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Influence of Arbuscular Mycorrhizal Fungus Inoculation and *Gliricidia sepium* leaves Mulch on the Vegetative Growth of *Telfairia* occidentalis (Hook F.)

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Abstract:
The effect of arbuscular mycorrhizal fungus, Glomus mosseae, and inoculation and Gliricidia sepium leaves mulch on the vegetative growth of Telfairia occidentalis was investigated in a field experiment. An initial 2.5 kg fresh weight of G. sepium green leaves were applied as mulch to T. occidentalis planted on beds with or without G. mosseae inoculation at planting with subsequent 5kg application at three-weekly intervals. Inoculation with G. mosseae enhanced luxuriant vegetative growth and together with G. sepium leaves mulch higher biomass yield was obtained. Nutrient yield and chlorophyll content were also highest in inoculated and mulched plants and lowest in uninoculated unmulched plants. Mulching increased arbuscular mycorrhizal fungi colonization of T. occidentalis roots more than what was obtained in the unmulched plants. The enhanced growth and the resulting higher biomass yield can be attributed to increased nutrient uptake and chlorophyll content caused by the G. mosseae and the additional nutrient to the soil from the G. sepium leaves mulch. The increased chlorophyll must have elevated the photosynthetic efficiency of the plant. The higher arbuscular mycorrhizal fungi colonization in mulched T. occidentalis roots indicates a possible conducive rhizosphere condition created by the G. sepium leaves mulch for the G. mosseae colonization.

Keywords: *Arbuscular mycorrhiza, mulch, Telfairia occidentalis, vegetative growth.*

1. Introduction

The greatest challenge facing the developing countries of the world nowadays is that of feeding their teeming population which keeps on increasing in an almost uncontrollable rate. Unfortunately most of the arable lands in these countries are either not fertile or have been acidified by excessive use of inorganic fertilizers. Besides, the use of inorganic fertilizers has come under serious attack due to their adverse effects on the environment and ecological food webs. Organic farming which negates the use of chemicals and is environmental friendly is rapidly gaining recognition as a system of agricultural production in most parts of the world (Amaya-Carpio et al., 2009; Roychowdhury et al.,2013). The presence of organic matter in the soil is of primary importance in maintaining its fertility, productivity and sustainability (Dick and Gregorich, 2004; Hu et al., 2014).

One inexpensive and less labour-intensive way of sourcing organic materials for a farm is obtaining it in situ as green manure from within or around the farm. Gliricidia sepium (Jacq.) Kunth ex Steud. (family: Fabaceae) is a medium sized leguminous shrub which can easily be raised as hedges around farms for green mulch production. G. sepium leaves mulch has been used to increase yield of maize and cassava (Okon, 2004; Okon et al., 2010; Okon, 2011), taro and rice (Kidd and Taogaga, 1985; Gonzal and Raros, 1998) as well as mung bean (Sangakkara et al., 2005).

Fluted pumpkin (Telfairia occidentalis Hook F.) is a popular highly priced and widely grown leafy vegetable in Nigeria. Its high nutritive and medicinal values as well as palatability make this vegetable a desirable requirement in most families' pots. The leaves contain high levels of copper, potassium, manganese, magnesium, iron and fibre (Taylor et al., 1983; Gupta et al., 1989; Olaofe et al., 1994), while the seeds have been shown to contain 53% fat and 27% crude protein (Olaofe et al., 1994). In order to get satisfactory vegetable and fruit yield, organic manure especially poultry droppings and inorganic fertilizers are usually applied by the farmers. Incidentally these are expensive to come by and their application is usually bi-weekly after nipping a frequency which makes them unaffordable to the peasant growers of this vegetable.

The inorganic fertilizer is used to provide the primary nutrients: nitrogen, phosphorus and potassium. Given that the inorganic fertilizers are to be avoided, then phosphorus which is a non-renewable resource will sooner or later be depleted in the near future (Cordell et al., 2009; Gilbert, 2009). Arbuscular mycorrhizal fungi (AMF) have been shown to enhance phosphorus extraction from the soil (Okon, 2004; Smith and Read, 2008). This they do by increasing the absorption surface area of the roots through the extra-radical hyphae which they spread extensively into the soil beyond the nutrient depletion zone to extract the immobile phosphorous (Smith and Read, 2008). In conjunction with G. sepium leaves mulch, AMF inoculation has increased yield in cassava (Okon et al., 2010). This research study was conducted to investigate the possibility of using AMF, Glomus mosseae, and a cheaper source of organic material, Gliricidia sepium leaves mulch, to increase the vegetative growth of Telfairia occidentalis in a nutrient poor soil.

2. Materials and Methods

This investigation was conducted in the University of Calabar, Department of Botany experimental plot located at 4° 54´N and 8° 20´E in Nigeria. The soil is typically a sandy coastal plain type. The experiment was laid out in a randomized complete block design with three replicates. Each treatment was represented by a bed of 1m×3m separated by another bed of similar size used as buffer against mycorrhizal contamination. Soil samples taken from the experimental site were pooled together and analyzed for nitrogen, phosphorus, potassium, calcium and magnesium using the method of Juo (1979) for soil analysis. Telfairia occidentalis seeds obtained from a fresh mature pod were planted three at a stand at a spacing of 1m×1m apart. Mycorrhizal inoculation was done at the time of planting by placing 30g of Glomus mosseae inoculum comprising of about 520-680 spore in planting holes before sowing the seeds. Mulch designated treatments were given an initial application of 2.5kg of fresh Gliricidia sepium leaves per 1m×3m bed. Subsequent applications were 5kg of fresh mulch at three weekly intervals.

2.1. Growth Measurements

Five weeks after planting (WAP), T. occidentalis from the different treatments were nipped as per replicate. Subsequent nipping was done at three weekly intervals. The shoots were separated into leaves and stem and separately oven dried at 70°C to constant dry weight for biomass yield. Thirteen weeks after planting, a final harvesting of the shoots at soil surface level was done and similarly treated to get the biomass yield. Representative fresh leaf samples were taken for chlorophyll content determination while some dried leaf samples from the different treatments were taken for nitrogen, phosphorus and potassium content analysis using the method of Juo (1979). At final harvest, representative feeder root samples were taken for arbuscular mycorrhizal fungi colonization assessment.

2.2. Determination of Chlorophyll Content

Chlorophyll content determination was done following the method of Arnon (1949). Chlorophyll was extracted in 80% aqueous acetone after homogenizing of leaf tissue. The supernatant was cleared by centrifugation at 1000rpm for 15minutes. Optical density was read in a Corning EEL colorimeter at 645mμ and 663μm. The amount of chlorophyll present in the extract was computed as mg of chlorophyll per gram fresh weight of tissue.

2.3. Arbuscular Mycorrhizal Fungi Colonization Assessment

Freshly harvested feeder roots were thoroughly washed in tap water and fixed in 50% ethanol. They were then cleared and stained after Koske and Gemma (1989) method. Colonization was determined by viewing under a dissecting microscope at × 45 magnifications using a grid intersection method of Giovannetti and Mosse (1980).

All data were subjected to a combined analysis of variance using the Windows version of Statistical Analysis System (1996) and Duncan's Multiple Range Test (DMRT) was used to separate the means at 0.05 level of probability when the F-ratio was significant.

3. Results

The initial site soil nutrient analysis is as shown in Table 1 below.

Organic C	Total N	Available P	Calcium	Magnesium	Potassium
10.1	0.7	55.1	21	11	0.9

Table 1 Initial experimental site nutrient status (mg g^{-1})

The initial nutrient status of the experimental soil showed that the site soil which is a typical acid sand soil derived from coastal plain sand is very poor in organic carbon and is very marginal for adequate growth of *T. occidentalis*.

Inoculation with *Glomus mosseae* greatly increased the vegetative growth of *Telfairia occidentalis* especially when in conjunction with *Gliricidia sepium* leaves mulch (Table 2, 3&4). Plant shoot biomass yield was highest in inoculated and mulched plants and lowest in uninoculated unmulched ones.

TREATMENT	**5WAP**	**8WAP**	**11WAP**	**14WAP**
M^+ mul^+	*11.00a	40.84a	104.59a	543.56a

M^+ mul^-	8.17b	30.03b	89.09b	340.67b
M^- mul^+	7.37bc	30.03b	85.67b	311.03bc
M^- mul^-	5.70d	8.53c	29.19c	145.41d

*Table 2: Effect of AMF inoculation and G. sepium leaves mulch on the leaves biomass of T. occidentalis (g plant^{-1}).M^+: mycorrhiza inoculated; M^-: mycorrhiza uninoculated; mul^+: mulch applied; mul^-: mulch not applied; *Means of three replicates. Means in the same column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range tests.*

TREATMENT	8WAP	11WAP	15WAP
M^+ mul^+	*18.26a	83.19a	95.90a
M^+ mul^-	13.56b	32.57b	56.88b
M^- mul^+	12.24bc	31.27b	55.18b
M^- mul^-	1.03d	8.00c	43.79c

*Table 3: Effect of AMF inoculation and G. sepium leaves mulch on the stem biomass yield of T. occidentalis (g plant-1). M^+: mycorrhiza inoculated; M^-: mycorrhiza uninoculated; mul^+: mulch applied; mul^-: mulch not applied; *Means of three replicates. Means in the same column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.*

Arbuscular mycorrhizal fungi colonization was highest in inoculated and mulched plants (Table 4). Mulching increased colonization by the indigenous mycorrhizal fungi in uninoculated plants which gave higher root percentage colonization than inoculated unmulched plants. There was a positive relationship between percentage mycorrhiza colonization and plant biomass yield, chlorophyll content and phosphorus uptake.

TREATMENT	AMF (%)	CHLOROPHYLL (mg/g-fw)	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	SHOOT DWT (g plant^{-1})
M^+ mul^+	*43a	340.1a	30.7a	8.58a	14.90a	639.46a
M^+ mul^-	26b	274.46c	16.3c	7.68b	7.68bc	397.55b
M^- mul^+	20bc	306.17b	25.3b	6.44c	8.71b	366.21bc
M^- mul^-	13d	219.22d	6.71d	2.01d	3.35d	189.20d

*Table 4 Effect of AMF inoculation and G. sepium leaves mulch on the growth of T. occidentalis. M^+: mycorrhiza inoculated; M^-: mycorrhiza uninoculated; mul^+: mulch applied; mul^-: mulch not applied; *Means of three replicates. Means in the same column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range tests.*

Chlorophyll content was highest in inoculated and mulched plants (Table 4). Nutrient uptake especially phosphorus was highest in inoculated plants (Table 4). Inoculation with G. mosseae in all cases gave higher phosphorus uptake in T. occidentalis. In the absence of mulch, chlorophyll content was lower in uninoculated plants. Mulching without inoculation increased nitrogen and potassium uptake in plants over inoculation without mulching.

4. Discussion

The higher percentage colonization in inoculated plants is in support of some earlier research findings (Oyetunji and Osonubi, 2007). This is to be expected as a result of inoculation with G. mosseae adding to and increasing the arbuscular mycorrhizal fungi propagules in the soil thereby increasing colonization potential. Some earlier work has reported an increase in colonization resulting from increasing inoculum (Daft and Nicolson, 1969). The highest percentage AMF root colonization in mulched plants is similar to the findings of Gryndler et al. (2009), with organic matter decomposition. Addition of organic matter has been shown to have beneficial effects on the growth of AM fungi (Caravaca et al., 2002; Gaur Adholeya, 2002). Thus the G. sepium leaves mulch must have enhanced extra-radical hyphae proliferation (Joner and Jakobsen, 1995), consequently leading to increased colonization of roots

(Muthukumar and Udaiyan, 2000). Friberg (2001) and Bautista-Cruz et al., (2014) have reported the highest growth of AMF extra-radical mycelia in soil amended with organic matter.

The positive relationship between AMF root colonization and biomass yield observed in this work is an indication of the beneficial effects of G. mosseae and the indigenous AMF to the growth and biomass yield of T. occidentalis. Similar relationship has been reported for other plants by Podeszfinski et al. (2002). This relationship is possibly due to higher AMF colonization resulting in enlarged root absorbing surface area caused by extra radical fungi mycelia. This then would have increased nutrient uptake and subsequently enhanced plant growth. It is worthy to note that without mulch application, the increase in biomass yield caused by AMF inoculation is not significantly different ($P < 0.05$) from that of mulched uninoculated plants. This shows that the growth enhancing effect of G. mosseae is through improved nutrient uptake especially phosphorus, which the mulched plants readily got within easy reach. Besides, the increased leaf chlorophyll content in mycorrhizal plants could have increased photosynthetic activities in inoculated plants with the resulting photosynthates increasing the biomass yield. Arbuscular mycorrhizal fungi inoculation has been shown to increase leaf chlorophyll content by some earlier studies (Mathur and Vyas, 2000; Ekanayake et al., 2004; Amanullah et al., 2012; Tanwar et al., 2013). This increase can probably be attributed to increased uptake of phosphorus which is an important element required during chlorophyll synthesis. Fattahpour et al. (2012) have observed a positive relationship between the phosphorus and chlorophyll contents in Zea mays. On the other hand, the higher chlorophyll content in mulched uninoculated plants over inoculated unmulched plants is an indication that the mulch must have supplied additional nutrients particularly phosphorus, nitrogen and magnesium to the plants. Some earlier studies have reported significantly high nitrogen, phosphorus, potassium and magnesium contribution to soil fertility by Gliricidia sepium leaves mulch (Okon, 2004; Okon et al., 2010). These can explain why uninoculated plants could perform favorably well with G. sepium leaves mulch application.

5. Conclusion

The results of this investigation have shown that *G. sepium* leaves mulch can be used to boost *T. occidentalis* vegetative growth and in conjunction with appropriate arbuscular mycorrhizal fungus inoculation, this vegetable can be abundantly produced to meet its high consumption demand in nutrient deficient or marginal soil.

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