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Selection of Indigenous Rhizobia Associated to Peanut Crop and Their Agronomic Performance Evaluation in Northen Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Microbial bioinoculation in plants is considered essential, as it helps reduce pollution levels and enhances crop productivity. The aim of this study is to evaluate the effect of microbial inoculation with selected rhizobia on the agronomic parameters of peanut plants. Peanut (*Arachis hypogaea*)

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root nodules, harvested in situ, were surface disinfected and crushed in petri dishes. Isolations were carried out from nodular crushed and the characteristic rhizobia colonies were purified. After a nodulation test of the purified isolates, the nodulating strains were subjected to an efficiency test. The first five strains showing the most satisfactory results on growth parameters were selected for the actual inoculation test. The growth, nodulation and mycorrhization parameters, as well as the number and weight of plant pods, were determined, after 3 months of crop. Results show a significant improvement in most of the parameters studied. Indeed, plant height was significantly improved from 38.33±2.86 (control) to 49.73±10.90 cm by inoculum R1 and from 38.33±2.86 to 59.22±9.87 cm by inoculum R2 ($p = 0.05$). These same inocula also significantly improved shoot dry weight with values ranging respectively from 4.43±0.86 (control) to 5.41±0.87 g (R1) and from 4.43±0.86 to 6.07±1.38 g (R2). Except for the mixed inoculum, all treatments applied significantly improved the nodules number. The values varied from 72.33±7.47 to 109.89±15.86 ; 109.33±21.18 ; 110.00±18.49 ; 105.67±16.9 and 113.11±20.63 nodules respectively for inocula R1, R2, R3, R4 and R5 ($p = 0.05$). Pods weight was significantly improved from 2.15±0.73 to 4.00±1.01 ; 4.02±0.97 and 3.80±0.96 g respectively by the inocula R3, R1, R2 ($p = 0.05$). The best results were obtained with inocula R1 and R2. These results confirm once again the stimulatory effect of rhizobia on the agronomic parameters of peanut. These inocula can be used as a biofertilizer to improve peanut crop yield.

Keywords: Arachis hypogaea; rhizobia; efficiency test; peanut plants; growth parameters.

1. INTRODUCTION

The economy of most developing countries, such as Côte d'Ivoire, is based primarily on agriculture. This activity is widely practiced in rural areas and constitutes the main source of income for local populations. Peanut (*Arachis hypogaea* L.) is one of the main food crops grown in Côte d'Ivoire, more acurately in the northern area of the country. This plant is cultivated for its richness in proteins and its high content of unsaturated oils, carbohydrates and mineral salts (Asibuo et al., 2008). Peanuts are used in confectionery and are recommended for vegetarian diets. They contain as much protein as meat and unlike meat, they do not contain uric acid or cholesterol (Asibuo et al., 2008). It is one of the most important oilseed crops worldwide. This plant is also cultivated in rotation to improve the nitrogen content of soil and thus contributes to its fertility (Koffi et al., 2021).

Despite the importance of this crop, it is faced with low yields due to unpredictable and unfavorable climatic conditions as well as the loss of fertile and cultivable soils. Furthermore, the current national production estimated at 112,698 tonnes in 2020, is insufficient to cover the annual needs of the population. It is therefore necessary to produce in sufficient quantity. Thus, in order to improve yields, chemicals substances are used excessively in agriculture. However, these substances cause the biodiversity loss and risks to human health (Ugwuoke et al., 2024). It is necessary to develop environmentally friendly methods to preserve the environment and human health. Thus, several alternatives are recommended, including biological processes using biofertilizers based on soil microorganisms (Ibrahim et al., 2025).

In fact, there is an influential community of microorganisms in the soil that associate with plant roots. These microorganisms form a symbiosis with most terrestrial plants (Smith et al., 2008). They thus contribute to improving the growth of the host plant and to combating biotic and abiotic stress. In addition, these symbionts are environmental friendly. Among these microorganisms, there is a genus called rhizobia which is specifically associated with legumes. These legumes, often called "green manures", contribute to soil fertility and plant productivity (Rani et al., 2021). This feat is due to their ability to fix atmospheric nitrogen thanks their association with soil rhizobia. In this symbiosis, rhizobia participate in improving the plant growth by providing it mainly nitrogen. In fact, they induce nodules on the roots of their hosts and it is within these compartments that the biological fixation of nitrogen naturally takes place (Coulibaly et al., 2021). Rhizobia mobilize nutrients for their hosts and in return the latter provide them carbon substances that they need for their activities. Several investigations showed the stimulatory effect of rhizobia on the growth of legumes and in particular on growth parameters in peanuts. Indeed Ding et al., (2024) showed that inoculation of peanut with selected rhizobia significantly improved growth and nodulation parameters in the plant. Koffi et al., (2025) also showed the stimulatory effect of rhizobia in synergy with selected CMA on peanut growth parameters.

Furthermore, very few studies have been carried out on the inoculation of peanut plant with rhizobia selected in Côte d'Ivoire, particularly in the northern area of the country. The objective of this present study is to select efficient rhizobia associated with peanut cultivation in the Korhogo region (north of the CI) and to evaluate their agronomic performance on the growth parameters of peanut seedlings under controlled conditions.

2. MATERIALS AND METHODS

2.1 Soil Sampling

Soil used as substrate was collected in peanut field in Takali (9°25 N ; 5°35 W) located in Korhogo area in northern Côte d'Ivoire. Soil collection was done at ten points of the plot, to obtain a representative composite soil sample of the targeted plot.

Physical and chemical parameters of this composite soil were determined at the Centre de Recherche en Océanographie (CRO) of Abidjan, Côte d'Ivoire. The pH (water) was measured in the supernatant of a soil / distilled water mixture in a ratio of 1/2.5. Organic and mineral matters content were determined according to Moreno et al. (2001). Contents of total nitrogen (N) and phosphorus (P) were quantified according to Kalambe (2021) and Kara et al. (1997). respectively by atomic absorption spectrometer after digestion with concentrated sulfuric acid. Potassium (K) was analyzed by means of argon plasma ionization source mass spectrometer (ICP-MS) according to Rao and Talluri (2007) method.

2.2 Root Nodules Sampling

The nodules were collected in situ from peanut roots in 3 different localities of study area. 15 peanut plants were collected from each locality. The roots were cut and the nodules were collected from each root. They were air-dried and then packaged in bags at room temperature.

2.3 Isolation and Purification of Rhizobia Strains

90 nodules at a rate of 2 nodules per plant were crushed individually, for the isolation of rhizobia. These nodules were first surface disinfected in a calcium hypochlorite solution $CaCl_2O_2$ (70%) for 3 min, then in 70% alcohol for 3 min. They were then rinsed 6 times with a series of sterile water for 3 min also after which the nodules were rehydrated. At the end of rinsing, each nodule was crushed in a drop of sterile water and a suspension has been sown onto Yeast Mannitol Agar (YMA). The petri dishes were then incubated at 28°C for 1 to 5 days depending on the growth rate of the colonies. The colonies characteristic (white, rounded, creamy colonies…) of the rhizobia were subsequently purified by successive subcultures, until homogeneous or pure colonies were obtained in each Petri dish.

2.4 Nodulation Test of Rhizobia Isolates

This test was conducted in Gibson tubes. The aim is to ensure that the isolates obtained on YMA agar are authentic strains of rhizobia. Indeed, there is no selective medium for the culture of rhizobia. The nodulation test will allow us to select the isolated rhizobia strains able to reinfect the host plant (peanut). This test was conducted in a crop chamber and made it possible to preselect around thirty strains of rhizobia after observing nodules on the root of the peanut plant (FAO 2020).

Peanut seeds, CNRA-ara 8-20 variety, were provided by the Centre National de Recherche Agronomique (CNRA) of Abidjan. This variety have a short cycle of 90 days. Seeds were surface-scarified in 70% calcium hypochlorite solution ($CaCl_2O_2$) for 8 min and then rinsed several times with sterile water (Gottardi and Nagl, 1998). They were pre-germinated in petri dishes containing 0.9% agar and incubated for 72 h at 28°C in the dark, in an oven.

The resulting seedlings were transferred into Gibson tubes containing Jensen's slanted medium for chamber crop. After transplanting the seedlings, they were covered with blotting paper which was watered so that the seed coats remained moist and made it easier to detach. This acclimatization phase lasts on average 2 to 4 days, the time needed for the seed coats to detach (Tang et al. 2023).

Liquid cultures of rhizobia made from each purified isolate were set up and used as inoculum for the nodulation test. These cultures were incubated under shaking at room temperature until a turbid medium with an optic density (OD) of approximately 0.7 at 600 nm was obtained. After 5 to 7 days of crop chamber, the young seedlings were inoculated with 1 ml of bacterial suspension.

After inoculation, the tubes containing the seedlings were transferred into wooden racks and then kept in a growth chamber at a controlled temperature of 28°C and under photosynthetic light. 4 replicates were performed per strain and an uninoculated negative control was added. The seedlings were watered with sterile water if necessary. After 1 month of crop, the appearance of at least one nodule on each plant was noted and the nodulating strains were selected and stored at -80°C for efficiency tests and for molecular identification.

2.5 Selection Test of Efficient Rhizobia Strains

2.5.1 Experimental design

The aim of this test was to select five best rhizobia strains and use them as inoculum in the actual peanut inoculation test. This test was carried out on 26 purified peanut nodulating strains selected during the nodulation test. The experiment was conducted in a greenhouse located at Nangui Abrogoua University in Abidjan, Ivory Coast (geographic coordinates 5°23 North latitude and 4°0 West longitude). The crop substrate is a soil from the site, sterilized at 120 °C for 24 h. Only one factor, the effect of inoculation, was studied. 27 treatments were carried out, namely 26 strains selected during the nodulation test and a control without inoculum. For each treatment, five (5) replicates were carried out. Peanut seeds were previously disinfected in a calcium hypochlorite solution before being germinated on 0.9% agar water. After 2 to 3 days of incubation in the dark, the seedlings were transferred into the sheaths containing approximately 1 kg of sterilized soil. The rhizobia strains were grown in YM (yeast mannitol) liquid medium with shaking at 150 rpm at 28°C for 2 to 4 days, until an OD of approximately 0.7 at 600 nm was obtained. This OD corresponds to a density of approximately 10^7 bacteria per mL. Five (5) to 7 days after the seedlings were transferred into the sheaths, they were inoculated with 5 mL of the bacterial suspension placed drop by drop around the rootlet. The plants were watered with demineralized water every day until harvest.

2.5.2 Measurement of growth and nodulation parameters

Every two weeks, the plant height was measured and after 45 days of crop, growth and nodulation parameters were evaluated. These are the dry aerial and root biomass, the number and weight of nodules. The plants were harvested and the aerial part was cut from the root and then the two entities were preserved in envelopes separately. The nodules of each plant were detached, counted and weighed per treatment using a precision balance. The aerial and root parts were dried in an oven at 70°C for 48 h, then weighed for biomass determination.

2.5.3 Determination of chlorophyll content

Chlorophyll content was determined according to the method of Makeen et al., (2007). For each sample, 100 mg of young leaves were collected and ground in 10 mL of 80% acetone diluted in phosphate buffer at pH 7.8. The resulting extract was centrifuged at 6000 rpm at 4 °C for 10 min, then incubated at 4 °C in the dark for 24 h. Then, the supernatant was collected and the OD was measured at 645 nm for chlorophyll b and at 663 nm for chlorophyll a using a spectrophotometer. The concentrations of total chlorophyll (ChlT) (chlorophyll a and b), expressed in mg/g of fresh matter, were obtained by the following formula: ChlT = [8.02 x OD (663) + 20.2 x OD (645)] V/M where V denotes the volume of the total extract in liters and M the mass of the ground fresh matter in grams (Trouvelot et al. 1986).

The averages of each parameter per treatment were taken into account and allowed to select the five (5) best rhizobia strains showing good performances with respect to the parameters studied. This selection was made after the statistical analysis of the data with the XLSTAT 2010 software. These strains were used as biological inoculum for the study of the effect of inoculation with selected rhizobia on the growth and yield parameters of peanut.

2.6 Peanut Inoculation Test with Five Selected Efficient Rhizobia Strains

2.6.1 Experimental design

The experiment was conducted in a controlled environment; in the same greenhouse whose characteristics were given above. A completely randomized experimental design was set up in the said greenhouse for this test. A single crop (peanut) and a single factor (inoculation) were studied. 5 inoculation treatments with rhizobia strains (R1, R2, R3, R4, R5) selected and the mixed Rm, as well as a control without inoculum were carried out. 9 repetitions were carried out per treatment.

Each rhizobial inoculum was prepared as described above. The mixed inoculum was prepared by adding equivalent volume (v/v/v/v/v) of each inoculum. Inoculation was carried out after 5 days of transfer of the young seedlings and 5 mL of each bacterial suspension was added drop by drop around the young rootlet. The bacterial inoculum was used during the exponential phase of bacterial growth which corresponds to an OD of 0.7 at 600 nm with approximately a number of 10^7 bacteria/mL.

2.6.2 Measurement of growth and nodulation parameters and pods characteristics

Every two weeks, plant height was measured. After three months of crop, plants were harvested according to the treatments and growth (height and weight) and nodulation parameters (number and weight) were measured as described previously. After harvesting, the aerial part was cut from the root and the two entities were packaged in envelopes separately, then dried in an oven at 70°C for 48 h. Both entities were weighed for the determination of shoot, root and total dry weight. Peanut pods and nodules were detached from roots before the drying.

Pods characteristics and nodulation parameters were also determined through the number and weight of pods.

2.7 Data Analysis

The data obtained were analyzed using STATISTICA 7.1 software. An analysis of medians was performed using the Kruskal-Walis test and revealed significant differences between the inoculation treatments applied. The multiple comparison test of the average ranks of the different treatments, at the 95% threshold, was applied. The aim was to evaluate the influence of different treatments on plant growth and to compare the intensity of these effects between these different treatments. The values of the percentage mycorrhization rate were first transformed into arcsine before analysis.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physic and chemical parameters of soil used as substrate

Soil physico-chemical parameters are shown in Table 1. Soil used as substrat has an acid pH (5.25) and poor in phosphorus and nitrogen.

Table 1. Physicochemical parameters of soil

Parameters	Values
pH	5.25
Organic matters (%)	4.23
Mineral matters (%)	2.53
Total nitrogen (mg/kg)	1.01
Total phosphorus (mg/kg)	4.57
Potassium (mg/kg)	244.27

3.1.2 Efficiency of rhizobia inoculating peanut on growth parameters of young seedlings olded 45 days

3.1.2.1 Efficiency on height, number and weigth of nodules of peanut seedlings

The heights of plants inoculated with rhizobia isolates were significantly different from that of the negative control at the end of the experiment (Table 2). The values ranged from 30.42 cm (negative control) to 38.06 cm (strain R11). The five best height values were obtained with strains R11, R3, R4, R1, R5 with respective increase rates of 25.12; 21.30; 20.51; 18.67 and 15.38% compared to the uninoculated negative control. These rhizobia strains therefore significantly improved the height of peanut plants.

Fresh nodule weight ranged from 103.8 mg to 11.6 mg. Except strain R3, no significant difference was observed in this parameter. However, a significant difference was observed between the nodule numbers of inoculated plants compared to that of the uninoculated control. Strain R3 had the highest nodule number (37.8 nodules), followed by strains R2 (27 nodules); R1 (25.2 nodules); R4 (23.4 nodules) and R5 (23.2 nodules) (Table 2). Nodulation parameters were the first criteria in choosing the five (5) best rhizobia strains selected.

3.1.2.2 Efficiency of rhizobia on aerial, root and total biomass of inoculated peanut plants

No significant differences were observed in the shoot, root and total dry weight of plants inoculated with the rhizobia strains, compared to those of the control (Table 3). The shoot dry weight (SDW) values ranged from 3.09 g (in strain R3) to 1.64 g (in strain R19). Root dry weight (RDW) ranged from 0.94 g in the control to 0.61 g in strain R19. Total dry weight (TDW) ranged from 3.76 g in strain R3 to 2.24 g in strain R19. Total dry weight is the sum of the SDW and RDW of the same plant.

3.1.2.3 Chlorohyll content of plant inoculated

Significant differences were also observed between the chlorophyll content of the leaves of plants inoculated with the rhizobia strains and that of the uninoculated control. The best chlorophyll levels were obtained with strains R5, R21, R1, R13 and R17 (Table 4). Generally, most of the strains improved the chlorophyll levels of the leaves compared to that of the uninoculated control. However, except for strains R5 and R1 which improved both plant height, nodulation and chlorophyll rates, the other three top strains improving height (R3, R11, R4) and nodule number (R3, R2, R4) did not improve chlorophyll rate. All these observations helped to select the following five best strains: R1, R2, R3, R4 and R5 based mainly on nodulation parameters.

Table 2. Height, number and weight of nodules of 45-day-old peanut (*Arachis hypogaea*) seedling inoculated with rhizobia

Rhizobia isolates	Height (cm)	Nodules number	Nodules weight (mg)
R6	33,06±5,11^{ab}	8,00±2,00^{ab}	21,40±3,78^{a}
R7	33,30±2,00^{ab}	5,80±1,09^{ab}	12,40±5,12^{a}
R2	33,94±2,22^{ab}	27,00±6,52^{ab}	76,20±0,16^{a}
R4	36,66±2,24^{bc}	23,40±4,88^{ab}	70,00±31,00^{a}
R8	32,02±3,67^{ab}	13,20±1,92^{ab}	36,6±9,00^{a}
R9	33,00±0,57^{ab}	16,60±3,85^{ab}	50,00±10,00^{a}
R10	34,56±2,09^{b}	10,00±1,58^{ab}	24,40±7,02^{a}
R11	38,06±2,20^{c}	17,20±9,01^{ab}	41,20±25,30^{a}
R3	36,9±1,88^{bc}	37,80±14,60^{b}	103,8±56,44^{b}
R12	32,18±2,30 ^{ab}	13,00±2,00^{ab}	30,00±10,00^{a}
R13	31,20±1,24^{ab}	6,80±1,09^{ab}	14,00±2,39^{a}
R1	36,10±1,25^{bc}	25,20±7,60^{ab}	93,2±17,30^{ab}
R5	35,10±2,41^{bc}	23,20±3,11^{ab}	82,2±10,00^{ab}
R14	33,64±2,02^{ab}	15,00±3,00^{ab}	27,00±10,00^{a}
R15	34,42±1,63^{ab}	12,40±2,41^{ab}	25,00±6,80^{a}
R16	31,60±2,16^{ab}	17,00±3,46^{ab}	47,20±7,10^{a}
R17	33,40±2,93^{ab}	14,60±3,65^{ab}	33,00±7,68^{a}
R18	34,96±1,00^{bc}	15,60±3,65^{ab}	33,60±7,27^{a}
R19	32,96±1,48^{ab}	5,80±0,84^{ab}	11,60±3,13^{a}
R20	32,00±1,20^{ab}	11,00±1,58 ^{ab}	20,60±5,13^{a}
R21	33,92±1,24^{ab}	22,8±4,76 ^{ab}	56,20±27,90^{a}
R22	34,02±1,02 ^{ab}	18,00±7,38 ^{ab}	39,80±16,01^{a}
R23	32,48±0,75^{ab}	14,80±3,27^{ab}	37,60±19,70^{a}
R24	33,86±2,00^{ab}	15,00±4,06^{ab}	34,00±14,01^{a}
R25	34,8±2,00^{ab}	14,80±3,27^{ab}	39,40±6,84^{a}
R26	34,04±2,06^{ab}	19,40±7,44^{ab}	55,00±26,70^{a}
Control	30,42±2,70^{a}	0,00±0,00^{a}	0,00±0,00^{a}

Ri = inoculum i. Values followed by the same letters in the columns are not statistically different (P > 0.05) according to Kruskal Walis test

Table 3. Shoot, root and total dry weight of 45-day-old peanut (*Arachis hypogaea*) plants inoculated during the efficiency test

Rhizobia isolates	SDW (g)	RDW (g)	TDW (g)
R6	2,06±0,56^{a}	0,65±0,04^{a}	2,71±0,58^{a}
R7	1,97±0,46^{a}	0,78±0,28^{a}	2,74±0,71^{a}
R2	2,54±0,66^{a}	0,78±0,19^{a}	3,32±0,71^{a}
R4	2,84±0,67^{a}	0,65±0,07^{a}	3,49±0,71^{a}
R8	2,01±0,70^{a}	0,67±0,08^{a}	2,68±0,75^{a}
R9	2,20±0,62^{a}	0,72±0,07^{a}	2,92±0,66^{a}
R10	2,13±0,86^{a}	0,79±0,28^{a}	2,92±1,11^{a}
R11	2,38±0,59^{a}	0,78±0,20^{a}	3,17±0,80^{a}

Rhizobia isolates	SDW (g)	RDW (g)	TDW (g)
R3	3,09±1,02^{a}	0,68±0,19^{a}	3,76±1,10^{a}
R12	2,06±0,46^{a}	0,75±0,16^{a}	2,81±0,54^{a}
R13	2,16±0,14^{a}	0,94±0,09^{a}	3,10±0,08^{a}
R1	2,51±0,69^{a}	0,70±0,11^{a}	3,20±0,66^{a}
R5	2,74±0,74^{a}	0,62±0,10^{a}	3,36±0,84^{a}
R14	2,55±0,62^{a}	0,81±0,15^{a}	3,36±0,56^{a}
R15	2,15±0,67^{a}	0,64±0,27^{a}	2,79±0,92^{a}
R16	2,78±0,45^{a}	0,89±0,39^{a}	3,67±0,81^{a}
R17	2,04±0,65^{a}	0,79±0,24^{a}	2,83±0,70^{a}
R18	3,02±0,70^{a}	0,66±0,23^{a}	3,68±0,76^{a}
R19	1,64±0,17^{a}	0,61±0,28^{a}	2,24±0,43^{a}
R20	2,05±0,35^{a}	0,82±0,21^{a}	2,86±0,45^{a}
R21	2,40±0,77^{a}	0,62±0,12^{a}	3,02±0,88^{a}
R22	3,10±0,59^{a}	0,87±0,22^{a}	3,97±0,79^{a}
R23	2,00±0,58^{a}	0,79±0,17^{a}	2,79±0,68^{a}
R24	2,53±0,35^{a}	0,88±0,30^{a}	3,40±0,50^{a}
R25	2,45±0,70^{a}	0,61±0,13^{a}	3,07±0,79^{a}
R26	2,94±0,55^{a}	0,77±0,11^{a}	3,704±0,49^{a}
Control	2,36±0,58^{a}	0,94±0,15^{a}	3,30±0,71^{a}

Values followed by the same letters in the columns are not statistically different (P > 0.05) according to the Kruskal Walis test. SDW: shoot dry weight, RDW: root dry weight, TDW: total dry weight

Table 4. Chlorophyll content of peanut plant leaves

Rhizobia isolates	Chlb (mg/g)	Chla (mg/g)	ChlT (mg/g)
R6	0.78±0.01^{ab}	0,86±0,05^{ab}	1,64±0,04^{ab}
R7	0.67±0.03^{a}	0,76±0,06^{ab}	1,43±0,08^{ab}
R2	0.67±0.08^{a}	0.80±0.08^{ab}	1,48±0,16^{ab}
R4	0.46±0.05^{a}	0.58±0.08^{a}	1,04±0,13^{a}
R8	0,49±0,01^{a}	0,56±0,01^{a}	1,06±0,03^{a}
R9	0,78±0,08^{ab}	0,87±0,02^{ab}	1,65±0,06^{ab}
R10	0,32±0,03^{a}	0,34±0,03^{a}	0,66±0,05^{a}
R11	0,40±0,05^{a}	0,52±0,09^{a}	0,92±0,14^{a}
R3	0,57±0,09^{a}	0,70±0,04^{ab}	1,27±0,11^{ab}
R12	0,65±0,05^{a}	0,74±0,02^{ab}	1,39±0,05^{ab}
R13	0,82±0,01^{b}	0,91±0,09^{b}	1,73±0,08^{b}
R1	0,84±0,08^{b}	0,93±0,09^{b}	1,77±0,16^{b}
R5	0,86±0,04^{b}	0,95±0,05^{b}	1,82±0,09^{b}
R14	0,37±0,03^{a}	0,46±0,10^{a}	0,83±0,13^{a}
R15	0,54±0,03^{a}	0,60±0,03^{a}	1,14±0,06^{a}
R16	0,60±0,04^{a}	0,68±0,10^{ab}	1,28±0,14^{ab}
R17	0,90±0,09^{b}	0,80±0,07^{ab}	1,70±0,16^{b}
R18	0,49±0,02^{a}	0,54±0,06^{a}	1,03±0,06^{a}
R19	0,58±0,02^{a}	0,68±0,03^{ab}	1,26±0,04^{ab}
R20	0,54±0,05^{a}	0,62±0,06^{a}	1,16±0,11^{a}
R21	0,87±0,01^{b}	0,94±0,05^{b}	1,81±0,05^{b}
R22	0,29±0,03^{a}	0,36±0,04^{a}	0,65±0,07^{a}
R23	0,50±0,01^{a}	0,60±0,01^{a}	1,10±0,01^{a}
R24	0,49±0,02^{a}	0,59±0,01^{a}	1,08±0,03^{a}
R25	0,56±0,06^{a}	0,63±0,06^{a}	1,19±0,11^{a}
R26	0,60±0,04^{a}	0,72±0,08^{ab}	1,32±0,13^{ab}
Control	0,36±0,04^{a}	0,45±0,09^{a}	0,81±0,09^{a}

Values followed by the same letters in the columns are not statistically different (P > 0.05) according to the Kruskal Walis test. Chl a: chlorophyll a; Chl b: chlorophyll b; Chl T: total chlorophyll

3.1.3 Effect of inoculation with five selected rhizobia on few agronomic parameters of peanut

Five rhizobial strains were selected after efficiency test and used for the inoculation test in non-sterile soil in controlled conditions.

3.1.3.1 Effect on height and biomass of plant

Growth parameters (height and biomass) of peanut plants were significantly improved upon inoculation with rhizobia (Table 5). The height was significantly improved by the strains R1 (49.73 cm) and R2 (59.22 cm) (α = 0.05) compared to the non-inoculated control (38.33 cm); representing a respective increase rate of 29.74% and 54.50%.

The shoot dry weight (SDW) was increased by the same strains with respective values of 5.41 and 6.07 g, i.e. respective improvement rates of 35.25% and 51.75%. Total dry weight (TDW) was significantly improved only by strain R2 (6.66 g), an increase of 41.1% compared to the control.

However, no significant improvement was observed in root dry weight biomass (RDW). In addition, the RDW of the control was higher than those of inoculation treatments, but without significant difference.

3.1.3.2 Effect on nodulation parameters and pods characteristics of peanut plants

The results on nodulation parameters and pods characteristic of peanut plants are shown in the Table 6.

The number of nodules varies from one strain to another. A significant improvement (at the threshold α = 0.05) in the nodules number of plants inoculated with rhizobia in monoculture was observed with all rhizobia strains.

The best results for the number and weight of nodules were obtained respectively with strains R2 (113.11) and R4 (262 mg) with a respective improvement of 56.38% and 53.28% compared to the non-inoculated control. However, the mixed inoculum still had no significant effect on either the number or the mass of nodules. No significant improvement in pod number was also observed. In contrast, pod mass was significantly improved with monoculture treatments R3 (4.00 g); R1 (4.02 g) et R2 (3.80 g), with rates of 76.74% (R2) to 86.98% (R1) compared to the control. The mixed inoculum had no significant effect on these different parameters (Table 6).

Table 5. Values of growth parameters of plants inoculated with selected rhizobia

Rhizobia strains	Height (cm)	SDW (g)	RDW (g)	TDW (g)
R5	45.56 ± 6.78^{ab}	4.78 ± 0.80^{ab}	0.60 ± 0.16^{a}	5.37 ± 0.93^{ab}
R4	43.61 ± 1.54^{ab}	4.22 ± 1.11^{ab}	0.57 ± 0.16^{a}	4.79 ± 1.19^{a}
R3	44.19 ± 4.02^{ab}	4.36 ± 0.89^{ab}	0.56 ± 0.12^{a}	4.92 ± 0.95^{a}
R1	49.73 ± 10.90^{b}	5.41 ± 0.87^{bc}	0.66 ± 0.09^{a}	6.07 ± 0.91^{ab}
R2	59.22 ± 9.87^{c}	6.07 ± 1.38^{c}	0.58 ± 0.15^{a}	6.66 ± 1.47^{b}
Rm	44.69 ± 2.71^{ab}	4.43 ± 0.86^{ab}	0.57 ± 0.14^{a}	5.00 ± 0.90^{ab}
Control	38.33 ± 2.86^{a}	4.00 ± 0.94^{a}	0.71 ± 0.14^{a}	4.72 ± 1.05^{a}

Values followed by the same letter, in the columns, are not statistically different (P > 0.05) according to the Kruskal-Walis test. SDW: shoot dry weight, RDW: root dry weight, TDW: total dry weight

Table 6. Values of nodulation and pods parameters of peanut plants inoculated with selected rhizobia

	Nodules		Pods	
Rhizobia strains	**Number**	**Weight (mg)**	**Number**	**Weight (g)**
R5	109.89 ± 15.86^{b}	255.33 ± 50.29^{ab}	1.78 ± 0.44^{a}	3.24 ± 0.72^{ab}
R4	109.33 ± 21.18^{b}	262.11 ± 39.14^{b}	1.89 ± 0.78^{a}	3.06 ± 0.75^{ab}
R3	110.00 ± 18.49^{b}	243.00 ± 30.00^{ab}	$2,22\pm0,97^{a}$	4.00 ± 1.01^{b}
R1	105.67 ± 16.90^{b}	260.33 ± 72.20^{b}	1.78 ± 0.44^{a}	4.02 ± 0.97^{b}
R2	113.11 ± 20.63^{c}	220.11 ± 77.97^{ab}	2.11 ± 0.78^{a}	3.80 ± 0.96^{b}
Rm	81.89 ± 8.33^{ab}	199.00 ± 63.02^{ab}	1.78 ± 0.67^{a}	2.88 ± 0.81^{ab}
Control	72.33 ± 7.47^{a}	171.00 ± 11.84^{a}	1.44 ± 0.53^{a}	2.15 ± 0.73^{a}

Values followed by the same letter in the columns are not statistically different (P > 0.05) according to the Kruskal-Walis test. Rm: mixed rhizobia inoculum

3.2 Discussion

Inoculation with selected rhizobia strains significantly improved the growth and nodulation parameters of peanut plants. These results confirm again the stimulatory effect of these symbionts, described by several authors. Indeed, Mohammad and Alobaidy (2023) and Doha et al. (2024) showed positive effects of inoculation with selected rhizobia on the growth of Acacia senegal seedlings in non-sterile soils. Similar effects of rhizobial inoculation were also observed by Touroumgaye et al. (2025), on peanut plants. A significant improvement in the height of inoculated plants was observed, mainly with rhizobial treatments R1 and R2. The improvement in growth parameters could be due to an improvement in the mineral and water nutrition of the plants provided by the symbionts (Ollivier et al., 2011). Dry aerial biomass (SDW) and total dry weight (TDW) were significantly improved by the same rhizobial treatments R1 and R2.

Similar results on SDW were also found by (Allito et al., 2021) in Faba bean inoculated with rhizobia strains. This improvement of SDW and plant height would be due to the introduction of rhizobia strains able to improve the mineral nutrition of plants, mainly that of nitrogen, their symbiotic efficiency and salt tolerance behaviours.

Indeed, several studies have highlighted the improvement in the growth of legumes during symbiosis with rhizobia (de Carvalho et al., 2012). Renganathan et al. (2025) reported that shoot dry weight (SDW) is a good indicator of the relative efficacy of strains.

However, no improvement in root dry weight (RDW) was observed, the inoculation with selected rhizobia even attenuated the RDW compared to that of the control. Low DRW values following inoculation were also observed by Yaremko et al., (2024) who found no improvement in either RDW or SDW. These low biomasses recorded could be due to a strong presence of nodules on the roots of the inoculated plants, which constitute a part of the root biomass given that these were detached before drying.

Compared with uninoculated control plants and inoculum-treated plants, the mixed inoculum had no significant effect on plant nodule number and weight. This would be due to the competition effect between the different strains present in the mixed inoculum.

The high number of nodules in the control suggests that the soil substrate harbors a sufficient indigenous population of rhizobia able of nodulating the peanut plant. However, the number of nodules observed in the non-inoculated control remains lower than that of the inoculated plants. This could be justified by the fact that indigenous strains are less efficient than those used for inoculation, which were more competitive with the latter. Sajid et al. (2011) also found a high number of nodules when peanut was inoculated with selected rhizobia.

Chlorophyll content results show that inoculated plants have higher chlorophyll content than non-inoculated plants. Rhizobia strains improve also chlorophyll content of peanut plant leaves chlorophyll. Begum (2022) claimed that an increase in chlorophyll content in inoculated plants could be due to the presence of large numbers of chloroplasts in the leaves. Similar results were found by dos Santos et al., (2023) who noted an increase in chlorophyll content in leaves of cowpea inoculated with selected rhizobia. This increase in chlorophyll content may be due to increased stomatal conductance, photosynthesis, transpiration and improved plant growth.

4. CONCLUSION

At the end of this study, microbial inoculation with selected rhizobia showed beneficial effects on several agronomic parameters of peanuts. The selected rhizobia significantly improved the height, shoot dry weight, nodule number, and pod weight. Strains R1 and R2 showed the best results following microbial inoculation. Therefore, it is important to select the most efficient strains to maximize plant productivity. These selected strains could be used as inoculum in peanut crop. Looking ahead, we plan to conduct the experiment in field conditions and select rhizobia strains resistant to drought and salt.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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