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Symbiotic Efficiency of *Acacia cyanophylla* Lindl and *Acacia pycnantha* Benth in Four Soil Types

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Authors' contributions

This work is a part of the PhD of the author AI and was carried out in collaboration between all authors. Authors AI and IM wrote the first draft of the manuscript, managed the literature searches, analyses of the study. Authors IJ and NL help designed the study and managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims to evaluate the interaction between soil rhizobia and legume plant, by comparing two *Acacia* species (*Acacia cyanophylla* Lindl and *Acacia pycnantha* Benth) grown in four different Moroccan soils, by measuring plant dry matter yield, and nodule numbers and weights. Plants were harvested 9 month after seeding. We observed that *Acacia cyanophylla* Lindl nodulated in all the four soils. Plant nodule numbers and yield were significantly enhanced by native soil rhizobia. *Acacia pycnantha* Benth nodulated only in one type of soil. After nodulation and isolation of nodules, *A. cyanophylla* was nodulated by rhizobia strains. In this experiment, the statistical results revealed a significant correlation between the number of nodules per plant and plant height ($r = .87$, $P < .05$), these results show that adequate nodulation improves the establishment and the plant growth. We recommend using rhizobial biodiversity in arid and desert areas if the plants do not have the ability to establish a symbiosis with native rhizobia. Inoculation of *Acacia* seeds during the establishment in nurseries improves growth and N2 fixation. The symbiotic nitrogen-fixing bacteria have been selected and conserved in Moroccan collection. These rhizobia may be used to inoculate wild, as well as, crop legumes, cultivated in reclaimed desert lands.

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1. INTRODUCTION

Legumes play a significant role in both agricultural and natural ecosystems by having a determinant role on the nitrogen cycle. The beneficial microorganisms that are present in the soil rhizosphere help crops to maintain growth and sustain productivity. The bacteria infect the plant root and induce the formation of nodules [1].

Symbiosis is well known to be highly specific plant-microbe interaction. This interaction is beneficial for plant growth, yield and crop quality. Indeed, this symbiotic relationship between legumes and rhizobia represents the most important nitrogen-fixation association. According to the early surveys of symbiotic specificity [2], legumes were suggested to comprise a range of taxonomically restricted cross-inoculation groups. Trees and shrubs of the genus *Acacia* from Australia have been widely introduced in tropical and subtropical regions [3,4]. Legume trees are gaining considerable interest in agroforestry in the arid and Mediterranean region. *Acacia* is a tree or shrub, spiny or spineless, with a bark of varying colors depending on the species. A majority of the *Acacia* microsymbionts are classified within the genera *Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* [5-9].

In our study, we have chosen a very promising legume*: Acacia cyanophylla,* which is a spreading shrub forage, with an average height of 4 to 5 m and can reach 7-8 m deep soil. The stem is usually much branched. The bark is smooth greenish gray color, and the foliage is evergreen. The roots of this legume are very powerful in both area and depth. The species is a native of Western Australia and was introduced in the southern Mediterranean basin, especially Morocco and Libya, for soil stabilization purposes, *A. cyanophylla* is a species that can produce an important biomass (woody and leafy), can be used as green manuring to enrich the soil, fodder for cattle and domestic energy [10-12]. It protects the environment against erosion and produces multi-use wood. *A. pycnantha* is *a* shrub or small tree with simple 'leaves' that are bright green or dull green in color. This species grows naturally in dry sclerophyll forests, open woodlands, shrub lands, heathlands and grasslands. It is mainly found growing in sandy and stony soils, but also occurs in red loams.

However, the aims of the present study were to evaluate the effect of soil and its native *Rhizobium* on growth and nodulation of *A. cyanophylla; A. pycnantha* and isolate *Rhizobium* strains from them.

Consequently, it is important to isolate and characterize bacteria from local conditions that can be used as potential inoculant in the same area where they are obtained. The advantage of soil isolates was shown to be more adapted and successful when used as inoculum.

2. EXPERIMENTAL DETAILS

2.1 Soil

The soil samples were collected from 4 sites of morocco at a depth 0-30 cm, each sample of soil was mixed with sand washed and sterilized twice for 30 min (30% sol - 70% sand), then filled in plastic pots. The physico-chemical analysis of each soil (Table 1).

2.2 Seed

The seeds of *A. cyanophylla* and *A. pycnantha* were delivered by DREF *(Regional Forestry Service)*, these seeds were previously sterilized with concentrated H_2SO_4 for 30 min and then rinsed several times with sterile water and processed in boiling water for 24 hours, were sowing in soil from four different sites in Morocco.

2.3 Experimental Design

The experiments were conducted in a greenhouse in the Faculty of Science, Meknès, Morocco. Under natural light conditions, maximum temperatures varied between 30-42°C and minimum of 15-28°C, maximum relative humidity of 80-95% and a minimum of 25 to 45%. The pots were arranged in a completely randomized design with four replications. Watering was done regularly with tap water. Nutrient solution devoid of nitrogen for plant growth [13] was applied once a week to compensate for losses of nutrients.

Table 1. Physical and chemical properties of experimental soil (0-30 cm)

		Soil 1	Soil 2	Soil 3	Soil 4
pH	H_2O	7,8	8,0	7,35	8,30
	KCl	7,4	7,6	7,10	7,6
TOTAL CARBON %		1,07	0,92	1,13	0,46
ORGANIC MATTER %		1,86	1,58	1,95	0,79
TOTAL NITROGEN %		0,08	0,07	0 ,20	0,06
AVAILABLE PHOSPHORUS P PPM		15,28	6,55	8,73	15,28
CA MEG /100		40,5	42,25	12,75	21,00
MG MEG/100		4,5	1,0	1,5	4,00
CLAY		23,4	24,4	10,6	7,6
FINE SILT		18,90	13,50	13,30	10,10
COARSE SILT		25,20	27,00	8,70	14,80
FINE SAND		20,80	23,30	22,40	21,30
COARSE SAND		11,90	10,80	44,30	45,80a

Soil 1 and Soil 2 were collected near Safi with rainfall of 178 mm / year; Soil 3 and 4 were collected near Youssoufia 90 km east of Safi with rainfall of 385 mm / year

2.4 Nodule Collection

Nine months after sowing, the fresh roots of *A. cyanophylla* and *A. pycnantha* plants were washed under running water with a screen underneath to catch the detached nodules. In the laboratory the roots with intact and undamaged nodules are cut, 3-5 mm on each side of the nodules. Before isolation, nodules were surface sterilized by immersing them in 95% ethanol for 5–10 s, then transferred to a solution of mercuric chloride ($HgCl_2$) acidified at 0.1% (w / v) for 3 minutes, at the end the nodules were washed for 5–10 times by sterilized distilled water [14]. *Acacia* fresh nodules per plant and per site were preserved, and the corresponding *Rhizobium* was isolated and purified for further applications.

2.5 Isolation and Purification of *Rhizobium* Strains

The sterile nodule is crushed individually in a drop of sterile distilled water in a sterile tube with sterile glass rod. The slurry is than diluted in 3 ml of sterile water and then streaked on the surface of YMA (Yeast-Mannitol Agar) in the petri dishes. Then, the petri dishes were incubated at 28±2°C for 2–3 days. The sufficient growth was showed after 5 days of incubation. The isolate from a single rhizobial colony was selected and re-streaked on fresh YMA [14]. Subculturing was carried out 4 – 5 times until the culture became pure. The pure culture was considered as *Rhizobium*.

3. RESULTS AND DISCUSSION

The ability of indigenous strains of soils used to infect *A. cyanophylla* and *A. pycnantha* was evaluated by two parameters: the number of nodules and dry matter of nodules per plant.

3.1 Nodulation

The results of the analysis of variance showed that seedlings of *Acacia* issues from sterilized seeds and not having received any inoculum differed significantly with the following probabilities P<,0001 for the parameter numbers nodules by plant and P =,0001 for the parameter dry matter of the nodules by plant (Fig. 1).

A. cyanophylla formed an average of 25 nodules/plant of dry weight 0.11 g per plant. *A. pycnantha* has been poor since it formed only three nodules of dry weight 10 mg per plant (Fig. 1). The effect of soil treatment was not significant at the 5% risk of error for both parameters. However, the performance of each species differed significantly according to the soil used. Not all legumes are able to nodulate and the effectiveness of nodulation concerns 88% of the species examined [15], which explains the absence of nodulation on the roots of the *A. pycnantha*. Therefore, this species has specific requirements for *Rhizobium*. In general, a limited number of strains of rhizobia can infect each legume. For example, the legume *Medicago truncatula* can be nodulated by *Sinorhizobium meliloti* and *S. medicae*. Regarding rhizobia, while some are very constricted specificity, others have against a broad host specificity.

The study clearly shows that the *A. cyanophylla* was nodulated in the four soils used. This species is therefore, infected with a wider range of *Rhizobium* strains. Indeed, it has been reported that *A. cyanophylla* is able to nodulate with both strains of slow-growing *Rhizobium* strain with quick growth [16]. The results of our study, in agreement with that of Dreyfus and Dommergues [17] and Soumare [18] show that both, *Acacia* are associated with a significant diversity to rhizobia. *A. cyanophylla* is associated with rhizobia rapid growth (*Mesorhizobium* and *Rhizobium*) and to slow-growing rhizobia (*Bradyrhizobuim*).

For both species of *Acacia*, we found that the dry matter of nodules did not differ significantly regardless of the soil used. However, the differences observed for this parameter allows us to suggest distinct categories of *Rhizobium*. We deduce that for *A. cyanophylla* the *Rhizobium* of soil 2, 3 and 4 were more efficient than soil 1. Dreyfus and Dommergues [17] studied the requirements to *Rhizobium* of 13 *Acacia spp* and found that they could be classified into three groups based on their effective nodulation by *Rhizobium* spp (fast-growing *Rhizobium*) *Bradyrhizobium* spp. (slow-growing *Rhizobium*) or both.

3.2 Plant Growth

3.2.1 Measurement of height and number of leaves per plant

The results showed that there was a clear difference between the two species of *Acacia* following analysis of variance in regard to the total height measured, and the number of leaves per plant (p <, 0001) (Fig. 2).

We noted that *A. cyanophylla* had a height (25.5 cm) two times greater than *A. pycnantha, which* had a height of (10.81 cm) (Fig. 2).

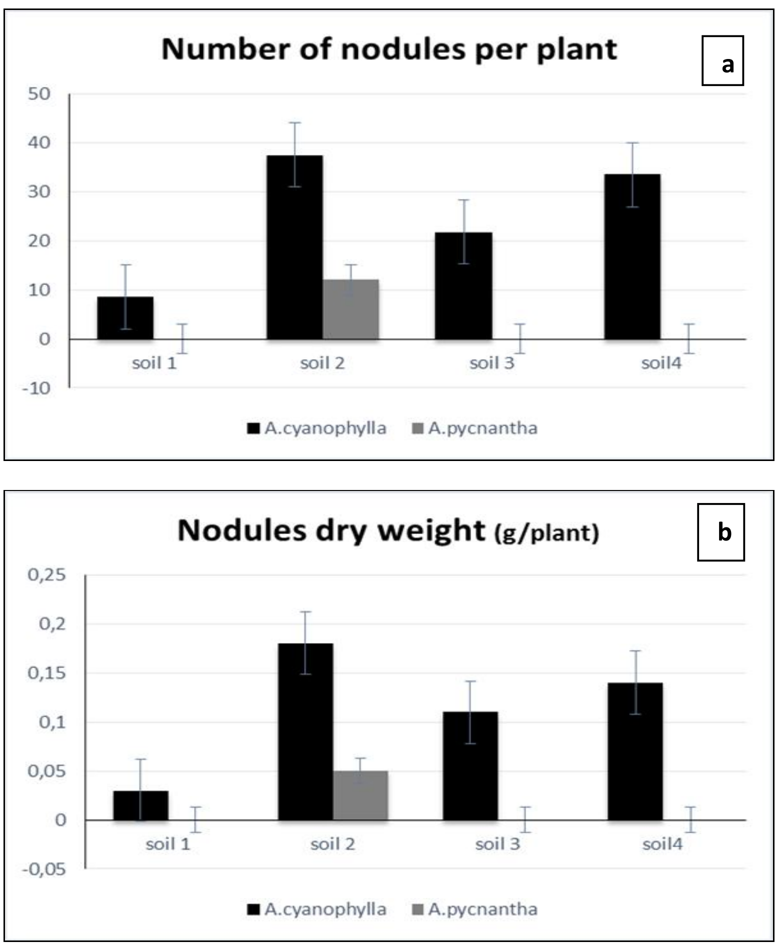

Fig. 1. The effect of soil type on nodulation number (a) and dry weight (b) of two *Acacia* species

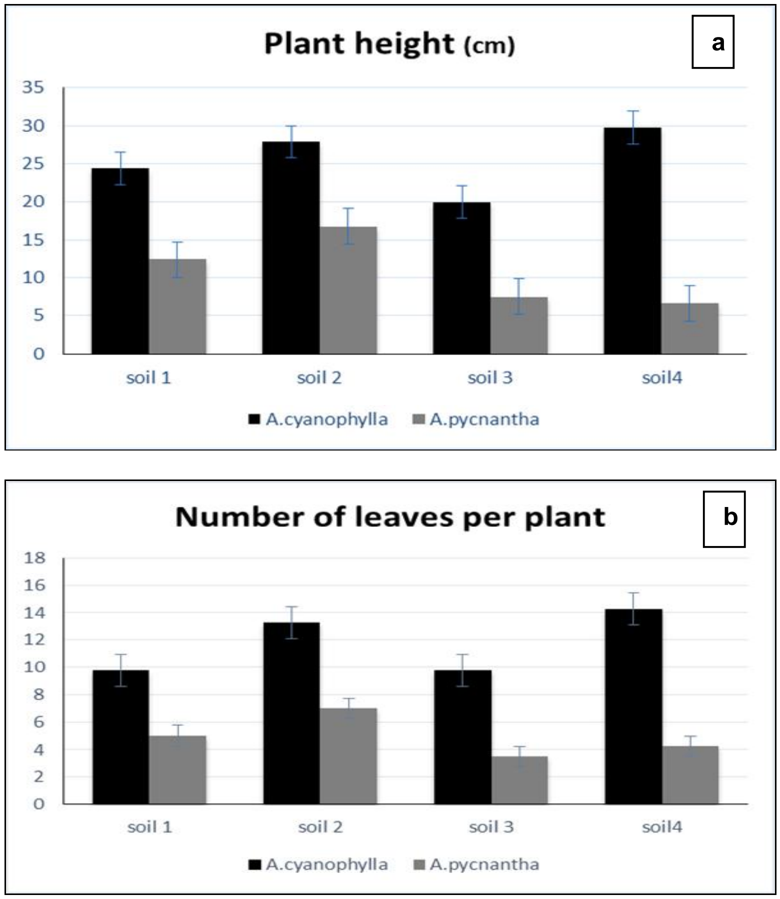

Fig. 2. The effects of soil type on plant height (a) and number of leaves (b) of two *Acacia* species

The effect of soil type was significant on number of leaves per plant with superiority of soil 2 and soil 4 statistically that were similar. The soils 3 and 1 were the lowest.

Symbiosis efficiency can be revealed by either the fixed nitrogen level measured by one of the known methods, or by vegetative development of the plant dry matter and total amount of N cumulated.

3.2.2 Shoot and root dry matter

The analysis of the variance showed that the effect of the species was significant for shoot (P = .0002) and root dry matter (P = .0001). Concerning the first parameter, it has proved that *A. cyanophylla* (2.16 g) differed significantly from *A. pycnantha*, which produced only 0.70 g. *A. cyanophylla*, had a more developed root system (1,43 g) than the *A. pycnantha* (0.45 g) had (Fig. 3).

The treatment (soil type) was also found to have a significant effect on shoot and root growth of the two species, with the following probabilities P = 0.047 and P = 0.033.

According to Mulongoy et al. [19] in a review of nodulation to Vigna (cowpea), a strain of *Rhizobium* isolated from a particular locality does not necessarily represent the best inoculum for this location rather than the other isolates from other environment.

The soil 1 may include *Rhizobium* that may be inefficient in this locality. Statistical results showed a significant correlation between the number of nodules per plant and plant height ($r = 0.87$, $P < 0.05$). These results demonstrate that adequate nodulation enhances the establishment and the plant growth.

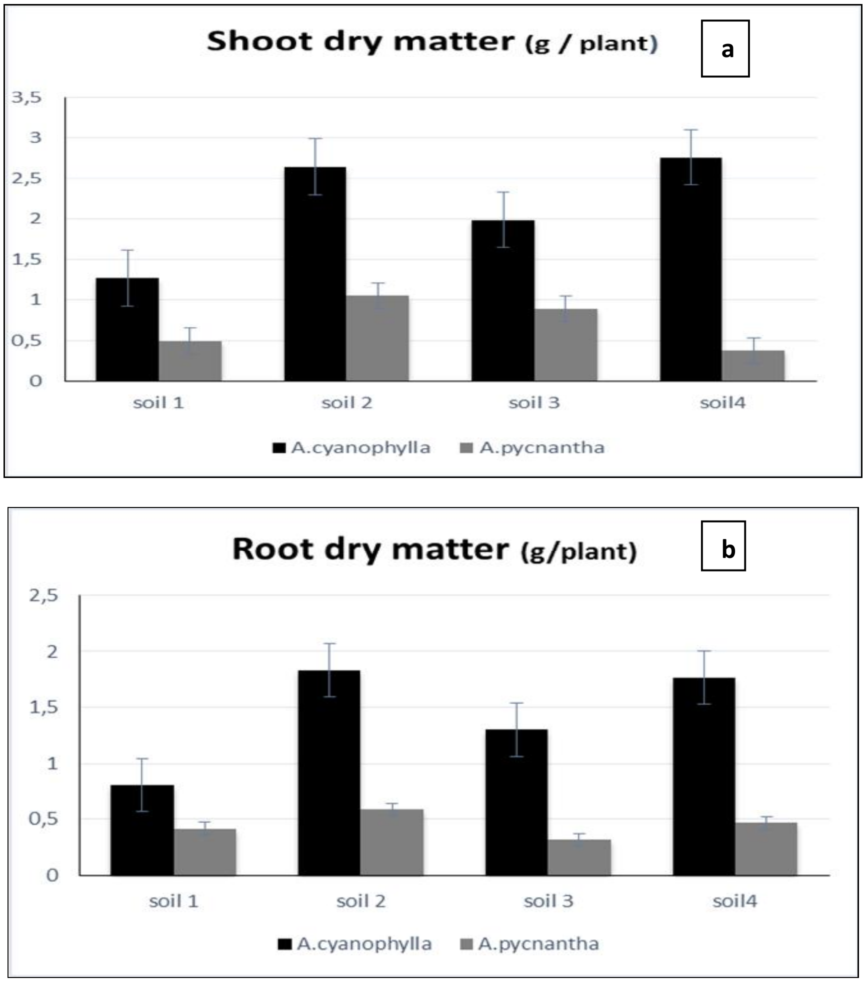

Fig. 3. The effects of soil type on the shoot (a) and root dry matter (b) of two *Acacia* species

4. CONCLUSION

Since nitrogen is commonly the most limiting plant nutrient in arable farming in the tropics and the most expensive element as a mineral fertilizer, Biological nitrogen fixation is the process of capturing atmospheric nitrogen by biological processes. Certain microorganisms and plant-microbe interactions accomplish it. *Acacia* are important nitrogen-fixing plants, which constitute an additional source of nitrogen in the ecosystem. Therefore, the results of this study revealed the existence of interspecific variability between the two plant species. *A. cyanophylla* has nodulated in the same way in all soils used, so it can be inoculated by a broad range of *Rhizobium* and is considered more promising in agroforestry. *A. pycnantha* has nodulated only in the soil 2 so this species quite specific requirement inoculation.

The soil 2 is considered favorable for both species of *Acacia* with high performance of *A.cyanophylla* regarding nodulation. It can be concluded that the second treatment contains a wide variety of *Rhizobium*. On the other hand, the selection of rhizobial strains is an effective way to evaluate their efficiency, competitiveness and genetic link.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ferguson BJ, Indrasumunar A, Hayashi S, Lin MH, Lin YH, Reid DE, Gresshoff PM. Molecular analysis of legume nodule development and autoregulation. Journal of Integrative Plant Biology. 2010;52:61-76.
2. Fred EB, Baldwin IL, McCoy E. Root nodule bacteria and leguminous plants. University of Wisconsin Press, No. 5, Madison; 1932.
3. Midgley SJ, Turnbull JW. Domestication and use of Australian *Acacia*: Case studies of five important species. Aust. Syst. Bot. 2003;16:89–102.
4. Brockwell J, Searle S, Jeavons A, Waayers M. Nitrogen Fixation in *Acacia*. ACIAR, Canberra; 2005.
5. Lajudie P, de Willems A, Nick G, Moreira F, Molouba F, Hoste B, Torck U, Neyra M, Collins MD, Lindström K, Dreyfus B, Gillis M. Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. International Journal of Systematic Bacteriology. 1998;48:369–382.
6. Lafay B, Burdon JJ. Small-subunit rRNA genotyping of rhizobia nodulating Australian *Acacia* spp. Applied and Environmental Microbiology. 2001;67:396–402.
7. Marsudi NDS, Glenn AR, Dilworth MJ. Identification and characterization of fast- and slow-growing root nodule bacteria from South-Western Australian soils able to nodulate *Acacia saligna*. Soil Biology and Biochemistry. 1999;31:1229–1238.
8. Nick G, Lajudie P de, Eardly BD, Suomalainen S, Paulin L, Zhang X, Gillis M, Lindström K. *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov, isolated from leguminous trees in Sudan and Kenya. Journal of Systematic Bacteriology. 1999a;49:1359–1368.
9. Nick G, Jussila M, Hoste B, Niemi M, Kaijalainen S, Lajudie P de, Gillis M, Bruijn FJ de, Lindström K. Rhizobia isolated from root nodules of tropical leguminous trees characterized using DNA-DNA dot-blothybridisation and rep-PCR genomic fingerprinting. Systematic and Applied Microbiology. 1999b;22:287–299.
10. Baumer M. Notes on trees and shrubs in arid and semi-arid Regions. FAO/UNEP Program "Ecological Management of Arid and Semi-Arid Rangelands in Africa, Near and Middle East". (EMASAR Phase II). 1983;270.
11. Ammari Y. Contribution to the study of allometric and ecological responses of *Acacia cyanophylla* Lindl, operating techniques in a valuation purposes, forestry and livestock management and planning, pastoral area Ennaser Governorate of Kairouan, Tunisia, graduate thesis, ENFI , Morocco; 1995.
12. Dommergues YR, Galiana A, Chaumont J, Diem A. Nitrogen fixing potential of *Acacia mangium* and *Acacia auricularformis seed* lings inoculated with *Bradyrhizobium and Rhizobium ssp.* Biology and fertility of soils. 1990;9;261-267.
13. Munns D. N Mineral nutrition and the legume symbiosis. In: A Treatise on Dinitrogen Fixation. Section IV - Agronomy and Ecology. Hardy RWF, Gibson AH. (eds), wiley-interscience, New York. 1977;353 – 391.
14. Vincent JN. A manual for the practical study of the Root Nodule Bacteria. International Biological Program. Handbook N°15, Blackwell Oxford, UK. 1970;164.
15. Graham PH, Vance CP. Legumes: Importance and constraints to greater use. Plant Physiol. 2003;131:872-877.
16. James KL, Paul WS, Paul GS, Dulal B. Symbiotic diversity in the cosmopolitan genus Acacia; SYMBIOSIS. 2006;41:107–117.
17. Dreyfus B, Dommergues Y. Nodulation of *Acacia* species by fast-and slow-growing tropical strains of *Rhizobium*. Applied and Environmental Microbiology. 1981;41(1): 97.
18. Soumare, et al. Impact of *Eucalyptus camaldulensis* on the diversity of rhizobium associate to *A. senegal* and *A. seyal* in Senegal. Journal of Applied Biosciences. 2013;67:5183–5193.

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19. Mulongoy K. Ayanaba A, Asanuma S, Rango Rao V. Cowpea nodulation and response to inoculation First OAU/STRC Inter-African conference on Biofertilizers. In: Effectivity of indigenous rhizobia for nodulation and early nitrogen fixation with *Leucaena leucocephala* grown in Nigerian soils. Soil Biol. Biochem. 1989;21(2):31-235.

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