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GROWTH RESPONSE ON-FARM OF TWO JATROPHA CURCAS (L.) VARIETIES AS AFFECTED BY MYCORRHIZAE AND ORGANIC MANURE IN THE LOCALITY OF DANG-NGAOUNDERE (ADAMAWA-CAMEROON)

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ABSTRACT

In view of contributing to the establishment and diversification of Jatropha curcas cultivation in the Guinea –savannah of the Adamawa region of Cameroon, a study was carried out to see the effect of organic manure, mycorrhizae and chemical fertilizer on the growth performance of Jatropha curcas in the locality of Dang (Ngaoundere). The experimental design was a split plot with two factors, the first being the origin or accession at two levels (Chad and Cameroon), the second being 4 treatments (organic manure, mycorrhizae, positive control=chemical fertilizer (NPK-20 10 10) and negative control) in three replicates. Data was collected on growth parameters as influenced by the different treatments, total biomass yields, carbon sequestration and root mycorrhizal colonization. Results indicate that fertilizers used have a highly significant effect ($P < 0.001$) on the growth, development and dry biomass produced at 180 days after sowing, as well as on the level of carbon sequestration. Accessions of local origin (Cameroon) adapted well in the Ngaoundere environment. Organic manure was the most effective in the improvement of growth and development of plants, followed by chemical fertilizers and mycorrhizae. Chemical fertilizer had a negative effect on root mycorrhizal colonization. Organic fertilizers followed by mycorrhizae are seemingly advantageous for the promotion of Jatropha curcas cultivation in the Ngaoundere locality.

Keywords: Jatropha curcas, mycorrhizae, organic fertilizer, growth, development, Dang-Ngaoundere..

1. INTRODUCTION

Sustainable development policies, the fight against climate change, the high cost of hydrocarbons and the populations' dependence on fossil fuels have motivated research interest on renewable energy resources which are non-polluting and accessible to all (Antonios, 2002). The use of petroleum products by industries produces more carbon dioxide, which is harmful to the environment and health (Péan *et al.*, 2005). Industries are in search of new sources to substitute fossil fuel whose world reserve can run out by the year 2050 (Chisti, 2007).

The use of plant species in the production of biogas is envisaged. This can contribute in lowering high cost of fuel prices, the world's dependence on fossil fuels and especially contribute to the reduction of green-house gas emission (Fall, 2007). Bio-fuels are renewable energy sources with no negative impact on the environment (Sall, 2007).

In order to ensure food security, research in the domain of bio-energy should be oriented towards non-edible, oil-producing plant species with potentials for bio-fuel production (Dieye, 2007; Jacquet *et al.*, 2009). It is therefore important to produce bio-fuels without compromising agricultural crops destined for human consumption (Bellefontaine *et al*., 2001; Nwaga, 2009).

Jatropha curcas (pourghère), a shrub belonging to the family Euphorbiaceae, seems a likely alternative to solve the problem of bio-energy without competing with food crops. This plant can be grown in association with other food crops (Dauriat *et al*., 2001). The toxic end-products (oil and cake) are neither used by humans or livestock, but are appreciated in traditional pharmacopeia (Bellefontaine *et al.*, 2001; Olivier, 2007). The plant adapts to tropical and sub-tropical climate and the semi-arid regions with annual rainfall of 500 or 700 mm. It withstands long periods of drought (3 years), all soil types and requires very little maintenance. It is used for life-fencing and along livestock corridors (Bellefontaine *et al.*, 2001 Olivier, 2007 Tchobsala *et al*, 2008). The plant has a plethora of other virtues: oil extracted from the seeds can serve as bio-fuels or for traditional soap making, rubbing oil, as well as insecticide or nematocide in crop protection (Hammaoui, 2006). The cake obtained after oil extraction is excellent organic manure for crops and when detoxified, can be used as feed for livestock (Kasuya *et al.*, 2013).

The practice of sustainable fertilization with mycorrhizae inoculation and the use of organic manure constitute an alternative for chemical fertilizers which carry serious risks of environmental pollution (Lachance et Rouleau, 2004; Bunch, 2004; Megueni *et al*., 2011). Organic manures (compost, fowl droppings) and bio-fertilizers (mycorrhizae and rhizobia) permit not only the cleansing of the environment but also increases soil nutrients which are important for the growth of plants (Nwaga, 2000a; 2009b; Tremier *et al*., 2002).

The general objective of this work is to contribute to the establishment of *J. curcas* in the guinea-savannah region of Ngaoundere (Cameroon). Specifically, this work will consist of:

1) evaluating the impact of mycorrhizae on the growth and development of *J. curcas* on-farm at Dang;
2) compare the impact of organic manure and mineral fertilizers on the development of the plant;
3) compare the adaptation rate of two accessions of *J. curcas* in the environment of the Adamawa-Cameroon.

2. MATERIAL AND METHODS

The trial was carried out on-farm, around the University campus at Dang-Ngaoundere, situated at an altitude of 1084 m, latitude 7°41'132''N and longitude 13°52'374''E. This is a locality in the Adamawa region (Cameroon), characterized by a guinea-savannah climate with two seasons: a rainy season (mid-March to mid-September) and a dry season (mid-September to mid-March). Rainfall varies between 1200 and 2000 mm, with an average of 1479 mm with a coefficient of variation of 9.8%. The minimum temperature (10°C) is registered in the months of December to January and the maximum (34°C) in the months of March-April. The mean annual temperature

varies between 22°C and 24°C and the relative humidity is highest (80%) in the months of July to August. The soils are basaltic and granites, favorable for agriculture and animal husbandry.
The planting materials are ripe seeds of *Jatropha curcas* harvested in the locality of Moundou (South of Chad) and from Garoua (North Cameroon). There is no noticeable morphological difference between the seeds from the different origins (Figure 1).

Figure 1: *Jatropha curcas* seeds from Cameroon (a) and from Chad (b).

The trial site was cleared and cleaned, tilled and demarcated into 10 by 6 m plots. Three beds separated with a space of 2 m each were made on each plot. 5 holes with 2 m spacing were dug in the middle of each bed.
The experimental design was a split plot with factor 1 being the origin of the seeds and factor 2 being 4 levels of fertilization (organic manure, mycorrhizae, chemical fertilizer and control) with three repetitions. *J. curcas* plants were placed 2 m apart in between lines and holes. Organic manure (OM) consisted of dried cow dung (2.5kg/hole). Chemical fertilizer (CF) applied was NPK (20-10-10) at a dose of 250g/hole). These two inputs were applied two weeks after seed germination. Mycorrhizae (M) inoculum obtained from the Biotechnology Centre in Yaoundé (Cameroon) was a mixture of *Glomus clarum, Gigaspora margarita* and *Scutellespora sp.* at a concentration of 20 spores/gram of substrate. 20 g of this substrate mixture was introduced into each hole during planting (Nwaga, 2009).
Seeds were pre-treated by soaking in cold water for 12 hours. Three healthy pre-treated seeds were put in each hole at a depth of 3 – 5 cm. Thinning was done 14 days after germination to leave one plant per hole. Plots were maintained clean by hand weeding.
Data were collected on germination and survival of plants, growth parameters and fresh weight of plants as well as root mycorrhization (nodulation) from a sample of 30 plants. The mean leaf surface area was calculated using the formula by RAUNKIAER (Ngatchou, 1987) as:

$$\text{Leaf Surface} = 2/3(L^* l)$$

(Where L = length of limb, and l = width of limb)
Dry matter (DM) was determined from plant samples dried under ambient temperature (air dried) for two days, followed by the oven at 105°C until constant dry weight was attained.
The rate of mycorrhizal colonization was evaluated as per Kormanick and Mc Graw (1992).

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The statistical package «Statgraphic plus 5.0» was used for analysis of variance (ANOVA) and for determining interactions between treatments. The Duncan test was used to compare the means.

3. RESULTS AND DISCUSSION

3.1 Germination rate of seeds at two weeks after planting

Seeds of *J. curcas* germinated between 6 to 14 days after sowing (DAS). Konaté (2006), Sharma (2007) and Nenwôla (2009) also observed that germination in *J. curcas* was averagely 10 days. There was a significant difference ($p < 0.05$) in the impact of the different treatments on the germination rate of the seeds originating from Chad (Figure 2). The germination rate for the seeds originating from Chad were 49.0 ± 7.2%; 54.0 ±16.1% and 48.0 ±13.8 % respectively, on plots that received organic manure, mycorrhizae and control. These values were 83.0±16.6; 96±19.5 and 82±13.5 % respectively for the same treatments with the seeds originating from Cameroon. And for the latter, the difference is highly significant ($p < 0.001$) between treatments. Seeds originating (accessions) from Cameroon, had a high germination rate as compared to those from Chad. Mycorrhizae stimulated the germination of seeds from both origins; 96.0±19.5 % for Cameroon accessions and 54.0±16.1 % for Chad accessions. This was likely due to water retention at the level of the radicles at the end of germination, a phenomenon commonly produced by fungi mycorrhizae. Earlier studies confirm the beneficial role of mycorrhizae in water and mineral supply/conduction in plants on dry land (Perret *et al.*, 2000; Thuries *et al.*, 2000).

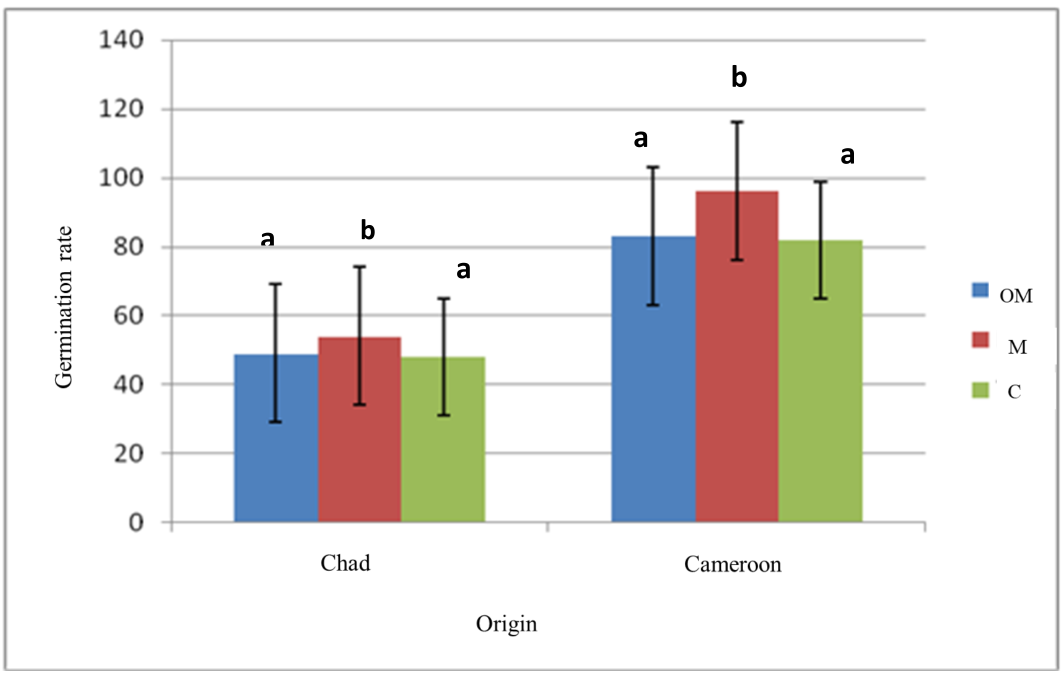


Figure 2: Germination rate of *Jatropha curcas* seeds at 14 DAS as per the different treatments
OM: organic manure; M: Mycorrhizae; C: control; DAS: Day after sowing
Values on the bars from the same accession followed by the same letter is not significantly different ($p < 0.05$) for Chad and $p < 0.001$for Cameroon).

3.2 Effect of different treatments on the survival rate of the plants

Compared to chemical fertilizers and control, mycorrhizae and organic manure significantly (P< 0.05) increased the survival rate of the plant at 180 DAS (Figure 3). The rates were 91.0±7.4; 95.0±3.2; 88.0±4.2; 88.0±2.5 % respectively for the plots enriched with organic manure, mycorrhizae, chemical fertilizer and control for the Cameroon accessions. These values were 95.0±1.1; 91.0±1.1; 80.0±1.1 and 86±2.5 % for the Chad accessions. The high rate of survival observed on plots enriched with organic manure and mycorrhizae were probably due to the uptake of mineral salts by the plants and its positive impact during the dry season (November – December). On the other hand, this result could be due to the capacity of environmental stress resistance conferred to the plant by the mycorrhizae (Bourou *et al*., 2011; Lounès-Hadj Sahraoui, 2013).

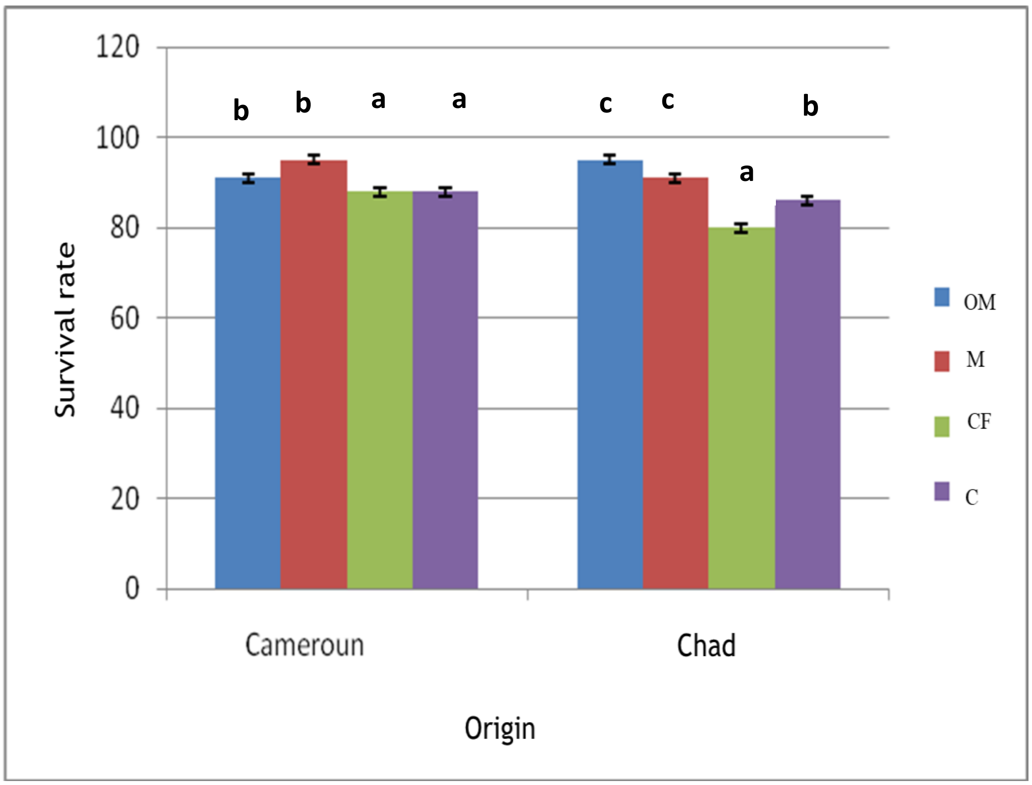


Figure 3: Suvival rate of *Jatropha curcas* plants at 180 DAS

OM: organic mqnure; M: Mycorrhizae; CF: Chemical fertilizer; C: control; DAS: Day after sowing

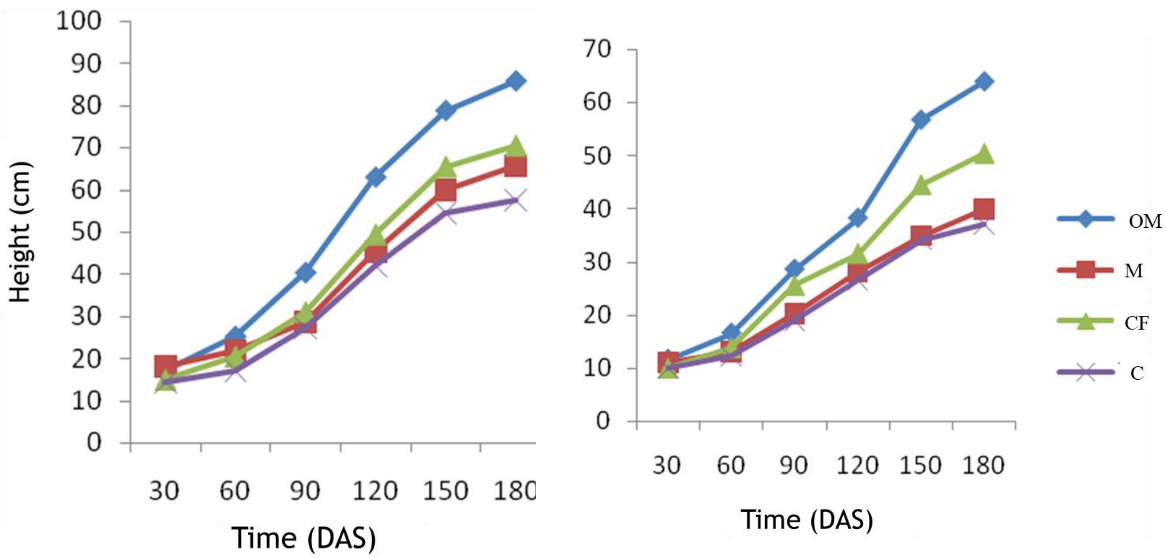
Values on the bars from the same accession followed by the same letter are not significantly different (p < 0.05).

3.3 Effect of different fertilizing agents on the growth of *Jatropha curcas*

The difference was highly significant (P<0:001) between the different treatments. Slow in the beginning, the growth rate (vertical) increased progressively with time (Figure 4). A gradual slow down was observed at 150 DAS for all two accessions. At 180 DAS, the height for

Cameroon accession were 85.9±5.2, 65.8±6.9, 70.5±5.5 and 57.6±7.3 respectively for the treatments organic manure, mycorrhizae, chemical fertilizer and control. Corresponding values for plants from Chad were 63.9±1 cm, 39.9±4.7 cm, 50.4±4.3 cm and 37.8±4.5. There was no significant difference between mycorrhizae treated plants and the control as of this date for the Chad accessions.

All the allocated treatments stimulated a vertical growth in all the *J. curcas* plants. Ouédraogo (2000) and Singh & Rathod (2002) obtained similar results on the growth and development of *J. curcas*. These results are close to that of Tchobsala *et al.* (2008) who observed a positive influence of chemical fertilizers and organic manure on the vertical growth of *J. curcas* in the northern region of Cameroon, as well as that of Tchuenteu (2009) who noted a profitable effect of mycorrhizae and organic manure on the vegetative development of *Ricinus communis* at Bini-Dang (Ngaoundéré-Cameroon). When comparing the two accessions (Chad and Cameroon), these results show that the local accession (Cameroon) performed better in terms of vertical growth parameters.



a. Origin Cameroon **b.** Origin Chad

Figure 4: Impact of the different treatments on the variability of *Jatropha curcas* height at different points in time.

DAS : Day after sowing ; OM: organic manure; M: Mycorrhizae; CF: Chemical fertilizer; C: control.

3.4 Effect of the different treatments on the number of leaves of *J. curcas*

The different treatments had highly significant (P< 0:001) effects on the increase in leaf number at 180 DAS (Figure 5). The values were 65.0±3.4, 51.0±5.3, 53.0±2.27 and 46±3.76 respectively for organic manure, mycorrhizae, chemical fertilizer and control. For the Chad accession, the values were 51.0±5.5, 40.0±5, 42.0±3.2 and 33.0±2.2 respectively, for the same treatments at 180 DAS.

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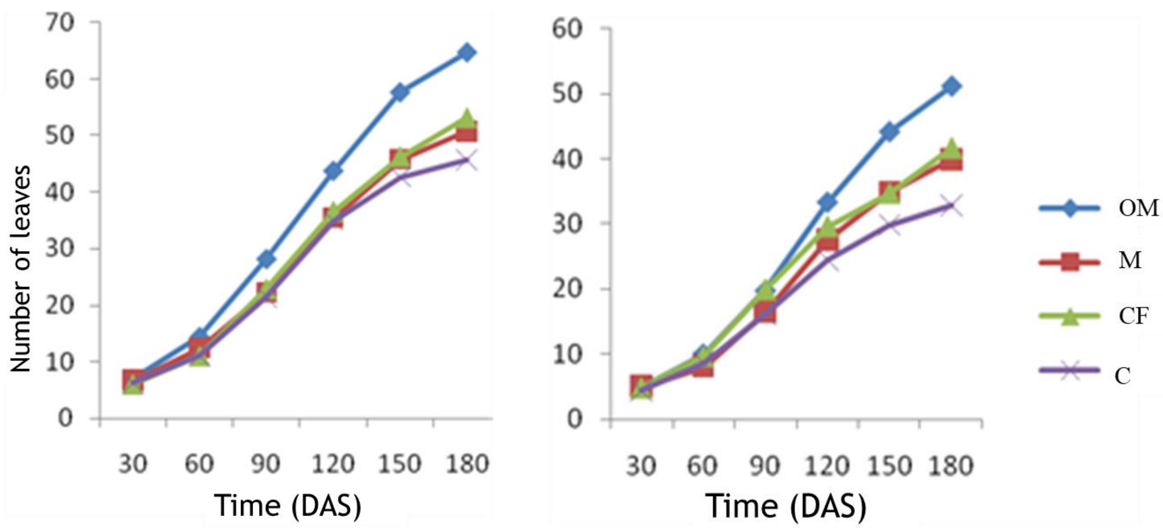
Leaf formation was slow between 120 and 180 DAS. This period corresponds to the dry season months of November and December, when plants are stressed as a result of lack of water. The number of leaves on the control treatment from both accessions remained low throughout the trial period.

Increase in the number of leaves on the treated plots suggests that fertilization (organic and mycorrhizae) enhances photosynthetic activity which is necessary for effective physiological functioning of the plant. This leaf increase could have an important role in the fight against climate change in the Adamawa region of Cameroon, given their importance in Carbon sequestration through photosynthesis, thus limiting soil exposure to harmful solar radiation and consequently increasing soil relative humidity and reducing erosion (Wiesenhiitter, 2003). Bunch (2004) and Sall (2007) have shown that leaves represent recyclable biomass and organic matter which could produce mineral elements necessary for plant nutrition, with *J. curcas* having remarkable physiological potentials. Leye *et al.* (2009) reported that the inoculation of *J. curcas* with *Glomus sp* led to a significant increase in leaf number. Organic manure provides nutrients which help in increasing the organic matter, stabilizing the pH, improving the structure and the water retention capacity of the soil; this explains the increase in the development of *J. curcas* as observed in all the plots amended with organic manure.

There was a highly significant correlation between plant height and leaf number in both accession ($R=0.839$; $P< 0.001$).



a. Provenance Cameroun **b**. Provenance Tchad

Figure 5: Variations in the number of leaves at different points in time as affected by the different treatments.

DAS : Day after sowing ; OM: organic manure; M: Mycorrhizae; CF: Chemical fertilizer; C: control

3.5 Effects of the different treatments on the leaf surface area (cm^2) of *Jathopha curcas* Leaf surface area showed a significant ($P< 0.001$) increase with the input of the various fertilizing agents and attained maximum from 90 to 120 DAP (Fig. 6). From 120 DAP, leaf surface area reduced greatly in both accessions. This reduction period corresponds to the dry season months

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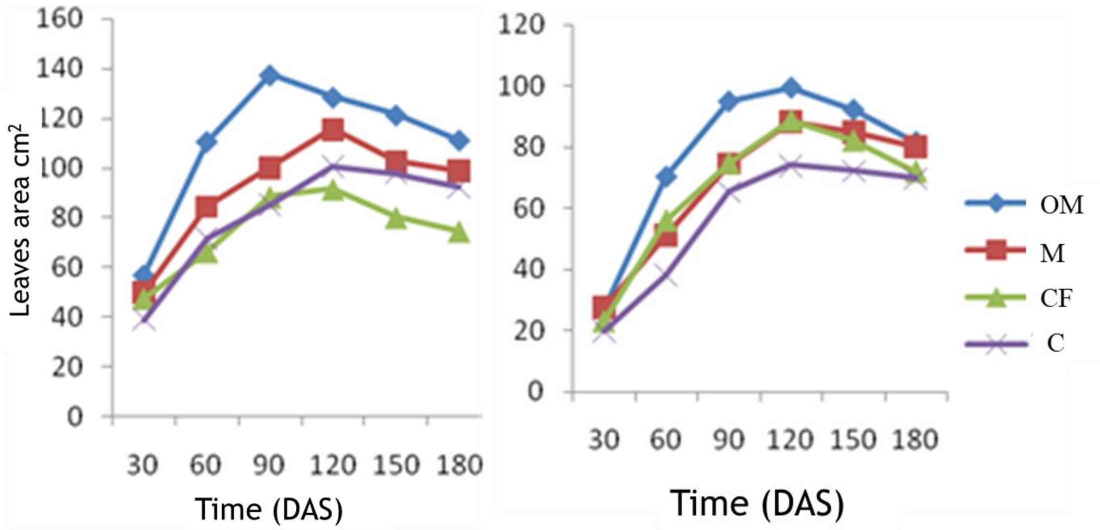
of November and December when most plants loose considerable amounts of their leaves in order to withstand the dry season. This work is similar to that of Ouédraogo (2000), which showed that under stress conditions, *J. curcas* reduced surface leaf area or sheds leaves in order to withstand the drought.



a. Origin Cameroon **b.** Origin Chad

Figure 6: Influence of the different treatments on the variation of leaf surface area of *Jatropha curcas* at different points in time.

DAS : Day after sowings; OM: organic manure; M: Mycorrhizae; CF: Chemical fertilizer; C: control

3.6 Effect of the treatments on plant biomass yield

The effect of organic manure, chemical fertilizer and mycorrhizae on the dry weight of *Jatropha curcas* plants is presented on figure 7. The different treatments significantly (P< 0,001) influenced values of this parameter.

Total dry biomass produced by the Cameroon accession was highest in organic manure (239±9.6 g) followed by chemical fertilizer (129.9±3.1 g), then by mycorrhizae (114.7±0.4 g). The lowest dry biomass yield was obtained from control (99.2±1.9 g). These values from the Chad accession were 176.4±3.6, 74.2±12.4, 46.5±9.5 and 43.2±2.8 g respectively for the same treatments. These results are in accordance with those of Tchuenteu (2009) who noted that organic manure increased biomass yields in *Ricinus communis*. Fertilization has a beneficial effect on dry biomass yields in *J. curcas* plants (Can *et al.*, 2004**;** Janaludin *et al.*, 2006).

There is a strong and positive correlation (R=0.854; P< 0.001 for Chad accession and R=0.892; P< 0.001 for Cameroon) between dry weight and height.

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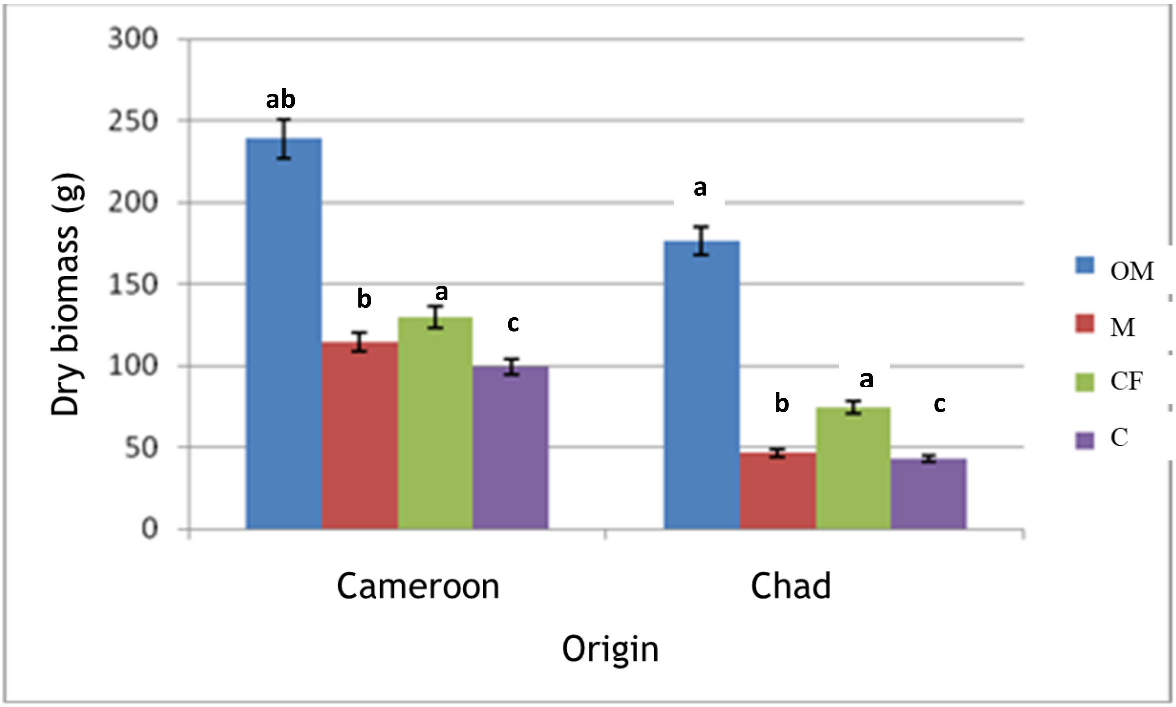

Figure 7 : Impact of treatments on dry biomass yields at 180 DAS

DAS: Day after sowings; OM: organic manure; M: Mycorrhizae; CF: Chemical fertilizer; C: control.

Values on the same set of bars followed by the same letters are not significantly different.

3.7 Effect of treatments on water content of the plants

Results obtained from the Cameroon accession shows high water content (74.1±3.1%) of plants on Mycorrhizae treated plots compared to plants on organic manure (70.7±7.3 %), control (71.7±4.3 %) and chemical fertilizer (68.5±5.3) treated plots. The effect of these treatments on the Chad accession was completely different from that of Cameroon accession (Figure 8). The high water content in plants of the Cameroon accession could be due to a more favorable response to environmental conditions. Mycorrhizae contribute more in water uptake and resistance to drought, which justifies the higher water content in plants in the mycorrhizae inoculated plots (Perrin, 1991**;** Megueni *et al.*, 2011).

According to Ouédraogo (2000), *J. curcas* retains three major adaptive characteristics which enhance its resistance to drought: a strong root development, a cuticle protection of above-ground structures and a reduction or shedding of leaves, which highly limits loses through transpiration. Actually, leaf loss (defoliation) was more pronounced on the organic manure treated plots (61%) and on the mycorrhizae treated plots (32%) for the Cameroon accession.

Improvement with organic manure and mycorrhizae conferred to the plant potentials for resistance to environmental stress.

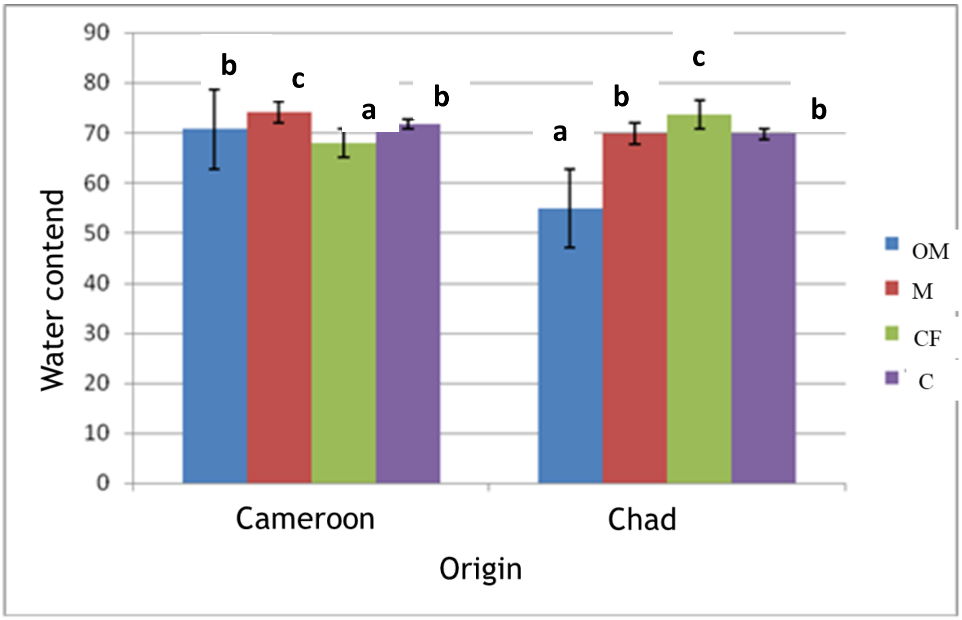

Figure 8: Effect of treatments on water content of *Jatropha curcas* plants at 180 DAS

DAS: Day after sowing; OM: organic manure; M: Mycorrhizae; CF: Chemical fertilizer; C: control

Values on the same set of bars followed by the same letters are not significantly different

3.8 Effect of different treatments on the mycorrhization rate of the *J. curcas* roots

Root colonization by mycorrhizae showed a highly significant difference (P< 0,001) between the seeds from the two origins (Table 4). The roots mycorrhization rate was lowest on chemical fertilizer treated plots compared to the other treatments. This shows that chemical fertilizer pollutes the environment and disturbs the development of soil microflora (Nwaga, 2000 ; Tremier *et al.*, 2002 ; Lachance and Rouleau, 2004). The sustainable management of soils for crop production would require the use of organic and biological fertilizers (Estève, 1996; Fresco, 2003 and Megueni *et al.*, 2011).

Tableau 4: Effect of the different treatments on the mycorrhization rate of *Jatropha curcas* root at 180 DAS

Origin	OM	M	CF	C	P
Cameroon	28.80 ± 0.40^{c}	40.49 ± 0.23^{d}	10.13 ± 0.21^{a}	20.57 ± 25^{b}	0.001
Chad	26.31 ± 1.5^{c}	40.23 ± 1.11^{d}	13.28 ± 1.14^{a}	$20.15\pm2{,}55^{b}$	0.001

OM: organic manure; M: Mycorrhizae; CF: chemical fertilizer; C: Control. DAS: Day after sowing

Line means with the same superscripts are not significantly different (p<0.001)

4. CONCLUSION AND PERSPECTIVES

At the end of this study, it is worth retaining that fertilizers significantly improved the growth, yield and the rate of root mycorrhizal colonization in the two accessions (Cameroon and Chad) of *Jatropha curcas*.

Germination in the two accessions was attained at two weeks after planting. The germination rate was higher for the Cameroon accession. Mycorrhizae improve the seeds germination rate and survival of the plants of the two accessions, as well as the water content. Mycorrhizae confer on *J. curcas* plants drought resistance properties such as defoliation and increase in water content. The Cameroon accession was better adapted to the study site than the accession from Chad. As concerns yields, plant dry weight was highest in the organic manure treated plants, followed by the chemical fertilizer, mycorrhizae and control in both accessions (Cameroon and Chad). The mycorrhization rate was lower on roots of plants in chemical fertilizer treated plots than in the control, which affirms the destruction of soil microflora by chemical fertilizers.

Good development of *J. curcas* requires input of organic material or mycorrhizae. In terms of agronomy, the local accession of *J. curcas* can be integrated in the vulgarization/extension program in the Guinea-savannah region of the Adamawa (Cameroon).

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