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ARTICLE

Improvement of tree growth in salt-affected soils under greenhouse conditions using a combination of peanut shells and microbial inoculation

Dioumacor Fall, Niokhor Bakhoum, Fatoumata Fall, Fatou Diouf, Mathieu Ndigue Faye, Cheikh Ndiaye, Valérie Hocher and Diégane Diouf
Full length Research Paper

Improvement of tree growth in salt-affected soils under greenhouse conditions using a combination of peanut shells and microbial inoculation

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This study aimed at selecting an effective microbial inoculum to enhance the performance of Senegalia senegal, Vachellia seyal and Prosopis juliflora and assessing the combination effect of microbial inoculation and peanut shells amendment on their growth under salinity in greenhouse conditions. In the first experiment, seedlings were individually cultivated in plastic bags containing non-sterile sandy soil. Seedlings were inoculated at transplantation with rhizobial and arbuscular mycorrhizal fungi (AMF) strains. Four inoculation treatments were performed: control, inoculation with rhizobia, inoculation with AMF and dual-inoculation with rhizobia and AMF. After one month, seedlings were gradually watered with four saline solutions (0, 86, 171 and 257 mM NaCl) for 4 months. ANOVA showed that inoculation treatments significantly increased seedlings growth particularly in saline conditions and the best performance was obtained with dual inoculation. In the second experiment, seedlings were grown under the same experimental conditions on a mixture of non-sterile sandy soil and 6 tha1 (169.56 g per bag) of peanut shells var 73-33. Results showed that inoculation, peanut shells and their combination significantly improved seedlings growth. The higher performance was obtained with the combination of microbial inoculation and peanut shells.

Key words: Mycorrhiza, rhizobia, organic amendment, Senegalia senegal, Vachellia seyal, Prosopis juliflora, salt tolerance.

INTRODUCTION

Soil salinity is a worldwide environmental problem, which negatively affects soil properties (Sumner, 2000; Kahlown and Azam, 2003). Salt-affected soils contain high exchangeable Na+ percentage, which leads to swelling and dispersion of clays, as well as breaking soil aggregates. Consequently, both water infiltration and water-holding capacity could be reduced, which leads to decreased plant growth and productivity, particularly in...
Revegetation of salt-affected soils is very challenging because, in addition to their high Na⁺ content, they are deficient in nutrients such as nitrogen (N), phosphorus (P) and devoid of organic matter (OM) (Asma et al., 2009). However, their physical, chemical and biological properties can be improved with the application of OM (Wang et al., 2014). These soils can be improved by providing a source of calcium (Ca²⁺) to replace excess sodium (Na⁺) from the cation exchange sites (Qadir et al., 2007). Therefore, the application of OM rich in calcium for soil remediation is important for sustainable land use and plant productivity under salinity (Choudhary et al., 2004; Wong et al., 2009). Recent studies show that peanut shells of variety 73-33 contains high proportion of calcium and could be used to improve saline soils characteristics (Fall, 2016).

In addition to their role in improving soil properties, enhancing plant salt tolerance facilitates growth in saline conditions. The role of biofertilizers such as rhizobia (Diouf et al., 2005; Thrall et al., 2008), Frankia (Dagne et al., 2013; Ngom et al., 2016), arbuscular mycorrhizal fungi (Evelin et al., 2009; Kohler et al., 2010) and plant growth-promoting rhizobacteria (Paul and Nair, 2008; Kohler et al., 2010) in plants tolerating salt stress has been well established in many studies. The bacterial-mycorrhizal-legume tripartite symbiosis is currently being suggested as a possible solution to reforestation (Kohler et al., 2010; Diagne et al., 2013; Soliman et al., 2014; Ngom et al., 2016; Zhu et al., 2016).

Biological nitrogen fixation, which consist of assimilation of atmospheric N in form of organic compounds by rhizobia or Frankia, is a sustainable source of N in cropping systems, as fixed N can be used directly by plants (Jensen and Hauggard-Nielsen, 2003; Garg and Geetanjali, 2007). Phosphorus availability is another limiting factor in salt-affected soils. Due to an extended network of fine hyphae, the arbuscular mycorrhizal fungi (AMF) can considerably improve the uptake of mineral nutrients particularly P and water in host plants (Aggarwal et al., 2011). AMF help plants to alleviate salt stress by enhancing mineral nutrition to compensate for nutrient deficiencies, compensating for nutritional imbalances, improving plant water status, and reducing salt uptake into the host plant (Weissenhorn, 2002).

The improvement of soil physical, chemical and biological properties and the strengthening of plant’s salt tolerance would contribute to better reforestation of salt-affected areas. Senegalia senegal (Syn. Acacia senegal), Vachellia seyal (Syn. Acacia seyal) and Prosopis juliflora are multi-purpose forest legumes widely found in arid and semi-arid zones. They have huge potential in agroforestry systems, fuel wood production, forage, medicinal products and gum production except for P. juliflora (Von Maydell, 1986). Thus, the rehabilitation of salt-affected soils with these species would allow local populations to benefit from their ecosystem services. Thus the objectives of this study are to; select an effective microbial inoculum (rhizobial alone, mycorrhizal alone or dual inoculation) to enhance the performance of S. senegal, V. seyal and P. juliflora under salt conditions and evaluate the combined effect of microbial inoculation and peanut shells amendment on seeding growth of these species in salty soils under greenhouse conditions.

**MATERIALS AND METHODS**

**Plant material**

Seeds of *S. senegal*, *V. seyal* and *P. juliflora* collected in 2013, respectively, at Daha and Ndiaffate for *V. seyal* and *P. juliflora*, were provided by the Centre National de Recherches Forestières (CNRF) of the Institut Sénégalais de Recherches Agricoles (ISRA). Seed scarification and pre-germination were done as described by Fall et al. (2009).

**Rhizobial and fungal inocula preparation**

Pre-germinated seedlings were transplanted into plastic bags (12 cm × 25 cm) filled with non-saline and none sterile soil (Table 1) collected from Sadioga (Centre of Senegal Peanut Basin, 16°23'18"W; 14°03'53"N) at 0 to 25 cm depth. For rhizobial inocula, two strains selected for their symbiotic infectivity and effectiveness in controlled conditions were used for each species. The strains ORS 3574 and ORS 3593 were used for *S. senegal* (Bakhoum et al., 2012), whereas ORS 3356 to ORS 3359 and Pj 34 and Pj 36 were used for *V. seyal* (Diouf et al., 2010) and *P. juliflora* (Dagne and Ingleby, 2003), respectively. Each strain was cultured in glass flasks containing liquid yeast extract mannitol medium (Vincent, 1970) at 30°C for an orbital shaker. For each plant species, the two individual cultures were adjusted to contain approximately the same rhizobial cells number. These cultures were mixed in equal proportions (v/v) to obtain the rhizobial inoculant (approximately 10⁸ cells/ml). For mycorrhizal inocula, a mixture of three AMF species *Golusm fasciculatum* (Thaxter sensu Gerdemam DAOM 227 130), *Rhizophagus irregularis* (Schneck and Smith DAOM 197 198) and *Glomus aggregatum* (IR 27) were used. They were obtained from the LCM collection (Laboratoire Commun de Microbiologie, IRD/ISRA/UCAD, and Dakar, Senegal). Equal amounts of each AMF inoculum were mixed to obtain a composite AM fungal inoculant with an average of 40 spores/g of soil and 80% colonized mycorrhizal roots (Ndoye et al., 2015).

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Table 1. Physical and chemical characteristics of soil collected at Sadioga (Central part of Senegal) in non-saline zone.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical characteristic</td>
<td></td>
</tr>
<tr>
<td>Clay (%)</td>
<td>05.5</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>11.5</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>83.0</td>
</tr>
<tr>
<td>pH 1 H2O</td>
<td>5.5</td>
</tr>
<tr>
<td>Electrical conductivity (at 25°C)</td>
<td>27</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>0.00</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total carbon (%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Total phosphorus (mgkg⁻¹)</td>
<td>52.00</td>
</tr>
<tr>
<td>Calcium (Ca) (méq%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Magnesium (Mg) (méq%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Sodium (Na) (méq%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Potassium (K) (méq%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Cationic exchange capacity (méq%)</td>
<td>2.99</td>
</tr>
</tbody>
</table>

Seedlings inoculation

For each salinity level, four inoculation treatments were performed: non-inoculated or control plants, plants inoculated with rhizobia, plants inoculated with AMF and plants dual-inoculated with rhizobia and AMF. Seedlings were inoculated at transplantation with 5 ml of rhizobial suspension for rhizobial inoculation alone, 20 g of AMF for mycorrhizal inoculation alone and 5 ml of rhizobial suspension and 20 g of AMF for dual inoculation. The control plants received equal amounts of autoclaved inocula.

Salt stress and experimental design

Salt stress was performed one month after transplantation. Plants were gradually exposed to NaCl in order to minimize any salinity shock. Four NaCl concentrations (0, 86, 171 and 257 mM) were tested. NaCl concentrations were increased by 43 mM per day until the required final concentration was reached. The electrical conductivity of the leachate from representative pots was monitored regularly with a salinometer (Digit 100 ATC Salinity pocket refractometer, CETI, Optical Instruments, Belgium) to ascertain actual NaCl concentrations within the rooting medium. Plants were arranged in a randomized complete block with two factors (salinity and inoculation). Each treatment was replicated ten times. Four months after salt stress application, plant height and collar were measured. The plants were harvested and the shoot (leaves + stem + branches) and root dry biomass were evaluated.

Evaluation of the combination of peanut shells and microbial inoculation on plants growth under salinity

After selection of the best inoculum (dual inoculation), the combined effect of microbial inoculation and peanut shell were evaluated under the same experimental conditions. Four NaCl concentrations (0, 86, 171 and 257 mM) were studied. For each species and each salinity level, four treatments with ten replicates were performed: control plants without inoculation and peanut shells, plants with peanut shells alone, plants dual inoculated alone, and plants with peanut shells and dual inoculated. For the supply of peanut shells, they were crushed and mixed with soil at dose of 6 tha⁻¹ corresponding to 169.56 g per bag (12 cm × 25 cm). Chemical characteristics of peanut shells variety 73-33 are presented in Table 2. Pre-germinated seedlings were transplanted into plastic bags with the different treatments. For microbial inoculation, the inocula (rhizobial and AMF) were prepared as described above for each plant species. Seedlings were dual inoculated at transplantation with 5 ml of rhizobial suspension and 20 g of AMF inoculants. Salt stress was gradually applied one month later and plants were arranged in a randomized complete block as described above. The experiment was carried out in greenhouse conditions (30°C / 25°C day / night, 16 h photoperiod) at the LCM-Laboratoire Commun de Microbiologie IRD/ISRA/UCAD in Dakar-Senegal (certified ISO 9001: 2015). Four months after salt stress application, the plant height, collar diameter, shoot (leaves + stems), root and total dry weight were evaluated as described above. The number of nodules per plant was counted. For the assessment of mycorrhizal root colonization (MRC), a subsample of total root mass were cleared at 90°C for 30 min in 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). MRC (corresponding to the proportion of cortex colonized by AMF) was evaluated microscopically using the notation scale described by Trouvelot et al. (1986).

Statistical analyses

Three replicates (three seedlings) per treatment were used for statistical analyses. Two-way-ANOVA analysis was used to assess the effect of salinity, microbial inoculation and their interaction on measured variables in the first experiment. In each experiment, Student-Newman-Keuls’s post-hoc test was used to determine significant differences (P ≤ 0.05) among treatments for various traits. All analyzes were carried out with XLSTAT™ software package (version 2009, Addinsoft, Paris, France).

RESULTS

Selection of microbial inoculum to improve S. senegal, V. seyal and P. juliflora plants growth under salinity

The ANOVA showed that salinity stress and inoculation had a significant (P < 0.05) effect on plant growth parameters (height, collar diameter, shoot and root dry weight) except for the height and collar diameter of V. seyal (Table 3). In the same way, the interaction between salinity and inoculation had a significant effect on these parameters except for height and collar diameter of S. senegal and P. juliflora. Results showed that salinity reduced the growth (height, collar diameter, shoot and root dry weights) of S. senegal, V. seyal and P. juliflora plants (Table 4) whereas microbial inoculation significantly increased plant growth.

This positive effect of microbial inoculation depends on NaCl concentration, the species and type of microorganism used as inoculum. No significant effect of rhizobial, mycorrhizal or dual inoculation was observed on the collar diameter of S. senegal seedlings and the height of V. seyal regardless of NaCl concentrations (Table 4). A significant effect of AMF inoculation and dual inoculation was observed on the collar diameter and shoot dry weight of S. senegal seedlings.
Table 2. Chemical characteristics of peanut shells (var 73-33) used in the study.

<table>
<thead>
<tr>
<th></th>
<th>N total (%)</th>
<th>C total (%)</th>
<th>C/N</th>
<th>P total (g kg⁻¹)</th>
<th>Ca (g kg⁻¹)</th>
<th>K (g kg⁻¹)</th>
<th>Na (g kg⁻¹)</th>
<th>Cl (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>46.24</td>
<td>46</td>
<td>0.61</td>
<td>6.9</td>
<td>4.73</td>
<td>1.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 3. Significance level from two-way ANOVA analysis testing the effects of salinity and inoculation on height, collar diameter (Col. diam.), shoots dry weight (SDW) and root dry weight (RDW) of *S. senegal*, *V. seyal* and *P. juliflora* plants grown on non-sterile soil for four months under greenhouse conditions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Factor</th>
<th>Height</th>
<th>Col. diameter</th>
<th>SDW</th>
<th>RDW</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. senegal</em></td>
<td>Salinity</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>S (p=0.001)</td>
<td>NS (p=0.381)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Salin*Inoc</td>
<td>NS (p=0.480)</td>
<td>NS (p=0.285)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td><em>V. seyal</em></td>
<td>Salinity</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>NS (p=0.590)</td>
<td>NS (p=0.574)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Salin*Inoc</td>
<td>S (p=0.048)</td>
<td>NS (p=0.002)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td><em>P. juliflora</em></td>
<td>Salinity</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>S (p=0.001)</td>
<td>S (p=0.001)</td>
<td>S (p&lt;0.0001)</td>
<td>NS (p=0.847)</td>
</tr>
<tr>
<td></td>
<td>Salin*Inoc</td>
<td>NS (p=0.524)</td>
<td>NS (p=0.200)</td>
<td>S (p&lt;0.0001)</td>
<td>S (p=0.039)</td>
</tr>
</tbody>
</table>

Salin*Inoc = interaction between salinity and inoculation. S = Significant, NS = Not significant.

Inoculation were observed on collar diameter and height in *P. juliflora* plants. No significant difference was observed between AMF inoculation alone and dual inoculation except for the collar diameter of *V. seyal* and the height of *S. senegal* plants grown at 257 mM of NaCl. An increase of 20 and 37% were observed with dual inoculation on collar diameter of *V. seyal* and height of *S. senegal* seedlings at 257 mM NaCl, respectively.

For biomass production, results showed that inoculation with rhizobia, AMF or dual inoculation significantly increased shoot dry weight (SDW) and root dry weight (RDW) of seedlings for all species. However, there were no significant differences between inoculation treatments, the best growth was obtained with dual inoculation. For *S. senegal*, SDW increased by 23, 26 and 49%, respectively with rhizobial, AM fungal and dual inoculation at 257 mM NaCl. RDW of *V. seyal* increased by 11, 64 and 67%, respectively with rhizobial, AM fungal and dual inoculation at the same NaCl concentration (Table 4).

Combined effect of microbial inoculation (dual inoculation) and peanut shells application on plant growth under salinity

Results showed that microbial inoculation, peanut shells or the combination of both improved the growth of *S. senegal*, *V. seyal* and *P. juliflora* plants under salinity conditions (Table 5). The positive effect of these treatments was more pronounced with an increase in NaCl concentration. The best performance was observed with the combination of peanut shells and microbial inoculation, irrespective of species. Collar diameter of *S. senegal* increased by 21, 34 and 126%, respectively, with microbial inoculation, peanut shells alone and combination peanut shells and inoculation at 257 mM NaCl whereas increases of 55, 162 and 169% in the height of *P. juliflora* were observed with the same treatments. The same trend was obtained on collar diameter and height of *V. seyal* plants. All treatments significantly increased shoot dry biomass under salinity, except inoculation in *S. senegal* at 86 mM NaCl (Table 5). No significant difference was observed between peanut shells alone and its combination with microbial inoculation. However, the best aerial growth (SDB) was obtained with combination peanut shells and microbial inoculation. SDW of *S. senegal* plants was increased by 133, 187 and 267%, respectively, with microbial inoculation alone, peanut shells alone and their combination at 257 mM NaCl. The same trend was observed in *V. seyal* with an increase of 126, 409 and 417%, respectively, and in *P. juliflora* with 417, 1280 and 1367% at the same NaCl concentration (Table 5). Peanut shells alone and its combination with microbial inoculation significantly increased root dry weight especially at high level of NaCl concentrations (171 and 257 mM). The best root growth was obtained with the combination of peanut shells and microbial inoculation for all species.
Combined effect of microbial inoculation and peanut shells on nodulation and mycorrhizal root colonization of plants

Table 6 showed the number of nodules and the mycorrhizal root colonization (MRC) of S. senegal, V. seyal and P. juliflora plants grown on non-sterile soil in response to dual inoculation (rhizobia and AMF), amendment with peanut shells and their combination. Results showed that all treatments increased nodulation and mycorrhization of plants compared to control plants.

However, peanut shell application significantly decreased the number of nodules and MRC of plants compared to microbial inoculation alone and the combination of peanut shells and microbial inoculation. Although no significant difference was noted, the number of nodules and MRC seemed to be higher in inoculated plants compared to inoculated and amended ones.

DISCUSSION

The results of the study show a positive significant effect of all microbial inoculation treatments on plant growth. However, the best growth was obtained with dual inoculation (rhizobia + AMF). The beneficial effect of dual inoculation can be attributed, in particular, to enhancement of P and N nutrients uptake (Smith and Read, 2008) which are lacking in salty soils (Hu and Schmidhalter, 2005; Asma et al., 2009), and improved plant water status (Aggarwal et al., 2011).

An improvement in P uptake through mycorrhizae under salt stress increases the plant's ability to reduce the negative effects of Na⁺ and Cl⁻ ions (Feng et al., 2002). Furthermore, mycorrhizal and rhizobial symbioses often act synergistically on infection rate, mineral nutrition and plant growth (Patereze and Cordeiro, 2004; Rabie, 2005) which increases plant tolerance of
Table 5. Height (cm), collar diameter (mm), shoot dry weight (g plant$^{-1}$) and root dry weight (g plant$^{-1}$) of *S. senegal*, *V. seyal* and *P. juliflora* plants grown in non-sterile sandy soil amended with peanut shells and/or inoculated with rhizobia and mycorrhiza, and exposed for four months to four salinity levels under greenhouse conditions.

<table>
<thead>
<tr>
<th>Salinity (mM NaCl)</th>
<th>Inoculation treatment</th>
<th><em>S. senegal</em></th>
<th><em>V. seyal</em></th>
<th><em>P. juliflora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height</td>
<td>Diamet er</td>
<td>SDW</td>
<td>RDW</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>35.3a</td>
<td>4.30b</td>
<td>1.53a</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>36.0a</td>
<td>4.27a</td>
<td>1.57a</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>58.3b</td>
<td>6.23b</td>
<td>4.04b</td>
</tr>
<tr>
<td></td>
<td>Ino+Pea shells</td>
<td>57.0b</td>
<td>5.96b</td>
<td>3.93b</td>
</tr>
<tr>
<td>86</td>
<td>Control</td>
<td>22.3a</td>
<td>4.65b</td>
<td>0.85a</td>
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<tr>
<td></td>
<td>Inoculation</td>
<td>26.3a</td>
<td>4.46b</td>
<td>0.98b</td>
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<td></td>
<td>Peanut shells</td>
<td>33.0b</td>
<td>6.26b</td>
<td>2.44b</td>
</tr>
<tr>
<td></td>
<td>Ino+Pea shells</td>
<td>39.0b</td>
<td>6.39b</td>
<td>2.45b</td>
</tr>
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<td>171</td>
<td>Control</td>
<td>14.3a</td>
<td>3.73a</td>
<td>0.41a</td>
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<td>Inoculation</td>
<td>18.3a</td>
<td>4.71a</td>
<td>0.74b</td>
</tr>
<tr>
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<td>Peanut shells</td>
<td>25.0b</td>
<td>6.20c</td>
<td>1.58c</td>
</tr>
<tr>
<td></td>
<td>Ino+Pea shells</td>
<td>27.0b</td>
<td>6.79b</td>
<td>1.63b</td>
</tr>
<tr>
<td>257</td>
<td>Control</td>
<td>12.3a</td>
<td>3.41a</td>
<td>0.30a</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>20.0b</td>
<td>4.12b</td>
<td>0.70h</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>21.3b</td>
<td>4.58b</td>
<td>0.86bc</td>
</tr>
<tr>
<td></td>
<td>Ino+Pea shells</td>
<td>25.0b</td>
<td>7.72b</td>
<td>1.10c</td>
</tr>
</tbody>
</table>

Diameter = collar diameter; SDW = Shoot Dry Weight; RDW = Root Dry Weight; Ino = dual inoculation (AMF + rhizobia); Ino+Pea shells = combination of microbial inoculation with peanut shells (6 that-1); For each NaCl concentration, values in column sharing the same letter comparing treatments are not significantly different at P<0.05 (Student-Newman-Keuls test). Each value is the mean of three replicates.

salinity stress (Rabie and Almadini, 2005). Similar results have been obtained on *Vigna radiata* by Singh et al. (2011), *Acacia mangium* and *Acacia auriculiformis* (Diouf et al., 2005), *Acacia* spp. (Thrali et al., 2008).

The study further shows that microbial inoculation, peanut shells or their combination significantly improved the growth of *S. senegal*, *V. seyal* and *P. juliflora* plants under salinity conditions. However, the best performance was obtained with the combination of peanut shells and microbial inoculation. The positive effect of this combination could be due to both improvement of soil properties through peanut shells amendment (Fall, 2016), mineral nutrition and water status of plants through microbial symbioses (Parniske, 2008; Ollivier et al., 2011). Indeed, the application of peanut shells increased total carbon, nitrogen, phosphorus, cation exchange capacity in addition to reducing soil salinity (Oo et al., 2013; Wang et al., 2014). The results also showed that dual inoculation (rhizobia + AMF), peanut shells and their combination significantly improved the nodulation and mycorrhization of *S. senegal*, *V. seyal* and *P. juliflora* plants grown under saline conditions. The low negative effect of peanut shells on seedlings
Table 6. Nodules number and mycorrhizal root colonization (%) of *S. senegal*, *V. seyal* and *P. juliflora* plants grown in non-sterile sandy soil amended with peanut shells and/or inoculated with rhizobia and mycorrhiza, and exposed for four months to four salinity levels under greenhouse conditions

<table>
<thead>
<tr>
<th>Salinity (mM NaCl)</th>
<th>Peanut shells (tha⁻¹)</th>
<th><em>S. senegal</em></th>
<th><em>V. seyal</em></th>
<th><em>P. juliflora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NN</td>
<td>MRC</td>
<td>NN</td>
<td>MRC</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>5ᵃ</td>
<td>10ᵃ</td>
<td>4ᵃ</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>30ᵇ</td>
<td>35ᵇ</td>
<td>17ᵇ</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>11ᵃ</td>
<td>24ᵇ</td>
<td>9ᵃ</td>
</tr>
<tr>
<td></td>
<td>Ino+ Pea shells</td>
<td>27ᵇ</td>
<td>30ᵇ</td>
<td>15ᵇ</td>
</tr>
<tr>
<td>86</td>
<td>Control</td>
<td>0ᵃ</td>
<td>3ᵃ</td>
<td>0ᵃ</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>8ᵇ</td>
<td>27ᶜ</td>
<td>10ᶜ</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>3ᵇ</td>
<td>16ᵇ</td>
<td>4ᵇ</td>
</tr>
<tr>
<td></td>
<td>Ino+ Pea shells</td>
<td>6ᵇᶜ</td>
<td>25ᶜ</td>
<td>7ᵇᶜ</td>
</tr>
<tr>
<td>171</td>
<td>Control</td>
<td>0ᵃ</td>
<td>3ᵃ</td>
<td>0ᵃ</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>4ᵇ</td>
<td>19ᵇ</td>
<td>7ᵇ</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>1ᵇ</td>
<td>9ᵃ</td>
<td>2ᵃ</td>
</tr>
<tr>
<td></td>
<td>Ino+ Pea shells</td>
<td>3ᵇ</td>
<td>17ᵇ</td>
<td>4ᵇ</td>
</tr>
<tr>
<td>257</td>
<td>Control</td>
<td>0ᵃ</td>
<td>0ᵃ</td>
<td>0ᵃ</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>1ᵇ</td>
<td>14ᶜ</td>
<td>2ᵇ</td>
</tr>
<tr>
<td></td>
<td>Peanut shells</td>
<td>1ᵇ</td>
<td>4ᵇ</td>
<td>1ᵇ</td>
</tr>
<tr>
<td></td>
<td>Ino+ Pea shells</td>
<td>1ᵇ</td>
<td>13ᵇ</td>
<td>2ᵇ</td>
</tr>
</tbody>
</table>

NN= Number of nodules; MRC= mycorrhizal root colonization; Ino = dual inoculation (AFM + rhizobia); Ino-pea shells = combination of microbial inoculation with peanut shells. For each species, values within a column sharing same letter comparing treatments are not significantly different at P<0.05 (Student-Newman-Keuls test). Each value is the mean of three repetitions.

nodulation and mycorrhization compared to inoculation alone could be explained by the slight inhibition of microbial symbiosis caused by addition of peanut shells. Thus, it is known that relatively high soil nutrient content such as N and P reduce nodulation (Hellsten and Huss-Danell, 2001; Gentili and Huss-Danell, 2002, 2003) and mycorrhization in plants (Nouri et al., 2014). Nodulation and mycorrhization observed in control plants grown in the absence (0 mM) and presence of low (86 mM) NaCl concentrations could be related to the presence of native fungi and rhizobia strains in soil.

Conclusion

The results of this study has shown that microbial inoculation with rhizobial and mycorrhizal selected strains improved the growth of *S. senegal*, *V. seyal* and *P. juliflora* plants under salinity conditions. However, the best growth was obtained with dual inoculation (rhizobia + AMF). The combination of dual microbial inoculation and peanut shells significantly improved height, collar diameter, shoot and root biomass, nodulation and mycorrhization of plants. Indeed, it is well established that the behavior of plants under greenhouse conditions is crucial for survival after
transplantation to natural conditions. However, it is necessary to conduct studies in natural environments to confirm the findings obtained under controlled conditions in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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