EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION ON SOIL PROPERTIES AND YIELD OF SELECTED RICE VARIETIES

**Christopher J. Okonji1, Olalekan S. Sakariyawo 2, Kehinde A. Okeleye2, Adedayo G. Osunbiyi 3 and Emmanuel O. Ajayi4[[1]](#footnote-2)**

1Department of Crop Science and Horticulture, Federal University Oye-Ekiti, Nigeria

2Department of Plant Physiology and Crop Production,

Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

3Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Ogun State, Nigeria

4National Horticultural Research Institute, Idi-Ishin, Jericho Reservation Area, Ibadan, Oyo State, Nigeria

**Abstract:** Plant growth can be stimulated by a symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and bacteria within the rhizosphere region. These interactions are crucial for increasing soil fertility, which leads to increased productivity and sustainability, as well as food security considering a high level of malnutrition. Six rice varieties were grown with (M+) or without (M-) AMF inoculation in a randomised complete block design with three replicates. The soil physic-chemical properties were determined using standard procedures. Bacteria were isolated from the soil samples and the colony count was determined during the early and late cropping seasons of rice. Specific soil properties (phosphate, pH, organic matter) increased dramatically in the presence of AMF, which led to significant rice yield in both seasons. Bacterial species isolated included *Lactobacillus* spp., *Klebsiella aerogenes, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*, *Azospirillum brasilense, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae,* and *Micrococcus* sp. Rice exudates increased the bacterial population in the early season, while AMF treatment increased the bacterial population in the late season and generally increased the bacterial species richness in both seasons. Although the actual mechanism that increased the bacterial species richness was not accessed, this study, however, shows that AMF-bacteria interaction increased and sustained soil fertility which consequently increased rice yield. A further study is necessary to determine the mechanism of the interaction observed between AMF inoculation and bacterial population.

**Key words:** mycorrhizal, phenolic compounds, NERICA, colonisation.

**Introduction**

Rice is the most widely consumed staple food for a large part of the world’s human population, especially in Asia and the West Indies. Since a large portion of maize crops are grown for purposes other than human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by the humans ([www.integratedbreeding.net](http://www.integratedbreeding.net)). It is predicted that a 50% to 60% increase in rice production will be required to meet demand from population growth by 2025 (Zhang and Wang, 2005). Interestingly, sub-Sahara Africa has the greatest concentration of high-value minerals and the highest concentration of degraded soils. It has the fastest growth in agriculture and the greatest level of agricultural imports. Sub-Sahara Africa has the highest proportion of the rural and poor smallholders in agriculture notwithstanding it has the fastest growth in agriculture and the greatest level of agricultural imports (Livingston et al., 2011). An ever increasing population and lifestyle have contributed to a demand for rice within the region making it the most imported food commodity (Livingston et al., 2011). Having huge resources for rice production, most African governments have put in a great deal of efforts to develop the local rice sector as an important component of national food security, economic growth, and poverty alleviation which has receiving great attention but is still confronted with low yield (Balasubramanian et al.,2007; FAOSTAT, 2015).

Several challenges have been encountered by numerous local farmers, which include: low soil fertility, biodiversity, poor management, climate change and policy issues. These challenges have immensely affected the productive capacity of rice within Nigeria and sub-Sahara Africa to meet the demands of growing populace, hence research and development preceded the development of rice breeds capable to withstand disease, pests, drought and soil infertility and low yield. In so doing, rice breeders utilised biotechnology to perform cross-fertilisation and back-crossing between rice species *Oryza sativa* and *Oryza glaberrima* which are the Asian and African historic species of rice, respectively to produce several traits of the ‘New Rice for Africa’ (NERICA) (West Africa Rice Development Association, 2001). These strains of rice happened to be effective in suppressing weeds, drought tolerant, resistant to pests and diseases, mature early, have favoured qualities, more grains per panicle, non-shattering grains and higher protein content. With such rice species and strains, there is the potential for Nigeria and other counterpart countries to be rice self-sufficient and when effectively managed with government support, to upgrade to become exporters of rice to the globe.

There would be a prolific implementation of the hybrid rice species, hence significant knowledge is required to understand what occurs below the ground where microorganisms within the rhizosphere interact with the rice species through the root via the symbiotic relationship. The arbuscular mycorrhizal fungi (AMF) represent a vital component of the majority of all terrestrial ecosystems. They form a symbiotic relationship with the roots of most plants by increasing plant phosphate (P) uptake and growth, whilst the plants supply the AM fungi with exudates for metabolism (Smith et al.,2003). Seal et al. (2004) quantified common compounds secreted by the rice plants within the rhizosphere which include: thiol acid, phenol, phenolic acid, indole, terpenic acid and phenylalkanoic acids. Transgenic rice species possessing proteins that confer resistance to pests have been shown to release root exudes containing the said protein pesticide into the soil microenvironment (Saxena et al., 2004). In return, AMF possesses distinct mycelium that provides a pathway for translocation of photosynthetic-derived carbon to soil microenvironments. This happens when there is a rapid turnover of the mycelium and exudation (glucose, starch, oligosaccharides) of living hyphae can enhance bacterial growth and composition within the soil (Staddon et al.,2003). An increased knowledge of the responses of bacteria composition and growth in the presence of AMF during cultivation of the newly developed rice species in soil is required to understand the effects on soil properties. The objectives of this study were to investigate (i) the effects of AMF treatment on soil properties and plant yield in soils containing different species of rice and (ii) the effect of AMF treatment on the indigenous bacterial population and composition within the rhizosphere of rice varieties.

**Materials and Methods**

Description of experimental site and sample collection

The experiment was conducted at the upland section of the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The early season trial was conducted between May 26th and September 4th, 2012, while the late season planting was done between September 8th and December 8th, 2012. The site is located between the rainforest and derived savannah agroecological zone of Nigeria (7°9′ 38.9″ N lat., and 3°21′ 53.9″ E long.; 140 m a.s.l). The rice experiment was grown twice in a year during the early and late season rainfall, respectively. The experiment comprised twelve treatments which were combinations of six rice varieties with (M+) or without (M-) arbuscular mycorrhizal fungi (AMF) inoculation. Random soil samples were taken from the field before planting to determine the soil physical and chemical properties. In addition, random soil samples were collected from the rhizospheric region in each plot for the determination of bacterial count in the laboratory.

Experimental design and treatment

Each experiment was a 6 x 2 factorial experiment in a randomised complete block design with three replications. Six varieties of rice (Moroberekan, NERICA 1, NERICA 2, NERICA 3, NERICA 4, and WAB 56-104) were combined with (M+) and without (M-) AMF treatment,. The AMF inoculum was obtained from the Department of Plant Physiology and Crop Production of the Federal University of Agriculture, Abeokuta. The pure inocula (*Funneliforms* sp.) were isolated and enriched from rice fields across Nigeria, identified as *Glomus mosseae* and *Glomus geosporum* and mixed according to Brundrett (2004). The mixed inocula were then applied to the hole (50 g per hole) made for rice seeds to be planted and referred to as the AMF treated (M+) plot, and the plots containing the rice variety without the AMF treatment were referred to as the AMF non-treated plots (M-). Rice was then planted at a spacing of 20 cm x 20 cm by the dibbling method on the plot of 4 m x 3 m (12 m2) giving rise to 250,000 plants/ha at the rate of 3 seeds/hole.

Five plants were randomly selected from the net plot for the determination of major agronomic parameters (growth, yield and yield components). Plant height, number of tillers and dry matter accumulation were recorded at 4, 8 and 12 weeks after planting (WAP). Days to 50% flowering and 90% maturity were also determined. The number of panicles per square meter was determined with the aid of quadrant (one meter square) by counting the number of panicles in the quadrant three weeks prior to harvest (Sakariyawo et al., 2012). Other yield component parameters were determined by standard procedures (Sakariyawo et al., 2012).

Soil analysis

Soil samples were collected from the individually treated soils (before planting, M+, M-) before and after harvest for analysis. Soil properties including pH, organic carbon and organic matter contents (%), N (%), P, Mg, Ca, and K (mg kg-1) were determined. Briefly, soil pH was determined in water (1:2.5) and KCl solution (1:1) using the glass electrode pH meter. The soil organic matter was determined according to the Walkey and Black (1934) method. The determination of total nitrogen (N) in the soil was analysed using the Kjeldahl method (Bremner, 1960), whilst the available phosphorus (P) was extracted using the Olsen’s extract and P in the extract was determined by using a spectrophotometer. The exchangeable cations (Mg, Ca, K) were extracted with 1 N ammonium acetate, K in the extract was determined by flame photometry, whilst Ca and Mg were determined using an atomic absorption spectrometer (AAS). The QA/QC was ensured by replicate digestion, use of blanks and high percentage recovery of elements.

Bacterial isolation, identification and count

A serial dilution technique was employed on samples obtained by collecting soils attached to the roots. Six-fold serial dilutions were prepared, and appropriate dilutions were plated on nutrient agar. Similarly, 2.8 g of nutrient agar was dissolved in 100 ml of sterile distilled water in a conical flask and corked with cotton wool and foil paper. It was sterilised at a temperature of 121ºC for 15 minutes in an autoclave. After autoclaving, it was allowed to cool down at a hot to touch temperature before using it for culturing. After culturing, biochemical identification of the isolates was done, in which each organism was identified according to the Cowan and Steel method of bacteria identification (Barrow, 2003), by their colonial appearance such as size, shape, consistency, colour, elevation, and Gram staining was done to further identify the isolate. The following biochemical tests were performed to further characterise the isolates through: sugar fermentation test**,** oxidase test**,** catalase test**,** citrate utilisation test, urease test, methyl red test, coagulase testand indole test.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using PROC GLM of Statistical Analytical System package (SAS, 2001). Bacterial count data were log-transformed before analysis. Means that were significantly different at   
*P* < 0.05 were separated using the least significant difference (LSD).

**Results and Discussion**

Soil properties

The physical and chemical properties of the soils (M+ and M-) were determined prior to planting, during the early and late cropping seasons (Table 1). The early cropping season was associated with more frequent rainfall than the late cropping season (Figure 1). It was observed that the soil was originally slightly acidic prior to planting and during the cropping season in the absence of AMF inoculation. During the late cropping season, the acidity of the soil increased, which resulted in a lower pH condition than the initial state. However, the addition of AMF into the soil resulted in a marked increase (*P* < 0.01) in pH to the neutral condition during the early and late cropping seasons, which resulted in improved soil condition. Similarly, the organic matter content of the soil was initially 2.88% and further decreased at the late cropping season (2.34%), but the addition of AMF resulted in a subsequent increase in OM content owing to AMF biomass. Although OM decreased at the late cropping season, the reduction was higher in AMF-treated soils. The concentration of macronutrients (N, Mg, K, Ca) within the soil seemed to be higher in AMF-treated soils compared to AMF-untreated soils, but was not significant (*P* > 0.05). However, in regards to P concentration in soil, the planting of rice led to a significant (*P* < 0.05) reduction in the amount of P owing to utilisation of P by the rice plant. Noticeably, there was a remarkable increase (*P* < 0.001) in concentration of P within the soil that was AMF-treated compared to untreated soils in both early and late cropping seasons. Unsurprisingly, a minimum of 35% P loss (abiotic + biotic) of the initial concentration of the available P was discovered following AMF treatment.

Table 1. Chemical properties of the experimental site.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil treatments | pH | Organic carbon | Organic matter (%) | Total nitrogen (%) | Phosphorus (mg kg-1) | Potassium (mg kg-1) | Magnesium (mg kg-1) | Calcium (mg kg-1) |
| Before planting | 5.7 | 1.55 | 2.88 | 0.13 | 33.22 | 0.26 | 1.90 | 16.10 |
| Early season cropping after the harvest of rice | | | | | | | | |
| -AMF | 5.8 | 1.86 | 2.57 | 0.15 | 28.45 | 0.18 | 1.10 | 15.40 |
| +AMF | 7.4 | 2.10 | 3.29 | 0.19 | 195.79 | 0.24 | 2.00 | 15.30 |
| Late season cropping after the harvest of rice | | | | | | | | |
| -AMF | 5.2 | 1.11 | 2.34 | 0.12 | 22.84 | 0.11 | 1.30 | 12.50 |
| +AMF | 7.2 | 1.99 | 3.06 | 0.18 | 125.32 | 0.19 | 2.00 | 12.40 |

Note: -AMF means without AMF; +AMF means with AMF.

Figure 1. Total rainfall distribution in 2012.

Rice yield

It was observed that all the growth and yield parameters were significantly affected (*P* < 0.05) by the treatment both in early and late cropping seasons   
(Table 2). In the early season, application of AMF significantly shortened (*P* < 0.05) the number of days to 50% flowering and days to 95% physiological maturity. Moreover, panicle length, panicle weight, number of seeds per panicle and yield of rice plant with AMF were significantly higher (*P* < 0.05) than those without AMF. During the late season, the rice plant without AMF did not survive after flowering and no data could be obtained from the plots (Table 2).

Table 2. The effect of AMF inoculation on growth, yield components and yield of upland rice during early and late cropping seasons.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Soil treatments | Days to 50% flowering | Days to 95% maturity | Panicle length (cm) | Panicle weight (g) | Panicles per m2 | Seeds per panicle | 100-seed weight (g) | Grain yield (kg/ha) |
| Early season | | | | | | | | |
| - AMF | 85.17 | 108.67 | 16.64 | 2.82 | 75.1 | 110.5 | 2.63 | 825 |
| + AMF | 75.56 | 96.72 | 20.75 | 3.44 | 128.0 | 139.1 | 3.33 | 2484 |
| SE ± | 0.48 | 0.67 | 0.45 | 0.07 | 4.44 | 2.31 | 0.06 | 70.4 |
| Late season | | | | | | | | |
| - AMF | 74.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| + AMF | 53.5 | 71.67 | 16.39 | 2.79 | 91.33 | 104.8 | 2.91 | 1586 |
| SE ± | 0.66 | 0.32 | 0.12 | 0.02 | 0.88 | 2.71 | 0.04 | 19.91 |

Note: -AMF means without AMF, +AMF means with AMF.

Bacterial count, composition and occurrence

The bacterial count ranged between 11 × 106 and 43 × 106 colony forming units (cfu) during the early season and from 14 × 106 to 82× 106 cfu in the late season (Figures 2 and 3). Different bacterial counts were observed at the rhizosphere of the rice variety while AMF inoculation affected the bacterial count in both seasons (Tables 3 and 4). Different isolates were observed in AMF-treated and AMF-non-treated plots. It was also observed that in the early season, in plots treated with AMF, ten (10) different bacterial isolates were identified, while seven (7) isolates were identified in AMF-non-treated plots (Table 3). During the late cropping season, the numbers of observed bacterial isolates were different among the treated plots and non-treated plots as seven (7) different bacterial isolates were identified in both AMF-treated and non-treated plots (Table 4).

Figure 2. The effect of AMF inoculation on bacterial counts of rice in the early season.

Figure 3. The effect of AMF inoculation on bacterial counts of rice in the late season.

Table 3. Bacterial isolates observed under different treatments in the early season.

|  |  |  |
| --- | --- | --- |
| Variety | With mycorrhizae | Without mycorrhizae |
| Nerica 1 | *Lactobacillus* spp*, Micrococcus* spp*,*  *Enterobacter cloacae* | *Bacillus subtilis,*  *Escherichia coli* |
| Nerica 2 | *Proteus mirabilis,*  *Pseudomonas fluorescens* | *Proteus mirabilis, Klebsiella aerogenes, Bacillus subtilis, Escherichia coli* |
| Nerica 3 | *Azospirillum brasilense, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae* | *Staphylococcus aureus,*  *Escherichia coli,* |
| Nerica 4 | *Pseudomonas aeruginosa,*  *Pseudomonas fluorescens* | *Proteus mirabilis, Escherichia coli,*  *Micrococcus* spp*,* |
| Moroberekan | *Enterobacter cloacae, Klebsiella aerogenes, Bacillus subtilis,Escherichia coli,*  *Pseudomonas fluorescens* | *Escherichia* *coli* |
| WAB 56-104 | *Staphylococcus* *aureus, Bacillus*  *subtilis, Pseudomonas fluorescens* | *Pseudomonas fluorescens, Klebsiella aerogenes, Escherichia coli* |

Table 4. Bacterial isolates observed under different treatments in the late season.

|  |  |  |
| --- | --- | --- |
| Variety | Mycorrhizae + | Mycorrhizae - |
| Nerica 1 | *Bacillus subtilis, Pseudomonas flourescens, Enterobacter cloacae* | *Bacillus subtilis, Micrococcus* sp.*,*  *Escherichia coli, Pseudomonas fluorescens* |
| Nerica 2 | *Bacillus subtilis, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens* | *Micrococcus* sp.*, Bacillus subtilis* |
| Nerica 3 | *Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae,*  *Escherichia coli* | *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* |
| Nerica 4 | *Escherichia coli, Proteus mirabilis, Pseudomonas fluorescens* | *Bacillus subtilis,*  *Proteus mirabilis* |
| Moroberekan | *Pseudomonas fluorescens,*  *Escherichia coli* | *Klebsiella aerogenes,*  *Escherichia coli* |
| WAB 56-104 | *Staphylococcus aureus,*  *Bacillus subtilis* | *Escherichia coli, Proteus mirabilis, Pseudomonas fluorescens* |

It was realised that the frequency of bacterial strains was not consistent as some bacteria species were more associated with the presence of AMF than without AMF. For instance, *Pseudomonas* sp. was often observed to be identified in AM treated soils during both early and late cropping seasons in plots having at least four (4) rice varieties, especially NERICA 2, 4 and Moroberekan, but they were often associated with WAB 56 variety in AMF non-treated plots. In contrast, *E. coli* was identified more in AMF non-treated plots containing NERICA 1, 3, Moroberekan and WAB 56 rice varieties, but only found consistently in the late cropping season of AMF-treated plots (Table 4).

Soil properties

The introduction of AMF into the soil was shown to increase certain soil physical and chemical properties in this study. Previous studies have shown that AMF inoculation in soils can increase the absorption of such macronutrients such as Mg and K (Singh et al., 2004; Halder et al., 2015). Although the uptake of macronutrients was not measured, it is suggested that AMF improved the availability and accessibility of these nutrients to plants. Notably, this study shows that AMF treatment drastically increased the concentration of P in the soils by releasing extracellular phosphatase enzymes (Koide and Kabir 2000; Hamel, 2004). P is a limiting factor in most agricultural soils, and this requires the incorporation of inorganic fertilizer into the soil. Similarly, Carreón-Abud et al. (2007) observed a dramatic increase in P in the aerial part of the blackberry plant (*Rubus fruticosus* var. brazos) treated with AMF. Abdel Latef and Chaoxing (2011) also observed increased P concentration and uptake into tomato when AMF was inoculated into saline soils. Even though AMF has the capability to mineralise P, Jonker et al. (2000) have reported that it is only to a minimal level. Furthermore, the fungus is capable to translocate P from the soil to the interior of the plant root system to ensure release to the plant (Smith et al.,2001; Villegas and Fortin, 2001; Toljander et al.,2007). Although P is vital for plant growth, it is immobile and plants find it difficult to access and acquire it, but accessibility is increased through a symbiotic relationship with AMF (Schachtman et al., 1998; Smith et al., 2011). More P was found in AMF treated soils in the early cropping season than in the late cropping season due to both biotic and abiotic losses (35%) via P uptake and utilisation by the plant (rice) and water erosion losses during the raining season of June and July. Unsurprisingly, despite the low concentration of P (28.45 mg kg-1) in AMF non-treated soils, there was still considerable loss (20%) through biotic and abiotic uses, since uptake is normally faster than replacement (Smith et al., 2011).

In addition to improvement to soil chemical properties (macronutrients) in AMF treated soils, it was discovered that there was a significant increase in the organic matter (OM) content and pH of the soils. In line with the symbiotic relationship between AMF and interacting biota, it happens that through the hyphae, AMF releases a range of compounds into the soil environment (Kögel-Knabner, 2002; Rillig 2004; Verbruggen et al., 2013) that can increase the OM content of soils. Obviously, OM contents increased considerably in AMF treated soils compared to non-treated soils. The exudate compounds released by AMF range from glucose, polysaccharides (glycogen, oligosaccharides) and organic acids to polymeric substances (gellan gum), some of which are recalcitrant and contribute to the OM content of the soil (Toljander et al.,2007; Verbruggen et al.,2013). In addition, the decomposition of plant litter has been observed to increase in the presence of AMF (Cheng et al.,2012) and dead AMF can also add to the OM content of soils. An increase in contact time during interaction amongst AMF and soil biota and components led to a considerable loss in the OM content (7%) which was lower than OM loss (9%) in non-treated soils during the cropping seasons. This shows that the replenishment and maintenance of OM in soil containing AMF was higher than in non-treated soils, but would be governed by the soil properties, AMF exudates and climatic conditions. In regards to pH, the addition of AMF to soils increased soil pH from 5.8 to 7.4 in the early cropping season, which supports the observation of Shukla et al. (2013) that AMF species richness was higher at higher pH levels and decreased with an increase in depth and a decrease in pH. An increase in pH is suggested to be phosphate-induced, as previous studies have shown that the incorporation of P at high rates elevates soil pH and reduces concentration of toxic aluminium compounds by forming insoluble variscite-like material [Al (OH)2 H2 PO4] precipitate in soil (Habte and Soedarjo, 1996; Manoharan, 1997). Similarly, an increase in contact time led to a decrease in the pH levels, owing to the loss of P through abiotic and biotic processes.

Rice yield

Previous studies have shown that AMF inoculation into agricultural soils improves plant growth, productivity and yield even under stress conditions (Clark and Zeto, 2000; Adesemoye et al.,2008; Zarea et al.,2009; Abdel Latef and Chaoxing, 2011). More precisely, Mäder et al. (2011) observed approximately 30% increase in wheat yield following AMF inoculation, whilst Khan and Zaidi (2007) observed over 50% higher wheat yields. However, when the AMF strain *Glomus intraradices* was inoculated to soils to investigate rice yields, Khan and Zaidi (2007) witnessed remarkable rice yield only in the second year of planting, whilst there was no significant yield earlier when compared to non-inoculated soils and they attributed it to inappropriate selection of AMF for rice. On the contrary, in this current study, there was highly significant rice yield (> 200%) during both seasons and was highly remarkable in the later season, this supports previous works by Fernández et al. (1997), Solaiman and Hirata (1997a) and Jhra et al. (2009).

After inoculating soil with AMF, the AMF colonises the rhizosphere region (Kaya et al.,2008) and then increases plant uptake of immobile nutrients, such as P by absorbing it from soil pool and other macronutrients (Tinker and Gildon, 1983; Bohrer et al.,2003; Abd-Alla et al.,2014), and consequently improves plant productivity. With the favourable pH condition, OM content and improved availability, uptake and translocation of P to the plant enhanced rice yield in support of Mohammed et al. (2014) and Pellegrino et al. (2015). This was also sustained in the late cropping season despite a minimal reduction in yield owing to a reduction in pH, OM and P concentration. However, the improved plant yield and maintenance of soil nutrients by AMF is highly dependent on the level of soil salinity, drought, soil properties, climatic and environmental conditions (Fernández et al.,1997; Solaiman and Hirata, 1997b; Abdel Latef and Chaoxing, 2011; Mäder et al.,2011; Pellegrino et al.,2015).

Bacterial count, composition and occurrence

The rhizosphere (i.e., the zone of soil influenced by plant roots and characterised by vital microbiological activities) represents a highly dynamic region governed by a complex mosaic of interactions between plants and microorganisms (Kennedy and De Luna, 2004). Varietal differences can affect the communities of microorganisms living in the rhizosphere, especially when there are differences in root secretions and exudates that are released by the plant. During the early season, NERICA 2 plot without AMF inoculation had the lowest bacterial count, but during the late season, the bacterial count increased. This shows consistency with the literature on the interactions between plants and microorganisms in the rhizosphere, where the former release compounds such as carbohydrates, phenolics, acetic acids, indoles, carboxylic acids, abietic acid and amino acids (Baudoin et al.,2003; Bacilio-Jiménez et al.,2003). The concentration and composition of these compounds vary based on the rice variety, soil properties and plant response to soil conditions (Bacilio-Jiménez et al.,2003; Seal et al.,2004). These were probable reasons why microbial counts were higher in the late cropping season without AMF treatment as plants would have reduced exudation or altered exudate composition which significantly favoured certain bacterial strain growth. Hence, the composition of the exudates can be either antagonistic or stimulatory to several bacteria species during either of the cropping seasons.

Mycorrhizal treatment, on the other hand, affected the bacterial count in the first season as a higher bacterial count was observed in non-treated rice but not in treated rice varieties. Studies have shown that AMF can have a significant impact on bacterial community composition and activity through competition for inorganic nutrients and enhancement of AMF species-specific bacterial stimulation (Artursson et al.,2006). Therefore, the reduction in the bacterial count may be as a result of the competition between the introduced AMF and the microorganisms in the rhizosphere or fungal exudation. However, following an increase in contact time, a striking increase in the bacterial count was observed in Moroberekan and WAB 56 AMF-treated soils which were of statistical similar population compared to the AMF non-treated NERICA 2 and NERICA 3 soils, thus suggesting a species-specific association of bacteria with AMF. This association is mainly governed by soluble factors or via physical contact between bacteria and AMF hyphae (Bianciotto et al.,1996; Artursson et al.,2006).

Rice root exudates and AMF colonisation also inﬂuenced the bacterial species composition by increasing some groups and decreasing others. For instance, during the early cropping season in AMF non-treated soils, *E.coli* was a common bacterium found in all rice varieties, but in the late cropping season it competed with *Bacillus* *subtilis* suggesting that the former was attracted by these rice varieties but its dominance was affected following the competition and reduction in particular rood exudates. The introduction of AMF led to an increased specific bacterial species composition and richness even though the population did not exceed that in AMF non-treated soils owing to the competition and diversity of exudates. More precisely, *Bacillus* *subtilis* and *Pseudomonas* *fluorescens* dominated in both early and late cropping seasons due to specific interactions with AMF hyphae or exudates. Previous studies have equally shown interactions (root colonisation, phosphate solubilisation, fungal growth, inhibition of plant pathogenic fungi) between *Pseudomonas* sp. and *Bacillus* sp. with *Glomus* sp. (Toro et al.,1997; Barae et al.,1998). *Bacillus subtilis,* in particular, had interaction in the rhizosphere with or without the presence of AMF. Synergistic interactions have been found between AMF and other beneficial soil microorganisms such as nitrogen-fixers (Sreenivasa and Bagyaraj*,* 1989; Biró et al.,2000).

Changes in bacterial community structure were shown to be driven by complex interactions between plant species (or genotype) and fungal species involved (Marschner and Baumann, 2003; Marschner and Timonen, 2005). However, bacterial communities in the mycorrhizosphere in turn influence the mycorrhizal or plant development in various ways, further complicating our understanding of bacterial-mycorrhizal interactions (Toljander, 2007). Due to the complex nature of the mycorrhizosphere, it has been difficult to trace the effects to specific factors/organisms. For example, the changes in microbial communities can be due to direct (e.g. belowground carbon allocation) or indirect influences of mycorrhizal colonisation (e.g. root exudation) or the combined effects of the mycorrhizal fungi and their biotic and abiotic environments (Toljander, 2007).

**Conclusion**

Rice production in Nigeria has encountered significant challenges, due to pests, diseases and soil fertility. A number of rice varieties that can withstand rice diseases have been developed and deployed in various places but the cost of fertilisation and the environmental impact of inorganic fertilizer have been one of the major setbacks in widespread productivity. This study investigated the deployment of six varieties of rice in the field with and without the inoculation of arbuscular mychorrizal fungi (AMF) consortium to determine if it can sustain soil fertility for rice production in two planting seasons and the corresponding influence on soil microbial richness. The application of AMF not only increased important soil properties (pH, organic matter, total NPK) for plant growth, but it remarkably increased soil microbial population and activity. In addition, AMF inoculation increased plant yield and development parameters for optimum rice growth during both seasons. This study, however, requires further tests in other soils for extensive deployment.

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UTICAJI INOKULACIJE ARBUSKULARNO-MIKORIZNIH GLIVA NA OSOBINE ZEMLJIŠTA I PRINOS ODABRANIH VARIJETETA PIRINČA

**Christopher J. Okonji1, Olalekan S. Sakariyawo 2, Kehinde A. Okeleye2,**

**Adedayo G. Osunbiyi 3 i Emmanuel O. Ajayi4[[2]](#footnote-3)\***

1Odsek za ratarstvo i hortikulturu, Federalni univerzitet Oye-Ekiti, Nigerija

2Odsek za fiziologiju biljaka i ratarsku proizvodnju,

Federalni poljoprivredni univerzitet, Abeokuta, Država Ogun, Nigerija

3Odsek za biološke nauke, Koledž za prirodne i primenjene nauke,

Univerzitet Crescent, Abeokuta, Država Ogun, Nigerija

4Nacionalni institut za istraživanja u hortikulturi, Idi-Išin,

Oblast rezervata Jerihon, Ibadan, Država Ojo, Nigerija

R e z i m e

Rast biljke može se stimulisati simbiotskom vezom između arbuskalarnih mikoriznih gljiva (engl. *arbuscular mycorrhizal fungi* ‒ AMF) i bakterija unutar regije rizosfere. Ove interakcije su ključne za povećanje plodnosti zemljišta, što vodi ka povećanoj produktivnosti i održivosti, kao i do prehrambene sigurnosti uzimajući u obzir visok nivo neuhranjenosti. Šest sorti pirinča uzgajane su sa (M+) ili bez (M-) inokulacije arbuskalarno-mikoriznih gljiva po metodi slučajnog blok sistema sa tri ponavljanja. Fizičko-hemijske osobine zemljišta određene su uz pomoć standardnih metoda. Bakterije su izolovane iz uzoraka zemljišta i broj kolonija je određen tokom rane i kasne vegetativne sezone pirinča. Specifične osobine zemljišta (fosfat, pH, organska materija) su se znatno povećale u prisustvu arbuskalarno-mikoriznih gljiva, što je vodilo do značajno većeg prinosa pirinča u obe berbe. Izolovane vrste bakterija obuhvatale su *Lactobacillus* spp., *Klebsiella aerogenes, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*, *Azospirillum brasilense, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae* i *Micrococcus* sp. Eksudati pirinča su povećali populaciju bakterija u ranoj žetvi, dok je tretman arbuskalarnim mikoriznim gljivama povećao populaciju bakterija kod kasne žetve i generalno povećao brojnost bakterijskih vrsta u obe sezone. Iako stvarni mehanizam koji je povećao brojnost bakterijskih vrsta nije bio poznat, ovim istraživanjem, međutim, pokazuje se da je interakcija arbuskalarno- mikoriznih gljiva i bakterija povećavala i održavala plodnost zemljišta, što je zatim povećalo prinos pirinča. Dalja istraživanja su neophodna kako bi se odredio mehanizam interakcije koji je uočen između inokulacije arbuskalarno-mikoriznih gljiva i populacije bakterija.

**Ključne reči:** mikoriza, fenolna jedinjenja, NERICA, kolonizacija.

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1. Corresponding author: e-mail: [oluwakayodefunmi@gmail.com](mailto:oluwakayodefunmi@gmail.com) [↑](#footnote-ref-2)
2. \*Autor za kontakt: e-mail: [oluwakayodefunmi@gmail.com](mailto:oluwakayodefunmi@gmail.com) [↑](#footnote-ref-3)