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Influence of Mycorrhizae and Rhizobium Inoculation on Growth, Nutrient Uptake and Proximate Composition of Upland Rice Cultivars

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Abstract
The activities of microbial rhizospheric organisms have been well documented in non-leguminous plants such as wheat, rice and maize. Such activities include nitrogen fixation, phosphate solubilization and mineralization that are beneficial for the overall growth and development of the plant. Field experiments were carried out in the early and late cropping season of 2013 in the tropical rainforest agro-ecology of southwest Nigeria to study the growth promotion, nutrient uptake, proximate composition of upland rice and harvested grains based on single inoculation of *Mycorrhizae* and *Rhizobium.* A 5 x 3 factorial experiment was conducted; five upland rice varieties selected with and without the application of mycorrhizae and rhizobium, laid out in split plot arrangement, with mycorrhizae and rhizobium in the main block, while variety was in the sub-plot. Each treatment was replicated three times. In both seasons, result show significant ($P < 0.05$) effect of single mycorrhizae and rhizobium inoculation on plant growth (plant height, numbers of tillers and leaves) and plant biomass except for shoot weight in the late season. Also, result show significant ($P < 0.05$) effect of single mycorrhizae and rhizobium inoculation on nutrient uptake in both seasons. Significant ($P < 0.05$) effect of single mycorrhizae and rhizobium inoculation was observed in proximate composition of rice grains in the early season, with rhizobium inoculated rice having the highest protein content. It was concluded that upland rice inoculation with mycorrhizae and rhizobium improved their nutrient uptake, proximate composition and growth.
Keywords: Mycorrhizae, rhizobium, upland rice, nutrient uptake, growth, proximate composition

1. Introduction

Rice (*Oryza sativa* L.) is the staple diet of over 40% of the world's population, making it the most important food crop (Hossain and Fischer, 1995). It is difficult to increase the production area as the cultivable lands in many countries are decreasing due to rapid urbanization. National demand for rice in Nigeria is rising because of population growth, increasing affluence and changing dietary habits therefore, it is necessary to increase the production capacity per unit area which currently requires high input (costly chemical fertilizers and pesticides) to achieve the potential. But peasant rice farmers cannot afford this hence the need to develop a technology which can fulfill rice production and protection requirements economically and on sustainable basis. Rice requires heavy nutrients for higher growth and yield, especially nitrogen (N) and phosphorus (P) application which is effective for increased growth and yield. But intensive input of organic and inorganic fertilizers degrade the environment, causing water pollution and soil contamination through phytotoxicity, ammonia volatilization and nitrate accumulation (Bremner, 1995). Therefore, it is necessary to promptly establish a sustainable rice production system with a lowered chemical input.

Soil microorganisms and their activities play important roles in transformation of plant nutrients from unavailable to available forms and also have many metabolic qualities related to soil fertility improvement. This is where mycorrhizae and rhizobium come into play; Arbuscular mycorrhizae fungi (AMF) are obligate symbionts that colonize the roots of about 80% extra-terrestrial plant species. In this relationship, AMF improve the host plant growth by increasing the uptake of water and minerals, especially the uptake of phosphorus (P) which is readily fixed in soil, and in return, they obtain photosynthates from the host plants recently, the beneficial effect of AMF on nitrogen (N) uptake by crop has been extensively studied. Also, *Rhizobium* association has been extensively explored in the root nodules of legumes where they fix atmospheric nitrogen but recent studies also suggest that *rhizobium* can exhibit plant growth promoting (PGP) activities with non-legumes (Yanni *et al*., 1997).It was reported in the studies conducted by Humphry *et al*., 2007 Mehboob *et al*., 2008 that, minute living creatures in association with certain non-legumes (cereals) after isolating them from the root nodules of local legumes possess some beneficial effects . Therefore, altering the rhizosphere micro flora by seed, soil or root inoculation with specific organism is considered a sensible opportunity. This can lead to the establishment of a large rhizobium population in the plant rhizosphere, improved nodulation and N_2-fixation even under adverse soil N conditions (Peoples et al., 1995). Sometimes, yields are not increased by inoculation, but N concentration in seed or plant parts may be increased over that of non-inoculated plants (Wani et al., 1995). In cases where both types of responses are not observed, the result might simply be a saving of soil N for succeeding crops. Certain mechanisms are attributed towards *rhizobium* which may be involved in their PGP activities i.e. mobilization and efficient uptake of nutrient (Biswas *et al*., 2000a, enhancement in stress resistance (Mayak *et al.,* 2004), solubilization of insoluble phosphates (Alikhani *et al*., 2006), production of phytohormones and vitamins (Dobbelaere *et al*., 2003). Several studies have been done on the biotrophic interaction between AMF, rhizobium and rice, but scarce information and knowledge of the influence of single inoculation of AMF and rhizobium on growth, nutrient uptake and proximate composition of upland rice in Nigeria has not been studied. With these experiments, it is expected that establishment of symbiotic relationship between rice and biofertilizers would not only increase upland rice yield by stimulation of nutrients uptake but also improve the quality of rice by enhancing the grain nutrient content. The objectives of this study were to verify the influence of mycorrhizae and rhizobium inoculation on growth, nutrition uptake, proximate composition and yield of upland rice under field conditions.

2. MATERIALS AND METHODS

2.1.1 Description of Location and Experimental Site

Field experiments were conducted at the Teaching and Research farm of the Federal University of Technology, Akure (7^0 17^1 N 5^0 10^1 E) in the southwest Nigeria between December 2012 – April 2013 (early season) and July 2013 – October 2013 (late season). The soil at the site of experiment is an alfisol (FAO, 1998) derived from the basement complex rock and it is sandy loam.It has an average annual rainfall range of about 1613mm per annum and the annual mean temperature is 27°C. The vegetation is tropical rain forest with an average relative humidity of between 56 and 59% during the dry season and 51 - 82% during the wet season (IITA, 2002).

2.1.2Seedling Nursery.

Prior to transplanting, the nursery stage was conducted at the screen house of the Department of Crop Soil and Pest Management of the Federal University of Technology, Akure. Small polythene pots of about 5cm in diameter and 10cm in length were filled with topsoil and Mycorrhizae/Rhizobium strains at 50g per pot; this was done to ensure maximum colonization of the roots before transplanting. Rice seeds were sown at 2-3 seeds per pot, the pots were made moist and maintained for about 21-25 days before germinated seedlings were transplanted to the field.

2.1.3Determination of soil chemical properties:

Soil samples were collected for the determination of chemical properties before planting and after harvest, Composite surface (0 to10cm) soil samples were collected using auger over each site before commencement of experiment and bulked. The soil samples were air-dried and sieved using a 2mm sieve before making the determinations. Soil organic carbon was determined by the procedure of Walkley and Black using the dichromate wet oxidation method (Nelson and Sommers, 1996), total N was determined by micro-Kjeldahl digestion method (Bremner, 1996), available P was determined by Bray-1 extraction followed by molybdenum blue colorimetry (Frank et al., 1998). Exchangeable K, Ca and Mg were extracted using 1.0 N ammonium acetate. Thereafter, K was determined using a flame photometer and Ca and Mg were determined by atomic absorption spectrometer (AAS). Soil pH was determined in soil-water (1:2) medium using the digital electronic pH meter (Ibitoye, 2006)

2.1.4 Determination of soil physical properties

At harvest three steel core samples were collected to 10 cm depth on heaps per plot and used for determination of gravimetric moisture content. Particle-size analysis was done using Bouyoucos hydrometer method (Sheldrick and Hand Wang, 1993). Soil bulk density was determined using the core method (Campbell and Henshall, 1991). The soil temperature at 15:00hr was determined to 5cm depth and three readings were taken per plot at 4 weeks interval from 4weeks after planting (WAP)

2.1.5 Experimental Design and Treatment

The experiment was a 3 x 5 factorial experiment with *Arbuscular mycorrhizae*, *Rhizobium* and without *AMF/Rhizobium* (control) and five varieties of upland rice (N-U-1, N-U-8, WAB 56-104, OFADA GR and MOROBEREKAN). The study was a split plot arrangement in Randomized Complete Block Design (RCBD), with AMF and rhizobium inoculation in the main plot, while varieties were in the sub-plot, replicated three times.

2.1.6 Source and Application of planting materials

The different rice seeds (varieties) were acquired from Africa Rice Centre, International Institute of Tropical Agriculture, Ibadan (IITA). The varieties are the improved high yielding varieties commonly grown by farmers in the ecological zone. Prepared *Arbuscular mycorrhizae fungi (Glomus intaradices*) inoculum with soil as carrier and cultured *Rhizobium* strains (RACA 3/5/12) were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The inoculants were applied to the potting medium where rice seeds were planted at the rate of 50 g per pot just after the seeds were planted.

2.1.7Field Layout and planting

There were three main plots, each plot consist of 15 sub-plots with a size measurement of 2m x 1m and inter sub-plot spacing of 0.5m in between plots. A total of 132 plants were planted per sub plot and each sub-plot consists of 21 plants per row. Transplanted seedlings were planted with the ball of earth into planting holes in the field at two seedlings per stand, according to their respective plot at a spacing of 25cm x 25cm^2.

2.1.8 Cultural Practices

Manual weeding was done twice during the duration of the experiment to prevent weed infestation on the field.

2.2.1Sampling and Data Collection

Five plants per plot were randomly selected from the net plot (2 x 2 m) for the collection of agronomic parameters (growth and development components) of upland rice varieties. Plant height, number of leaves and number of tillers were recorded at 4, 8 and 12 weeks after planting (WAP). Plant height was determined using a ruler from soil surface to the tip of the tallest leaf. Other growth components parameters were determined by standard procedures.

2.2.2Nutrient analysis of plant sample

For nutrient analyses, the N content was determined by semi-micro Kjeldahl procedure (Bremmer, 1960). Total P and K content were determined by HNO3 − HClO4 treatment and were measured by vanadomolybdate spectrophotometry and flame atomic-absorption spectrometry, respectively. Fresh leaf samples of rice plant were collected from each cultivar and treatments prior to flowering to determine the total nutrient content (N, P and K) using methods mentioned above.

2.2.3 Chlorophyll extraction of plant sample

Chlorophyll extraction was done with spectrophotometer at the soil analysis laboratory of the Department of Crop, Soil and Pest Management, Federal University of Technology Akure. Ondo State. 1 gram fresh plant samples (leaves) were cut into small pieces and inserted into test tubes. Then 100 ml (80%) acetone was added in air tight condition to avoid losses. The test tubes were kept for 24 to 48 hours in normal temperature. Data was recorded by Absorption Spectrophotometer. Total chlorophyll = Absorbance (chlorophyll a + chlorophyll b) × Correction factor

2.2.4 Proximate composition of grain samples

Harvested grains were carefully threshed, cleaned and freed from dirt, stones, chips and other extraneous material, then ground for proximate analysis. The proximate analyses of threshed rice grains for moisture, total ash was determined by the method described by Kadan *et al*. (1997) and AACC (2000). Nitrogen was determined by the micro Kjeldahl method (AACC, 2000) and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Crude fibre content was determined using soxhlet apparatus (AOAC, 1997) and carbohydrate content was determined by difference. All the proximate composition values were reported in percentage.

2.3.1Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA), mixed model at 5% probability level. All data were checked prior to statistical analysis for the violation of ANOVA assumption, Means were separated using DMRT. Genstat statistical package was used for the analysis (17th Edition).

3. Results and Discussions

Table 1. Physio-chemical properties of the soil before planting

Soil properties	Values	
	Season 1	Season 2
Sand (%)	82.78	60.4
Silt (%)	6.38	26
Clay (%)	10.84	13.6
Nitrogen (%)	0.47	0.28
Organic Carbon (%)	2.78	0.91
Organic Matter (%)	4.79	0.71
Calcium (cmol/kg)	5.5	2.51
Magnesium(cmol/kg)	2.9	2.13
Potassium(cmol/kg)	1.08	2.04
Phosphorus(cmol/kg)	4.35	2.65
pH	5.94	5.03
CEC	11.54	12.35
Al2	0.7	
H	0.47	

*mean values are presented in the table.
Legend:
Season 1 = Early Season
Season 2 = Late Season.

3.1.1 Influence of mycorrhizae and rhizobium inoculation on plant biomass

Tables 2 and 3 showed that mycorrhizae and rhizobium inoculation increased shoot and root growth more in the early season than in the late season. The highest rice shoot and root development was attained in rice plants inoculated with mycorrhizae, (50 % increased shoot weight over un-inoculated control plants). In the early season, single inoculation of mycorrhizae and rhizobium improved shoot and root growth; length, fresh weight and number of roots. No varietal variability was observed across the varieties examined in both seasons. In the early season, there were no significant interaction ($P < 0.05$) of rhizobium and the cultivar observed across varieties in the root weight, no significant interaction ($P < 0.05$) of mycorrhizae and cultivar observed as per root weight as well, while it was observed in the late season. Significant interaction ($P < 0.05$) of mycorrhizae and cultivar was observed in shoot growth, root length and number of roots in both seasons, except for lateral root length and number of roots in the late season. In the early season no significant interaction ($P < 0.05$) of rhizobium and cultivar was observed in root growth, shoot growth and root length except in number of roots. While in the late season it was only observed in root weight and tap root length. In the early season, N-U-8 had the highest fresh root and shoot weight but was not significantly different from other varieties while in the late season, WAB 56-104 had the highest root and shoot weight. WAB 56-104 had the highest tap root and lateral root length in both seasons and number of roots but not significantly different from other varieties, while N-U-1 had the highest number of roots in the late season. Plant biomass response was slightly different when comparing the two seasons, with the early season performing better and this could have been influenced by factors such as favourable soil parameters, climatic conditions, water stress and microbial interactions. The rice plants inoculated with mycorrhizae showed significant beneficial effect, this is because mycorrhizae improves the growth and biomass of wide host range including rice plant and is an efficient phosphate solubilizer and transporter. AMF is able to enhance the absorption of nutrients from the soil which could have moved to the roots principally by mass flow, in addition to those, which could have diffused through the soil slowly. Its beneficial effect on host plant as a result of mycorrhizae infection is usually associated with improved plant nutrition, especially phosphorus by virtue of extensive root system that extend the functional mycelium into surrounding soil, making a greater pool of nutrients available to the plant. This leads to increased plant growth, often as high as several hundred-fold increases in biomass (Menge 1983). The positive response observed with inoculation of rhizobium in all growth parameters (viz. shoot length, root length, shoot fresh weight, root fresh weight of both vegetative and reproductive stages of rice plants can be attributed to its ability to fix atmospheric nitrogen. under N-limited conditions, plant roots excrete compounds with high C/N ratios, favoring rhizospheric N2 fixation (Klein *et al.* 1990). Their performance (rhizobium) when inoculated with rice plants may be due to synergistic effects of several factors.

Table 2. Influence of Mycorrhizae and Rhizobium inoculation on plant biomass of upland rice cultivars (early season)

Treatments	Fresh root weight(g)	Fresh shoot weight (g)	Taproot length (cm)	Lateral root length (cm)	No of roots
Mycorrhizae	60.41b	113.62c	15.11c	11.97c	77.72c
Rhizobium	44.95a	64.71b	13.55b	10.10b	68.51b
Control	42.50a	50.49a	10.92a	8.80a	37.12a
Varieties					
WAB 56-104	48.88a	70.86a	12.97a	11.11a	60.11a
MOROBEREKAN	41.35a	55.71a	12.44a	9.83a	52.00a
OFADA GR	46.40a	68.83a	12.39a	9.73a	59.82a
N-U-1	49.46a	74.05a	12.59a	9.71a	56.94a
N-U-8	54.34a	80.62a	12.67a	10.22a	67.41a
M*V	ns	*	*	*	*
R*V	ns	ns	ns	ns	*

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

Table 3. Influence of Mycorrhizae and Rhizobium inoculation on plant biomass of upland rice cultivars (late season)

Treatments	Fresh root weight(g)	Fresh shoot weight (g)	Taproot length (cm)	Lateral root length (cm)	No of roots
Mycorrhizae	33.98a	38.70a	11.19c	7.56b	78.59c
Rhizobium	45.67b	38.42a	10.07b	7.86b	69.05b
Control	31.58a	32.10a	7.58a	6.33a	35.60a
Varieties					
WAB 56-104	38.98a	41.36a	12.52a	8.49a	58.70a
MOROBEREKAN	34.98a	40.19a	11.55a	8.03a	57.67a
OFADA GR	35.36a	32.12a	10.73a	7.06a	62.37a
N-U-1	38.12a	34.22a	10.30a	7.26a	63.52a
N-U-8	37.93a	34.19a	10.46a	7.22a	63.15a
M*V	*	*	*	ns	ns
R*V	*	ns	*	ns	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

3.1.2 Influence of mycorrhizae and rhizobium inoculation on plant growth

Rice plants inoculated with mycorrhizae and rhizobium were significantly ($P < 0.05$) taller than those un-inoculated (control) in both seasons (Tables 4 and 5). In the early and late season, significant ($P < 0.05$) varietal variability was observed in both seasons in plant heights across all the period of investigation. Moroberekan a local grown cultivar had significantly higher plant height than other varieties in both treatments and seasons and it was closely followed by WAB 56- 104. Significant interaction ($P < 0.05$) of mycorrhizae and cultivar was observed in both seasons on plant height. Significant interaction ($P < 0.05$) of rhizobium and cultivar was also observed in both seasons on plant height, except at 4WAP in the late season. Number of tillers was significantly ($P < 0.05$) increased by mycorrhizae application at 8 and 12 WAP in both seasons, while number of tillers was not significantly ($P < 0.05$) increased by rhizobium application in the early season but was significantly increased in the late season at 12WAP. There was no significant varietal variability ($P > 0.05$) observed in the number of tillers among the varieties in the two seasons. N-U-1 had the highest number of tillers in rice plants inoculated with rhizobium in the early season, while Moroberekan and N-U-8 had the highest number of tillers among plants inoculated with mycorrhizae. In the late season, N-U-8 had the highest number of tillers among varieties inoculated with rhizobium, while N-U-1 had the highest number of tillers amongst varieties inoculated with mycorrhizae. Number of leaves was significantly ($P < 0.05$) increased by mycorrhizae and rhizobium application at 8 and 12 WAP in both seasons. No significant difference was observed between the treatments in the early season, significant difference was only observed in the late season at 12WAP. N-U-1 had the highest number of leaves among varieties inoculated with rhizobium in the early season and late season, while Moroberekan had the highest number of leaves among varieties inoculated with mycorrhizae in the early season and WAB 56-104 had the highest number of leaves among varieties inoculated with mycorrhizae in the late season. Significant interaction ($P < 0.05$) of rhizobium x cultivar was observed in the early season on number of leaves at 4WAP and 8WAP, while Significant interaction ($P < 0.05$) of mycorrhizae and cultivar was observed at 4WAP. Growth response was higher in the early season than the late season and this was influenced by factors such as favourable soil parameters, climatic conditions and microbial interactions. It was observed that, arburscular mycorrhiza was significantly affected when compared with non mycorrhizae plants. Mycorrhizae plants performed better than non-mycorrhizae plants (control) in terms of plant height, number of tillers and number of leaves. This is due to the enhanced mitotic activity of the plant stem cells, increased soil nutrients which influences growth and leads to high photosynthesis rate. The results are in good agreement with that of (Sakariyawo et al., 2013) who also reported that upland rice inoculated with mycorrhizae performed better than non-mycorrhizae plants. Plants inoculated with rhizobium also performed better when compared with the un-inoculated plants, especially in the late season, this is due to their growth promoting mechanisms including mobilization and efficient uptake of nutrients (Biswas *et al*., 2000a, enhancement in stress resistance (Alami *et al*., 2000), solubilization of insoluble phosphates (Alikhani *et al.,* 2006), induction of systemic disease resistance (Tuzun and Kloepper, 1994), inhibition of fungal growth (Nautiyal, 1997), production of phytohormones (Dakora, 2003), vitamins (Dobbelaere *et al.,* 2003), siderophores (Neiland and Leong, 1986) and increased availability of nutrients and phytohormones like indole acetic acid and ethylene. These results are in comformity with Biswas *et al*. (2000b) who have reported increase in growth response of rice plants when inoculated with rhizobium.

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Table 4. Influence of Mycorrhizae and Rhizobium inoculation on growth parameters of upland rice cultivars. (Early season)

Treatments	Plant height (cm)			Number of tillers			Number of leaves		
	4WAP	8WAP	12WAP	4WAP	8WAP	12WAP	4WAP	8WAP	12WAP
Mycorrhizae	43.53a	57.18ab	87.64a	4.17a	9.38b	12.50b	13.98a	29.78b	43.58b
Rhizobium	46.97b	70.04b	90.59a	3.70a	7.13a	9.47a	12.93a	24.05a	40.70b
Control	46.12b	66.52a	77.22b	3.33a	7.23a	8.25a	12.93a	21.50a	27.22a
Varieties									
WAB 56 -104	46.67bc	74.80d	95.22c	3.28a	7.83a	8.61a	12.00ab	21.06a	31.78ab
MOROBEREKAN	49.67c	78.78e	98.89d	4.50a	8.00a	10.17a	17.44b	28.00a	34.72ab
OFADA GR	47.68bc	70.84c	93.22c	3.61a	7.00a	9.17a	12.17ab	23.83a	29.06a
N-U-1	46.23bc	67.12b	87.72b	3.11a	7.50a	9.67a	10.28a	21.67a	32.33ab
N-U-8	44.96bc	66.28b	86.28b	3.94a	8.50a	10.78a	13.22ab	24.56a	33.00ab
M*V	*	*	*	*	ns	ns	*	ns	ns
R*V	*	*	*	ns	ns	*	*	*	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test.
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

Table 5. Influence of Mycorrhizae and Rhizobium inoculation on growth parameters of upland rice cultivars. (Late season)

Treatments	Plant height (cm)			Number of tillers			Number of leaves		
	4WAP	8WAP	12WAP	4WAP	8WAP	12WAP	4WAP	8WAP	12WAP
Mycorrhizae	49.02b	72.33b	92.80b	1.12a	4.51a	7.19b	7.29ab	14.81a	25.19b
Rhizobium	46.85ab	69.03ab	90.07b	2.18b	5.01a	8.57c	8.28b	14.99a	22.69ab
Control	45.49a	65.80a	85.62a	2.03b	4.61a	6.23a	6.09a	12.55a	21.22a
Varieties									
WAB 56 -104	47.36a	70.17b	90.00b	2.33b	5.27a	6.89a	7.84a	15.17a	24.33a
MOROBEREKAN	51.68b	75.94c	98.11c	2.51b	4.97a	6.44a	7.99a	15.02a	22.22a
OFADA GR	46.28a	67.54ab	87.56ab	1.37a	4.73a	7.82a	7.22a	13.84a	24.56a
N-U-1	44.16a	66.03a	86.00a	1.24a	4.22a	7.91a	6.38a	12.40a	22.07a
N-U-8	46.13a	65.59a	85.81a	1.42a	4.37a	7.58a	6.69a	14.14a	22.00a
M*V	*	*	*	*	ns	*	ns	ns	*
R*V	ns	*	*	*	ns	ns	ns	ns	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test.
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

Table 6: Post planting soil nutrient content soils at the experimental sites.

Treatments	N		OM		OC		K		P		Mg		Ca		Na		pH	
			(%)							cmol/kg								
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Mycorrhizae innoculation	0.71	0.82	5.13	1.32	3.21	0.76	0.03	0.03	3.89	3.27	2	1.2	4.1	2.3	0.17	0.03	6.04	5.5
Rhizobium innoculation	0.83	0.81	5.48	1.79	3.18	1.04	0.02	0.03	4.69	1.94	1.2	1.1	2.3	2.1	0.04	0.01	5.55	5.37
Control	0.63	0.66	2.3	1.62	1.33	0.94	0.05	0.02	2.73	1.79	0.8	0.9	1.7	1.9	0.02	0.01	5.27	4.79

N: Nitrogen, **P**: Phosphorus, **K**: Potassium, **Na**: Sodium, **Ca**: Calcium, **Mg**: Magnessium, **OC**: Organic carbon, **OM**: Organic matter.
MHI:Mycorrhizae inoculation, **RHI**: Rhizobium inoculation, **NI**: Not-inoculated. **S1** Early Season **S2** : Late Season.
*mean values are presented in the table.

3.1.3 Influence of mycorrhizae and rhizobium inoculation on nutrient uptake

As summarized in Table 7, mycorrhizae and rhizobium inoculation significantly ($P < 0.05$) increased nutrient uptake in rice plants in the early season. Rice plants with the highest nutrient and chlorophyll contents was reached in rice plants inoculated with mycorrhizae, (50 % of increase, especially in chlorophyll content over un-inoculated control plants), slightly higher than plants inoculated with rhizobium. Plants inoculated with rhizobium did have higher phosphorus content than mycorrhizae inoculated plants and significantly ($P < 0.05$) different from un-inoculated control plants. Significant ($P < 0.05$) mycorrhizae x cultivar interaction was observed in nitrogen content and potassium content, while significant ($P < 0.05$) rhizobium and cultivar interaction was only observed in nitrogen content. In Table 8 (late season), mycorrhizae inoculated plants were significantly different from the control and rhizobium inoculated plants in relation to chlorophyll and phosphorus contents of rice plants. While rhizobium inoculated plants was significantly different from control and mycorrhizae inoculated plants only in potassium contents of rice plants. Slight cultivar variability was also observed in chlorophyll and potassium contents of rice plants. Significant ($P < 0.05$) mycorrhizae x cultivar interaction were observed in all nutrient uptakes, while significant ($P < 0.05$) rhizobium and cultivar interaction was also observed except in potassium content of rice plants. Maximum uptake of nutrient attained by mycorrhizae inoculated plants in our result is in good agreement with that of George (1995) who reported that in addition, mycorrhizae inoculation results in an increase in the uptake of other macro and micronutrient. This results is supported by past works of (Lukiwati *et al.,* 1997b, Lukiwati & Hardiosoewignjo, 1998, Singh *et al.,* 2004) who reported that nutrient uptake of mycorrhizae plants was higher compared to non-mycorrhizae one. One of the most dramatic effects of mycorrhizae infection on the host plant is the increase in (N, P, K and S) uptake mainly due to the capacity of the mycorrhizae fungi to absorb nutrients from soil and transfer it to the host plant roots (Asimi *et al*., 1980). Mycorrhizae have been shown to improve immobile nutrients uptake such as P, Zn and Cu and other nutrients due to ability of the fungal hyphae to extend into the rooting zone and absorb mineral nutrients of low mobility, mainly phosphorus, but also copper and zinc (Kothari *et al.,* 1990; Tarfdar and Marschner, 1994). Also, increased chlorophyll content in both mycorrhizae and rhizobium inoculated plants bears a positive correlation with the increase in soluble sugars. The promotion of chlorophyll formation in inoculated plants may presumably reflect more photosynthesis to meet the carbon requirements of mycorrhizae fungi and rhizobium, since both micro- symbionts depend on the host plant for their carbon source. The increase in the nutrient content of plants inoculated with rhizobium is mainly due to the changes produced in the morphology of the roots by the phyto-hormones that are synthesized by many rhizobium and that result in an increase in root surface area (Bashan *et al.*, 2004). However, rhizobium may also enhance mineral uptake, not only as a consequence of the increase in root surface area but also by stimulating proton efflux activity (Bashan, 1990). Maximum N content observed in plants inoculated with rhizobium could be the result of better nitrogen fixation and uptake of N from the soil. The slight variation in results of both seasons may be due to variations in factors such as soil parameters, climatic conditions, microbial interactions or competition between introduced and native microbial populations.

Table 7 Influence of Mycorrhizae and Rhizobium inoculation on leaf nutrient analysis of upland rice cultivars (Early season)

Treatment	Chlorophyll content (mg/g)	Nitrogen content (%)	Phosphorus content (cmol/g)	Potassium content (cmol/g)
Mycorrhizae	0.55c	4.90c	2.64c	31.29c
Rhizobium	0.37b	4.65b	2.48b	29.58b
Control	0.15a	3.07a	1.52a	24.41a
Varieties				
WAB 56-104	0.34a	4.20a	2.15a	27.90a
MOROBEREKAN	0.30a	3.98a	2.11a	27.51a
OFADA GR	0.34a	4.16a	2.13a	27.93a
N-U-1	0.34a	4.22a	2.21a	28.37a
N-U-8	0.35a	4.24a	2.25a	28.50a
M*V	ns	*	ns	*
R*V	ns	*	ns	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

Table 8. Influence of Mycorrhizae and Rhizobium inoculation on leaf nutrient analysis of upland rice cultivars (late season)

Treatment	Chlorophyll content (mg/g)	Nitrogen content (%)	Phosphorus content (cmol/g)	Potassium content (cmol/g)
Mycorrhizae	35.58b	2.24a	0.59b	30.65a
Rhizobium	26.99a	2.38a	0.43a	36.39b
Control	22.02a	2.24a	0.42a	27.69a
Varieties				
WAB 56-104	30.52ab	2.55a	0.52a	32.33ab
MOROBEREKAN	23.09a	2.31a	0.43a	28.22a
OFADA GR	24.47a	2.43a	0.49a	30.77ab
N-U-1	34.18b	2.47a	0.50a	31.39ab
N-U-8	28.72ab	2.41a	0.47a	35.19b
M*V	*	*	*	*
R*V	*	*	*	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

3.1.4 Influence of mycorrhizae and rhizobium inoculation on proximate composition

Harvested grains from yield were subjected to proximate analysis, the proximate composition of rice varieties inoculated with mycorrhizae, rhizobium and un-inoculated (control) is presented in Tables 9 and 10. In the early season, inoculation with mycorrhizae and rhizobium significantly ($P < 0.05$) increased grain moisture content over un-inoculated (control), but there was no significant difference observed across cultivars. Rhizobium and mycorrhizae inoculation did not significantly affect ash content and no significant difference was observed amongst cultivars. With the exception of rhizobium inoculation and control, mycorrhizae inoculation significantly (P < 0.05) increased the fat content of rice grains. Significant difference was observed in the fat content amongst rice cultivars with N-U-8 having the highest fat content 4.99%. Rhizobium and mycorrhizae inoculated treatments significantly ($P < 0.05$) increased the protein content of grains over the un-inoculated (control); the range varied from 8.50% and 7.65% to 6.65% according to treatment. N-U-1 had the highest protein content 8.43% amongst cultivars. The treatments applied did not significantly ($P < 0.05$) increase the crude fibre percentage of grains, however mycorrhizae treated plants did have higher crude fibre content 5.77%. There was no significant difference observed in carbohydrate content of rice grains across all treatments, with carbohydrate content varying between 65.31% and 68.47%. No significant difference was observed across all cultivars as well. In the late season, treatments applied did not significantly affect moisture, ash, fat, protein and carbohydrate content of rice grains except crude fibre content, where rhizobium inoculated grains had the highest crude fibre content 7.61% and significantly different from other treatments. No cultivar variability was observed across all cultivars in moisture, ash, fat, protein and carbohydrate content except in crude fibre content where cultivar variability was observed with N-U-1 having the highest crude fibre content 8.02%. The crude fibre content, in general, is influenced by the environmental conditions and the cultivar characteristics. Cultivars vary in their crude fibre content according to cultivar, location and time of harvest; this could be an important factor with respect to the late season result. The fibre content of grains is important for food and animal feed and it is needed in a reasonable proportion as it gives bulk to the diet and helps in the movement of food through the digestive tract; also the crude fibre can contribute as a source of energy to the animal body (Elsiddig et al., 1996). The high ash content observed in the late season in the treatments and cultivars was due to the low moisture content of grains; reverse was the case in the early season. Also high ash content could be attributed to the increase in grain weight and size as a result of improved physical, chemical and nutritional properties of the soil. High fat content could be attributed to the improved nutritional status of the plant through microbial inoculations which enhances nutrient uptake. Rhizobium inoculation increased the grain protein content of rice, and this is due to their ability to fix atmospheric nitrogen and increasing nitrogen nutrient made available to rice plant. This inevitably leads to increased protein content of grains and also encourages the photosynthetic process and other physiological factors that increase protein synthesis.it is well known protein content of grains increases with improved plant nutrition and the application of rhizobium could results in a high exchangeable capacity, hence a considerable quantity of phosphorus is diverted to available form and thus increased protein. (Elsiddig et al.,1996). Balogun and Olatidoye (2012) reported that rice based protein are easily digested and provides the necessary calories in diet of many segment of the world population and therefore promote the utilization of dietary fat and reduce wastage of protein. Mycorrhizae and Rhizobium inoculation significantly decreased the carbohydrate content of rice grains. This could be attributed to the high concentration of nitrogen available to the plant which increased the protein content in treated plants. The low carbohydrate content in inoculated treatments is directly related to the high protein content of rice grains compared to the un-inoculated control.

Table 9: Influence of Mycorrhizae and Rhizobium inoculation on proximate composition of upland rice cultivars (early season)

Treatments	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fibre (%)	Carbohydrate (%)
Mycorrhizae	13.58c	4.05a	4.70b	7.65b	5.77a	65.31a
Rhizobium	12.39b	3.86a	4.06a	8.50c	5.70a	66.27a
Control	11.42a	3.99a	3.88a	6.65a	5.32a	68.47a
Varieties						
WAB 56 - 104	12.04a	4.50a	4.80bc	7.78abc	5.99a	65.83a
MOROBEREKAN	12.81a	4.26a	4.42abc	6.79ab	6.24a	65.76a
OFADA GR	12.55a	4.22a	4.69bc	8.03bc	5.13a	66.88a
N-U-1	12.56a	3.88a	3.25a	8.43c	5.10a	66.68a
N-U-8	12.46a	3.87a	4.99c	7.40abc	5.61a	66.55a
M*V	ns	ns	ns	ns	ns	ns
R*V	ns	ns	ns	*	ns	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

Table 10.Influence of Mycorrhizae and Rhizobium inoculation on proximate composition of upland rice cultivars (late season)

Treatments	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fibre (%)	Carbohydrate (%)
Mycorrhizae	3.42a	11.96a	3.46a	5.54a	7.49a	68.64a
Rhizobium	3.25a	11.80a	3.76a	5.55a	7.61b	68.15a
Control	3.30a	11.86a	3.55a	5.69a	6.30a	69.32a
Varieties						
WAB 56 - 104	3.26b	11.41a	3.91a	5.54a	6.73ab	69.24a
MOROBEREKAN	3.26ab	11.70ab	3.97a	5.80a	5.91a	69.97a
OFADA GR	3.22ab	11.52a	3.67a	5.56a	7.42bc	68.38a
N-U-1	3.02a	12.22ab	3.16a	5.65a	8.02c	67.93a
N-U-8	3.49ab	12.50b	3.25a	5.41a	7.58bc	67.99a
M*V	ns	ns	ns	ns	*	ns
R*V	ns	ns	ns	ns	*	ns

For each variable, means followed by the same letter in the column are not significantly different by DMRT test
ns: no significant interaction at ($P < 0.05$)
*: significant interaction at ($P < 0.05$)

4. Conclusions

This study has shown that there are significant influences of microbial sources (mycorrhizae and rhizobium inoculation) on the growth, nutrient uptake and content of upland rice plant. All rice cultivars inoculated with single inoculants of mycorrhizae and rhizobium showed better performance in respect of plant growth, biomass, nutrient uptake and proximate composition of rice grains over un-inoculated control. Therefore the application of mycorrhizae and rhizobium to upland rice cultivars had a positive effect on the growth and nutrition of the crop irrespective of the cultivars. It is possible therefore, to obtain good response with the exploitation of these microbial sources in the tropical rainforest agro-ecology of south-west Nigeria similar to those established in this study.

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