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Effect of indigenous arbuscular mycorrhizal fungi (AMF) on tree seedling growth in semi-arid soils of Borno State, Nigeria

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ABSTRACT: Inoculation of trees and crops with arbuscular mycorrhizal fungi (AMF) leads to growth improvement through nutritional benefit, among others. Thus, inoculation of *Faidherbia albida* and *Balanites aegyptiaca* seedlings with their rhizosphere AMF inocula or when cross inoculated with the other's AMF in the semi-arid soils of Borno State, Nigeria, resulted in both trees benefiting more than when un-inoculated. For instance, the 20 week old seedlings i8noculated with their own AMF recorded significantly ($P<0.05$) better growth in terms of heights (of 53.5cm and 48.8cm, as against the corresponding values of 37.0cm and 33.5cm for the un-inoculated plants) of *F. albida* and *B. aegyptiaca*, respectively. Again, shoot dry weights of 2.68g/plant and 2.82g/plant were obtained for the inoculated plants against 1.04g/plant and 1.27g/plant for the un-inoculated plants, respectively. The shoot nutrient contents (N, P,K, Mg and Ca) of the trees under the influence of their own AMF were also higher than when un-inoculated or even when cross inoculated. Root dry weights of inoculated plants were, however, lower than those of the un-inoculated plants or those inoculated with the other's inoculum. This result has, thus, demonstrated that semi-arid soils of Northeastern Nigeria do contain active AMF propagules in the rhizopheres which are effective in improving tree seedling growth, especially, in the nursery.

Key words: Innoculation; Growth improvement; Arbuscular mycorrhizal fungi; Semi-arid soils.

Introduction

The effect of AMF fungi on growth improvement of trees, especially, legumes, in low P soils is well documented. From most of these studies, growth improvement of trees was attributed to the improved uptake of water, nutrients such as P, N, Ca and Zn from the soil as well as increased N-fixation activities by the *Rhizobium* as a result of association with mycorrhizal fungi. Some of these results indicate that mycorrhizal fungi enhance not only dry matter yield, and total N yield of the plants but also the amount of N derived from the soils and from fixation (1). These benefits, according to these researchers are even more than those brought about by B fertilization.

In Nigeria, particularly in the Sudan-Sahelian region of Northeastern Nigeria, leguminous trees such as *F. albida* and non-leguminous trees such as *Balanites ageyptiaca*, commonly left on farmlands sustain improved crop growth under them. They also produce fodder for livestock consumption during the driest

part of the year as well as other products, which are consumed by human beings. These trees are, however, generally slow growing, especially in the field. The application of mycorrhizal fungi to these trees before out planting in the field can considerably facilitate their establishment and growth as well as transfer of nutrients or mycorrhizal infection to adjacent crop plants.

From an earlier study (AMF Survey of Tress) these trees were found to be heavily endomycorrhizal, a condition that has been built over several years of development. However, despite the apparent importance of mycorrhiza in improving tree establishment and growth, especially of these Sahelian species, there is very limited information on their mycorrhizal benefits, especially the non-legumes. It is, therefore, necessary to study the growth performance of these trees under the influence of mycorrhizal fungi. It can also be used in afforestation and agroforestry programmes in the region, since these two programmes are very crucial in the area due to its denuded nature as well as the rapidly increasing need for more food production for the over growing population.

Materials and Methods

Balanites aegyptiaca and *Faidherbia albida* seeds were locally collected. The seeds were surface sterilized in 5%, H_2O_2 for 5 minutes after which they were washed in several changes of distilled water.

Breaking of seed dormancy: Surface-sterilized *B. aegyptiaca* seeds were soaked in distilled water in a beaker for two days. The soaked seeds were then removed and kept in a damp sterile cloth, kept on a tray and covered with another tray. This was left at room temperature (37°C) until the endocarp had split open (after 5 days of damp keeping).

Dormancy in *F. albida* seeds was broken by making a small cut on each surface-sterilized seed (part of the testa removed, away from the end that would produce the radicle), using a sterile razor blade.

Pot and potting mixture sterilization: Earthen pot (22cm diameter and 20cm depth) were surface-sterilized with cloth moistened with 5% H_2O_2 after which they were rinsed with distilled water. Potting mixture consisted of topsoil, which were steam sterilized for 45 minutes at 100°C in mint sterilizers. Sterilized soil was left for 2-3 days before being used for planting.

Soil Inoculation with AMF: Each surface-sterilized pot was filled to about two-thirds of its volume with sterilized soil. One hundred grams (100g) of the crude inoculum collected from under *F. albida* or *B. aegyptiaca*, was spread over the soil surface and covered with more sterilized soil. The control pots received no mycorrhizal inoculation.

Sowing of seeds: Three *F. albida* seeds prepared as described above were sown in pots containing sterilized soil with or without mycorrhizal inoculum. At six weeks after sowing, the seedlings were thinned to one per pot. For *B. aegyptiaca*, one germinating seed was also planted in each pot containing 2.5kg sterilized soils, inoculated with 100g each of crude AMF inoculum from *Balanites* or *F. albida* trees while the controls received no mycorrhizal inoculation. The treatments were replicated four times (24 stands) and arranged in a complete randomized design on wooden tables (150cm height). These were kept in a screen house under natural light with maximum photosynthetic active radiation (PRA) of 1800-2000+ mol m^2 s^{-1} and day/night temperature of 39°C ± 2°C and relative humidity that varied between 74%and80%. The pot were all irrigated twice daily until six weeks after germination, when watering regime was reduced to once daily.

Plant heights, number of leaves and number of branches were recorded on monthly basis starting at six weeks after germination. At the end of 20 weeks of growth, the shoots were harvested, oven dried at 60°C for 48 hours and dry weights determined. Shoot nutrient contents were determined using the oven dried shoots. These were ground in a mortar and pestle to powder form fine enough to pass through 1mm sieve mesh. Nitrogen content was determined by Micro-Kjeldah method Jackson, (2), Phosphorus content by using the Vanadomolybdate yellow method (Murphy and Rikey, (3). Potassium by theflame photometric method, Black, (4), Magnesium and Calcium contents by the Titrimetry method (5).

Roots were reclaimed from the soils by thorough washing under tap water until they were freed from all particles. Small portions of the root systems (fine roots) were removed, cut into 2cm segments and fixed in

formalin acetic acid (FAA) in Mccartney bottles. These were later cleared for 2 minutes at 21°C in 10% KOH and stained with Chlorazole Black E stain in lactic acid and glycerin and examined microscopically for the presence or absence of vesicles and/or arbuscules (evidences of AMF colonization). The remaining roots were oven-dried at 60°C for 48 hours and then weighed.

Calculations

$$\% \text{ AMF Colonization} = \left[\frac{N}{NT}\right] \times 100$$

Where NI = Number of Intersections with Arbuscules and vesicles; NT = Total number of Intersections.

$$\% \text{ Weight Gain or Loss} = \left[\frac{DI - DUI}{DUI}\right] \times 100$$

Where DI = Dry weight of inoculated plants; DUI = Dry weight of un-inoculates plants.

Statistical Analysis

The data for all the parameters assessed were analyzed by a two-way analysis of variance (ANOVA) where the sources of variation were the trees and the inoculum.

Results

Effect on some morphological features studied

A. Effect on height

The mean heights of the trees as influenced by the different mycorrhizal inocula are presented in Table 1. Applying the LSD test showed that the mean height for *F. albida* is significantly ($P<0.05$) greater when inoculated with its own AMP inoculum than when AMF *Balanites* was used. For *Balanites*, however, even though inoculation with its own AMF produced taller seedlings than the AMF-*albida* inoculated ones, there was no significant difference between the effects of the two mycorrhizal inocula ($P>0.05$).

The height of the tree seedlings at different days after germination is presented in Figure 1. Figure 1A represents height of *B. aegyptica* while Figure 1B is that of *F. albida*. Both figures reveal better height increase when inoculated with the other's inoculum.

B. Effect on branch production

The means for the number of branches, obtained from a two-way ANOVA is also contained in Table 1. It shows, as with the height analysis, that inoculation of either tree species with their own AMF inoculum resulted in the production of significantly ($P<0.05$) more branches compared to the un-inoculated seedlings but the differences between the effect of the two inocula in both trees were not significant.

C. Effect on number of leaves

The means of the number of leaves is also presented in Table1. The LSD test showed that the un-inoculated plants produced significantly ($P<0.05$) fewer leaves than plants of both tree species inoculated with both mycorrhizal inocula. It further showed that for *F. albida*, inoculation with AMF from *Balanites* led to the production of fewer leaves than inoculation with its own AMF. The opposite is true for *Balanites* trees.

Effect on shoot and root dry weights

The result of the effect of mycorrhizal inoculation on shoot dry weight of the tree species showed a significant ($P<0.05$) difference between inoculum treatment and the tree type (Table 2). It also shows that

the differences between the two inocula only reaches significance for *F. albida* seedlings, with dry weights being higher for AMF *albida* inoculated plants. For both tree species, however, the result showed that un-inoculated plants produced lower shoot weight than the AMF inoculated ones.

Table 1: Effect of mycorrhizal inoculation on morphological features of 20 week-old *F. albida* and *B. aegyptiaca* seedlings. (Figures in brackets indicate percentage difference from control).

	INOCULUM		
	Control	**AMF (alb)**	**AMF (Bal)**
(a) Height (cm)			
F. albida	37.0a	53.5b(45)	50.0c(35)
B. aegyptiaca	33.5a	47.0b(40)	48.8b(46)
LSD at 5%	3.11		
(b) Number of branches			
F. albida	0.8a	2b(150)	1.5ab(88)
B. aegyptiaca	0.8a	1.5ab(88)	2.3b(188)
© Number of leaves			
F. albida	35.3a	45.8b(30)	43.4b(23)
B. aegyptiaca	32.3a	44.3b(37)	46.0b(42)
LSD at 5%	2.48		

Data in each row followed by the same letters are not significantly different.

The means of the root dry weights (Table 2) shows that for *F. albida* trees, the mean root dry weight for the control is significantly ($P<0.05$) greater than roots of those under both inoculum treatments but with no significant difference between the effects of the two inocula. For *Balanites*, however, the mean root dry weight for the control is significantly ($P<0.05$) greater than that of AMF *Balanites* un-inoculated plants, which is also significantly ($P<0.05$) lower than that of AMF *albida* inoculated ones.

Percentage AMF colonization of the tree seedlings

As there was zero infection for all control plants, the homogeneity of variance assumption underlying the ANOVA procedure is not satisfied. As such, the control treatments were excluded from the analysis. Thus, Figure 2 contains the actual levels of AMF colonization of the two tree species. It revealed as with the other parameters, that the trees were more colonized when inoculated with their own inoculum than with the other's inoculum.

Effect on shoot nutrient content

The means of the different shoot nutrients of the trees obtained from the ANOVA output are presented in Table 3. It shows a significant ($P<0.05$) difference between P and n contents of the two tree species, with content being larger for *balanites*. It also showed that application of inoculum significantly ($P<0.05$) increased N content, but there was no evidence of a significant difference between the effects of the two inocula ($P>0.05$). Cross inoculation with the other's AMF also did not reach any significant level. Using the LSD test (5% level) to compare the means, it was observed that the trees inoculated with their own AMF produced higher shoot P and n than when inoculated with the other's inoculum (Table 3).

Fig. 1: heights of 20 week old potted *B. aegyptiaca* (A) and *F. albida*(B) seedlings inoculated with either their own AMF or the other's AMF.
VAM = Vesicular Arbuscular Mycorrhiza
Alb. = *albida*
Bal. = *Balanites*

Fig. 2: PercentageAMF colonization of tree seedlings
VAM = Vesicular Arbuscular Mycorrhiza
Alb. = *albida*
Bal. = *Balanites*

The result of shoot K content revealed that its content in *Balanites* trees is significantly (P<0.001) greater than that of *F. albida*. Inoculation also significantly (P<0.05) increased shoot K content with an indication that the inoculum effect was most apparent for *Balanites* However, the result still demonstrates the greater response of the tree species to inoculation with their AMF than with the other's AMF.

Using the LSD test (5% level) to compare the means of shoot Mg content contained in table 3, it was observed that its content for the un-inoculated *F. albida* plants was significantly lower than those of *Balanites* plants inoculated with either of the inocula. There was, however, no significant difference (P>0.05) between the effects of the two AMF inocula on this nutrient in the two tree species.

For shoot Ca content, comparisons made using the LSD test (5% level) showed similar results as with magnesium content, except that *Balanites* seedlings inoculated with AMF from the parent tree contained significantly (P<0.05) greater shoot Ca than those inoculated with AMF from *F. albida.*

Table 2: Mean* Shoot and Root dry weights (g/plant) of *B. aegyptiaca* and *F. albida* seedlings under the influence of mycorrhizal inoculation.

Tree species/Treatment	**Inoculum**			**% difference**	
	Control	**AMF (alb.)**	**AMF (Bal.)**	**AMF (alb.)**	**AMF (Bal.)**
(a) Shoot Dry weight					
B. aegyptiaca	1.27a	2.72b	2.82b	114	122
F. albida	1.04a	2.68b	2.37c	158	128
LSD at 5%					
(b) Root Dry Weight					
B. aegyptiaca	1.94a	1.67a	1.34b	-14	-31
F. albida	1.97a	1.27a	1.53b	-36	-22
LSD at 5%	0.31				

* = Means from 4 replication. Data in the same row and having the same letters are not significantly different from each other.

Discussion

The result of the present study has shown that inoculation increased heights of both tree species compared to non-inoculation, with the effect of the inocula being dependent on tree type. This is not unexpected since plant species are known to differ in their degree of response to mycorrhizal colonization. Thus, the greater heights observed for *F. albida* than *Balanites* may well be due to the physiological differences rather than due to response to AMF inoculation.

The height increase in *F. albida* of 45% over un-inoculated ones compares with result of Ducousso and Colonna (6) where *Faidherbia albida* inoculated with *F. mosseae* recorded height increase of 34% over non-inoculated ones.

AMF inoculation was also found to significantly increase the number of branches, number of leaves and shoot dry weight of the trees over the un-inoculated plants. What is interesting in these observations is the fact that the increases were more when the trees were inoculated with their own AMF inoculum than when inocula with the other's inoculum. It is possible that this preferential response is due to the

mycorrhizal fungi being more compatible with the parent tree than with a different species. These general growth improvement, are likely to be as a result of nutritional improvement.

Nonetheless, the AMF from both trees were able to improve each other's growth to some extent which signifies that the two tree species can be colonized by either group of AMF inoculum.

Table 3: Mean* nutrient contents of 20 week old potted *B. aegyptiaca* and *F. albida* seedlings after inoculation with their own AMF or with the other's AMF.

Tree species/AMF Treatment	**Nutrient Content (%)**				
	N	**P**	**K**	**Mg^{-2}**	**Ca^{-2}**
B. aegyptiaca control	1.31a	0.15a	2.55a	0.32a	0.53a
B. aegyptiaca AMF-alb	1.57b	0.18b	3.40b	0.59b	0.66b
B. aegyptiaca AMF-Bal	1.77b	0.21b	3.95b	0.77b	0.73c
F. albida control	1.25d	0.12d	1.10d	0.49d	0.29d
F. albida AMF-alb	1.44d	0.17e	1.8e	0.54e	0.31e
F. albida AMF-Bal	1.34d	0.14ed	1.23ed	0.29ed	0.25d
LSD at 5%	0.33	0.05	0.72	0.27	0.10

Results represent the means from 4 replications. Data for each tree species, in the same column followed by the same letters, are not significantly different.

The effect of inoculation on root production showed that the mycorrhizal plants produced fewer roots or at least, roots with smaller weights compared with those produced by the non-mycorrhizal plants. Even though this observation contrasts with those of other workers (Bagyyaraj and Manjunath, (7); Huang *et al* (18); Ducousso and Colonna, (6), it is in agreement with others (9,10,11). These scientists explained the negative effect of AMF on root production as being attributed to utilization of carbohydrates meant for root formation, for the development and propagation of the saprophytic AMF fungi. According to Read (12), root extension, branching and root hair development is strongly inhibited in the presence of AMF mycelia. The others believe that greater portion of the carbohydrates released by the plant for the purpose of root formation, is taken up by the fungus for its own development, thus, resulting in less roots being produced than in a situation where the AMF fungi are lacking. Moreover, since AMF fungal hyphae take up the role of root hairs as water and nutrient absorbers, the urge for such plant to produce many roots will be reduced.

The result also showed that for *F. albida*, there was no significant difference between the effects of the two inocula on root weight while for *B. aegyptiaca*, seedlings inoculated with AMF from the parent tree produced significantly lower root weight than the AMF-*albida* inoculated ones. The result of the latter was, however, not significantly different from those of the un-inoculated plants. This indicates that *Balanites* is more compatible with its own inoculum while *F. albida* can be equally colonized by either of the AMF to bring about similar reduction in root production. Moreover, AMF from *F. albida* did not significantly reduce root weight of *Balanites* even though it was able to increase height and shoot weight of the tree. Also, judging from the values of the % colonization, it showed that *Balanites* was highly colonized by AMF from *albida* (68%), a value that should have brought about differences between the root weight of the inoculated and the un-inoculated seedling. IT, therefore, appears that some other factors, such as alteration in biochemical activities of the plant, may be involved in the process of root production by mycorrhizal plants. This will require further investigation.

The nutrient contents (N, P, K, Mg and Ca) of the inoculated trees were also found to be higher than those of the un-inoculated plants. For *B. aegyptiaca*, it was also observed that there was no significant

difference in the nutrient contents of the seedlings as a result of inoculation with the different AMF, except for calcium, whose content was significantly higher under AMF-Bal inoculation than under AMF-*albida* inoculation.

For *F. albida*, only inoculation with its AMF inoculum significantly improved the nutrient content of the seedlings with the exception of nitrogen whose content was not significantly increased over the un-inoculated by any of the AMF inocula. This is, however, unexpected, especially, as mycorrhizal benefit in facilitating N-fixation by leguminous plants is well-documented (13). However, since these were pot experiments lasting only 20 weeks, they might not have grown long enough for this influence to be manifested. The higher values observed for *B. aegyptiaca* treatments might have been as a result of the greater amount of shoot produced; thus, indicating differences due to their genetic constitution rather than differences due to response to mycorrhizal inoculation.

Nevertheless, it is generally assumed that growth improvements observed in mycorrhizal plants are as a result of nutritional improvement. For instance, increases in shoot P content of pepper plants have been closely correlated with stimulation of flower bud production (14). It is also documented that the photo-respiratory activities of plants are increased by mycorrhizal fungi (15, 16).

The failure of AMF from *Balanites* to bring about significant increase in the nutrient content of *F. albida* seedlings over the un-inoculated ones, even though it recorded high colonization (7%%) can only be explained on the basis of retention of the nutrients by the fungus.

On the whole, both tree species revealed that inoculation with their own AMF resulted in better growth and higher nutrient content than when not inoculated. It has also shown that the soil inocula contained AMF propagules, which differed in their ability to colonize the different tree species.

Conclusion

The effect of inoculation of the two tree species *F. albida* and *B. aegyptiaca* with their rhizophere soils resulted in significant (P, 0.05) growth improvement (height and shoot weight) over non-inoculation, with inoculation with AMF from parent tree enhancing growth more than cross-inoculation with the other's AMF. Contrastingly, AMF inoculation was found to have negative effect on root production (less roots produced by mycorrhizal plants). The lack of significant difference between the effect of the two AMF sources on the root weight of *F. albida* but not for *B. aegyptiaca* implies that the former can be equally colonized by either of the AMF while the latter may be more compatible with its own AMF. Moreover, AMF from *F. albida* could not effect significant changes in the root weight of *B. aegyptiaca* despite the fact that it highly colonized it (68%) and increased its height and shoot weight. It appears, that some other factors apart from the stimulating effect of AMF may be involved in the process of root production by mycorrhizal plants, an assumption, which will require further investigation.

The result of the investigation on nutrient contents (N, P, K, Mg and Ca) of the trees revealed that they were improved by inoculation than when the plants were not inoculated, except for *F. albida* where none of the AMF sources improved its N content to a significant level. The failure of AMF from *B. aegyptiaca* to bring about significant increase in the nutrient contents (N, P, Mg and Ca) of *F. albida* seedlings over those of the un-inoculated plants despite the high root colonization (75%), can only be assumed to be due to the retention of the nutrients by the fungus. It also shows the extent to which variations can occur with AMF inoculation, an area tat will require future research attention.

Recommendations

There is need to further investigate other factors that may be stimulating root production by mycorrhizal plants other than AMF. Further research attention is also needed in determining level of AMF colonization and nutrient uptake, particularly, N by tree seedlings.

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