant weight.

Table 4. Means of five accessions of *Amaranthus spp.* for five agronomic parameters evaluated

Accession	Total leaf weight (g)	Leaf size (cm^2)	Root weight (g)	Plant weight at six weeks(g)	Plant Height at six weeks(cm)
BUAM 001	6.13a	41.07b	1.99bc	13.79a	26.20b
BUAM 002	6.78a	27.89c	4.04a	10.42b	19.35c
BUAM 003	6.19a	38.29b	3.69a	11.77b	26.25b
BUAM 004	6.99a	51.88a	2.93ab	14.30a	30.95a
BUAM 005	0.19b	13.66d	0.84c	3.34c	11.34d

Key: Means with same letter are not significantly different along the column

Plant height at six weeks (Table 4) was significantly highest in accession BUAM 004 with a significant mean value of 30.95cm. This was followed by accessions BUAM 003 (26.25cm) and BUAM 001 (26.20cm) which were not significantly different from each other but significantly different from accessions BUAM 004. BUAM 005 also recorded the least mean value in plant height (11.34cm). Generally this suggests that accession BUAM 005 was poor and least in yield improvement qualities, whereas accession BUAM 004 will make a good parent for leaf yield improvement.

4. CONCLUSION

Treatment of *Amaranthus* with 400kg $ha^{-1}$of poultry manure (PM) gave a better leaf weight yield as well as better root weight and total plant weight. These characters constitute the bulk of economic yield in *Amaranthus.* Combination of AMF-PM performed adequately better than single treatment of AMF and inorganic fertilizer alone and therefore could be considered as a possible alternative to inorganic fertilizer. Combination of *Glomus* (*mosseae, deserticola, intaradices*) and *Acaulospora* (*colossica, scrobicurlata*) fungi species produced repressive interaction on crop performance on the characters observed. Accession BUAM 004 performed best in the entire yield characters studied and can be considered as possible putative parent for *Amaranthus* yield improvement, whereas accession BUAM 005 is the poorest of the accessions of *Amaranthus* considered.

5. LIMITATION OF RESEARCH

Root colonization study will be conducted to ascertain the mechanism involved in the interaction of AMF and AMF-PM in order to ascertain if there was AMF colonization and the extent if there was any. Furthermore, this will provide information of the mechanism surrounding the positive interaction of AMF-PM with *Amaranthus.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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