

Supply of Compost and Arbuscular Mycorrhizal Fungi for Enhancing Quality of *Ceiba pentandra* (Kapok Tree) Seedlings

B. L. A. Anguiby^{1*}, E. L. Bomisso¹, K. S. B. N'goran² and S. Ake¹

¹Université Félix Houphouët-Boigny, UFR Biosciences, Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources Biologiques, 22 BP 582 Abidjan 22, Côte d'Ivoire.

²Université Félix Houphouët-Boigny, UFR Biosciences, Centre National de Floristique, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Author BLAA conducted the study under the supervision of the authors ELB, KSNB and SA. Authors ELB and BLAA designed the study.

Author BLAA wrote the protocol and the first draft of the manuscript under the supervision of authors ELB and SA. Authors BLAA and ELB managed the literature searches. All authors collaborated to the revision and improvement of the initial draft submitted. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2020/v32i930329

Editor(s):

(1) Dr. Kofi Agyarko, University of Education, Ghana.

Reviewers:

(1) Marliane de Cássia Soares da Silva, Federal University of Viçosa (UFV), Brazil.

(2) Olajire Fagbola, Agronomy University of Ibadan, Nigeria.

(3) Swati Saha, Indian Agricultural Research Institute, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57843>

Original Research Article

Received 01 May 2020

Accepted 05 July 2020

Published 06 August 2020

ABSTRACT

The success of reforestation depends on the production of quality seedlings in nurseries. Initial fertilization and mycorrhization are determinants that improve the growth of plants. The purpose of this study was to improve the quality of *Ceiba pentandra* seedlings using fertilization and mycorrhization in nurseries. Seedlings of *C. pentandra* were subjected to various treatments (T1: Sterilized forest soil (100 %), T2: 90 % T1 + sterilized mycorrhizal inoculum (10 %); T3: 90 % T1 + mycorrhizal inoculum (10 %); T4: 90 % T1 + compost (10 %); T5: 80 % T1 + mycorrhizal inoculum (10 %) + compost (10 %)). The results showed an increase of 117.35 % in the dry stem weight of young *Ceiba pentandra* mycorrhizae (T3) seedlings compared to non-mycorrhizae (T2) seedlings. Growth parameters, roots, total leaf area were better with the treatment combining mycorrhization

*Corresponding author: E-mail: bossombra1909@yahoo.fr;

and compost application. Furthermore, the sturdiness quotient was identical for all substrates. Seedlings of *Ceiba pentandra* are highly dependent on mycorrhization. For rapid production of quality seedlings, fertilization could be coupled with mycorrhization.

Keywords: *Ceiba pentandra*; arbuscular mycorrhizal fungi; forest seedlings; sturdiness quotient; fertilization.

1. INTRODUCTION

Ceiba pentandra (Kapok tree) is a tropical tree that can be used as fibre and timber. Today, production and trade is centred on timber, mainly for the production of plywood [1]. *Ceiba pentandra* is one of the largest trees in the Ivorian forest where it can reach 50 meters in height and 2 meters in diameter [2]. However, the massive harvest of timber due to the decline of noble species and strong market demand is contrasted by a limited supply from wild plantations. These spontaneous stands hardly guarantee a sustainable and consistent supply to industries. It is therefore necessary to think about a better management of this forest resource by reforestation. In Côte d'Ivoire, in order to achieve successful reforestation, the structures in charge of forest management have long relied on the choice of fast-growing species such as *Tectona grandis*, *Cedrela odorata* and *Acacias sp* [3,4]. Numerous studies have shown that the success of this operation depends on the production of quality seedlings and knowledge of the factors influencing their growth [5,6,7,8]. Indeed, the success of forest plantations depends on many genetic determinants inherent to the environment to be reforested, but also on the morpho-physiological quality of the plants [9]. As a result, morpho-physiological criteria for nursery-produced plants have been developed for successful reforestation programs [10,9,8]. With this in mind, nursery care such as fertilization and the use of adequate substrate is carried out [11,12, 13,14]. However, the use of these plants does not necessarily guarantee survival after planting. For good reason, the constraints inherent in environments such as humidity, temperature and physical stress are sometimes detrimental to the recovery and survival of seedlings [12]. Studies conducted in this direction have shown that nursery association of plants with mycorrhizal fungi improves growth in the nursery and in the field, while ensuring their survival after transfer to natural environments [15-20]. These advantages have led to the inclusion of mycorrhization of plants at the exit of the nursery among the quality criteria for forest plants [10,21]. Since then, mycorrhizal symbiosis

has been considered a key factor in the success of reforestation programs [15].

Most environments are generally supplied with mycorrhizal propagules. However, degradation of the environment leads to a reduction in this potential [22,23]. The rarity and incompatibility of the strains encountered can constitute a real obstacle to the installation of new plants [24]. Also, the time required to establish a functional and effective association after transfer to the field can be detrimental to the survival of the young plant [25]. The production of mycorrhized plants in the nursery is therefore necessary. This operation can be carried out either by association with a well-defined strain (controlled mycorrhization), or through the management of mycorrhizal potential in situ using a mycotrophic plant [26,25,27]. According to the work of Khasa et al. [28] and Anguiby et al. [29], *Ceiba pentandra* is associated with endomycorrhizal fungi.

The aim of this work was to assess the influence of compost and arbuscular mycorrhizal fungi supply on the performance of *Ceiba pentandra* seedlings under nursery conditions.

2. MATERIALS AND METHODS

2.1 Soil Sampling and Study Sites

Soil samples were collected under kapok trees at the Botanical Garden of Bingerville (JBB) in Côte d'Ivoire. The diameter at breast height (dbh) of these trees was equal to 1.36 ± 0.17 m. This garden is situated at 5°21'50.7"N latitude and 3°53'20.7"W longitude. The nursery was established under a shaded area covered with transparent polyethylene plastic and protected by a fine mesh fabric built at the National Floristic Center of the Félix Houphouët-Boigny University in Cocody (Côte d'Ivoire). The National Floristic Center is located at 5°20'47.9" N latitude and at 3°59'02.0" W longitude. The average temperature is between 25 and 29°C and rainfall is 1650 mm/year for both sites.

2.2 Production of Mycorrhizal Inoculum

The production of the inoculum consists of growing two mycotrophic plants on soil taken from under *Ceiba pentandra* tree. Through this process, the mycorrhizogenic potential (spore, mycelial hyphae) is improved. Spore abundance and diversity of arbuscular mycorrhizal fungi in this soil have already been determined [29]. Sorghum plants (*Sorghum bicolor*) and Niebe (*Vigna unguiculata*) were chosen on the basis of their mycotrophic character. The seeds are of commercial origin. The growing medium consists of a 1:1 mixture of rhizospheric soil of *Ceiba pentandra* and sandy soil sterilized twice at 120 °C for 30 min at a pressure of 1 bar per 24 h interval. On these substrates, pre-germinated Sorghum and Niebe seeds were sown at a rate of two seeds/ container. The experimental unit is a pot (5 litres), filled with 4 Kg of substrate. One treatment is represented by two containers. The trial lasted 90 days for *Sorghum* and 60 days for Niebe.

Watering was carried out 48 hours apart with tap water. Density of spores and plant mycorrhization indices (intensity and frequency) obtained with the grid of Trouvelot *et al.* [30] governed the choice of growth substrate used as mycorrhizal inoculum for the nursery inoculation of the kapok plants.

2.3 Compost Production

The fertilizer was made from a litter taken from a poultry farm. This litter was subjected to aerial composting for three months. Before use, the compost produced was sieved with a sieve with a mesh size of 2 mm and then sterilised in an oven maintained at 90 °C for 1 hour.

2.4 Treatments and Experimental Design

The nursery soil, a forest soil which was also sterilized had soil characteristics shown in Table 1. The total nitrogen and phosphorus in the soil were determined by the Kjeldahl method [31] and by colorimetry [32], respectively. The soil was sterilized in an oven maintained at 120 °C for 01 hour and 30 min. The soil was distributed in polyethylene bags (2 kg per bag). The experimental design is a Fisher block consisting of five treatments and three replicates:

T₁: Sterilized forest topsoil only (100 %);
T₂: 90 % T₁ + sterilized mycorrhizal inoculum (10 %);

T₃: 90 % T₁ + mycorrhizal inoculum (10 %);
T₄: 90 % T₁ + compost (10 %);
T₅: 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %). This last treatment is called mixed treatment.

For T₂, T₃, T₅ treatments, the amount of mycorrhizal or non-mycorrhizal soil applied corresponds to one tenth of nursery soil (1:10) to minimize the effect of the minerals content on plant growth [33].

2.5 Inoculation and Fertilization

Ceiba pentandra seeds were collected from one tree so as to limit intrinsic variation (Seed diameter= 4.39 ± 0.03 mm). These seeds were disinfected by soaking in 10 % sodium hypochlorite solution for 15 min and, then rinsed 3 times for 10 min with distilled water. They were pre-germinated for three days in a Petri dish (diameter= 19 cm) lined with a slice of blotting paper. Before sowing the seeds, the mycorrhizal inoculum was placed in a crater made in the center of the substrate contained in the bag. The pre-germinated seed was then sown in the centre of the nursery bag, taking care that the entire hypocotyl is immersed in the inoculum. The inoculum was covered with sterile forest soil. The compost was added two weeks after transplanting the seedlings (0.2 kg per plant).

2.6 Variables Assessed

2.6.1 Assessment of *Ceiba pentandra* seedlings roots colonized by AMF

The Philips and Hayman [34] staining method was used to stain fungal structures. The fine roots of three months seedlings were collected carefully. These roots were washed and then cut into fragments of at least 1 cm in length. They were then placed in tubes and covered with 10 % potassium hydroxide solution in a double boiler maintained at 70°C for 30 minutes. This treatment removed the cellular content of the roots. Then the structures formed by the fungi in the roots were colored with an ink blue solution. Mycorrhizal frequency and intensity were calculated as described by Trouvelot *et al.* [30].

2.6.2 Mycorrhizal Dependency Index (MDI)

This index was determined with the T₂ and T₃ treatments. It was determined with the following formula [35].

$$MDI (\%) = \frac{[SdMyc (T3) - SdnMyc (T2)] \times 100}{SdnMyc (T2)}$$

SdMyc: Stem dry weight of mycorrhizal seedlings; **SdTnMyc:** Stem dry weight of non-mycorrhizal seedlings.

To get the dry weight of the stems, the fresh stems were placed in a drying oven maintained at 80 °C for three days and weighed.

2.6.3 Growth assessment

The growth variables considered were height, and diameter of seedlings. These variables were taken weekly for three months commencing at one month after sowing. The height was, expressed in cm and, was measured from the collet to the apical meristem of the stem. The diameter was determined at two centimetres above ground level with a 0.01 mm precision electronic calliper. Leaf numbers were counted manually.

2.6.4 Morphological indices

The sturdiness quotient reflects the stocky or hail nature of the seedlings. It has been determined by measuring the height (h) in centimetres divided by the stem diameter (d) in millimetres (h/d) [10,14]. The ratio of stem (FWS) to root (FWR) fresh weight was also calculated. It measures the difference between the transpiration surface (stem), and the water absorption surface (root) of the plants produced.

2.6.5 Measurement of fresh roots weight of seedlings

The fresh weights of the stems (FWS) and roots (FWR) of the three-month-old seedling were determined by weighing.

2.6.6 Leaf area

This productivity parameter was determined by referring to the method described

by Zraibi et al. [36]. The leaf blade was detached from the petiole and then weighed to obtain the fresh weight (LFW). Then one square centimetre (la) of leaf blade was cut off and weighed to obtain lfw.

The leaf area of a leaf limb (La) was obtained by the following relationship :

$$La = \frac{la * LfW}{lfw}$$

Total leaf area was estimated by multiplying La by the number of leaves in each seedling. For this variable, the leaf in the middle position was selected.

2.6.7 Chlorophyll content

The chlorophyll content was measured with a device, the SPAD-502 Plus (KONICA MINOLTA). This instrument allows non-destructive measurement of chlorophyll pigment content in plants [37].

2.6.8 Leaf nutritional variables

The minerals determined were nitrogen, phosphorus, potassium, calcium and magnesium. The Kjeldahl method was used to determine nitrogen. Potassium and calcium were determined with the flame photometer [38]. Magnesium and phosphorus were measured by atomic absorption spectrometry (AAS) [38] and colorimetry respectively.

2.7 Statistical Analyses

Spore density, frequency and intensity of mycorrhization were treated with the Student t test. For the variables measured on the kapok seedlings, the Fisher's test (ANOVA) was used to test the hypothesis of equality of means. The Newman and Keuls test was used to determine groups of homogeneous means.

Table 1. N and P contents of forest soil and compost

Mineral elements	Nursery soil	Compost
N (%)	0 < 1	3.15 ± 0.20
P (%)	1.44 ± 0.14	1.26 ± 0.05

3. RESULTS

3.1 Spores Density, Frequency and Intensity of Mycorrhization in *Sorghum bicolor* and *Vigna unguiculata*

An increase in spore density was noted after growing by counting. From 2.27 ± 0.22 spores per gram of original soil, the density increased to 7.47 ± 0.39 and 4.05 ± 0.12 for *Sorghum bicolor* and *Vigna unguiculata*, respectively (Table 2). The number was doubled for *Sorghum*. The difference between the densities of spores isolated under the two plants was significantly marked in favour of Sorghum. Root colonization was more intense in Sorghum roots (Table 2). As a result, the culture medium of *S. bicolor* was subsequently chosen as mycorrhizal inoculum.

3.2 Effects of Compost Supply and Mycorrhization on the Growth of *C. pentandra* Seedlings

3.2.1 Seedlings susceptibility to mycorrhization and Mycorrhizal Dependency Index (MDI)

The roots of *Ceiba pentandra* seedlings from treatments T₁, T₂ and T₄ did not show any signs of mycorrhization. However, those from T₃ and T₅ showed susceptibility to mycorrhization. This is

evidenced by the presence of spores, vesicles and mycelial hyphae that attest to arbuscular mycorrhization observed in the root cortex of the seedlings (Fig. 1).

The dependency index was assessed with T₂ and T₃ treatments. The mean dry weight of the stem was 1.21 ± 0.10 g and 2.64 ± 0.06 g for T₂ and T₃ respectively. The dry weight of young plants from T₃ was almost double that of T₂. Thus, the mycorrhization dependence (DMI) of the kapok seedlings was 117.35 % under the conditions of the study.

3.2.2 Effects of compost supply on plant mycorrhization

The results showed that the application of the compost involved in the treatment T₅ did not significantly affect the frequency and intensity of root colonization of young plants (Table 3). In fact, the frequency and intensity of mycorrhization were statistically identical for T₃ and T₅ treatments. However, the proportion actually mycorrhized was higher for treatment T₃.

3.2.3 Effects of treatments on average plant diameter

The mean diameter of the plants obtained at the end of the nursery monitoring period varied significantly ($p < 0.01$) with respect to the nature of the treatment (Fig. 2). Plants from the T₁

Table 2. Spores density under Sorghum and Niebe, frequency (F) and intensity (I) of mycorrhization of these plants

Plants	Density (Spore g ⁻¹ of soil)	Frequency (%)	Intensity (%)
<i>V. unguiculata</i>	4.05 ± 0.12 b	100	34.10 ± 4.85 a
<i>S. bicolor</i>	7.47 ± 0.39 a	100	15.17 ± 1.97 b
Statistics test	$t=69.09$ ($df: 1$); $p=0.000$	-	$t=2.31$ ($df=1$); $p= 0.003$

Means with different letter(s) in columns are significantly different at $p \leq 0.05$

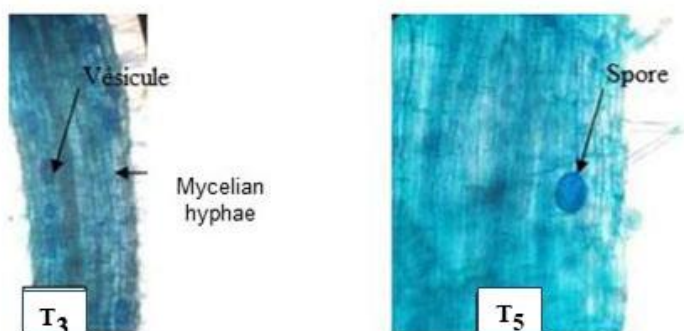


Fig. 1. Roots cortexes of *C. pentandra* seedlings from T₃ and T₅ treatments coloured with ink blue

Table 3. Frequency (F) and intensity of mycorrhization (I) of *C. pentandra* seedlings

Traitements	F (%)	I (%)
T ₁	-	-
T ₂	-	-
T ₃	85.90 ± 4.90 a	25.18 ± 7.76 a
T ₄	-	-
T ₅	88.80 ± 10.96 a	22.36 ± 6.79 a
test statistic	t (df : 1)=0.54 ; p= 0.6	t (df : 1)=-0.64 ; p= 0.54

Means with different letter(s) in columns are significantly different at $p \leq 0.05$. T₁ : Sterilized forest soil (100 %), T₂ : 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃ : 90 % T₁ + mycorrhizal inoculum (10 %); T₄ : 90% T₁ + compost (10 %); T₅ : 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

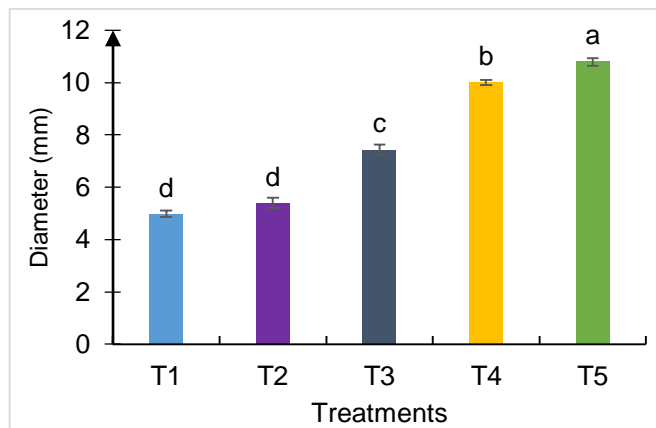


Fig. 2. Effect of treatments on the average diameter of *Ceiba pentandra* seedlings
 T₁ : Sterilized forest soil (100 %), T₂ : 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃ : 90 % T₁ + mycorrhizal inoculum (10 %); T₄ : 90% T₁ + compost (10 %); T₅ : 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

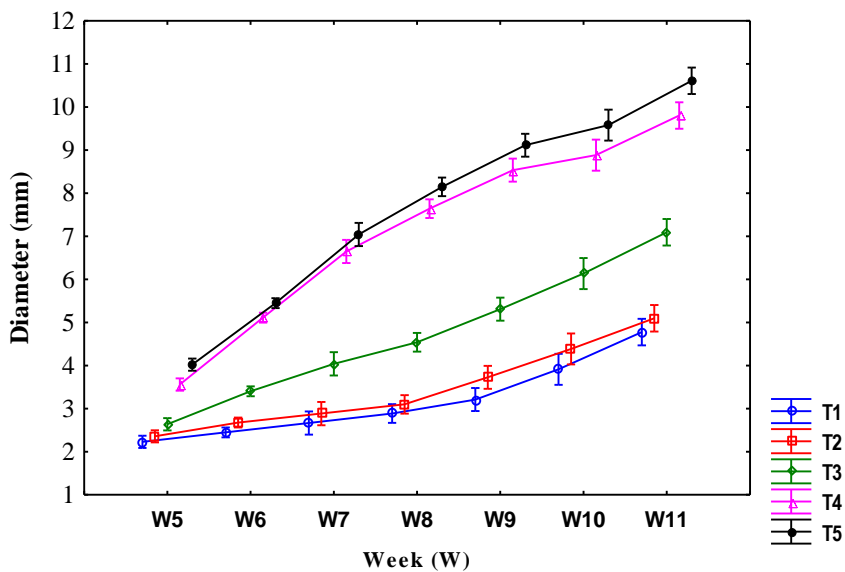


Fig. 3. Growth of the diameter of *C. pentandra* seedlings
 T₁ : Sterilized forest soil (100 %), T₂ : 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃ : 90 % T₁ + mycorrhizal inoculum (10 %); T₄ : 90% T₁ + compost (10 %); T₅ : 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

treatment showed the lowest diameter values. Treatments T₂ and T₃, which received the sterilized mycorrhizal inoculum and unsterilized inoculum respectively, produced plants of different sizes. Indeed, those from T₃ are significantly larger in diameter (7.43 ± 0.20 mm) than those from T₂ (5.40 ± 0.19 mm). The mixed treatment, T₅ resulted in the best radial growth (10.79 ± 0.15 mm) followed by T₄ (10.01 ± 0.10 mm). The T₅ treatment stimulated radial growth of seedlings by 116.81 % compared to the T₁ treatments. From week 8 to the end of the study (week 12), the diameter of T₅ plants was greater than that of the other treatments (Fig. 3).

3.2.4 Effect of treatments on plant height

The average height of three-month-old *Ceiba pentandra* seedlings as affected by the applied treatments is shown in Figs. 4 and 5. Plant height varied significantly ($p < 0.01$) between treatments. The mixed treatment (T₅), combining fertilizer application and unsterilized mycorrhizal soil, gave the best results (73.28 ± 2.79 cm) followed by compost supply treatment alone T₄ (66.52 ± 2.06 cm). Also the plants receiving the T₃ treatment showed significantly higher values than the T₂ and T₁ treatments. The height of the seedlings increased by 12.72 %, 62.92 %, 62.92 %,

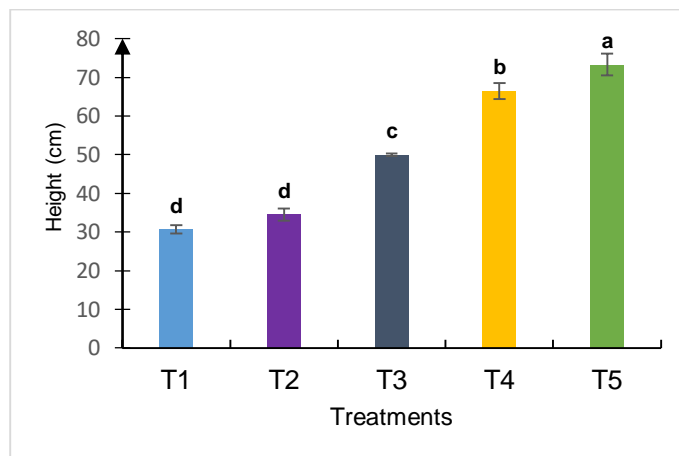


Fig. 4. Effect of treatments on the average height of *Ceiba pentandra* seedlings

T₁ : Sterilized forest soil (100 %), T₂ : 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃ : 90 % T₁ + mycorrhizal inoculum (10 %); T₄ : 90% T₁ + compost (10 %); T₅ : 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

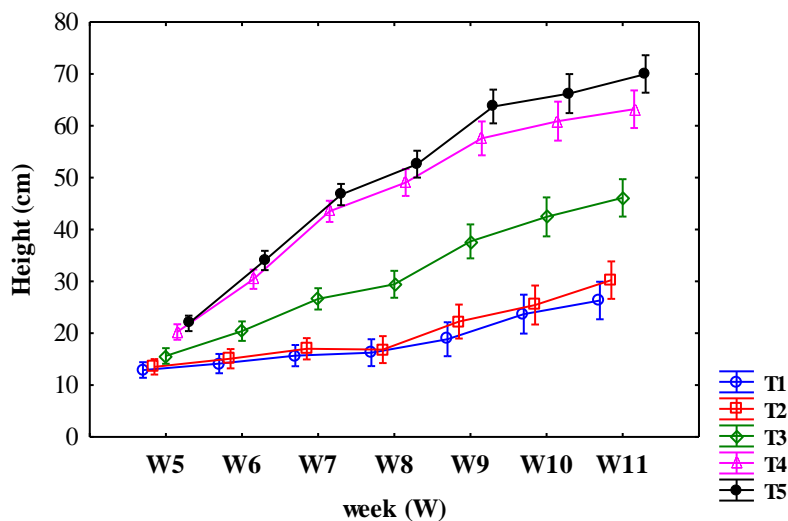


Fig. 5. Growth in height of *C. pentandra* seedlings

T₁ : Sterilized forest soil (100 %), T₂ : 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃ : 90 % T₁ + mycorrhizal inoculum (10 %); T₄ : 90% T₁ + compost (10 %); T₅ : 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

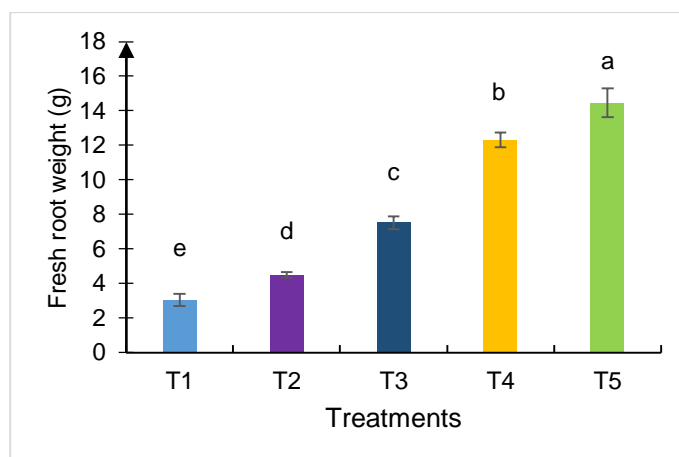


Fig. 6. Effect of treatments on the fresh root weight of *Ceiba pentandra* seedlings

*T*₁ : Sterilized forest soil (100 %), *T*₂ : 90 % *T*₁ + sterilized mycorrhizal inoculum (10 %); *T*₃ : 90 % *T*₁ + mycorrhizal inoculum (10 %); *T*₄ : 90% *T*₁ + compost (10 %); *T*₅ : 80 % *T*₁ + mycorrhizal inoculum (10 %) + compost (10 %)

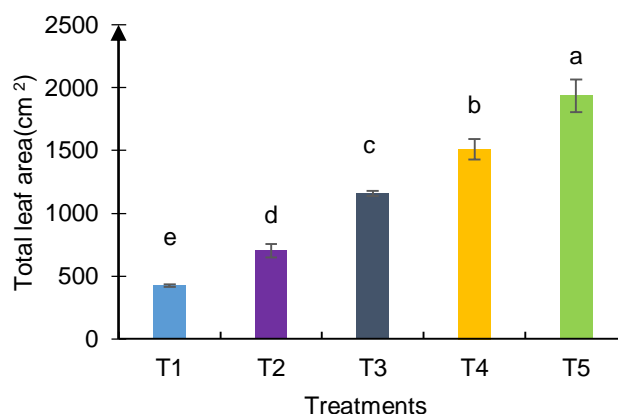


Fig. 6. Effect of treatments on the total leaf area of *Ceiba pentandra* seedlings

*T*₁ : Sterilized forest soil (100 %), *T*₂ : 90 % *T*₁ + sterilized mycorrhizal inoculum (10 %); *T*₃ : 90 % *T*₁ + mycorrhizal inoculum (10 %); *T*₄ : 90% *T*₁ + compost (10 %); *T*₅ : 80 % *T*₁ + mycorrhizal inoculum (10 %) + compost (10 %)

116.90 % and 138.97 % respectively for the sterilized mycorrhized soil (*T*₂), unsterilized mycorrhized soil (*T*₃), compost application (*T*₄) and compost + unsterilized mycorrhized soil (*T*₅) compared to the treatment (*T*₁).

3.2.5 Effect of treatments applied on the fresh weight of plant roots

Fresh root weight values ranged between 3.06 and 14.46 g (Fig. 6). The type of treatment applied significantly influenced the rooting of young plants [F (*df*: 4)= 140.54; *p* < 0.01]. In ascending order the fresh root weight followed the trend *T*₁ < *T*₂ < *T*₃ < *T*₄ < *T*₅. Thus, treatments consisting of the sterilized substrate alone (*T*₁) and that consisting of the addition of sterilized mycorrhizal soil (*T*₂) produced plants with low fresh root weights.

3.2.6 Effect of treatments on the total leaf area of *Ceiba pentandra* seedlings

The leaf area of the plants from *T*₃ was higher than that of *T*₁ and *T*₂ plants (Fig. 7). On all the treatments studied, the highest total leaf area was obtained in the plants subjected to treatment *T*₅.

3.2.7 Sturdiness quotient and ratio of Fresh Weight of Stems (FWS) to Fresh Weight of Roots (FWR)

Plant height divided by diameter gave values between 6 and 7 (Table 4). Analysis of these data showed that this quotient was significantly impacted by substrate composition (*p* = 0.003). The mixed treatment gave the highest values

Table 4. Sturdiness quotient (h/d), fresh stem weight ratio, root system (FWS/FWR) and chlorophyll content of *Ceiba pentandra* seedlings

Treatments	h/d	FWS/FWR	Chlorophyll content (SPAD unit)
T ₁	6.16 ± 0.20 c	2.09 ± 0.10 ab	50.76 ± 1.02 a
T ₂	6.41 ± 0.24 bc	1.57 ± 0.10 b	48.10 ± 3.57 a
T ₃	6.72 ± 0.22 ab	1.68 ± 0.08 b	53.44 ± 1.79 a
T ₄	6.64 ± 0.16 ab	2.63 ± 0.26 a	51.23 ± 1.73 a
T ₅	6.79 ± 0.16 a	2.38 ± 0.36 a	47.18 ± 2.75 a
Test statistics	F (df: 4)= 9.88, p < 0.01	F (df: 4)=7.29; p < 0.01	F (df: 4)= 0.92; p > 0.05

Means with different letter(s) in columns are significantly different at $p \leq 0.05$. T₁: Sterilized forest soil (100 %), T₂: 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃: 90 % T₁ + mycorrhizal inoculum (10 %); T₄: 90% T₁ + compost (10 %); T₅: 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %). FWS : fresh weight of stem ; FWR : fresh weight of root

Table 5. Minerals contents of the leaves of kapok seedlings

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
T ₁	4.91±0.00 a	0.48 ± 0.02 b	3.83 ± 0.05 a	0.96 ± 0.00 b	1.37 ± 0.02 bc
T ₂	4.96 ± 0.00 a	0.46 ± 0.01 b	4.01 ± 0.03 a	0.95 ± 0.00 b	1.25 ± 0.00 d
T ₃	5.51 ± 0.33 a	0.48 ± 0.00 b	3.46 ± 0.12 b	1.06 ± 0.05 a	1.33 ± 0.03 c
T ₄	5.15 ± 0.11 a	0.56 ± 0.00 a	3.99 ± 0.14 a	0.94 ± 0.00 b	1.38 ± 0.00 b
T ₅	5.14 ± 0.06 a	0.46 ± 0.01 b	3.97 ± 0.06 a	1.06 ± 0.01 a	1.50 ± 0.00 a
Test statistics	F (df: 4) =2.25 ; p =0.13	F (df: 4) =13.26 ; p < 0.01	F (df: 4) = 6.12 ; p < 0.01	F (df: 4) = 7.60 ; p < 0.01	F (df: 4) = 37.07 ; p < 0.01

Means with different letter(s) in columns are significantly different at $p \leq 0.05$. T₁: Sterilized forest soil (100 %), T₂: 90 % T₁ + sterilized mycorrhizal inoculum (10 %); T₃: 90 % T₁ + mycorrhizal inoculum (10 %); T₄: 90% T₁ + compost (10 %); T₅: 80 % T₁ + mycorrhizal inoculum (10 %) + compost (10 %)

(6.79 ± 0.16) and the control treatment T₁ gave the lowest values (6.16 ± 0.20). The fresh weight ratio between stem and root (FWS/FWR) was also significant between treatments (Table 4). Values ranged from 1.57 to 2.38. The treatments were grouped into two main groups on the basis of the observed differences. The T₄ and T₅ group and the T₃ and T₂ group with high and low ratios respectively. The T₁ treatment presented intermediate values to both groups.

3.2.8 Chlorophyll content

The chlorophyll pigment concentration measured with SPAD-502 Plus was between 47.18 and 53.44 SPAD units (Table 4). The chlorophyll content of plants from the T₃ treatment (mycorrhized soil) was observed to be the highest (53.44 ± 1.79 SPAD units). Nevertheless, the chlorophyll pigment richness of the leaves was not statistically different with all the treatments applied [F (df:4) = 0.924; p < 0.495].

3.2.9 Nutritional variables

Unlike N content of the leaves, P, K, Ca Mg contents were influenced by the treatments that

were applied (Table 5). Phosphate content was higher with the addition from T₄ treatment. Potassium content (3.46±0.12 %) was lower in the T₃ treatment. Also, the leaves from T₃ and T₅ seedlings showed the highest calcium contents .Compared to T₁ (sterilized forest soil) and T₂ (T₁+ sterilized mycorrhizal inoculum), the leaves of the seedlings from treatment T₃ (T₁ + mycorrhizal inoculum) showed highest values of minerals contents (N, P, Ca). However, except for the phosphorus content, the leaves of seedlings from T₅ treatment showed the highest mineral content.

4. DISCUSSION

Mycotrophic plants have a "nurses" might, they are capable of creating an environment suitable for the growth of the following crop [39,40,41]. In this study, after planting of *Sorghum bicolor* and *Vigna unguilata* in soils collected under *Ceiba pentandra* trees, results showed that the number of spores doubled. However, spores of arbuscular mycorrhizal fungi were more enhanced by *S. bicolor* than by *V. unguilata*. Indeed, according to Houngnandan et al. [42]

findings, Poaceae roots are suitable for the proliferation of mycorrhizal fungi. Thus in this study, the sorghum (Poaceae) seedlings were allowed to increase a density of AM fungal spores compared to *V. unguiculata*. These results showed that the choice of the mycotrophic plant in the improvement of the mycorrhizal potential of soils is a crucial step.

The results obtained showed the best growth variables with seedlings from the nursery substrate supplemented with unsterilized mycorrhized soil (T₃) compared to the substrate supplemented with the same mycorrhized but sterilized soil (T₂). This difference would be due to the presence of growth-promoting microorganisms, particularly endomycorrhizal fungi in the added soil. The soil used as an inoculum contains arbuscular mycorrhizal (AM) fungi dominated by the genus *Glomus* [29].

In addition, the present study showed that the roots of plants that received only the mycorrhizal inoculum (T₃) were endomycorrhized. A functional symbiosis was therefore established between the young plants and the AM fungi. These associations stimulate plant growth even in environments with constraints [43,44,45]. The improvement in the seedling growth resulted from a better absorption of nutrients, particularly phosphorus, by the mycorrhized roots [18]. Indeed, the mycelial hyphae of AM fungi increase the surface area for nutrient uptake. In addition, through enzymatic activities (phosphatases, phytases), mycorrhizae contribute to the release of insoluble minerals in the soil [46, 47,48,49]. Mrabet *et al.* [33] obtained similar results on the growth of *Argania spinosa* seedlings, after cultivation on soil with added mycorrhizal soil. Similarly, Droh *et al.* [50] noted improved nursery growth of cocoa plants following inoculation with 65 g of mycorrhizal soil from a cocoa orchard. Thus, native fungi are potentials which are to be explored to improve plant growth [51]. The 117 % increase in stem dry weight of mycorrhized seedlings (T₃) compared to non-mycorrhized seedlings (T₂), demonstrates that *Ceiba pentandra* seedlings are highly dependent on mycorrhization. Above all parameters evaluated, the treatment with the mycorrhized soil alone (T₃) allowed the best growth compared to T₂. Also, the mycorrhizal structures observed in the root system of the T₃ and T₅ treatments showed that three months were sufficient for effective root colonization under the conditions of the study. Thus, the seedlings of *Ceiba pentandra* were found to be receptive to mycorrhizal fungi.

C. pentandra seedlings, like those of *Argania spinosa* [33] and *Tetraclinis articulata* [15] were able to associate with AM fungi during their initial growth. However, there were no differences in the level of mycorrhizal root colonization between treatments with fertilizer (T₅) or without fertilizer (T₃). It is accepted that a soluble fertilizer application increases solubility but strongly reduces and even suppresses mycorrhizal symbiosis [52]. In the present study, the degree of root colonization was not impacted despite the application of organic fertilizer. This result is similar to that of Gosling *et al.* [53]. According to these authors, fertilizers that slowly release minerals (compost) do not seem to have negative effects on AM fungi and may even stimulate them. In addition, a synergistic effect of compost and mycorrhized soil resulting in improved plant growth was reported in this study. Compared to the mycorrhized soil treatment (T₃) and the compost treatment alone (T₄), the seedlings resulting from the composite treatments (T₅) showed the best values in terms of growth variables, rooting and above-ground dry biomass. This synergistic effect was observed by Guissou *et al.* [54] on the height of *Zizyphus mauritiana* seedlings but not on the total biomass. Jan *et al.* [55] also demonstrated this effect following inoculation with AM fungi combined with the addition of compost and phosphorous on the growth of *Trifolium alexandrinum*. This growth is also due to the high root biomass highlighted in this study and probably to the availability of mineral elements. The mineralization of the compost enriched the environment in mineral elements, while the mycelial hyphae facilitated their absorption. Through improved growth performance, the mixed treatment (T₅) reduced the production time of *Ceiba pentandra* seedlings. According to Adou [2], the required height at the nursery exit of *C. pentandra* seedlings should be between 20 and 30 cm. It took three months for the control plants (T₁) to attain that height. It took six weeks for T₄ and T₅ and eight weeks for T₃ to attain that height.

The sturdiness quotient and the FSW/FRW ratio are morphological indices that can predict seedlings survival after transfer to the wild [10, 56]. In this study, the robustness ratio values of *Ceiba pentandra* seedlings ranged from 6 to 7. For Lamhamedi *et al.* [57], this ratio should be less than 7 for *Acacia cynaphylla* plants. However, according to Thomson [10], a ratio higher than six is harmful to the growth of *Epicea* plants in water-deficient environments. In view of

the results obtained with regard to the ratio of fresh stem/root weight, the absorption area (fresh root weight) of the *C. pentandra* seedlings produced is lower than that of transpiration regardless of the treatment. However, the high stem/root fresh weight ratio for plants from T₅ and T₄ treatments suggests better stem growth to the benefit of the root. This could be explained by the container (nursery bag) which is a potential obstacle to root system growth.

5. CONCLUSION

Young plants of *Ceiba pentandra* are highly dependent on arbuscular mycorrhizal fungi for their growth. Mycorrhization combined with the use of compost was responsible for the best values of growth and rooting variables. Also, these results reveal that soils collected under the trees of the species are potential sources of mycorrhizal inoculum. This method can be used in forest nurseries to reduce the production time of the seedlings while guaranteeing their quality. Given the importance of the sturdiness quotient, the seedlings produced should be sent to a natural environment to determine its value.

ACKNOWLEDGEMENTS

The authors would like to thank the commandant of the Botanical Garden of Bingerville for allowing sampling in the garden and the manager of the National Floristic Center of the Felix Houphouët Boigny University (Côte d'Ivoire) for nursery site.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Duvall CS. *Ceiba pentandra* (L.) Gaertn. [Internet] PROTA4U's file. Brink, M. & Achigan-Dako, E.G. (Publishers). PROTA (Plant Resources of Tropical Africa), Wageningen, The Netherlands ; 2011. Available:<http://www.prota4u.org/search.asp> Accessed on 26 March 2020.
- Adou K. Bibliographic study on *Ceiba pentandra*. SODEFOR Abidjan, Côte d'Ivoire. 2010;12.
- Galiana A, Kanga NA, Gnahoua G, Pity B, Dupuy B, Domenach A, Mallet B. Nitrogen fixation in *Acacia mangium* in plantations. Bois & Forêts Des Tropiques. 1996;249(249):51–62.
- Maldonado G, Louppe. The village plantations of *Tectona grandis* in Côte d'Ivoire, Bois et Forêts Des Tropiques. 1999;12.
- Onguene NA, Tsimi JPM, Balla MJE. Mycorrhizal status of Okoume (*Aucoumea klaineana* Pierre) in artificial regeneration in southern Cameroon. *Tropicultura*. 2002;20(3):104–108.
- Davis AS, Jacobs DF. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. *New Forests*. 2005;30(2-3):295-311.
- Tchinmegni FI, Tsobeng AC, Ngonkeu MEL, Tchoundjeu Z. Evaluation of the mycorrhizal status of *Allanblackia floribunda* with a view to its domestication in rainforest areas of Cameroon. *Revue Scientifique et Technique Forêt et Environnement du Bassin du Congo*. 2016;6(Janvier): 81-83.
- Grossnickle S, MacDonald J. Seedling Quality: History, application and plant attributes. *Forests*. 2018;9(283):23.
- Aussenac G, Guehl JM, Kaushal P, Granter A, Grieu Ph. Physiological criteria for assessing the quality of forest seedlings before planting. *Revue Forestière Française*. 1988;40:131-139.
- Thomson BE. Seedling morphological evaluation, In Durvea ML (ed.). *Proceedings: Evaluating seedling quality: Principles, procedures and predictive abilities of major tests*. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis. 1985;59-71. [ISBN 0-87437-000-0]
- Guehl J, Falconnet G, Gruez J. Physiological characteristics and survival after planting of container grown *Cedrus atlantica* seedlings on different types of growing substrates. In *Annales des Sciences forestières*, EDP Sciences. 1989;46:1-14.
- Haase DL. Morphological and physiological evaluations of seedling quality. Riley LE, Dumroese RK, Landis TD (tech. cords) National proceedings: Forest and Conservation Nursery Associations-2006. Proc. RMRS-P-50. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 2007;3–8.

13. Ammari Y, Lamhamedi MS, El Abidine A, Akrimi N. Production and growth of resinous plants in different compost-based substrates in a modern forest nursery in Tunisia, *Révue Forestière Française*, 2007;59(4) :339-358.
14. El Abidine, Abdenbi Z, Mohamed B, Abdelmonim B, Lamhamedi, ABBAS Y. Growth and development of seedlings of two cork oak origins produced in the nursery in containers of different depths.. *Forêt Méditerranéenne*. 2016;37:137-150.
15. Abbas Y, M Arabou, Duponnois R, M Abourouh M Effect of Arbuscular Mycorrhizae on the Growth and Nutrition of Three Provenances of *Tetraclinis Articulata* Vahl Masters. *Annales de la recherche forestière au Maroc*. 2013;7:7-16.
16. Mrabet Said El, Ouahmane L, El Mousadik A, Msanda F, Younes. The Effectiveness of Arbuscular Mycorrhizal Inoculation and Bio-Compost Addition for Enhancing Reforestation with *Arganiaspinosa* in Morocco, *Open Journal of Forestry*. 2014; 4(1):14-23
17. Gagnon J. Performance of mycorrhized plants after 4 to 21 years of growth in 15 plantations established in several ecological regions of Quebec. *Forestry Research Paper*. 2016;178:34.
18. Guissou T, Babana AH, Sanon KB, Bâ A M. Effects of Arbuscular Mycorrhizae on the Growth and Mineral Nutrition of Fruit Trees of Diverse Geographical Origins Grown in Greenhouses. *Biotechnologie, Agronomie, Société et Environnement*, 2016;20(3):417-426-426.
19. Gaba-Chahboub H, Lamhamedi MS, Abrous-Belbachir O. Effect of ectomycorrhizal inoculation in nurseries on the growth and nutrition of atlas cedar plants in Algeria. *Bois & Forêts Des Tropiques*. 2017;330(330):57-67.
20. Sfairi Y, Mohamed N Al F, Issam J, Ouahmane L. Responses of Atlas Cypress seedlings, *Cupressus atlantica* Gaussen, to the inoculation of arbuscular mycorrhizae from various origins in the western High Atlas region of Morocco. *Bois et Forêts Des Tropiques*. 2018;337:27–38.
21. MousainD, Plassard C, Argillier C, Sardin T, Leprince F, El Karkouri K, Arvieu JC, Cleyet-Marel JC. Strategy for improving the quality of Mediterranean forest plants and reforestation through the use of controlled mycorrhization in tree nurseries. *Boccone*. 1996;5(1):376-387.
22. Duponnois R, Bâ, Plenchette C, Thioulouse J, Cadet P. Effect of fallowing on populations of arbuscular mycorrhizal fungi in Senegal. *Fallows in tropical Africa*, 2000;1:325.
23. Park H, Eun-Hwa L, Kang HK, Eom AH. Community structures of arbuscular mycorrhizal fungi in soils and plant roots inhabiting abandoned mines of Korea. *Mycobiology*. 2016;44(4):277-282.
24. Duponnois R, Hafidi M, Ndoeye I, Galiana A, Dreyfus B, Prin Y. Management and enhancement of soil microbial resources for sustainable revegetation of Sahelian environments In: Dia A. (ed.), Duponnois Robin (ed.), Wade A. (pref.) *The major African project of the Great Green Wall: concepts and implementation*. Marseille : IRD. 2010;171-182. (Synthesis).
25. Henry C, Selosse MA, Richard F, Ramanankierana H, Ducousso M. To understand the dynamics of mycorrhizal communities during plant succession. First part: study methods, characterizations and functioning (bibliographical review). *Revue Forestière Française*. 2014;2:125-150.
26. Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science*. 2005;85(1):31-40.
27. Ndonda AM, Mahungu NT, Moango AM, Yandju MC. Effect of arbuscular mycorrhizal fungi on phosphorus in tropical soils and involvement in the biosynthesis of cassava carotenoid. *Journal of Applied Biosciences*. 2019;135(5):13750–13764.
28. Khasa P, Furlan V, Lumande K. Roots symbiosis in some important forest species in Zaire. *Bois & Forêts Des Tropiques*. 1990;224(224):27-33.
29. Anguiby BLA, Ouattara G, Bomisso EL, N'goran B, Ouattara B, Coulibaly SA, Aké S. Evaluation of the mycorrhizal status of trees of *Ceiba pentandra* (L), Gaertn and *Tieghemella heckelii* (A. Chev), Pierre, from Bingerville Botanical Garden in Côte d'Ivoire. *Journal of Applied Biosciences*. 2019;138(1):14092–14105.
30. Trouvelot A. Measurement of the VA mycorrhization rate of a root system. Search for estimation methods with functional significance. *Physiological and Genetical Aspects of Mycorrhizae*. 1986; 217-221.
31. Kirk PL. Kjeldahl method for total nitrogen. *Anales Chemical*. 1950;22:354-358.

32. Dabin B. Application of automatic dosing to soil analysis. Cahier Orstom Serie Pedologie. 1967;3(4):335-366.
33. Mrabet Said El, Msanda F, El Mousadik A, Ouahmane L. Evaluation of the mycorrhizogenicity of rhizospheric soils of : *Chamaecytisus albidus* and *Ononis natrix* in the production of high-performance plants of *Argania spinosa* L. Skeels, American Journal of Innovative Research and Applied Sciences. 2017;4(1):44-51.
34. Phillips JM, et Hayman DS. Improved Procedures for Clearing Roots and Staining Parasitic and Vesicular-Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. Transactions of the British Mycological Society. 1970;55(1):158-IN18
35. Echairi A, Nouaim R, Chaussod R. Interest of controlled mycorrhization for the production of argan (*Argania spinosa*) plants in nursery conditions. Science and global changes / *Sécheresse*. 2008;19(4): 277-281
36. Zraïbi L, Nabloussi A, Merimi J, El Amrani A, Kajeiou M, Khalid A, Caid H S. Effect of saline stress on physiological and agronomic parameters of different varieties of *Carthamus tinctorius* L. Al Awamia. 2012;125(126):15-40.
37. Ling Q, Huang W, Jarvis P. Use of a SPAD-502 Meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynthesis Research. 2011; 107(2):209-214.
38. Pinta M. Reference methods for the determination of mineral elements in plants. Determination of the elements Ca, Mg, Fe, Mn, Zn and Cu by atomic absorption (1973). In : Crop Feeding Control. Budapest (HON) ; s.l: Académie des Sciences de Hongrie ; CII, 1972, p. 143-158. Contrôle de l'Alimentation des Plantes Cultivées : Colloque, 2., Séville (ESP); 1968.
39. Ouahmane L, Duponnois R, Hafidi M, Kisa M, Boumezouch A, Thioulouse J, Plenchette C. Some Mediterranean Plant Species (*Lavandula* Spp. and *Thymus Satureioides*) Act as Potential 'Plant Nurses' for the Early Growth of *Cupressus Atlantica*. Plant Ecology. 2006;185(1):123-34.
40. Wahbi S, Sanguin H, Oufdou K, Hafidi M, Galiana A, Domergue O, Baudoin E, Prin Y, Duponnois R. Influence of mixed bean/wheat crops on the mycorrhizal potential of soils and the structure of mycorrhizal microflora in Morocco. In: 3rd Francophone mycorrhizal days (JFM), Nancy, France, 05-07 September 2012. Abstract, 2012; 84p.
41. Manaut N, Hafidi M, Ouahmmou A, Baudoin Ezékiel, Chaffii K, Prin Y, Ouahmane L, Sanguin H, Galiana A, Boumezough A, Duponnois Robin. Nurse plant: propagation vector of mycorrhizal fungi to optimize the performance of reforestation operations in Morocco. In: Duponnois Robin (ed.), Hafidi M. (ed.), Ndoye I. (ed.), Ramanankierana H. (ed.), Bâ A.M. (ed.) Symbiotic fungi against desertification: Mediterranean, tropical and island ecosystems. Marseille: IRD. 2013; 391-409.
42. Houngnandan P, Yemadje R G H, Kane A, Boeckx P, Van Cleemput O. Native glomerales of the clear forest at *Isoberlinia doka* (Craib and Stapf) in Wari-Marou in central Benin. 2009;27(2):83-87.
43. Zougari-Elwedi B, Sanaa M, Labidi S, Sahraoui AL. Evaluation of the impact of arbuscular mycorrhization on the mineral nutrition of *Phoenix dactylifera* L. var. Deglet Nour seedlings. Etude et Gestion des Sols. 2012;19(3):193-202.
44. Leye EHM, Ndiaye M, Ndiaye F, Diallo B, Sarr A S, Diouf M. Effect of mycorrhization on the growth and development of *Jatropha curcas* L. Revue des Energies Renouvelables. 2009;2(12):269-278.
45. Diallo B, Samba A N S, Djibril S. Effects of arbuscular mycorrhizal fungi on the growth and development of *Ricinus communis* plants grown under saline stress under semi-controlled conditions 19 (janvier): 2016;1-59.
46. Mousain D, Matumoto-Pintro P, Quiquampoix H. The role of mycorrhizae in the phosphate nutrition of forest trees. *Revue Forestière Française*. 1997;49:67-81.
47. Founoune H, Duponnois R, Ba A M, Sall S, Branget I, Lorquin J, Chotte J L. Mycorrhiza helper bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. New Phytologist, 2002;153(1), 81-89.
48. Duponnois R, Colombet A, Hien V, Thioulouse J. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of

- Acacia holosericea*. Soil biology and Biochemistry. 2005;37(8):1460-1468.
49. Hinsinger P, Ndour NYB, Becquer T, Chapuis-Lardy L, Masse D. Issues related to phosphorus in tropical soils: 267-278, In Restoration of the productivity of tropical and Mediterranean soils: Contribution to agroecology, IRD (Edit). 2015;540.
 50. Droh G, Kouassi A, Kouadjo ZGC. Effects of two types of AMF on growth of cocoa seedlings (*Theobroma cacao* L.) in greenhouses, *Global Journal Of Advanced Research*, 2016 ; (3), 157-164.
 51. Ouahmane La, Hafidi M, Plenchette C, Kisa M, Boumezzough A, Thioulouse J, DuponnoisR. *Lavandula* species as accompanying plants in Cupressus replanting strategies: Effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. *Applied Soil Ecology*. 2006b;34(2):190- 99.
 52. Bâ A, Guissou T, Duponnois R, Plenchette C, Sacko O, Sidibé D, Windou B. Controlled mycorrhizal and phosphate fertilization: applications to Jujube tree domestication. *Fruits*, 2001, 56(4), 261-269.
 53. Gosling P, Hodge A, Goodlass G, Bending GD. Arbuscular Mycorrhizal Fungi and Organic Farming. *Agriculture, Ecosystems & Environment*, 2006; 113 (1- 4): 17- 35.
 54. Guissou T, Moustapha A, Plenchette C, Guinko S, Duponnois R. Effects of arbuscular mycorrhizae on water stress tolerance of four fruit trees: *Balanites aegyptiaca* (L.) Del., *Parkia biglobosa* (Jacq.) Benth., *Tamarindus indica* L. and *Zizyphus mauritiana* Lam. *Science et changements planétaires/Sécheresse*. 2001;12(2):121-7.
 55. Jan B, Amjad A, Fazli W, Shah SNM, Asif K, Farmanullah K. Effect of Arbuscular mycorrhiza fungal inoculation with Compost on Yield and Phosphorous Uptake of Berseem in Alkaline Calcareous Soil. *American Journal of Plant Sciences*. 2014;9(5):1359-69.
 56. M'Sadak Y, Mohamed AE, Rim El K. Evaluation of substrates and plants produced in forest nurseries. *Bois & Forets des Tropiques*. 2012;313:61-71.
 57. Lamhamedi MS, Fortin JA, Ammari Y, Ben J, Poirier M, Fecteau B, Godin L. Evaluation of composts, substrates and the quality of plants (*Pinus pinea*, *Pinus halepensis*, *Cupressus sumpervirens* and *Quercus suber*) grown in containers. Technical report: Execution of the development works of three pilot nurseries in Tunisia. Publication DGF de Tunisie and Pampev Internationale Ltée, Quebec., Canada. 1997;121.

© 2020 Anguiby et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/57843>