Nigerian Journal of Soil Science

Journal homepage:www.soilsjournalnigeria.com

Vegetative growth and yield response of *Amaranthus hybridus* to mycorrhizal fungi (*Glomus mosseae*) inoculation, organic and micronutrient fertilizer application

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ARTICLE INFO

Article history:
Received December 25, 2019
Received in revised form December 30,2019
Accepted January 14, 2020
Available online April 29, 2020

Keywords:
Micronutrient,
Amaranthus hybridus,
Mycorrhizal inoculum,
Organic amendment

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https://doi.org/10.36265/njss.2020.300105

ISSN-1597-4488

ABSTRACT

This study was carried out at the Faculty of Agriculture Teaching and Research farm, University of Benin during the early planting season in April 2016 to evaluate the growth response of *Amaranthus hybridus* and some soil physical and chemical properties as influenced by organic and micronutrient fertilizers, mycorrhizal inoculum. In this experiment four treatments (poultry manure + cow dung + pig manure (PCP), poultry manure + mycorrhiza (Pm), poultry manure + mycorrhiza + foliar blend (PmMF) and control (C) were replicated four times and arranged in a randomized complete block design (RCBD) in a plot size of $70m^2$. Soil samples were taken prior and after the trial at a 0-15cm depth for analysis. Results revealed that the soil (sand, silt and clay) and the textural class remained the same (loamy sand) and the micronutrient had no significant effect on the treated plots; however, all the treatments showed a significant increase in the soil pH, organic carbon, organic matter, NPK and Mg and a slight decrease in Ca and Na when compared to the pre-trial soil indicating an increase in residual soil nutrient status. Nutrient content N, P, K, plant height (51.13cm), number of leaves (67.80), stem girth (5.61cm), leaf area ($194.87cm^2$) and yield (7.51 t/ha) were significantly higher in plots treated with PM when compared to other treatments PMF, PCP and control in that order, with control having the lowest values of plant height (19.13 cm), number of leaves (26.00) stem girth (2.80 cm) leaf area (57.76 cm^2) and yield (0.80 t/ha). Also, significantly higher spores, root arbuscules, root vesicle and root hyphae were recorded in PM (66.20,13.60,9.80,13.40) and PMF (74.40,10.50,11.10,10.50) treatment when compared to other treatments PCP and control.

1.0. Introduction

Amaranthus hybridus is a nutritious and leafy vegetable which belongs to the Amaranthaceae family and it is cultivated in most part of Nigeria as a cheap and readily available source of essential proteins, vitamins, minerals and essential amino acid (Akubugwo *et al.,* 2007). The leaves and the soft portions of the shoots are usually boiled in water and cooked with onions tomatoes, oil and or other additives of modern culinary delights and are rich in vitamin A and iron which are lacking in the diets of pregnant women and young children (Masariramb *et al.,* 2012). According to Sanni *et al.*, (2013), deficiencies in vitamins and minerals such as vitamin A and iron in developing countries like Nigeria are widespread with negative consequences on children's growth and development. Therefore there is a need for Nigerians to grow green vegetables like *Amaranthus hybridus* to supply the body with such nutrients.

Most soils in the tropics have low nutrient status because of the parent materials from which they are formed. Thus the production and nutritional values of these vegetables are limited due to the low fertility of native soils in most parts of Nigeria (Oyedeji *et al.,* 2014).

Dependence by farmers on inorganic fertilizers possess a constraint due to unavailability at the right time, high cost of inorganic fertilizers, lack of technical know-how, lack of access to loans and also the high cost of agricultural products (Mofunanya *et al.,* 2014). This negative effect has led to the search for more environmentally friendly means of soil fertility and the use of animal farmyard manure which are rich in the primary plant nutrient and have shown tremendous improvement in soil nutrient content characteristics (Imasuen *et al.,* 2013). Also, the use of mycorrhiza has been seen to enhance plant growth by forming a symbiotic relationship with plant roots and facilitate the uptake of water and nutrient, especially phosphorus which is low in the tropics and in turn benefiting from carbon compounds provided by the plant (Ofili *et al.,* 2014). Micronutrient deficiencies are on the increase in most soils due to continuous use without replenishment. According to Upinder *et al.,* (2016) the current micronutrient application to crops may need to be doubled by 2050 to meet the food demand of the increasing population of the country through intensive cultivation on marginal lands. Awareness has been created through literatures on the importance of *Amaranthus hybridus* as a leafy vegetable in the human diet for many developing countries because of its nutritional importance. Thus the objective of the study was to determine the influence of PM, Cd, Pm + Cd, Mycorrhizal, Mn on some soil physical and chemical properties as well as the growth of *Amaranthus hybridus*.

2.0. Materials and Methods

2.1. Description of the study area

This study was conducted at the Faculty of Agriculture Teaching and Research field, University of Benin, Benin City at the early period of the rainy season April 2016. The study area is located between latitude 6°41'N and longitude 5°63'E at altitude 110m above sea level**.** There are two major seasons the rainy and dry season, with the mean annual rainfall of 2500 mm and average mean temperature of 32°C**.** The site was previously cultivated with maize, and the dominant fallow plant species were *Panicum maximum* and *Mimosa pudica.*

2.2. Source of materials used

Seeds of *Amaranthus hybridus* were obtained locally from Edaiken market, while the mycorrhizal inoculum was from Rubber Research Institute of Nigeria Iyanomo, both in Edo State, Nigeria. The poultry manure was obtained from Ojemai farms at Ugbhioko, pig dung from University of Benin research farm, the cow dung from a nearby cattle ranch close to Capitol at the University of Benin, and the foliar blend were obtained from National Programme for Food Security at Abuja.

2.3. Land preparation, soil sampling and laboratory analysis

Vegetable beds were made, and seeds were sown by broadcasting to obtain seedling. The experimental plot which measured 70m^2 (14 m x 5 m) in size and divided into sixteen (16) subplots of 2 m x 1.25 m. A randomized complete block design (RCBD) was used with four treatments replicated four times. The treatments include control(C), poultry manure, cow dung and pig dung (PCP), poultry and mycorrhiza (PM), poultry, mycorrhiza and Micronutrient Fertilizer (MNF). The rates of application were 12.8 kg, 6.4 kg, 6kg of poultry, pig dung and cow dung were applied 2 weeks before transplanting at a sowing space of 30cm X 15cm. Soil samples were collected randomly before planting and after harvest at a depth of 0-15cm for analysis to determine the soil physical and chemical properties and mycorrhizal spore count, root arbuscules, vesicles and hyphae in the soil.

2.4. Physical and chemical analysis of soil samples

The particle size analysis was done using the particle size fractionation as described by Genrich and Bremner, (1974) while the soil pH was determined in a 1:1 soil to water suspension using a digital pH meter (Mclean 1982). The soils organic matter was calculated by first determining the soil organic carbon, and the values obtained were multiplied by a constant factor of 1.724 to obtain the organic matter. Micro Kjeldahl digestion procedure of Bremner and Mulvaney, 1982 was used to determine the total nitrogen in the soil. Available phosphorus was extracted using the method of Bray and Kurtz, (1945) and the exchangeable cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) was extracted by the method described by Thomas, (1982).

2.5. Data collected on plant parameters

Six individual plants were randomly sampled and tagged per plot to determine their growth parameters at 2, 4, and 6 weeks after transplanting (WAT). The agronomic parameters measured were; the plant height, number of leaves, leaf area and stem girth. The harvesting of the vegetables was done at the height of 10 cm above ground at the end of the sixth week, and the freshly harvested portion was weighed in a digital weighing balance to account for the fresh weight yield. After that, the fresh vegetative part was further wrapped in an aluminum foil paper, placed in an oven at a temperature of 65° c and allowed to dry to a constant weight to account for the dry matter content.

2.6. Statistical Analysis

Data on growth and yield parameters were analyzed using SPSS-Statistical Package. Measurable variables were tested for significance with the one-way analysis of variance (ANOVA) procedure of a Random Complete Block Design (RCBD). The treatment means were compared using Duncan Multiple Range Test at 5% level of significance.

3.0. Results and Discussion

3.1. Organic manure, pre-soil physical and chemical properties, micronutrient

Tables 1 and 2 shows the chemical properties of the organic manure (poultry, pig and cow) and the physical and chemical properties of the soil used for the trial, respectively. For the organic manure, chemical properties revealed high N, P, K, Ca and Mg, but poultry manure recorded the highest N, K but slightly lower in P compared to pig dung and cow dung. The soil of the experimental field was of loamy sand and pH of 4.61. At the same time, the nitrogen (1.10 g/kg) and organic carbon (11.50 g/kg) were lower than the established critical values of 1.5-2.0 g/kg and 20-30 g/kg respectively according to Orhue *et al.,* (2015), exchangeable bases (Ca 2.92, Mg 0.99, Na 0.51, K 0.40) cmol/kg and available Phosphorus (27.97 mg/kg) were higher than the established critical values.

3.2. Pre-soil mycorrhizal spore count analysis and Effect of Treatment on mycorrhizal spore count and root colonization

The mycorrhizal inoculum added to the soil contains 53 mycorrhizal spore counts. The mycorrhizal spore count from pre-trial soil was 15. Table 2 shows the result from post-trial soils: plots treated with mycorrhiza in combination with poultry manure- PM (66.20), PMF (74.40) had more mycorrhizal spore count, root arbuscules, root vesicles and root hyphae when compared with untreated mycorrhiza plots PCP (31.00) and control (19.05) respectively, indicating that the addition of mycorrhiza had a significant effect on the soil which is in agreement with Mahmood *et al.,* (2010).

Table 1: Some chemical properties of the organic manure and micronutrient used for the trial

PARAMETERS	Cow dung	Poultry Manure	Pig dung	Foliar blend	Values (%)
Nitrogen (%)	1.99	2.91	2.43	Boron (B)	0.03
Phosphorus (mg/kg)	820.7	1549.2	1598.3		
Potassium (cmol/kg)	7.70	12.80	12.80	Cobalt (Co)	0.002
Calcium (cmol/kg)	13.49	155.59	155.59		
Magnesium (cmol/kg)	21.70	53.50	53.50	Iron (Fe)	0.10
pH	6.6	6.1	6.9		
				Manganese (Mn)	0.10
				Molybdenum (Mo)	0.002
				Zinc (Zn)	0.05

Means with same letters along the column are not significantly different at 5% level of probability

Table 2: Some physical and chemical properties of the soil before and after trial

Treatment	Sand	Silt	Clay	pH	Org. Carbon	N	P	Ca	Mg	K	Na	Ex. Acidity	ECEC
	……….g/kg…… ….			($H_2$0)	……..g/kg……		mg/kg	……………….cmol/kg…………………………					
					Pre-trial soil								
	851.3	18.7	148.5	4.61	11.5	1.1	27..97	2.92	0.99	0.40	0.51	0.6	5.72
					Post- trial soil								
Control	821[a]	20[a]	159[a]	5.5[a]	13.4[a]	4.2[b]	20.8[c]	0.9[a]	1.3[a]	1.11[a]	0.10[d]	0.8[a]	4.21[a]
PCP	791[a]	21[a]	188[a]	5.5[a]	14.9[a]	7.5[a]	45.3[a]	0.7[a]	0.9[a]	1.08[a]	0.20[c]	0.8[a]	3.68[b]
PM	796[a]	20[a]	184[a]	5.4[a]	14.8[a]	7.0[a]	38.3[ab]	0.5[a]	0.9[a]	0.88[b]	0.32[b]	0.5[a]	3.10[b]
PMF	801[a]	20[a]	179[a]	5.6[a]	13.7[a]	7.2[a]	35.7[ab]	1.1[a]	0.7[a]	1.12[a]	0.40[a]	0.9[a]	4.22[a]

Means with same letters along the column are not significantly different at 5% level of probability

Figures 1 to 5 shows the effect of treatments on plant height, number of leaves, stem girth, leaf area and yield respectively. There was a significant difference (P<0.05) on the effect of treatments on yield, plant height, number of leaves, stem girth, leaf area throughout the experiment when compared with control and this was seen with the increase in the age of the plant. The highest number of

Table 2: Pre-soil mycorrhizal spore count analysis and Effect of Treatment on mycorrhizal spore count and root colonization

Treatment	Spores	Root arbuscules	Root vesicles	Root hyphae
Pre-soil	15.00			
Control	19.05^{d}	6.00^{c}	7.20^{b}	8.40^{c}
PCP	31.00^{c}	8.50^{bc}	5.70^{c}	9.20^{bc}
PM	66.20^{b}	13.60^{a}	9.80^{ab}	13.40^{a}
PMF	74.40^{a}	10.50^{b}	11.10^{a}	10.50^{b}

Means with same letters along the column are not

leaves, stem girth and leaf area were recorded in plots treated with PM for the 6WAT when compared with other treatments. For yield PM with 7.5 t/ha also recorded the highest yield of *Amaranthus hybridus,* when compared to other treatments and control Figure **6**, this is an indication that organic manure application among other cultural practices increases vegetable yield in poor tropical soils (Law-Ogbomo *et al*., 2009).

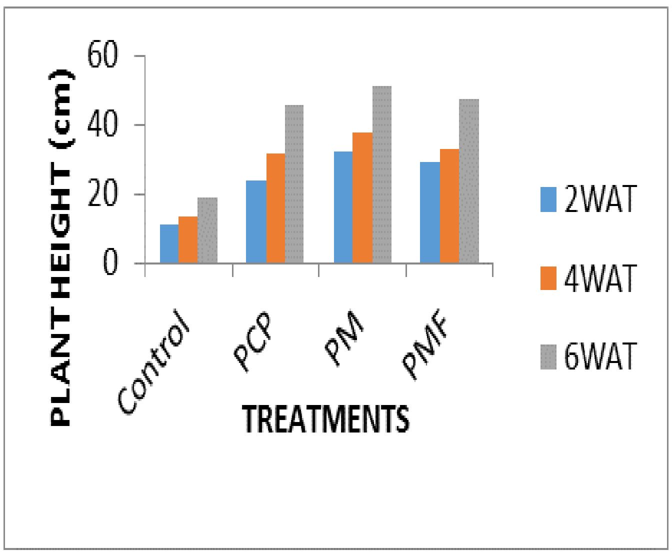

Fig. 1: Effect of the treatments on plant height

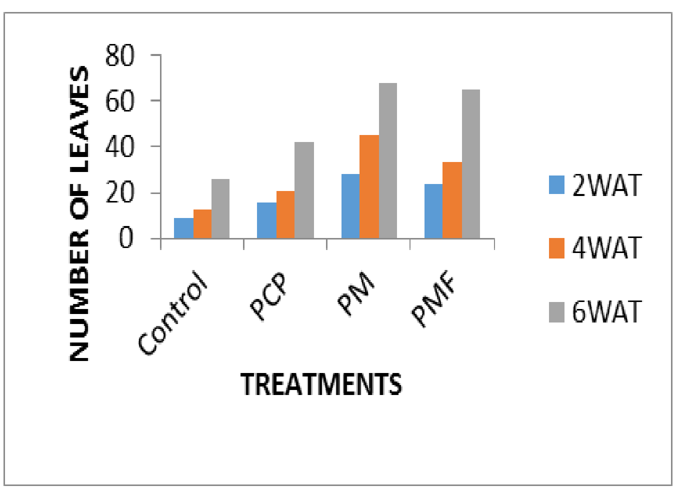

Fig. 2: Effect of treatment on the number of leaves.

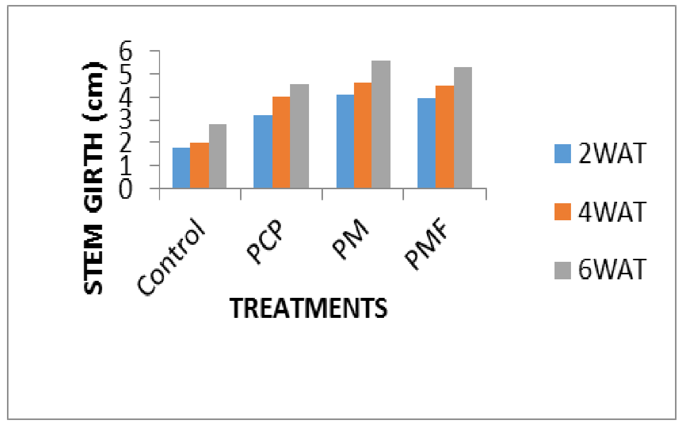

Fig. 3: Effect of Treatments on Stem Girth

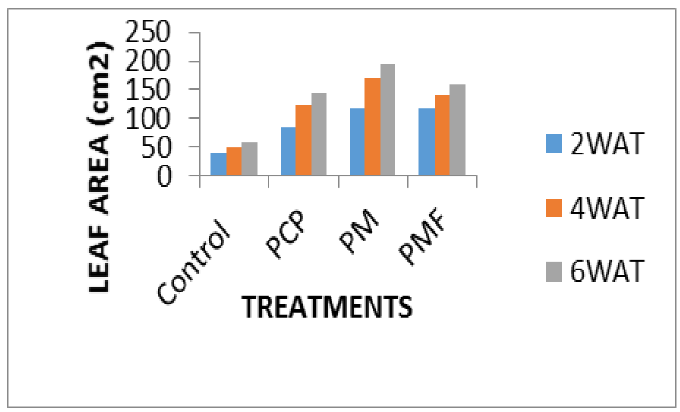

Fig. 4: Effect of Treatments on Leaf Area

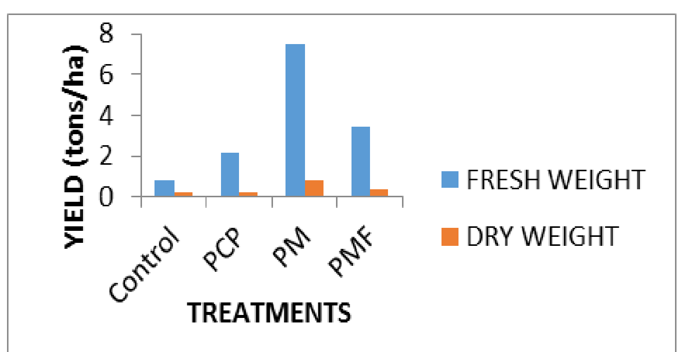

Fig. 5: Effect of Treatments on Yield.

Fig. 5: Effect of Treatments on Yield.

Table 3 shows the effect of treatment on some post-soil physical and chemical properties. The particle size distribution of the soil remained the same (loamy sand) after the trial. There was a significant increase ($p<0.05$) in soil pH, organic carbon, N, P and K in the soils amended when compared to the pre-trial soils, but no significant difference in soil pH and organic carbon amongst the treatments, while N, P and K showed some significant difference amongst treatments. The N and P content were above the critical levels (1.5-2.0 g/kg) and (10-16 mg/kg) respectively, while the organic matter content (25.68 g/kg) was within the established critical level (20-30 g/kg) reported by Orhue *et al.*, (2015). The Ca and Mg when compared with a critical level of 2.5 cmol/kg Ca was low and Mg above the critical level of 0.20-0.40 cmol/kg established by Udo *et al.,* (2009), while the sodium and potassium were high in all the treatments when compared with 0.02cmol/kg and 0.16-0.25cmol/kg critical levels reported by Akirinde and Obegbesan, (2000) respectively. Significantly higher ($p<0.05$) exchangeable acidity (0.90 cmol/kg) were recorded in soils treated with PMF, though not significantly different from control but higher than other treatments. All the treatments significantly increased the nutrient status of the soil.

Table 3: Post-soil physical and chemical properties

Treatment	Sand	Silt	Clay	pH	Org. Carbon	N	P	Ca	Mg	K	Na	Ex. Acidity	ECEC
	……….g/kg…… ….			(H_20)	……..g/kg……		mg/kg	……………….cmol/kg………………………					
Control	821^a	20^a	159^a	5.5^a	13.4^a	4.2^b	20.8^c	0.9^a	1.3^a	1.11^a	0.10^d	0.8^a	4.21^a
PCP	791^a	21^a	188^a	5.5^a	14.9^a	7.5^a	45.3^a	0.7^a	0.9^a	1.08^a	0.20^c	0.8^a	3.68^b
PM	796^a	20^a	184^a	5.4^a	14.8^a	7.0^a	38.3^{ab}	0.5^a	0.9^a	0.88^b	0.32^b	0.5^a	3.10^b
PMF	801^a	20^a	179^a	5.6^a	13.7^a	7.2^a	35.7^{ab}	1.1^a	0.7^a	1.12^a	0.40^a	0.9^a	4.22^a

Means with same letters along the column are not significantly different at 5% level of probability

3.3. Effect of treatments on proximate analysis and mineral composition and nutrient uptake

Tables 4: shows the proximate analysis and mineral composition and nutrient uptake of *Amaranthus hybridus.* There was no significant difference amongst treatments in the moisture content, crude protein, fat and oil, ash, crude fibre carbohydrate but a slight difference in vitamin C. At the same time, in the mineral composition and nutrient uptake of *Amaranthus hybridus,* there was a significant difference amongst treatment

Table 4: Effect of treatment on mineral composition and nutrient uptake and proximate analysis of Amaranthus hybridus plant

Treatment	Mineral composition (%)					Nutrient uptake (%)					Proximate analysis (%)						
	N	P	K	Ca	Mg	N	P	K	Ca	Mg	Mc	Cp	F/O	Cf	Ash	C	Vit C
Control	1.41^c	0.15^{ab}	0.12^a	1.22^a	$.132^c$	0.12^a	0.11^c	0.06^a	0.387^a	$.042^c$	5.0^b	8.8^a	10.3^a	19.7^a	10.3^a	45.9^a	12.85^{ab}
PCP	1.49^c	0.18^a	0.09^a	0.32^{ab}	0.156^c	0.15^a	0.10^a	0.12^a	0.096^d	$.047^c$	5.0^b	9.3^a	10.0^a	6.5^b	10.3^a	44.4^a	10.02^b
PM	1.81^a	0.22^a	0.13^a	0.44^{ab}	0.348^b	$.17^a$	0.20^a	0.11^a	0.136^c	0.108^b	6.0^b	11.3^a	10.0^a	15.2^b	10.0^a	37.0^b	12.60^{ab}
PMF	1.65^b	0.19^a	0.11^a	0.88^b	0.168^a	0.15^a	0.18^b	0.10^a	0.273^b	0.168^a	10.0^a	10.3^a	10.0^a	17.6^b	10.0^a	37.7^b	14.85^a

Means with same letters along the column are not significantly different at 5% level of probability

Conclusion

The results from this experiment showed that the combination of mycorrhiza with poultry manure (PM) significantly increased the plant height, number of leaves, stem girth, leaf area and yield of *Amaranthus hybridus.* Similarly, some soil physical and chemical properties were also improved as shown, with an increase in soil pH, organic carbon, nitrogen, phosphorus and potassium. Therefore the addition of mycorrhiza to the soil in combination with poultry manure is beneficial and suitable for *Amaranthus hybridus* cultivation and seen to improve some physicochemical properties of the soil.

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