EFFECTS OF INOCULATING SYMBIOTIC MICROORGANISMS ON *ACACIA MANGIUM* GROWN ON COCONUT FIBER.

Original Research Article

Abstract

The ability of *Acacia mangium* to grow on degraded soils, thanks to its ability to fix atmospheric nitrogen, makes it a very important species in agroforestry and in the restoration of impoverished soils. However, all the experiments on *Acacia mangium*, whether inoculated or not, have only been carried out on a single type of substrate, i.e. soil. The aim was to determine whether inoculation significantly increased the biomass and height growth of *Acacia mangium* grown on coconut fiber. The experimental design comprised 5 treatments (Ta, T, M, R, and MR) with 5 replicates. Characterizing the coconut fiber and the dry biomass of Acacia mangium above ground and roots consisted of determining the content of elements such as P, K, Ca, Mg, and N. The number of nodules was also counted The results showed that inoculation (M, R and, MR) had no significant effect on the height growth and biomass of *Acacia mangium*. Non-inoculated (Ta and T) plants showed greater growth, probably due to the richness of nutrients (N, P) in the coconut fiber. The presence of nodules in the non-inoculated plants indicates the presence of indigenous strains in the coconut fiber. Double inoculation (mycorrhizae/rhizobia) significantly stimulated the growth and nodulation of *Acacia mangium*. Double inoculation also increased substrate fertility, which had a positive effect on plant growth. The *Glomus-Bradyrhizobium* combination appeared to be the most effective in improving the growth and nodulation of *Acacia mangium*.

key words: Acacia mangium, inoculation, coconut fiber, mycorrhizae, rhizobia, Côte d'Ivoire

Introduction

The ability of *Acacia mangium* to grow on degraded, acidic, and nitrogen-deficient soils, mainly due to its ability to fix atmospheric nitrogen, makes it a very important species in agroforestry and the restoration of soils depleted by intensive cultivation (Hamad-Sheip and al., 2021; Bongoua-Devisme and al., 2019). In addition, several studies on *Acacia mangium* have produced convincing results in solving soil toxicity problems through phytoremediation (Majid and al., 2011; Cipriani and al., 2013; Bongoua-Devisme and al., 2019). Similarly, the forestry work of Hamad-Sheip and al. (2021) has demonstrated the effectiveness of *Acacia mangium* in improving soil quality. This species also appears to be useful in controlling endemic species such as *Imperata cylindrica* (Gnahoua and Louppe, 2003).

To date, much of the research on *Acacia mangium* has focused on its inoculation with symbiotic microorganisms (Diouf and al., 2002; N'goran and al., 2002; Jayakumar and Tan, 2006; Bongoua-Devisme and al., 2019). Authors such as Bulakali and al. (2000), Saar and al. (2005), and Redon (2010) have shown that inoculated symbiotic microorganisms significantly stimulate plant growth.

However, all the experiments carried out on *Acacia mangium*, whether inoculated or not, have been carried out on only one type of substrate, i.e. soil (N'guessan and al., 2006; Majid and al., 2011), although this is not the only substrate, despite its predominance. This is the case for hydroponics, where coconut fiber seems to be the ideal substrate. Given this observation, we felt it necessary to initiate this work to evaluate the effect of inoculating symbiotic microorganisms on the growth of *Acacia mangium* grown on coconut fiber. Our study aims to determine the effect of inoculating symbiotic microorganisms on *Acacia mangium* grown on coconut fiber. Ultimately, this work will enable us to demonstrate the effect of inoculated microorganisms on substrates other than soil.

Study site

The study was carried out at the National Center for Floristic Research (CNF), part of the Félix Houphouët Boigny University in Cocody, Abidjan. The CNF covers an area of 10.35 ha. There is an arboretum of 4.72 ha and a secondary forest of 5.63 ha. The CNF is located between 3°59'06' and 3°58'57' west longitude and 5°20'43' and 5°20'58' north latitude (Figure 1). This study was conducted in a greenhouse located in the secondary forest of the CNF. This greenhouse covers an area of 65 m2. The climate of our study site is the same as that of the city of Abidjan, i.e. a bimodal rainfall regime characterized by two rainy seasons and two dry seasons. The average annual temperature is 32°C. Geologically, the city of Abidjan is situated on a sedimentary basin, with mainly sedimentary and volcanic-sedimentary formations, namely sandstones, clays, conglomerates, sands, dolomites, and limestones (Yacé, 2002). There are also schistose metamorphic rocks (Perraud, 1971 in Kpangui, 2009). The soils are predominantly ferralsols (highly desaturated ferrallitic soils), developed on terminal continental sand deposits. However, Regosols are the most common (Guillaumet and Adjanohoun, 1971 in Kpangui, 2009). These soil types are characterized by the presence of a thin humus horizon and a thin gravel horizon (Kpangui, 2009).

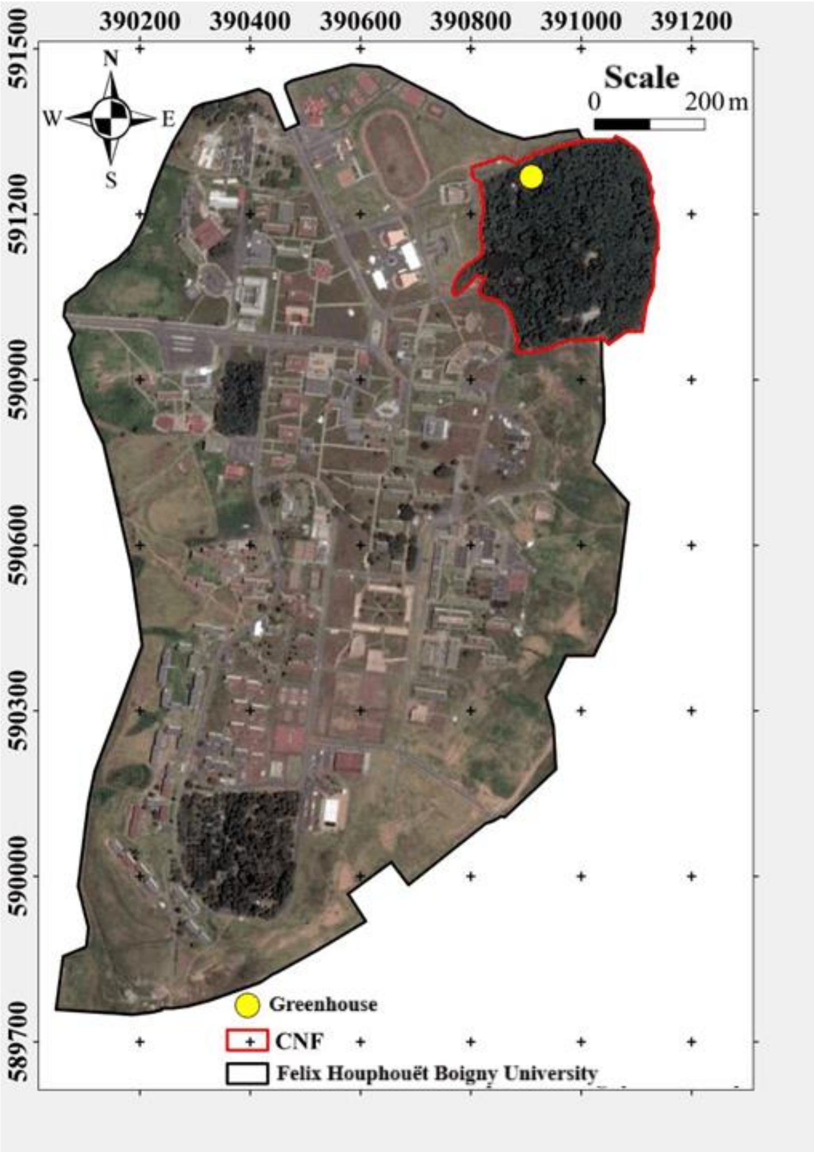


Figure 1 : Map of the National Center for Floristic Research (CNF) in Abidjan

Plant material

The plant material consisted of *Acacia mangium* seeds supplied by the National Center of Agronomic Research (CNRA) in Cocody (Figure 2a).

Biological material

The inocula were supplied by the Laboratoire Commun de Microbiologie (LCM) of the Bel Air Research Center in Senegal. They consisted of rhizobia (Bradyrhizobium/ORS 1785; Figure 2b) and mycorrhizae (*Glomus intraradice*; Figure 2c).

Cultivation substrate

This study was carried out on coconut fiber. The substrate was supplied by Cocosol Service in Bonoua (Figure 2d).

a) Seeds of *Acacia mangium*

b) Inoculum ORS 1785 (or 13C)

d) Coconut Fiber

c) *Glomus intraradice*

Figure 2: Materials used in the study

Methods

Preparation of culture media

- **Agar solution**

The agar solution was prepared by dissolving 8 g of agar in 1 L of distilled and sterilised water. The mixture was heated to 110°C for sterilisation, then poured into Petri dishes and left to solidify.

- **Buffer solution**

The phosphate buffer solution is a compound used to dissolve the rhizobial inoculum immobilised in the alginate beads. It consists of 23 g potassium hydrogen phosphate (K_2HPO_4), 14.6 g potassium dihydrogen phosphate (KH2PO4) and 1 L distilled water. The prepared solution was autoclaved at 120°C for 30 minutes.

- **Dissolution of alginate beads**

Five grams (5 g) of rhizobial inoculum was added to 1 L of buffer solution. Everything was mixed well and left overnight to obtain a homogeneous solution. The resulting rhizobial inoculum is used to inoculate the seeds.

- **Principle of pregermination of *Acacia mangium* seeds**

Acacia mangium seeds were surface disinfected with concentrated sulphuric acid (95%) for 1 hour. After three successive rinses with sterile distilled water, the seeds were left in sterile distilled water for 10 h. They were then wiped and dried in a fume hood for 30 min before being placed in petri dishes containing agar water (0.8%). The petri dishes were incubated in an oven at 30°C for 72 h for pre-germination.

Establishment of nurseries and inoculation of pre-germinated seeds

Two hundred (200) nursery bags (15 L X 40 L X 150 h cm) were filled with coconut fibre (450 g/bag). The coconut fibre nurseries were then watered with unsterilised distilled water.

Three types of inoculation were carried out on pre-germinated seeds of *Acacia mangium* grown on coconut fiber. These were mycorrhizal inoculation, rhizobial inoculation and double rhizo-mycorrhizal inoculation.

Twenty grams (20 g) of mycorrhizal inoculum (*Glomus intraradice*) was carefully poured and mixed into the three holes previously made in the coconut fiber. The pre-germinated *Acacia mangium* seeds in the petri dishes were then transplanted at a rate of three seeds per bag. The seedlings were watered with distilled water.

After transplanting the pre-germinated *Acacia mangium* seeds into the three holes previously made in the coconut fiber (three seeds/bag), 10 L of prepared rhizobial inoculum (ORS 1765) was injected around the seed rootlets. The nurseries were then watered with distilled water after inoculation of the pre-germinated *Acacia mangium* seeds.

Experimental design

The experimental design consisted of five isolated blocks of treatments to avoid contamination between treatments. Each block consisted of 25 replicates. The five treatments in this experiment are named as follows:

- Ta, absolute control, receives no inputs;
- T, sterile rhizobial and mycorrhizal buffer solution;
- M, mycorrhizal, receives mycorrhizal inoculum;
- R, rhizobium, receives rhizobial inoculum;
- MR, mycorrhiza + rhizobium, with two inocula (mycorrhizal and rhizobial).

Temperature and humidity were measured three times a day, in the morning, at noon, and at the end of the day, using a thermometer and a hygrometer, respectively, in the greenhouse. The average temperature in the greenhouse varied from 28 to 36°C and the average humidity from 37 to 67% throughout the day.

Plant characterization

The characterization of the coconut fiber and the dry biomass of *Acacia mangium* above ground and roots consisted of determining the content of elements such as P, K, Ca, Mg, and N. These analyses were carried out by the Plant and Soil Laboratory (LAVESO) of the Agronomy College (ESA) of the National Polytechnic Institute Houphouët Boigny (INPHB) of Yamoussoukro.

The principle was to destroy the organic compound by calcination, followed by solubilization of the elements by attack with a strong acid. The extract obtained was used to determine the essential elements: P, K, Ca, Mg, and Na.

Potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined by flame photometry on the extract obtained by mineralization. Phosphorus (P) was determined by molecular absorption spectrophotometry at 400 nm.

The number of nodules was counted using a binocular microscope. The nodules were sampled, observed, and then photographed using a binocular microscope. These organelles were generally ovoid and approximately 2 cm in size.

Statistical analysis

Data from the monthly monitoring of the plants were entered and organized using Excel software. Data on height, number of leaves, nodules, and aerial and root dry biomass of Acacia mangium were subjected to analysis of variance (ANOVA) to identify treatments that significantly affected plant growth. Statistical analysis was performed using Xlstat 2014.503 software, with a significance threshold of 5% according to the Newmann-Keuls test.

RESULTS

Characterisation of coconut fiber

The results of the chemical characterisation of coconut fibre are presented in Table I. We observed high levels of K (9610 $mg.kg^{-1}$), Ca (3960 $mg.kg^{-1}$), Mg (3200 $mg.kg^{-1}$), N (3100 $mg.kg^{-1}$) and P (800 $mg.kg^{-1}$). These values are good and considered satisfactory (> 2‰).

Table I: Chemical element content of coconut fiber in $mg.kg^{-1}$

Elements	K	Ca	Mg	N	P
Concentration ($mg.kg^{-1}$)	9610	3960	3200	3100	800

Height development in the treatments

Figure 3 shows the growth of the plants and the number of leaves/plants from 1 to 3 months. It can be seen that the highest plant heights were obtained in the Ta, T, and MR treatments.

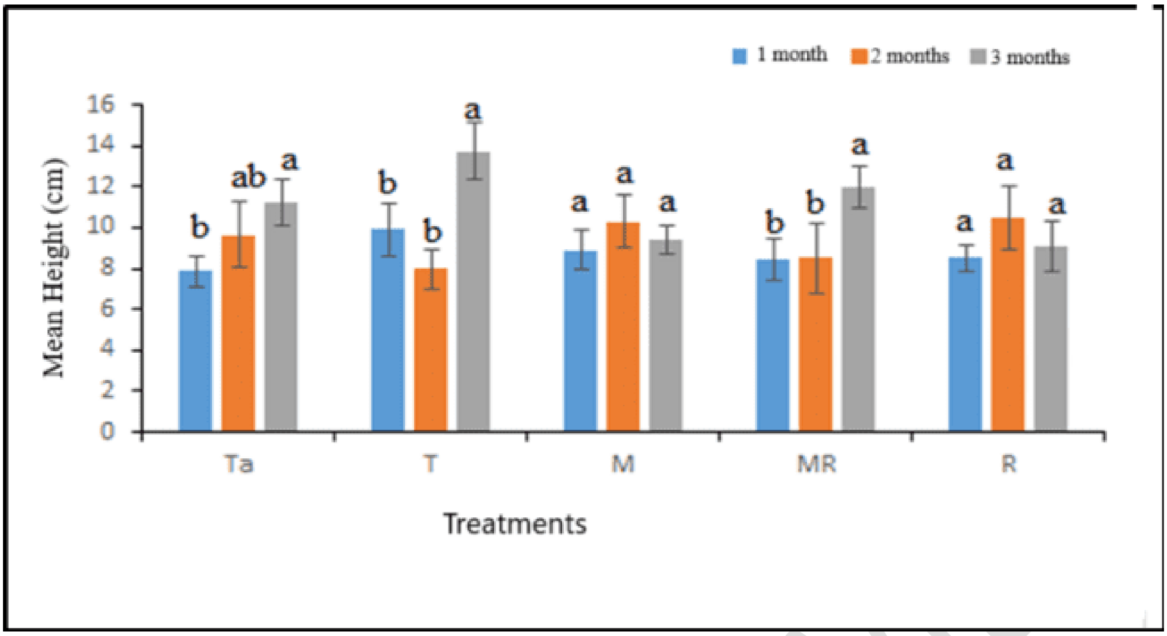
The statistical test carried out on the height of the plants from 1 to 3 months (Figure 4) showed that there was a significant increase in the height growth of the plants from the Ta and MR treatments. There was also a very significant increase in height growth between plants from the 1st month and those from the 3rd month in the T treatment. On the other hand, there was no significant difference between the height growth of plants from the 1st and 3rd month in treatments M and R.

1 month

2 months

3 months

Figure 3: Photographs of monthly growth parameters (height and leaf number) of *Acacia mangium*

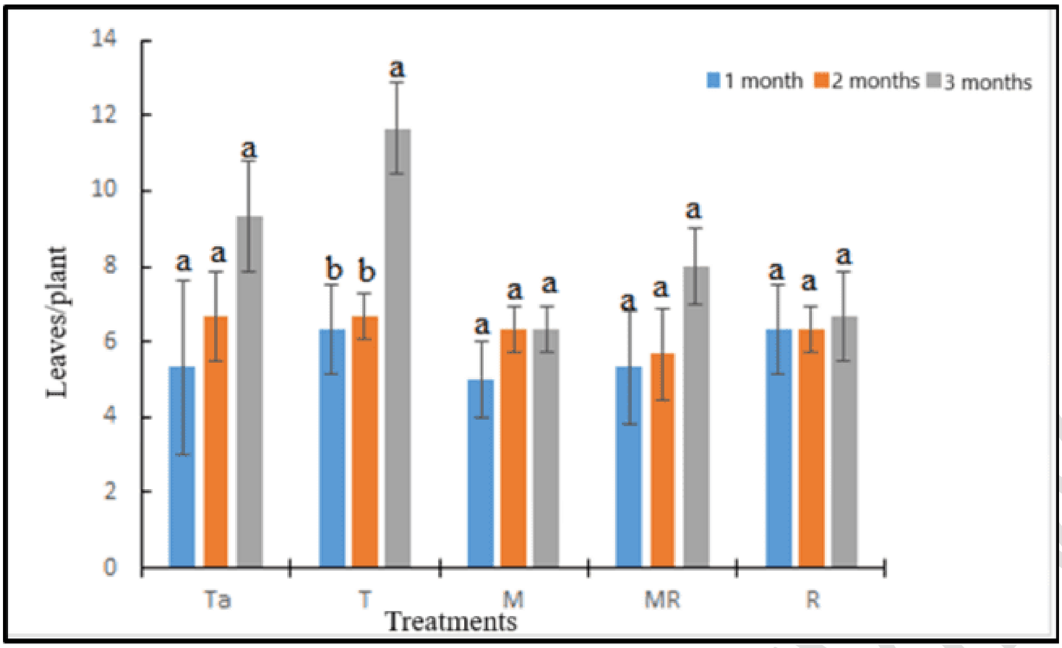


Vertical bars indicate the estimated standard deviations of the means. N = 3. Histograms followed by the same letter are not significantly different according to the Newmann-Keuls test at the 5% significance level. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

Figure 4: Diagram showing evolution of mean height (cm) of *Acacia mangium* from 1 to 3 months according to treatments.

Changes in the number of leaves in the treatments

Regarding the number of leaves counted on each plant, it can be seen that the number of leaves/plant increases significantly each month, regardless of the treatment applied (Figure 5). It increases from 5 leaves/plant at 1 month to 12 leaves/plant at 3 months, irrespective of the treatment used. However, the number of leaves is higher in the non-inoculated treatments (treatments Ta, and T) with 9 to 12 leaves/plant than inoculated treatments (treatments M, R, and MR) with 5 to 8 leaves/plant. The non-inoculated treatments (treatments Ta and T) therefore stimulate leaf development better than the inoculated treatments (treatments M, R, and MR).



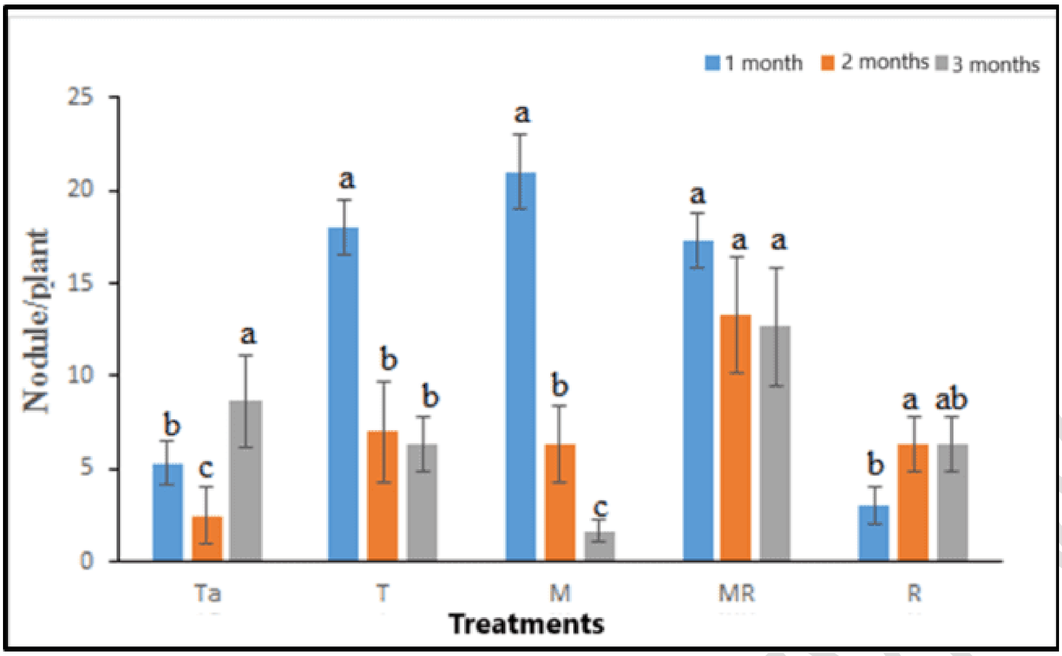
Vertical bars indicate the estimated standard deviations of the means. N = 3. Histograms followed by the same letter are not significantly different according to the Newmann-Keuls test at the 5% significance level. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

Figure 5 : Diagram showing the evolution of *Acacia mangium* leaf number from 1 to 3 months after treatments.

Changes in the number of nodules

Figure 6 shows a photograph of nodules taken with a binocular microscope. These organelles are generally ovoid and about 2 cm in size. Figure 7 shows the evolution of nodules per plant as a function of treatment from 1 to 3 months. There was a decrease in the number of nodules per plant. This ranged from 21 to 17.33 nodules/plant at month 1 compared to 12.67 to 1.67 nodules/plant at month 3 for treatments T, M, and MR. On the other hand, there was an increase in the number of nodules/plant at month 1 (3 to 5.33 nodules/plant), and month 3 (6.33 to 8.67 nodules/plant) for treatments Ta and R.

Figure 6: *Acacia mangium* nodules imaged under a binocular microscope



Vertical bars indicate the estimated standard deviations of the means. N = 3. Histograms followed by the same letter are not significantly different according to the Newmann-Keuls test at the 5% significance level. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

Figure 7 : Diagram showing the evolution of *Acacia mangium* nodules number from 1 to 3 months after treatments.

Effect of inoculation on growth parameters and Nodulation of *Acacia mangium* after three months of cultivation

Table II shows the growth and nodulation parameters of Acacia mangium after three months of cultivation.

The statistical test confirms that leaf development is greater in the non-inoculated treatments (treatment T) than in the inoculated treatments (treatments M, R, and MR). With the exception of treatment T, there was no significant difference between the number of leaves observed on non-inoculated plants compared to inoculated plants.

Similarly, we found that the height growth of uninoculated plants (treatment T) was very significant and greater than that of inoculated plants (treatments M, R, and MR).

However, the number of nodules observed in the roots of uninoculated plants (treatment Ta) was highly significant but lower than that observed in the MR treatment, except for treatment M. The number of nodules/plants observed in treatments T and R was highly significant but not statistically different in the two treatments. Thus, inoculation did not improve the leaf biomass and height of Acacia mangium but rather increased the number of nodules.

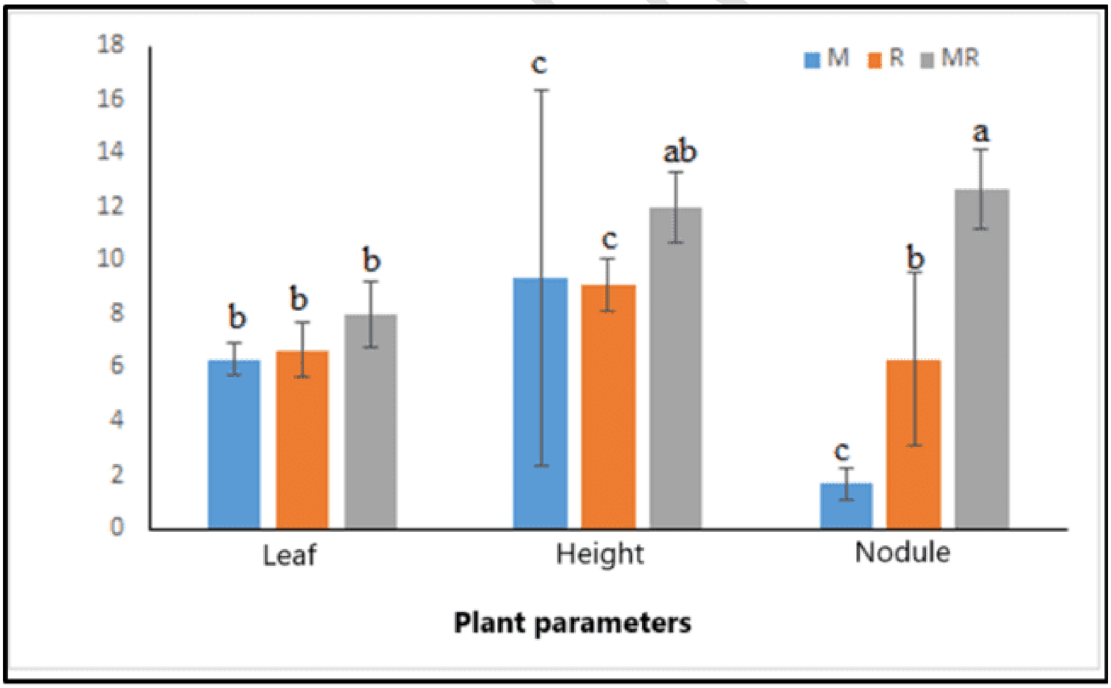
Table II: Growth and nodulation parameters of *Acacia mangium* after three months of cultivation

parameters	Ta	T	M	MR	R	Pr > F
Leaf/plant	9.33 ± 1.5b	11.67 ± 1,2a	6.33 ± 0.6b	8 ± 1b	6.67 ± 1.2b	0.0018**
Height (cm)	11.2 ± 1.1bc	13.73±1,4a	9.37 ± 0.7c	12 ± 1ab	9.1 ± 1,3c	0.0024**
Nodule/plant	8.67 ± 2.5b	6.33 ±1,5b	1.67 ± 0.6c	12.67 ± 3.2a	6.33 ± 1.5b	0.0011**

The letters a, b, and c following the mean values on the same line are statistically different according to the Newmann-Keuls test at the 5% significance level. **: A highly significant difference. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

Effect of double and single inoculation

Figure 8 shows the number of leaves and nodules/plant and the height (cm) of the plants after three months of cultivation. The results show that the number of leaves/plant, nodules/plant, and plant height growth are greater with double inoculation (MR treatment) than with single inoculation (M and R treatments).



Vertical bars indicate the estimated standard deviations of the means. N = 3. Histograms followed by the same letter are not significantly different according to the Newmann-Keuls test at the 5% significance level. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

Figure 8 : Diagram showing the influence of single (M and R), and double (MR) inoculation on height (cm), number of leaves, and nodules per plant after three months of cultivation.

Above-ground and root biomass of Acacia mangium

Table III shows the weight of above-ground and root biomass of *Acacia mangium* as a function of treatment. It can be seen that regardless of the treatment applied, the above-ground biomass (0.06 to 0.3g) is greater than the root biomass (0.02 to 0.05g). It can also be seen that above-ground and root biomass are significant and greater in uninoculated plants (T treatment) than in inoculated plants (MR treatment).

Table III: Above-ground and root biomass weights of *Acacia mangium* according to treatments after three months of cultivation

Treatments	Above-ground biomass (g)	Root biomass (g)
Ta	0.12 ± 0.05a	0.03 ± 0.01cd
T	0.29 ± 0.2cd	0.05 ± 0.01bc
M	0.08 ± 0.02b	0.02 ± 0.00d
MR	0.14 ± 0.01a	0.02 ± 0.01cd
R	0.06 ± 0.01bc	0.02 ± 0.00d

The letters a, b, and c following the mean values on the same line are statistically different according to the Newmann-Keuls test at the 5% significance level. **: A highly significant difference. Treatments: Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

N and P content of above-ground and root biomass

Table IV shows the nitrogen (N) and phosphorus (P) contents measured in the above-ground and root biomass of *Acacia mangium* under each treatment after three months of cultivation. There was a higher N content in the above-ground biomass (2 to 7 mg/plant) than in the root biomass (0.3 to 0.5 mg/plant), i.e. more than five times the N content in the root biomass, regardless of the treatment applied. However, the N content of above-ground biomass was higher in uninoculated plants (treatments T and Ta) than in inoculated plants (treatments M and R), except in the MR treatment. Similarly, the N content of root biomass was higher in uninoculated plants (treatments T and Ta) than in inoculated plants (treatments M, MR, and R).

The P content of above-ground biomass was higher in treatments T and MR. However, the P content of treatment M was about ten times lower than that of treatments MR and T. The P content of root biomass was not determined.

Table IV: N and P contents of *Acacia mangium* plants grown under different treatments after three months of cultivation

	Above-ground biomass Mg/plant		Root biomass Mg/plant	
	N	P	N	P
Ta	2.7	n a	0.47	na
T	6.5	0.5	0.51	na
M	1.9	0.05	0.37	na
MR	4.2	0.6	0.35	na
R	2.02	n a	0.43	na

na: not analysed. Treatments : Ta, absolute control; T, control; M, mycorrhiza; R, rhizobium; MR, mycorrhiza + rhizobium.

DISCUSSION

Effect of coconut fiber characteristics

Variations in aboveground and root biomass and plant height were observed in the non-inoculated and inoculated treatments.

Height was significantly higher in non-inoculated plants compared to inoculated plants (MR treatments), with $Pr < 0.05$ (according to the Newmann-Keuls test). The same was true for aboveground and root biomass. This result seems to indicate that inoculation with the micro-organisms did not significantly increase the growth and biomass of the inoculated plants compared to the controls (non-inoculated plants). This finding could be related to the high nutrient content of coconut fiber.

Indeed, the characterisation of coconut fiber revealed that this substrate is rich in nitrogen (content greater than 2‰) and phosphorus (content greater than 0.7 ‰) (Assa, 2002). Furthermore, according to Mouafek (2010), when the environment is rich in nitrogen, the symbiotic fixation of atmospheric nitrogen is slowed down and the symbiosis is only established when the nitrogen content in the environment becomes scarce. According to the work of Ribet (1995) and Mouafek (2010), nutrient deficiencies can limit symbiotic fixation by reducing infection. However, the high potassium and phosphorus content of coconut fiber could explain the strong growth observed in non-inoculated plants, as Tarekegn (2017) suggests that potassium only promotes nodulation in the presence of sufficient phosphorus.

Furthermore, the higher nitrogen and phosphorus content in the non-inoculated plants (treatments Ta and T) compared to the inoculated plants (treatments MR, M and R) suggests that the nitrogen nutrition of *Acacia mangium* was mainly by assimilation of the mineral nitrogen contained in the coconut fiber rather than by atmospheric nitrogen fixation.

According to L'Taief et al (2009), nitrogen nutrition in legumes results from two pathways: atmospheric nitrogen fixation and mineral nitrogen assimilation.

The presence of nodules in non-inoculated plants (treatments Ta and T) indicates the presence of autochthonous strains in the coconut fiber. This finding indicates that the coconut fiber is not healthy and that it would be desirable to sterilize it in inoculation studies. In addition, the low nodulation in treatments R (1st month) and M (3rd month) could be due to competition between the indigenous strains and the strains of inocula Bradyrhizobium (Abou-Shanab and al., 2017) and *Glomus intraradice*.

This result seems to indicate the presence of indigenous symbiotic bacterial and fungal strains that influence the effect of *Acacia mangium* inoculation on growth and nodulation, as indicated by the work of Diouf et al. (2005). The effectiveness of inoculation could be variable, depending on the characteristics of the coconut fiber (P and N content, type of strains present). The chemical composition and microbiology would therefore have a positive effect on the nodulation of *Acacia mangium*, but not on its root development.

Furthermore, the low root biomass compared to aboveground biomass under the different treatments could be related to the chemical composition of the coconut fiber. According to Redon (2009), when the environment is poor in limiting elements such as P and N, microorganisms improve the uptake of the limiting elements needed by the plant, thereby promoting an increase in above-ground and even root biomass.

Effect of double and single inoculation

The number of nodules was higher on double-inoculated plants (MR) than on single-inoculated plants (M and R). This suggests an effect of double inoculation on nodulation. In addition, there was greater growth in the double-inoculated plants compared to the single-inoculated plants.

These results indicate that double inoculation (MR) significantly increases the growth and nodulation of Acacia mangium grown on coconut fiber compared to single inoculation (M and R). These observations are consistent with those of Fall et al. (2009) and Isseu (2010). According to these authors, double inoculation with mycorrhizal and rhizobial strains increases the quality of soil fertility and consequently affects plant growth. For Lopes (2018), the inoculation of Racosperma auriculiformis with endomycorrhizal gives better results when combined with rhizobia.

In addition, the lower nodulation observed in treatment M compared to treatment R in the 3rd month of cultivation could be because rhizobia, thanks to its nitrogenase activity, produces nodulation factors that stimulate root nodulation.

The low nodulation of plants inoculated with *Glomus intraradice* indicates that the symbiosis between *Acacia mangium* and *Glomus intraradice* is not efficient. However, despite the low nodulation observed under the M treatment, strong growth was observed, not dissimilar to that observed under Bradyrhizobium. This result is in line with that of Redon (2009), who claims that the main role of mycorrhizae is to stimulate plant growth by providing the plant with the necessary nutrients.

Furthermore, this low nodulation may also be due to competition between the indigenous fungal strains in the coconut fiber and the added strain *(Glomus intraradice*). The *Glomus-Bradyrhizobium* association seems to improve the growth and nodulation of *Acacia mangium*.

The association of symbiotic microorganisms (rhizobia, mycorrhizal fungi) is a convenient and effective means of improving plant growth at a low cost (Isseu, 2010). However, how well this symbiosis works varies greatly depending on the species and the characteristics of the growing substrate.

Conclusion

This study investigated the effect of inoculating symbiotic microorganisms on *Acacia mangium* grown on coconut fiber.

The results showed an increase in the height of non-inoculated plants due to the richness of nutrients (N, P) in coconut fiber. This indicates that inoculation has no significant effect on the height growth and biomass of *Acacia mangium* grown on coconut fibre. The study also showed that the biomass of *Acacia mangium* was greater in the non-inoculated control treatments than in the inoculated treatments. The significance of above-ground and root biomass under non-inoculated plants compared to inoculated plants suggests that inoculation does not significantly increase the biomass of *Acacia mangium*. This finding therefore contradicts the hypothesis that inoculation with symbiotic microorganisms would significantly increase biomass and height growth of *Acacia mangium* grown on coconut fiber. The first hypothesis was therefore not supported.

Statistical analysis (ANOVA test) showed a significant effect of double inoculation (DII) on the growth parameters (height, leaf) of *Acacia mangium* grown on coconut fiber. Given these results, the second hypothesis that double mycorrhizae/rhizobia inoculation stimulates the growth of *Acacia mangium* grown on coconut fiber was verified.

In addition, the presence of nodules under the non-inoculated plants indicates that the coconut fiber contains indigenous bacterial and fungal strains that would either promote nodule formation under the control treatments or create an antagonistic effect with the strains supplied by the inoculation (the mycorrhizal or rhizobial strain).

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