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Evaluation of the effect of arbuscular mycorrhizal fungi on the growth of tomato (*Lycopersicum esculentum* Mill.) grown in Bangui (Central African Republic) under controlled conditions

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Abstract
Valuing mycorrhizae is a potential alternative to the use of fertilizers and phytosanitary products. This study aimed at evaluating the effect of Arbuscular Mycorrhizal Fungi (AMF) on the growth of tomato (*Lycopersicum esculentum* Mill.). The trial was conducted in a greenhouse for 4 months. Inoculation of tomato with the four AMF strains was compared to the control (non-inoculated plant) in a simple randomized design. Height growth of inoculated tomato plants varied among treatments with different strains of AMF. Glomus intraradices resulted in high stem growth (59.12±2.12 cm). The effect of AMF inoculation was significant on the growth of neck diameter of tomato plants. The Glomus fasciculatum strain showed a larger value (13.66±0.82 mm) followed by Glomus mosseae; (12.46±0.20 mm) and Glomus intraradices (11.99±0.42 mm) in diameter. The number of leaves produced per plant differed significantly between plants inoculated with the different strains of AMF and those not inoculated. The average number of leaves (32.2 and 30 leaves/plant) respectively with the plants inoculated with Glomus mosseae and Glomus intraradices. The best average number of branches (4.33±1.25) was obtained with the Glomus fasciculatum strain. The highest values were obtained with the Glomus aggregatum strain (149.57 g) for total fresh biomass and 36.56 g for total dry biomass.

Keywords: AMF, organic agriculture, tomato, Bangui, Central African Republic

Introduction

Today, conventional agriculture no longer inspires consumer confidence, largely due to various scandals related to the use of pesticides in agriculture (Philipp *et al.*, 2019) [24]. In the Central African Republic (CAR), agriculture is practiced by more than 80% of the rural, urban and peri-urban population. In this agriculture, market gardening occupies an important place 23% in the Central African economy. Among the vegetable crops: tomato, cucumber, eggplant, cabbage, amaranth, okra, carrot, spinach, etc. Tomato (*Lycopersicon esculentum* Mill.) is one of the most important crops from an economic and nutritional point of view. It is one of the most consumed vegetables in the world (FAO, 2012) [13]. It is considered the main crop capable of influencing the future of many farmers in the world (Peet *et al.*, 2004) [25]. Despite the importance of tomato in the country, its yield (0.5 t/ha) remains very low compared to the world's leading producer 56 t/ha and African 39 t/ha. One of the causes of this low yield is the poverty of the soil in organic and mineral matter and the intensification of production (Lompo *et al.*, 2009) [21]. Faced with this situation, market gardening must move towards sustainable and more productive cropping systems. The integrated soil fertility management (ISFM) approach is a solution to this decline in soil fertility. According to Bationo *et al* (2012) [4], it allows for the sustainability of production systems and can guarantee better product competitiveness. This approach can be carried out through the use of bio fertilizers such as arbuscular mycorrhizal fungi (AMF). The use of bio- fertilizers contributes to the balance of ecosystems, guarantees the environment and human and animal health, admitting that the products of the market gardening are consumed raw without minimum hygiene. They do not leave any pest in the soil and can be used on all crops including vegetables and fruit trees.

The AMF live in symbiosis with the roots of plants, they provide the plant with water and mineral salt and also allow the fixation of atmospheric nitrogen, in return the plant provides the AMF with sugar, product of photosynthesis (Bationo *et al*., 2012) [4]. Tomato is particularly susceptible to inoculation by AMF (Edathil *et al*., 1996) [11]. Its growth and quality are positively affected by this symbiosis; whether under salinity and drought stress (Al Karaki, 2000; Subramanian *et al*., 2006) [1, 29], interaction with pathogens (Fakhro *et al*., 2010; Steinkellner *et al*., 2012) [28], or in combination with NPK fertilizer or different P levels (Poulton *et al*., 2002; Mujica Perez *et al*., 2010; Conversa *et al*., 2013, Ziane, 2018) [23, 7]. In CAR, the application of AMF in agriculture is non-existent and little known. It is limited to few studies that have shown the beneficial potential of inoculation in certain crops. Therefore, the objective of this work is to evaluate the comparative effects of arbuscular mycorrhizal fungi (AMF) on the growth of tomato grown under controlled conditions.

Material and Methods

Plant Material: The plant material used for the experimentation was a tomato seed of variety Roma VF, a F1 generation with 97% germination power.

Fungal material: The fungal material was composed of inocula of AMF strains and a mycorrhized root obtained from the Laboratory of Fungi Biotechnology of the Check Anta Diop University of Dakar (Senegal). These are the following strains: G1: Glomus mosseae, (Nicholson, Gerd, Trappe DAOM 227 131); G2: Glomus aggregatum (Schenke, Smith, Emend, Koske) (DAOM 227 128); G3: Glomus fasciculatum (Thaxter, Sensu, Gere-mann, gerd) (DAOM 227 130); G4: Glomus intraradices (Schenker, Smith) (DAOM 127 198).

Growing medium: The culture substrate was a sterilized soil (1 h at 120°C) from Boukoko whose physico- chemical characteristics were recorded in Table 2 below.

Table 1: Physico-chemical characteristics of the soil of Mbaïki

Component elements	Content (100 g of sand)
Fine sand Medium sand	36.02% 44.18
Coarse sand	15.10%
Silt	0.25%
Lilt	1.19%
Clay	3.25%
Organic material	0,232%
Total Carbon	0,134%
Total nitrogen	0,014%
C/N ratio	10
Assimilable phosphorus Potassium	53,76 ppm 0.011 meq/100 g
pH (p/v: 1/2,5)	6,9

Source: Djamndo *et al*. (2021) [10].

Methods

Setting up the trial: The trial was conducted in a greenhouse for 4 months in the experimental field of the liaison office of the Higher Institute of Rural Development Rural (HURD) of Mbaïki located in the Faculty of Health Sciences (FACSS) of the University of Bangui (altitude: 436m, latitude: 4° 22'38" N and longitude: 18°33'37" E). The plants were grown under the following conditions: average temperature 30/ 25.9 ± 2°C and relative humidity above 50%. (Figure 1). The experimental setup used was the one described by Haro and Sanon (2020) [15] modified. The experimental set-up used was a simple randomized design. This setup consisted of 4 treatments [four inoculated treatments (G1, G2, G3, and G4mix) and one non-inoculated control)] and each treatment was repeated 4 times.

Setting up a nursery: The nursery was planted in the open ground on a 5 m long and 1 m wide bed, i.e. an area of 5m². The ground was amended with a wheelbarrow of sawdust associated with 15 kg of manure. Three days before sowing, the soil was regularly watered, the soil was loosened, a furrow of 1cm deep was traced spaced between them of 10 cm, traced in the direction of the length of the board. The seeds were placed in the furrow by small pinches at a rate of 5g/m² then the soil was mulched to keep the soil moist. The straw was removed after 4 days of sowing. The soil preparation and maintenance work was done manually.

Transplanting of tomato plants: The plants were transplanted 4 weeks after sowing in the nursery, when the plants reached an average height of 24 cm and an average diameter of 5 mm (pencil size). The nursery bed was copiously watered before the transplanting operation. The roots of the plants removed from nurseries were washed with tap water and dressed. The plants were transplanted bare-rooted in individual holes dug in each of the pots, sunk to the level of the collar. At the end of the operation, the plants were sufficiently watered to promote recovery. Maintenance was done every day until harvest.

Inoculation of tomato plants: The inoculation was done on the same day as the transplanting of the tomato seedlings. Each plant received 10g of inoculum. The inoculum was buried in the growing medium at a depth of 2 to 3 cm in contact with the root system of the tomato plant. The mycorrhizal inoculum was composed of a mixture of spores, fungal propagules, fragments of mycorrhizal roots and soil.

Parameter measurements: To evaluate the effect of AMF on tomato growth, height, crown diameter, average number of leaves, average number of branches were measured and counted every 30 DAT. Above-ground and root biomass were measured at 90 days. The experimental design used was totally randomized with two factors: inoculum and variety. The inoculum factor included uninoculated controls and plants inoculated with G1, G2, G3 and G4. The variety factor had only one level: variety 1.

Height measurements: Plant height measurements were taken in each experimental unit at 30, 60 and 90 days after transplanting (DAT) using a tape measure.

Measurement of crown diameter: Neck diameter was measured using an electronic caliper at the separation zone between the root system and the aerial part at 30, 60 and 90 days after transplanting.

Number of leaves: The number of leaves (NL) was counted on each plant of the experimental units every 30, 60 and 90 days after transplanting, the leaves of the main stem as well as the leaves of the branching stem were all counted.

Number of branches: The number of branching (NB), on each plant, the number of branching, primary, secondary, tertiary every 30, 60 and 90 days after transplanting was counted.

Measurement of aerial, root and total biomass: The method used for the measurement of aerial, root and total biomass was that described by Haro *et al*. (2020) [15] modified. At 90 days after transplanting, each seedling was carefully stripped to recover the aerial part and all roots of the seedlings. All these parts were oven-dried at 70 °C for 72 hours until a constant weight was obtained for measurement of above-ground, root, and total biomass. After the biomass measurement, the roots were used for the study of mycorrhizal infection.

Statistical analysis: All data obtained were analyzed by ANOVA using SPSS 20 software for Windows. Tukey's test was used to determine any significant difference between the different varieties at the threshold of $p<0.05$. Results were expressed as means ± standard deviation. All experiments were performed in four replicates.

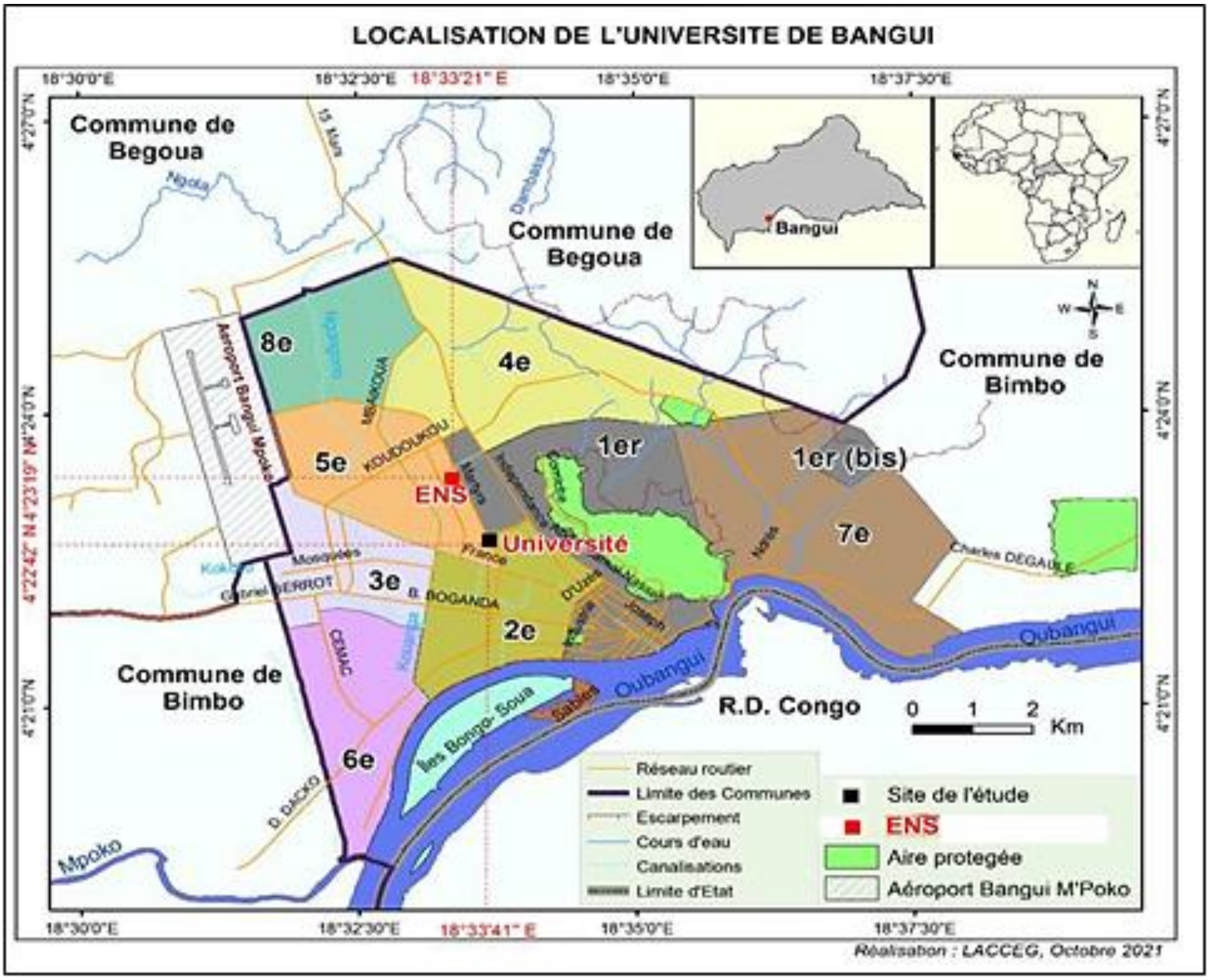

Fig 1: Map of the study area (University of Bangui) (LACCEG, 2021)

Results

Effect of inoculation on height growth of tomato plants

Height growth of inoculated tomato plants varied among treatments by strains of mycorrhizal fungi used (Figure 2). Glomus intraradices (G4) resulted in high stem growth (59.12±2.12 cm). The other strains on the other hand showed significantly higher height growth compared to non-inoculated plants (Figure 2).

Effect of AMF inoculation on neck diameter of tomato plants

Table 2 shows the values of neck diameter at harvest of the plants of the different at 90 DAT. The results showed that the effect of AMF inoculation was significant on the growth of neck diameter of tomato plants. The Glomus fasciculatum strain showed higher value (13.66±0.82 mm) followed by Glomus mosseae; (12.46±0.20 mm) and Glomus intraradices (11.99±0.42 mm) strains. It was noted that all the plants inoculated with the different strains showed a significant increase compared to the uninoculated plants (controls).

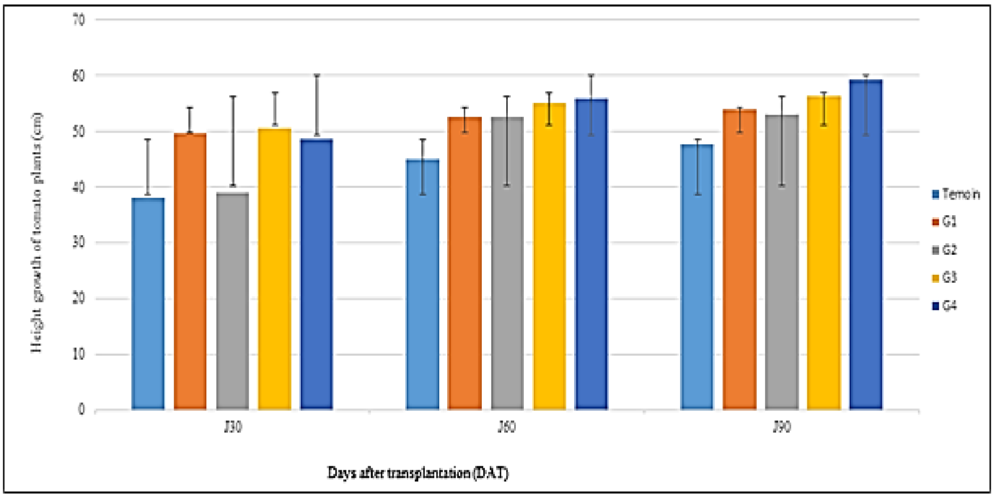

Fig 2: Effect of mycorrhizal inoculation on height growth of tomato plants inoculated with four strains of AMF. Control = not inoculated; **G1:** Glomus mosseae; **G2:** Glomus aggregatum; **G3:** Glomus fasciculatum; **G4:** Glomus intraradices. D30 =30 DAT; D60 = 60 DAT; D90 = 90 DAT. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

Table 2: Effect of mycorrhizal inoculation on crown diameter of tomato plants with four strains of arbuscular mycorrhizal fungi.

Treatments	**Average diameter at harvest of the plants (mm)**		
	D30	**D60**	**D90**
Control	5,50±1,16d	7,21±1,36d	7,52±2,12d
G1	7,52±1,06c	11,92±2,08b	12,46±0,20b
G2	8,07±1,58b	9,11±1,59c	9,78±1,25c
G3	9,46±0,41a	12,89±1,89a	13,66±0,82a
G4	8,20±0,86b	11,60±1,81b	11,99±0,42b

Control = not inoculated; **G1:** Glomus mosseae; **G2:** Glomus aggregatum; **G3:** Glomus fasciculatum; **G4:** Glomus intraradices. D30= 30 DAT; D60= 60 DAT; D90= 90 DAT. For the same column, values that share the same letter are not significantly different according to Tukey's test at the 5% threshold.

Effect of inoculation on the average number of leaves of tomato plants

The number of leaves produced per plant differed significantly between plants inoculated with the different strains of AMF and those not inoculated. It was recorded that plants inoculated with Glomus aggregatum and Glomus fasciculatum produced a higher average number of leaves (32.2 and 30 leaves/plant) than plants inoculated with Glomus mosseae, Glomus intraradices and the control, respectively (Figure 4).

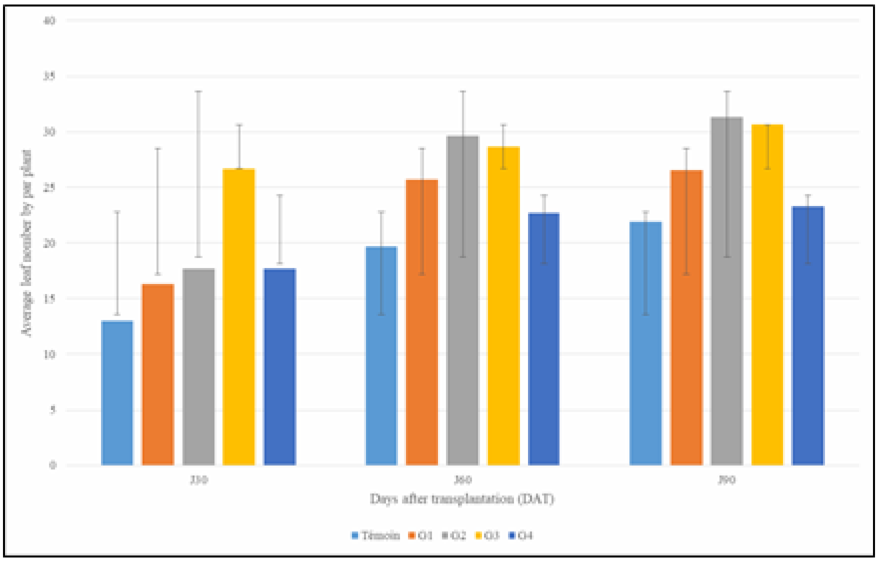

Fig 3: Effect of mycorrhizal inoculation on the average leaf number of tomato plants inoculated with four strains of AMF. Control = not inoculated; **G1:** Glomus mosseae; **G2:** Glomus aggregatum; **G3:** Glomus fasciculatum; **G4:** Glomus intraradices. D30= 30 DAT; D60= 60 DAT; D90= 90 DAT. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

Effect of inoculation on the average number of branching of tomato plants

The average number of branching varied among the different treatments. Statistical analyses showed significant differences between the different treatments. The best average number of branching (4.33±1.25) was obtained with the Glomus fasciculatum (G3) strain at 90 DAR. The other treatments gave lower results (Table 3).

Table 3: Effect of mycorrhizal inoculation on the average number of branching of tomato plants with four strains of arbuscular mycorrhizal fungi.

Treatments	Average number of branching		
	D30	**D60**	**D90**
Control	1,00±1,00d	2,33±1,52c	1,33±1,33d
G1	4,33±0,57b	3,00±2,64b	2,00±0,00c
G2	3,00±3,6c	4,00±1,60a	3,00±3,60b
G3	7,00±1,00a	4,83±2,08a	4,33±1,28a
G4	3,33±3,05c	3,33±1,00b	3,00±1,52b

Control = not inoculated; **G1:** Glomus mosseae; **G2:** Glomus aggregatum; **G3:** Glomus fasciculatum; **G4:** Glomus intraradices. D30= 30 DAT; D60= 60 DAT; D90= 90 DAT. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

Evaluation of aerial, root and total biomass: The results on the fresh or dry biomass areal, root and total showed variability according to the different treatments (Table 4). The results showed significant differences between the different treatments. The highest values were obtained with the Glomus aggregatum (G2) strain (149.57 g for total fresh biomass and 36.56 g for total dry biomass). It is noted that the Glomus intraradices strain (G4) presented a low total biomass (19.62 g) compared to the other treatments (Table 4).

Table 4: Effect of mycorrhizal inoculation on root, areal and total fresh and dry biomass of tomato plants at 90 DAR inoculated with four strains of CMA.

Treatments	Root biomass (g)		Areal biomass (g)		Total biomass (g)	
	Fresh	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**
Control	15,58c	4,448b	85,41c	17,9d	100,99d	22,34c
G1	18,818b	4,13b	114,99b	25,11b	133,81b	29,2b
G2	26,21a	6,71a	123,36a	29,84a	149,57a	36,56a
G3	16,548c	3,09c	96,70b	20,62c	113,25c	23,72c
G4	9,76d	2,16d	95,67b	17,45d	105,44d	19,62d

Control = not inoculated; **G1:** Glomus mosseae; **G2:** Glomus aggregatum; **G3:** Glomus fasciculatum; **G4:** Glomus intraradices. D30= 30 DAT; D60= 60 DAT; D90= 90 DAT. For the same column, values that share the same letter are not significantly different according to the Tukey test at the 5% threshold.

Discussion

The evaluation of the effects of arbuscular mycorrhizal fungi on the growth and development of tomato was carried out using the five quantitative parameters: height, diameter, average number of leaves, average number of branches and biomass. From the present comparison, it was found that there is variability among the different parameters to distinguish the effects of the strains on the tomato plant.

Tomato plant growth throughout the vegetative cycle is an intrinsic characteristic of the plant species. The tomato plant was sensitive to the effect of Glomus intraradices, it marked a positive effect on height growth and diameter at the crown of the plant. Inoculation with AMF significantly improved plant height and crown diameter. This is in agreement with the results of Chen *et al*, (2013) [6]. On cucumber, Baslam *et al*, (2013b) [3] on lettuce, Kaya *et al*, (2009) [17] on bell pepper, Copetta *et al*, (2011) and Ziane (2018) [8, 28] on tomato and M'pika *et al*, (2015) [20]. Who reported a significant effect on diameter growth and fruit yield of three tomato varieties (Roma, Mongol and Locale de Senegal) with Glomus strains.

Inoculation significantly increased the number of leaves in inoculated plants compared to non-inoculated plants. This increase was much more observed in Glomus aggregatum and Glomus afasciculatum which had 36 and 34 leaves/plant, respectively. These results are similar to the results of several authors including Daft and El-Giahmi (1976), Parvathi *et al*. (1985) [9, 22]. Who reported that inoculation of plants with CMA resulted in improved nutrition and a more significant increase in leaf number.

The number of branching was significantly higher in inoculated plants compared to non-inoculated plants, as the number of branching increases, more branches will bear fruits which is in agreement with the results of Caris *et al*. (2009) [5] On sesame. In Senegal, Glomus aggregatum increases the number of branches and buds that produce flowers and fruits (Bâ *et al*., 2001) [2]. However, tomato cultivation with Glomus aggregatum can be encouraged because it can increase its fruit production.

The biomass obtained with Glomus aggregatum is significantly different from other inoculated and non-inoculated plants. These results are lower than those of Kitabala *et al*. (2015) in Congo. These results are also contradictory to those of Cornel (2009) who reported that Glomus aggregatum inoculum significantly increased above-ground and root biomass. Similarly, Haro *et al*. (2015) [17]. Showed that inoculation of cowpea plants with the genus Glomus significantly improved growth and total biomass.

Conclusion

One of the challenges that the CAR and the rest of the world will have to face is to increase agricultural yields in order to satisfy the ever increasing food demand of the population, while lowering the dependence on chemical fertilizers. The objective of this work was to evaluate the comparative effects of arbuscular mycorrhizal fungi (AMF) on the growth of tomato grown under controlled conditions. Four strains of Glomus (Glomus mosseae, Glomus aggregatum, Glomus fasciculatum, Glomus intraradices) were tested on a tomato variety. The results showed that in terms of fertilizer value, AMF are more efficient in fixing atmospheric nitrogen (nitrogen element of plant growth) and provide the plant with water and mineral salt necessary for photosynthetic mechanisms. This work did not evaluate the chemical fertility of the soil after the experiment. It would have allowed a better understanding of the effect of AMF on the symbiosis and the growth of the plant.

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