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Development of Beneficial-Microbial-Based Biofertilizers for Future Generation of Agriculture (Bio-Agriculture) and Their Global Health Impacts in Cameroon

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Abstract

Over decades, chemical fertilizers have been used to increase crop production and meet increasing demands. However, the overuse of these products is known to be harmful to humans, animals and environmental health. In addition to air and water pollution, they are also responsible for the depletion of minerals from the soil and global warming. Furthermore, their residues in farm and animal products are the cause of metabolic and endocrinological disorders leading to cancers, cardiovascular failures, reproductive complications, congenital malformations and more. In this work, we have developed three types of biofertilizers (LanaRhizo, Lana-MSP and LanaMyco) based on soil bacteria and fungi isolated in Bockle (Garoua). The aims of these findings include the reduction of harmful health effects caused by chemical fertilizers with the promotion of good health to the multi-sectorial global One Health sector. Biofertilizers which are made up of beneficial microorganisms are a viable alternative technology to increase food production without jeopardizing human and environmental health. Biofertilizers include all organisms which supply or make different nutrients available to plants (nitrogen fixers, phosphorus solubilizers and mycorrhiza). Results demonstrate significant improvements in soybean and maize growth parameters, yield, protein content, oil content (soybean), and total soluble sugar content (maize) with biofertilizer application. Furthermore, the study explores the impact of Lana-MSP on human health by assessing flavonoid biosynthesis and reduced glutathione (GSH) production in maize, showing significant increases compared to the control and NPK treatments. Biofertilizers are important to organic farming

in that they are cost effective, completely environment friendly, and unharmful and they do not cause pollution but reduce Greenhouse Gases (GHG) emissions into the atmosphere. With the actual use of biofertilizers in applied agriculture, they could constitute a promising alternative to chemical fertilizers, offering benefits for future generations of agriculture and human health.

Keywords

Biofertilizers, Future Generation of Agriculture (Bio-Agriculture), LanaRhizo, Lana-PSM, LanaMyco, Global Health

1. Introduction

Since the industrial revolution of the 19th century, human activities have emitted Greenhouse Gases (GHG) into the atmosphere which causes a warming of the average temperature on the surface of the globe and disrupts the global climate [1]. If not significantly reduced, these emissions could totally endanger global health, the world economy, human societies and ecosystems. The agricultural sector represents 13.5% of global GHG emissions [1]. The advent of an industrial system of agriculture involving the use of chemicals, preservatives, hormones, and antibiotics resulted in increased food growth and production [2]. This new technique produces crops and livestock in larger quantities than the sustainable agriculture practiced in the past [3]. Industrial agriculture is characterized by mono cropping, in which the same crop is grown season after season. Mono cropping reduces the soil's ability to naturally eliminate pests and replenish nutrients [4]. To combat this menace, industrial agriculture uses heavy amounts of chemical fertilizers and pesticides [5]. Agrochemicals are commonly used in agricultural production to control or prevent diseases, pests and weeds in order to maintain high quality agricultural products and eliminate or reduce yield losses. With this industrialized system, food is produced at reduced costs and farmers therefore get higher profits from their farm but serious concerns were being raised about health risks resulting from residues in drinking water and food and from occupational exposure [2]. [6] reiterated that heavy doses of chemical fertilizer, although leading to self-reliance in food production, cause harmful impacts on living organisms and also depreciate the environment. The chemical contaminates the food produced and further alters the normal body functions of the consumer [7]. The residues of chemical fertilizers are responsible for several metabolic diseases such as cancers, cardiovascular failures, and reproductive complications with some leading to endocrinological disorders and more. [8] reported 75% of pesticide residues in conventionally grown produce. Water supplies are polluted by toxic insecticides, herbicides, and chemical fertilizers used [2]. These dangers can be reduced by the use of microbial biofertilizers (Phosphate Soluble Microorganisms (PSM), *Rhizobium* and *Mycorrhiza*) [9]. Biofertilizers contribute to the sustainable maintenance of human health, and improve the

nutritious properties of crops by increasing; the antioxidant activity, the total phenolic compounds, flavonoids and glutathione [10]. These secondary metabolites play preventive roles in cancer, neurodegenerative, and cardiovascular disorders [11]. Microbial biofertilizers are environmentally friendly and are a potential alternative to chemical fertilizers and pesticides [12]. Furthermore, the use of these beneficial microorganisms is a recommended solution for increasing productivity and crop yields, preserving global human, animal and environmental health at a lower cost than chemical fertilizers [9]. They contain living or latent cells of beneficial microorganisms for a symbiotic living of a plant by colonizing the rhizosphere or the interior of the plant that promotes their growth through the improvement of main nutrient absorption [13]. Among the biofertilizers produced, we have the Arbuscular Mycorrhizal Fungi (AMF), which constitute a group of root obligate biotrophs that exchange mutual benefits with about 80% of plants. They are considered natural biofertilizers, since they provide the host water, nutrients, and pathogen protection, in exchange for photosynthetic products [14]. Phosphate Solubilizing Bacteria (PSB) have the ability to liberate organic phosphates or to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. These bacteria make available the soluble phosphates to the plants, and in return, gain root borne carbon compounds, mainly sugars and organic acids, necessary for bacterial growth [15]. Current research suggests that the inoculation of crops with Phosphate Solubilizing Microbes (PSM) has the potential to reduce application rates of phosphate fertilizer by 50% without significantly reducing crop yield [16] [17]. Microbial biofertilizers play a fundamental role in plant access to mineral nutrients from the soil. Findings have shown that biofertilizers increase plant uptake not only of phosphorus and zinc in particular, but also of nitrogen, potassium, magnesium, calcium and sulphur [14] [18]. Our products yield good productivity in farming many types of agricultural crops such as rice, maize, millet, groundnuts, tomatoes, potatoes, yams, oignons and fruit plants. It is estimated that 20 to 25% of plants' phosphorus requirements are met by bacteria and fungi [19]. *Rhizobiums* are symbiotic atmospheric nitrogen-fixing bacteria, which are responsible for the formation of nodules on legumes with which they enter into symbiosis [20]. The use of rhizobia biofertilizers in tropical areas of the Sub-Saharan Africa (SSA) has relatively increased compared to the previous decades due to the agronomic benefits associated with biofertilizers such as yield increase, cost saving, and improved soil health [21]. It is for this purpose that at LANAVET, a group of scholars have developed and produced biofertilizers based on bacteria and fungi isolated from the Cameroon soils in Bockle (Garoua). This study examined the effects of inoculation of three types of biofertilizers (LanaRhizo, Lana-MSP and LanaMyco) developed at LANAVET based on beneficial microorganisms on increasing productivity, nutrient biosynthesis and yield of soybean and maize plants, as well as stimulating the production of antioxidant molecules to promote good human and animal health.

2. Material and Methods

We conducted this research work at the National Veterinary Laboratory (LANAVET) in Bockle, Garoua, Cameroon from 2021 to 2023.

2.1. Plant and Microbial Material

Soybean seeds (Houla 1 variety) and maize seeds (Maize 8501 variety) were acquired from the Institute of Agricultural Research for Development (IRAD). The microbial material consisted of an inoculum of *Rhizobium* and a Phosphorus Solubilizing Microorganism (PSM) inoculum were acquired from the Soil Microbiology Laboratory, Biotechnology Center, Faculty of Science at the University of Yaounde I, and multiplied at LANAVET. The Arbuscular Mycorrhizal Fungi (AMF) inoculum was obtained from GIC Agribiocam, Yaounde.

2.2. Formulation Preparation

The performance of the formulation greatly depends on multiple dynamics under field conditions, in particular the microbial composition, the support used for the preparation of the formulation, the method of administration, the application strategies and the subsistence of microbial strains in native soil and the plant ecosystem, which are selected during the development of formulations [22]. The development of an effective and efficient formulation depends mainly on the constituents used to prepare the formulation, which includes potentially beneficial microbial strains, a support and an adjuvant [23]. An appropriate support is an important element in the preparation of the formulation. It serves as a delivery material for living microbial strains during laboratory treatment in the field. Individually or compositely, inorganic/organic or appropriate synthetic supports can be used to support microbial growth and effective delivery of the desired microbes [24].

2.3. Quality Control of Bacterial Biofertilizers

2.3.1. Dilution of Bacterial Culture

Serial dilutions of the broth culture inoculated with *Rhizobium* or PSM were performed. To do this, we used 9 sterile tubes, each containing 9 mL of sterile diluent (physiological solution). Then, the number of living cells is determined by spreading the serial dilutions (10^{-7}, 10^{-8}, or 10^{-9}) on agar medium (depending on the concentration). Three repeated samples of 0.1 mL of the 10^{-8} or 10^{-9} broth are spread on Petri dishes containing Yeast Extract Mannitol Agar + red Congo. The Petri dishes are incubated at (30 - 35˚C) for 2 - 7 days.

2.3.2. Enumeration of Bacterial Colonies

The calculation of the number of bacterial colonies per mL was done as described by [25].

$$\text{Number of cells/mL} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{Inoculum volume}}$$

Regarding the quality control of the LanaRhizo and Lana-PSM biofertilizers, the following aspects were considered and verified: pH, water content and number of viable strains in the carrier-inoculant.

2.4. Distribution of Bacterial Cultures

The dilution of the final culture broth and the seeding of the carrier (powder) were done by adjusting the sterile air blow speed potentiometer of the ceiling blower, which was cleaned and disinfected the day before and then started at a low blow speed of 50 pascals of air pressure (air speed potentiometer set at 25 - 30). Then, the outer surface of the different bottles or sachets of products was cleaned using a household cloth and a piece of absorbent paper soaked in 70% alcohol. Broth culture of each of the two bacterial strains was inoculated and measured for 2,000 mL for each strain using a measuring cylinder and transferred into a sterile 5-liter flask. The mixture was stirred for 10 to 15 minutes. Then the inoculum was distributed into 500 mL Erlenmeyer flasks for inoculation into the carrier.

2.5. Inoculation of the Carrier

The surface of the bags was cleaned with a piece of absorbent paper soaked in a 70% alcohol. Using the syringe, the inoculum was injected into the bag containing the carrier. Subsequently, with the stickers, the orifice used to inoculate the carrier was sealed. Forthermore, the inoculum contained in the bags was spread by gently stirring using gloved- hands in a sterile condition. After this process, the bags were transported using a cart to the incubation chamber set between 28 - 30˚C for a period of 4 to 7 days depending on the dose. After the incubation period, those bags were transferred to +4˚C room.

2.6. Quality Control of Fungal Biofertilizers

2.6.1. Extraction of AMF Spores and Counting

To extract the spores from our LanaMyco biofertilizer, sieves of mesh sizes ranging from 700 µm, 200 µm, 100 µm and 45 µm were used. Spores were extracted according to the method described by [26] [27]. This consisted of pouring 300 mL of water into a jar containing 100 g of soil and then homogenizing them. This mixture was then poured into the sieve of the largest mesh size. This operation was repeated three times. The rinsing was then carried out using a water hose. Spores were retained on the meshes of the last four sieves likely as a clear water. The latter was poured into tubes previously labelled. The spores were observed at magnifications of 10 to 40 using a stereomicroscope. Calculate the number of spores/g according to the formula: $N = n \times 27.76/100$ with n the average of the number of spores of the three replications.

2.6.2. Evaluation of the Percentage of Root Colonization by AMF

The total root system of plants (harvested 6 to 8 weeks after sowing) was cut into pieces of about 1 cm in length (preferably the finest roots). These root fragments were then washed with tap water, and clarified in 10% potassium hydroxide

(KOH) for 15 minutes, in a water bath at 90˚C. After draining with a sieve, the root fragments were washed 3 times with tap water, then introduced into 10% hydrochloric acid (HCl) for 15 minutes. After partial removal of the HCl, the roots were stained with a mixture of acid fuchsin or methylene blue (0.01%), lactic acid-glycerol-water in proportions of 5/3/2 (v/v/v) in a water bath at 90˚C for 30 minutes. The root segments are then decolorized using the mixture of lactic acid-glycerol-water (5/3/2) for 24 hours. Once decolorized, the roots were mounted between a slide and cover slip on slides in groups of 10. Approximately 10 slides were made per treatment and then observed under a microscope. Mycorrhization was thus evaluated based on the presence or absence of characteristic structures of mycorrhizal fungi (mycelial filaments, spores, arbuscules, and vesicles) in the roots [28] [29].

2.7. Application Mode of LanaRhizo, Lana-PSM, and LanaMyco Biofertilizers

The inoculation of commercial biofertilizers was done by seed coating with the inoculant. The seeds were first sprayed with clean water, the contents of the biofertilizer packet were mixed with clean water in a container until a heavy pasty liquid is obtained. The seeds were then added to the container and mixed until they were stuck together. They were left to dry in the shade for at least 20 minutes before sowing. Then, seeds are manually sown at 5 cm depth and covered (**Table 1**). Refer to **Appendix** for the comparison of fertilizers needed per hectare according to the different methods of organic, chemical, and bioertilization.

Table 1. Mode of application of commercial biofertilizers.

Biofertilizers	Formulation/ carrier	Application mode	Dose and timing of application
Lana-PSM	Powder	Seed inoculation by coating, 10 kg for 25 kg of seeds/ha	1 kg for 2.5 kg of maize seeds at sowing
LanaRhizo	Powder	Seed inoculation by coating, 10 kg for 50 kg of seeds/ha	1 kg for 5 kg of soybean or peanut seeds at sowing
LanaMyco	Powder	Seed inoculation by coating, 10 kg for 25 kg of seeds/ha Inoculation by soaking roots or cuttings	1 kg for 2.5 kg of maize, wheat, cotton, rice seeds at sowing 20 - 50 g/plant of tomato, onion, yam, cassava, sweet potatoes at sowing

2.8. Growing Conditions

Microbial biofertilizers were inoculated into soybean seeds and maize seeds disinfected with 2.5% sodium hypochlorite for 3 minutes, followed by 5 washes in sterile distilled water. Four treated seeds were then sown in each hill. The plants were grown in a temperature range of 30 to 37˚C and were irrigated with tap water. Each experimental unit had a dimension of 3 m × 4 m, covering an area of 12

m^2. Manual cleaning of the trial was done twice.

2.9. Treatments and Experimental Design

The study was laid out in a randomized complete block design with three replications and two treatments for soybean crop and three treatments for maize crop in the field. The treatments were: *Rhizobium* biofertilizer and uninoculated control for soybean and PSM biofertilizer, uninoculated control and uninoculated with applied NPK at a rate of 200 kg·NPK·ha^{-1} for maize. The NPK was split applied; 100 kg·ha^{-1} at 1 week after planting and the other half at 50% flowering.

2.10. Evaluation of Food Quality

Approximately 50 g of undamaged seeds were randomly selected, and the protein and oil contents were measured using Fourier transform near-infrared spectroscopy (FT-NIR). Soluble sugar measurements were performed using the method described by [30]. A total of 100 mg of soybean powder was mixed with 50% acetonitrile, and the samples were shaken for 8h at room temperature in an incubator. Then 500 mL of the supernatant was transferred to a new tube containing 200 mL of acetonitrile, and the mixture was shaken to achieve protein precipitation. After 10 min at room temperature, the samples were centrifuged at 20˚C for 10 min, and the supernatant was filtered using a syringe filter (0.22 mm) before detection using a UPLC-RID [30].

2.11. Antioxidant Assay for Human Health

A spectrophotometric approach was used to evaluate antioxidants. Reduced glutathione (GSH) was according to Ellman's method [31]. A methanolic extract of corn dry seeds (1ml) was homogenized in 2 ml of 5% (w/v) sulfosalicylic acid under cold conditions. The homogenate was shaken, and 100µl of supernatants was mixed with 1500 µl of Ellman's reagent. After 1 hour, the absorbance was taken at 412 nm. The GSH level was expressed as mmol/g f.w: $GSH = \Delta DO/(\varepsilon \times L \times m)$. To measure the total flavonoid content of corn, 0.1 g of dry seeds obtained in all experimental plots (treatments) were ground with 5 mL of ethanol in a porcelain mortar and the centrifuged at 10,000 rpm for 5 min. Then, 500 µL was removed from the upper phase and 1.5 mL of ethanol, 100 µL of 10% aluminum chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water were added, and then was kept for 40 min at room temperature. Then the absorbance of the solutions was measured at 415 nm compared to the control without herbal extract [32]. Finally, by placing the absorption value of the samples in the standard curve equation of quercetin, the amount of total flavonoid was measured in terms of mg of quercetin per g of seeds dry weight.

2.12. Statistical Analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA). The experimental data met the assumptions of normality and variance homoge-

neity, and no transformation was needed. For comparison of the means, the Duncan's post-hoc test was used at $p \leq 0.05$. The data were analysed using SPSS version 16.0 package.

3. Results

3.1. Biofertilizers Produced to Increase the Growth and Yield of Crops

Table 2 below presents the 3 brands of biofertilizers produced with their different concentrations, validity periods, and packaging mode. These biofertilizers are applied to several crop speculations such as legumes (soybeans, peanuts, cowpeas...) which, through symbiotic association with rhizobium (nitrogen-fixing bacteria), fix atmospheric nitrogen, an essential limiting element for the growth and yield of seed legumes. The PSM (Phosphorus-solubilizing microorganism) solubilizes phosphorus by releasing protons and organic acids, thus making phosphate available for plant absorption. Mycorrhizae improve plant nutrient absorption, yield, and vegetal biomass production (Table 2).

Table 2. Characteristics of the biofertilizers Lana-PSM, LanaRhizo, and lanaMyco.

Characteristics	*PSM*	*Rhizobium*	*Mycorrhiza*
Code	PSM	*Rhizobium*	*Mycorrhiza*
Concentration (number of cells per g of inoculum	1.6×10^9 UFC/g	1×10^9 microorganisms per gram	60 ± 4 spores per gram
Validity period	1 year	> 6 months	2 to 3 years
Conditioning	1 kg	1 kg	2 kilograms
Target culture	All culture	Legumes	All culture

3.2. Impact of Microbial Biofertilizers on Crop Production and Yield

The field experiment (Figure 1) was carried out to evaluate the effect of inoculation of the *Rhizobium* biofertilizer on the soybean Houla 1 variety popularized in far North of Cameroon. The results in Figure 1 show that inoculation with *Rhizobium* increased the root nodules per plant by 51.7% and the nodules biomass by 46.4% compared to the control treatment. Regarding plant height, the results showed that inoculation with *Rhizobium* increased the height by 10.8% compared to the control treatment. There was also an increase in the biomass of soybean plants and grain yield of 38.7% and 39.9% respectively when inoculated with *Rhizobium* compared to the control treatment.

The field experiment (Figure 2) on high yielding maize 8501 variety was conducted to evaluate the effect of PSM biofertilizer inoculation on its grain yield.

Figure 2 described that inoculation of PSM biofertilizer significantly increases corn grain yields by 25.1% compared to the control treatment and by 17.7% compared to NPK treatment. The yields induced by the different treatments are statistically different. The difference is significant (ANOVA, $p < 0.05$).

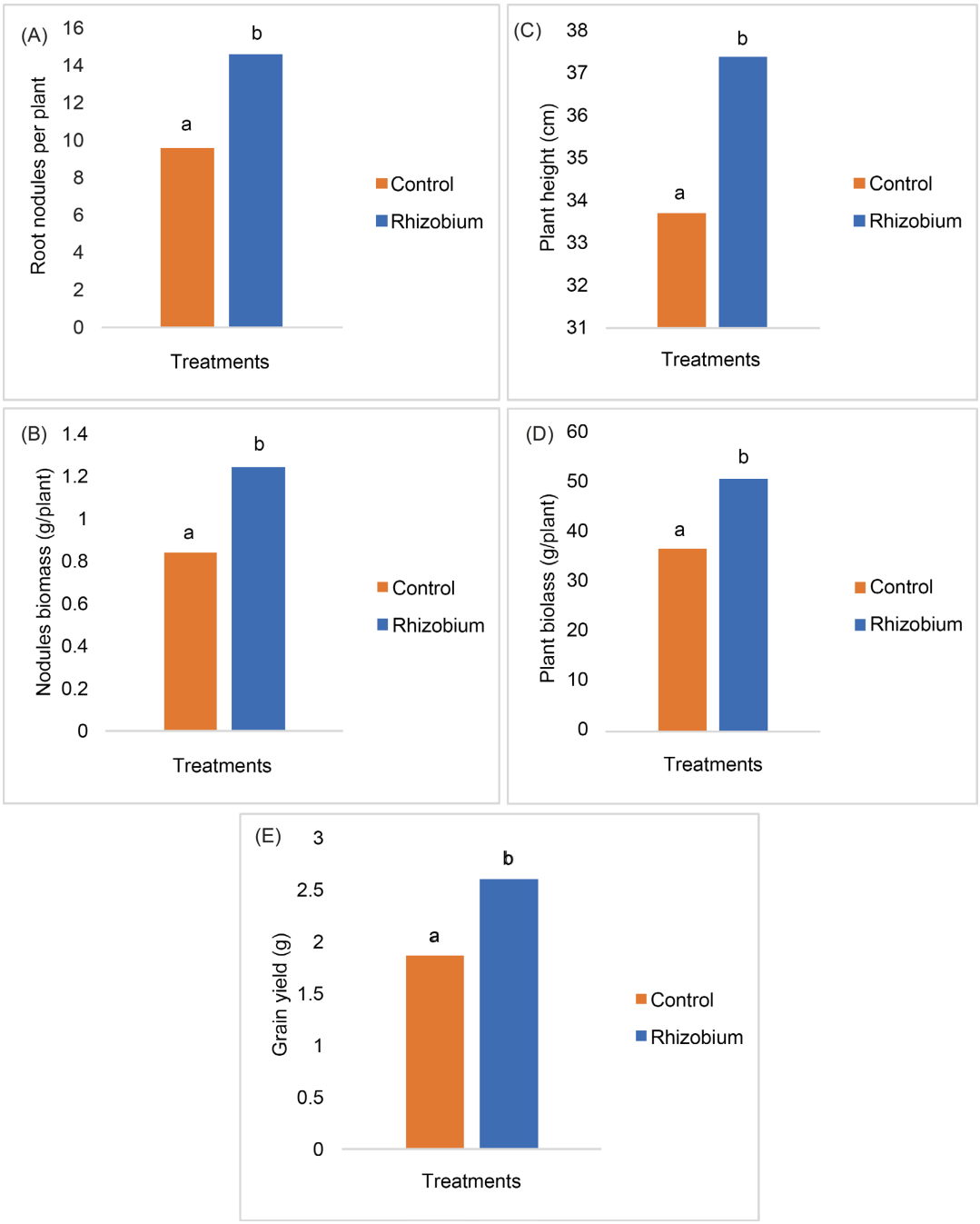

Figure 1. Effect of Rhizobium biofertilizer inoculation on soybean (*Glycine max*) in the field. (A) Root nodules per plant. (B) Nodules biomass (g/plant). (C) Plant height (cm). (D) Plant biomass (g/plant). (E) Grain yield (g). Control: Treatment without bacterial inoculation. Different letters indicate significant difference at $p \leq 0.05$ according to the Duncan's post-hoc test, means ± error.

3.3. Impact of Rhizobium and PSM Biofertilizers on Food Quality

The results of the determination of protein contents of soybean and maize seeds showed high protein contents of plants treated with *Rhizobium* and MSP biofertilizers (36 and 9.5%) compared to non-inoculated plants (31.3 and 7.6%; respectively) (Figure 3(A) and Figure 3(C)), which were increases of 15.1% and 25.6%

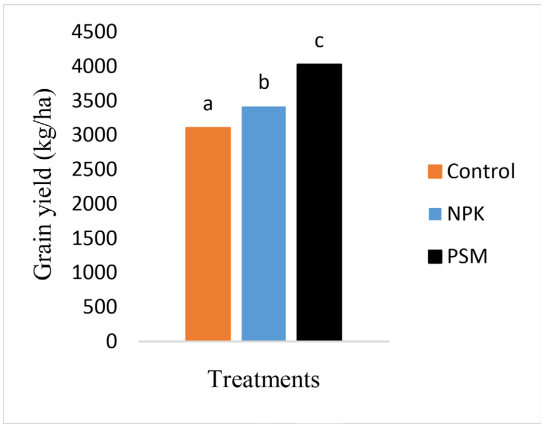

Figure 2. Effect of PSM biofertilizer inoculation on corn grain yield in the field. Control: treatment without bacterial inoculation; NPK: Treatment with chemical NPK fertilizer (20-10-10). Different letters indicate significant differences at $p \leq 0.05$ according to the Duncan's post-hoc test, means ± standard error.

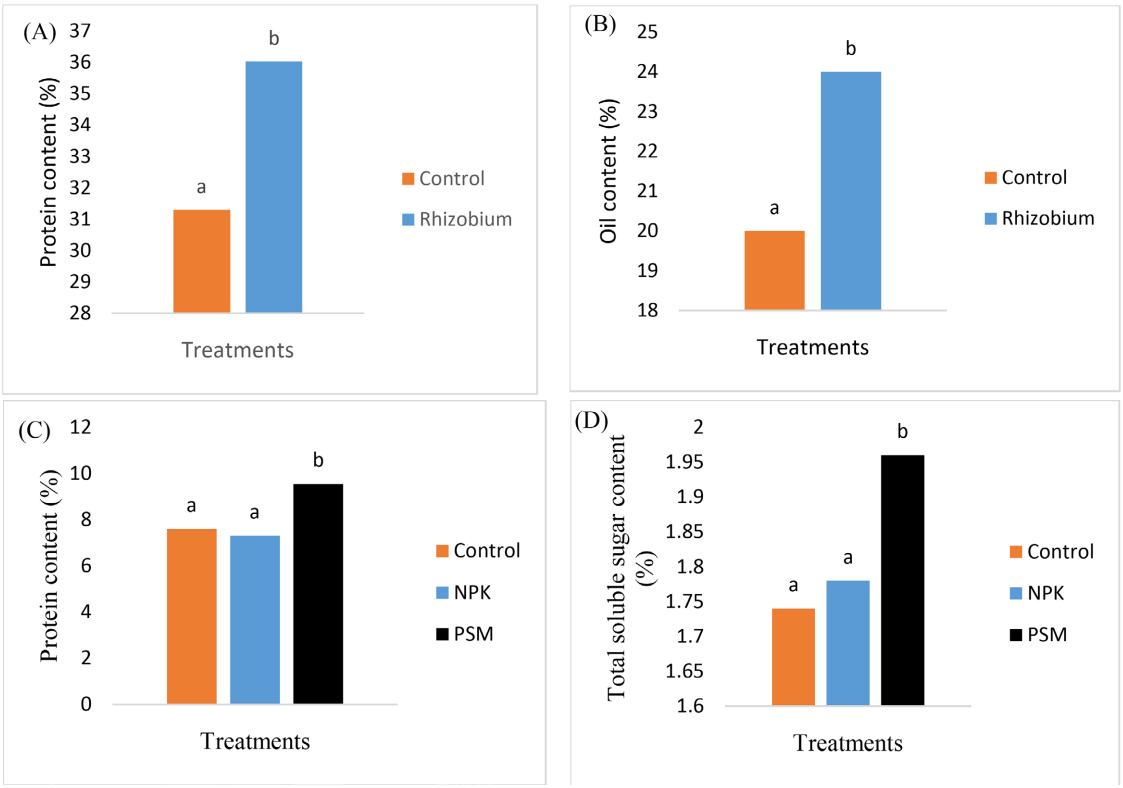

Figure 3. Effect of inoculation with biofertilizers *Rhizobium* and PSM on food quality (soybean and maize seeds). (A) Protein content (%) in soybean. (B) Oil content (%) in soybean. (C) Protein content (%) in maize. (D) Total soluble sugar content (%) in maize. Control: treatment without bacterial inoculation; NPK: Treatment with chemical NPK fertilizer (20-10-10); PSM and *Rhizobium* treatments with biofertilizers inoculation. Different letters indicate significant differences at $p \leq 0.05$ according to the Duncan's post-hoc test, means ± standard error.

compared to the controls. These results demonstrate the effectiveness of the two inoculated biofertilizers in increasing protein production in grain legumes such as soybean and in grasses such as maize.

Regarding the oil content of soybeans (Figure 3(B)), it was found that the oil content of seeds biofertilized with *Rhizobium* 24% was significantly higher than that of non-biofertilized soybeans 20%.

Total soluble sugar contents in corn were measured, with significant differences ($p < 0.05$) observed in sugar content of both MSP and control treatments. The percentage increase in sugar content was (12.6% and 10.1%) MSP compared to absolute control and control with chemical fertilizer (Figure 3(D)).

3.4. Effect of the Application of Biofertilizer PSM on the Human Health

The results obtained showed that MSP biofertilizer applied to corn plants caused a significant increase in the biosynthesis of flavonoids and glutathione (GSH). Flavonoids are found in fruits, cereals, flowers, roots and seeds of many plants and function as antioxidants. Glutathione (GSH) is a key defense mechanism against oxidative stress and cancer formation in the body. MSP biofertilizer applied to corn, positively influences the stimulation of flavonoid biosynthesis in corn seeds compared to controls with a value of 161.28 mg EQ/g, a significant increase of 44.5% and 92.9% compared to control and NPK respectively (Figure 4(A)). It is also noted that the application of the MSP biofertilizer promotes a better stimulation of GSH production compared to the controls with a value of 4.73 mmol/g against 4.36 and 4.35 mmol/g for the control and NPK respectively (Figure 4(B)).

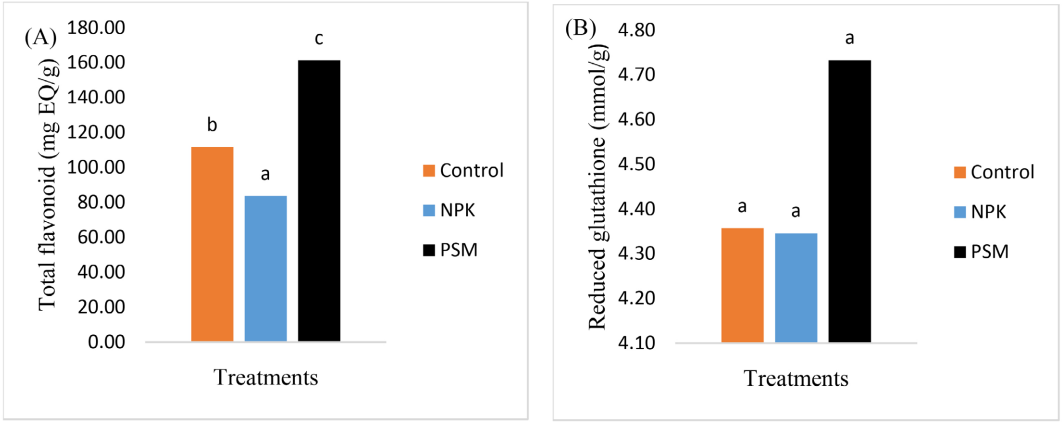

Figure 4. Total flavonoid content of maize seeds and reduced glutathione as influenced by Biofertilizer MSP and chemical fertilizer. Different letters indicate significant differences at $p \leq 0.05$. (A) Total flavonoid. (B) Reduced glutathione. Control: treatment without bacterial inoculation; NPK: Treatment with NPK chemical fertilizer (20-10-10); PSM: treatments with biofertilizer inoculation.

3.5. Root Colonization Percentage by AMF

Table 3 presents the results of the percentage of root colonization in hyphae and vesicles of the different strains used for the formulation of mycorrhiza inoculum. It appears from these results that the best strain was Glomus hoi with a colonization percentage of 87% in hyphae, followed by Rhizophagus irregularis, Scutellospora gregaria with 80% in hyphae, and Gigaspora margarita which had a rate of

68% hyphae. In general, we could conclude that all strains are above the average concentration indicating good colonization. Similarly, the percentage (30% - 53%) of vesicle colonization was good for the four strains (Table 3). The roots of Maize were colonized by the structures of AMF (Vesicles and Hyphae) (Figure 5).

Table 3. Root colonization rate of corn by the different strains of AMF used.

Strains	*Hyphes* %	*Vesicles* %
Glomus hoi	87	35
Gigaspora margarita	69	37
Rhizophagus irregularis	80	53
Scutellospora gregaria	80	42
Average	79	42

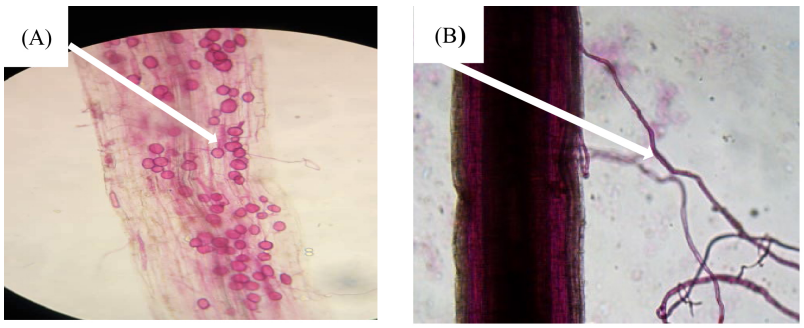

Figure 5. Structure of AMFs in the roots of Maize, stained using acid fuchsin method. (A)Vesicle. (B) Hyphae.

4. Discussion

To reduce the current negative environmental impacts from agriculture (e.g., high amounts of external inputs of fertilizer and/or agrochemicals), there is an urgent need to develop new and more sustainable agronomic management measures. These novel techniques should inter alia improve soil health, human health and environmental health [9]. Biofertilizers are living microbes that enhance plant nutrition by either mobilizing or increasing nutrient availability in soils with a low risk of toxic residues in farm products. They increase plant productivity by improving soil fertility and nutrient content. Following their important benefits in global health, we developed 3 types of biofertilizers for agricultural use. Encouraging findings were obtained from all the 3 brands of biofertilizers (LanaRhizo, Lana-PSM and LanaMyco) produced at LANAVET. Researchers show that the application of *Rhizobium* biofertilizer can replace expensive inorganic nitrogen fertilizers to improve the growth of legumes and hormone synthesis. These results revealed that the addition of rhizobial biofertilizers significantly improves certain growth attributes of soybeans. The superior performance of soybean plants inoculated with rhizobium is supported by [33], who also reported improved soybean growth and nitrogen fixation, as well as grain yield after inoculation with rhizobial

strains such as *Bradyrhizobium*. [34] also showed that bacterial endophyte isolates can increase soybean biomass by 94% to 120% and grain yield by 72% to 77% compared to the control treatment. In addition, the inoculated bacteria may have released ammonia or produced nitrogenase through their nifH genes to fix N2 and its absorption by plant roots to enhance growth and yield [35]-[37]. The high root nodulation achieved with inoculation of the *Rhizobium* biofertilizer suggests successful symbiosis between the inoculated *rhizobium* and soybean roots [38] [39]. Accordingly, improved soybean root nodulation and N_2 fixation were reported with the inoculation of *Bradyrhizobium* species [33] [40] [41]. The poor root nodulation in the control can be attributed to a low density of native N_2 fixing symbiotic *rhizobium* [39] [42].

The impact of PSM biofertilizer on corn plants in the field showed very encouraging results. The treatment inoculated with the PSM biofertilizer had a higher yield than those who received 200 kg/ha of chemical NPK fertilizer (20-10-10) and those who received no fertilizer application (control). This reveals that PSM improved maize nutrition. So PSM could replace chemical NPK fertilizer in corn cultivation. This biofertilizer based on phosphorus-solubilizing bacteria can not only help plants obtain insoluble forms of phosphate, but can also make phosphate available for plant uptake [43] [44]. Microorganisms have the ability to absorb immobile nutrients such as phosphorus from the soil and transfer them to their host plants, which is one of the main effects of microbial symbiosis. This result is in agreement with the work of [45] who also showed a significant increase in all growth parameters of maize varieties (ATP S4.syn Y and CMS 8501) treated with biofertilizers based on beneficial microorganisms such as AMF in comparison to non-maize plants treated. [46] also demonstrated that inoculation of bacterial strains of *Arthrobacter* sp. or *Bacillus* sp. promotes the growth of Cameroonian maize varieties CMS 8501 and CMS 8704 compared to control plants. Three *Arthrobacter* sp. (V54, V64, and V84) and three Bacillus sp. (V62, V39, and V1) strains have been successfully isolated from the rhizosphere of maize plants grown in Cameroon [47]. Each was associated with an individual set of plant growth-promoting traits, including the ability to solubilize rock phosphate, to fix atmospheric nitrogen, to produce siderophores, to tolerate salt, to encourage the germination of maize, and to support the growth of maize plants [47]. The observed high root colonization in AMF strains inoculated maize plants demonstrates the capacity of inoculated mycorrhiza to compete with other rhizosphere microbiota and survive, which is an important characteristic of efficient biofertilizers in enhancing soil fertility and productivity. Studies have reported that the application of single AMF species directly into agricultural soils often results in short-term, inadequate colonization rates of the cultivated plants [9] [48]-[50]. In constrast, the presented study inoculated maize seedlings with a consortium of different AMF species.

The protein content of soybean and maize seeds varies depending on the treatments applied. *Rhizobium* and MSP biofertilizers induce higher protein contents

by 15.1 and 25.6% compared to control treatment. These improvements in nutrient supply to crops could influence the quality of consumable food for humans and livestock. It appears from this study that commercially produced biofertilizers have a positive impact on the production of proteins (essential elements for the synthesis of essential amino acids). [51] also carried out greenhouse tests at the International Institute of Tropical Agriculture (IITA), Ibaban, Nigeria on different commercial biofertilizers based on beneficial microorganisms from South Africa using as culture testing a variety of maize (*Zea mays*, cv. TZE-Y POPDT STRC 4), the results of this study showed that inoculation of the biofertilizer Mazospiriflo-2 improved protein content significantly in maize cultivated.

The soybean seeds treated with Rhizobium biofertilizer had the highest oil content compared to the control treatment. Other studies on the application of biofertilizers such as mycorrhizal fungi have shown an improvement the plant-soil association by forming hyphae arouns the plant root, increase the absorption of nutrients such as nitrogen and phosphorus, and consequently improve the fatty acids biosynthesis and oil content [52] [53]. The total soluble sugar content of MSP biofertilised maize (1.96%) was higher than to non-maize seeds treated. This result demonstrated that the MSP biofertilizer has the ability to stimulate the biosynthesis of carbohydrate in maize seeds.

Concerning the effect of the application of PSM biofertilizer on the human health. In our study, MSP biofertilizer inoculated into maize plants causes a significant increase in flavonoid biosynthesis and stimulation of reduced glutathione (GSH) production compared to the control. MSP biofertilizer applied to maize, positively influences the stimulation of flavonoid biosynthesis in corn seeds compared to the control with a value of 161.28 mg EQ/g, a significant increase of 44.5% compared to the control. We also note a better stimulation of GSH biosynthesis compared to the control with a value of 4.73 mmol/g against 4.36 mmol/g for the control. [54] also showed that endophyte isolate biofertilizers could stimulate GSH production in Curcuma plants. Flavonoids are found in fruits, cereals, flowers, roots and seeds of many plants. They are related to plant growth and development, pigment formation, and protection against environmental stress. Flavonoids function as antioxidants and exert anti-inflammatory effects on the cardiovascular system by modulating classical inflammatory response pathways and other system diseases and are beneficial to human health [55]. They have various pharmacological effects, such as immunomodulatory, hypoglycemic, antibacterial and anti-inflammatory, tumor invasion and metastasis effects, therapeutic effects in many chronic diseases [56]. Glutathione (GSH) is a key defense mechanism against oxidative stress and cancer formation in the body [57]. Increasing glutathione levels is a proactive measure that people can take to promote good health and achieve healthy longevity [58]. Glutathione (composed of three amino acids: cysteine, glutamate, and glycine) is very effective for cardiovascular health due to its antioxidant properties, detoxification mechanism, and control of the endothelium and vascular walls [59]. As shown by various research and studies, heart fail-

ure, hypertension, and other cardiovascular diseases are associated with low glutathione levels or disruption of glutathione homeostasis, which only establishes the importance of maintaining adequate glutathione levels. Our study also supports the use of glutathione to reduce and combat oxidative stress, improve endothelial stability, and protect the myocardium.

5. Conclusion

Biofertilizers LanaRhizo, Lana-MSP and LanaMyco produced and applied to different crops have been found effective in improving most growth parameters and crop yields as well as food quality by enhancing the bioavailability of nutrients important for human and animal health compared to untreated crops. These beneficial microorganism-based biofertilizers are suitable to be an alternative to very expensive and environmentally hazardous chemical inputs. The use of chemicals in agriculture can be avoided and thus, they can be removed from human and animal diets by employing biofertilizers. The establishment of industrial biofertilizer production plants is strongly recommended for the development of large-scale agriculture. Further study is needed to optimize the needed quantities of biofertilizers versus chemical fertilizers. Biofertilizer technology will ensure healthy food security for the future population. We also suggest that those at the helm of authority should review biological fertilizers laws to enhance the effective supervision of biofertilizer quality and monitor existing laws on the use of these products. There is a need to also educate the farmers on the danger associated with the indiscriminate use of agrochemicals.

Author Contributions

WA, BM and ND designed the study. BM, HA and MLS conduced the experiment. BM analysed the data. WA and BM wrote the manuscript with contribution from all authors. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

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

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Appendix. Comparison between Organic, Chemical and Bio-Fertilization Fertilization Options

Fertilizers	Categorical	Quantity ($Kg \cdot ha^{-1}$)	Advantages	Disadvantages	Duration of action
Organics	- Biochar - Animal manure - Compost - Slurry	2-200.103	- Soil structure improvement - Water retention improvement - Nutrient supply - Low cost - Stimulates plant growth - Stimulates plant productivity and yield	- Availability issue - Low solubility - Short-lived action - May contain non-selective germs - Highly variable quality	Medium term
Chemical inputs	- Nitrogen - Phospho-rus - Potassium - Pesticides (herbicides, fungicides, insecti-cides, ne-maticides)	200 - 300 0.01 - 1.5	- Nutrient supply - Stimulates plant growth - Stimulates plant productivity and yield - Regulates the growth of insects, plant patho-genic fungi, weeds, and nematodes	- Environmental pollution - Long-term risk to human and animal health - Leaves toxic and poorly degradable debris - Soil depletion - Increases greenhouse gas emissions - High cost	Short term
Microbial biofertiliz-ers	- Mycorrhiza - PSM - Rhizobium	5 - 100	- Selective germs that multiply continuously for several years in soils - Spores resist harsh environmental conditions for a long time - Provides nutrients - Increases the taste quality of food - Increases nitrogen fixation - Increases plant productivity and yield - Reduces the need for nitrogen and phosphate fertilizers - After 4 years of application, additional input is no longer necessary - Higher rainfall promotes their multiplication further - Restores soil fertility - Stimulates plant defence - Enhances plant resistance to diseases, pests, and stress - Synthesize growth hormones - Solubilize phosphorus - Improves soil biodiversity - Low cost - Reduces greenhouse gas emissions	- Difficult mass production - Sensitive to pesticides - Slow action	Long term