Influence of the Beneficial Microorganisms Bioformulations on the Soil Physicochemical Parameters and the Nutritional Profile of *Abelmoschus Esculentus* Cultivated in Cameroon

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Authors' contributions

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

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ABSTRACT

Abelmoschus esculentus, a plant cultivated in tropical and temperate regions throughout the world, is highly appreciated for its various uses. Its culture encounters difficulties, particularly in Cameroon, due to soil infertility. In order to overcome this difficulty, the use of biological fertilizers in order to regenerate agricultural soils for more production is suggested. Three types of formulations were prepared after *in vitro* compatibilities tests, T1 (*B. amyloliquefaciens* and *T. harzianum*); T2 (*B. velezensis* and *B. amyloliquefaciens*) and T3 (*B. velezensis* and *T. harzianum*)

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with the aim of improving soil physicochemical; agromorphological and nutritional profile of okra. The plants were cultivated in 4.5 m² plots, arranged in complete randomized blocks design. ANOVA revealed significant differences between T1treatment and the other treatments and with the T0 control in plant height with the growth rate of 1.144cm/day, fruit length (11.53±0.49cm) and the average weight of fruits per treatment (1868.00±279.45g) at the significance level of $P<0.05$ (Tukey test). The productivity of treatment T1 per hectare (16.604±2.48t/ha) was highly significant compared to the other treatments and the control (8.53±1.49t/ha). Parameters such as disease resistance, leaf area and number of leaves were not significant between treatments but with the control. The values obtained with T1 treatment were high compared to the others. Regarding nutritional properties, the Na, K, Mg, Cu, Fe, Zn, carbohydrates and proteins contents were significantly increased compared to the control with contents values above the same okra variety. These different results may suggest the use of bioformulation with *B. amyloliquefaciens* and *T. harzianum* in order to improve soil fertility and to produce a sufficient quantity and quality of biofortified okra.

1. INTRODUCTION

Soils are a natural resource that is essential and non-renewable, providing important services to both ecosystems and human life. They play a fundamental role in crop production [1]. The quick spread of intensive agricultural systems, the use of fertilizers, and rapid human population growth have had negative effects on soil fertility, mainly decreasing the soil organic carbon and the total soil nitrogen, and changing the composition of carbon and nitrogen, owing to the loss of soil organic matter through erosion and leaching [2], and thus resulting in unsustainable soil degradation [3]. Almost 40% of the world's arable farmland are infertile (UNEP (United Nations Environment Program, ISRIC World Soil Information, www.unep.org/geo/geo3/french). This in turn minimizes the productivity of crops to satisfy the population needs. These soils require more suitable mechanisms to fertilize them. Improvement of soil fertility is far from being simple.

Okra (*Abelmoschus esculentus*), like other plant species cultivated under these conditions, has shown a low yield. Okra is valued for its therapeutic properties [4] and its industrial and artisanal use [5]. Its fruits have a high nutritional value in biologically active molecules such as nutrients and antioxidants. They are notably rich in calcium, iron, magnesium, vitamins A, C, E, K and lipids (oleic, linoleic and palmitic acids) [6]. It is a vegetable with high economic potential for poor communities because of its selling capacity in rural and urban markets [7]. Its low yield can be explained by insufficient soil nutrients that cause an imbalance in the microflora, leading to fungal, bacterial and viral diseases, and insect attacks at all stages of the plant's growth [8]. Between 2013 and 2018, global okra production increased from 6.48 million tons to 8.9 million tons, India, Pakistan and Iraq being important production centers [9]. In Africa, production is decreasing and varies according to the country. During the same periods, production dropped from 120.000t to 90.780t in Cameroon; from 100.000t to 57.721t in Egypt; from 71.350t to 66.360t in Ghana and 826.170t to 1.978.256t in Nigeria [9]. Under optimal growing conditions, okra productivity can reach up to 30 - 40t / ha [10]. However, there is a large gap between potential and effective yield [11]. This leads to a need to minimize the difference between the potential yield and the yield obtained by modern biological soil amendment techniques.

Biological soil fertility restoration techniques within the smallholder agroecosystems, in combination with other agronomic management practices, would provide the much-needed solutions for revitalizing the declining global food production [12]. Beneficial soil microbiota such as plant growth promoting microorganisms comprising fungi and bacteria maintain key agroecological cycle fundamental for soil nutrient enrichment, crop nutrient improvement, water uptake enhancement and biocontrol of pests and diseases [13]. This microbiota involves in healthy plant development and growth through secretion of hormonal growth regulators. Soils are identified to be abundant in millions of indigenous microorganisms known as beneficial bacteria and fungi, which play the role of biostimulants in the agricultural service and will intervene in phytostimulation, plant defense, and

soil activation and enhance in nutrient availability to the plants through biochemical processes such as mineralization, chelation, solubilization [14]. Microorganisms residing in plant tissues without causing any apparent symptoms, namely endophytes, are important resources for the discovery of biologically active compounds with promising agricultural and pharmaceutical applications [15]. Fungal species as *Trichoderma* species are recognized as mycoparasites and are widely used in agriculture as biofungicides and bioremediation agents [16]. The perfect symbiosis between beneficial micro-organisms creates important regenerative forces that can develop very surprising effects in different environments. What makes them remarkable is the mixture of several species of microorganisms that together have a structuring and antioxidant regenerative action that gives them extraordinary effects and a very wide and varied range of applications that are almost limitless [17]. Application of *B. amyloliquefaciens* as fungicide significantly reduces branch canker disease in tea plants under field conditions [18]. In a related study, the use of *B. amyloliquefaciens* together with other microorganisms has been shown to improve the strain's biocontrol potential. The vigor and functionality of plant growth promoting microorganisms depend on intrinsic soil properties as nutrient availability, temperature, soil pH; environmental and agronomic management factor [19]. This review is therefore exploring strategies involving in the use of microbial bioformulation as biostimulants in agricultural system which is still in progress in Africa. These could be effectively used to improve soil fertility and agricultural productivity as biofertilizers. In this context, the present study was carried out in order to evaluate the soil fertility and yield of *Abelmoschus esculentus* under the effect of biofertilizer formulations based on the use of plant growth promoting microorganisms.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out in Mbele II, a locality of OBALA Subdivision in LEKIE Division, Centre Region of Cameroon. The locality of Obala is located at 528m above sea level with geographical references 4°10'0" N and 11°31'60" E. Its climate is tropical and humid with an annual rainfall of about 1638 mm. The temperature is about 24.7°C [20].

2.2 Field Preparation

A plot of 229.5m² (25.5m x 9m) was cleared, spaded, crumbled and ridges formed in a complete randomized block design with three treatments and one control with five replications. The experimental design was organized of 20 elementary plots divided into 5 blocks 2m apart. Holes of 2 cm depth with a 50 cm spacing were formed on each ridge for seeding.

2.3 Acquisition of Microbial Strains

The microbial material used in this study was obtained from DORA AGRI-TECH Laboratories of H.K. Industrial CO., LTD in China. It was made of three strains of microorganisms, PGPR bacteria (*Bacillus amyloliquefaciens* 10^{11} CFU/g and *Bacillus velezensis* 10^{10} CFU/g) and fungi (*Trichoderma Harzianum* 2×10^9 CFU/g).

2.4 Preparation of Bioformulations

Three formulations T1 (*Trichoderma Harzianum* and *Bacillus amyloliquefaciens*); T2 (*Bacillus velezensis* and *Bacillus amyloliquefaciens*); T3 (*Trichoderma Harzianum* and *Bacillus velezensis*) were thus prepared by the association of two strains of microorganisms following the method modified by [21] (Fig.1). The design and formulation of effective microbial consortia as inoculants require evidence of the ability of the consortium members to coexist. Therefore, microbial strains (*B. amyloliquefaciens* and *B. velezensis*) were subjected to in vitro compatibility test applying the agar diffusion test as described by [22], with minor modifications. The co-culture of *T. harzianum* with *Bacillus* species was done on agar plate method as described by [23] was adopted with minor modifications. The preparation occurred after that, consisting of mixing 1kg of 2 microbial strains with 1kg of molasses in 8l of non-chlorine water to activate spores. The mixture was then fermented in a well-sealed bucket for 7days. This mixture was then mixed with 50kg of rice bran used as carrier material and taken into a barrel for another fermentation of 7days before application to the farm.

2.5 Bioinoculation and Seedling

Applications of bioformulations were made two weeks before seedling and three weeks after seedling at a concentration of 40g per hole.

Seedling was done by introducing 3 seeds per hole at 2cm from the soil and at a distance of 50cm. The weeds were removed manually every week until harvest period.

2.6 Evaluation of Morphological Parameters

Plant height was measured from the plant base to the first leaf stalk after each week from day 14 to day 35 using a tape measure and the mean was determined. The growth rate of plants in each treatment was determined according to the formula below:

$$\text{Growth Rate} = \frac{\text{Plant heigh difference}}{\text{Difference in Tme}}$$

The plant height difference is calculated from the last record at day 35 in cm to the first record at day 14. The time is also calculated during in days.

The number of leaves was counted on the plants of each treatment from day 14 to day 35 and the mean was after that calculated. The leaf area was also recorded by the planimetric method. Plant vigor was determined on day 35. Disease severity (Fig. 2) was observed based on the size of symptomatic lesions caused by the pathogens on the leaves before blooming by visual observation and by counting the infested plants per treatment. The determination of the percentage of disease-free plants was done according to the formula of [24].

$$\%\text{infestation} = \frac{\text{number of plants infested per treatment}}{\text{Total number of plants}} \times 100$$

2.7 Harvesting and Productivity Assessment

Harvesting was done at the week six after sowing. The harvest weight for each treatment was recorded until the eleventh week and the average calculated. Fruit length was assessed at the harvest time using a graduated ruler. Productivity per hectare for each treatment was estimated by extrapolating the average weight of production in each treatment according to the following formula:

$$\textbf{PHec} = \frac{Pmt}{Ac} \times (10.000 \text{m}^2)$$

PHec: production per hectare (tons);
Pmt: average production per treatment (tons);

Ac: Area of culture per treatment (m²).

2.8 Analysis of the Nutritional Profile of *Abelmoschus esculentus*

The assessment of mineral elements was done on young okra fruits at harvest, such as iron, calcium, zinc, magnesium, sodium, potassium and phosphorus according to the method described by [25]. The organic nutrients as:

- Total carbohydrates were determined according to [26].

% carbohydrates = Ms - % proteins - % lipids - % ash

- Total proteins

The nitrogen content was determined by the Kjeldahl method [27] and the protein content was deduced according to the formula:

% protein = % nitrogen*6.25.

- Total fiber

The fiber content was calculated according to the following formula [28]:

$$\textbf{Fibers} = \frac{M1 - M2}{M \times Ms} \times 100$$

Where:

-M1: mass of the sample dried at 105°C;
-M2: mass of the sample dried at 550°C;
-M: mass of the test sample;
-Ms: % dry matter.

2.9 Soil Sample Collection Prepared for Analysis

Soil samples were collected with a hand auger between 0 and 20cm soil depth from the control and treated plots according to the randomized complete block design. This was done one week before application of the bioformulations and four weeks after planting. The composite samples were predried for analysis of pH-water, pH-KCl, organic matter (OM), exchangeable bases (EB), cation exchange capacity (CEC), total nitrogen (Ntot), assimilable phosphorus (pass), total phosphorus (Ptot), exchangeable acidity (AE), granulometry, total calcareous (CaCO$_3$), following the international methods recommended by [29].
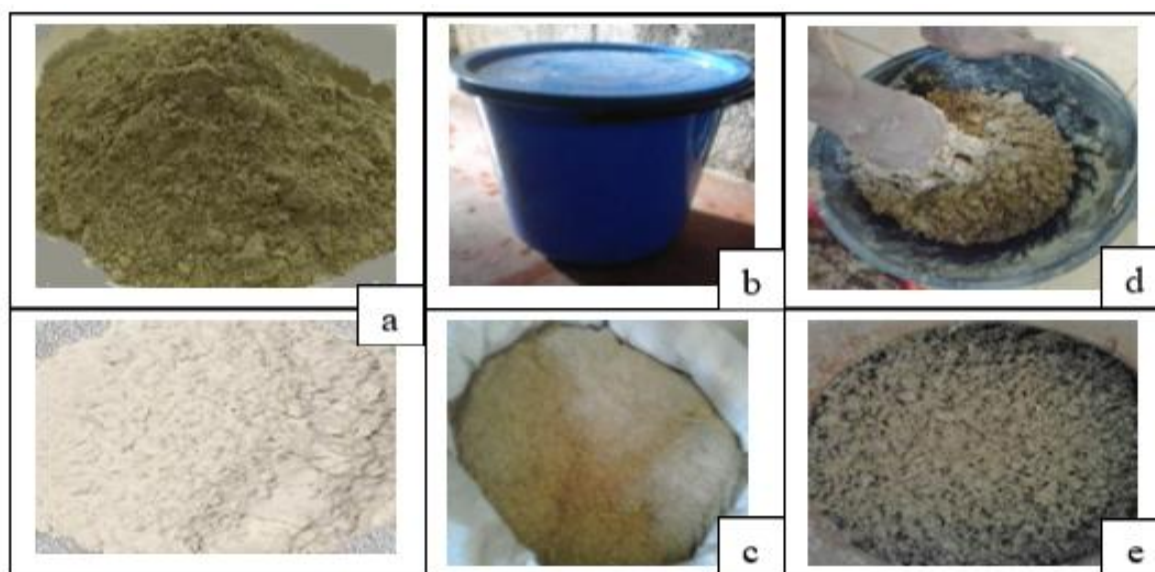
Fig. 1. Preparation of bioformulation manures: (a): microbial strains, (b): mixing of brown sugar+ microbial strain + non-chlorinated water = activated solution, (c): rice bran, (d): activated solution + rice bran, (e): Formulation to be used
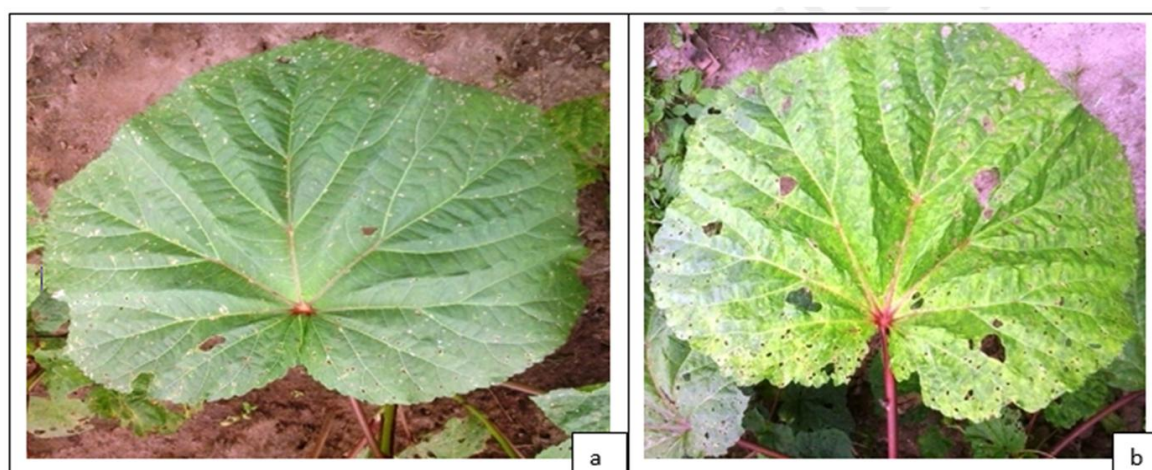
Fig. 2. (a) = plant disease resistance with T1 treatment; (b) = plant disease severity on the control

2.10 Data Analysis

The data were analyzed using the Rcmd package of the Rversion 3.6.3 software where the normality and homogeneity of the different data were tested using the Shapiro-Wilk and Bartlett's K-squared tests respectively. The TUKEY test was used to compare the different means through one-way's ANOVA in order to determine the significant differences between them. The FactoMineR package incorporated in Rcmd was used to perform a factorial analysis of mixed data for physicochemical and agromorphological parameters.

3. RESULTS

3.1 Variation of Plant Height

The okra plants height in the presence of the different treatments was progressive over time. The significant difference was only observed from day 21 to day 28 between the treatments and the control except with treatment T2 (Table 1). At day 35, all treatments showed a significant difference with the control. The difference was also significant between T1 and the other treatments and with the control at day 35.

The plants treated with T1 on day 35 had the best average height (32.56±1.45cm) followed by the plants of the T3 treatment (28.7±1.34cm) compared to the control whose plants showed an average height of 22.46±1.13cm.

The growth rate determines the average of daily growth of plants for each treatment. Treatment T1 showed a significantly higher growth rate (1.144cm/day) followed by T3 (0.915cm/day) and T2 (0.899cm/day) compared to the control T0 whose plants showed a growth rate of 0.720 cm/day.

3.2 Variation in Leaf Number

The results from day 14 after sowing showed no significant increase in leaf number between the different treatments compared to the control until day 35. The fluctuation of the number of leaves was roughly one between the 14th and the 21st day after sowing. After 21 days, this fluctuation increased slightly and was equal to the average of 02 leaves per week (Table 2).

3.3 Variation in Leaf Area

Fig. 1 reveals that there was a significant difference in the variation of plant leaf area between the treatments and the control on day 35. Treatment T1 had a high average leaf area (866.33±46.54cm²) compared to the other treatments and to the control (620,00±68,63 cm²) (Fig.3).

Table 1. Effect of the different treatments on plant height 14 days after of seedling

| Time (day)	T0	T1	T2	T3
14	7.33±0.58a	8.53±0.51a	7.46±0.64a	8.03±0.5a
21	9.76±85ab	13.86±0.45c	12.4±0.96bc	13.56± 0.85 c
28	17.86±1.26d	23.9±2.34fg	20.16±1.72 de	21.9 ± 0.95 ef
35	22.46±1.13ef	32.56±1.45i	26.33±1.8gh	28.7±1.34 h
DH	15.13cm	24.03 cm	18.87 cm	20.67 cm
GR	0.720 cm/day	1.144 cm/day	0.899 cm/day	0.984 cm/day

Means with the same letters on the same line are not significantly different according to the Tukey test P<0.05. GR = growth rate; DH = difference in height between day 14 and day 35

Table 2. Effect of the different treatments on the variation of the number of leaves with time

Time (day)	T0	T1	T2	T3
14	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±00.0a
21	4.33±0.57a	5.00±0.00a	5.00±0.00a	4.66±0.57a
28	6.33±0.57b	7.33±0.57bc	7.33±0.57bc	7.00±0.00b
35	8.33±0.57cd	9.33±0.57d	8.66±0.57d	9.00±0.00d

Means with the same letters on the same line are not significantly different according to the Tukey test P<0.05
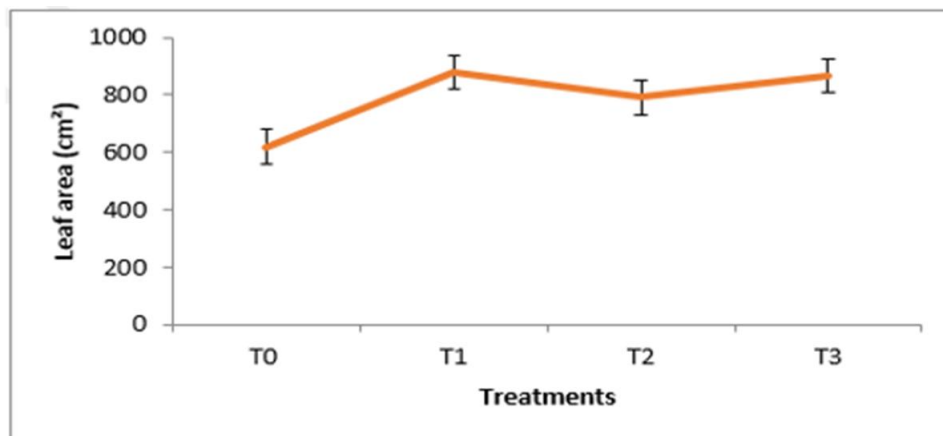
Fig. 3. Effect of the different treatments on the variation of leaf area at day 35 after seedling

3.4 Effect of Bioformulations on Plant Disease Resistance

The analysis of variance showed a significant effect in plant vigor of treatments T1 and T3 compared to the control. The percentage of healthy plants was found to be highest in treatment T1 (86.48%) followed by treatment T3 (83.94%) while the lowest percentage (71.77%) was obtained with the control (Fig. 4).

3.5 Variation in Fruit Length per Treatment

The average of fruits length in the different treatments showed a significant difference compared to the fruits from the control plants. There was no difference between treatments T1 and T3, nor between T2 and T3. The fruits of the plants in treatment T1 had a significantly higher mean value (11.53 cm) than those of other treatments. The lowest value was attributed to the fruits of the control (7.30 cm) (Fig. 5).

3.6 Evaluation of Fruit Yield per Treatment

The evaluation of the fruits weight per plant according to the treatments has shown no significant difference between the plants of treatment T1 and T3 but between them and the control. Plants in treatment T1 showed a high average weight compared to the other

treatments (1868g). The control plants gave a low average weight (960.66g). As the average weight is related to the production per hectare, treatment T1 (showed a higher yield (16.604t/ha) than the other treatments and compared to the control (8.53t/ha) (Table 3).

3.7 Analysis of Soil Physicochemical Parameters under Effect Treatments

Fig. 6 presents the factor analysis of the mixed data, which was used to group together the physicochemical parameters with a better affinity with the different treatments. This analysis on the treated soils and the control soil presented an overall coefficient of inertia of 87.7%, showing the percentage of parameters well represented on the correlation axis. The parameters grouped around each quarter circle are attached to the treatment linked to that quarter. Variables such as potassium (K), exchangeable base saturation (V), assimilable phosphate (Pass), magnesium (Mg), sodium (Na), sum of exchangeable bases (SBE) and calcium (Ca) refer to treatment T1; sand (S), exchangeable acidity (EC), cation exchange capacity (CEC) refer to the control T0, Clay content (A) and pH-KCl are more related to treatments T2 and T3. Variables such as carbon (C), organic matter (OM), nitrogen (N), total phosphorus (TP), silt (L) and pH-water are not significantly related to any treatment (Fig. 4). Calcareous content was found to be null in all treatments.
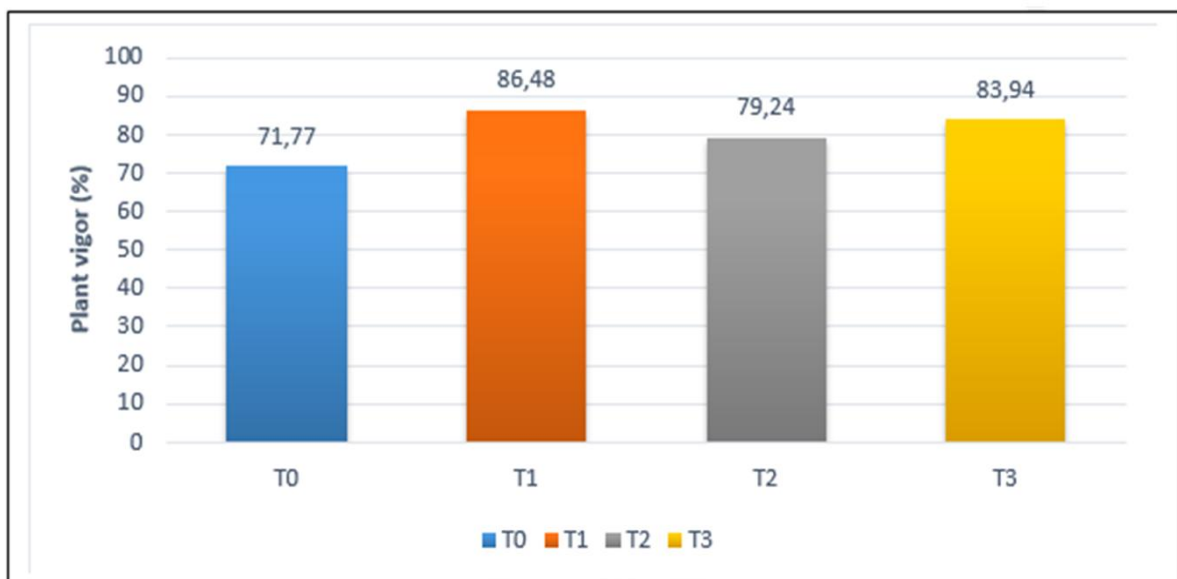

Fig. 4. Effect of the different treatments on the plants vigor on day 35 after sowing
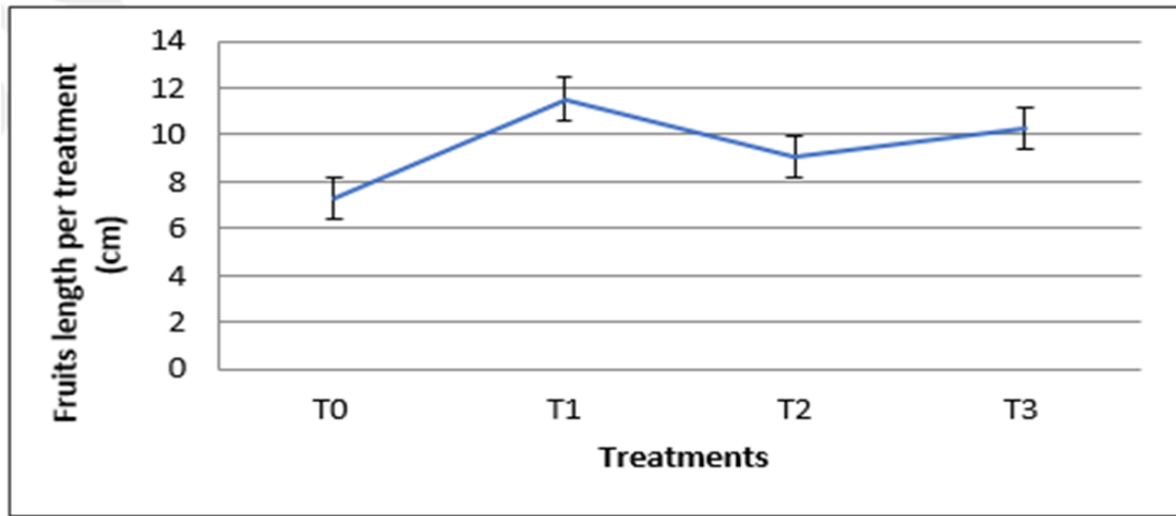
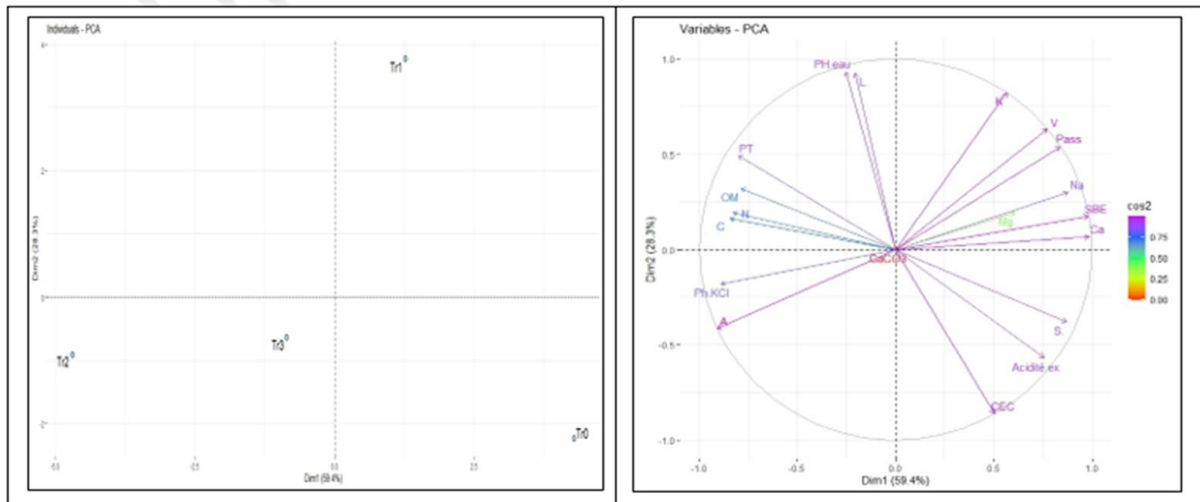**Fig. 5. Fruits length in treatments at harvest**

Fig. 6. Multifactor analysis of mixed data of soil physicochemical parameters of different treatments
A=Clay; S=Sand; L=Lime; OM=Organic matter; C=Carbon; Pass=Assimilable phosphate; Ptot=Total phosphorus; SBE=Sum of Exchangeable Bases; CEC=Cation Exchange Capacity; V=Saturation rate of Exchangeable Base

3.8 Correlation between Soil Physicochemical and Agromorphological Parameters of Okra Plants in the Different Treatments

The matrix correlation (Table 4) establishes the statistical relationship degree between the physicochemical soil parameters and the morphological parameters of okra. The question here was to see which of the soil physicochemical parameters influenced the growth and average production of okra per treatment.

The matrix showed that for okra plant growth, pH_{H2O} was positively correlated with plant height (r = 0.99***) and leaf area (r = 0.91***). The correlation was also positive between nitrogen level and leaf area (r=0.81**) and also between potassium level and plant height (r=0.61*) but negative and highly significant between CEC and leaf area (r=-0.84**).

In the case of the relationship between physicochemical and productivity parameters, the matrix showed a positive and highly significant correlation between pH_{H2O}, fruit length and average production per treatment (r=0.99***). It was also positive between fruit

length and total phosphorus level (r=0.78**); between nitrogen level and average production per treatment (r=0.52*); between potassium level and average production (r=0.6*) and between potassium level and fruit length (r=0.56*). The correlation was rather negative and highly significant between CEC and average production (r=-0.92***) and with fruit length (r=-0.8**).

3.9 Analysis of the Nutritional Profile of Okra under T1 Treatment

Table 5 shows the nutritional value of okra in relation to the different analyses carried out on the fruits from treatments T0 and T1 at harvest. Analysis of nutritional data related to calcium Ca (W = 0.91246, p = 0.4527); Copper Cu (W = 0.84356, p = 0.1395); Iron Fe (W = 0.90248, p = 0.3888); Magnesium Mg (W = 0. 81472, p = 0.07939); Sodium Na (W = 0.81555, p = 0.08074) and Zinc Zn (W = 0.8377, p = 0.1247) related to the T0 and T1 treatments are homogeneous and follow the normal distribution.

Analyses of variance revealed a highly significant difference for Ca, Cu, Mg, Na, Zn, carbohydrates, K and proteins (p<0.01) related to the different treatments. This difference is significant for Fe (p=0.03) and not significant for fibre (p=0.72) according to the treatments Table 4.

3.10 Correlations between Nutritional Parameters and Agromorphological Parameters of Okra of Treatment T1 and Control

The student's t-test highlights the correlation between the agromorphological and nutritional parameters by comparing their statistical means. The question was to determine which of the agromorphological parameters influenced the nutritional profile of okra. This analysis to compare the means of these different parameters was revealed in Table 6. It was found that there was no significant difference between leaf area (LA) and nutritional parameters (p>0.05). The difference was however highly significant between plant emergence and some of nutritional parameters (p<0.01) and highly significant (p<0.001) with sodium and protein. No difference was also found between fruit length (FL) and nutritional parameters (p>0.05) except for calcium and magnesium where it was highly significant (p<0.001). The difference was also significant between plant vigor (VP) and nutritional parameters such as copper, sodium, protein and zinc (P=0.05).

4. DISCUSSION

In the case of organic matter management technologies, less attention is paid to microbiology, and the physical, chemical, and engineering approach predominates. However, when using organic materials and fertilizers, the whole process must be considered. The present study was based on the influence of beneficial microorganisms' bioformulations on the agromorphological parameters and nutritional profile of *Abelmoschus esculentus* cultivated in Cameroon. Three types of bioformulations namely T1 (*Trichoderma Harzianum* and *Bacillus amyloliquefaciens*); T2 (*Bacillus velezensis* and *Bacillus amyloliquefaciens*); T3 (Trichoderma *Harzianum* and Bacillus *velezensis*) were prepared with the aim of improving soil parameters and okra productivity. The bacterial and fungal counts of each formulation were concentrated at 10^{11} CFU/g for *Bacillus amyloliquefaciens*; *Bacillus velezensis* 10^{10} CFU/g) and fungi (*Trichoderma Harzianum* 2x 10^{10} CFU/g). Previous findings have demonstrated that *B. amyloliquefaciens* and *B. velezensis* in a concentration of 10^6 CFU/mL and *T. harzianum* in concentration of $2x10^5$ provided significant protection to plant according to [30]. However, previous results have emerged, which reported that combined strains can reduce plants disease. [31,32] reported that *T. harzianum* when combined to another PGPM strain destroyed the external and internal hyphae continuity of *Glomus intraradices*, which resulted in regulation of P acquisition of mycorrhizal plants.

Table 3. Effect of different treatments on productivity at harvest

Treatments	Average weight per treatment (g)	Weight in hectare
T0	960.66± 167.28 a	8.53±1.49a
T1	1868.00±279.45 b	16.604±2.48b
T2	1338.66±145.40ab	11.897±1.292 ac
T3	1490.33±242.09ab	13.248±2.142bc

Means with the same letters in the same column are not significantly different according to the Tukey test P<0.05

Table 4. Matrix of correlation between physicochemical and agromorphological parameters of various treatments

	C	Ca	CEC	K	LF	Mg	N	Na	OM	Pass	Pf/t	pH_{H2O}	Ptot	SF	TP
C	1														
Ca	-0,76	1													
CEC	-0,59	0,43	1												
K	-0,33	0,61	-0,43	1											
LF	0,17	-0,25	-0,87	0,56	1										
Mg	-0,87	0,51	0,18	0,48	0,3	1									
N	0,47	-0,88	-0,55	-0,32	0,59	-0,06	1								
Na	-0,51	0,94	0,16	0,75	-0,11	0,31	-0,86	1							
OM	0,98	-0,68	-0,7	-0,17	0,29	-0,82	0,44	-0,41	1						
Pass	-0,66	0,85	-0,03	0,91	0,28	0,67	-0,55	0,87	-0,53	1					
Pf/t	0,23	-0,22	-0,92	0,61	0,99	0,22	0,52	-0,04	0,36	0,31	1				
pH_{H2O}	0,23	-0,22	-0,92	0,61	0,99	0,22	0,52	-0,04	0,36	0,31	0,99	1			
Ptot	0,58	-0,8	-0,8	-0,05	0,78	-0,12	0,94	-0,68	0,6	-0,38	0,75	0,75	1		
SF	0,3	-0,52	-0,84	0,28	0,94	0,19	0,81	-0,41	0,37	0,01	0,91	0,91	0,92	1	
TP	0,22	-0,23	-0,9	0,6	0,99	0,24	0,55	-0,065	0,34	0,29	0,99	0,99	0,76	0,93	1

OM=Organic Matter; Pass=Assimilable Phosphate; Ptot=Total Phosphorus; CEC=Cation Exchange Capacity; LF=Length of Fruit; SF=Foliar Area; Pf/t=Weight of Fruit per treatment; TP=Plant Height

Table 5. Nutritional parameters of *Abelmoschus esculentus* related to the treatments

		T0	T1
Organic molecules (g/100 g of content)	Fibers	3.13±0.12a	3.10±0.10a
	Proteins	2.10±0.10	4.40±0.20
	Carbohydrates	7.00±0.20a	9.00±0.00b
Mineral molecules (g/100 g of content)	Na	6.00±0.26a	8.07±0.15b
	Mg	56.00±0.00a	60.00±1.00b
	Ca	81.67±0.58a	86.33±1.53a
	K	291.67±3.06a	364.00±1.00b
	Zn	2.82±0.02a	3.00±0.02b
	Cu	1.89±0.01a	2.01±0.02b
	Fe	0.63±0.12a	0.87±0.06b

*Means with the same letters in the same column are not significantly different according to the Tukey test P<0.05

Table 6. Comparative table of nutritional and agromorphological parameters of okra under T1 treatment

	Plants emergence	LF (cm)	LA(cm^2)	VP(%)
Ca(mg/100g)	0.01	0.006	0.11	0.51
Cu(mg/100g)	0.02	0.14	0.1	0.05
Fe(mg/100g)	0.03	0.14	0.1	0.06
Fibres (g)	0.02	0.16	0.11	0.06
Glucides (g)		0.4	0.1	0.06
K(mg/100g)	0.07	0.06	0.13	0.07
Mg(mg/100g)	0.01	0.001	0.11	0.15
Na(mg/100g)	0.002	0.27	0.1	0.05
Proteins(g)	0.007	0.09	0.1	0.05
Zn(mg/100g)	0.01	0.16	0.11	0.05

Leaf area (LA), plant vigor (VP), fruit length (FL)

The carrier was rice bran in order to obtain solid formulation. [14] underlined in his previous research that, a good microbial inoculum should be packaged in a carrier material that provides and optimum microenvironment as pH, water and carbon content for microorganisms, maintain longer shelf life and microbial viability without the need for a special storage facility. The preparation principle is also corroborated to those of [33] who revealed that the carrier material should be cost-effective, readily available, eco-friendly, acquiescent to nutrient supplement and not harmful to the user. The rice bran as carrier material was used by [34] and [35] in their various agricultural formulations.

Bioinoculation was done one week before seedling for each treatment. The application before seedling on native soil was to activate soil macrobiota and destroy plant phytopathogens. This in turn to agree with the research of [36] who demonstrated that some of plant growth promoting microorganisms' formulation have no direct impact on plant growth but significantly affect the diversity and structure of native microbial communities which will therefore influence plant activities. The finding of [37] are opposite to this process and underline that the inoculation improves plant growth but had no influence on species diversity and richness of native microbe in the host plant roots. [30] also underlined in vitro activities of *Trichoderma harzianum* of plant grown in soil, and demonstrated that, application of the compounds on soil before the seedling emergence was less effective than after. This justifies the second microbial inoculant application three weeks after seedling in this study.

The multifactorial analysis of mixed data of physicochemical parameters according to the correlation circle allowed the combination of physicochemical parameters with a higher affinity with the different treatments. This analysis on the treatment and control soils showed an overall coefficient of inertia of 87.7%, highlighting the percentage of parameters well represented on the correlation axis. The parameters grouped around each quarter circle are attached to the treatment linked to that quarter.

The analyses have shown that more parameters were related to the T1 treatment with high contents compared to the other treatments. This variation would be related to the combined activity of the microorganisms involved in this bioformulation which have the ability to efficiently decompose soil organic matter in order to release the most soluble elements that would be absorbed by the plant. The work of [38] supports this by stating that some PGPR strains such as *Bacillus amyloliquefaciens* secrete a diverse spectrum of compounds that promote the degradation of organic matter in order to release nutrients for plant growth.

The organic matter content and near-neutral pHwater of the culture soil (6.70) related to the T1 treatment contributed to the successful growth of okra, which requires an optimal pH between 6 and 7 [39] justified by a positive and highly significant correlation between pHwater and agromorphological parameters such as plant height, fruit length and average fruits weight. The saturation rate (79.57%) of cation in the T1 treated soil was high, showing the ability of *Bacillus amyloliquefaciens* to release cations contained in the organic matter for their uptake by the plant. This release was justified by high levels of Mg (2.56meq/100g), potassium (1.90meq/100g) and other minerals compared to the other treatments. The relative humidity of the bimodal rainfall agro-ecological zone is about 1638 mm, which responds favorably to the water requirements of okra at the vegetative growth stage for an average need of about 900 to 1200 mm [39]. The temperature of the environment, which is 24.5°C, also matches the plant's needs for rapid growth at temperatures between 20 and 30°C [40]. The modification of edaphic parameters such as pH could be linked to the action of *T. harzianum* which secretes metabolites capable of ensuring chemical modifications of the environment; an assertion corroborated by [11] with a positive correlation between other chemical parameters such as total phosphorus, nitrogen and potassium. [41] demonstrated that *Bacillus amyloliquefaciens* contribute to the stability of soil aggregates determining both mechanical and physical properties by modifying the arrangement of soil particles. The increase in nitrogen levels in the treatments compared to the control would also be due to microbial action with the ability to fix atmospheric nitrogen and dissimilative reduction (transformation of nitrate into ammonia) [42]. Its availability in soil level for plants is due to the action of *B. amyloliquefaciens* and *B. velezensis*

capable of solubilizing total phosphorus into a plant-available portion [15]. This would also be linked to their capacity to secrete phytases, which are enzymes capable of breaking down phytates (organic phosphate) in order to release the phosphate ions thus available for plants [15]. Treatment T1 therefore has a significant amount of assimilable phosphate compared to the other treatments and the control; this is evidence that the microorganisms in this bioformulation have solubilized a significant amount of total soil phosphorus, mainly under the effect of *B. amyloliquefaciens*. A negative correlation was thus established between total phosphorus and assimilable phosphate, justifying the decrease in the level of one in favor of the other. Treatment T2 had more organic matter than the other treatments but its availability of exchangeable cations was low. This could be due to a temperature that is not suitable for *Bacillus velezensis* to break down extensively organic matter as far as its high temperature activity is 16°C [43], hence the non-synergistic effect of the two PGPRs in this bioformulation for this effect. This may also be due to a competitive inhibition in trophic sources between the PGPR composing this bioformulation since they live with root exudates to better sustain their activity [44]. This could also be caused by the high clay content in this treatment compared to the others; thus slowing down the mineralization of organic matter by these PGPRs [45]. The CEC is high in the T0 control soil compared to the other treatments but does not have the high mineral content. This difference would therefore be due to the presence of minerals that were already present in the soil before the okra culture and the application of formulations.

The results obtained on plant height presented the plants treated with T1 as those that significantly grew compared to the other treatments and the control. This would be due to the synergistic action of *B. amyloliquefaciens* and *Trichoderma harzianum* combination having colonized the rhizosphere of *Abelmoschus esculentus,* thus secreting phytohormones such as auxin and gibberellin which stimulate their root development and their vertical growth. The results are similar for the number of leaves and leaf area, despite the fact that this parameter did not vary significantly between the different treatments except with the control. This hypothesis is in the same way with the work of [45] on the involvement of these hormones in the growth and development of wheat. Multi-strain PGPMs mixtures, so called microbial consortia,

appear to have greater efficacy on improvement of plant-growth than single strains [46]. However, the work of [47] demonstrated that *Trichoderma harzianum* induced germination and growth in beans through its capacity to solubilize phosphate, zinc, manganese, copper and iron. It was also found that other soil physicochemical parameters under T1 treatment such as assimilable phosphate and potassium showed higher concentrations compared to the other treatments, which would contribute to high growth of okra compared to the other treatments. [48] Work supports the fact that PGPRs such as *Bacillus sp.* improve plant nutrient status by assisting them in nitrogen fixation and increasing root exchange surface area, and phosphorus solubilization for some plants such as oilseed rape, wheat and maize by improving root biomass. The results of this work established a high positive correlation between plant height and number of leaves. The high potassium content assessed in the soil in some treatments would also have contributed to plant growth by increasing the leaf area, although the correlation between plant morphological parameters and this rate was not strong but positive. The work of [30] showed that *Trichoderma Harzianum* fixation favored the uptake and concentration of some nutrients "dissolved" in the soil solution such as copper, iron, manganese, phosphorus and sodium.

Regarding the plants vigor, the results obtained placed the plants treated with T1 as those with the most resistance capacity to biotic factors during the vegetative and reproductive growth phase such as harmful microbial attacks. This could be justified on the one hand by the activity of *T. harzianum* due to its ability to synthesize elicitors such as peptaïboles (trichorzianins A and B and trichorzins) and exo-chitinases that will induce plant resistance by increasing their immunity and on the other hand by the activity of *Bacillus amyloliquefaciens* secreting bioactive molecules such as plantozolicin, polyketides with fungal and bactericidal actions. *Bacillus velezensis* for the T2 treatment would also have secreted amylocyclicin with bactericidal actions. This assertion is corroborated by the work of [14] where *Bacillus sp.* facilitated root colonization and interaction with host plant defense responses. [49] has also demonstrated that T. harzianum had antagonistic activity against *Fusarium proliferatum* and *Fusarium verticillioides*, with mycelial inhibition rates of 68.38% and 60.64%, respectively. Culture filtrates suppressive rates of T. harzianum strains

exhibited antifungal activity against an F. verticillioides strain (32.2%) that was stronger than mycelium (23.50%). In terms of productivity, all the harvesting established over six weeks put the biofertilizer effect of T1 in first position with an average production of 16.604t/ha higher than the other treatments and significant compared to the treatment T2 and the control (8, 53t/ha) productivities while okra production in [50] findings for the same variety ranged between 7.92 and 9.13 tons per hectare in control and beneficial microorganism biofertilizers plants respectively. The result of [51] for the same variety cultivated in the rainy season under the effect of the effective microorganisms is also law (14.807t/ha). This production was also higher than those obtained by [40] (11.70t/ha), who used a different okra variety and cultivated under the effect of organic manure. This difference may be related to the type and role of the microorganisms from each formulation used in these different crops in terms of their ability to release nutrients from the soil to the benefit of the plants and also to increase their immunity to microbial pathogen attacks for higher production. As a good biological control agent, *T. harzianum* has been widely used in vegetable production. [52] has shown that, the inoculation with *Trichoderma harzianum*, increased root weight in wheat, root length in beans, and greater yields in lettuce and tomato have been recorded. This difference was also related to plant height and fruits weight, hence the strong positive correlation between these two parameters (99%). Okra yield was also related to the number and weight of fruits, which is supported by the work of [8]. The correlation test between the different agromorphological parameters by factorial analysis of mixed data on the different treatments showed an overall percentage of inertia of 99.8%, thus justifying a positive correlation between fruit weight and fruit length. The early emergence of the plants as well as their vigor also justified a better production, hence the positive correlation between these different parameters. The control got a low productivity. It may be due to disease attack severity of okra at the growth stage. [53] underlined that both biotic and abiotic agents cause diseases in plants and pose a problem in agriculture by significantly affecting plant growth and crop yield at a global scale. From factorial analyses of mixed data, it appears that the bioformulation with Trichoderma harzianum and Bacillus amyloliquefaciens positively and significantly have influenced more physicochemical and agromorphological

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parameters than the other treatments and compared to the control.

The results obtained after analysis of the biochemical composition of the okra revealed that the fruits from the treatment and previously dried had a better nutritional constitution than the control. The levels of most of the components assessed were highly significant compared to those of the control, especially in protein, which doubled in the treated accession. Studies conducted by [54] showed that the contents of nutritional constituents of okra increase considerably due to the process of drying which leads to the decrease of their water content. The fiber content did not vary between the treatment and the control but however doubled compared to the standard value for the same variety (1.7g to 3.13g). This can be explained by the physico-chemical status of the soil, which contributed to increasing the fiber content of these plants during their growth in the different accessions, thus favoring a better absorption of nutritive elements. The low level of iron in these plants (>10% /100g) would be due to its use in the different metabolic pathways of the plant such as photosynthesis, respiration, nitrogen fixation and assimilation and DNA synthesis [55]. High magnesium levels are directly involved in plant growth, sugar synthesis, transport and storage, which explains the highly significant positive correlation between magnesium levels and fruit length, and the significant correlation with sugar levels in the fruit. There is also a correlation between zinc levels and sugar levels because its presence in plants favors the conversion of starch into sugars. The high potassium content is thought to be due to the stimulation of the okra roots by *Trichoderma harzianum* and *Bacillus amyloliquefaciens* for better absorption and thus storage was directed to the reproductive organs hence the positive correlation between fruit length and potassium concentration. The sodium content of okra fruits is correlated with plant emergence which means that this content could be due to root uptake at soil level, stimulated by the potential microorganisms constituting the T1 bioformulation because the contents differ between the treated accession and the control for a same type of soil. This is supported by the work of [56] on the molecular characterization of *Trichoderma harzianum* in olive. *Trichoderma harzianum* would therefore have the capacity to increase nutritional value [57]. Its synergistic effect with *Bacillus amyloliquefaciens* would thus amplify this content. The various nutritional analyses of okra show the ability of bioformulation T1 to stimulate the various pathways of secretion and absorption of nutrients by the okra plant which would make it an excellent source of energy for the food industry. The various nutritional analyses of okra show the ability of bioformulation with *Trichoderma harzianum and Bacillus amyloliquefaciens* to stimulate the various pathways of secretion and absorption of nutrients by the okra plant which would produce biofortified fruits due to their contents in various nutrients.

5. CONCLUSION

Soil fertility is a major asset in agricultural production for reducing hunger and poverty. The three types of bioformulations consisting of T1; T2 and T3 were analyzed and significantly influenced soil physicochemicals and agromorphological effects of okra relative to the control. The literature survey showed that several PGPMs can be used effectively to promote plant growth in normal and stressful environments; however, their real effectiveness under field conditions could hardly be evaluated due to the high variability in the efficacy and reproducibility in several environmental conditions. Initial observations made on the analysis of the physicochemical parameters of the culture soil show that it is acidic and moderately rich in minerals. Bioformulations have thus acted as soil correctors as chelating and complexing agents and also as nitrification inhibitors to reduce soil acidity. It has been observed that these parameters are most significantly influenced by the T1 bioformulation compared to the other formulations. The same observations were made concerning agromorphological parameters, placing the T1 bioformulation on top. It would have acted here as an agronomic additive. The nutritional properties evaluated showed that the T1 bioformulation finally tested doubled the levels of certain nutrients in the okra, such as proteins. Levels of other nutrients increased significantly. In view of all the above, the bioformulation based on Trichoderma harzianum and Bacillus amyloliquefaciens would be adapted to the correction of the soil in order to improve its fertility and improve the productivity of okra. The fruits are thus biofortified and would be adequate for various uses due to its contents.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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