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Effects of Beneficial Microorganisms and Organic Fertilization on Yields and Biochemical Markers Responses of Two Rice Cultivars (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) forms a vital part of the diet of almost three billion people worldwide. Increasing rice production is crucial to meeting the demands of this ever-growing population. This study examines how the agromorphological and biochemical characteristics of two rice varieties are affected by symbiosis with beneficial microorganisms. The study used a completely randomized block design with two factors and was conducted in the botanical garden of the Faculty of Sciences at the University of Yaoundé I in central Cameroon. To achieve this, the agronomic (plant height, stem diameter, number of thalli per plant, number of panicles per capsule, weight of ears per plant, and 1000-grain weight) and biochemical (contents in total chlorophyll, total soluble sugars, and proline) traits of the rice varieties (main factor) Nerica L8 (improved) and Tonga (local), were evaluated in response to the following treatments (secondary factor): T0 (control), T1 (mycorrhizae), T2 (endophyte), T3 (endophyte and charcoal), T4 (endophyte, charcoal, and mycorrhizae) and T5 (urea). This study showed that urea treatment (T5) was the most effective, with the highest values for all agromorphological traits. These included an average plant height of 105.45 ± 6.06 cm, an average stem weight per plant of 8.27 ± 2.80 g and an average weight of 1000 grains of 36.10 ± 0.10 g for Nerica L8. Conversely, the mycorrhizae-inoculated treatments (T1 and T4) had high root colonization rates ranging from 88.53% to 96%. The results revealed a higher number of spores (53 spores/g of soil for Nerica L8 and 42 spores/g for Tonga)

and greater mycorrhizal dependency in treatment T1 (+59.11% for Nerica L8 and +50.80% for Tonga). Furthermore, treatments T4 and T2, which contained mycorrhizae, resulted in a significant accumulation of total chlorophyll, total soluble sugars and proline in both rice varieties studied ($P < 0.05$). This study suggests that using mycorrhizae and urea, either separately or together, could be key to improving rice yields.

Keywords

Oryza sativa, Mycorrhizae, Endophyte, Urea, Yield, Biochemical Compounds

1. Introduction

Rice is a cereal grain belonging to the Poaceae family. Its starchy fruit has become one of the world's most widely consumed staple foods after wheat [1]. It is grown in many places around the world, including Asia, America and Africa. Rice is much more than just a basic food. It is a significant source of energy and has long been an integral part of the culinary traditions of many nations worldwide. It is also crucial in the fight against malnutrition, particularly in areas where access to a varied diet is limited. Junaidur-Rahman *et al.* [2] argue that rice is a universal food because it is accessible and affordable.

The Food and Agricultural Organization (FAO) estimates that 540.4 million tonnes of rice will be produced worldwide in 2025, over 90% of which will come from Asia. China is the world's largest producer and consumer of rice [3]. According to the World Trade Organization (WTO), the top exporting nations are India (~40%), Thailand (16% - 18%), Vietnam (10% - 14%), Pakistan (10% - 14%), and the United States (6% - 7%). Furthermore, 30% of rice imports originate from the Middle East and sub-Saharan Africa, particularly Ivory Coast, Nigeria, and Senegal. Cameroon is one of the nations most reliant on imports, and this crop is cultivated and consumed in around 40 of the 54 African countries [4]. According to the National Institute of Statistics (2024), Cameroon's rice imports have increased dramatically from 648,085 tons per year—around 20% more than in 2023—to a record bill of 320 billion CFA francs. Local production is only expected to meet a small percentage of the nation's demand, which is anticipated to have peaked at 140,710 tons [5].

However, the main causes of low rice production in Cameroon are agronomic vulnerability, such as depleted soils, improper input management and high exposure to drought, which limits the efficacy of farming practices, and climate variability, namely irregular rainfall and extreme temperatures, which lowers yields. Furthermore, Djomo *et al.* [6] highlight post-harvest losses caused by pests and deficiencies in agricultural management, including inadequate technical support, low mechanization and limited access to improved seeds. In light of these mounting issues, adopting more sustainable yield-increasing techniques is becoming es-

sential. An excellent example of this is forming a symbiotic relationship with beneficial microorganisms like endophytic bacteria and arbuscular mycorrhizal fungi (AMF) [7]. The research of Nwaga *et al.* [8] shows that this symbiosis is present in more than 80% of terrestrial plants. It is worth noting that the use of mycorrhizae as biofertilizers offers a promising way to enhance the performance and resilience of rice plants while protecting them from harmful substances. The use of biochar, a biofertilizer derived from animal products that modifies soil microbial activity and structure, frequently improves crop yields. Biochar raises the soil's CEC (cation exchange capacity) and pH, and supplies nutrients, thus demonstrating its unquestionable fertilizing potential [9]. In fact, a number of recent studies have demonstrated that AMF and endophytic bacteria enhance photosynthetic efficiency, promote growth and increase rice's tolerance to water deficiency by adjusting the osmotic balance and carbohydrate content favourably [10]. These helpful biofertilizers form a mutually beneficial relationship with the host plant, enhancing its access to water, phosphorus, nitrogen, iron, and other trace elements. They can also defend the plant from pathogen attacks [11] [12]. They are applied to grown plants to enhance yield and the production of bioactive substances, such as carbohydrates, proteins, lipids and polyphenols [13].

Therefore, to improve the performance of host plants in degraded environments, it is necessary to research the biochemical and physiological responses of inoculated plants [14]. For around 20 years, many authors have recognised the increase of proline and total sugars—products of photosynthesis—as a typical metabolic reaction in plants subjected to environmental perturbations [15]. According to Zerrad *et al.* [16], higher levels of total sugars at this stage correspond to the maximal vegetative activity observed in rice varieties and organs. Total sugars are primary carbon molecules produced and exported throughout the plant during photosynthesis [17]. Indeed, several authors have demonstrated that sugars strengthen cytoplasmic content, thereby raising the cell's osmotic pressure in relation to its environment [18].

Thus, integrating biofertilizers into agricultural practices could fully exploit the potential of the symbiosis between microorganisms, offering a sustainable way to meet food needs. The objective of this study was to highlight the potential impact of applying an organic fertilizer and arbuscular mycorrhizal fungi (AMF) alone or in combination on the yield traits and biochemical compounds of two rice varieties.

2. Materials and Methods

2.1. Experimental Detail

From January 3 to April 29, 2025, the experiment was conducted in the botanical garden of the Faculty of Sciences at the University of Yaoundé I (Central Region, Cameroon). The rainfall pattern at this experimental site is bimodal, with two dry seasons (a long one from mid-November to mid-March and a short one from July to August) and two wet seasons (a long one from mid-August to mid-September and a short one from mid-March to mid-June). The latitude is

3˚85'N, the longitude is 11˚49'E, and the average yearly temperature is approximately 25˚C ± 2˚C.

2.2. Plant Material

This study used two varieties of rain-fed rice supplied by the Agricultural Research Institute for Development (IRAD) in Nkolbisson, Cameroon. The first local variety, known as "Tonga", originates from western Cameroon and has a development cycle of around 110 - 115 days. The second improved variety, "Nerica L8" (formerly ADRAO), was created by Monty Jones of the Africa Rice Center and has a cycle duration of roughly 100 to 120 days.

2.3. Chemical Fertilizers and Symbiotic Materials

GIC AGRIBIOCAM (Organic Agriculture in Cameroon) provided the pure culture media used for the inoculants, which included: **i**) an inoculant based on arbuscular mycorrhizal fungi (AMF) consisting of a mixture of four strains (*Glomus hoi*, *Rhizophagus intraradices*, *Scutellospora gregaria*, and *Gigaspora margarita*) at a concentration of 50 spores per gram of soil; and **ii**) a culture of endophytic bacteria consisting of mixture of five bacterial strains (BNBL19Ca, SSL9, BNL, SDL4, and RRNBL5). A soil amendment known as charcoal (produced by pyrolysis at 300˚C - 800˚C on organic matter) comes from the Soil Microbiology and environmental Laboratory in the Department of Microbiology. The experiment also involved the use of urea, which was purchased from a local market. This chemical fertilizer, which was applied at three different stages: at the start of tillering, during panicle initiation and at heading.

2.4. Experimental Design and Treatment

The experiment was carried out using a mixture of fine sand and soil from the village of Fébé in the central region of Yaoundé. These soil samples were collected from the top layer (0 - 15 cm deep) and were then subjected to a physicochemical analysis (Table 1). To create a substrate with an appropriate balance of water retention, drainage and aeration, the sand and soil were separated using a 2 mm diameter brick sieve and then combined at a ratio of three parts soil to one part sand (3:1) [19]. To prevent contact with the ground—the main source of contamination—each bag (weighing 25 kg) was placed on a wooden surface. This wooden shelf measured approximately 46.5 cm in height, 31 cm in width and 4.9 m in length. It was made from planks and slats.

Table 1. Physicochemical analyses of the soil used.

	Elements	Contents
Texture (%)	Clay	42
	Silt	8.50
	Sand	49.50
	Textural class	Sandy Clay

Continued

Soil reaction	pH water	5.2
	pH KCl	4.3
	ΔPh	−0.9
Organic matter	Organic carbon	0.83%
	Organic matter	1.43%
	Total nitrogen (g/kg)	0.04
	Carbon/nitrogen	22
Exchangeable cations (meq/100g)	Calcium	5.76
	Magnesium	2.96
	Potassium	0.20
	Sodium	0.01
Cation exchange capacity (meq/100g)	Total bases	9
	CEC pH7	11
	Saturation	81%
Assimilable phosphorus	Bray II (mg/Kg)	4.51

A completely randomized block design with two factors was used to conduct an experimental trial in the form of a pot experiment. The main factor was the varieties (Nerica L8 and Tonga), and the secondary factor was the treatments [**T0**: no inoculum (negative control), **T1**: mycorrhizae, **T2**: bacterial endophyte, **T3**: endophyte-charcoal, **T4**: mycorrhizae-endophyte-charcoal, and **T5**: urea (positive control)]. On the other hand, the replicates (six for each treatment) were separated by around 25 cm within the same treatment. Four holes per pot and six seeds per hole were used in the meticulous sowing process. To leave only one plant per hole, thinning was done 14 days after seeding. Ultimately, 144 plants were collected and allocated as follows: 4 plants each bag of Baco, 12 plants per treatment, and 72 plants per variety. However, in each pot at a depth of 10 cm, 10 g of mycorrhizal inoculum and charcoal per plant were applied, along with 10 g of urea per plant. Additionally, a culture of phosphate-solubilizing endophytic bacteria was introduced into each seedling hole by adding 50 mL of inoculum. The plants were watered twice a daily (at 7:00 am and 5:30 pm) until the grains were collected from the experimental site, once all inoculations for the four treatments (T0, T1, T2, T3, T4, and T5) had been completed on the pots of the two varieties under investigation.

2.5. Agronomic Parameters Measurement

For each genotype and treatment tested, and in each repetition, the following data were recorded: plant height (cm), number of thalli per plant (No), and stem collar diameter (cm) were measured one, two, and three months after sowing with a tape measure and caliper. Also, after 140 days of sowing, the number of panicles per plant (g), 1000-grain weight (g), weight of ears (g) were evaluated.

2.6. Estimation of Mycorrhizal Dependency and Response to Mycorrhization

Following harvest, the *O. sativa* plants were weighed on a sensitive scale after the roots had been cleaned of waste with tap water. To determine the fresh biomass, the weight of each of the 12 plants in each treatment was recorded individually and the average weight computed. After sun-drying for a month to eliminate all water, the same plants were weighed individually again to determine their dry biomass. Each test was conducted twice for each variety studied.

Relative mycorrhizal dependency (RMD), which measures how much mycorrhizae can increase biomass production in plants, and response to mycorrhization (RM), which measures the impact of mycorrhization, can be calculated using the dry biomass collected. Plenchette *et al.*'s [20] formulas are used to determine these parameters.

$$RMD = \left[\frac{DBMT - DBNMT}{DBMT}\right] \times 100$$

$$RM = \left[\frac{DBMT - DBNMT}{DBNMT}\right] \times 100$$

where: $DBMT$ = Dry biomass from mycorrhizal treatment; $DBNMT$ = Dry biomass from non mycorrhizal treatment.

Mycorrhizal dependency is assessed using the scale developed by [21], which classifies it as follows: X = 0 (no dependency); 0 < X < 25 (marginal dependency); 25 < X < 50 (moderate dependency); 50 < X < 75 (high dependency); and X > 75 (extreme dependency).

2.7. Root Colonization and Microscopic Observations

Root staining involved collecting root fragments, measuring 1 - 2 cm in length, from both types and their various treatments. These fragments were placed in test tubes and processed using the Kormanik and McGraw [22] technique. For this purpose, the roots were first chopped and then cleaned with tap water. Next, they were bleached in a water bath containing 5% KOH at 90˚C for 30 min. Following three rinses with tap water, the roots were acidified in a 0.01% acid Fuchsin solution containing a 5:3:2 mixture of lactic acid, glycerol and water, at 90˚C for 30 min. Following three rinses with tap water, the roots were immersed in a 1% hydrochloric acid solution at 90˚C for 30 min. Then, they were placed in a 0.01% acid Fuchsin solution in a 5:3:2 mixture of lactic acid, glycerol, and water for a further 30 min. Once the staining solution had been removed from the test tube, the roots were decoloured for a full day in a solution of lactic acid, glycerol and water (5:3:2). The stained root pieces (30 fragments per treatment, or 10 fragments per replication) were placed parallel to one another on slides and covered with coverslips in groups of ten. They were then examined under a microscope (SWIFT M28) at 10× and 40× magnification. There were three replicates. The frequency of root colonization can be determined based on the presence or absence of mycorrhizal structures such as mycelial filaments, spores and vesicles.

$$TC(\%) = \left[\frac{n}{N}\right] \times 100$$

where: TC = rate or frequency of racial colonization; N = total number of racial fragments (10 fragments) found on the lame-lamelle mountain; and n = the number of racial fragments observed with one or more mycorhizian structures.

2.8. Extraction of AMF Spore

Spores were extracted using the technique of Schenck and Perez [23]. An Erlenmeyer flask fitted with a filter was filled with 100 grams of soil. After adding approximately 300 mL of tap water, the mixture was homogenised. After standing for a few seconds, the mixture was successively sieved through columns of sieves with progressively smaller mesh sizes: 45 µm, 125 µm and 250 µm. After at least three rounds of washing and settling, the contents of each sieve were placed individually onto 9.4 cm Petri dishes. All analyses were performed three times for each variety studied. A stereo microscope (ZEISS) was used to see the spores at magnifications between 10x and 40x. Using the formula:

$$N = \frac{n \times 27.76}{100}$$

where: n = average number of spores in three repetitions.

2.9. Biochemical Parameters

2.9.1. Photosynthetic Essay

Total chlorophyll was extracted from the mesophyll cells of the leaf blades of three-month-old fresh leaves of the two rice varieties in each treatment. Three replicates were made for each sample. For each replicate, 1 g of fresh leaf was crushed in a mortar, followed by the addition 20 mL of 70% ethanol. The mixture was then homogenized and left to rest outside for three minutes. After filtration (n˚4 Whatman filter paper), the concentration of total chlorophyll in the rice leaf sample extracts, expressed in milligrams per milliliter, was calculated in triplicate using the method described by Arnon [24]. The formula used to determine the total chlorophyll concentration is given below:

$$\left[Total\ chlorophyll\right] = \left(0,0202 \times DO\ 645\right) - \left(0,0802 \times DO\ 663\right)$$

2.9.2. Total Soluble Sugar Extraction and Quantification

Total soluble sugars were extracted in triplicate using the method described by Babu *et al.* [25], with slight modifications. 0.5 g of the each sample was homogenized with 80% ethanol and the mixture was then centrifuged for 10 minutes at 3500 rpm. The resulting crude extract was then gathered in Eppendorf tubes and stored at −20˚C.

The anthrone method was used to determine the total soluble sugars [26]. The reaction mixture was prepared by combining 5 mL of anthrone reagent with 15 mL of the alcoholic extract. This was then homogenised and boiled in a water bath at 80˚C for 20 minutes. Once cooling melting ice, the absorbance of the green

complex at 620 nm was measured (Hitachi Spectrometer U-200). The total soluble sugar content was calculated in milligrams of glucose equivalent per gram of fresh weight (mg GluEq/g FW). Each test was conducted twice.

2.9.3. Proline Extraction and Quantitation

Proline extraction was performed using the technique described by Troll and Lindsley [27]. In a mortar, 500 mg of freshly crushed rice leaves were mixed with 10 mL of 3% sulfosalicylic acid. After that, the homogenate was centrifuged for 10 to 15 minutes at room temperature at 3500 rpm. All analyses were per formed three times for each variety studied.

For each sample, 1 mL of the previously obtained extract is collected, and it is combined with 1 mL of a solution composed with 120 mL of distilled water, 300 mL of acetic acid, and 80 mL of orthophosphoric acid. Next, 2 mL of acetic acid and twenty-five milligrams of ninhydrin are added. For 30 min, the mixture is heated to 100˚C in a water bath (SALVIS). After letting it cool, use a vortex mixer to combine 5 mL of toluene—let them stand. After adding a teaspoon of Na_2SO_4 to the upper phase, used a spectrophotometer (Hitachi Spectrometer U-200) to measure the optical density at 528 nm.

2.10. Data Analysis

The data obtained for the two analysed parameters were subjected to a one-way analysis of variance (ANOVA) using the 26th version of IBM SPSS software (SPSS, Inc., Chicago, IL, USA). The difference between the various means ± standard deviation was compared using Tukey's HSD (Honestly Significant Difference) test at the 0.05 level. Multiple linear regression (MLR, software R version 4.1.2 (R Development Core Team 2022)) and Principal component analysis (PCA, SPAD version 5.5-Monoposte, Decisia, Pantin, France) were used to investigate the effect of the biofertilizer on the agromorphological and biochemical parameters of the two rice varieties. Correlations between each analysis were conducted by Pearson test.

3. Results

3.1. Effects of Treatments on the Agronomic Parameters of Rice Varieties

The effects of the treatments on the growth and yield characteristics of the two rice varieties under study changed over the first, second and third months. **Table 2** shows that, regardless of variety, treatment T0 (control) produced the lowest values for both growth parameters examined when compared to treatments T1, T2, T3, T4 and T5 three months after sowing. Treatments T3 and T5 produced the greatest average plant height values, measuring 100.10 ± 9.02 cm (T3) and 105.45 ± 6.06 cm (T5) for Nerica L8, and 96.20 ± 18.02 cm (T3) and 101.90 ± 40.12 cm (T5) for Tonga (**Table 2**). With values of 5.95 ± 0.50 cm for Nerica L8 and 5.77 ± 1.36 cm for Tonga, the mycorrhizae treatment (T1) had the greatest impact on the average stem diameter trait (**Table 2**).

Table 2. Growth and yield parameters of two rice varieties in response to different treatments three months after sowing.

Varieties	Tr	Growth parameters		Yield parameters			
		Plant height (cm)	**SD (cm)**	**NTP (No.)**	**NPP (No.)**	**WEP (g)**	**1000-GW (g)**
Nerica L8	T0	88.90 ± 7.45 **a**	4.00 ± 0.25 **a**	1.58 ± 0.51 **a**	6.50 ± 1.44 **a**	3.17 ± 0.52 **a**	28.40 ± 0.40 **a**
	T1	94.97 ± 5.46 **ab**	5.95 ± 0.50 **b**	2.85 ± 0.42 **b**	10.42 ± 2.10 **b**	4.34 ± 1.75 **b**	32.34 ± 0.04 **c**
	T2	98.84 ± 7.94 **b**	5.10 ± 0.84 **b**	2.20 ± 0.15 **b**	12.00 ± 2.76 **c**	4.80 ± 2.17 **b**	31.09 ± 2.00 **bc**
	T3	100.10 ± 9.02 **c**	5.62 ± 0.59 **b**	2.24 ± 0.44 **b**	12.50 ± 2.74 **c**	5.06 ± 2.20 **c**	32.90 ± 1.00 **c**
	T4	90.63 ± 5.60 **a**	5.45 ± 0.56 **b**	2.63 ± 0.28 **b**	12.91 ± 2.93 **c**	4.65 ± 1.64 **b**	35.04 ± 3.00 **d**
	T5	105.45 ± 6.06 **c**	5.66 ± 0.58 **b**	3.09 ± 0.52 **c**	13.66 ± 2.38 **cd**	8.27 ± 2.80 **d**	36.10 ± 0.10 **d**
Tonga	T0	78.28 ± 25.31 **a**	4.52 ± 0.41 **a**	0.53 ± 0.52 **a**	7.08 ± 2.90 **a**	4.85 ± 2.26 **c**	27.90 ± 2.00 **a**
	T1	82.64 ± 10.11 **ab**	5.77 ± 1.36 **b**	1.52 ± 0.15 **b**	12.25 ± 3.05 **b**	2.72 ± 1.36 **a**	30.56 ± 1.00 **b**
	T2	86.65 ± 27.30 **b**	4.63 ± 1.12 **a**	1.83 ± 0.57 **b**	12.08 ± 2.71 **b**	5.13 ± 1.65 **c**	30.20 ± 4.00 **b**
	T3	96.20 ± 18.02 **c**	4.88 ± 0.49 **a**	1.75 ± 0.45 **b**	13.07 ± 2.67 **bc**	6.76 ± 2.05 **d**	31.50 ± 0.50 **b**
	T4	83.63 ± 13.35 **ab**	4.99 ± 1.99 **a**	1.77 ± 0.44 **b**	11.91 ± 2.46 **b**	3.75 ± 1.37 **b**	33.00 ± 3.00 **c**
	T5	101.90 ± 40.12 **cd**	4.83 ± 0.67 **a**	2.33 ± 0.57 **c**	13.42 ± 3.14 **bc**	8.77 ± 2.57 **e**	33.96 ± 2.00 **c**
F-value		3.135	3.682	4.255	2.187	2.969	4.631
***P* (>*F*)**		0.00433**	0.0061**	0.00372**	0.01812*	0.00588**	0.00886**
Variety		*	ns	ns	*	**	*
Treatment		***	ns	*	*	***	**
Interactions		***	*	**	**	***	***

PH: average plant height; **SD**: average stem diameter; **NTP**: average number of thalli per plant; **NPP**: average number of panicles per capsule; **WEP**: average weight of ears per plant; **1000-GW**: average weight of 1000 grains; **No.**: number. **Tr:** treatment; **T0**: control; **T1**: mycorrhizae; **T2**: endophyte; **T3**: endophyte-charcoal; **T4**: endophyte- charcoal-mycorrhizae; **T5**: urea. **df error** =12. *Values with the same letter in the same column and same variety are not significantly different in the Turkey HSD test at the* 5% ($P < 0.05$). ***P* < 0.05; ****P* < 0.01; *****P* < 0.001; **ns** = not significant.

In contrast to the Tonga variety, the Nerica L8 variety recorded the highest values for the four yield parameters under study (Table 2). With the exception of treatments T1 (2.72 ± 1.36) and T4 (3.75 ± 1.37) for the average weight of ears per plant trait in the Tonga variety (Table 2), treatment T0 had the lowest values of the six treatments examined (0.53 to 1.58 for average number of thalli per plant, 6.50 to 7.08 for average number of panicles per capsule and 27.90 to 28.40 for average weight of 1000 grains). Conversely, the urea treatment resulted in values much below the 5% threshold for both varieties (Table 2).

3.2. Impact of Biofertilizers on Symbiotic Parameters

3.2.1. Root Colonization and Arbuscular Mycorrhizal Fungi (AMF) Sporulation

Root colonization was evaluated after harvesting the roots in accordance with the

treatment. It was found that the variety and treatment [plants inoculated with mycorrhizae (T1 and T4) and plants not inoculated with mycorrhizae (T0, T2, T3 and T5)] varied (Table 3). As shown in Table 3, the two treatments inoculated with mycorrhizae (T1 and T4) recorded the highest rates of root colonization, regardless of the variety studied. The Nerica L8 and Tonga varieties displayed the highest rates of root colonization under treatment T4 (96.67% and 92.88%, respectively), followed by treatment T1 (92.29% and 88.33%, respectively) (Table 3). Treatment T0 exhibited the lowest rates (53.34% for Nerica L8 and 58.89% for Tonga) (Table 3).

Both variety and treatment impacted sporulation. With 53 and 42 spores/g of soil respectively, the Nerica L8 and Tonga cultivars displayed the greatest sporulation levels in the mycorrhizal treatment (T1) (Table 3 and Figure 1). This was followed by the endophyte-charcoal-mycorrhizae consortium (T4), which produced 41 and 35 spores/g of soil (Table 3 and Figure 1). However, non-mycorrhizal plants exhibited the lowest values: 13 spores/g of soil for Nerica L8 and 11 spores/g of soil for Tonga in treatment T5; and 15 and 14 spores/g of soil, respectively, for Nerica L8 and Tonga in treatment T0 (control) (Table 3 and Figure 1). The different spore morphotypes are observed based on their color (black, white, brown, yellow, light golden, rough light brown, light golden, light yellow, dark brown, rough black), size (large, average and small), shape (oval and round), and number of spores per gram (Table 3 and Figure 1).

However, mycorrhizal structures composed of spores (resistance), vesicles (storage) and mycelial hyphae (transport structures) were found in both rice varieties examined by optical microscopy of root fragments (Figure 2). These structures are known as endomycorrhizal fungi.

Table 3. Root colonization (%) and sporulation (spores/g of soil) in two rice varieties.

Parameters	***Varieties***	**Non-mycorrhizal**				**Mycorrhizal**	
		T0	T2	T3	T5	T1	T4
Root colonization	**Nerica L8**	$53.34 \pm 2.87^{a*}$	$71.62 \pm 1.74^{b*}$	$87.77 \pm 3.28^{c*}$	$86.66 \pm 1.10^{c**}$	$92.29 \pm 2.58^{d**}$	$96.67 \pm 2.32^{de**}$
	Tonga	$58.89 \pm 1.32^{a**}$	$71.10 \pm 1.06^{b*}$	$87.43 \pm 2.41^{c*}$	$67.77 \pm 2.54^{b*}$	$88.33 \pm 4.12^{c*}$	$92.88 \pm 2.76^{d*}$
Sporulation	**Nerica L8**	$15.00 \pm 0.63^{a*}$	$26.00 \pm 0.11^{b**}$	$25.00 \pm 0.71^{b**}$	$13.00 \pm 0.07^{a*}$	$53.00 \pm 2.65^{d**}$	$41.00 \pm 1.14^{c**}$
	Tonga	$14.00 \pm 0.21^{a*}$	$21.00 \pm 0.39^{b*}$	$23.00 \pm 1.06^{b*}$	$11.00 \pm 0.12^{a*}$	$42.00 \pm 1.02^{d*}$	$35.00 \pm 0.85^{c*}$
Variety		**	**	*	**	***	***
Treatment		***	***	***	***	***	***
Interactions		**	**	**	**	***	***

The stars indicate the level of significance between each variety in the same column for a given treatment, for each parameter* ($P < 0.05$). *Values with the same letter in the same line are not significantly different at the* $P < 0.05$ *threshold.* **PH: average plant height; **SD**: average stem diameter; **NTP**: average number of thalli per plant; **NPP**: average number of panicles per capsule; **WEP**: average weight of ears per; **1000-GW**: average weight of 1000 grains. **T:** treatment; **T0**: control; **T1**: mycorrhizae; **T2**: endophyte; **T3**: endophyte-charcoal; **T4**: endophyte-charcoal-mycorrhizae; **T5**: urea. *These symbols* (***** $P < 0.05$; ****** $P < 0.01$; ******* $P < 0.001$; **ns** = not significant) *refer to the variety effect, the treatment effect and their interactions.*

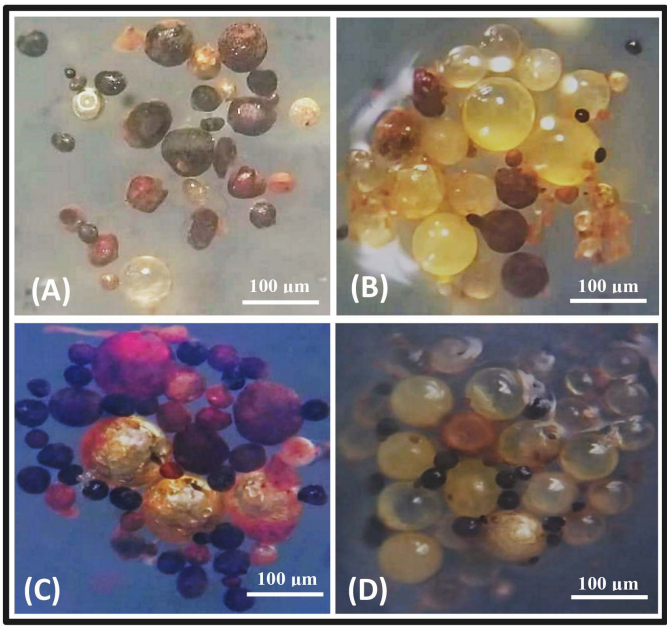

Figure 1. Diversity of AMF spore morphotypes observed by stereomicroscope in the two varieties according to the treatment conditions. (A) Black, white, light brown, light golden colors. (B) light yellow, light, light brown, black colors. (C) Golden, light golden, dark brown, light, black and rough black colors. (D) White, black, brown, light yellow colors.

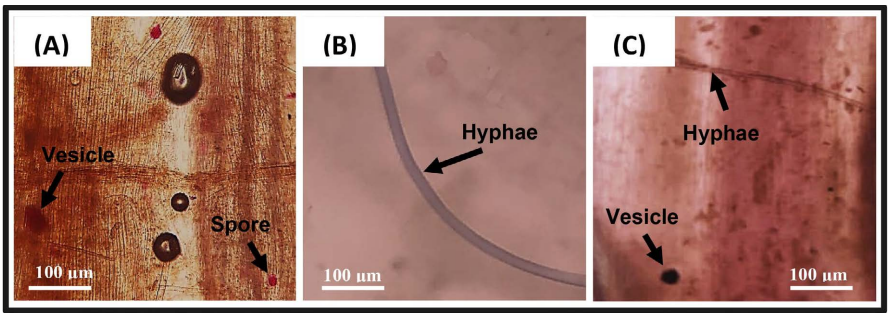

Figure 2. Structures of AMFs observed in rice root fragments inoculated or not after root colonization. (A) Vesicle and spore; (B) Hyphae; (C) Vesicle and hyhae.

3.2.2. Relative Mycorrhizal Dependency and Response to Mycorrhization

Different rice cultivars respond differently to mycorrhization (RM) and have different levels of relative mycorrhizal dependency (RMD) (Table 4). The treatments inoculated with mycorrhizae of the Nerica L8 variety showed the highest dependency and responsiveness, with +49.11% and +64.23% for treatment T1, respectively, and +22.37% (RMD) and +28.83% (RM) for the endophyte-charcoal-mycorrhizae consortium (Table 4). The Tonga variety exhibited somewhat lower levels, but similar findings. Conversely, the DMR and RM rates for treatments not inoculated with mycorrhizae (T0, T2 and T5) ranged from −6.22% to 0% and from −8.74% to 0%, respectively (Table 4). Additionally, the DMR and RM rates for the treatment combining endophyte-charcoal were extremely low, yet still displayed positive values, as seen with the Nerica L8 variety (Table 4).

Table 4. Dry biomass (g/plant), relative mycorrhizal dependency and response to mycorrhizal of two rice varieties subjected to different treatment conditions.

Varieties		Non-mycorrhizal			Mycorrhizal		
		T0	T2	T3	T5	T1	T4
Nerica L8	Dry biomass	4.12 ± 0.67^{a**}	3.90 ± 0.84^{a*}	4.49 ± 0.49^{a**}	4.39 ± 0.11^{a*}	5.89 ± 2.57^{c*}	4.91 ± 0.86^{ab*}
	RMD (%)	0	−6.22	+8.04	−5.46	+49.11	+32.37
	RM (%)	0	−8.11	+12.17	−8.74	+64.23	+48.83
Tonga	Dry biomass	3.03 ± 0.73^{a*}	4.26 ± 0.13^{b**}	3.51 ± 0.33^{a*}	4.43 ± 1.24^{b*}	5.68 ± 0.25^{c*}	4.70 ± 0.82^{b*}
	RMD (%)	0	−5.83	+8.73	−5.09	+28.80	+21.60
	RM (%)	0	−6.32	+10.87	−7.63	+31.84	+18.16
Variety		*	*	*	ns	ns	ns
Treatment		*	*	*	*	***	***
Interactions		ns	ns	*	ns	**	**

RM: Response to mycorrhization; **RMD**: relative mycorrhizal dependency. **The stars indicate the level of significance between each variety in the same column for a given treatment, for each dry biomass* ($P < 0.05$). *Values with the same letter in the same line are not significantly different at the* $P < 0.05$ *threshold. These symbols* (*****$P < 0.05$; ******$P < 0.01$; *******$P < 0.001$; **ns** = not significant) *refer to the variety effect, the treatment effect and their interactions.*

The average dry biomass is measured in grams per plant (g/plant) and changes with treatment. For the two treatments inoculated with mycorrhizae (T1 and T4), the average dry biomass of the Nerica L8 and Tonga varieties ranged from 4.70 ± 0.82 g/plant (T4) to 5.89 ± 2.57 g/plant (T1) (Table 4). Additionally, treatments T0 (3.03 ± 0.73 g/plant) and T3 (3.51 ± 0.33 g/plant) for Tonga and T2 (3.90 ± 0.84 g/plant) for Nerica L8 showed the lowest values (Table 4).

3.3. Impact of Biofertilizers on Biochemical Components under Treatment Conditions

Treatments T4 and T1, which had mycorrhizae, showed the highest total chlorophyll levels (Figure 3(A)), with 17.33 mg/g fresh weight (FW) for the Nerica L8 variety and 17.16 mg/g FW for the Tonga variety, respectively. In contrast, treatments T5 (6.02 mg/g FW for Tonga) and T2 (8.24 mg/g FW for Nerica L8) revealed the lowest total chlorophyll levels (Figure 3(A)). However, the Tonga variety showed extremely high amounts of total chlorophyll in both the endophyte treatment (T2: 15.50 mg/g FW) and the control treatment (T0: 12.55 mg/g FW). Compared to the Tonga variety, the Nerica variety exhibited notably higher total chlorophyll concentrations in treatments T3 (12.67 mg/g FW) and T5 (10.55 mg/g FW) (Figure 3(A)).

Figure 3(B) depicts that, for treatments T0, T1, T2, T3 and T4, the Nerica variety accumulated more total soluble sugars than the Tonga variety. Thus, the concentration of total soluble sugars was significantly higher for treatment T5 (776.25 mg GE/g FW). In contrast to the other treatments, there were no significant differences between treatments T1, T2 and T3 of the two varieties ($P < 0.05$).

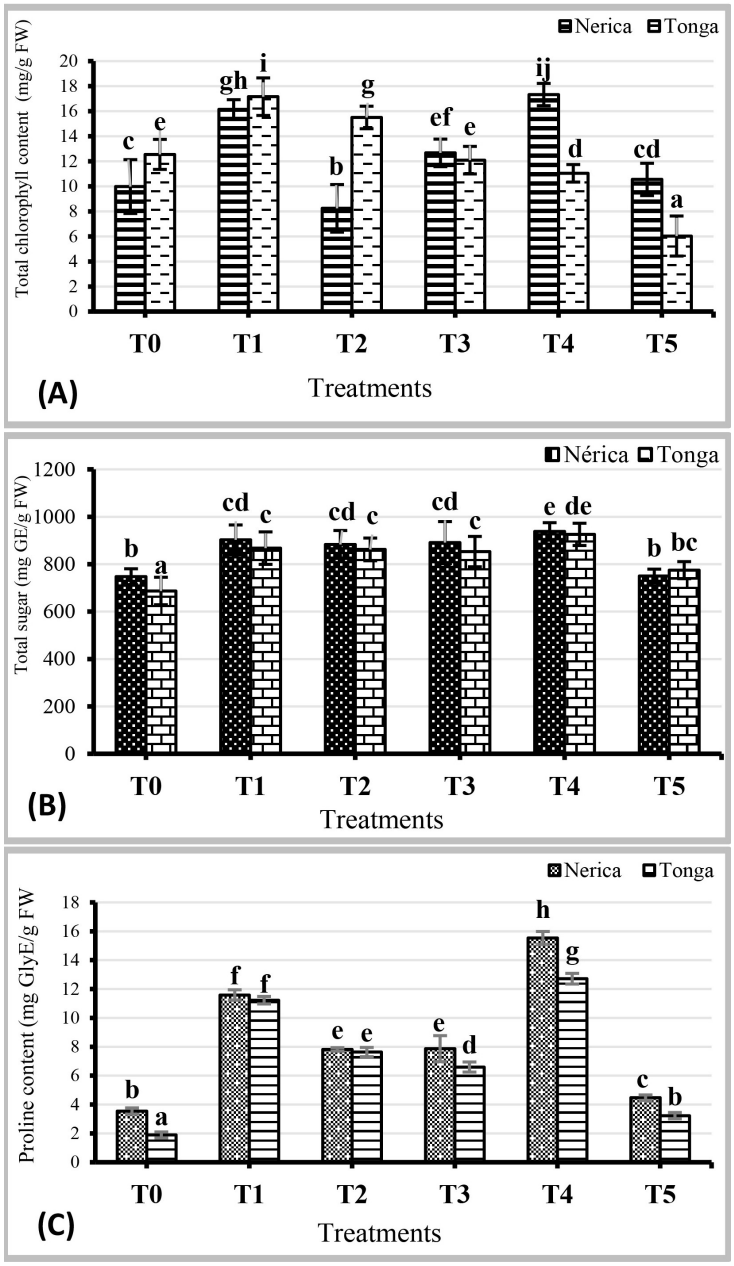

Figure 3. Variation of biochemical compounds of fresh rice leaves in response to different treatments. *Histogram bars followed by the same letters for each variety and for each treatment are not significantly different at the* 5% *threshold* (*Tukey test*, *HSD*).

With 15.53 mg GlyE/g FW and 12.72 mg GlyE/g FW, respectively, the varieties Nerica L8 and Tonga indicated the highest levels of proline for treatment T4, which is composed of a consortium of endophyte-charcoal-mycorrhizae (**Figure 3(C)**). Conversely, the lowest concentrations were found in the first treatment (T0: between 1.89 and 3.54 mg GlyE/g FW) and the second treatment (T5: between 3.22 and 4.47 mg GlyE/g FW) for both varieties (**Figure 3(C)**). Unlike the other treatments, treatments T1 and T2 for the two varieties did not show any significant differences (**Figure 3(C)**).

3.4. Relationship between Parameters Studied

3.4.1. Multiple Linear Regression (MLR)

As shown in Table 5, the variance inflation factor (VIF) values for all the agronomic and biochemical parameters studied were recorded as less than 5. The effect of inoculation with organic fertilisers and arbuscular mycorrhizal fungi on plant height, stem diameter, number of panicles per plant, weight of 1000 grains, sugar content and proline content was found to be positive and highly significant at the 1% threshold (Table 5). The coefficient of determination (R^2) values for the explained effects of the treatments studied were 0.732 for agronomic parameters and 0.541 for biochemical parameters (Table 5).

Table 5. Multiple linear regression (MLR) analysis performed on the study variables (growth, yield and biochemical parameters) of two rice varieties in response to different treatments (T0, T1, T2, T3, T4 and T5).

Agronomic parameters				Biochemical parameters			
Variables	Estimate	$P(>\|t\|)$	VIF	**Variables**	Estimate	$P(>\|t\|)$	VIF
Intercept	23.405	<0.0001***		Intercept	52.670	<0.0027***	
PH (cm)	5.595	0.0209**	1.18	T. Chl	0.322	2.3537*	2.32
SD (cm)	0.021	0.0621**	1.37	Sugar	0.888	5.9955**	4.03
NTP (No)	0.325	0.0033*	2.09	Proline	0.776	6.3881**	2.67
NPP (No)	3.494	0.0780**	1.11				
WEP (g)	0.655	0.0112*	1.17				
1000-GW (g)	19.129	0.0484**	3.23				
R^2	**0.732**			R^2	**0.541**		

***** = Significant; ****** = highly significant; **ns** = not significant; **VIF** = variance inflation factor; **R^2** = coefficient of determination; **PH**: plant height; **SD**: stem diameter; **NTP**: number of thalli per plant; **NPP**: number of panicles per plant; **WEP**: weight of ears per plant; **1000-GW**: weight of 1000 grains. **T. Chl**: Total chlorophyll.

3.4.2. Principal Component Analysis and Correlation between Parameters Studied

To demonstrate the affinities between the two rice varieties and the relationships between all the evaluated characteristics (growth, productivity, biochemical compounds, root colonization and sporulation), a principal component analysis was performed (Figure 4). The first two principal components explained 88.43% and 91.68%, respectively, of the total variability of the examined variables in Nerica L8 (PC1: 55.17%; PC2: 33.26%) and Tonga (PC1: 69.84%; PC2: 21.84%) (Figure 4(A)-(B)). In both varieties, however, the highest values for stem diameter, root colonization, sporulation and the three biochemical parameters (total chlorophyll content, total soluble sugar content and proline content) were observed in mycorrhizae-inoculated plants (T1 and T4) (Figure 4). The average values for the traits of number of panicles per capsule, number of thalli per plant, ear weight per plant, and plant height were also recorded for treatments T3 (endophyte) and T4 (endo-

phyte-charcoal) (Figure 4). Conversely, these characteristics were crucial for the urea treatment (T5); however, independent of variety, biochemical compound accumulation was minimal (Figure 4). On the other hand, for every parameter examined, the control treatment (T0) had the lowest values.

In our trials, the number of thalli per plant and the number of panicles per capsule ($r = 0.947$, $P < 0.01$), as well as the weight of 1000 grains ($r = 0.888$, $P < 0.01$), showed positive and highly significant correlations for the productivity trait (Table 6). Regarding biochemical parameters, we also found positive correlations that were highly significant between sugar content and proline concentration ($r = 0.921$, $P < 0.01$), as well as between sugar content and total chlorophyll content ($r = 0.889$, $P < 0.01$) (Table 6). Similar findings were observed for root colonization and sporulation, as well as for root colonization and stem diameter, with respective values of 0.667 and 0.724 ($P < 0.01$) (Table 6). However, root colonization and the three biochemical compounds exhibited a positive and significant correlation ($P < 0.05$) (Table 6). Table 6 also shows that significant correlations were found at the 5% threshold between sporulation and sugar content ($r = 0.816$) and proline content ($r = 0.854$), as well as between 1000-grain weight and the number of panicles per capsule ($r = 0.885$).

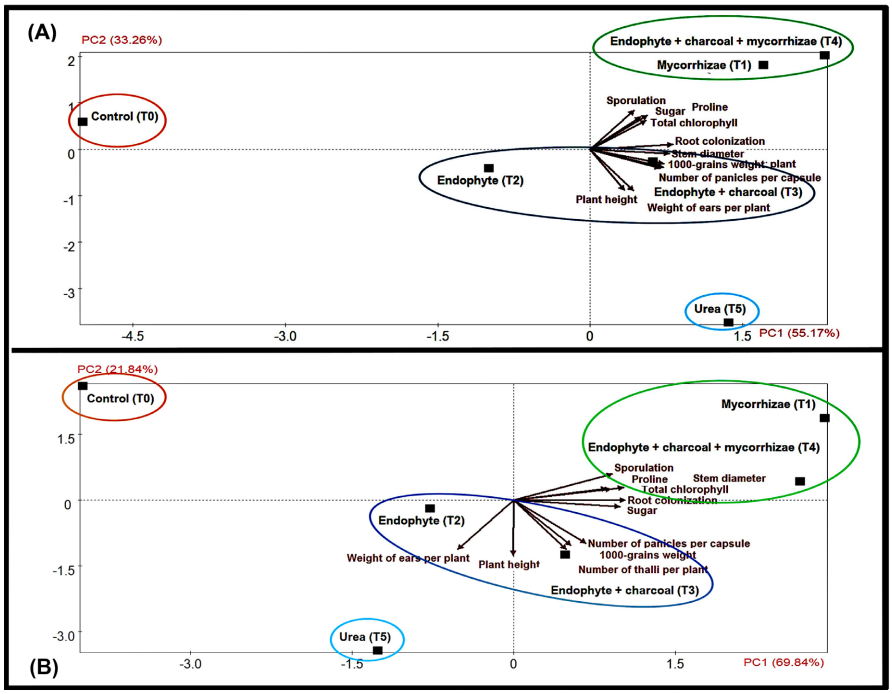

Figure 4. Principal component analysis based on growth traits, productivity, biochemical compounds, root colonization, and sporulation from two rice varieties under different treatments. A. Nerica L8 variety. B. Tonga variety.

Table 6. Correlation between growth traits, yield, biochemical compounds, root colonization and sporulation.

	PH	SD	NTP	NPC	WEP	1000-GW	Root C.	Sporulation	Sugar	T. Chl	Proline
Plant Height	1										

Continued

SD	0.221	1									
NTP	0.671	0.522*	1								
NPC	0.713	0.572	0.947**	1							
WEP	0.865*	0.009	0.610*	0.344	1						
1000-GW	0.633	0.479	0.888**	0.885*	0.622	1					
Root C.	0.162	0.724**	0.824*	0.668	0.003	0.639*	1				
Sporulation	−0.393	0.686*	0.148	0.145	−0.575	0.023	0.667**	1			
Sugar	−0.075	0.510	0.375	0.530	−0.377	0.351	0.885*	0.816*	1		
T. Chl	−0.239	0.644	0.287	0.254	−0.389	0.459	0.843*	0.753	0.889**	1	
Proline	−0.316	0.584	0.320	0.402	−0.447	0.386	0.881*	0.854*	0.921**	0.809	1

significant* ($P < 0.05$) ***highly significant* ($P < 0.01$, *Pearson's correlation tests*) **PH: average plant height; **SD**: average stem diameter; **NTP**: average number of thalli per plant; **NPC**: average number of panicles per capsule; **WEP**: average weight of ears per plant; **1000-GW**: average weight of 1000 grains. **Root C.**: Root colonization; **T. Chl**: Total chlorophyll.

4. Discussion

To understand the soil's ability to provide and alter the mineral elements necessary for plant growth, we assessed its physicochemical properties. The results showed that the soil used in this experiment had an acidic pH of 5.2, raising concerns about its structural stability due to the high likelihood of compaction and destruction. These results corroborate those of Ngo-Nkot *et al.* [28], who demonstrated that ferralitic soils in Cameroon's Central, Littoral and Eastern regions have an acidic pH ranging from 3.96 to 6.00. The use of chemical fertilizers, pesticides, and slash-and-burn methods, which change microbial communities and lower soil stability, are thought to be the cause of this acidity [28]. The low levels of organic carbon (0.83%) and organic matter (1.43%) found in the soil examined in this study indicate low biological fertility and limited nutrient storage capacity. Factors affecting soil microbial activity include available phosphorus, organic matter, organic carbon, pH and nitrogen. In addition to the physicochemical characteristics, the significance of microflora, especially mycorrhizal fungi, must be considered. Furthermore, the naturally occurring amount, mycorrhizal fungi had to be added because the soil had not been sterilized.

The effects of organic fertilizer, bacteria and arbuscular mycorrhizal fungi (AMF) inoculants on the growth, productivity and capacity to accumulate biochemical compounds associated with nutrition and stress resistance were assessed in two rice cultivars. Yield, an essential indicator of agricultural crop, is impacted by the variety grown, climate, fertilization techniques, and soil fertility. According to our findings, the yields traits (PH, NTP, NPP, WEP and 1000-GW) were significantly impacted by urea-based treatments (T5) and treatments involving a combination of endophytes and charcoal (T3). The ability of these two varieties to use mineral nitrogen effectively in this type of soil, due to improved physiological adaptation

and closer interaction with the present microflora, possibly accounts for this finding, which contradicts our predictions (after employing AMF). For this reason, urea is a nitrogen fertilizer that is made up of 46% organic nitrogen. Nitrogen is an essential component that helps all plant structures to grow and develop. It is also necessary for the production of chlorophyll (photosynthesis) and the formation of cells. According to Chu *et al.* [29], nitrogen may account for up to 75% of production increases. Yameogo *et al.* [30] argue that placing nitrogen deep in the soil is key to increasing its efficacy in rice farming. Their research shows that placing nitrogen deep in the soil enhances its efficiency by 50% and ensures the plant has access to it throughout its growth cycle. The decrease in competition for space between rice plants encourages tillering, panicle density and the number of grains per panicle—and consequently, yields. This may be the primary cause of rising urea levels in the pots. Pérez-Jaramillo *et al.*'s research [31], which showed that some rice varieties attracted more varied and useful microbial communities than others, may further support this conclusion. This would explain their ability to enhance agronomic performance and nutrient absorption in certain situations. An analysis of the soil's composition showed that the important effect of the T3 treatment (which combines endophytes and charcoal) on the yield of the two rice varieties under study was due to the presence of organic matter (3.81%) and organic carbon (2.21%). This is most likely due to various microbial activities that recycle specific nutrients. These findings are comparable to those reported by Mefo [32] for soils in central Cameroon. By contrast, we found that the various rice varieties cultivated on mycorrhizae-containing soil had larger stem diameters than those planted on non-mycorrhizae-containing soil. The stem diameters of plants with mycorrhizae were noticeably larger than those without AMF (5.95 cm for Nerica L8 and 5.77 cm for Tonga, compared to 4 cm for T0 and 5.66 cm for T5). This variation is thought to be caused by beneficial microbes, especially AMF. These findings align with those of Anozie and Orluchukwu [33], who found that mycorrhizal maize plants grew thicker than non-mycorrhizal ones. Campo *et al.* [34] emphasized the relevance of the findings further, which show that rice producers benefit from arbuscular mycorrhizal symbiosis, by highlighting the potential of using AMF to increase rice height growth, yields and improve stress tolerance. The study considered how well AMFs increased the height of the improved plant (Nerica L8) compared to the local plant (Tonga). However, the control group (T0) had the lowest yield, demonstrating the significance of nutrient inputs—chemical or biological—in raising rice production in this study's experimental setup.

The analysis of this mutually beneficial association revealed differences between the two varieties of rice. In treatment T4, which combined an endophyte-mycorrhizae-charcoal, the root coloration rate—a measure of the extent of mycorrhizal fungal colonization—was considerably higher (96.67%), particularly in the improved variety Nerica L8, indicating the establishment of a functional root symbiosis [35]. It is likely that the microenvironmental conditions in the rhizosphere

were enhanced by the combination of charcoal and microorganisms. This increased moisture retention and nutrient availability, as well as providing anchorage surfaces that supported the growth of spores and the establishment of fungal hyphae [9]. Additionally, as has been observed in several other improved cultivars, the higher responsiveness of Nerica L8 may be related to its thicker root system or greater sensitivity to microbial signals [35]. In contrast, the control (T0), which had no inoculum, exhibited minimal coloration, highlighting the importance of biological soil enrichment in establishing symbiosis. Similarly, treatments T1 (mycorrhizal inoculum at a concentration of 53 spores/g of soil for Nerica L8 and 42 spores/g of soil for Tonga) and T4 (a consortium of endophytes-mycorrhizae-charcoal) at a concentration of 41 spores/g of soil for Nerica L8 and 36 spores/g of soil for Tonga exhibited the highest sporulation, indicating strong fungal activity and successful symbiosis. By enhancing soil conditions and acting as a substrate for spore growth, we see that charcoal may have contributed to this result, whereas the Tonga variety's treatments T0 and T5 displayed extremely poor sporulation. This reveals the vital role of microbial inoculation in stimulating symbiotic microflora [35]. These findings are consistent with those of Rivaton [36], who suggested that organic fertiliser could significantly increase soil spore numbers and the potential for mycorrhizal colonization. However, several authors have also pointed out that plants can grow in moist, organic-rich soils even in the absence of mycorrhizae, which is often the case. Indeed, heavily fertilized plants can manage without mycorrhizae too. Conversely, mycorrhizal fungi require a relationship with plants to access the carbon-rich products of photosynthesis [37].

Mycorrhizal dependency is mostly related to soil fertility, and is the degree to which a plant relies on mycorrhizal condition to produce maximum yield at a given level of fertility [38]. Rice varieties cultivated in soil with mycorrhizae have an average dry root biomass per plant of 5.89 g compared to 3.03 g in soil without mycorrhizae. Mycorrhizal colonization of rice roots may contribute to the significant increase in dry root biomass in mycorrhizal soil. Our findings are consistent with those of Plenchette *et al.* [20], who demonstrated that mycorrhizal root colonization increases dry root biomass. Therefore, crop root growth and development are affected by plant species, soil texture and agricultural management techniques [34]. Given to Zrig *et al.* [39], inoculating *Lactuca sativa* with mycorrhizae resulted in higher root biomass than in control plants. This could be explained by enhanced development of the root system or its richer root secretion, which encourages the recruitment and colonization of a greater variety of AMF and endophytic bacteria. Recent work by Abdouraman *et al.* [7] has revelead that mycotropic plants—as onions—depend on fungal structures for transport (hyphae), transfer (arbuscules), resistance (spores) and storage (vesicles). However, it seems that the roots of non-mycorrhizal plants are highly resistant to mycorrhizal fungi and are usually not colonized by them. To determine the extent to which rice relies on mycorrhizal symbiosis to enhance its growth, the relative mycorrhizal dependency (RMD) of rice was evaluated. According to the findings of our study, the

RMD was 28.56% for Tonga and 49.11% for Nerica L8. These results are consistent with those of Oni *et al.* [40], who studied the mycorrhization of various food crops and showed that inoculating them with AMF increased productivity. The results suggest a greater receptivity to fungal inoculation, possibly due to the characteristics of the roots or greater compatibility with AMF. According to Martin-Cardoso *et al.* [41], Poaceae rely on arbuscular mycorrhizae. A high level of dependency on these fungi suggests that they enhance rice plant biomass and height growth. Ghosh *et al.* [42] asserts that plants with a high level of dependency on mycorrhizae can promote the proliferation of AMF in the soil. We assessed the effect of symbiosis, or mutualistic association, between rice and endophytes using important biochemical markers. Our findings showed that mycorrhizal and microbial treatments significantly impacted chlorophyll concentration. The greatest amounts were found in Tonga and Nerica L8, at around 17 mg/g FW for treatments T1 (mycorrhizae) and T4 (mycorrhizae-endophyte-charcoal), indicating increased photosynthetic efficiency. Similar findings were reported by Singh *et al.* [43], who demonstrated that greater affinity of mycorrhizal fungi for rice enhances chlorophyll production. We observed that plants colonized by multiple AMF species appear to distribute their carbon specifically to the fungi that deliver the most mineral elements to them, reducing photosynthesis [44].

However, the plant synthesizes total sugars, which are the products of photosynthesis. The fact that photosynthesis is one of most fundamental and significant physiological processes in plants and can be susceptible to changes in the environment. Our results showed that a significant accumulation of total soluble sugars occurred in both rice varieties studied, depending on the treatment conditions. This increase was higher for treatments involving microbial (T2 and T3) and mycorrhizal (T1 and T4) inoculation ($P < 0.05$). This increase could be linked to more active photosynthesis and better carbon assimilation, a process which is often stimulated by beneficial microorganisms. These results are consistent with those obtained by Rouphael *et al.* [45], who demonstrated that AMF, when combined with other biofertilisers, promote the accumulation of energy reserves, such as sugars. Conversely, treatments T0 (control) and T5 (urea), which contain the least amount of sugar, demonstrate the positive impact of mycorrhizal and microbial inoculations on carbon metabolism. According to earlier research by Manck-Gotzenberger and Requena [46], the transport of glucose that has previously been cleaved in the cytoplasm of the colonized cortical cell may be facilitated by glucose transporters encoded by SWEET genes in groups 1 and 2, which are also overexpressed during mycorrhizal symbiosis. Doidy *et al.* [47] stated that AMF uses monosaccharide transporters to absorb fructose and glucose. The increased need for carbohydrates and nitrogen molecules, such as proline (one of the most easily mobilisable chemicals), may explain these levels [16].

Proline acts as a reservoir for free radicals and a sink for carbon and nitrogen. In response to salt stress, it regulates cellular redox potential and stabilises subcellular structures, such as membranes and proteins [48] [49]. According to our find-

ings, proline content varied greatly depending on the treatment. The Nerica L8 variety inoculated with the endophyte-mycorrhizae-charcoal consortium, showed the highest accumulation (15.54 mg/g FW), followed by Tonga (12.72 mg/g FW) with the same treatment. These results imply that proline, a key osmoprotectant involved in stress tolerance, accumulates as a result of microbial interactions. An improvement in the physiological state of the treated plants may be linked to the increase in proline levels. These findings are consistent with those of Sarma *et al.* [50], who highlighted that endophytes and mycorrhizae can induce proline accumulation in rice under various stress conditions. Similarly, recent research by Ngumbi *et al.* [51] showed that microbial inoculations significantly increased proline levels in cereal crops, improving their adaptive response and metabolic resilience. Conversely, the low proline levels in the T0 control group for both varieties suggest the absence of microorganisms, which could inhibit the plant's osmoregulatory response.

5. Conclusion

This research aimed to evaluate the impact of symbiosis with beneficial bacteria on the yield, resilience to water stress (proline concentration) and photosynthesis (chlorophyll and sugar content) of two varieties of *O. sativa*. Contrary to expectations, the soil was acidic and sandy-clay-loam with low fertility and low organic matter content. The improved variety Nerica L8 was found to be most susceptible to symbiosis with arbuscular mycorrhizal fungi (AMF) and biofertilizers (both chemical and organic). Unlike mycorrhizal plants, which showed mixed development, our research suggested that the use of biofertilizers resulted in discernible improvements in biomass, plant height, number of tillers and productivity. Urea treatment produced the highest grain yield, demonstrating the instantaneous efficacy of chemical fertilizers without the symbiotic advantages of biological treatments. Effective colonization was demonstrated through symbiotic studies (root colonization, sporulation, RMD, etc.), particularly in treatments T1 (mycorrhizae) and T4 (endophyte-charcoal-mycorrhizae). Furthermore, increased levels of proline, chlorophyll, and total soluble sugar indicate better physiological condition and cultivar adaptation following microbial and mycorrhizal inoculation. This study highlights the potential of charcoal-associated microbial consortia as a sustainable method of enhancing rice performance and development, particularly in poor, untreated soils. Therefore, it can be concluded that AMFs and urea improve rice growth, yield and nutrition in fields and nurseries.

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Author Contributions

NAFACK T.V.: Writing original draft, methodology, writing—review and editing. **MANGA N.J.** and **TOBOLBAI R.**: Conceptualization, writing review and editing, investigation, and formal analysis. **NADJILOM Y.**: Methodology, writing review and editing. **NYANGA A.C.B.**: Methodology and investigation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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