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Impact of *Arbuscular Mycorrhizal* Fungi (AMF) on Colonization Rate and Growth of *Crotalaria Retusa* and *Senna Occidentalis* under Nitrogen Stress

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Abstract

This study examines the impact of *Arbuscular Mycorrhizal* Fungi (AMF) on the colonization rates and growth of *Crotalaria retusa* and *Senna occidentalis* under varying nitrogen levels (low, medium, high). Conducted at Ahmadu Bello University, Zaria, the research used sterilized soil from a degraded site, filled into perforated buckets. AMF was applied using the Trench method. Root samples were collected, stored in 50% ethanol, and analyzed for *mycorrhizal* colonization following Brundett *et al*. (1994). Results indicated that the percentage rate of colonization (PRC) of AMF on *C. retusa* roots across different nitrogen levels was not statistically significant ($p \leq 0.218$). Colonization was highest at low nitrogen (76.67±1.91%), followed by medium (72.83±3.89%) and high nitrogen levels (70.00±1.07%). Similarly, for *S. occidentalis*, the PRC was also not significant ($p = 0.987$) across nitrogen levels, with highest colonization at low nitrogen (76.50±2.39%), followed by high (76.00±3.02%) and medium (76.00±2.08%) levels. Overall, the study found that AMF colonization rates in *C. retusa* and *S. occidentalis* increased as nitrogen application decreased, regardless of

whether the plants were inoculated with AMF. This suggests that lower nitrogen levels may favor higher AMF colonization in these species.

INTRODUCTION

Soil degradation is a critical and growing global problem with implications for key policy areas, including food security, climate change, and biodiversity resources (Sawa et al 2015). Human activities such as massive deforestation, overgrazing, over cultivation, bush burning, and general land misuse exacerbate soil degradation. The nitrogen-fixing abilities of legumes can be enhanced not only by *Rhizobium* spp. but also by AMF fungi colonizing their roots (Smith and Smith, 2011). Although *Arbuscular mycorrhizal* fungi are present in Guinea Savannah soils (Smith and Read, 2008; Arumugan *et al*., 2010), their potential to improve crop yields through management practices is not fully realized. AMF facilitates energy and matter flow between plants and soil (Cardon and Whitbeck, 2007) and improves plant tolerance to stress by altering morpho-physiological traits (Alqarawi *et al*., 2014a; Hashem *et al*., 2015). Considered natural growth regulators of terrestrial flora, AMFs can act as bio-inoculants and are promoted as prominent bio fertilizers for sustainable productivity (Barrow, 2012). AMF-inoculated soils form more stable masses and have significantly higher extra-radical hyphal mycelium than non-AMF-treated soils (Syamsiyah *et al*., 2018). Integrating leguminous cover crops into existing farming systems has been successful due to their high agronomic benefits (Loos *et al*., 2001; Fosu *et al*., 2004). Legumes can replenish soil nitrogen, a critical growth factor, especially under high nitrogen-fixing stress. Selecting the ideal plant species is essential for restoring degraded land (Mukhopadhyay *et al.,* 2013).

Crotalaria, belonging to the *Fabaceae* family, comprises approximately 600 species in tropical and subtropical areas (Polhill, 1982). Known for high dry matter production, *Crotalaria* can grow in poor soils with low nitrogen content (Daimon *et al*., 1998). These species are effective fallowing cover crops for soil regeneration (Muller- Samann and Kotschi, 1994) and bind significant amounts of nitrogen (Fischier *et al*., 1999). Their slow decomposition rate over the dry season improves soil physical properties, regardless of tillage regime. *Crotalaria* species, cultivated as food crops and used in traditional medicine worldwide, are found abundantly in

Zaria, including *Crotalaria renaria*, *Crotalaria falcata*, *Crotalaria* intermedia, *Crotalaria retusa*, *Crotalaria lachnosema*, *Crotalaria macrocalyx*, and *Crotalaria naragutensis* (Nuhu *et al*., 2009).

Senna occidentalis, from the *Leguminosae* family, is commonly known as coffee senna in English and *Raidore* in Hausa. These wild plants are crucial primary producers in Nigeria's savanna ecosystem, providing shade and enhancing soil fertility (Tambari *et al*., 2015). Medicinally important species in this genus include *Senna angustifolia, Senna acutifolia, Senna occidentalis*, *Senna javanica, Senna biflora, Senna fistula*, and *Senna sophera* (Kaur *et al.*, 2016).

MATERIALS AND METHODS

The study took place in the experimental garden of the Department of Botany at Ahmadu Bello University, Zaria, located at latitude 11°N, longitude 7°42'E, and an altitude of 660 meters. This area experiences a tropical climate, with the highest temperatures occurring in April and cold, dry harmattan winds blowing between November and January. The research was carried out during the 2018 and 2019 growing seasons.

Preparation of Soil, Planting Buckets, Fertilizer, and AMF

Soil samples were collected from a degraded site at the Institute of Agricultural Research (IAR), sieved through a 6 mm mesh, and sterilized at 120 °C for 2 hours. Perforated buckets were washed with tap water and filled with 7-kg of sterilized, degraded soil.

The trench method was used to inoculate the soil with *Arbuscular mycorrhizal* fungi (AMF). Five grams of inoculum were added to each of the 36 buckets with perforated covers to facilitate water drainage. An additional 36 buckets were prepared without the inoculum. Seeds of *C. retusa* and *S. occidentalis* were planted, both singly and in combination. The buckets were regularly watered and observed until germination was achieved.

Fertilizer Application

Nitrogen fertilizers were applied at three levels, low 0.9g , medium 6g, and high 12g per bucket.

Determination of AMF Percentage Root Colonization

Healthy root samples of *C. retusa* and *S. occidentalis* were collected from each treatment, replicated during harvesting, washed in running tap water, and stored in glass vials containing 50% ethanol until analysis. The roots were then rinsed with water at least four times to remove the ethanol. The percentage rate of colonization in *C. retusa* and *S. occidentalis* roots was then determined according to the method outlined by Brundrett *et al*. (1994). The roots were cleared in a 10% potassium hydroxide (KOH) solution in a water bath at 900 °C for 1 hour, which removes cytoplasm from most root cells, leaving root structure and fungal elements to interact.

This was then drained and rinsed with running tap water. The roots were then bleached with 10% hydrogen peroxide (H_2O_2) at room temperature for 30 minutes to remove the pigments. The solution was drained and washed thoroughly in running tap water. The cleared roots were then acidified by immersion in 10% hydrochloric acid (HCl) for 5 to 10 minutes to neutralize the alkalinity due to KOH, and then drained. A trypan blue stain was added to the roots in running tap water. The roots were then stained with 5.0% glycerol over night to remove coloration from root cells.

The stained roots were cut into pieces 1 cm long, picked randomly, arranged on the slide, and observed under a microscope to study the hyphae, vesicles, and arbuscules. The slide was scanned microscopically, and the percentage root length colonized by *Mycorrhizal* endophyhe (% RLC) was determined by scoring the presence or absence of *Arbuscular* endophyhe touched by other graticle axes that cross the root each time a root is encountered. For a reasonable and accurate estimate of RLC, 50 intersections per slide were observed, counted, and carefully recorded.

The percentage % root length colonization (% RLC) was calculated using the formular below:

$$\text{RLC} = \frac{Number\ of\ root\ bits\ showing\ colonizaiton}{Total\ number\ of\ root\ bits} \text{ x } 100$$

($p = 0.218$). The colonization rate was high (76.67 ±1.91% at low level follow by mean and high nitrogen levels.

Result and Discussion

The percentage rate of colonization (PRC) of AMF on the root of C. *retusa* treated with three nitrogen levels was not significant with average mean values of 72.83±3.89% and 70.60±1.07% respectively.

Table 1: Percentage rate of colonization (PRC) of AMF on the roots of *C. retusa* and *S. occidentalis* inoculated with AMF during the application of three nitrogen levels

	Nitrogen Levels			
Plant	**High (%)**	**Medium (%)**	**Low (%)**	**P-value**
C. retusa	70.00±1.07[a]	72.83±3.89[a]	76.67±1.91[a]	0.218
C. retusa* + *C. retusa	76.83±2.61[a]	78.67±2.11[a]	72.33±7.32[a]	0.622
S. occidentalis	76.00±3.02[a]	76.00±2.08[a]	76.50±2.39[a]	0.987
S. occidentalis* + *S. occi.	69.33±0.99[b]	73.67±2.60[b]	81.33±1.33[a]	0.001
S. occidentalis* + *C. retusa	72.33±1.41[b]	78.67±1.33[a]	69.33±2.29[b]	0.005

Low = 0g, Medium = 6g and High = 12g

**Means sharing the same superscript (down the column) are not significantly different from each other ($p > 0.05$)

The result also showed that the PRC of AMF on the roots of S. *ocdidentalis* was not significant ($p = 0.987$) across the three nitrogen levels. The rate of colonization was observed to be high (76.50±2.39%) at low nitrogen followed by high 76.00±3.02%) and medium 76.00±2.0%) level of nitrogen application.

Table 2: Percentage rate of colonization (PRC) of AMF on roots of *C. retusa* and *S. occidentalis* un-inoculated with AMF during three nitrogen application levels

	Nitrogen Levels			
Plant	**High (%)**	**Medium (%)**	**Low (%)**	**P-value**
C. retusa	55.00±2.11[a]	55.00±1.69[a]	60.33±1.20[a]	0.068

C. retusa + C. retusa	56.00±1.71[b]	61.67±1.20[a]	57.33±1.52[ab]	0.042
S. occidentalis	52.00±1.03[b]	52.83±1.08[b]	61.33±1.52[a]	0.000
S. occidentalis + S. occi.	50.17±1.42[ab]	53.50±1.52[a]	48.83±1.17[b]	0.078
S. occidentalis + C. retusa	51.83±1.17[b]	59.00±0.86[a]	59.00±1.65[a]	0.001

Low = 0g, Medium = 6g and High = 12g

**Means sharing the same superscript (down the column) are not significantly different from each other ($p > 0.05$)

The study also revealed that across the three nitrogen levels, the PRC values ranges between 55.00±2.11% to 60.33 1.20% in *C. retusa* un-inoculated with AMF.The findings revealed that the percentage rate of colonization increased as nitrogen application levels decreased in both inoculated and un-inoculated conditions for *C. retusa* and *S. occidentalis*. Although this effect was higher in inoculated conditions, it was not statistically significant in either case. This result aligns with Treseder, (2004) findings, which reported that soil nitrogen addition decreased the AMF colonization rate. Conversely, other researchers, such as Eom *et al*. (1999), found that increasing nitrogen levels led to higher colonization rates. Thus, the impact of nitrogen on AMF colonization remains contradictory. The study also showed that during intraspecific competition between *S. occidentalis* species, the PRC was highly significant ($p = 0.00$) across all three nitrogen levels, with colonization rates increasing as nitrogen levels decreased (Table 1). This finding is consistent with Eom *et al*. (1999). Under un-inoculated conditions, PRC increased at high and medium nitrogen levels but decreased suddenly at low nitrogen levels. Numerous studies, such as those by Camenzind *et al*. (2014), have investigated changes in AMF colonization rates under nitrogen addition in natural ecosystems, with contradictory results (Yang *et al*., 2018).

Similarly, during intraspecific competition between C. retusa species, the colonization rate was not significant. The highest colonization rate occurred at medium nitrogen levels for both inoculated and uninoculated conditions .

In interspecific competition between *C. retusa* and *S. occidentalis*, the lowest PRC was observed at low nitrogen levels, while the highest PRC occurred at medium nitrogen levels, as shown in Table 1. This is in line with Aliyu, (2019). Several researchers have reported that AMF colonization is plant species-specific and

generally decreases with nitrogen addition. level. (Liu *et al*., 2014), Liu et al., (2012) and Jiang *et al*., (2018) found that fertilization (with nitrogen) reduced the AMF colonisation rate and attend AMF community composition in the roots of E. mutants in alpine Meadows. In contrast, Yang *et al*., (2018) did not find any significant changes in AMF colonization in *E. mutants* following nitrogen addition which contradict our finds. The discrepancy might be related to the differences in the sensitivity of AMF species to the amount of available nitrogen (N) levels added or sensation to different plant roots.

Conclusion

The percentage rate of colonization (PRC) of AMF increases with decreased nitrogen levels in the roots of *C. retusa* and *S. occidentalis* inoculated with AMF. During the interspecific competition between the two species, the colonization rate was high only at the medium nitrogen application level. Under un-inoculated conditions, a similar observation was made (i.e., colonization rate increases with a decreased level of nitrogen in both *C. retusa* and *S. occidentalis* . However, during interspecific competition between the two species, the highest colonization was observed at medium and low nitrogen levels.

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