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Research Article
Natural Nodulation and AMF Colonization of *Retama raetam* and their Impact on Soil Microbial Properties in Arid Regions of Tunisia

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Abstract

Background and Objective: *Retama raetam* is a wild shrub that plays an important role in the restoration and the maintenance of soil fertility. To our knowledge, this legume has not been examined for its root Arbuscular Mycorrhizal Fungi (AMF) colonization. The aims of this study were to evaluate the natural nodulation and AMF colonization of *Retama raetam* growing in five soils from arid regions of Tunisia and their Impact on soil microbial properties. **Materials and Methods:** Legume nodulating rhizobia associated with *R. raetam* was estimated using the Most Probable Number (MPN) method. The plant mycorrhizal status was assessed by microscopic observation of root fragments after trypan blue staining. The spore density was quantified and was expressed as the total number of spores per 100 g of soil. The effect of soil microorganisms (rhizobia and mycorrhizal fungi) on soil properties was also investigated. **Results:** *Retama raetam* was colonized by both Arbuscular Mycorhizal Fungi (AMF) and rhizobia in all studied sites. The mycorrhizal status and nodulation intensity varied between sites. The highest mycorrhizal and nodulation intensity were observed in Bou Hedma National Park. The AMF colonization was positively correlated with soil microbial properties arbuscular mycorrhizal fungi could effectively participate in the rehabilitation of arid ecosystems of Tunisia. **Conclusion:** Arbuscular mycorrhizal fungi could effectively participate in the rehabilitation of arid ecosystems of Tunisia. *Retama raetam* could be an important alternative to increase soil fertility and to prevent erosion in degrade lands of Tunisia.

Key words: Arbuscular mycorrhizal fungi, arid regions, nodulation, *Retama raetam*, soil microbial properties

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tunisia lies entirely within the arid and semi-arid climate range with an average annual rainfall less[1] than 100 mm annum^{-1}. Most regions of Tunisia are frequently subjected to high temperature and drought spells which destroy natural vegetation and lead to soil erosion and the advance of sand dunes. The use of legume plants can be an alternative to preserve these ecosystems and slow desertification processes. Legumes produce extensive, deep root systems which can develop mycorrhizal and rhizobial symbioses capable of fixing atmospheric nitrogen and absorbing soil nutrients. These associations play a vital role in preserving and even restoring fertility of poor and eroded soils.

Legume plants, because of their symbiotic associations with nitrogen-fixing bacteria (rhizobia) and mycorrhizal fungi can grow on highly degraded soils, contribute to soil stabilization and erosion prevention[2,3]. Legumes are also a major source of timber, phytochemicals, phytomedicines and nitrogen fertility in agrosystems because of their capacity to establish a nitrogen fixing symbiosis with soil bacteria known as rhizobia[4]. Arbuscular Mycorrhizal Fungi (AMF) have been shown to enhance the growth of roots and to help plants to use more efficiently soil nutrients and to grow under hard conditions such as drought, salinity and nutrient deficiency. Additive and sometimes synergistic effects on legume performance are frequently seen when both rhizobia and AMF are present[5]. It is assumed that AM fungi are able to increase nodulation and N fixation by supplying high levels of P to the nodules[6]. Most studies have concentrated on indirect relationships between AMF and rhizobia but there is evidence that arbuscular mycorrhizae may directly and preferentially stimulate nodule function[7].

It is known that microorganisms participate in the maintenance of soil quality and structural stability[8] due to their role in the biogeochemical cycling of elements[9]. Changes on soil quality can be assessed through physical, chemical and biological processes. Biological indicators including, enzymes activities, microbial biomass and soil respiration have been suggested as good indicators of soil quality and productivity[10].

Retama raetam is a wild shrub legume belonging to the Fabaceae. This evergreen legume is well adapted to drought stress and is a good contributor and to preserve fragile ecosystems. So far several studies are available about the rhizobia nodulating *R. raetam*[11-13]. However, little is known concerning the degree of root mycorrhizal colonization of this legume and their effects on soil properties. The purpose of this study is to explain la capacity of this green legume to survive in arid regions of Tunisia and specially the role of their symbiotic associations with nitrogen-fixing bacteria (rhizobia) and mycorrhizal fungi in this adaptation. Therefore, the objective of this study was to evaluate AMF colonization, occurrence of nodulation and to determine to the number of viable rhizobia nodulating *R. raetam* growing in five soils from arid regions of Tunisia. The second objective of this study was to evaluate the influence of *R. raetam* on soil microbiological parameters such as soil microbial biomass and soil respiration.

MATERIALS AND METHODS

Occurrence of nodulation and sampling procedure: During January, 2014 we prospected in the Southern area of Tunisia, corresponding to five arid regions (Table 1). The intensity of nodulation (No. of nodules per plant) and the morphology of nodules were investigated visually. Soil samples were obtained from the top 15-25 cm at each site from both under *R. raetam* and away from canopies. The soil was passed through a 2 mm sieve and stored in the cool shade prior to the experiment. Roots samples were collected to a depth of 25 cm of at least five individual plants of *R. raetam* and stored at 4°C until analyzed.

Soil analysis: The pH values and Electrical Conductivity (EC) were measured by pH meter and conductivity meter, respectively. Soil organic carbon (Corg) was determined by the Walkley and Black method. Total Nirogen (TN) was determined by the Kjeldahl method. Total soil microbial biomass C (Cmic) evaluation were determined by the chloroform fumigation extraction method[14]. Soil respiration was detected by the titration of CO_2 emission as described by

Table 1: Soil characteristics of the study sites

Sites	pH	EC	Corg (%)	Total N (%)	C:N
Site 1: Zarate (33°41′ N, 10°23′ E)	7.28±0.067	1.530±0.035	0.70±0.055	0.10±0.007	6.90±0.64
Site 2: Bou Hedma National Park (34°42′ 47″ N, 9°28′ 27″ E)	7.63±0.056	1.380±0.037	0.60±0.025	0.10±0.009	5.91±0.38
Site 3: Matmata (33°28'60" N et 10°4'0" E)	7.46±0.055	1.390±0.042	0.74±0.016	0.11±0.009	6.84±0.46
Site 4: Bengardenne (33°7 59″ N, 11°12 58″ E)	7.53±0.096	1.632±0.048	0.59±0.022	0.10±0.009	5.74±0.67
Site 5: Nafta (33°52' 23.12" N, 7°52' 39.54" E)	7.63±0.081	1.320±0.050	0.67±0.029	0.12±0.009	5.77±0.67

C:N: Carbon nitrogen ratio, Corg: Organic carbon, EC: Electrical conductivity, TN: Total nitrogen

Ohlinger[15] and qCO_2 was calculated by diving CO_2-C released from the sample by Cmic content.

Estimation of legume-nodulating rhizobia populations: The enumeration of legume nodulating rhizobia associated with *R. raetam* was estimated using the Most Probable Number (MPN) method. Sterilized seeds were germinated in petri dishes containing soft agar (1%) as described by Mahdhi *et al.*[12]. One seedling was aseptically transplanted into plastic pots filled with autoclaved vermiculite. Inoculation was performed 48 h after transfer with 2 mL of soil diluted suspensions. Four replicates were considered. The pots were placed in a growth chamber at 23°C with a 12-16 h photoperiod and watered daily with sterilized distilled water. Thirty days latter, the presence or absence of nodules were recorded and the MPN was calculated according to Bennett *et al.*[16].

Assessment of root colonization by AM fungi: In the laboratory plant roots were washed with sterile water, cleared by heating in 10% KOH at 90°C for 1 h, bleached by immersion in 10% H_2O_2 for 5 min, acidified in dilute HCl and stained with 0.05% trypan blue in lactophenol[17]. The duration of staining depended upon the respective root diameter. Stained roots were checked for AMF infection by examination under a compound microscope and the mycorrhizal colonization percentage was obtained[18]. A minimum of 300 root segments per plant were counted. Mycorrhizal intensity (M%) is defined as the proportion of the root invaded by endomycorrhizal fungi:

$$M\ (\%) = \frac{95n5+70n4+30n3+5n2+n1}{N}$$

with, n is number of fragments assigned with the index 0, 1, 2, 3, 4 or 5 and N is number of the observed fragments.

Isolation, enumeration of AM fungal spores: The AMF was extracted from 100 g soil samples. The AMF spores were isolated by wet-sieving and sucrose centrifugation[19]. Quantification was carried out in petri dishes under a stereoscopic microscope. The spore density was expressed as the total number of spores per 100 g of soil[20].

Statistical analyses: Statistical analyses were performed with a SAS statistical package. The data were subjected to ANOVA and the statistical effects of sites were tested. Comparisons among means were made using the Least Significant test at the 5% levels of significance ($p<0.05$).

RESULTS

Soil chemicals properties: The soil characteristics of the study sites are given in Table 1. Data showed that the pH and Electrical Conductivity (EC) levels were remarkably similar and all soils were basic (pH>7). The high total nitrogen was observed in site 5 (0.12%). Carbon contents appeared to be higher in the site 1 and 3. The high carbon:nitrogen ratio was also observed in site 1 (6.9 ± 0.64).

Occurrence of nodulation: Result show that *R. raetam* was nodulated in the five studied sites and there was not significant variation between sites studied ($F = 3.227$, $p = 0.088$). The high number of nodules (7.5 ± 0.424) was observed in the site 4 (Fig. 1).

Nodules from *R. raetam* were elongate with continuing apical meristematic (indeterminate) growth. The size of the nodules varied from 10-15 mm. The external colour of the nodules is white or white-brown and pink in cross-section indicative of the presence of leghaemoglobin.

Estimation of legume-nodulating rhizobia populations: Figure 2 presents the MPN of rhizobia that able to nodulate *R. raetam* in the soil. The populations of indigenous LNB nodulating *R. raetam* varied significantly between sites ($F = 89.854$, $p<0.001$). The values ranged between 2×10^2 to 10^4 bac g^{-1} soil, the highest values were observed in site 2.

Soil microbiological properties: Mean values of microbial properties are presented in Fig. 3. The results indicate that rhizosphere of *R. raetam* in site 2 (Bouhedma) contains the highest Cmic (386.3 ± 8.13 µg C g^{-1} soil) which is an indicator of good soil quality. In all sites the values were also lower in

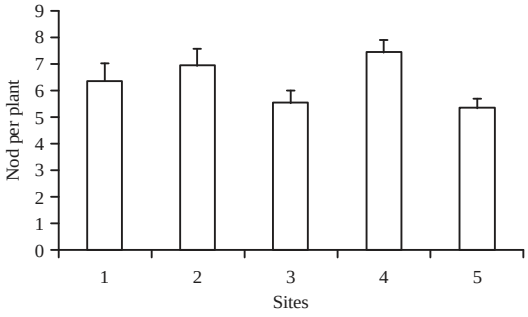

Fig. 1: Number of nodules for *Retama raetam* in natural conditions. Error lines correspond to standard deviation (n = 12)

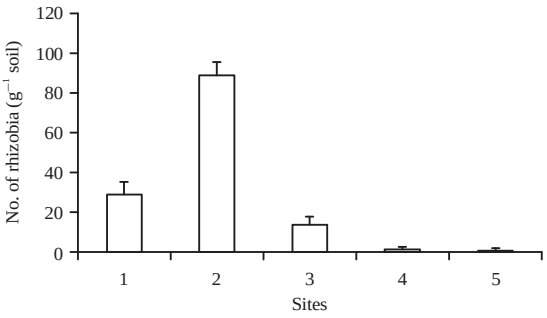

Fig. 2: Most Probable Number (MPN) of rhizobia that able to nodulate *Retama raetam* in the soil. Error lines correspond to standard deviation (n = 3)

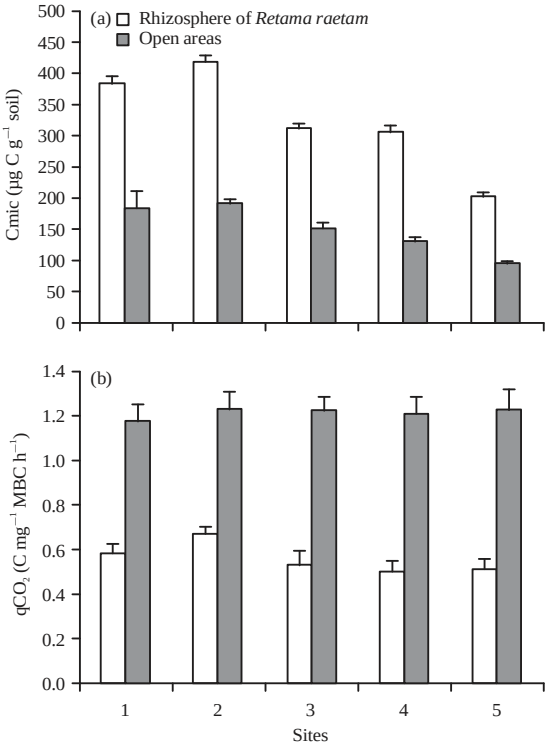

Fig. 3(a-b): (a) Rhizosphere soil microbial biomass carbon (Cmic) and (b) Metabolic quotient (qCO_2) in the studied soil. Error lines correspond to standard deviation (n = 3)

open areas, compared to canopied soils. The analysis of variance was found to be highly significant between sites and Cmic (F = 170, 735, p<0.001).

As regards metabolic quotient, we found that values beneath *R. raetam* were lower than bare soils. The metabolic quotient (qCO_2) was similar for all evaluated sites. There was not significant variation in qCO_2 between studied sites (F = 2.8, p = 0.085). The highest value was observed in site 2 (0.676±0.027).

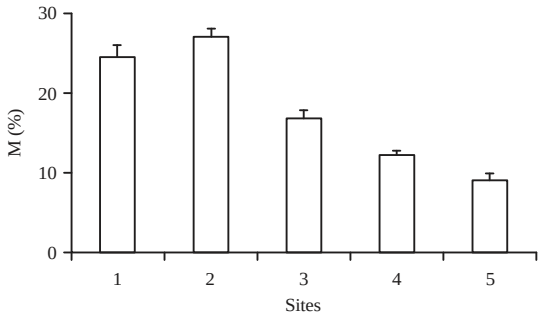

Fig. 4: Intensity of arbuscular mycorrhizal colonization (M%) of *Retama raetam* in the studied soils. Error lines correspond to standard deviation (n = 3)

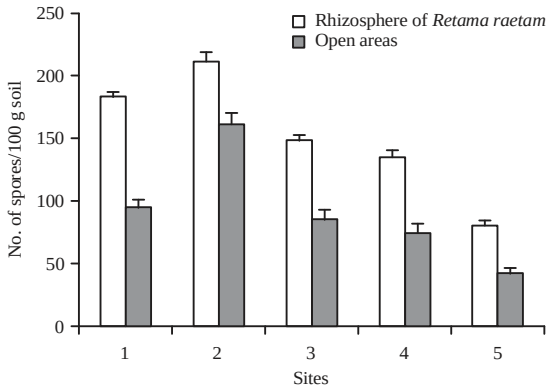

Fig. 5: Spores number of the AM fungal in the studied soils. Error lines correspond to standard deviation (n = 3)

Assessment of root colonization by AM fungi: The intensity of arbuscular mycorrhizal colonization (M%) of *R. raetam* are presented in Fig. 4. The AMF colonization values differed significantly among sites ($F_{4,10}$ = 83.432, p<0.001). The maximum percentage of root infection was recorded in site 2 (M% = 27.1±0.88%), whereas the minimum infection from site 5 (M% = 9.26±0.62%). In all sites observed *R. raetam* root segments contained typical hyphae, arbuscules and vesicules in the root cortex, although not necessarily in the same root segment. Most commonly, AMF hyphae were intercellular, but we also observed with a lower frequency, intracellular hyphae. Extra-radical spores have also been observed in some root segments.

Number of spores: The AMF spores were present in all soil samples (Fig. 5). The AM fungal spores numbers significantly varied in the rhizospheres of *R. raetam* growing in studied sites in this study ($F_{4,10}$ = 112.832, p<0.001) and ranged from 81.33±2.90 (site 5) to 211.66±7.11 spores per 100 g

Table 2: Pearson correlation coefficient between different studied parameters

Parameters	Cmic	qCO_2	MPN	No. of spores	C:N	TN	Corg	M (%)	pH	EC	Nod
Cmic	1.000	0.596[a]	0.792[c]	0.980[c]	-0.534[a]	0.855[c]	0.784[c]	0.931[c]	ns	ns	ns
qCO_2		1.000	0.697[b]	0.610[a]	-0.621[a]	0.692[b]	ns	0.631[a]	ns	ns	ns
MPN			1.000	0.822[c]	-0.592[a]	0.812[c]	0.622[a]	0.837[c]	ns	ns	ns
No. of spores				1.000	-0.545[a]	0.857[c]	0.766[c]	0.951[c]	ns	ns	ns
C:N					1.000	-0.760[c]	ns	-0.590[a]	ns	ns	ns
TN						1.000	0.797[c]	0.861[c]	ns	ns	ns
Corg							1.000	0.745[c]	ns	ns	ns
M%								1.000	ns	ns	ns
pH									1.000	ns	ns
EC										1.000	ns
Nod											1.000

[a]Significant at p<0.05, [b]Significant at p<0.01, [c]Significant at p<0.001, ns: Non significant, M%: Intensity of mycorrhization, Cmic: Microbial biomass, qCO_2: Metabolic quotient, C:N: Carbon nitrogen ratio, Corg: Organic carbon, TN: Total nitrogen, MPN: Most probable number, EC: Electrical conductivity, Nod: No. of nodules

dry soil (site 2). In all sites spores number in the rhizosphere of *R. raetam* was high than control soil (open areas).

Correlation between different parameters: The correlation matrix (Table 2) showed that the intensity of arbuscular mycorrhizal colonization was positively correlated with spores number (r = 0.951, p<0.001) with soil microbial biomass (r = 0.931, p<0.001) and with the MPN of rhizobia (r = 0.837, p<0.001). The Cmic was positively correlated with MPN (r = 0.792, p<0.001), spores number (R = 0.980, p<0.001) and with the metabolic quotient (qCO_2) (r = 0.596, p = 0.019). Number of spores was also positively correlated with MPN (r = 0.822, p<0.001) and with qCO_2 (r = 0.610, p = 0.016). Correlations between pH, Electrical Conductivity (EC), number of nodules and other variables were not significant at p<0.05.

DISCUSSION

Many legumes have the possibility to associate with both rhizobia and AMF. This makes legumes as an important plant functional group since they can form a tripartite symbiosis with nitrogen-fixing bacteria and phosphorus-acquiring arbuscular mycorrhizal fungi[21,22]. In extreme ecosystemic conditions, rhizobia and AMF have been shown to enhance the growth of roots and to help plants to use more efficiently soil nutrients and to grow under hard conditions such as salinity, drought and nutrient deficiency. In this study the occurrence of natural nodulation, the MPN of rhizobia that able to nodulate *R. raetam* in the soil, spores number and root colonization by AMF were determined. In addition the objective of this study was to determine whether *R. raetam* modi ed soil quality.

The MPN technique is a means to estimate microbial population sizes[23]. The technique is widely used to enumerate rhizobia based upon the ability of rhizobia to nodulate appropriate host legume plants. This test is based on the assumption that bacteria are randomly distributed and that one or more rhizobia are capable to form roots nodules on an appropriate host[24]. The low number of MPN were recorded in all sites (Fig. 2). This could be explained by the sensitivity of the MPN assay employed that was not high enough to detect the low population of rhizobia nodulating *R. reatam.* In addition rhizobial populations may be affected by several conditions. This reflected in the lowest MPN estimate with *R. raetam* in the site 4 and 5 characterized by high soils temperatures. Results of occurrence of nodulation show that *R. raetam* was nodulated in the five studied sites but the number of nodules is very low (<8 nodules per plant). This can be explained by the effect of several environmental conditions.

Zahran[25] showed that legume rhizobia association and especially nodule formation are very sensitive to salt and osmptic stress. In arid regions, several environmental conditions affects both the free-living and symbiotic life of rhizobia themselves. According to Becquer and Prevost[26] diverse factors can affect the symbiosis and prevent the appearance of nodules. All sites reported in this study are in arid area of Tunisia, where rainfall does not exceed 180 mm and soil temperature exceeds 35°C. In addition, phosphorus deficiency of the Tunisian arid soils could be considered the main barriers for the formation of nodules in the natural macrosymbionts.

This study reveals that AM fungi are present in wide range of Tunisian soils. The AM fungi colonization was observed in the roots of *R. raetam* in all sites prospected in this study. This suggested that AMF may take an important role in the developing and sustaining of *R. raetam* in arid regions of Tunisia. In addition, AM fungal spores numbers in the rhizospheres of *R. raetam* where higher than open area in all sites. These results agree with those by Bagayoko *et al.*[27] and Sun *et al.*[28] which found that the legumes plants were much

mycotrophic that Poaceae and other families. The highest intensity of arbuscular mycorrhizal colonization (M%) of *R. raetam* was observed in site 1 and 2 characterized by a high vegetation biomass (protected zones and park). Facelli *et al.*[29] showed that AMF colonization levels can decrease with increasing vegetation biomass because of increased competition for infective AMF units. In all sites, root colonization was positively related to the availability of infective AMF units (e.g., spores) in the soil as described by Garrido *et al.*[30].

The spore densities found in this study are relatively high, ranging from 81-211 spores per 100 g dry soil. The abundance of AMF spores in the different soils prospected in this study suggests that these soils may be characterized by high diversity of AMF. Microscopic observation of spore suspensions in our study showed a dominance of small spores, compatible in shape and size to those of Glomus. The dominance of small spores may be a selective adaptation to water stress[31]. The number of spores in the rhizosphere of *R. raetam* is high than the open areas. This is consistent with other studies showning that some plant species have the ability to promote the development of fungal propagules in their rhizosphere and the infected roots producing a diversity of spores[32].

For soil fertility and dynamics analyses, many parameters such as soil chemical, soil microbial biomass has potential to be used as indicators of soil quality. Therefore, the second objective of this study was to evaluate the influence of *R. raetam* cover on soil microbiological parameters such as soil microbial biomass and soil respiration.

Compared with open areas, *R. raetam* soils were characterized by a low C:N ratio, which demonstrated the higher soil enrichment with nitrogen N within the *Retama* habitat. This could be explained by the high N content of *R. raetam* litter favored by their capacity to fix atmospheric nitrogen that improves the N status of soil. The high soil nitrogen and organic carbon content showed in the soil under *R. reatam* gives a particular interest of this legume to the fertility of the studied sites.

In this study, a higher soil microbial biomass values were recorded under *R. raetam* canopies. Similar results were reported by Traore *et al.*[33], Cao *et al.*[34] and Bhattarai *et al.*[35] showed that showed plants favor microbial growth in soil and then improve soil microbial. Microbial respiratory activity is an indication of carbon utilization efficiency[36]. The qCO_2 is a measure of how effective microorganisms are in the studied sites. Thus, significant decreases in qCO_2 values with open area reflected a more efficient use of organic substrates by microbial communities associated to *Acacia tortilis*[37]. In contrast for open areas, higher metabolic quotient indicated that important quantities of carbon per unit biomass were lost through respiration of substrates incorporated into microbial biomass.

The analysis of our results showed also a clear, significant correlation between nodulation, mycorrhization and soil microbial properties. This positive correlation can be explained by the important roles of the symbiotic organisms essentially rhizobia and AMF when they influence soil structure, fertility and therefore facilitate the development of vegetation cover[38]. Soil Cmic and qCO_2 are used as indicators of soil development or degradation and changes in soil quality[39].

CONCLUSION

In conclusion, this study is the first report on the importance and the natural diversity of AM fungi in the rhizosphere of *R. raetam* in Tunisia. The results reported the presence of tripartite symbiosis (*R. raetam*-rhizobia-AMF) in all studied sites. The highest mycorrhizal and nodulation intensity were observed in Bou Hedma National Park. The present study reported also that soil microbial properties in different studied sites were improved with the presence and levels of AMF colonization roots. Thus, *R. raetam* could be an important alternative to increase soil fertility and to prevent erosion in degrade lands of Tunisia.

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REFERENCES

1. Ferchichi, A., 1996. La lutte contre l'ensablement et pour la stabilisation des dunes: Essai de la fixation biologique des dunes en Tunisie presaharienne. Recherches sur la desertification dans la Jeffara. Rev. Tunis. Geogr., 12: 49-102.
2. Fagg, C.W. and J. L. Stewart, 1994. The value of *Acacia* and *Prosopis* in arid and semi-arid environments. J. Arid Environ., 27: 3-25.
3. Werner, G.D.A., W.K. Cornwell, J.H.C. Cornelissen and E.T. Kiers, 2015. Evolutionary signals of symbiotic persistence in the legume-rhizobia mutualism. Proc. Natl. Acad. Sci. USA., 112: 10262-10269.
4. Laranjo, M., A. Alexandre and S. Oliveira, 2014. Legume growth-promoting rhizobia: An overview on the *Mesorhizobium* genus. Microbiol. Res., 169: 2-17.

5. Lekberg, Y. and R.T. Koide, 2005. Arbuscular mycorrhizal fungi, rhizobia, available soil P and nodulation of groundnut (*Arachis hypogaea*) in Zimbabwe. Agric. Ecosyst. Environ., 110: 143-148.
6. George, E., H. Marschner and I. Jakobsen, 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. Biotechnol., 15: 257-270.
7. Xie, B., W.N. Davidson III and P.J. DaDalt, 2003. Earnings management and corporate governance: The role of the board and the audit committee. J. Corporate Finance, 9: 295-316.
8. Bastida, F., J.L. Moreno, T. Hernandez and C. Garcia, 2007. The long-term effects of the management of a forest soil on its carbon content, microbial biomass and activity under a semi-arid climate. Applied Soil Ecol., 37: 53-62.
9. Roldan, A., F. Caravaca, M.T. Hernandez, C. Garcia, C. Sanchez-Brito, M. Velasquez and M. Tiscareno, 2003. No-tillage, crop residue additions and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). Soil Tillage Res., 72: 65-73.
10. Garcia, C., T. Hernandez and F. Costa, 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal., 28: 123-134.
11. Mosbah, M. and M. Mars, 2006. Genotypic diversity of rhizobia isolated from *Retama raetam* in arid regions of Tunisia. Ann. Microbiol., 56: 305-311.
12. Mahdhi, M., A. Nzoue, P. de Lajudie and M. Mars, 2008. Characterization of root-nodulating bacteria on *Retama raetam* in arid Tunisian soils. Progr. Nat. Sci., 18: 43-49.
13. Farida, B., D. Geraldine, B. Abdelghani, B. Djellali, B. Said and L. Gisele, 2009. *Retama* species growing in different ecological-climatic areas of Northeastern Algeria have a narrow range of rhizobia that form a novel phylogenetic clade within the *Bradyrhizobium* genus. Syst. Applied Microbiol., 32: 245-255.
14. Amato, M. and J.N. Ladd, 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem., 20: 107-114.
15. Ohlinger, R., 1995. Soil Respiration by Titration. In: Methods in Soil Biology, Schinner, F., E. Kandeler, R. Ohlinger and R. Margesin (Eds.). Springer, Berlin, pp: 95-98.
16. Bennett, J.E., P.L. Woomer and R.S. Yost, 1990. Users manual for MPNES most-probable-number enumeration system, Version 1.0. NifTAL Project and University of Hawaii, Honolulu, Hawaii, USA.
17. Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.
18. Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.
19. Gerdemann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235-244.
20. McKenney, M.C. and D.L. Lindsey, 1987. Improved method for quantifying endomycorrhizal fungi spores from soil. Mycologia, 79: 779-782.
21. Tajini, F., M. Trabelsi and J.J. Drevon, 2011. Co-inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases P use efficiency for N_2 fixation in the common bean (*Phaseolus vulgaris* L.) under P deficiency in hydroaeroponic culture. Symbiosis, 53: 123-129.
22. Abd-Alla, M.H., A.W.E. El-Enany, N.A. Nafady, D.M. Khalaf and F.M. Morsy, 2014. Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol. Res., 169: 49-58.
23. Ben-David, A. and C.E. Davidson, 2014. Estimation method for serial dilution experiments. J. Microbiol. Methods, 107: 214-221.
24. Woomer, P., P.W. Singleton and B.B. Bohlool, 1988. Ecological indicators of native rhizobia in tropical soils. Applied Environ. Microbiol., 54: 1112-1116.
25. Zahran, H.H., 1998. Structure of root nodules and nitrogen fixation in Egyptian wild herb legumes. Biol. Plant., 41: 575-585.
26. Becquer, C.J. and D. Prevost, 2014. Nodule formation potential in forage and grain legumes from rhizobia indigenous to Sancti Spiritus, Cuba. Cuban J. Agric. Sci., 48: 301-307.
27. Bagayoko, M., A. Buerkert, G. Lung, A. Bationo and V. Romheld, 2000. Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: Soil mineral nitrogen, mycorrhizae and nematodes. Plant Soil, 218: 103-116.
28. Sun, X., W. Hu, M. Tang and H. Chen, 2016. Characterizing and handling different kinds of AM fungal spores in the rhizosphere. World J. Microbiol. Biotechnol., Vol. 32. 10.1007/s11274-016-2053-0.
29. Facelli, E., J.M. Facelli, S.E. Smith and M.J. McLaughlin, 1999. Interactive effects of arbuscular mycorrhizal symbiosis, intraspecific competition and resource availability on *Trifolium subterraneum* cv. Mt. Barker. New Phytol., 141: 535-547.
30. Garrido, E., A.E. Bennett, J. Fornoni and S.Y. Strauss, 2010. Variation in arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to above-ground defoliation. J. Ecol., 98: 43-49.
31. Picone, C., 2000. Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. Biotropica, 32: 734-750.
32. Azcon-Aguilar, C., J. Palenzuela, A. Roldan, S. Bautista, R. Vallejo and J.M. Barea, 2003. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. Applied Soil Ecol., 22: 29-37.

33. Traore, S., L. Thiombiano, J.R. Millogo and S. Guinko, 2007. Carbon and nitrogen enhancement in Cambisols and Vertisols by *Acacia* spp. in Eastern Burkina Faso: Relation to soil respiration and microbial biomass. Applied Soil Ecol., 35: 660-669.
34. Cao, C.Y., D.M. Jiang, X.H. Teng, Y. Jiang, W.J. Liang and Z.B. Cui, 2008. Soil chemical and microbiological properties along a chronosequence of *Caragana microphylla* Lam. plantations in the Horqin sandy land of Northeast China. Applied Soil Ecol., 40: 78-85.
35. Bhattarai, A., B. Bhattarai and S. Pandey, 2015. Variation of soil microbial population in different soil horizons. J. Microbiol. Exp., Vol. 2. 10.15406/jmen.2015.02.00044.
36. Saviozzi, A., R. Levi-Minzi, R. Cardelli and R. Riffaldi, 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant Soil, 233: 251-259.
37. Fterich, A., M. Mahdhi and M. Mars, 2011. Impact of grazing on soil microbial communities along a chronosequence of *Acacia tortilis* subsp. *raddiana* in arid soils in Tunisia. Eur. J. Soil Biol., 50: 56-63.
38. Caravaca, F., M. Alguacil, P. Torres and A. Roldan, 2005. Plant type mediates rhizospheric microbial activities and soil aggregation in a semiarid Mediterranean salt marsh. Geoderma, 124: 375-382.
39. Kaschuk, G., P.A. Leffelaar, K.E. Giller, O. Alberton, M. Hungria and T.W. Kuyper, 2010. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. Soil Biol. Biochem., 42: 125-127.