Proposal Title: **Biofertilizer Development from Nigeria's Semi-Arid Tree Crop's Rhizosphere for Sustainable Agriculture and National Economic Growth**

Research Team Members:

Professor Abubakar Gidado, Department of Biochemistry, Faculty of Life Sciences, University of Maiduguri (Principal Investigator, PI)
Professor Sunday P. Madakan, Department of Biological Science, Faculty of Life Sciences, University of Maiduguri (Co-PI)
Dr. Rakiya Abdullahi, Department of Soil Microbiology, Faculty of Agriculture, University of Maiduguri, (Co-PI)
Dr. Ali Abubakar, Department of Forestry and Wildlife Management, Faculty of Agriculture, Modibbo Adama University, Yola. (Co-PI)
Prof. Aliyu B. Mustapha, Department of Crop Production and Horticulture, Faculty of Agriculture, Modibbo Adama University, Yola (Co-PI)
Jummai T. Kaldapa, Centre for Biotechnology, University of Maiduguri (Co-PI)
Mwajim Bukar, Centre for Biotechnology, University of Maiduguri (Co-PI)
Aisha Idris Danaski, Centre for Biotechnology, University of Maiduguri (Research Mentee)
Mohammed Yusuf Idris, Department of Soil Microbiology, Faculty of Agriculture, University of Maiduguri (Research Mentee)

PROJECT SUMMARY

Estimated Nigeria's population in 2025 stood at over two hundred and thirty million (Worldometer 2025), driving greater demand for food amidst cultivable land shrinkage and lost of soil fertility. To address these growing challenges, chemical fertilizers are employed, and they play a pivotal role in improving soil fertility, thereby supporting sustained food production and meeting the rising demands of an expanding population. Nevertheless, chemical fertilizers have been linked to substantial environmental and health hazards. Plant Growth Promoting Rhizobacteria (PGPR) are currently viewed as viable alternatives to conventional agrochemicals. The interest in these beneficial microbes is due to their ability to enhance plant growth through multiple mechanisms, including nitrogen fixation, biosynthesis of phytohormones, stimulation of root development, suppression of phytopathogens, biodegradation of organic contaminants, and solubilization of nutrient forms that are otherwise inaccessible to plants. Trees thriving in nutrient-deficient soils have evolved various adaptive mechanisms to sustain growth and productivity under challenging environmental conditions. One such strategy involves establishing symbiotic associations with soil-dwelling microorganisms that facilitate nutrient acquisition, particularly nitrogen. Building on this premise, we recently reported the presence of Plant Growth Promoting Rhizobacteria (PGPR) in the rhizosphere soils of trees naturally occurring in the arid and semi-arid regions of North-East Nigeria, with strong biofertilizer potential. The objectives of this project are to Identify and optimize the most effective carrier materials for the bacterial inoculants to ensure viability, shelf-life, and ease of application in agricultural settings, conduct comprehensive pot and field trials of the developed biofertilizers to assess their performance across different soil types, crop species, and environmental conditions, analyse the physical and chemical properties of soils used in the trials to establish baseline fertility levels and monitor changes induced by biofertilizer application and to finally characterize the soil bacterial communities using both conventional microbiological techniques and molecular methods to understand the changes induced by the biofertilizer application. Standard scientific methods will be adopted in achieving the objectives of the project. The project is aimed at transition from laboratory-scale application to scalable agricultural application of the identified bacterial inoculants.

OBJECTIVES OF THE RESEARCH PROJECT

The project objectives are to:

1. Identify and optimize the most effective carrier materials for the bacterial inoculants to ensure viability, shelf-life, and ease of application in agricultural settings.
2. Conduct comprehensive pot and field trials of the developed biofertilizers to assess their performance across different soil types, crop species, and environmental conditions.
3. Analyse the physical and chemical properties of soils used in the trials to establish baseline fertility levels and monitor changes induced by biofertilizer application.
4. Characterize the soil bacterial communities using both conventional microbiological techniques and molecular methods to understand the changes induced by the biofertilizer application.

Statement of Research Problem

We recently developed biofertilizer candidates derived from rhizosphere soils of key tree crops in semi-arid and arid regions of North-East Nigeria although we have not validated their efficiency under diverse agronomic conditions. The previous project laid a strong foundation by uncovering microbiological and molecular insights and identifying promising microbial strains. However, the transition from laboratory-scale innovation to scalable agricultural application demands rigorous evaluation of these microbial consortia in real-world environments.

There is a critical need to assess the performance, stability, and synergistic interactions of these biofertilizer candidates as consortia under controlled pot trials and open field conditions. This will help determine their adaptability across climatic variations and crop species, and ensure their reliability as eco-friendly alternatives to synthetic fertilizers. Without such validation, the potential for commercialization, policy integration, and contribution to sustainable agriculture and national economic growth remains largely untapped.

Commercial Viability

The commercial viability of biofertilizers in Nigeria and across Africa is increasingly promising, driven by the urgent need for sustainable agricultural practices, rising environmental awareness, and the economic imperative to reduce dependence on synthetic inputs.

The Nigeria fertilizer market, which includes both chemical and biofertilizers, is valued at approximately USD 1.2 billion in 2024, with projections to reach USD 1.4 billion by 2030. Biofertilizers are emerging as high-growth segment due to increasing demand for eco-friendly inputs. Across Africa, the biofertilizer market is projected to grow from USD 246.8 million in 2025 to USD 387.4 million by 2030.

This surge is fuelled by expanding organic farming practices, government support for sustainable agriculture, and technological advancements in microbial formulations.

RESEARCH PROJECT METHODS

1. Identification and optimization of the most effective carrier materials for the bacterial inoculants

To support inoculants growth and effective delivery into the field, suitable carriers such as coal, clays, charcoal, cellulose, lignite, vermiculite, sawdust, wheat bran, and rice husk are going to be tested. Shelf life, bioavailability, release rate, and overall performance of the bioinoculants-based bio-formulation will be used as selection criteria.

2. Pot and field trials of the developed biofertilizers

Suitable food crops will be evaluated for the biofertilizer effect using both pot and field trials. The pot experiment will be carried out at the screen house of the Faculty of Agriculture; University of Maiduguri and the field trials will be conducted in at least three states in North-East Nigeria. The treatments will consist of the inoculants (TI), Inoculants plus different rates of inorganic fertilizer (TF), NPK (positive control) and Normal Control (C) arranged in a completely randomized block design (CRD), with five (5) replications. For pot experiments, soil collected from the experimental sites will be analysed for physical and chemical properties, then steam sterilized to eliminate weed seeds and pathogens.

Growth measurements, including plant height and leaf count, will be recorded at two-week intervals, while stem girth will be measured at harvest. The pot experiments will last for six weeks, after which fresh and dry biomass, along with nutrient uptake, will be analysed. For field experiments, growth parameters, grain quality, yield and other important parameters will be analysed post harvest.

3. Soil Physical and Chemical Properties Analysis

Particle size distribution, soil pH, organic carbon content, total nitrogen, available phosphorus, exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}) will all be analysed using standard methods.

4. Characterization of soil Bacterial Communities

Standard microbiological techniques will be used for the isolation of the total viable bacteria. Species specific media will then be used for the reviving and isolation of the nitrogen-fixing and phosphate solubilizing bacterial strains. Characteristics of the isolates on specific media will be used for the determination of the strain's morphology, gram staining and biochemical characteristics.

Bacterial DNA will be isolated using Bacteria Genomic DNA Extraction Kit using the manufacturer's instructions. The DNA will be eluted with TE Buffer (pH 6.8) and quantified using a *NanoDrop* spectrophotometer. The concentration and purity of the DNA will be determined based on absorbance at 260 nm and the A260:A280 ratio.

The *16S rRNA* gene will be amplified using primers will targeting a 1465 bp segment of the 1500 bp gene. The polymerase chain reaction (PCR) will be performed with FIREPol Master Mix, and Aliquots of PCR products will be electrophoresed and visualized in 1.8% agarose gels using standard electrophoresis procedures.

The *16S rRNA* and *nif* genes of isolates will be sequenced using sangers sequencing method and the sequences will be compared with that of other microorganisms by way of BLAST (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

Statistical Analysis

The impact of biofertilizer isolates on the food crops will be assessed using analysis of variance (ANOVA). Treatment means are to be expressed as Mean ± Standard Error (SE), and statistical significance will be determined at $P < 0.05$. Sequence editing will be conducted using BioEdit software, while alignment and phylogenetic analysis will be performed using Clustal X and MEGA 11.

Research Timelines

RESEARCH MILESTONE	TIMELINE
Research Flag-off	January (Year 1)
Procurement of Equipment, Chemicals, Reagents and Laboratory Consumables	January-March (Year 1)
Identification and optimization of the most effective carrier materials for the bacterial inoculants	February-July (Year 1)
Experimental pot trial of Biofertilizer Candidates	June-July (Year 1)
First Year Field Trials with Biofertilizer candidates	June-October (Year1)
Physico-chemical Analysis, Conventional and Molecular Characterization of Bacterial Samples from Pot Trials	August-November (Year 1)
DNA Sequencing, of Isolates from Pot Trials and Analysis	November-December (Year 1)
Physico-chemical Analysis, Conventional and Molecular Characterization of Bacterial Samples from Field Trials	January- April (Year 2)

DNA Sequencing, of Isolates from Field Trials and Analysis	April (Year 2)
First Year Data Analysis, Report Writing and Dissemination	April-June (Year 2)
Second Year Field Trials	June-October (Year 2)
Physico-chemical Analysis, Conventional and Molecular Characterization of Bacterial Samples from Second Year Field Trials	October-December (Year 2)
Second Year Data Analysis, Report Writing and Dissemination	January-May (Year 3)
Third Year Field Trials	June-October (Year 3)
Physico-chemical Analysis, Conventional and Molecular Characterization of Bacterial Samples from Third Year Field Trials	October-December (Year 3)
Third Year Data Analysis, Report Writing and Dissemination	January- March (Year 4)
Final Report Writing	April-June (Year 4)

Host Institution Laboratory Space and Facilities

The Biotechnology Centre, University of Maiduguri will be the host for the project. The Centre is equipped with two functional Laboratories, the Plant Tissue culture Laboratory, and the Molecular biology Laboratory. The Molecular Biology laboratory will host most of the molecular and microbiological activities of the project. The Molecular Biology Lab has a general lab space of 26ft x 48ft and Nine (9) laboratory suites within the laboratory, comprising the preparation room, weighing room, PCR and spectrometric suite, electrophoresis suite, Microscopy room, spectroscopy rooms and extraction room all measuring about 15ft x 12ft. All suites and general work area are well furnished with epoxy standard work surfaces and furniture. The laboratory has a technologist office and two restrooms. The laboratory is fully air conditioned and has an overhead water reservoir. The Centre has an experimental farm, a greenhouse and functional 100kva generator power back up. The Centre has adequate office space for administrative and technical personnel and a conference room. There are seven (7) trained laboratory personnel.

Equipment and Materials in the Centre

S/N	EQUIPMENT DESCRIPTION & MODEL	MANUFACTURER	QTY
1	Thermal Cycler (*MasterCycler* Nexus, Gradient & *MasterCycler* Nexus X2)	Eppendorf	2
2	Micro Centrifuge (5430)	Eppendorf	1
3	*NanoDrop* Spectrophotometer (2000C)	Thermos Scientific	1
4	Bio Spectrometer with µCuvette G1.0	Eppendorf	1
5	ELISA reader (MULTISKAN FC)	Thermos Scientific	1
6	Refrigerated Centrifuge (Sigma 4-16K)	Sigma	1
7	Electrophoresis power pack with mini, midi & maxi agarose gel tank and Horizontal PAGE tank	Cleaver Scientific	2
8	Chemical Balance 0.0001g (ADAM PW254)	ADAM	1
9	Top Loading Balance (0.1g) (Stuart pro)	Ohaus	1
10	UV-transilluminator	Cleaver Scientific	1
11	Gel Documentation System with computer set	Cleaver Scientific	1
12	Workstation (LF series, Air Science)	Air Science	1
13	PCR Cabinet (Isocide)	ESCO	1
14	pH Meter H12550 (Require servicing and replacement of electrode)	Hanna Instruments	2
15	Heating plate/ Magnetic Stirrer (RCT-Basic)	IKA	2
16	Homogenizer (T25 ultra-Turrax)	IKA	2
17	Water purification System (NW-Series)	Heal-force	1
18	Heating Bath (Clifton)	Clifton	1
19	Shaking Water Bath (Medline 7RS-06)	Medline	1
20	Water Bath (DK420) 1		1
21	Oven (Memmert)	Memmert	1
22	Hybridization Oven (Binder)	Binder	1
23	Shaking incubator (Stuart)	Stuart	1
24	Colony Counter (Reichert, QueBeck)	Reichert	1
25	Vortex (Benchmark)	Benchmark	1
26	Biomixer (Benchmark)	Benchmark	1
27	Ultra-low Freezers (-850C)	Haier	1
28	Deep freezer (200C)	Haier	2
29	Laboratory Fridge -40C	Haier	1
30	Dissecting Microscope (Amscope)	AMScope	2
31	Epi-florescence Microscope (Evos)	EVOS	1
32	Autoclaves		2
33	Hot Air Oven (Blue bird) Model 33159 1	Blue bird	1
34	Flame Photometer PFP7	Jenwey	1
35	Ice maker (Hoshizaki)	Hoshizaki	1
36	Dry sterilizer (Germinator)	Germinator	1
37	Automatic Pipettes	Eppendorf	5 each

	a. Eppendorf Research Plus (100-1000ul) b. Eppendorf Research Plus (20-200ul) c. Eppendorf Research Plus (10-100ul) d. Eppendorf Research Plus (0.5-10ul) e. Eppendorf Research Plus (0.1-2.5ul)		
38	Pipette Aid Drummond	Drummond	5
39	Adequate Glass wares		

Budget

		Unit	**Cost**
1.0 Personnel Costs/Allowance			
1.1 Principal researcher	~~₦~~ 3,625,000.00	1	~~₦~~ 3,625,000.00
1.2 Team Members	~~₦~~ 4,518,750.00	6	~~₦~~ 4,518,750.00
1.3 Technical Support	~~₦~~ 2,550,000.00	6	~~₦~~ 2,550,000.00
1.4 Field Staff	~~₦~~ 180,000.00	6	~~₦~~ 1,080,000.00
Sub-Total			**11,773750.00**
2.0 Equipment			
2.1 Multi-platform Orbital Shaker (Fisherbrand)	~~₦~~ 4,500,000.00	1 Unit	~~₦~~ 4,500,000.00
2.2 Benchtop Bioreactor (BioFlo® 120)	~~₦~~ 12,000,000.00	1 Unit	~~₦~~ 12,000,000.00
2.3 PH Meter (HANNA 2211)	~~₦~~ 2,500,000.00	1 Unit	~~₦~~ 2,500,000.00
2.4 Pipette Calibrations	~~₦~~ 75,000.00	15pcs	~~₦~~ 1,125,000.00
Sub- Total			**20,125,000.00**
3.0 **Supplies/Consumables**			
3.1 Sterile Falcon tubes (50mL x 500 x5)	~~₦~~ 75,000.00	X5	~~₦~~ 375,000.00
3.2 Sampling bags/tube	~~₦~~ 580,000.00	X2carton	~~₦~~ 1,160,000.00
3.3 Microbial DNA extraction Kit (DNeasy ultra-clean microbial kit, 250 prep)	~~₦~~ 450,000.00	X2	~~₦~~ 900,000.00

3.4 Qiaquick PCR clean-up kit (250prep)	₦ 593,580.00	x2	₦ 1,786,740.00
3.5 PCR Primers (16S rRNA, *nif* genes)	₦ 75,000.00	x3	₦ 225,000.00
3.6 DNA Ladder (NEB Quickload 1kb, 100bp & 50 bp)	₦ 135,609.00	X2	₦271,218.00
3.7 Invitrogen SYBR green I nucleic acid stain	₦ 430,375.00	X2	₦ 860,750.00
3.8 PCR Master Mix (NEB QuickLoad, 250 preps X 5)	₦ 528,750.00	X2	₦ 1,057,500.00
3.9 Phusion® High-Fidelity PCR Master Mix with HF Buffer (500rxn)	₦ 678,240.00	X2	₦ 678,240.00
3.10 Agarose powder (500g)	₦ 450,500.00	X1	₦ 450,500.00
3.11 Boric Acid (500g)	₦ 154,125.00	X1	₦ 154,125.00
3.12 Trizma Base (Fisher Chemical)	₦ 355,000.00	X1	₦ 355,000.00
3.13 Ethanol (DNA grade) 2.5L	₦ 55,000.00	X2	₦ 110,000.00
3.14 Ethanol (Disinfection Grade) 2.5L	₦ 27,500.00	X4	₦ 110,000.00
3.15 Safe lock Eppendorf tubes (PK/500)	₦ 45,000.00	X5	₦ 180,000.00
3.16 Eppendorf™ Thin-Walled PCR Tubes (1,000 pcs)	₦ 209,500.00	X2	₦ 419,000.00
3.17 Yellow barrier pipette tips (20-200uL), Case of 10	₦ 21,000.00	X15	₦ 315,000.00
3.18 Blue barrier pipette tips (100-1000uL), case of 10	₦ 25,000.00	X15	₦ 375,000.00
3.19 White Barrier pipette tips (0.1-20uL case of 10	₦ 25,000.00	x5	₦ 125,000.00
3.20 Nitrile Gloves (Medium & Large Sizes) case of 10	₦ 50,000.00	X6	₦ 300,000.00
3.21 Petri Dishes 100/carton	₦ 20,000.00	X10	₦ 200,000.00
3.22 Glass slides & coverslip 20pcs/pack x10	₦ 6,000.00	X10	₦ 60,000.00
3.23 Grams Stains set 1L each	₦ 25,500.00	X5	₦ 127,500.00
3.24 Wire loop (5)	₦ 2,500.00	X2	₦ 5,000.00
3.25 Bench Wipes	₦ 6,000.00	X20	₦ 120,000.00
3.26 TSA media (merck), 500g	₦190,000.00	X1	₦ 190,000.00

3.27 LB broth (merck) 500g	₦151,250.00	X2	₦ 151,250.00
3.28 Biofertilizer Carrier (Clay, charcoal, cellulose, sawdust, wheat bran, and rice husk, lignite, vermiculite)	₦1,500,000.00	X4	₦ 7,500,000.00
3.29 Bioreactor reagents and Consumables	₦2,500,000.00	X4	₦10,000,000.00
3.30 Pikovskayas Agar (merck) 500g	₦ 229,560.00	X1	₦ 229,560.00
3.31 Peptone (merck) 500g	₦ 125,000.00	X1	₦ 125,000.00
3.32 Disposable Sterile Pasteur pipette	₦ 25,0000.00	X5	₦ 125,000.00
3.33 N-free malate medium (500g)	₦ 251,250.00	X2	₦.502,500.00
3.34 Azospirillum medium A & B (500g)	₦ 250,000.00	X1	₦ 250,000.00
3.35 250mL Culture flask (Carton of 12)	₦ 86,525.00	X2	₦ 173,050.00
3.36 Biosafety Waste Bags	₦ 123,240.00	X3	₦ 369,720.00
3.37 Nursery polybags	₦ 10,000.00	X10	₦ 100,000.00
3.38 Watering can, trowels, shovels	₦ 105,000.00	X3	₦ 315,000.00
3.39 Seed	₦10,000.00	X3	₦ 30,000.00
Sub-total	**₦**		**₦ 30,781,153.00**
4.0 Data Collection and Analysis			
4.2 Sanger Sequencing	₦ 40,000.00	X75	₦ 3,000,000.00
4.3 Soil-Physicochemical Analysis	₦ **1,**225,000.00	X6	₦ **7,**350,000.00
4.4 Bioinformatics Data Analysis	₦ 1,500,000.00	X1	₦ **1,**500,000.00
4.5 Statistical Data Analysis	₦ **1,**250,000.00	X1	₦ **1,**250,000.00
4.6 Laptop Computer	₦ 2,000,000.00	X3	₦ 6,000,000.00
Sub-Total			**₦ 19,100,000.00**
5.0 Field Trial			
5.1 Field trial site, demarcation, markings, beddings, plant growth measurement tools	₦ 500,000.00	3 regions x3 plots x 3 years	₦ 4,500,000.00
4.2 Technical assistants (field Trial) x9	₦ 180,000.00	X9	₦ 1,620,000.00
Sub-total			**₦ 6,120,000.00**
6.0 Dissemination			

6.1 Publication	₦ 5,000,000.00		₦ 5,000,000.00
6.2 Patent Application	₦ 500,000.00		₦ 500,000.00
6.3 Conference attendance	₦ 2,000,000.00		₦ 2,000,00.00
Sub-total			**₦ 7,500,000.00**
Travels and Research Meetings			
7.1 Quarterly researchers team meeting	₦ 250,000.00	6	₦ 3,000,000.00
7.2 Research secretariat	₦ 250,000.00		₦ 250,000.00
7.3 Internet Services	₦ 500,000.00		₦ 500,000.00
Sub-total			**₦ 3,750,000.00**
Grand total			

Budget Summary

S/N	Item	Sub-Total
1.0	Personnel Costs/Allowance	₦ 11,773,750.00
2.0	Equipment Cost	₦ 20,125,000.00
3.0	Supplies and Laboratory Consumable	₦ 30,781,153.00
4.0	Data Collection and Analysis	₦ 19,100,000.00
5.0	Field Trial	₦ 6,120,000.00
6.0	Dissemination	₦ 7,500,000.00
7.0	Travels and Meetings	₦ 3,750,000.00
	Sub-Total	**₦ 99,249,903.00**
	Indirect Cost (5% of total direct cost)	₦ 4,962,495.15.00
Grand Total		**₦ 104,212,398.15**