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Enhancing Coating Inoculation for Improved Nitrogen-Fixing Efficiency of Rhizobia in Soybean (*Glycine max* L.)

Anne Nora Ndokon Bikang[1], Liliane Meguekam Tekam[2*], Alain-Martial Sontsa-Donhoung[3], Dieudonné Nwaga[1,4*]

[1]Soil Microbiology Laboratory, Biotechnology Centre, University of Yaounde I, Yaounde, Cameroon
[2]Department of Biological Sciences, Higher Teacher Training College of Yaounde, University of Yaounde I, Yaounde, Cameroon
[3]Laboratory of Regional Biological Control and Applied Microbiology, Institute of Agricultural Research for Development (IRAD), Yaounde, Cameroon
[4]GIC AGRIBIOCAM, Yaounde, Cameroon
Email: *dnwaga@yahoo.fr, *fmeguekame@yahoo.fr

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Abstract

Plant biofertilization involves introducing compounds containing living microorganisms into the coating medium to sustainably enhance plant production and soil health. This is a complex process that undergoes multiple stages of development before yielding a final product. The final biofertilizer is used by legumes-protein-rich crops in symbiosis with rhizobia to enable biological nitrogen fixation increasing natural soil fertility. This study aims to determine the optimal formulation of a rhizobial biofertilizer to improve the performance of soybean (*Glycine max* L. cv. Docko). To this end, soybean seeds obtained from IRAD were coated with different formulations derived from locally sourced materials. Palm kernel oil was used as an adhesive in one group, while corn powder served as an adhesive in another. The coated seeds were then sown in the field. The results indicate that the combination of pigeon pea powder + sugarcane molasses, with palm kernel oil as an adhesive, produced the best nodulation (nitrogen fixation). This formulation also led to significant improvements in growth (+350%) and total nitrogen content (+1100%) compared to the bacterial broth inoculum control (B0) ($P \le 0.01$). These findings represent a significant advancement in improving nitrogen-fixing bacterial inoculants and enhancing soil fertility for the sustainable cultivation of soybeans in this tropical soil.

Keywords

Formulation, Biofertilizer, N-Fixing, Rhizobia, Coating, Growth

1. Introduction

Climate change now imposes on farmers a resilient and less expensive production technique to meet consumer needs like organic fertilization which guarantees human health and that of agricultural soils. Confused with a wide range of products such as green or animal manure, intercrops, or even chemical fertilizers enriched with organic matter [1], biofertilizer is a support containing soil microorganisms such as rhizobium that are applied to plants to promote their growth [2]; They provide an added benefit in both nutrient uptake and plant performance when inoculated into the rhizosphere [3]. The micro-organisms they contain directly affect plant development by producing the growth hormones, or reducing the influence of pathogens [1]. Biofertilizers are of great economic importance and are less expensive than synthetic fertilizers [4] and their application is perceived as an innovative and environmentally friendly technology to improve soil fertility and development [5]. When these biofertilizers are based on rhizobium, they accentuate the biological fixation of nitrogen by several species of cultivated legumes such as soybeans [6]. These symbioses play an important socio-ecological role in environmental protection and improve agricultural yields [7]. The annual production of rhizobial inoculants is estimated at about 2000 tons worldwide [8]. Rhizobial strains are available in the market as bioinoculants [9] and meet about 79% of the global demand for biofertilizers. Biofertilizers constitute about 5% of the total fertilizers available in the market [10] with an estimated value of $2.3 billion. This economic value is estimated to reach $3.9 billion by 2025, with an annual growth rate of 11.6% during the forecast period [3]. However, biological analyses show that some biofertilizers have alarming results in terms of their quality [11]. This is how approximately 63% of commercialized biofertilizers are contaminated by one or more bacterial strains [12] and on average 90% of legume inocula are recognized as having no effect on the productivity of the legume for which they are used [13] in general and soybeans in particular.

The soybean is an herbaceous plant of the legume [14] whose pod contains 2 to 4 seeds high in proteins, carbohydrates, and fats [15]. Its nodular root system is organized around a taproot that branches into rootlets improving the nitrogen composition of the soil before growing other plants such as wheat or corn. Warm climate plants have a pH between 5.5 and 6.5 and optimal temperatures are between 20 and 30˚C. Its global production is approximately 340 million of the tonnes since 2017 and the first place is occupied by the United States of America with a production of 123,664,230 tonnes [16]. In Cameroon, soybean cultivation already weighed around 550 million FCFA in 2015 [17] with producing basins in the far North, West, and North-West. However, it is subject to biotic and abiotic constraints. Thus, this work aims to seek a better formulation of biofertilizer based on nitrogen-fixing rhizobia for improving the performance of soybean (*Glycine max* L.) in the field.

2. Materials and Methods

This study was conducted in semi-controlled conditions in the experimental garden of the University of Yaoundé I (3˚59'10''N and 11˚49'59''E), from March to July 2023, with an average temperature of 29˚C. Microbiological and biochemical tests were carried out at the Soil Microbiology Laboratory of the Biotechnology Centre (CTB).

2.1. Plant Material

The plant material used in this study consists of seeds of *Glycine max* L. cv. Docko, sensitive to NaCl [18], from the IRAD of Wakwa in the Adamawa region. The choice of products (organic and inorganic) is based on their low cost and accessibility, as well as their ability to provide nitrogen and carbon for rhizobial growth [19]. Thus, the materials used for the formulation of biofertilizers are composed of by-products of the agro-food industry (digestate), shell powder, and yeast mannitol broth (YMB) media; the different associations of these formulations made it possible to classify them into three groups (**Table 1**). The coating material for soybeans is palm kernel oil and maize powder. The BOSD rhizobial consortium, provided by the Soil Microbiology Laboratory of the Biotechnology Centre of Yaoundé I, consists of 4 strains isolated in the soybean rhizosphere, namely Bosd1, Bosd2, Bosd4 and Bosd6 [20].

2.2. Methods

2.2.1. Determination of the Physicochemical Parameters of the Formulation Materials

The physicochemical parameters are determined on the one hand on the solid supports used: biochar, charcoal, coating substrate, white powder, soil sample from the Garoua field (production site of the Docko variety), Cajun weight and cow dung, and on the other hand on liquid supports: digestate and sugarcane molasses at the Laboratory of the Soil Analysis and Environmental Chemistry Research Unit (URASCE) of the University of Dschang using the methods of [21].

2.2.2. Biofertilizer Formulations

The biofertilizer formulations based on the combinations of these different media mentioned above are listed in **Table 1**. The Yeast Mannitol Broth (YMB) medium noted B0 is prepared according to the standard method of [22]. As for the formulations noted B1 and B2, the solid materials are ground, passed through an 80 µm diameter sieve, and mixed in the proportions indicated in **Table 1** for a final mass of 100 g autoclaved at 121˚C for 30 min; each of these two formulations is added to 40 ml of consortium inoculum [23] were the standard YMB medium (yeast mannitol brother) was prepared from the protocol developed by [22]; Subsequently, four colonies of each isolate (bosd1, bosd2, bosd4 and bosd6) are added to this medium. Formulation B3 is made by the modified method of [24]; thus, approximately 2 ml of sugarcane molasses is added to 98 ml of pigeon pea extract. Formula B4 is obtained by diluting 80 ml of digested with 20 ml of distilled water.

Formulations B5 and B6 are B3 and B4 respectively with the addition of 1 g of white powder in each of them. The pH of the formulations, namely B3, B4, B5, and B6, are adjusted to 6.8; they are also autoclaved at 121˚C for 30 minutes and finally, four colonies are inoculated. All these formulations are divided into three groups (**Table 1**) and stored at room temperature during the evaluation of the growth and viability of the rhizobia.

Table 1. Composition of biofertilizer formulas based on local products.

Types of formulations	Codes	Materials used
Liquid media	B0	YMB
	B3	Cajan extract (98 ml) + sugar cane molasses (2 ml)
	B4	Digestate.
Solid media	B1	YMB + biochar (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%).
	B2	YMB + charcoal (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%).
Semi-solid media	B5	Cajan extract (98 ml) + sugar cane molasses (2 ml) + 1% white powder.
	B6	Digestate +1% white powder.

2.2.3. Evaluation of the Growth and Viability of the Rhizobial Consortium in the Different Formulations

The consortium's growth is evaluated four days after inoculation for 56 days, followed by its monthly viability using the counting method after decimal dilution described by [25]. The individualized colonies are evaluated by manual counting after 24 h of incubation, and the bacterial load (CN) is obtained by calculating the number of rhizobia per ml of solution and expressed in log CFU/ml.

CN (log CFU/ml) = Number of colonies counted × Dilution factor

The volume of inoculum deposited

The viability of the consortium in the different formulations is evaluated after the growth of the bacteria every 7 days (7th, 14th, 21st, 28th, 35th, 42nd, 49th, and 56th day) over 2 months [26].

2.2.4. Evaluation of the Development of the Rhizobial Consortium by Alternative Growth Media

The consortium development assessment presented in **Table 2** is carried out according to the modified method of [24] described by [27].

Yeast extract is prepared using sterile distilled water and the solution obtained is brought to the boil. As for the soybean, Cajun pea and bean extracts, approximately 100 g of power of each material are mixed with 1000 ml of water and mixed. The mixture is then boiled for two hours, cooled and autoclaved at 121˚C for 30 minutes.

From the previous Petri dishes, four colonies of each strain are taken and inoculated into the different growth media (Table 2).

Optical densities are read using a UV-visible spectrophotometer (Jenway 6305) at 600 nm every 24 hours for 4 days, at the end of which the media are centrifuged [26]. The pellet obtained after centrifugation is seeded in Petri dishes containing Yeast Extract Mannitol Agar (YEMA) medium to determine the fresh biomass (mg/ml) of the consortium used.

2.2.5. Soybean Seed Coating

Each previously prepared nitrogen-fixing rhizobia biofertilizer formulation is used for coating apparently healthy and morphologically similar soybeans with palm kernel oil as an adhesive for one block and corn powder for the other by the effluent method. This method consists of mixing 0.05 g (solid) or 0.05 ml (liquid) of each biofertilizer formulation with 0.15 ml of palm kernel oil or 0.15 g of maize powder, then adding 5 g of soya beans. The mixture is transferred to a container, stirred carefully and spread on a clean surface in the shade between 26 ± 2˚C, 55% and 64% humidity. These coated seeds are finally sown in the field 24 hours later.

Table 2. Composition of nitrogen-fixing rhizobia growth media based on local products. A: Mannitol; B: Sucrose; C: Sugarcane molasses; D: Milk powder; E: Soybean extract; F: Pig pea extract; G: Bean extract; H: Yeast extract; I: NaCl (50 mg·ml^{-1}); J: $MgSo_4$ (10 g·l^{-1}); K: $CaCl_2$ (40 g·l^{-1}); L: $FeCl_3$ (4 g·l^{-1}) mg·ml^{-1}; *: Stock solutions.

For 11 elements	Medium															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	10	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	10	10	10	10	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	10	10	10	10	-	-	-	-
D	-	-	-	-	-	-	-	-	-	-	-	-	10	10	10	10
E	100	-	-	-	100	-	-	-	100	-	-	-	100	-	-	-
F	-	100	-	-	-	100	-	-	-	100	-	-	-	100	-	-
G	-	-	100	-	-	-	100	-	-	-	100	-	-	-	100	-
H	-	-	-	40	-	-	-	40	-	-	-	40	-	-	-	40
I*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
J*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
K*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

2.2.6. Sowing Soybeans

The seeds coated with these different formulations were sown in triplets on 7 kg of substrate consisting of a mixture of black earth and sand with a diameter of 0.7^{-1} mm in the ratios (3 v/v) contained in 5 L culture pots. The pots were arranged in two completely randomized factorial blocks corresponding to the two coating adhesives (palm kernel oil and corn powder), and each block consists of 10 treatments corresponding to the different formulations with six repetitions each. The treatments carried out were: T0 (negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil). The same treatments were repeated with corn powder (m), *i.e.* 20 treatments in total. The plants were irrigated every day with tap water and once a week with this water enriched with 3 g/l of NaCl until harvest.

2.2.7. Evaluation of the Effect of Formulations Based on Nitrogen-Fixing Rhizobia and the Soybean Seed Coating Substrate on Growth

Growth parameters are measured during flowering [22]. Thus, the number of leaves is determined by counting, the height of the stems by direct measurement using a tape measure, the average leaf area [28] method, $S = 2/3 * L * l * n * 0.80 * 0.662$ (cm^2) where n is the number of leaves, L is the length of the leaf, and l is the sum of the maximum widths of its leaflets. The nodules are collected and counted after harvest, and the masses of the different organs were determined using a precision balance (KERN 572) to 0.001 g.

2.2.8. Evaluation of the Effect of Formulations Based on Nitrogen-Fixing Rhizobia and Soybean Seed Coating Substrate on Physiological Parameters

Chlorophyll is measured in non-senescent adult leaves harvested 4 weeks after sowing by the modified [29] descried by [30]. The total nitrogen in these leaves is measured according to the method described by [31].

The obtained effect of each result was the difference in quotient between treated and control blocks by control block multiplied by 100.

2.2.9. Statistical Analysis

The data for each parameter has been collected and treated into SPSS version 23.0 software for analysis of variances (ANOVA) using Duncan's test at the 1% threshold. Microsoft Excel 2016 software was used to present the results in the form of histograms and curves with means ± standard deviation.

3. Results and Discussion

3.1. Physicochemical Characteristics of Formulation Materials Based on Nitrogen-Fixing Rhizobia

The data recorded during the characterization of the biofertilizer formulation materials are recorded in **Table 3**. The analysis of this table shows that: some materials used are acidic because of their pH between 4.82 and 6.7 while others are basic with a pH between 7.8 and 9.68. The carbon and total nitrogen contents are higher in sugarcane molasses compared to the coating substrate. The phosphorus content is more abundant in Ox dung (**Table 3**). The major components of the biofertilizer are material supports of organic, inorganic, or synthetic nature which have appropriate physicochemical properties to maintain microbial strains in good physiological storage conditions and the field [32]; Bacterial growth considers several factors including an appropriate pH buffer, high water holding capacity, and chemical and physical uniformity [33]. Thus, the pH of different materials used for biofertilizer formulation ranges from 6 (Garoua soil) to 9.68 (digestat), this growth pH is generally close to neutral, is known to support a large population of rhizobia and maintain their viability [34].

Table 3. Physicochemical properties of materials for the formulation of biofertilizers. Biochar (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%); B2: YMB + charcoal (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%); B3: Pigeon pea extract (98 ml) + sugarcane molasses (2 ml); B4: Digestate; B5: Pigeon pea extract (98 ml) + sugarcane molasses (2 ml) + 1% white powder; B6: Digestate +1% white powder; N: total nitrogen; OM: organic matter; C: carbon; P: phosphorus; Nd: undetermined.

Parameters	Solid supports							Liquid supports	
	Biochar from peanut shells and litter leaves	Charcoal	Ox dung	White powder (shell)	Cajun pea powder	Coating substrate	Garoua soil	Digestate	Sugarcane molasses
pH-water	8.2	7.8	8.4	8.3	6.3	6.7	6.0	9.68	4.82
Organic materials									
OM (%)	94.60	76.30	64.90	3.70	95.50	2.76	3.31	Nd	Nd
C (%)	47.30	38.15	32.47	1.85	47.75	1.60	1.92	17.05	49.60
N (%)	1.67	0.84	2.11	0.13	3.11	0.12	0.14	74.67	1520.40
Exchangeable cations									
Calcium	1290	890	1770	1050	610	7.60	2	Nd	Nd
Magnesium	72	403	184	62	378	10	1.20	Nd	Nd
Potassium	1526	6628	3091	56	5268	2.26	0.71	Nd	Nd
Sodium	96	574	245	80	392	0.21	0.05	Nd	Nd
Phosphorus available ($mg \cdot Kg^{-1}$)	29.06	9.26	323.66	1.20	116.7	2.13	3.89	Nd	Nd

3.2. Effects of Formulations Based on Nitrogen-Fixing Rhizobia on the Growth of the BOSD Rhizobial Consortium

The growth of the consortium in the different formulations is shown in **Figure 1**. It emerges from its analysis that the loads in the different formulas increase exponentially and there is no significant difference between treatments B1, B4 (8.73 and 8.74 log CFU/ml) and the control treatment B0 (8.72 log CFU/ml). Formula B3 (8.89 log CFU/ml) has the highest load compared to the other formulas. The Bradyrhizobium strain would grow better in alternative media compared to the standard YMB medium [35]. This observation is made by an increase of + 30 to + 36% in these media over 4 days of observation. The formula enriched with Cajun pea extract supplemented with sugarcane molasses has the highest loading (8.89 log CFU/ml) compared to the standard YMB medium (8.71 log CFU/ml). Media enriched with sugarcane molasses would significantly increase the growth and viability of rhizobial strains [36].

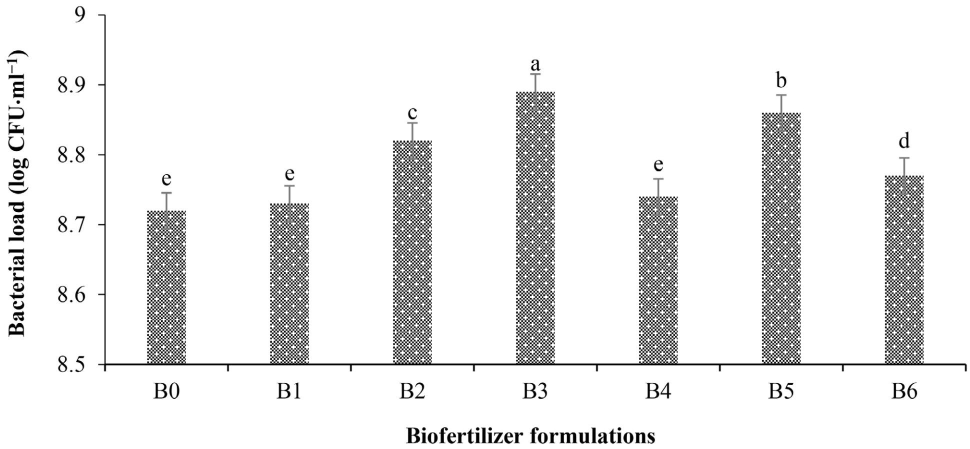


Figure 1. Bacterial load of the consortium in the different formulations after 4 days of incubation. Different letters indicate a difference at the 1% threshold. B0: YMB; B1: YMB+.

3.3. Effects of Formulations Based on Nitrogen-Fixing Rhizobia on the Viability of the BOSD Rhizobial Consortium

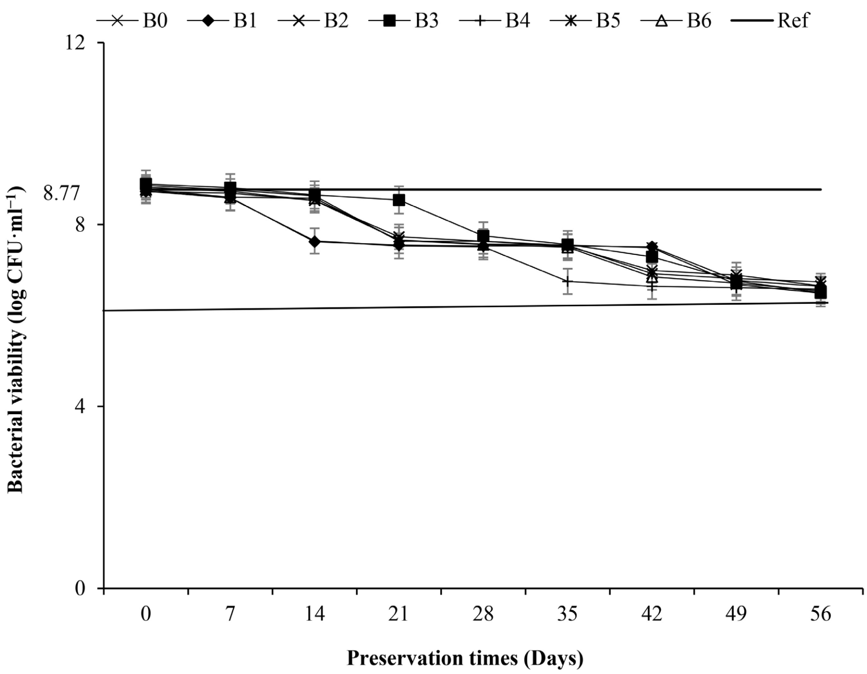


Figure 2. Bacterial viability in formulations as a function of storage time. B0: YMB; B1: YMB + biochar (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%); B2: YMB + charcoal (5%) + coating substrate (10%) + white powder (5%) + Garoua soil (20%) + cow dung (60%); B3: Pigeon pea extract (98 ml) + sugarcane molasses (2 ml); B4: Digestate; B5: Pigeon pea extract (98 ml) + sugarcane molasses (2 ml) + 1 % white powder; B6: Digestate +1% white powder.

The viability of the rhizobial consortium in the different formulations is shown in Figure 2. Its analysis shows that: the curves have a generally decreasing shape; this is reflected by a progressive decrease in the viability of the consortium in the different biofertilizer formulations depending on the shelf life. All formulas (B1 to

B6) have loads that vary between 6.5 and 6.74 log CFU/ml compared to the control B0 (6.49 log CFU/ml) after 56 days of counting. Formula B5 (6.74 log CFU/ml) has the highest bacterial load compared to the others and formula B3 (6.5 log CFU/ml) is the lowest. Determining the lifespan of a microbial strain in a formulation is an important step in the development of biofertilizers [37] and prolonged survival of inocula during storage is a desirable effect capable of increasing the application of biofertilizer formulations in industry [38]. In addition, variations in the charges in these formulations differ depending on the nature of the latter. They would probably be due to differences in the physicochemical properties of the transport materials used [39].

3.4. Effects of Formulations Based on Nitrogen-Fixing Rhizobia on Alternative Growth Media of the Rhizobia BOSD Consortium

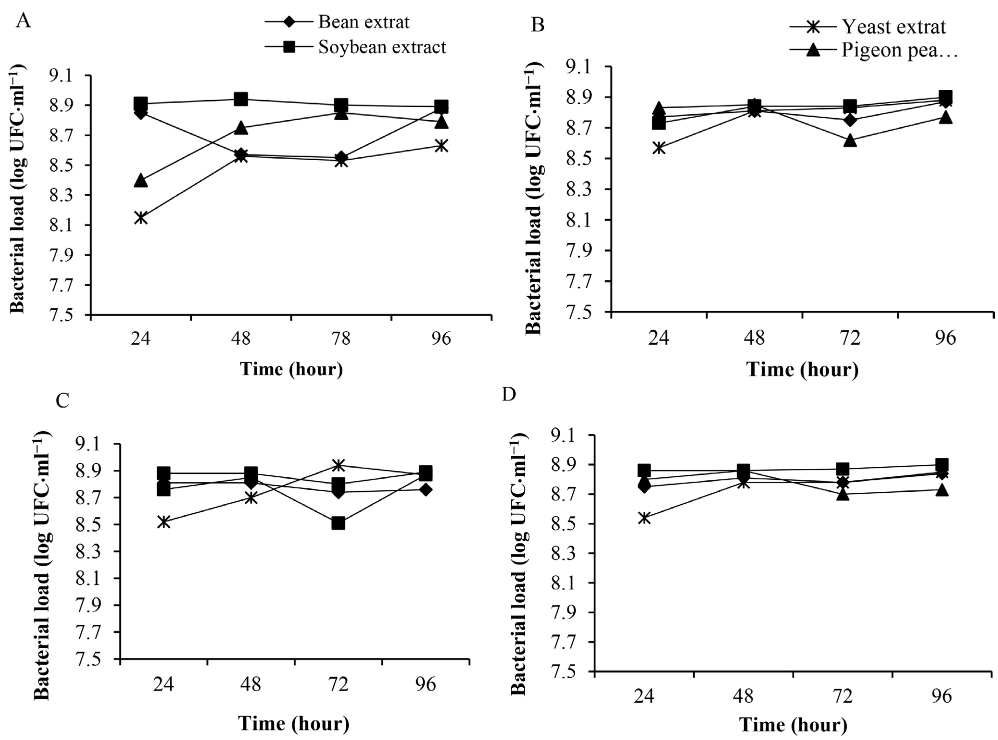


Figure 3. Growth of BNL strains in different alternative culture media. A: sucrose; B: mannitol; C: milk powder and D: sugarcane molasses.

The growth of the BOSD rhizobial consortium in alternative media is shown in **Figure 3**. Its analysis shows that: the supply of nitrogen sources or extracts enriched with nitrogen sources allows an increase in the bacterial load in all the consortium media during the evaluation period except soybean extract, the loads of which remain constant. Alternative media thus appear to be beneficial due to their local availability and affordable cost [27]. The growth of the consortium in the developed media is reflected by its development with a preference for the medium enriched with soybean extract as a nitrogen source and powdered milk as a carbon source compared to the yeast extract/mannitol medium, which is the standard

YMB medium. Indeed, the VUID1 strain would use all the alternative sources of carbon available (sucrose, glycerol, and cow's milk) to develop [27]. Fast-growing and intermediate-growing species of rhizobia have been reported to be able to grow in media containing sucrose as a carbon source [40].

3.5. Effect of Biofertilizer Formulations and Coating Method on Soybean Growth Parameters in the Field

Table 4. Effects of biofertilizers and the coating method on the growth parameters of soybean plants. T0 (Negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil).

Treatments	Growth parameters		
	Plant sizes (cm)	Number of leaves/Plant	Leaf area (cm^2)
T0	25.67 ± 0.44 h	14 ± 0.57 cd	9.48 ± 0.42 e
T1	33.41 ± 0.71 de	13.83 ± 0.40 cd	13.78 ± 0.33 b
T1 + h	33.33 ± 0.54 de	15.83 ± 0.70 abc	11.77 ± 0.64 bcde
T1 + m	32.67 ± 0.65 de	15.83 ± 0.70 abc	13.49 ± 0.66 bc
T1 + Pb	28.75 ± 0.30 fgh	14.67 ± 0.33 bcd	9.82 ± 0.61 de
T2 + h	30.08 ± 0.93 ef	14.83 ± 0.54 cdefg	9.40 ± 0.74 hi
T2 + m	34.58 ± 0.78 bc	15.33 ± 0.49 bcdefg	13.29± 0.63 bcde
T3 + h	28.67 ± 0.66 f	14.67 ± 0.49 defg	10.45 ± 0.74 fgh
T3 + m	35.91 ± 0.35 ab	16.83 ± 0.16 abcd	14.44 ± 0.36 b
T4 + h	37.25 ± 0.87 a	18.17 ± 0.98 a	14.99 ± 0.91 a
T4 + m	33.5 ± 0.84 bcd	16.33 ± 0.49 abcdef	13.26 ± 0.56 bcde
T5 + h	33.33 ± 1.09 cde	17.17 ± 0.60 abc	12.53 ± 0.33 bcdef
T5 + m	32.91 ± 0.49 cd	15.83 ± 0.54 qbcdefg	14.75 ± 0.42 ab
T6 + h	31 ± 0.51 def	16.67 ± 0.49 abcde	11.63 ± 0.55 cdefgh
T6 + m	25.25 ± 0.47 g	12.33 ± 0.49 g	7.81 ± 0.26 i
T7 + h	31.25 ± 0.44 def	17.33 ± 0.42 ab	11.30 ± 0.25 defgh
T7 + m	28.75 ± 0.30 f	14.33 ± 0.66 efgh	10.96 ± 0.46 efgh

Values in the same column followed by the same letter are not significantly different at the 1% level.

The responses of soybean plants in the different formulations and coating methods are presented in Table 4. Analysis of the table shows that all morphological parameters of soybean plant growth significantly increased ($P \leq 0.01$) in the treatment (T4 + h) with the highest and lowest values in the treatment (T6 + m) compared to the control T0. The contribution of rhizobial strains in the soybean rhizosphere would promote hydromineral and nitrogen nutrition. This supply of water and nutrients would instantly stimulate the phenomenon of photosynthesis, thus inducing good growth and development of these crops, namely the size, number of leaves, and their leaf surface [40]. This is also the case for Cowpea (*Vigna unguiculata* L.) after fertilization with consortium biofertilizer (CBF) [41].

3.6. Effect of Formulations and Coating Method on Nodulation of Soybean Plants

Table 5. Effect of biofertilizers and coating method on nodulation of soybean plants. T0 (negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil).

Treatments	Number of nodules/plant	Effet/T1 (%)	Nodules weight (mg.plant^{-1})
T0	0 j	-	0 n
T1	3.67 ± 0.21 ghi	-	62.17 ± 0.40 j
T1 + h	4.5 ± 0.22 dfgh	+54	70.33 ± 0.21 i
T1 + m	5.5 ± 0.22 def	+50	73.17 ± 0.3 h
T1 + Pb	3 ± 0.36 i	−18	28.83 ± 0.3 m
T2 + h	5.67 ± 0.3 de	+23	84.33 ± 0.21 f
T2 + m	10 ± 0.51 ab	+163	164.58 ± 0.45 ab
T3 + h	4.83 ± 0.3 efg	+32	81.5 ± 0.42 g
T3 + m	10.67 ± 0.33 a	+191	181.33 ± 0.66 a
T4 + h	8 ± 0.36 c	+178	179 ± 0.25 ab
T4 + m	4 ± 0.25 fghi	+89	99.5 ± 0.61 e
T5 + h	5.33 ± 0.33 def	+45	149.58 ± 0.63 bc
T5 + m	6.5 ± 0.42 d	+77	59.58 ± 0.73 k
T6 + h	3.17 ± 0.3 hi	−14	57.5 ± 0.42 l
T6 + m	5.17 ± 0.47 def	+41	80.33 ± 0.66 g
T7 + h	6 ± 0.36 de	+63	114.33 ± 0.35 d
T7 + m	8.67 ± 0.66 bc	+145	164.5 ± 0.5 ab

Values in the same column followed by the same letter are not significantly different at the 1% level.

The different values of the assessment of the effect of formulations on the number, and weight of nodules as well as the effect on soybean plants are presented in **Table 5**. The analysis of this table shows that the contribution of formulated biofertilizers leads to a significant increase in the number and weight of nodules regardless of the coating method (**Table 5**). As for the effect of nodulation on soybean plants, treatments (T2 + m), (T3 + m) and (T4 + h) have more significant effects namely: +163%, +191%, and +178% respectively) then the other formulations and compared to (T1). Legumes in general and beans in particular produce a large number of nodules at the root or stem level when the appropriate symbiotic partners are provided [42] [43]. This symbiotic association is used in several legume species, thus increasing their productivity [19] [20]. The growth parameters of soybean plants all show a significant increase in formulations with palm kernel oil as a coating adhesive. However, the nodulation effects are greater on the one hand with charcoal (+191%) and on the other hand with pigeon pea powder sugarcane molasses (+178%); this result would reflect the beneficial effect of biofertilizers on plant nodulation [27].

3.7. Effect of Formulations and Coating Method on Soybean Plant Biomass

Table 6. Effects of biofertilizers and coating method on soybean plant biomass. T0 (negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil).

Treatment	Parameters									
	BAF (g/plant)	Effet (%)	BRF (g/plant)	Effet (%)	BAS (g/plant)	BRS (g/plant)	BTF (g/plant)	Effet/T0 (%)	Effet/T1 (%)	BTS (g/plant)
T0	0.47 ± 0.03 f	-	0.37 ± 0.02 f	-	0.28 ± 0.03 cde	0.24 ± 0.02 cde	0.83 ± 0.05 h	-	-	0.54 ± 0.04 def
T1	1.01 ± 0.11 bcd	+115	1.12 ± 0.11 de	+200	0.37 ± 0.02 bcd	0.3 ± 0.02 abcd	2.14 ± 0.16 cde	+150	-	0.67 ± 0.02 bcd
T1 + h	0.94 ± 0.006 cdef	+100	0.29 ± 0.03 f	−15.7	0.32 ± 0.21 cde	0.1 ± 0.004 f	1.27 ± 0.08 fgh	+187	+37	0.39 ± 0.03 f
T1 + m	0.9 ± 0.01 cdef	+93.3	0.42 ± 0.02 f	−16.2	0.31 ± 0.01 cde	0.15 ± 0.009 ef	1.21 ± 0.05 fgh	+176	+26	0.57 ± 0.04 cde
T1 + Pb	0.53 ± 0.04 ef	+13	0.44 ± 0.03 f	+19	0.31 ± 0.02 cde	0.25 ± 0.01 bcd	0.97 ± 0.08 gh	+14	−136	0.56 ± 0.04 de
T2 + h	1.1 ± 0.07 bcd	+143	1.31 ± 0.02 cd	+254	0.28 ± 0.02 cde	0.23 ± 0.02 cde	2.45 ± 0.27 cd	+190	+40	0.52 ± 0.02 def
T2 + m	1.39 ± 0.13 bc	+196	0.97 ± 0.06 de	+162	0.46 ± 0.05 ab	0.29 ± 0.02 abcd	2.36 ± 0.25 cd	+200	+50	0.76 ± 0.04 ab
T3 + h	1.25 ± 0.11 bc	+166	2.24 ± 0.17 a	+500	0.33 ± 0.03 bcde	0.28 ± 0.01 abcd	3.49 ± 0.35 ab	+310	+160	0.61 ± 0.02 bcd
T3 + m	1.44 ± 0.14 ab	+206	1.29 ± 0.12 cd	+249	0.52 ± 0.03 a	0.34 ± 0.03 ab	2.73 ± 0.09 c	+220	+70	0.87 ± 0.06 a
T4 + h	1.85 ± 0.12 a	+294	2.01 ± 0.08 ab	+443	0.52 ± 0.02 a	0.38 ± 0.02 a	3.87 ± 0.11 a	+350	+200	0.9 ± 0.02 a
T4+ m	0.69 ± 0.06 def	+47	0.84 ± 0.07 e	+127	0.2 ± 0.01 e	0.21 ± 0.03 de	1.55 ± 0.05 efg	+80	−70	0.42 ± 0.04 ef
T5 + h	1.21 ± 0.07 bc	+157	1.67 ± 0.13 bc	+351	0.35 ± 0.02 bcd	0.28 ± 0.01 abcd	2.88 ± 0.16 bc	+240	+90	0.64 ± 0.04 bcd
T5 + m	1.03 ± 0.12 bcd	+121	0.84 ± 0.07 e	+127	0.35 ± 0.02 bcd	0.24 ± 0.01 bcde	1.83 ± 0.04 def	+120	−30	0.6 ± 0.04 bcd
T6 + h	1.17 ± 0.09 bcd	+149	1.68 ± 0.15 bc	+354	0.38 ± 0.04 bc	0.28 ± 0.01 abcd	2.85 ± 0.22 bc	+240	+90	0.66 ± 0.05 bcd
T6 +m	0.9 ± 0.08 cdef	+91	0.89 ± 0.08 de	+140	0.23 ± 0.01 de	0.31 ± 0.02 abcd	1.79 ± 0.05 def	+110	−40	0.54 ± 0.04 def

Continued

T7 + h	0.97 ± 0.25 bcde	+106	0.91 ± 0.13 de	+146	0.34 ± 0.07 bcd	0.24 ± 0.04 bcde	1.88 ± 0.1 def	+120	−30	0.59 ± 0.006 bcde
T7 + m	1.25 ± 0.12 bc	+166	1.07 ± 0.11 de	+189	0.39 ± 0.01 abc	0.33 ± 0.02 abc	2.46 ± 0.34 cd	+172	+22	0.74 ± 0.04 abc

Values in the same column followed by the same letter are not significantly different at the 1% level.

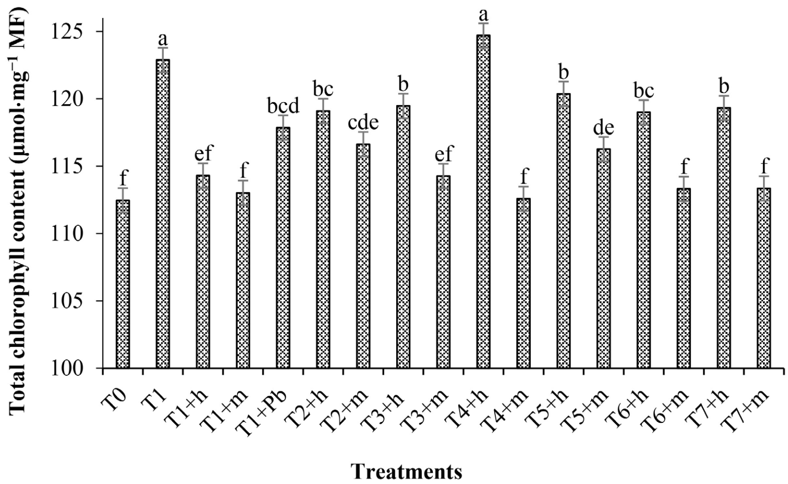
The effect of formulations and coating methods on the total biomass of soybean plants harvested in an open environment is presented in **Table 6**. Its analysis shows that: plants of different formulations have a similar biomass effect (+200%) to that of the positive control medium (T1) and +350% compared to that of plants of the negative control medium (T0). The biomass effect is more significant with the Cajun pea powder + sugarcane molasses associated with palm kernel oil (+350%). [27] also showed the beneficial effect of biofertilizers on the biomass and nodulation of peanut plants (*Arachis hypogaea* L.). The rhizobia-legume symbiosis at the root level is materialized by the formation of nodules, the seat of the biological fixation of nitrogen essential for the growth of the plant. Nodulation and biomass production depend on the compatibility between the plant genotype, as in the case of faba bean where the rhizobium strain and its interaction with the biophysical conditions of the soil had led to an increase of between 69 and 114% [42]; similar responses were observed in cowpea with rhizobia strains [20].

3.8. Effects of Biofertilizers and Coating Method on Total Chlorophyll Content of Soybean Plants

The total chlorophyll contents determined 4 weeks after sowing are presented in **Figure 4**. The analysis of this shows that: the T4 + h treatment has a high total chlorophyll content (125) with an effect of (+11%) compared to the control (T0), while the T4 + m treatment represents the lowest content (113) compared to T0. However, in each treatment, the leaves of soybean plants whose seeds had been coated with palm kernel oil have the highest total chlorophyll content compared to those of plants whose seeds had been coated with corn powder, highlighting the importance of stickiness on the sowing quality of soybeans. The palm kernel oil used causes sufficient humidification of the seeds, reducing physiological accidents and improving the germination quality and good growth of the plant.

3.9. Effects of Biofertilizers and Coating Method on Total Nitrogen and Protein Contents of Soybean Plants

The total nitrogen (N) contents determined after harvest in soybean leaves are reported in **Table 7**. It emerges from his analysis that the contribution of biofertilizers leads to a significant increase ($P \leq 0.01$) in the nitrogen content with an effect of +861% for the treatment (T4 + h) compared to the positive control (T1) and +1102% compared to the negative control (T0). The total nitrogen abundance is observed at this level of the treatment added to palm kernel oil. The biological fixation of nitrogen by bean, soybean, and cowpea plants has a positive effect on the nitrogen content compared to the control according to the work of [44]. These beneficial microorganisms are capable of fertilizing the soil enriched with all micro and macronutrient elements via nitrogen fixation, solubilization or mineralization of phosphorus and potassium, plant growth regulation factors, and degradation of organic matter in the soil [45]. The same is true for the protein content which presents a significant ($P \leq 0.01$) high value in the treatment (T4 + h), namely 195.31 which presents effects of +1165% and +935% respectively compared to T1 and T0.



Values in the same column followed by the same letter are not significantly different at the 1% level.

Figure 4. Effects of biofertilizers on total chlorophyll content of soybean leaves. T0 (negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil).

Table 7. Effects of biofertilizers and the coating method on the nitrogen and total protein contents of soybean plants for the effluent method. T0 (negative control), T1 (treatment with bacterial inoculum), T1 + h (bacterial inoculum + oil), T1 + pb (bacterial inoculum + white powder), T2 + h (B1 + oil), T3 + h (B2 + oil), T4 + h (B3 + oil), T5 + h (B4 + oil), T6 + h (B5 + oil) and T7 + h (B6 + oil).

Treatment	Parameters					
	Total nitrogen (mg/g)	Effets/T0 (%)	Effets/T1 (%)	Total protéin content (mg/g)	Effets/T0 (%)	Effets/T1 (%)
T0	2.60 ± 0 j	-	-	16.28 ± 0 j	-	-
T1	8.68 ± 0.87 h	+241	-	54.25 ± 5.42 h	+230	-
T1 + h	13.02 ± 0 f	+400	+159	81.38 ± 0 f	+400	+170
T1+ m	10.42 ± 0 g	+301	+60	65.10 ± 0 g	+300	+70
T1 + Pb	5.21 ± 0 i	+100	−141	32.55 ± 0 i	+100	−130
T2 + h	14.76 ± 0.87 e	+500	+259	92.23 ± 5.2 e	+467	+237

Continued

T2 + m	13.02 ± 0 f	+400	+159	81.38 ± 0 f	+400	+170
T3 + h	28.85 ± 0.2 b	+1010	+769	180.31 ± 1.25 b	+1008	+778
T3 + m	2.60 ± 0 j	0	−241	16.28 ± 0 j	0	−230
T4 + h	31.25 ± 0 a	+1102	+861	195.31 ± 0 a	+1165	+935
T4+ m	3.47 ± 0.87 j	+33.4	−208	21.70 ± 5.42 j	+35	−195
T5 + h	26.24 ± 0.2 c	+909	+668	164.02 ± 1.26 c	+907	+677
T5 + m	7.61 ± 0.2 h	+193	−48	47.57 ± 1.25 h	+192	−38
T6 + h	18.23 ± 0 d	+601	+360	113.93 ± 0 d	+600	+270
T6 +m	7.81 ± 0 h	+200	-41	48.83 ± 0 h	+200	−30
T7 + h	13.02 ± 0 f	+400	+159	81.38 ± 0 f	+400	+170
T7 + m	5.21 ± 0 i	+100	−141	32.55 ± 0 i	+100	−130

Values in the same column followed by the same letter are not significantly different at the 1% level.

4. Conclusion

Biofertilizers are combinations of microorganisms and other components that boost plant growth and nutrition, thus achieving satisfactory results after their application. They allow good conservation of microbial strains, particularly the rhizobial consortium used in their formulation. The evaluation of the formulations obtained as well as the soybean seed coating technique on its growth showed that pigeon pea powder + sugarcane molasses has better nodulation and growth in this species with an increase of +350%. Similarly, the total nitrogen content increases significantly by +1100% compared to the bacterial inoculum (B0) thus, these formulations allow an exponential improvement in the growth parameters of soybean plants in the field. The local substrates used showed an important potential as local culture media and carriers for the production of soybean nodulating bacterial inoculants. These results constitute considerable progress in the improvement not only of inoculants based on nitrogen-fixing bacteria but also in the fertility and sustainable production of grain legumes. The treatments applied contain nutrients and mineral elements essential for the growth of these plants. However, it would be necessary to co-produce the method with farmers to ensure the transfer of skills in order to sustainably improve their farming practices.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bhattacharyya, P.N. and Jha, D.K. (2011) Plant Growth-Promoting Rhizobacteria (PGPR): Emergence in Agriculture. *World Journal of Microbiology and Biotechnology*, **28**, 1327-1350. https://doi.org/10.1007/s11274-011-0979-9

[2] Herrmann, L. and Lesueur, D. (2013) Challenges of Formulation and Quality of Biofertilizers for Successful Inoculation. *Applied Microbiology and Biotechnology*, **97**,

8859-8873. https://doi.org/10.1007/s00253-013-5228-8

[3] Kumar, S., Diksha,, Sindhu, S.S. and Kumar, R. (2022) Biofertilizers: An Ecofriendly Technology for Nutrient Recycling and Environmental Sustainability. *Current Research in Microbial Sciences*, **3**, Article 100094. https://doi.org/10.1016/j.crmicr.2021.100094

[4] Zhang, J., Cook, J., Nearing, J.T., Zhang, J., Raudonis, R., Glick, B.R., *et al*. (2021) Harnessing the Plant Microbiome to Promote the Growth of Agricultural Crops. *Microbiological Research*, **245**, Article 126690. https://doi.org/10.1016/j.micres.2020.126690

[5] Fasusi, O.A., Cruz, C. and Babalola, O.O. (2021) Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management. *Agriculture*, **11**, Article 163. https://doi.org/10.3390/agriculture11020163

[6] Hara, F.A.D.S. and Oliveira, L.A.D. (2005) Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos de Iranduba, Amazonas. *Pesquisa Agropecuária Brasileira*, **40**, 667-672. https://doi.org/10.1590/s0100-204x2005000700007

[7] Lupwayi, N.Z., Clayton, G.W. and Rice, W.A. (2006) Rhizobial Inoculants for Legume Crops. *Journal of Crop Improvement*, **15**, 289-321. https://doi.org/10.1300/j411v15n02_09

[8] Ben Rebah, F., Prévost, D., Yezza, A., *et al*. (2007) Agro-Industrial Waste Materials and Wastewater Sludge for Rhizobial Inoculant Production: A Review. *Bioresource Technology*, **98**, 3535-3546. https://doi.org/10.1016/j.biortech.2006.11.066

[9] O'Callaghan, M. (2016) Microbial Inoculation of Seed for Improved Crop Performance: Issues and Opportunities. *Applied Microbiology and Biotechnology*, **100**, 5729-5746. https://doi.org/10.1007/s00253-016-7590-9

[10] Verma, M., Mishra, J. and Arora, N.K. (2018) Plant Growth-Promoting Rhizobacteria: Diversity and Applications. In: *Environmental Biotechnology*: *For Sustainable Future*, Springer, 129-173. https://doi.org/10.1007/978-981-10-7284-0_6

[11] Herridge, D.F., Peoples, M.B. and Boddey, R.M. (2008) Global Inputs of Biological Nitrogen Fixation in Agricultural Systems. *Plant and Soil*, **311**, 1-18. https://doi.org/10.1007/s11104-008-9668-3

[12] Herrmann, L., Atieno, M., Brau, L., *et al*. (2015) Microbial Quality of Commercial Inoculants to Increase BNF and Nutrient Use Efficiency. In: de Bruijn, F.J., Ed., *Biological Nitrogen Fixation*, Wiley-Blackwell, 1031-1040. https://doi.org/10.1002/9781119053095.ch101

[13] Catroux, G., Hartmann, A. and Revellin, C. (2001) Trends in Rhizobial Inoculant Production and Use. *Plant and Soil*, **230**, 21-30. https://doi.org/10.1023/a:1004777115628

[14] Yinhui, L., Rong, X.G., Zhangxiong, L., *et al*. (2008) Genetic Structure and Diversity of Cultivated Soybean (*Glycine max* (L.) Merr) in China. *Theoric Applied and Genetic*, **117**, 857-871. https://doi.org/10.1016/S2095-3119(15)61289-8

[15] Doré, C. and Varoquaux, F. (2006) History and Improvement of Fifty Cultivated Plants. Institut national de la recherche agronomique.

[16] FAOSTAT (2022) Base de données statistiques agricoles.

[17] FAO (2015) Statistiques de production alimentaire.

[18] Taffouo, V.D., Meguekam, T.L., Amougou, A., *et al*. (2010) Salt Stress Effects on Germination, Plant Growth and Accumulation of Metabolites in Five Leguminous Plants. *Journal of Agricultural Science and Technology*, **4**, 27-33.

[19] Nwaga, D., Jansa, J., Angue, M.A. and Frossard, E. (2010) The Potential of Soil Beneficial Micro-Organisms for Slash-And-Burn Agriculture in the Humid Forest Zone of Sub-Saharan Africa. In: *Soil Biology*, Springer, 81-107. https://doi.org/10.1007/978-3-642-05076-3_5

[20] Bahdjolbe, M., Sontsa-Donhoung, A., Abdouraman, H., Wade, A., Tobolbaï, R., Okiobe, S.T., *et al*. (2023) Isolation, Characterization, and Selection of Bacterial Endophytes from Soybean (Glycine Max) Nodules and Roots in Some Soils of Cameroon for Promoting Growth of Forage Legume Plants. *Microbiology Research Journal International*, **33**, 51-67. https://doi.org/10.9734/mrji/2023/v33i11-121417

[21] Pauwels, J.M., Van Ranst, E., Verloo, M., *et al*. (1992) Soil Science Laboratory Manual. Methods of Soil and Plant Analysis, Equipment, Management of Glassware and Chemicals. Agricultural Publications, No. 28, 180 p.

[22] Vincent, J.M. (1970) A Manual for Practical Study of Root Nodule Bacteria. Blackwell Scientific Publishers, 164.

[23] Pastor-Bueis, R., Sánchez-Cañizares, C., James, E.K. and González-Andrés, F. (2019) Formulation of a Highly Effective Inoculant for Common Bean Based on an Autochthonous Elite Strain of Rhizobium Leguminosarum Bv. Phaseoli, and Genomic-Based Insights into Its Agronomic Performance. *Frontiers in Microbiology*, **10**, Article 2724. https://doi.org/10.3389/fmicb.2019.02724

[24] Somasegaran, P. and Hoben, H.J. (1985) Methods in Legume-Rhizobium Technology. NifTAL Project and MIRCEN. Department of Agronomy, 2nd Soil Science Hawaii Institute Tropical Agriculture Human Research, University of Hawaii at Manoa.

[25] Arora, N.K., Tiweri, S. and Singh, R. (2014) Comparative Study of Different Carriers Innoculated with Ndule Forming and Free-Living Plant Growth Promoting Bacteria Suitable for Subtainable Agricture. *Journal Plant Pathology Microbiology*, **5**, Article 229. https://doi.org/10.4172/2157-7471.1000229

[26] Nwaga, D. and Ngo Nkot, L. (1998) *In Vitro* Tolerance of Rhizobia Isolated from *Vigna unguiculata* in Cameroon Compared to *Bradyrhizobium japonicum*. *Cahiers Agricultures*, **7**, 407-410.

[27] Ngo Mimb, J.A., Boyomo, O., Ngo Nkot, L., *et al*. (2014) The Use of Local Substrates in the Production of Legume Nodulating Bacteria Inoculants for SubSaharan Africa: Preliminary Results. *Journal of Applied Biosciences*, **75**, 6183-6191. http://dx.doi.org/10.4314/jab.v75i1.2

[28] Kumar, N., Krishnamoorty, V., Nalina, L., *et al*. (2002) A New Factor for Estimating Total Leaf Area in Banana. *Revista Brasileira de Fruticultura*, **44**, 42-43. https://doi.org/10.1590/0100-29452022119.

[29] Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W. and Strickland, J.D.H. (1965) Fluorometric Determination of Chlorophyll. *ICES Journal of Marine Science*, **30**, 3-15. https://doi.org/10.1093/icesjms/30.1.3

[30] Choula, F., Meguekam, T.L. and Wamba, F.O. (2023) Effect of Salinity on Physiological, and Biochemical Traits of Two Forage Species: *Albizia lebbeck* and *Faidherbia albida*. *African Journal of Agricultural Research*, **19**, 537-546. https://doi.org/10.5897/AJAR2023.16314

[31] Devani, M.B., Shishoo, C.J., Shah, S.A. and Suhagia, B.N. (1989) Spectrophotometric Method for Microdetermination of Nitrogen in Kjeldahl Digest. *Journal of AOAC International*, **72**, 953-956. https://doi.org/10.1093/jaoac/72.6.953

[32] Malusá, E., Sas-Paszt, L. and Ciesielska, J. (2012) Technologies for Beneficial Microorganisms Inocula Used as Biofertilizers. *The Scientific World Journal*, **2012**, 1-12. https://doi.org/10.1100/2012/491206

[33] Raimi, A., Roopnarain, A. and Adeleke, R. (2021) Biofertilizer Production in Africa: Current Status, Factors Impeding Adoption and Strategies for Success. *Scientific African*, **11**, e00694. https://doi.org/10.1016/j.sciaf.2021.e00694

[34] Sahu, P.K. and Brahmaprakash, G.P. (2016) Formulations of Biofertilizers—Approaches and Advances. In: *Microbial Inoculants in Sustainable Agricultural Productivity*, Springer, 179-198. https://doi.org/10.1007/978-81-322-2644-4_12

[35] Erdiansyah, I., Taufika, R., Widodo, T., Damanhuri Jannah, D.D. and Prayitno, H. (2022) Viability of Biofertilizer Bacteria *Rhizobium spp* Based on Household Waste. *IOP Conference Series: Earth and Environmental Science*, **980**, Article 012009. https://doi.org/10.1088/1755-1315/980/1/012009

[36] Arfarita, N., Imai, T. and Prayogo, C. (2022) Utilization of Various Organic Wastes as Liquid Biofertilizer Carrier Agents towards Viability of Bacteria and Green Bean Growth. *Journal of Tropical Life Science*, **12**, 1-10.

[37] Lobo, C.B., Juárez Tomás, M.S., Viruel, E., Ferrero, M.A. and Lucca, M.E. (2019) Development of Low-Cost Formulations of Plant Growth-Promoting Bacteria to Be Used as Inoculants in Beneficial Agricultural Technologies. *Microbiological Research*, **219**, 12-25. https://doi.org/10.1016/j.micres.2018.10.012

[38] Bashan, Y., de-Bashan, L.E., Prabhu, S.R. and Hernandez, J. (2013) Advances in Plant Growth-Promoting Bacterial Inoculant Technology: Formulations and Practical Perspectives (1998–2013). *Plant and Soil*, **378**, 1-33. https://doi.org/10.1007/s11104-013-1956-x

[39] Sohaib, M., Zahir, Z.A., Khan, M.Y., Ans, M., Asghar, H.N., Yasin, S., *et al*. (2020) Comparative Evaluation of Different Carrier-Based Multi-Strain Bacterial Formulations to Mitigate the Salt Stress in Wheat. *Saudi Journal of Biological Sciences*, **27**, 777-787. https://doi.org/10.1016/j.sjbs.2019.12.034

[40] Meguekam, T.L., Moualeu, D.P., Taffouo, V.D. and Stützel, H. (2021) Changes in Plant Growth, Leaf Relative Water Content and Physiological Traits in Response to Salt Stress in Peanut (*Arachis hypogaea* L.) Varieties. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **49**, Article 12049. https://doi.org/10.15835/nbha49112049

[41] Kaur, G., Kaur, J. and Walia, S.S. (2024) Effect of Integrated Nutrient Management on Soil Health, Soil Quality, and Production of Cowpea (*Vigna unguiculata* L.). *Journal of Basic Microbiology*, **64**, e2400225. https://doi.org/10.1002/jobm.202400225

[42] Allito, B.B., Ewusi-Mensah, N., Logah, V. and Hunegnaw, D.K. (2021) Legume-rhizobium Specificity Effect on Nodulation, Biomass Production and Partitioning of Faba Bean (*Vicia faba* L.). *Scientific Reports*, **11**, Article 3678.

[43] Masson-Boivin, C. and Sachs, J.L. (2018) Symbiotic Nitrogen Fixation by Rhizobia—The Roots of a Success Story. *Current Opinion in Plant Biology*, **44**, 7-15. https://doi.org/10.1016/j.pbi.2017.12.001

[44] Htwe, A.Z., Moh, S.M., Soe, K.M., Moe, K. and Yamakawa, T. (2019) Effects of Biofertilizer Produced from Bradyrhizobium and Streptomyces Griseoflavus on Plant Growth, Nodulation, Nitrogen Fixation, Nutrient Uptake, and Seed Yield of Mung Bean, Cowpea, and Soybean. *Agronomy*, **9**, Article 77. https://doi.org/10.3390/agronomy9020077

[45] Sinha, R.K., Valani, D., Chauhan, K. and Agarwal S. (2010) Embarking on a Second Green Revolution for Sustainable Agriculture by Vermiculture Biotechnology Using Earthworms: Reviving the Dreams of Sir Charles Darwin. *Journal Agriculture Technology Sustainable Development*, **2**, 113-128. https://doi.org/10.5897/JABSD.9000017