



RESEARCH PAPER

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Effects of dual inoculation with arbuscular mycorrhizal fungi and rhizobia on *Acacia senegal* (L.) Willd. seedling growth and soil enzyme activities in senegal

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Abstract

Acacia senegal (L.) Willd. is a multipurpose legume that is economically and ecologically important in Sahelian areas, especially in Senegal. It has long been used for arabic gum production. However, drought and overexploitation lead to decreased soil fertility and tree productivity. An experiment was conducted to examine the response of *A. senegal* seedlings to inoculation with mixed arbuscular mycorrhizal fungi (*Glomus fasciculatum*, *Rhizophagus irregularis* and *Glomus verrucosum*) and rhizobial strains (ORS 3574, ORS 3593, ORS 3607 and ORS 3628) in glasshouse conditions. After 6 months of culture under non-sterilized field soil from Dahra (northern part of Senegal), plant height, arbuscular mycorrhizal root colonization rate and soil acid phosphatase activity were significantly enhanced by the combined inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia. However, plant biomass, soil spore density and hyphal length were significantly improved by the single inoculation with mycorrhizal or rhizobial strains. The number of nodules was higher for rhizobial inoculated plants. No significant increase in shoot nutrient contents was observed after inoculation. The microbial inoculation enhances the soil acid phosphatase activity whereas no positive effect was noticed on soil total microbial activity. These results indicate that the single inoculation with AMF or rhizobia improves *A. senegal* seedling growth under this non-sterilized field soil better than the dual inoculation. It is suggested that, for the success of a dual inoculation, a careful selection of effective combinations of microsymbionts is necessary to enhance plant growth and soil bio-functioning and for restoration of soil fertility in a given environment.

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Introduction

Acacia senegal (L.) Willd. is a leguminous tree belonging to the *Acacia* family which are used in reforestation processes (Midgley and Bond, 2001). *Acacia senegal* widely distributed through arid and semiarid regions of Africa, is the main species in the world producing the internationally-traded arabic gum. This tree is very important for the livelihoods of many rural populations and has potential for wider use (fire wood production, animal forage, medicinal products, agroforestry systems, etc.). Its incorporation into farming systems will help diversifying agriculture, stimulate income generation and contribute to land improvement, soil fertility replenishment and biodiversity preservation. However, in Senegal several decades of drought and over exploitation of natural resources led to a dramatic deforestation (Floret *et al.*, 1993) and to a decreased arabic gum production.

Increasing costs of chemical fertilizers and environmental concerns limit their use to improve plant growth and yield. In this context, soil symbiotic microorganisms are known to be a promising alternative to the use of chemical N and P fertilizers and for improving soil fertility, especially in the arid regions of Africa where phosphorus and nitrogen soil deficiency constitute the major factors limiting plant growth and mineral nutrition (Duponnois *et al.*, 2001; Sarmiento *et al.*, 2006). In fact, soil microorganisms and enzymes are the primary mediators of soil biological processes including organic matter degradation, mineralization and nutrient cycling (Sardans *et al.*, 2008). Soil microbial activities can indicate the relative availability or limitation of particular energy or nutrient sources in the environment (Shaw and Burns, 2006) and have been used as indicators of soil health (Zhang and Xu, 2008). Symbiotic microorganisms may help maintain good health and fertility which contribute to greater plant growth and productivity (Sheng *et al.*, 2012).

As a leguminous tree species, *A. senegal* is able to establish symbiotic associations with soil microorganisms such as rhizobia for improving

atmospheric nitrogen fixation. This latter helps replenish N in the soil through N-rich litter-fall, along with root and nodule turnover which are necessary for plant growth and protection (Scheublin and Vander, 2006). Studies demonstrated the beneficial effects of inoculating legumes with rhizobia (de Carvalho *et al.*, 2012; Ravikumar, 2012). Ceccon *et al.* (2011) found that the inoculation with *Sinorhizobium americanum* could improve the *Acacia farnesiana* growth and the re-establishment of important plant-soil interactions in degraded areas. In another study, Ahmed and co-authors (2014) reported that the inoculation of *Cajanus cajan* with rhizobia increased plant growth and performance.

Benefits of *A. senegal* could also result from its ability to form symbiotic association with arbuscular mycorrhizal fungi (AMF), therefore affecting soil mycorrhizal potential and contributing to plant growth through increase of phosphorus nutrition (Smith and Read, 2008). The work of Sultana and Miao (2014) revealed that AMF stimulates the growth of the legume, *Trofolium incarnatum*. The positive impact of AMF inoculation was also reported by the study of Hassan and Abakeer (2013) on *Vicia faba*. The beneficial effects of dual inoculation with AMF and rhizobia on plant growth were well documented (Mortimer *et al.*, 2008; Franzini *et al.*, 2010). Larimer *et al.* (2014) have found a positive effect of dual inoculation with AMF and rhizobia on the growth of prairie legume *Amorpha canescens*. So more, the response of plant growth to dual inoculation depended on several factors such as microbial strains, plant species and varieties, environmental conditions, as well as the compatibility among them (Azcon *et al.*, 1991).

Previous works on study area have focused on the effects of single inoculation with rhizobia or AMF on *Acacia senegal* growth (Ndoye *et al.*, 2013; Bakhoun *et al.*, 2012; Ndiaye *et al.*, 2011). However, very less research work has been conducted on the effects of dual inoculation with symbiotic microorganisms on *A. senegal* growth and soil bio-functioning, mainly under low fertile tropical soil conditions of Senegal.

Hence, this present study aimed to evaluate the effects of dual inoculation with selected arbuscular mycorrhizal fungi and rhizobia on the growth parameters of *Acacia senegal* and on enzyme activities in a non-sterilized field soil of Senegal.

Material and methods

Field soil collection

The soil used was collected in June 2010 from a 0-25 cm depth in an *A. senegal* plantation located at Dahra (northern part of Senegal, 15° 21' N, 15° 29' W). The soil samples were sieved (4 mm) to remove large stones and then thoroughly homogenized. The physicochemical characterization (Table 1) of a composite soil sample was analyzed at the laboratory LAMA (Laboratoire des Moyens Analytiques, IRD, Dakar, Senegal, certified ISO 9001, version 2000).

The total amount of carbon and nitrogen was quantified by using the combustion system ThermoFinnigan Flash EA 1112 (ThermoFinnigan, France). The colorimetric determination of total and available phosphorus was performed according to the method of Dabin (1965). Soil pH values were measured in 2 M KCl suspensions at a solid liquid ratio of 1:2.5. Soil physical characteristics were determined according to the method of Gee and Bauder (1986), and exchangeable cations following the method of Thomas (1982).

Production of AM fungal and rhizobial inoculants

The three AMF species used: *Glomus fasciculatum* (Thaxter sensu Gerdemann DAOM 227 130), *Rhizophagus irregularis* (Schenck and Smith DAOM 197 198) and *Glomus verriculosum* (Blaszkowski and Tadych DAOM 227 115) were obtained from the LCM collection (Laboratoire Commun de Microbiologie, IRD/ISRA/UCAD, Dakar, Senegal). These AMF strains were multiplied individually on roots of maize plantlets (*Zea mays* L.) during 3 months in sterilized sandy soil under glasshouse conditions. After 3 months, for each AMF inoculum, the maize plants were uprooted, and the roots cut into 0.5 cm pieces were mixed with the soil from trap culture. Equal amounts of each AMF inoculum were mixed to get a composite AM fungal inoculant with an average of 40

spores/g of soil and 80% colonized mycorrhizal roots. Four rhizobial strains selected for their symbiotic infectivity and effectiveness on *Acacia Senegal* growth in controlled conditions (Bakhoun *et al.*, 2012) were used for the experiment. They were isolated from rhizospheric soils of Dahra and Goudiry (Senegal) either by trapping or *in situ*. The strains, affiliated to *Mesorhizobium plurifarum* with their accession number after submission to Genbank were ORS 3574 (JQ039729), ORS 3593 (JQ039736), ORS 3607 (JQ039737) and ORS 3628 (JQ039740). Each strain was grown in a YEM liquid medium (Vincent, 1972) for 5 days at $28 \pm 2^\circ$ C and their culture was adjusted to get approximately 10^9 cells/ml. The four individual grown cultures were mixed in equal proportions (v/v/v/v) to obtain the rhizobial inoculant (approximately, 10^9 cells/ml).

Experimental design and inoculations

The experiment was conducted in a glasshouse ($30^\circ\text{C}/25^\circ\text{C}$ day/night, 16 h photoperiod) at IRD institute (Bel Air experimental station, $14^\circ44'\text{N}$ - $17^\circ30'\text{W}$) in Dakar (Senegal). Seeds of *A. senegal* collected from Dahra, were scarified and pre-germinated according to Fall *et al.* (2008). Pre-germinated seeds were sown into a Polyethylene bag (23.5 cm x 9.5 cm) containing 800 g of soil substrate slightly moistened with tap water. Seedlings were inoculated with 20 g of AMF inoculant (containing an average of 40 spores/g of soil, mycelia and 80 % of root colonization rate), at sowing. One week after culture, each plant received 5 ml of rhizobial inoculant. The control plants (without mycorrhizal and rhizobial inoculants) received equal amounts of autoclaved inoculum. Four treatments were performed: non-inoculated control (C), plants inoculated with AM fungi (AMF), plants inoculated with rhizobia (R) and plants co-inoculated with AMF and rhizobia (AMF+R). Ten 10 replicates per treatment were arranged in a completely randomized design and plants were watered every 2 days (without nutrient addition) for 6 months.

Analyses

Plant growth and shoot nutrient contents

Plant height was measured every month. After 6 months of culture, plants were harvested and the dry weight of shoots and roots (at 70° C for 48 h) were determined.

Phosphorus (P), Nitrogen (N) and Potassium (K) contents were quantified in shoot dry matter at the laboratory LAMA (Laboratoire des Moyens Analytiques, IRD, Dakar, Senegal, certified ISO 9001, version 2000). After drying, leaf tissues of each plant were ground, ashen (500°C), digested in 2 ml HCL (6N) and 10 ml HNO₃, then analyzed by colorimetry for P and by flame emission for K. Plant tissues were digested in 15 ml H₂SO₄ (36 N) containing 50 g.l⁻¹ salicylic acid for N (Kjeldahl) determination (John, 1970).

Evaluation of nodulation and mycorrhizal parameters

The number of nodules per plant was counted. Mycorrhizal root colonization rate was estimated after clearing and staining with 0.05 % Trypan blue (Phillips and Hayman, 1970; Trouvelot *et al.*, 1986). The density of soil AMF spores was determined according to Gerdemann and Nicolson (1963). The soil hyphal length was measured on membrane filters according to Jakobsen *et al.* (1992), and the total hyphal length was estimated using the Gridline intersects method (Hanssen *et al.*, 1974).

Measurements of soil enzyme activities

Total microbial activity in soil samples was measured using the Fluorescein Diacetate [(3',6'-Diacetylfluorescein), FDA] Hydrolysis Assay (Alef, 1998). The total microbial activity was expressed as µg of product corrected for background fluorescence per hour and per gram of soil.

Phosphatase acid activity was measured in soil samples with 5 mM Disodium p-Nitrophenyl Phosphate Tetrahydrate solution following Tabatabai's (1969) procedure and expressed as µg p-nitrophenol g⁻¹ h⁻¹ at 37° C.

Statistical analysis

All data were subjected to a one-way ANOVA using the XLSTAT software version 2010. Mean values were compared using the Student-Newman-Keuls multiple range test ($p \leq 0.05$). Percentage root mycorrhizal colonization data were arcsine transformed prior to analysis. Using the same software, a Principal Component Analysis (PCA) was carried out for grouping the treatments with their similar characters. The measured parameters with the treatments were projected on the first two axes of the PCA factorial plane. Correlation analysis was carried out to test the covariance among dependent variables by means of the Pearson coefficient using the same software.

Results

Acacia senegal seedling growth after inoculation with AMF and rhizobia

From 4 to 6 months after sowing, the inoculation of *A. senegal* seedlings with AMF and/or rhizobia resulted in a significant increase in plant height (Fig. 1). Six months after transplanting, the plants inoculated either with AMF or rhizobia improved significantly their shoot, root and total dry weight compared to the non-inoculated control ones. The treatments with AMF and rhizobia augmented the total dry weight of plants by 53.80 % and 79.11 %, respectively, whereas the dual inoculation decreased it by 20.25 % (Fig. 2).

Table 1. Physicochemical characteristics of soil used in this study.

Components	Contents
Clay (%)	2.60
Coarse silt (%)	7.56
Fine silt (%)	2.74
Coarse sand (%)	35.83
Fine sand (%)	51.27
Total C (mg/kg)	0.41
Total N (mg/kg)	0.05
C/N ratio	9.00
Total P (mg/kg)	33.12
Available P (mg/kg)	12.66
Ca meq (%)	0.56
Mg meq (%)	0.29
K meq (%)	0.09
Na meq (%)	0.19
pH (H ₂ O)	5.71

Table 2. Mineral nutrition of *Acacia senegal* plants in response to mycorrhizal and rhizobial inoculations after 6 months of culture under non-sterilized soil substrate.

Treatments	N (mg/plant)	P (mg/plant)	K (mg/plant)
Control	19.72 ^{ab} ±0.84	9.36 ^{ab} ±0.40	11.04 ^a ±0.47
AMF	20.97 ^a ±0.91	10.03 ^a ±0.44	11.33 ^a ±0.49
R	19.47 ^{ab} ±1.08	9.52 ^{ab} ±0.53	9.77 ^a ±0.54
(AMF+R)	16.81 ^b ±0.69	8.20 ^b ±0.34	10.43 ^a ±0.43

AMF: arbuscular mycorrhizal fungi; R: rhizobia; N: nitrogen; P: phosphorus, K: potassium

Means (n=10) ± standard errors are given. In column, values followed by the same letter are not significantly different according to the Student Newman-Keuls test ($p \leq 0.05$).

Effect of inoculation with AMF and rhizobia on shoot mineral nutrition

Results on shoot nutrient contents were recorded in Table 2. The inoculation with AMF tended to enhance the amounts of nutrients (N, P and K) in shoots. However, the shoots of plants inoculated with the combination AMF and rhizobia had lower nutrient contents than the controls even if the difference was not significant.

Nodulation and mycorrhization of A. senegal after inoculation with AMF and rhizobia

The results on number of nodules, mycorrhizal root colonization rate and soil mycorrhizal potential were presented in Table 3. The number of nodules per plant was significantly increased by the rhizobial inoculation. The inoculation with both types of microorganisms increased significantly the mycorrhizal root colonization rate compared to their single application and the control plants. The colonization rate was low in the controls and the

plants inoculated with rhizobial strains. The single and co-inoculations with both microorganisms enhanced significantly the spore density by 78.43 % (AMF), 53.93 % (R) and 129.06 % (AMF+R). Between treatments, the highest value was observed with the dual inoculation (AMF+R) and the lowest with rhizobia. In contrast, the effect of single inoculation with AMF or rhizobia on soil hyphal length was significantly positive and more marked than that of the dual inoculation.

Effect of arbuscular mycorrhizal fungal and rhizobial inoculations on soil enzyme activities

The activity of soil acid phosphatase was positively increased by the application of the symbiotic microorganisms as follow: Control < AMF < Rhizobia < (AMF+R) with enhancement of 4.40 %, 170.54 % and 1052.80 %, respectively. In contrast, the soil total microbial activity was not influenced by the single and co-inoculations with the two microsymbionts (Table 4).

Table 3. Nodulation and mycorrhizal parameters of *Acacia senegal* plants in response to mycorrhizal and rhizobial inoculations after 6 months of culture under non-sterilized soil substrate.

Treatments	Rhizobial parameter		Mycorrhizal parameters	
	Nodule Number/plant	Root colonization (%)	Spore density (/50 g ⁻¹ of soil)	Hyphal length (cm.g ⁻¹ of soil)
Control	0.4 ^a ±0.16	16.61 ^a ±2.06	262.67 ^d ±7.62	4.00 ^b ±1.15
AMF	1 ^a ±0.47	19.53 ^a ±1.91	468.67 ^b ±8.51	19.33 ^a ±5.46
R	4.6 ^b ±0.76	17.56 ^a ±1.34	404.33 ^c ±4.10	18.00 ^a ±1.15
(AMF+R)	1.3 ^a ±0.47	50.96 ^b ±3.94	601.67 ^a ±4.63	7.33 ^b ±0.67

AMF: arbuscular mycorrhizal fungi; R: rhizobia

Means (n=10) ± standard errors are given. For spore density and Hyphal length, n=3.

In column, values followed by the same letter are not significantly different according to the Student Newman-Keuls test ($p \leq 0.05$).

Correlation matrix and relationships between parameters and treatments

The results of the correlation matrix (Table 5) showed that plant growth responses (shoot, root and total dry weight) were positively and significantly correlated each other. Shoot N content was significantly and

positively correlated to shoot P content and negatively to soil acid phosphatase activity. The correlation between root mycorrhizal colonization rate and soil acid phosphatase activity was significantly positive whereas that between soil hyphal length and FDA activity was significantly negative.

Table 4. Soil enzyme activities in response to *Acacia Senegal* mycorrhizal and rhizobial inoculations after 6 months of culture under non-sterilized soil substrate.

Treatments	Fluorescein Diacetate Activity ($\mu\text{g FDA h}^{-1} \text{g}^{-1}$ of soil)	Acid Phosphatase Activity ($\mu\text{g pNPP h}^{-1} \text{g}^{-1}$ of soil)
Control	1.55 ^a ±0.07	21.59 ^b ±1.59
AMF	1.28 ^b ±0.07	22.54 ^b ±7.94
R	1.37 ^{ab} ±0.05	58.41 ^b ±23.56
(AMF+R)	1.49 ^{ab} ±0.01	248.89 ^a ±89.78

AMF: arbuscular mycorrhizal fungi; R: rhizobia

Means (n=3) ± standard errors are given. In column, values followed by the same letter are not significantly different according to the Student Newman-Keuls test ($p \leq 0.05$).

A principal Component Analysis (PCA) was performed on the correlation matrix of all data studied (Fig. 3). The first two axes explained about 83.89 % of the total variability. Plant growth (shoot, root and total dry weight), nodule number, shoot nutrient contents (N and P), root mycorrhizal colonization rate, soil hyphal length and enzyme activities were linked to the first axis which explained 57.76 % of the variability. Shoot height and spore density were linked to the second axis which explained 26.14 % of the variability. The treatments

with the parameters can be divided into 3 clusters. The group 1 encompassed the control plants and those inoculated with AMF and were linked to shoot nutrient contents. The plants inoculated with rhizobial strains formed the second group and were correlated to their biomasses and nodulation and to soil hyphal length. The group 3 assembled the plants inoculated with the combination (AMF+R) and was linked to shoot height, root mycorrhizal colonization rate, soil spore density and enzyme activities.

Table 5. Correlation coefficients between plant growth and nutrition parameters, root mycorrhizal datasets and soil microbial activities.

	SH	SDW	RDW	TDW	Nodules	N	P	K	RMC	Spores	Hyphae	FDA	AcP
H	1	-0.493	-0.365	-0.387	-0.455	-0.503	-0.497	0.152	0.825	0.939	0.061	-0.208	0.740
SDW		1	0.968*	0.979*	0.917	0.568	0.674	-0.443	-0.666	-0.264	0.804	-0.658	-0.567
RDW			1	0.999*	0.800	0.686	0.783	-0.244	-0.675	-0.192	0.906	-0.811	-0.617
TDW				1	0.829	0.661	0.761	-0.289	-0.671	-0.201	0.893	-0.787	-0.605
Nodules					1	0.209	0.333	-0.763	-0.420	-0.142	0.626	-0.427	-0.269
N						1	0.990*	0.467	-0.900	-0.590	0.537	-0.559	-0.952*
P							1	0.354	-0.900	-0.538	0.640	-0.640	-0.933
K								1	-0.178	-0.185	-0.150	-0.053	-0.353
RMC									1	0.831	-0.367	0.298	0.983*
Spores										1	0.205	-0.282	0.804
Hyphae											1	-0.973*	-0.352
FDA												1	0.326
AcP													1

*, Correlation is significant at the 0.05 levels (2-tailed)

H: shoot height, SDW: shoot dry weight, RDW: root dry weight, TDW: total dry weight, N: shoot nitrogen content, P: shoot phosphorus content, K: shoot potassium content, Nodules: nodule number, RMC: root mycorrhizal colonization, Spores: spore density, Hyphae: hyphal length, FDA: total microbial activity, AcP: acid phosphatase activity.

Discussion

Most of the previous studies on the effects of symbiotic microorganisms and their interaction in growth and nutrient uptake by legume plants were done under sterilized conditions where competitions with indigenous soil microbiota is limited. In general, the results from these works lead to better increase of single and dual inoculations on plant growth and productivity (Arumugam *et al.*, 2010). However, under natural conditions, several factors including the presence of native microorganisms may alter or reduce the positive impact of the introduced inoculants on plant growth. The aim of this study was to assess the effects of inoculation with selected AMF and rhizobial strains on *A. senegal* seedlings and on soil enzyme activities under unsterilized soil conditions in Senegal.

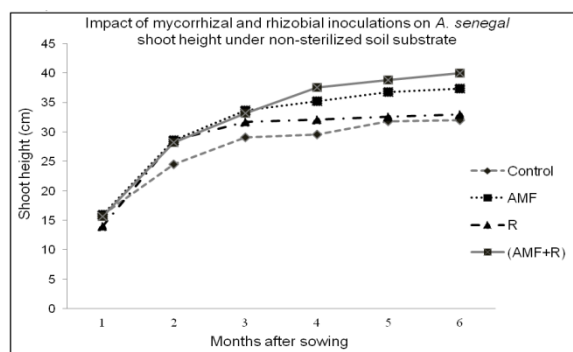


Fig. 1. Impact of mycorrhizal and rhizobial inoculations on *A. senegal* shoot height from 1 to 6 months after growing under non-sterilized soil substrate.

AMF: arbuscular mycorrhizal fungi; R: rhizobia

Our results showed that inoculation of *A. senegal* seedlings with AM fungi improved significantly *A. senegal* plant growth under unsterilized field soil. These confirmed results of several reports on increased plant growth due to AMF inoculation (Gogoi and Singh, 2011; Ndoeye *et al.*, 2012; Kadian *et al.*, 2014). Arbuscular mycorrhizal fungi are known for their beneficial effects even under stress conditions (Ndiaye *et al.*, 2011), and influence plant growth in several manners (Klironomos, 2003). In this study, mycorrhizal inoculated plants recorded higher root colonization rate, nodule number and shoot nutrient (N, P and K) contents. The AM hyphal length and spore density were also higher in soil of

mycorrhizal inoculated plants. Thus, the positive impact of AMF might be attributed to better proliferation of spores and hyphae which extend in several centimeters into the soil and help the plant to uptake mineral nutrients such as P, N, Zn and Cu (Atul-Nayyar *et al.*, 2009; Parkash *et al.*, 2011). AMF contribute to plant growth by improving mineral phosphate solubilization and phosphorus nutrition (Dudhane *et al.*, 2011). Beneficial effects of mycorrhizal fungi in shoot P content might be attributed to its role in phosphorus uptake and translocation through the involvement of phosphatases in the transport of phosphorus (Kadian *et al.*, 2013).

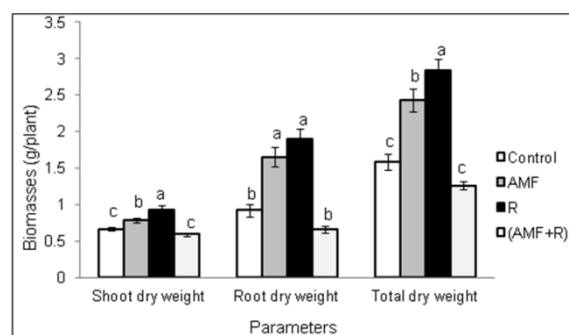


Fig. 2. Growth of *Acacia senegal* in response to mycorrhizal and rhizobial inoculations under non-sterilized soil substrate.

AMF: arbuscular mycorrhizal fungi; R: rhizobia

For each parameter, bars followed by the same letter are not significantly different according to the Student Newman-Keuls test ($p \leq 0.05$).

In this experiment, plants inoculated with rhizobial strains increased their growth, nodulation and mycorrhizal parameters compared to the controls. Similar to our observations, Sajid *et al.* (2011) in groundnut cultivars and recently Bakhoun *et al.* (2012) in *A. senegal* provenances, reported beneficial effects of rhizobial inoculation on plant growth that might be attributed to the rhizobial inoculant used and its highest performance in comparison to the indigenous microorganisms. However, the rhizobial inoculation did not improve the nutrient contents of shoots. This effect of rhizobial inoculation on nutrient contents might be due either to the fact that the formation of nodules requires additional energy and nutrient substances from the plant (Turk *et al.*, 1993),

or the higher plant biomass which can dilute the concentration of nutrients in shoots. The growth improvement might be explained by Plant Growth Promoting Rhizobacteria (PGPR) effect of rhizobia on inoculated plants.

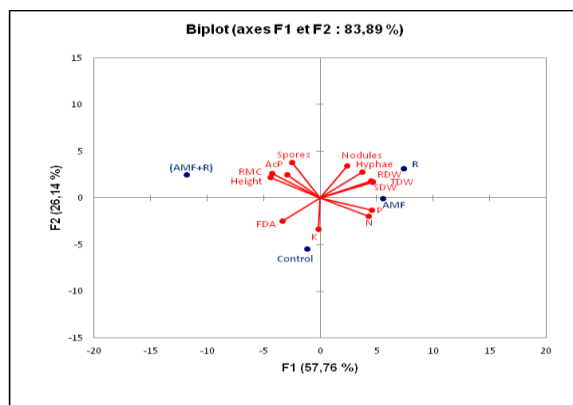


Fig. 3. Principal component analysis among parameters, non-inoculated and inoculated treatments.

SDW: shoot dry weight, RDW: root dry weight, TDW: total dry weight, N: shoot nitrogen content, P: shoot phosphorus content, K: shoot potassium content, Nodules: nodule number, Spores: spore density, Hyphae: hyphal length, RMC: root mycorrhizal colonization, FDA: total microbial activity, AcP: acid phosphatase activity

Furthermore, the dual combination of AMF and rhizobia usually enhanced the growth of legumes (Tajini and Drevon, 2012). Surprisingly, there were no synergistic effects of the dual inoculation on plant biomasses and shoot nutrient contents, while the single application of AMF and rhizobia enhanced all measured parameters except shoot mineral nutrition of rhizobial plants. Similar trends on the negative effect of dual inoculation were observed by Ballesteros-Almanza *et al.* (2010) and by Franzini *et al.* (2010). This result might be assigned to a competition between introduced and native microorganisms. Our results contradicted those of studies which demonstrated synergistic microbial interactions between AMF and rhizobia on plant growth and nutrition (Pindi, 2011; Salahedin *et al.*, 2013). We observed that the dual inoculation produced the highest plants but with less biomass. In the same way, Sgrott *et al.* (2012) found that AMF had no effect on plant height, but increased total

plant biomass. Nevertheless, the dual inoculation increased significantly height and root colonization of plants, and also soil spore density and acid phosphatase activity. This positive correlation was observed by Stancheva *et al.* (2008) in Alfalfa. The significant increase in root mycorrhizal colonization and AMF spore density with the dual inoculation observed in our study is in agreement with reports of Lalitha and Santaguru (2012) on some legume trees. The high acid phosphatase activity recorded in dual inoculation was in accordance with the results of Geneva *et al.* (2006) in pea, and may be due to the commutative effect of the three patterns (plant root, AMF and rhizobia), which possessed acid phosphatase activity (Abd-Alla, 1994).

Our results suggest that root mycorrhizal colonization was not closely related to plant biomass (Wang *et al.*, 2006). Thus, high colonization rates may not be a prerequisite for growth responses in all plants inoculated with AMF (Requena *et al.*, 1996). All these results showed that the mechanisms controlling interactions of bacteria with AMF and plant roots in the mycorrhizosphere and their activities are very difficult to generalize because these interactions vary with microbial species, plant cultivars (Aysan and Demir, 2009) and also with soil and environmental conditions.

Phosphatases are believed to be important for P scavenging and remobilization in plants (Shane *et al.*, 2014). We also observed that the significant increase in soil acid phosphatase activity following dual inoculation was positively correlated to spore density and root mycorrhizal colonization rate, and negatively to plant biomass and mineral nutrition. In fact, Phosphatases are enzymes that help bound P mineralize into soluble forms and make it available to the plants. This P is then absorbed by the plants through the AM colonized roots, thus absorbing maximum soil P. Similar correlations were observed by He *et al.* (2010) after inoculation of *Artemisia ordosica* krasch with AMF. But, these contradicted results of Subramanian *et al.* (2011) who observed higher acid phosphatase activity in rhizobial

inoculated treatment compared to the dual inoculation and control. This indicated that rhizobia are able to solubilize both organic and inorganic phosphates (Alikhani *et al.*, 2006). Also, soil inoculated with either AMF or rhizobia recorded high hyphal length but low acid phosphatase activity whereas the contrary was observed in soil from the dual inoculation. These findings may be due to the efficiency of the microorganisms able to solubilize precipitated P components (Chabot *et al.*, 1996).

Conclusion

The results of the present work demonstrated the beneficial effects of single inoculation with AMF and rhizobia on *A. senegal* seedling growth under non-sterilized soil conditions in Senegal. It is suggested that under certain conditions, the dual inoculation may be deleterious to plant growth; and that, its effects depend on plant species, symbionts involved, and also on soil and environmental conditions. Thus, suitable combinations of AMF and rhizobia may increase plant growth and P use efficiency, enhancing N₂ fixation under limited P supply conditions. Further experiments should be carried out in natural conditions to confirm the beneficial effects of single inoculation with mycorrhizal and rhizobial strains, and also to test the impact of other AMF inoculants alone and in combination with rhizobia in order to facilitate a better growth of *A. senegal*.

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