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Comparative Effect of Endogenous Mycorrhizal Fungi Consortia in Improving *Gossypium hirsutum* L. Growth and Yield

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Abstract: Cotton growers from Northern Cameroon use chemical fertilizers to improve soil fertility. However, the use of chemical fertilizers exhibits an immediate solution to decline of soil fertility problem, but its exclusive use causes an increase of soil degradation. With the aim of sustainably increasing of cotton growth in Cameroon, study was conducted to evaluate effects of combinations of various strains of endogenous mycorrhizal fungi from Sudano-Sahelian savannahs of Cameroon on cotton productivity. Colonization rate, AMF diversity, cotton growth and yield were assessed according to suitable methods. Regarding the assessment of cotton productivy a 8×3×2 experimental design with 08 types of treatments, 03 experimental sites (Djalingo, Djabi and Djaba), and 02 cotton varieties (IRMA Q302 and IRMA L457) were used. Results shown that the colonization rate of cotton plant roots is higher in the Division of Mayo-Rey (93.33%). The cotton plants growth varies according to fertilizer and experimental area. There is no significant difference between cotton varieties relative to seeds yield and fibers yield. Endogenous mycorrhizal strains from Northern Cameroon better improve cotton productivity in our study areas than exogenous mycorrhizal strains in the range of 5 to 70%. Cotton fibers yield from Djaba site were 1.85 and 2.02 folds greater than that from Djalingo and Djabi respectively. Based on these results, the domestication and application of endogenous mycorrhizal fungi in North Cameroon for cotton growth is a contribution to increase cotton productivity and to limit the use of chemical inputs, as well as a guarantee of sustainable agriculture.

Keywords: Endogenous Mycorrhizal Fungi, Diversity, *Gossypium hirsutum*, Growth, Yield

1. Introduction

Cotton (*Gossypium hirsutum* (L.)) belongs to Malvaceae family and as become a very popular crop in North Cameroon. Today, African cotton producing countries in the CFA franc zone constitute one of the largest cotton producing zones in the world. According to the International Cotton Advisory Committee (ICAC), the supply from 12-15% of the world's fiber exports. In 2018-2019, these zones supplied 4.5% of the 26 million tons of the world's cotton fiber, almost 3.3 million tons of seed cotton [1]. Cotton growing remains the main economic pillar of the Northern

regions of Cameroon (Adamawa, North and Far-North). It directly supports nearly 200.000 farming families representing more than 2 million people. In this production area, cultivation is practiced on approximately 90% of farms and contributes to the monetization of rural areas by providing nearly 60% of net agricultural income. SODECOTON is the leading employer in Northern regions of Cameroon with approximately 1.900 permanent employees and 3.000 contracted seasonal workers each year. At the national level, cotton accounts for 6% of non-oil exports and 14.1% of the GDP of the industrial agriculture and export branch [2]. Cotton crop is the engine of economy in Sudano-Sahelian zone of Cameroon.

In the Northern Cameroon, cotton is one of crops whose the growth requires the use of large quantities of chemical fertilizers [3], consequently a gradual degradation of natural resources such as water, soils fertility and biodiversity [4]. The cotton sector faces challenges such as the decline of soil fertility, pests and the high cost of inputs, which affect production and impact the economy [5]. Farmers of Northern Cameroon are spending a lot of money to buy chemical inputs in order to obtain better agricultural yields. However, the using of chemical fertilizers exhibits an immediate beneficial effect on crop productivity and provides an immediate solution to declining of soil fertility problem, their high cost makes them almost inaccessible to small farmers [6-7, 8]. Furthermore, its exclusive use causes an increase in acidity, a degradation of the physical status and a decrease in soil organic matter [9]. In this context, it seems necessary to consider in local farming communities, the management methods that allow rational and sustainable exploitation of bioresources [10] and also to increase agricultural production while protecting the natural ecosystems [11]. In this respect, recent work relative to cotton in Northern Cameroon focused to improve plant productivity while ensuring sustainable agriculture [12, 13]. To the best of our knowledge, no work has been carried out for the application of endogenous mycorrhizal fungi for cotton productivity in North Cameroon. For this purpose, produce and use this biofertilizer for cotton growth, will contribute not only to improve cotton productivity, but also to valorize endogenous mycorrhizal fungi associated to cotton as well as to ensure sustainable agriculture. Mycorrhizae are symbiotic associations between mycorrhizal fungi and plant roots [14, 15]. Indeed, mycorrhizal inoculum improves plant production by promoting plants hydromineral nutrition [16].

The main objective of this study was to compare the effect of native strains of endogenous mycorrhizal fungi to other fertilizers. Specifically, it consisted to: (1) evaluate colonization rate of cotton plant roots by Endogenous Arbuscular Mycorrhizal Fungi; (2) assess the effect of endogenous mycorrhizal fungi from Northern Cameroon on cotton growth and yield. The usefulness of this work is based on the fact that native strains of endogenous mycorrhizal fungi that better improves cotton productivity will be promoted in order to reduce the use of chemical fertilizers in the Northern Cameroon.

2. Materials and Methods

2.1. Study Site

The study was carried out in three Divisions of North Cameroon (Benoue, Mayo-Louti and Mayo-Rey) and nine (09) localities, three (03) from each Division: Benoue (Djalingo, Ngong, Ouro-Kessoum), Mayo-Louti (Bidzar, Djabi, Guider) and Mayo-Rey (Djaba, Dogba, Guidjiba). North Cameroon is from Sudano-Sahelian climate with a long dry season (9 months: October - June) and a short rainy season (3 months: July - September) [17].

2.2. Material

2.2.1. Cotton Seeds

Cotton seed varieties IRMA Q302 and IRMA L457 are used for this work. These seeds were provided by the Institute of Agronomic Research for Development (IRAD) of Maroua, Cameroon. Both cotton varieties used present a short life cycle (120 days) and are very used by cotton growers of Great North Cameroon. Using varieties presented short reproduction cycle is advantageous for farmers because they may have several harvests per year if they have off-season crops methods.

2.2.2. Mycorrhizal Inoculum

Endogenous and exogenous mycorrhizal fungi were used. Endogenous mycorrhizal fungi were obtained by multiplication of mycorrhizal fungi spores associated with the cotton rhizosphere in North Cameroon. It contains 17 species (Figure 1) previously identified [18]. The production of endogenous mycorrhizal used was carried out according to the Tropical Agrosystem Research Unit method of INRA in France. Indeed, *Vigna unguculata* L. use as a trap plant was grown in a pot. A mixture of 500 g of soil from sites of extracted spores in Benoue, Mayo-Louty and Mayo-Rey of North Cameroon and 1500 g of sand were put in each pot. The sand was sterilized using a wood fire for 3 hours. Plants of *Vigna unguculata* were watered every three days [19]. 65 days after sowing, growing soil in pot was used as endogenous mycorrhizal fungi. It consists of a mixture of sand, mycelium, roots and with a concentration of 10 spores/g of substrate.

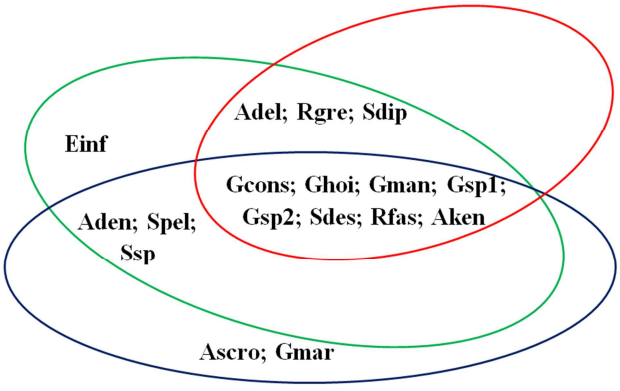


Figure 1. *Endogenous mycorrhizal fungi species identified and contain in inoculum in relation with site of study.*

Exogenous mycorrhizal fungi were supplied by the Laboratory of Microbiology and Soils of the Biotechnology Center of the University of Yaounde I. It contains infective spores and propagules of three genera: *Glomus*, *Scutellospor*a and *Gigaspora* species with a concentration of 20 spores/g of the substrate [20].

Green circle: Endogenous mycorrhizal fungi of Bénoué (BEN); Red circle: Endogenous mycorrhizal fungi of Mayo-Louti (MAL); Blue circle: Endogenous mycorrhizal fungi of Mayo-Rey (MAR).

Glomus constrictum (Gcons); *Glomus hoi* (Ghoi); *Glomus manihotis* (Gman)*; Glomus sp. 1* (Gsp1)*; Glomus sp. 2* (Gsp2)*; Septoglomus desorticola* (Sdes); *Rhizophagus fasciculatus* (Rfas)*; Acaulospora denticulata* (Aden)*; Acaulospora delicata* (Adel)*; Acaulospora scrobiculata* (Ascro)*; Acaulospora kentinensis* (Aken)*; Entrophospora infrequens* (Einf)*; Gigaspora margarita* (Gmar)*; Racocetra gregaria* (Rgre)*; Scutellospora dipurpurascens* (Sdip)*; Scutellospora pellucida* (Spel)*; Scutellospora* sp. (Ssp).

2.2.3. Chemical Inputs

The chemical fertilizer (NPKBS of formula 21.08.12.3.1. and zinc sulfate) and the chemical insecticide used is CONQUEST C 176 EC from the firm Arysta Lifescience, of which the active ingredients are acetamiprid (32g/l) and cypermethrin (144g/l) [21]. They were purchased at the Cotton Development Society (SODECOTON) from Garoua Cameroon. These chemical inputs are suitable for cotton growing and are commonly used by cotton growers of Northern Cameroon.

2.3. Methods

2.3.1. Determination of Mycorrhizal Status of the Cotton Plant in the Field

A 100 % flowering, 76 Day After Emergence (JAL), the fine roots of *Gossypium hirsutum* L. was found at the root system of a cotton plant chosen at random. The fine roots are washed and conserved in 70% alcohol for microscopic observation.

The 1-2 cm fine root fragments are introduced into the test tube and treated according to the method of [22]. The procedure is as follows: the harvested roots are cut and then washed with tap water; they are then clarified in 5% KOH for 15 min at 90°C in a water bath. The roots are again washed 3 times with tap water, then acidified in 1% HCl for 15 min at 90°C. They are immersed for 30 min at room temperature on the one hand in a 0.05% acid fuchsin solution added to the solution consisting of lactic acid-glycerol-water (5-3-2) and on the other hand with the solution of 5% methylene blue, then the staining solution is emptied from the test tube and the discoloration is carried out in a solution of lactic acid-glycerol-water (5-3-2) for at least 24 hours. The stained root fragments (30 fragments/site) are mounted parallel on slides and covered with coverslips in groups of 10 and observed at magnifications 10 and 40 using the ZEISS brand stereo microscope in the laboratory of the Biotechnology Center of the University of Yaoundé 1. This observation made it possible to determine the rate of root colonization (TC) by observing fungal structures, such as arbuscules, hyphae, spores and vesicles [22]:

$$TC\ (\%) = (n/N) \times 100$$

with (n) the number of observed root fragments possessing one or more mycorrhizal structure(s) and (N) the number of total root fragments (10 fragments) found on the slide-coverslip assembly;

2.3.2. Evaluation of Mycorrhizal Inoculum on Cotton Productivity

(i). Land Preparation and Experimental Design

The sowing method adopted is direct sowing, because soil disturbances such as plowing and displacement of topsoil can lower the mycorrhizal potential in an agricultural system [23]. Direct sowing has the characteristic of facilitating the infiltration of water into the soil while reducing evaporation and above all creating an environment favorable to the development of biological activity [24]. An 8×3×2 experimental design with 8 types of fertilizers (Unfertilized plot; chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; endogenous mycorrhizal fungi of study site; a combination of mycorhizal fungi from Benoue and Mayo-Louti; a combination of mycorrhizal fungi from Benoue and Mayo-Rey; a combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; a combination of mycorrhizal fungi from Benoue; Mayo-Louti and Mayo-Rey; exogenous mycorrhizal fungi), three experimental sites (Djalingo, Djabi and Djaba) and two cotton varieties (IRMA Q302 and IRMA L457) was used. Each experimental unit has 3 seedlings lines and each line has 10 poquets. There are 30 plants per experimental unit.

(ii). Sowing

Sowing took place on July 2019. 05 seeds are placed per poquet at 3 cm depth [25] and thinning was carried out 02 weeks after sowing so as to leave 02 plants/poquet. Mycorrhizal inoculum was applied at sowing time and put in direct contact with seeds and over the entire cavity of the hole. The chemical fertilizer was applied on the 14th day after sowing [26]. The chemical insecticide was sprayed on the aerial part of cotton plants pre-fertilized with chemical fertilizer using an Ulva brand rotating disc at flowering time (45 days after sowing).

(iii). Studied Parameters and Statistical Analysis

During the vegetative phase, the cotton plant's height, number of leaves per plant and flowering are evaluated at regular intervals. At fruit maturity, the number of capsules per plant was noted; cotton fibers and cotton seeds yields were evaluated. Cotton seeds yield and cotton fibers yield were evaluated according to the following formula:

$Rh = (RS \times 10000)/S$ where Rh = yield/ha; RS = Yield on S area and S = area occupied by an experimental unit (ie 3.6 m^2).

All data were statistically analyzed using the «Statigraphic Plus 5.0» software. The significance of differences was determined using Duncan's test.

3. Results

3.1. Mycorrhizal Status of the Cotton Plant in the Field

(i). Endomycorrhizal Structures

Observation of the roots of *Gossypium hirsutum* L. revealed the presence of structures (hyphae and vesicles) characteristic of mycorrhizae (Figure 2), attesting to the existence of mycorrhizal symbiosis in this plant.

(ii). Colonization Rate

Figure 3 presents the colonization rate (TC) of the cotton roots of all the sites of study. The analysis of variance reveals a significant difference ($p= 0.05$) between these rates. In general, TC is higher in the Divisions of Mayo-Rey (Djaba 100%; Dogba 93%, Guibadji 87%) and Benoue (Djalingo 83%; Ouro Kessoum 77%; Ngong 60%) than that of the Division of Mayo-Louti (Guider 57%; Djabi 47%; Bidzar 40%), with a significant difference ($p<0.05$). These differences in colonization would be due to the physico-chemical properties of the soil and in particular the higher phosphorus content in the Mayo-Louti [27]. Indeed, Duponnois R. *et al.* [28] state that the higher the phosphorus content in the soil, the metabolites necessary for the initiation and formation of the plant-mycorrhizal fungus association would not be exuded in sufficient quantities following a drop in membrane permeability roots due to their high phosphorus content. The colonization rate values obtained in the present study conducted in the field are higher than the frequency of mycorrhization of the work of [29] grown in pots with regular watering. These authors reported that the frequency of mycorrhization of potted maize roots is 3.66% for the Division of Benoue, 4.33% for the Division of Mayo-Rey and 4.66% for the Division of Mayo-Louti. This would be due to water stress because [30] claim that the importance of Arbuscular Mycorrhizal Fungi (AMF) is also evidenced by water deficit which induces an increase in mycorrhizal infection.

Thus, the high rate of root colonization in the field reveals that the endomycorrhizal structures of AMFs develop better there than in pots.

The sites with the best colonization rates would also have the best growth and the best yield due to the ability of AMF spores to improve the hydromineral nutrition of the plants, but this remains to be verified.

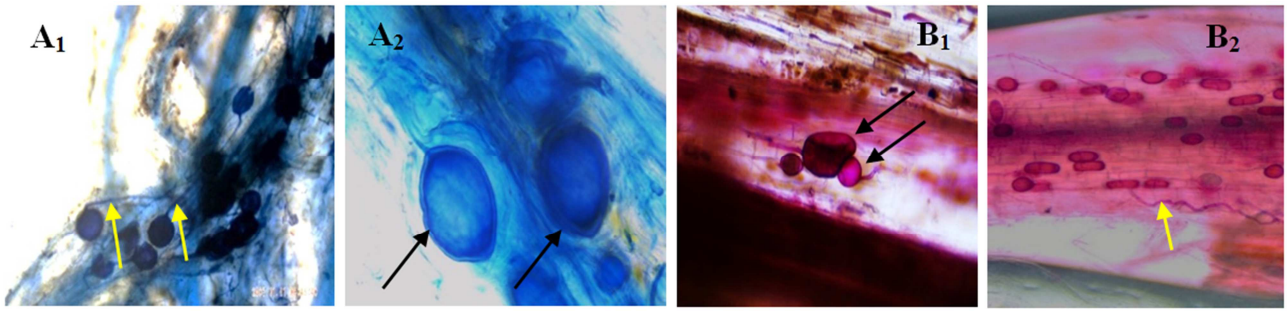


Figure 2. *Cotton plant roots stained to observe endomycorrhizal structures.*

Yellow stick: hypha; Black stick: vesicle.
(A_1 and A_2): Roots stained with methylene blue; (B_1 and B_2): Roots stained with acid fuschin

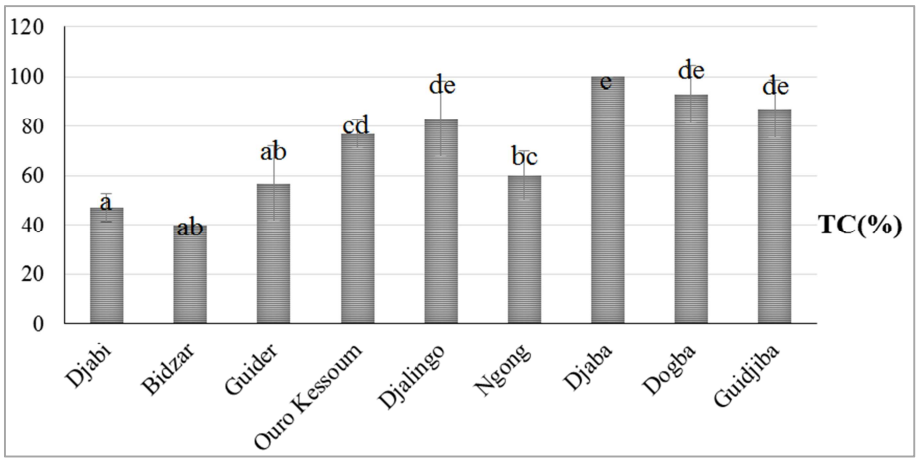


Figure 3. *Root colonization of Gossypium hirsutum L. in relation with sites of study. Values of bands affected by the same letter(s) are not significantly different according to the Duncan's test at the 5% threshold.*

3.2. Cotton Production

3.2.1. Number of Leaves Per Cotton Plant in Relation to the Site of Study, Varieties and Treatments

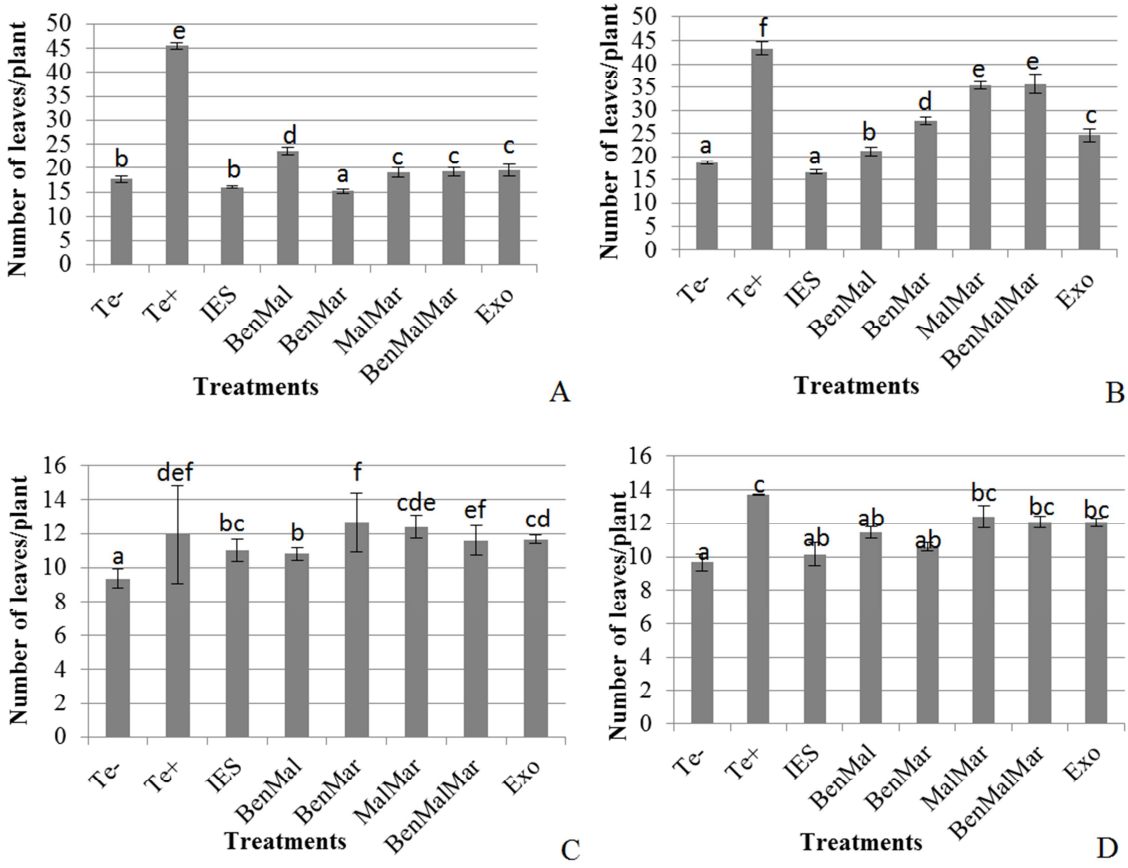
At 60 JAL the number of leaves per cotton plant for the two varieties was noted and there is globally significant differences at all sites between treatments ($p<0.05$). Regarding consortia for the Q302 variety from the Djalingo site, Benoue Division (Figure 4 A), the highest values are obtained with the composite mycorrhizal consortium BenMal (23.56±2.26 leaves/plant). For the L457 variety (Figure 4 B), the composite mycorrhizal consortia MalMar and BenMalMar have induced better leaf biomass with respectively 35.40±11.54 leaves/plant and 35.70±11.41 leaves/plant.

Figure 4 (C&D) presents the number of leaves per plant of the two varieties at Djabi site, Mayo-Louti Division. For the Q302 variety (Figure 4 C), above all the treatments, only the composite consortium MalMar (12.40±0.69 leaves) and the exogenous mycorrhizal fungi Exo (11.66±0.23 leaves) allowed a real growth of leaf biomass at 60 JAL. Regarding biofertilizers for the L457 variety (Figure 4 D), the composite mycorrhizal consortia MalMar (12.4±0.69 leaves/plant), BenMalMar (12.1±0.34 leaves/plant) and SI (12.06 ±0.23 leaves leaves/plant), induce a better leaf biomass which, however, remains very low on this site of study.

Djaba site number of leaves per plant for the two varieties, Mayo-Rey Division is presented in Figure 4 (E&F). It emerges from Figure 4 E (Q302 variety) that the composite mycorrhizal consortium BenMal (25.1±2.22 leaves/plant) and the exogenous mycorrhizal fungi Exo (23.46±0.7 leaves/plant) induced a better number of leaves per plant for this variety compared to other treatments. For the L457 variety (Figure 4 F), there is no significant difference ($p=0.5617$) between the treatments. Nevertheless, the number of leaves in composite mycorrhizal consortia and the exogenous mycorrhizal fungi Exo is higher.

In general, the composite mycorrhizal consortia BenMal, MalMar and the exogenous mycorrhizal fungi Exo have leaf biomass 1.32 to 1.92 times higher than that of the negative control for the different varieties and on the different sites. There is a better improvement by mycorrhizal consortia, which would be explained by the presence of native species of AMF spores contained therein. Indeed, these native species would be better adapted to the pedo-climatic conditions of the different study sites because [15] state that arbuscular mycorrhizal fungi improve leaf production and that the contributions of native strains induce more effects than the introduced strains.

These results corroborate those of [31], who observed that mycorrhizal inoculation in plantain produced approximately twice as much biomass as non-mycorrhizal plants. The work of [32] also reports a 1.61 times significantly higher number of leaves in date palm seedlings inoculated with AMF spores compared to non-inoculated seedlings.



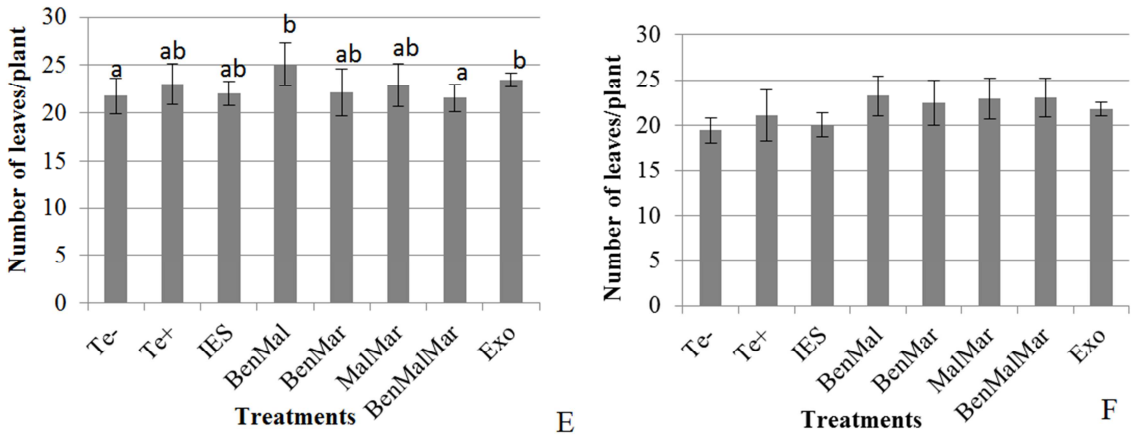


Figure 4. *Number of leaves per cotton plant at 60 JAL in relation with treatments and cotton varieties of Djalingo, Djabi and Djaba sites.*

(A; C; E) Var Q302: Q302 Variety of cotton; (B; D; F) Var L457: L457 Variety of cotton; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; Exo: exogenous mycorrhizal fungi. For each period, values of bands affected by the same letter(s) are not significantly different according to the Duncan's test at the 5% threshold.

The low number of leaves per plant at Djabi site, Mayo-Louti Division can be attributed to agricultural practices, since continuous cultivation leads to enrichment with chemical fertilizer (NPK) regularly applied in cotton-maize rotations [33], which is a recurrent practice in our study area. Several studies have shown the influence of the physico-chemical properties of soils on endomycorrhizal symbiosis [34-35]. Indeed, even if mycorrhizal fungi show hardly any host specificity, mycorrhizal populations seem adapted to given edaphic and climatic conditions [15].

Leaf biomass is not the only parameter that benefits from the efficiency of mycorrhizal symbiosis. The latter acts on other parameters such as the height of the plant.

3.2.2. Height of the Cotton Plant in Relation to the Site of Study, Varieties and Treatments

The height of the cotton plant at 60 JAL varies significantly according to each site and statistical analyzes show globally significant differences ($p<0.05$) (Figure 5). At Djalingo site, regarding biofertilizers for variety Q302 (Figure 5A), the highest values are obtained with the composite mycorrhizal consortium BenMal (53.40±2.26 cm) and the exogenous mycorrhizal fungi Exo (51.87±2.72 cm). For the L457 variety (Figure 5B), the composite mycorrhizal consortia BenMar and MalMar provide better plant height with 51.46±2.92 cm and 48.10±11.54 cm respectively.

It emerges from figure 5C (Q302 Variety for Djabi site) that the composite mycorrhizal consortium MalMar with 41.80 ± 3.46 cm induced the largest height compared to Te+ (41.60 ± 7.94 cm), at MYCO-MS SI (36.73±4.13 cm) and Te- (31.46±1.11 cm). For the L457 variety (Figure 5D), the composite mycorrhizal consortium BenMal has the best height 46.40±7.87 cm, followed by MalMar at 45.80±4.10 cm and the exogenous mycorrhizal fungi Exo at 42.60±3.50 cm, with no significant difference.

For the variety Q302 (Figure 5 E) at the Djaba site, it emerges that the composite mycorrhizal consortia BenMar (46.76±2.05 cm), BenMal (46.03±1.66 cm) and MalMar (45.06±1.28 cm) improved the height plants compared to the exogenous mycorrhizal fungi Exo (42.96±1.87cm), with no significant difference. For the L457 variety (Figure 5 F), the composite mycorrhizal consortia BenMal (47.16±0.68 cm), BenMar (46.46±3.26 cm), IES (45.36±4.13 cm), MalMar (45.53±1.53 cm) and the exogenous mycorrhizal fungi Exo (44.83±1.65 cm) had a better effect on plant height compared to Te+ (40.4±2.26 cm) and Te- (37.32 ±2.01cm) with a significant difference ($p<0.05$).

In general, except at the Djalingo site where Te+ provides the best height, the native mycorrhizal consortia improved the height of the cotton plant from 26.37 to 93.97% compared to Te-. There is a particular dominance of the endogenous mycorrhizal consortium MalMar for all varieties and on all sites compared to other treatments and particularly to the exogenous mycorrhizal fungi Exo. This would be explained by a better adaptability of native species reintroduced into their original natural habitat. Indeed, [30] observed better growth in maize plants inoculated with an endogenous population of mycorrhizal fungi compared to non-inoculated plants. In addition, [36] observed that the growth of *Leymus arenarius* plants is significantly improved by an endogenous CMA inoculum compared to monospecific commercial inocula of exogenous origin.

The results obtained in this research work are similar to those of [37], in their work on two types of plantain plant material (*Musa* AAB), indicating that the height of the plants increased respectively by 85.7% on plants from fragments mycorrhizal stem and 75.8% on mycorrhizal tissue culture plants compared to those not mycorrhizal.

The growth and development of the cotton plant are also marked by flowering, a parameter on which it would also be important to evaluate the effect of mycorrhizal inoculation.

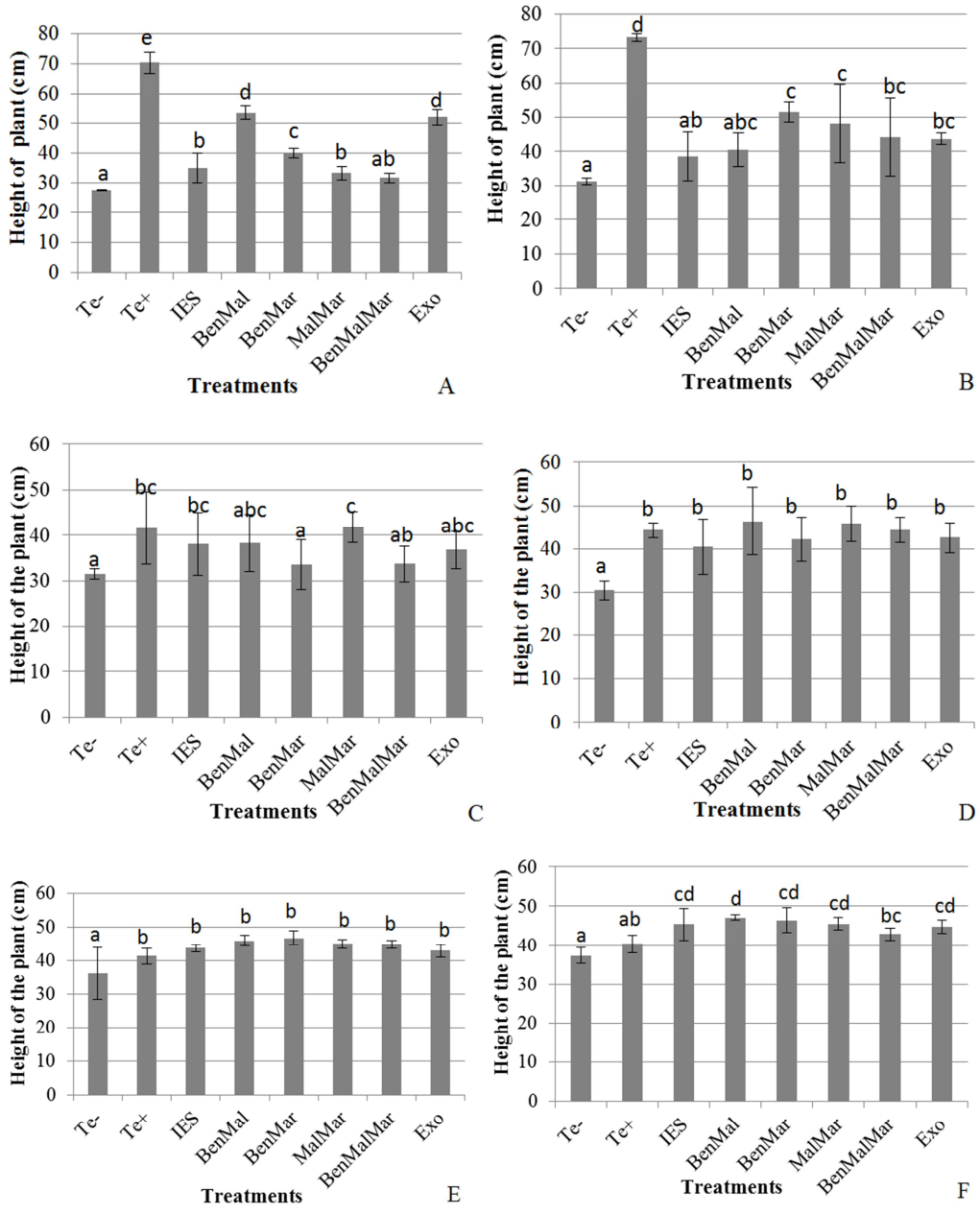


Figure 5. *Height of the cotton plant at 60 JAL depending on the treatments at the Djalingo, Djabi and Djaba sites.*

(A; C; E)Var Q302: Q302 Variety of cotton; (B; D; F)Var L457: L457 Variety of cotton; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; Exo: exogenous mycorrhizal fungi. For each period, values of bands affected by the same letter(s) are not significantly different according to the Duncan's test at the 5% threshold.

3.2.3. Cotton Flowering Dates in Relation to the Site of Study, Varieties and Treatments

The flowering at the Djalingo site is presented in Table 1. There is a significant difference between the different treatments ($p<0.05$). The best performing native mycorrhizal consortia BenMar and BenMal gave their first flowers respectively at 40.66±1.15 JAL and 41.33±1.15 JAL earlier than the exogenous mycorrhizal fungi Exo (42±0 JAL), all with variety L457, with no significant difference. In general, for the two varieties, the negative control of the L457 variety reaches 100% flowering (83.66±2.88 JAL) last, about ten days after the native mycorrhizal consortia and the exogenous mycorrhizal fungi Exo with significant difference ($p<0.05$).

Table 1. *Flowering of the two varieties of cotton in relation with treatments on Djalingo site.*

Treatments	1st Flowering (JAL)		100% of Flowering (JAL)			
	Var Q302	Var L457	Var Q302		Var L457	
			M	P (days)	M	P (days)
Te-	53,66±4,16^{d}	56,00±3,60^{c}	83,00±2,00^{d}	0	83,66±2,88^{e}	0
Te+	39,66±0,57^{a}	40,33±0,57^{a}	60,00±1,00^{a}	-23	58,33±1,15^{a}	-24
IES	46,66±0,57^{c}	45,00±2,00^{b}	72,33±2,30^{c}	-11	71,00±1,73^{d}	-12
BenMal	44,00±1,00^{bc}	41,33±1,15^{a}	64,33±2,51^{b}	-19	62,66±1,52^{b}	-21
BenMar	42,66±0,57^{ab}	40,66±1,15^{a}	67,33±2,88^{b}	-16	64,66±1,52^{bc}	-19
MalMar	42,66±2,08^{ab}	42,00±0,00^{a}	65,66±2,08^{b}	-18	63,00±2,00^{b}	-20
BenMalMar	44,00±1,00^{bc}	42,33±0,57^{ab}	72,00±0,00^{c}	-11	66,33±2,30^{c}	-17
SI	43,00±2,00^{b}	42,00±0,00^{a}	66,00±1,00^{b}	-17	63,66±1,15^{bc}	-20
P-value/Mean	0,0000	0,0000	0,0000	-16±4	0,0000	-19±4

Var Q302: Q302 Variety of cotton; Var L457: L457 Variety of cotton; M: Maturity; P: Precocity; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; SI: exogenous mycorrhizal fungi. Values followed by the same letter(s) on the same column are not significantly different according to the Duncan's test at the 5% threshold.

At the Djabi site, the effect of the different treatments on flowering indicates that there is a significant difference (p 0.05) between the different treatments (Table 2). For the two varieties, Q302 and L457, the Te- treatment produced its first flower in the last position respectively 58.00±1.00 JAL and 57.00±1.00 JAL. In general, for the two varieties, the best treatments MalMar, BenMar, and BenMal each produce their first flowers respectively 47.00 ± 0.00 JAL, 47.33 ± 0.57 JAL, 47.33 ± 1.52 JAL and 48.33±0.57 JAL. The Q302 variety reached 100% flowering before the L457 variety, by the exogenous mycorrhizal fungi Exo (86.00±1.00 JAL) and the composite consortium MalMar (88.00±1.00 JAL), with no significant difference.

Table 2. *Flowering of the two varieties of cotton in relation with treatments on Djabi site.*

Treatments	1st Flowering (JAL)		100% of Flowering (JAL)			
	Var Q302	Var L457	Var Q302		Var L457	
			M	P (days)	M	P (days)
Te-	58,00±1,00^{e}	57,00±1,00^{e}	106,00±1,00^{e}	0	105,00±2,64^{e}	0
Te+	47,00±0,00^{a}	47,00±0,57^{a}	87,00±0,00^{ab}	-19	92,00±1,52^{b}	-13
IES	50,33±0,57^{c}	49,66±0,57^{b}	88,33±1,52^{b}	-18	88,66±1,15^{a}	-17
BenMal	48,66±0,57^{abc}	48,33±0,57^{a}	89,00±1,00^{b}	-17	95,00±1,00^{c}	-10
BenMar	47,33±1,52^{ab}	50,33±0,57^{b}	97,33±2,08^{d}	-9	100,00±1,00^{d}	-5
MalMar	49,00±0,00^{bc}	47,33±0,57^{a}	88,00±1,00^{ab}	-18	89,00±1,73^{a}	-16
BenMalMar	52,66±2,08^{d}	54,00±1,00^{d}	91,66±1,52^{c}	-15	96,33±1,15^{c}	-9
Exo	52,66±1,52^{d}	52,00±1,00^{c}	86,00±1,00^{a}	-20	90,33±0,57^{ab}	-15
P-value/Mean	0,0000	0,0000	0,0000	-17±4	0,0000	-12±4

Var Q302: Q302 Variety of cotton; Var L457: L457 Variety of cotton; M: Maturity; P: Precocity; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; SI: exogenous mycorrhizal fungi. Values followed by the same letter(s) on the same column are not significantly different according to the Duncan's test at the 5% threshold.

Table 3 presents the flowering at the Djaba site. There appears to be a significant difference between the different treatments ($p<0.05$). Overall, for both varieties, the first flowers are observed in plot units treated with: the exogenous mycorrhizal fungi Exo (44.66±0.57 JAL) and the native mycorrhizal consortia BenMal (45.00±0.00 JAL); MalMar (46.00±1.00 JAL) and IES (46.33±1.52 JAL), with no significant difference. The Q302 variety reached 100% flowering at 68.00±3.00 JAL and at 69.00±1.00 JAL respectively with the composite consortia BenMal and BenMar. The L457 variety reaches 100% flowering 5 to 6 days later with the same consortia and in the same order. In general, the exogenous mycorrhizal fungi Exo and the Te+ treatment reach 100% flowering at least six (06) days after the composite mycorrhizal consortia BenMal and BenMar, with a significant difference ($p<0.05$). This early flowering of inoculated plants would be due, in general, to the activity of AMF and in particular to that of native species, because [38] reported that inoculated plants promote early flowering. Furthermore, [39] state that adding AMF to plants can reduce the sowing-flowering date. [40], likewise showed that AMF allowed a gain in flower growth in groundnut of 90% for inoculated plants compared to plants in the control soil, in Algeria.

These results partially corroborate those of [41] on the effect of AMF inoculation on flower growth and quality in *Chrysantheme morifolium* Ramat, who showed that inoculated plants completed flowering between 98 and 104 days compared to uninoculated plants which were completed after 112 days, a time saving of 8 to 14 days. In addition, the

work of [42] on *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* revealed that inoculated plants flowered fifteen (15) days before non-inoculated plants.

Subsequently, these flowers fall, then begins the appearance of the capsules.

Table 3. *Flowering of the two varieties of cotton in relation with treatments on Djaba site.*

Treatments	1st flowering (JAL)		100% of Flowering (JAL)			
	Var Q302	Var L457	Var Q302		Var L457	
			M	P (days)	M	P (days)
Te-	49,66±1,15^{c}	49,00±0,00^{d}	94,00±1,73^{e}	0	96,00±1,00^{d}	0
Te+	47,66±0,57^{bc}	47,33±0,57^{bc}	79,33±2,51^{cd}	-15	81,00±2,00^{b}	-15
IES	46,33±1,52^{ab}	47,00±1,00^{bc}	81,00±4,00^{d}	-13	86,00±3,00^{c}	-10
BenMal	45,00±0,00^{a}	45,00±0,00^{a}	68,00±3,00^{a}	-26	73,00±4,00^{a}	-23
BenMar	48,33±0,57^{bc}	47,33±1,52^{bc}	69,00±1,00^{a}	-25	75,00±2,00^{a}	-21
MalMar	46,66±1,52^{ab}	46,00±1,00^{ab}	75,00±2,00^{b}	-19	79,00±2,00^{b}	-17
BenMalMar	48,33±2,3^{bc}	48,33±1,52^{cd}	78,00±1,00^{bcd}	-16	81,00±1,00^{b}	-15
Exo	45,00±1,00^{a}	44,66±0,57^{a}	76,66±0,57^{bc}	-18	79,00±0,00^{b}	-17
P-value/Mean	0,0032	0,0004	0,0000	-19±5	0,0000	-17±4

Var Q302: Q302 Variety of cotton; Var L457: L457 Variety of cotton; M: Maturity; P: Precocity; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with CONQUEST C 176 EC; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; SI: exogenous mycorrhizal fungi. Values followed by the same letter(s) on the same column are not significantly different according to the Duncan's test at the 5% threshold.

3.2.4. Number of Capsules Per Plant

Globally, there was not a significant difference between fertilizers used relative to cotton capsules production in our area of study. However, negative control exhibited significantly (p 0.05) the lowest cotton capsules production (Figure 6).

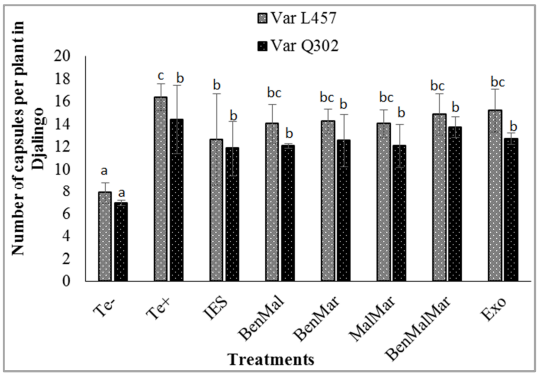
The number of capsules per plant of L457 cotton variety of Te-, Te+, IES, BenMal, BenMar, MalMar, BenMalMar and Exo treatments were 6.97±1.90, 13.49±5.84, 9.89±3.18, 11.61±3.47, 11.03±3.85, 11.54±3.70, 11.62±4.77 and 11.66±4.17 respectively. Between biofertilizer used in the current study, Exo exhibited not significantly the highest capsules production of L457 cotton variety and it increased this production parameter nearly twice (1.67) higher than the negative control with significant difference.

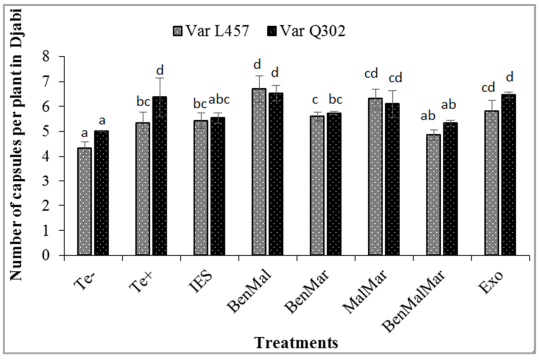
The number of capsules per plant of Q302 cotton variety from Te-, Te+, IES, BenMal, BenMar, MalMar, BenMalMar and Exo treatments were 7.39±2.17, 13.57±6.42, 11.46±4.70, 12.17±4.65, 12.15±5.09, 13.23±6.35, 12.22±5.12 and 11.52±3.74 respectively. Between biofertilizer used in this work, MalMar exhibited not significantly the highest capsules production of Q302 cotton variety and it increased this parameter nearly twice (1.79) higher than the negative control with a significant difference.

Relative to the negative control, the sites of study affected significantly (p 0.05) the number of capsules per plant. 7.38±0.45, 4.66±0.33 and 9.51±0.75 were the number of capsules per plant from the Djalingo, Djabi and Djaba sites respectively. Djaba site exhibited the highest capsule production. The number of capsules per cotton plant from the Djaba site was 1.29 and 2.04 folds greater than that from Djalingo and Djabi sites respectively.

Results obtained in the current work on cotton capsule production corroborate data found in the literature. Indeed, [43] studied the effects of four strains of Arbuscular Mycorrhizae Fungi on soybean growth and revealed that the endogenous strain of mycorrhizal fungi increased at 2.26 folds the soybean pods production compared to the negative control.

According to several authors, there is a positive and significant correlation between cotton capsule production and cotton seed yield, and between cotton capsule production and cotton, fibers yield. In this regard, we expected the greatest cotton seeds yield and cotton fibers yield from BenMalMar or MalMar biofertilizer. Also, we expected the highest cotton seed and fiber yields from the Djaba site. But these need to be investigated.





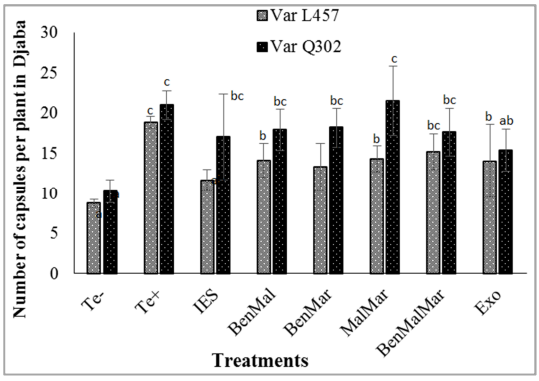


Figure 6. *Number of capsules per cotton plant in relation with treatments and cotton varieties of Djalingo, Djabi and Djaba sites.*

Var L457: L457 Variety of cotton; Var Q302: Q302 Variety of cotton; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with tropfort; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; Exo: exogenous mycorrhizal fungi. For a cotton variety, values of bands affected by the same letter are not significantly different according to the Duncan's test at the 5% threshold.

3.2.5. Seed and Fiber Yields

Globally, there was not a significant difference between biofertilizers used relative to cotton capsule production (seeds and fiber yields) in our study area. However, negative control exhibited significantly ($p<0.05$) the lowest cotton capsules production (Tables 4, 5 and 6). Also, studied production parameters varied significantly ($p<0.01$) between the sites. Cotton variety affected not significantly studied production parameters.

Seeds yield of L457 cotton variety of Te-, Te+, IES, BenMal, BenMar, MalMar, BenMalMar and Exo treatments were 39.39 ± 11.15, 158.27 ± 91.02, 84.39 ± 40.70, 118.80 ± 30.31, 101.34 ± 46.50, 102.59 ± 32.21, 112.48 ± 61.54 and 115.59 ± 59.98 kg/ha respectively. Between the biofertilizer used in the current study, BenMal exhibited not significantly the highest seeds yield of L457 cotton variety and it increased this production parameter at 201.60% relative to the negative control with significant difference ($p<0.05$).

Seeds yield of Q302 cotton variety of Te-, Te+, IES, BenMal, BenMar, MalMar, BenMalMar and Exo treatments were 38.98 ± 12.26, 128.53.57 ± 58.19, 79.26 ± 23.91, 86.52 ± 36.06, 93.08 ± 36.04, 116.04 ± 41.56, 95.38 ± 27.96 and 72.30 ± 19.76 Kg/ha.respectively. Between the biofertilizers used in this work, MalMar exhibited not significantly the highest seeds yield of Q302 cotton variety and it increased this parameter at 197.72% relative to negative control plot with significant difference (p 0.05).

Relative to the negative control plots, the sites of study affected significantly ($p<0.01$) the cotton seeds yield. 33.26 ± 1.96, 28.84 ± 0.54 and 55.47 ± 0.81 Kg/ha were the cotton seeds yield from Djalingo, Djabi and Djaba sites. The Djaba site exhibited significantly the highest seeds yield. The cotton seeds yield from Djaba site were 1.67 and 1.92 folds greater than that from Djalingo and Djabi sites respectively.

Between the biofertilizers used, the highest value (91.47 ± 27.14 Kg/ha) of fiber yield of L457 cotton variety were from BenMal generally with significant difference ($p<0.05$), IES treatment while exhibiting the lowest value (68.36 ± 36.49 Kg/ha). Fibers yield from IES plot was 2.16 folds greater than that from negative control plot (31.57±11,65 Kg/ha). Fibers yield of Q302 cotton variety varied from 61.10 ± 22.61 Kg/ha for exogenous biofertilizer to 85.13 ± 45.60 Kg/ha for MalMar treatment. Cotton fibers yield from treated plants by MalMar was 2.78 folds greater than from the negative control plot (30.57 ± 13.93 Kg/ha) with significant difference ($p<0.05$).

Cotton fiber yield concerning negative control plots from the Djalingo, Djabi and Djaba sites were 24.77 ± 1.51, 22.65 ± 1.75 and 45.80 ± 1.15 Kg/ha affected by significant difference ($p<0.01$). The highest cotton fibers yield was from the Djaba site while the lowest was from the Djabi site. Cotton fibers yield from the Djaba site were 1.85 and 2.02 fold greater than that from Djalingo and Djabi respectively.

The results obtained in this seed cotton yield study are almost similar to those obtained by [44] on the response to mycorrhizal inoculation of some old varieties of wheat in France. This author observed values at least 2 times higher for inoculated plants compared to uninoculated plants for seed / plant weight. In addition, the results obtained by [13] on the response of two cotton varieties to exogenous mycorrhizal fungi inoculation in the Sudano-Sahelian zone of Cameroon are less than those of the present study with a cotton-lint yield in inoculated plants 1.36 times greater than the yield in cotton-fiber at the negative control.

Mycorrhizal inoculation and in particular the composite endogenous inocula MalMar, BenMal and BenMalMar significantly improved overall yield of different varieties and at all sites study. This is not surprising, because in this mutually beneficial combination, the benefits of the plants can be characterized agronomically by increased growth and better yield [23]. This is self-evident, given that AMFs are likely to confer better growth on their hosts thanks to improved hydromineral nutrition, particularly phosphate nutrition, and reduced parasite pressure [45-11, 29]. In addition, [15] have shown that the use of endogenous mixed strains would be more suitable than the reference monospecific AMF strains.

Table 4. *Cotton yield in Djalingo site in relation with treatments and cotton varieties.*

Treatments	L457 cotton variety		Q302 cotton variety	
	SY (Kg/ha)	FY (Kg/ha)	SY (Kg/ha)	FY (Kg/ha)
Te-	35.22±5.12^{a}	25.84±4.01^{a}	31.29±6.90	23.70±9.28
Te+	245.60±22.17^{c}	167.18±15.17^{b}	91.21±47.04	68.41±37.62
IES	137.83±79.72^{b}	104.55±62.59^{b}	57.64±58.52	43.08±43.58

Treatments	L457 cotton variety		Q302 cotton variety	
	SY (Kg/ha)	FY (Kg/ha)	SY (Kg/ha)	FY (Kg/ha)
BenMal	160.82±81.24^{bc}	121.14±60.16^{b}	51.76±17.39	39.37±12.50
BenMar	155.84±47.48^{bc}	114.22±38.19^{b}	76.49±42.66	54.47±35.44
MalMar	145.98±48.36^{b}	110.56±34.69^{b}	100.52±72.35	39.09±21.16
BenMalMar	189.39±77.16^{bc}	138.32±54.24^{b}	99.50±5.19	67.11±13.89
Exo	197.53±27.86^{bc}	147.24±17.75^{b}	64.28±17.23	50.98±16.38
P-value	0.0164	0.0288	NS	NS

Table 5. *Cotton yield in Djabi site in relation with treatments and cotton varieties.*

Treatments	L457 cotton variety		Q302 cotton variety	
	SY (Kg/ha)	FY (Kg/ha)	SY (Kg/ha)	FY (Kg/ha)
Te-	28.30±1.08^{a}	23.89±3.64^{a}	29.38±7.81^{a}	21.41±6.46^{a}
Te+	32.70±10.05^{a}	24.52±9.18^{a}	83.68±19.55^{b}	65.17±15.93^{b}
IES	39.15±9.81^{a}	31.56±8.17^{a}	67.56±15.27^{ab}	50.78±11.51^{ab}
BenMal	90.42±5.75^{d}	67.90±2.47^{d}	71.59±36.89^{b}	52.62±27.54^{ab}
BenMar	42.20±9.53^{ab}	33.85±4.09^{ab}	59.63±12.41^{ab}	46.16±9.28^{ab}
MalMar	68.73±13.45^{c}	55.31±10.86^{c}	74.71±33.45^{b}	69.05±40.06^{b}
BenMalMar	38.73±10.81^{a}	29.93±6.73^{a}	59.27±24.21^{ab}	45.52±27.54^{ab}
Exo	55.60±6.25^{bc}	42.45±6.65^{b}	53.12±3.93^{ab}	39.89±3.83^{ab}
P-value	0.0000	0.0000	0.045	0.048

SY: cotton seeds yield; FY: cotton fibers yield; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with tropfort; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; Exo: exogenous mycorrhizal fungi. Values followed by the same letter(s) on the same column are not significantly different according to the Duncan's test at the 5% threshold.

Table 6. *Cotton yield in Djaba site in relation with treatments and cotton varieties.*

Treatments	L457 cotton variety		Q302 cotton variety	
	SY (Kg/ha)	FY (Kg/ha)	SY (Kg/ha)	FY (Kg/ha)
Te-	54.66±12.36^{a}	44.98±9.08^{a}	56.29±12.11^{a}	46.61±8.41^{a}
Te+	196.53±17.53^{d}	176.81±6.00^{d}	210.72±10.77^{f}	189.64±9.08^{g}
IES	76.20±12.01^{ab}	68.97±13.48^{b}	112.60±8.57^{bc}	102.14±14.10^{bc}
BenMal	105.15±10.76^{c}	85.37±16.03^{bc}	136.22±6.19^{d}	125.71±6.25^{de}
BenMar	105.99±21.81^{c}	92.48±7.98^{c}	143.12±11.12^{d}	131.89±4.01^{e}
MalMar	93.38±13.76^{bc}	80.52±13.04^{bc}	172.91±7.81^{e}	147.27±7.13^{f}
BenMalMar	109.33±8.56^{c}	90.64±5.94^{c}	127.39±7.55^{cd}	111.18±5.09^{cd}
Exo	93.66±17.87^{bc}	76.90±16.63^{bc}	99.50±9.12^{b}	92.44±9.66^{b}
P-value	0.0000	0.0000	0.0000	0.0000

SY: cotton seeds yield; FY: cotton fibers yield; Te-: Unfertilized plot (negative control); Te+: chemical inputs: combination chemical fertilizer with tropfort; IES: Endogenous mycorrhizal fungi of study site; BenMal: combination of mycorhizal fungi from Benoue and Mayo-Louti; BenMar: combination of mycorrhizal fungi from Benoue and Mayo-Rey; MalMar: combination of mycorrhizal fungi from Mayo-Louti and Mayo-Rey; BenMalMar: combination of mycorrhizal fungi from Benoue, Mayo-Louti and Mayo-Rey; Exo: exogenous mycorrhizal fungi. Values followed by the same letter(s) on the same column are not significantly different according to the Duncan's test at the 5% threshold.

4. Conclusion

The aim of this study was to help improve cotton productivity in Northern Cameroon while limiting the use of chemical inputs. Cotton plant growth varied according to fertilizer and study site. There was no significant difference between cotton varieties L457 and Q302 in terms of seed and fibre yield. Endogenous mycorrhizal strains from the Sudano-Saharan savannahs of northern Cameroon improve the growth, seed and fiber yields of cotton plants. The Djaba study site is more favorable to cotton growth.

The endogenous strains of mycorrhizal fungi used in the present work improve cotton productivity better than exogenous strains of this biofertilizer, suggesting that by producing and applying endogenous mycorrhizal fungi from the Sudano-Sahelian savannahs of Northern Cameroon for cotton growth, we are not only helping to increase cotton growth, but also limiting the use of chemical inputs and the importation of biofertilizers, as well as ensuring sustainable agriculture.

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All authors contributed equally to the conception and design of the study.

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Conflicts of Interest

The authors declare no conflicts of interest.

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