American Journal of Plant Sciences, 2024, 15, 492-518
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Effect of Biostimulants Based on Natural Products on the Growth and Nutritional Value of Bean (*Phaseolus vulgaris* L.)

Aimé Giresse Tzeuton[1], Flore Eliane Eyenga[2,3], Arouna Meshuneke[1,4], Gaston Mbang Elock[1,4], Fabrice Damien Wassom[1,4], Walter Jospin Timma Kom[1,4], Marie Paule Djam Kengoum[1,4], Erica Cabrelle Damtse Damtse[1], Landry Silatsa Fotsing[1], William Asah Che[1], Marlyne Josephine Mananga[2,3], Nicolas Niemenak[1,5], Cécile Annie Ewané[1,2,4*]

[1]Laboratory of Plant Physiology and Biochemistry, Higher Teachers' Training College, University of Yaoundé 1, Yaoundé, Cameroon
[2]Department of Biochemistry, Faculty of Sciences, University of Yaoundé 1, Yaoundé, Cameroon
[3]Laboratory of Food Sciences and Metabolism, University of Yaoundé 1, Cameroon
[4]Laboratory of Phytoprotection and Valorisation of Genetic Resources of the Biotechnology Centre, University of Yaoundé 1, Yaoundé, Cameroon
[5]Department of Biological Sciences, Higher Teachers' Training College, University of Yaoundé 1, Yaoundé, Cameroon
Email: *cecile-annie.ewane@facsciences-uy1.cm

How to cite this paper: Tzeuton, A.G., Eyenga, F.E., Meshuneke, A., Elock, G.M., Wassom, F.D., Kom, W.J.T., Kengoum, M.P.D., Damtse, E.C.D., Fotsing, L.S., Che, W.A., Mananga, M.J., Niemenak, N. and Ewané, C.A. (2024) Effect of Biostimulants Based on Natural Products on the Growth and Nutritional Value of Bean (*Phaseolus vulgaris* L.). *American Journal of Plant Sciences*, **15**, 492-518.
https://doi.org/10.4236/ajps.2024.157035

Received: May 7, 2024
Accepted: July 21, 2024
Published: July 24, 2024

Abstract

Beans (*Phaseolus vulgaris* L.) are widely grown in Cameroon and play a key role in the fight against food insecurity, malnutrition and poverty. However, its cultivation encounters problems due to abiotic and biotic stresses, which leads to the use of synthetic fertilizers and pesticides, which cause significant damage to the environment and human health due to the presence of synthetics residues in the seeds, pods and in the leaves that are eaten. Promoting the use of natural products is becoming a necessity for organic and eco-responsible agriculture that limits contamination problems and improves people's purchasing power. This study aims to assess the effect of biostimulants based on natural products on the growth and nutritional value of common bean (*Phaseolus vulgaris* L.). Bean seedlings from white variety (MEX-142) and red variety (DOR-701) were treated every seven days in the field from their pre-emergence, emergence and growth to their maturation under a randomized block experimental design. Six treatments and three repetitions with the biostimulants based on natural products and controls were thus performed and the agromorphological parameters were measured. After 120 days, the contents of growth biomarkers and defense-related enzymes were evaluated in leaves, while the contents of macromolecules, minerals and antinutrients were evaluated in seeds. These biostimulants significantly increased ($P < 0.0001$) agromorphological parameters, induced significant accumulation of growth biomarkers (chlorophylls, phenols and proteins) and defense-related enzymes (peroxidase, polyphenol oxidase and phenylalanine ammonia lyase) in leaves compared to controls (T+ and T−). They also promoted the accumulation of proteins, lipids, total sugars, iron, zinc and a significant decrease ($P < 0.0001$) of antinutrients including oxalates, phytates, tannins and saponins in seeds compared to controls (T+ and T−). Treatment with biostimulants, in particular BS4, improves the performance of bean plants in the field as well as the biofortification of seeds regardless of the variety.

Keywords

Phaseolus vulgaris L., Biostimulants, Biofortification, Growth and Defense Biomarkers, Nutritional Value

1. Introduction

The bean (*Phaseolus vulgaris* L.) is a species of annual plant in the family *Fabaceae*, genus *Phaseolus*, commonly grown as a vegetable and native to Latin and Central America [1]. It is considered the most consumed species in the genus *Phaseolus* and among "beans" in the broad sense, due to its high proteins content (20% - 28% dry product), carbohydrates (46% of the dry product), fibers (15% of the dry product), micronutrients (iron and zinc), and its low lipids content (1.5% of the dry product) [2] [3], important in human nutrition in many developing countries. Beans are experiencing renewed interest and improving levels of consumption as an important source of proteins for households in Africa due to the outrageous increase in the kg of meat in the markets [4]. In addition, bean consumption reduces the risk of cardiovascular disease and other effects such as anti-obesity, anti-oxidants and anti-inflammatory [5].

Cameroon produces about 390.816 tons of beans per year (17th world producer) on an area of 230.000 hectares, i.e. a yield of 870 kg/ha [6]. This production is provided by poor small farmers and intended for local, national and cross-border consumption [7]. However, this bean production is increasingly facing many difficulties related to soil fertility, diseases and pests that affect yield, which make its cost more and more high in the markets.

To this end, farmers have turned to the use of chemical fertilizers in order to overcome these difficulties and increase their production yields. However, a lack of knowledge on the properties of the soil, and the use of industrial fertilizers can affect the long term physico-chemical and microbiological properties of the soil [8]. In addition, an abusive use of synthetic agricultural inputs in agriculture during the cultivation and conservation of crops leads to adverse effects on the environment i.e. as source of water and soil pollution, human health and sometimes the presence of pesticide residues in the seeds [9]. Indeed, this bean cultivated in Cameroon is increasingly exported to Europe and is facing a crucial problem of pesticide residues that can lead to blocking any export to European

Union.

This is why it is important to adopt eco-responsible agricultural techniques that limit the use of synthetic fertilizers and pesticides for organic agricultural inputs. Thus, the use of organic agricultural imputs is recommended because it reduces the number of residues present in plants, crops and the environment. Faced with concerns about growing poverty and the decline in the purchasing power of populations in sub-Saharan Africa, it is essential to innovate processes such as the biofortification of food through the action of biostimulants. Biofortification is the process of increasing nutritional value through conventional breeding, good agronomic practices or biotechnological modification [10].

Recent studies have highlighted the biostimulating action of natural plant-based products, in particular *Tithonia diversifolia* and biological tissues such as clams, in improving the agronomic performance of several plants [11]-[14]. These products showed positive biofertilizing, biostimulating and preventive biopesticide effects in banana, cocoa and vegetable plants compared to control plants. However, they have not been used in beans. This study aims to evaluate the effect of biostimulants based on natural products on the growth and nutritional value of beans (*Phaseolus vulgaris* L.).

2. Materials and Methods

2.1. Biological Materials

The biological materials used for the realization of this work consisted of the seeds of two (02) varieties of bean and (04) biostimulant products. Red bean mainly cultivar DOR-701 (small dark red bean commonly called Meringue) and white bean mainly cultivar MEX-142 (small white bean) collected at the Agricultural Research Institute for Development (IRAD) of the station multipurpose school in Foumbot, a town located in the Noun division, West region of Cameroon.

Biostimulant products in particular BS1, BS2, BS3 and BS4 based on natural products were provided by the BioTid Research Team of the Laboratory of Plant Physiology and Biochemistry (LPBV) of the Higher Teachers' Training College of the University of Yaoundé 1.

2.2. Chemical Material

NPK chemical fertilizer (20-10-10) was used as an amendment for the positive control treatment (T+) and was purchased at the Mokolo market in Yaoundé 2nd district.

2.3. Methods

2.3.1. Field Experiment

The plots were cleared manually and loosened using a pickaxe, then 12 blocks were formed using a hoe, the blocks were separated from each other by an interval of 60 cm. The experimental device was that of randomized blocks with 6 treatments and 3 repetitions, in non-sterile conditions. The treatments were:

T− = plot treated with water (negative control).

T+ = plot treated with NPK 20-10-10 chemical fertilizer (positive control).

F1 = plot treated with BS1 (based on *Tithonia diversifolia* + Emilia *Coccinea*).

F2 = plot treated with BS2 (based on *Tithonia diversifolia* + *Clams*).

F3 = plot treated with BS3 (based on *Sonchus Oleraceus*).

F4 = plot treated with BS4 (based on *Urtica* sp.).

The replicates were separated from each other by an interval of 25 cm.

2.3.2. Sowing and Maintenance

A good quantity of seeds of the two varieties of beans were soaked in water 24 hours before sowing, in order to accelerate their germination. Seeds were sown manually using a dibbler and 5 seeds were sown in holes of 3 cm deep, at 25 cm intervals on the lines and between the lines. Treatments (biostimulants and chemical fertiliser) were carried out once a week and watering every two days. When the plants reached 15 to 20 cm in height, they were hoeed and the soil ridden up to the first two leaves; then every 30 cm, the oars were planted and angled inwards then were tied at the top. Thinning took place 14 days after sowing, and weeding took place 30 days after sowing using a hoe.

2.3.3. Collection of Data

The effect of the various treatments on the development of the plants was evaluated by taking agronomic parameters every 7 days from the end of the second week after sowing and extended over the following 12 weeks until at plant maturity. The agromorphological measurements taken were leaf number, plant size, stem diameter, branching number, pH, sunshine, soil humidity and temperature. After flowering, the number of flowers and pods was counted every two days until maturation.

- The number of sheets was counted by hand.
- The height of the plant was measured using a tape measure.
- The ramifications were counted by hand.
- The diameter of the pod was measured using the vernier caliper.
- The number of flowers and pods was counted by hand.
- Soil pH, sunlight, humidity and temperature were measured using a thermo-probe (soil survey instrument 4 in 1).

The maturation of the pods began on the 60th day and on bean plants having reached maturity, a good quality of leaves harvested in the different modalities were sent to the Laboratory of Plant Physiology and Biochemistry (LPBV) of the Higher Teachers' Training College of the University of Yaoundé 1 for the purpose of analyzes. While the harvested seeds were later sent to the Laboratory of Food Sciences and Metabolism (LabSAM) of the University of Yaoundé 1 for analysis purposes as well. For each variety, two types of samples were used, namely: treated raw beans and control raw beans. All analyses on samples were carried out in triplicate.

2.3.4. Evaluation of the Effect of Treatments on the Accumulation of Biomarkers

For each treatment 0.5 g of fresh bean leaves was used for sample's analyses. The extraction and quantification of samples were carried out according to the method reported by [15]-[22] modified respectively for total chlorophylls (652 nm), total phenols content (760 nm), total proteins (595 nm), phenylalanine ammonia-lyase (290 nm), peroxidase (470 nm), polyphenol oxidases (330 nm). The total phenols were measured in mg per g of fresh weight while that of the total proteins concentration was expressed in mg equivalent (Eq) of bovine serum albumin (BSA) per g of fresh weight (FW). The phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase specific activity were expressed in µmol/min/mL.

2.3.5. Evaluation of the Effect of Treatments on the Nutritional Value of the Bean Seed

For each treatment a mass of 5 g fresh bean was used for sample's analyses of the determination of water and dry matter content carried out according to the method reported by [23], while a mass of 0.1 g of dry bean powder was used for sample's analyses of the total nitrogen content that was determined after mineralization of the samples according to the Kjeldahl method [24] and monitoring of the assay by the method of [25]. The total amount of nitrogen was determined by the method [26]. The extraction and quantification of samples were carried out according to the method reported by [27]-[29] respectively for total lipids, total sugars, iron (Fe) and zinc (Zn) minerals. The dry matter and water content were expressed as percentage of fresh matter (g/100g FW), while the total protein content in the samples were expressed in g/100g dry matter (DM), with the conventional coefficient of conversion of nitrogen into protein used, i.e. 6.25 according to [26]. The total lipids and the total sugars were expressed in g/100g of DM, while the mineral content was expressed in mg/100g of dry matter.

2.3.6. Determination of Antinutrients Content in the Bean Seed

Bean powder was used to determined the oxalates content (1 g), phytates content (2 g), tannins content (1 g) and saponins content (5 g) according to the method reported by [30]-[34]. The oxalates, phytates, tannins and saponins content were expressed in g/100g of dry matter.

2.4. Statistical Analysis

The analysis of variance (ANOVA) was performed using Tukey's test at the 5% probability threshold using XLSTAT software (version 21.0 for Windows) to compare the different modalities and make the graphic representations. A four-factor ANOVA (modality, variety, day and repetition) was carried out for the analysis of agromorphological parameters (diameter and height of the stem, number of leaves, branching, flowers, pods) and physicochemical characterization of substrates during cultivation, and to two factors (modality and variety) for the biochemical analysis of seeds (total proteins, total sugars, total lipids, iron, zinc, oxalates, phytates, tannins, saponins) and leaf extracts of bean (total chlorophyll, total phenols, total proteins, peroxidases, polyphenol oxidases and phenylalanine ammonia-lyases). The Principal Components Analyzes (PCA) were carried out using the Pearson correlation test.

3. Results

3.1. Effect of Biostimulants on Bean Growth

Analysis of variance of agromorphological parameters of bean plants throughout this experiment revealed that the variable "modality", and "day" (of taking the measurements) each had highly significant effects ($P < 0.0001$) on the plant responses (the height of the plant, the diameter of the stem, the number of leaves, the branching, the number of flowers, and the number of pods). The variable "variety" and the interaction "modality*variety" showed highly significant effects ($P < 0.0001$) on height, number of leaves, branching, number of flowers and number of pods; and the interaction "modality*variety*day" also showed a highly significant effect ($P < 0.0001$) on these parameters except for branching. The coefficient of determination (R^2) for all the vegetative variables is close to 100% indicating that the model used is reliable and reproducible (**Table 1(A)** and **Table 1(B)**). Amongst these variables, the one that most influences plant growth in terms of height, diameter, number of leaves, branching, number of flowers is the variable "day", while the variable "variety" is the most influential for the number of pods.

Table 1. Analysis of variance of the effects of different biostimulants on the agromorphological parameters (diameter, number of leaves, branching, height, number of flowers and number of pods) in the leaves of bean plants.

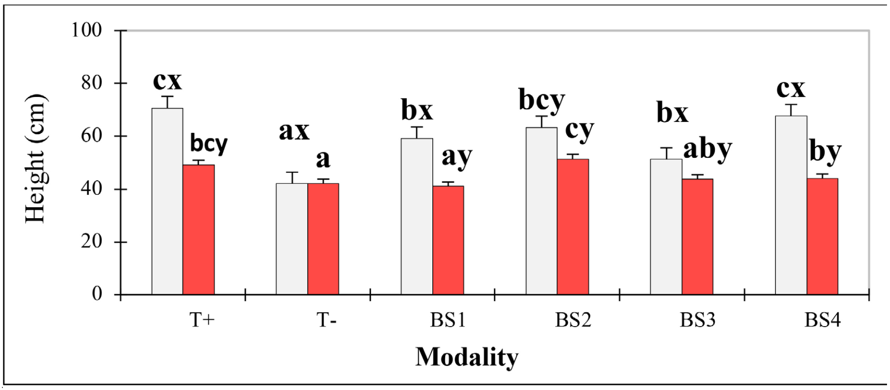
A		Diameter (cm)		Number of leaves		Branching	
		$R^2 = 99\%$		$R^2 = 97\%$		$R^2 = 95\%$	
Source	df	F	*P*	F	*P*	F	*P*
Modality	5.000	150.370	**0.000**	339.847	**<0.000**	131.706	<**0.000**
Variety	1.000	**<0.0001**	1.000	1646.276	**<0.000**	527.721	<**0.000**
Day	7.000	4859.111	**0.000**	567.847	**<0.000**	429.795	**<0.000**
Repetition	2.000	**<0.0001**	1.000	6.993	**<0.001**	3.220	0.042
Modality*Variety	5.000	**<0.0001**	1.000	16.896	**<0.000**	7.726	<**0.000**
Modality*Variety*Day	35.000	6.260	**0.000**	3.040	**<0.000**	1.403	0.076

B	Height (cm)			Number of flowers			Number of pods		
	$R^2 = 99\%$			$R^2 = 90\%$			$R^2 = 94\%$		
Source	df	F	*P*	df	F	*P*	df	F	*P*
Modality	5.000	8.453	<**0.000**	5.000	75.201	**<0.000**	5.000	114.023	**<0.000**
Variety	1.000	52.109	<**0.000**	1.000	399.028	**<0.000**	1.000	991.797	**<0.000**
Day	2.000	255.866	<**0.000**	9.000	148.571	**<0.000**	5.000	174.637	**<0.000**

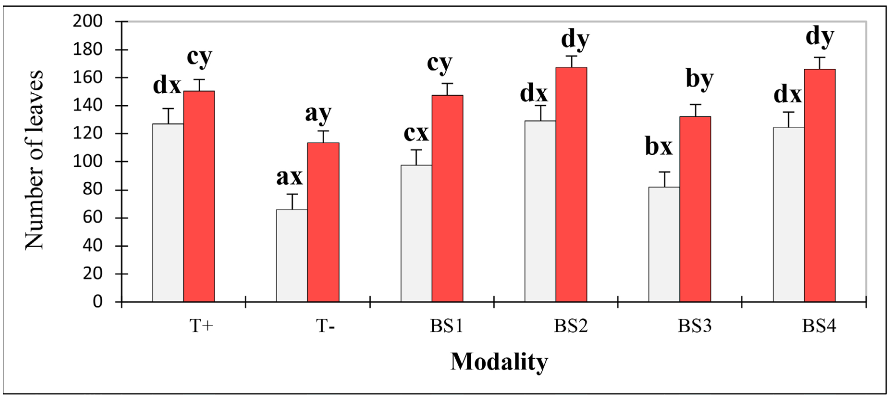
Continued

Repetition	2.000	0.435	0.648	2.000	4.824	**<0.009**	2.000	6.450	**<0.002**
Modality*Variety	5.000	3.804	<**0.004**	5.000	31.310	**<0.000**	5.000	29.543	**<0.000**
Modality* Variety*Day	10.000	2.821	<**0.005**	45.000	5.202	**<0.000**	25.000	6.516	**<0.000**

Values in bold correspond to tests so the null hypothesis is rejected with a significant level alpha = 0.05. *DF is the degree of freedom*; *F is the F test value and P is the probability.*



(A)



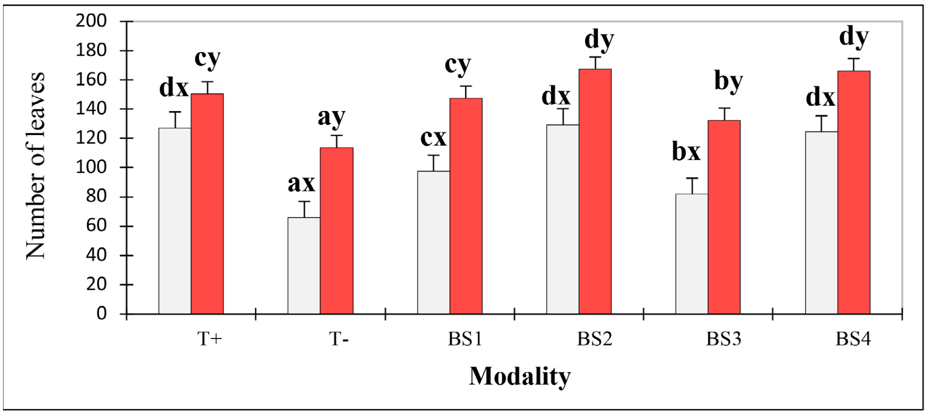
(B)

Each bar represents the standard deviations of three repetitions for each modality and the letters (*a*, *b and c*) *represent the means between statistical groups of different modalities and between varieties* (*x and y*) *according to the ANOVA test* $P \leq 0.05$).

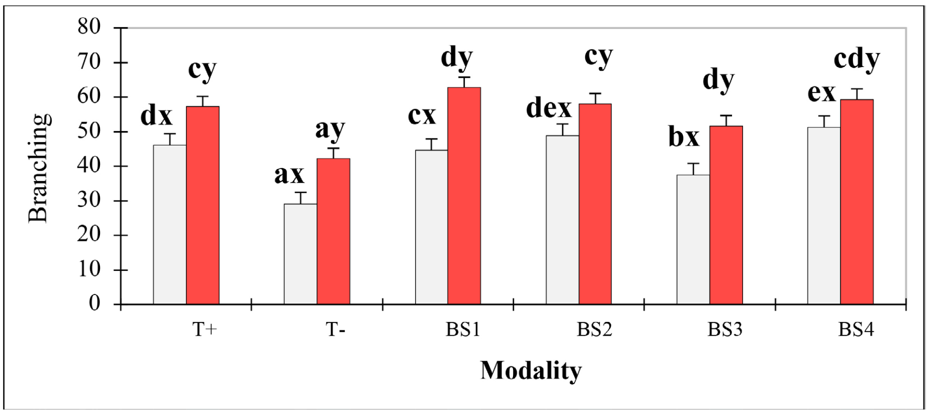
Figure 1. Effect of biostimulants on the growth of white bean (Grey) and red bean (Red) plants in terms of height (A) and diameter (B) during the experiment.

Plants treated with biostimulants (BS1, BS2, BS3 and BS4) showed higher growth in agromorphological parameters (height, stem diameter, number of leaves, branching, number of flowers, number of pods) compared to the negative control (Figure 1 and Figure 2). However, the modalities that showed the most efficiency in terms of rapid increase in height, number of leaves, number of branches, number of flowers and pod were the BS2 and BS4 modalities compared with the positive control (T+) (Figure 1 and Figure 2). The number of pods was three (03) times greater in the BS2 and BS4 modalities compared to the negative control (T−). A similarity was noted in the increase in stem diameter between the treated plants and the positive control (Figure 1(B)).

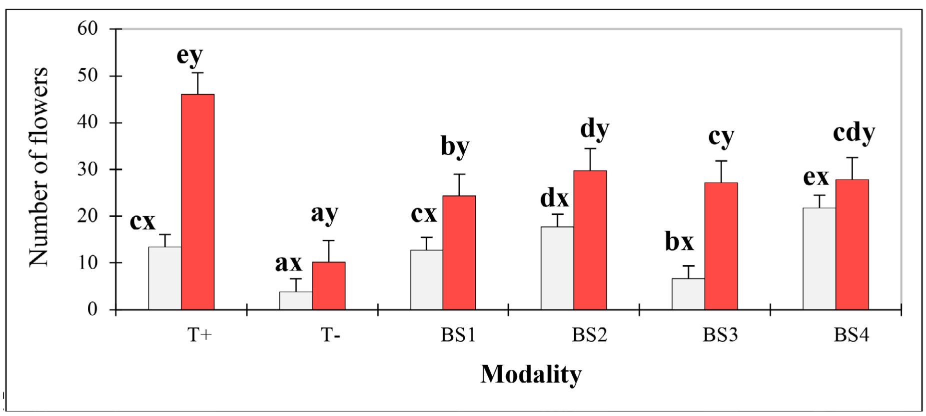
Regardless the modality, the number of leaves, branches, flowers and the number of pods were greater in red bean plants compared to white bean plants. However, the white bean plants were more important in terms of height compared to the red bean plants except for the negative control. The leaves and pods of plants treated with biostimulants (BS1, BS2, BS3 and BS4) were larger compared to the negative control (Figure 2).



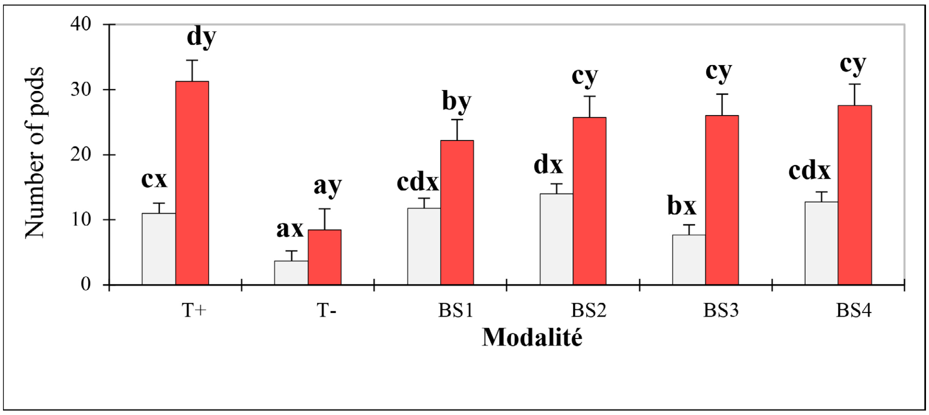
(A)



(B)



(C)



(D)

Each bar represents the standard deviations of three repetitions for each modality and the letters (a, b, c, d et e) represent the means between statistical groups of different modalities and between varieties (x and y) according to the ANOVA test $P \leq 0.05$).

Figure 2. Effect of biostimulants on the vegetative growth of white bean (Grey) and red bean (Red) plants in terms of number of leaves (A), branching (B), number of flowers (C), number of pods (D) during the experiment.

3.2. Physicochemical Characterization of Substrates

The analysis of variance (ANOVA) of the physicochemical characteristics of the substrates (pH, temperature, insolation and relative humidity) in the field during the growth of bean plants revealed that the variables "modality" and "day", the interaction "modality*variety" each had a significant ($P < 0.001$) effect on soil pH and a highly significant ($P < 0.0001$) effect on field soil temperature (Table 2). The coefficient of determination R^2 for all the variables is close to 100% indicating that the model is reliable and reproducible. Among these variables, the one that most influences soil pH was the interaction "modality*variety", while the variable "day" has the most influence on soil temperature (Table 2).

Low insolation (Low^-) and high humidity (Wet^+) relative to the soil in the field were recorded throughout the experiment. There exists a significant difference variation in soil temperature and pH between the treated modalities and the control modalities (Figure 3). Overall, the modalities (BS1, BS2, BS3 and BS4) improve the soil temperature and pH more than the controls.

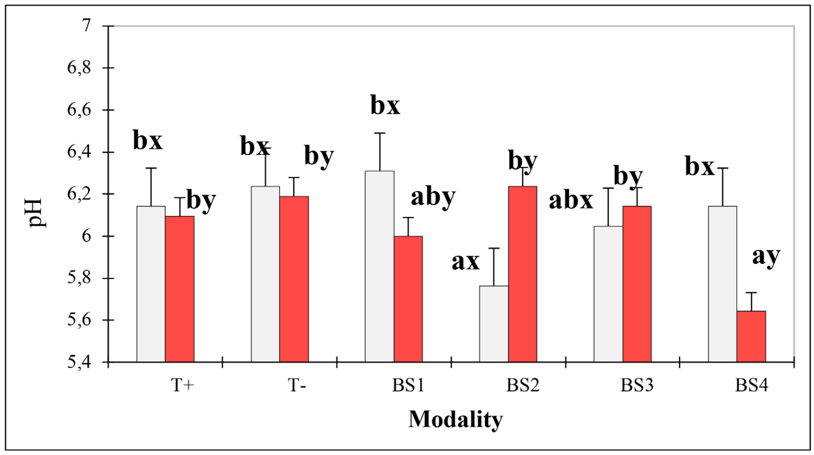
Table 2. Analysis of the variance of the evolution of the average pH and average temperature of the bean growth substrate during the experiment.

		pH		Temperature (°C)	
		$R^2 = 36\%$		$R^2 = 90\%$	
Source	df	F	*P*	F	*P*
Modality	5.000	3.173	**<0.009**	32.864	**<0.000**
Variety	1.000	1.100	0.296	0.949	0.331
Day	6.000	2.667	**<0.016**	248.701	**<0.000**
Repetition	2.000	4.247	**<0.016**	9.038	**<0.000**

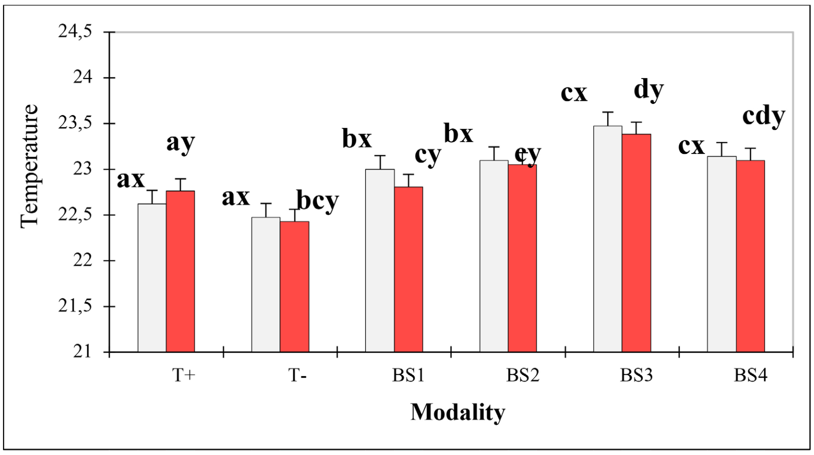
Continued

Modality*Variety	5.000	6.741	**<0.000**	0.822	0.535
Modality*Variety*Day	30.000	1.207	0.223	2.180	**<0.001**

Values in bold correspond to tests so the null hypothesis is rejected with a significant level alpha = 0.05. *DF is the degree of freedom; F is the F test value and P is the probability.*



(A)



(B)

Each bar represents the standard deviations of three repetitions for each modality and the letters (*a*, *b and c*) *represent the means between statistical groups of different modalities and between varieties* (*x and y*) *according to the ANOVA test P* ≤ 0.05).

Figure 3. Evolution of the pH and average temperature of the bean growth substrate during in the experiment in the field.

3.3. Biochemical Characterization of Bean Plant Extracts

Analysis of variance of biomarkers accumulation in bean leaves revealed that overall, the variables "modality" and/or "variety" each had a highly significant effect ($P < 0.0001$) on the content of total chlorophylls, total phenols, total proteins, polyphenol oxidases, peroxidases and phenylalanine ammonia-lyases. The coefficient of determination R^2 for all the variables was between 89% and 100%, indicating thus that the model used was reliable and reproducible (**Table 3**).

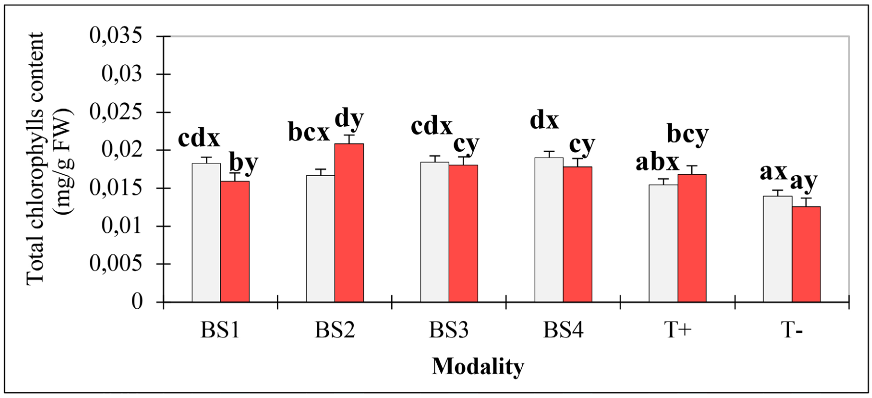
There was a highly significant effect of the interaction "modality*variety" ($P < 0.0001$) on the accumulation of total chlorophylls, total phenols and defence-related enzymes. Amongst the two variables ("modality" and "variety"), the one that most influences the accumulation of total chlorophylls, peroxidases and phenylalanine ammonia-lyases was the "modality", while the variable "variety" has the most influence on the accumulation of total phenols, total proteins and polyphenol oxidases.

Table 3. Analysis of variance of the effects of different biostimulants on the accumulation of biomarkers (total chlorophylls, total phenols, total proteins, PPO, POX and PAL) in the leaves of bean plants.

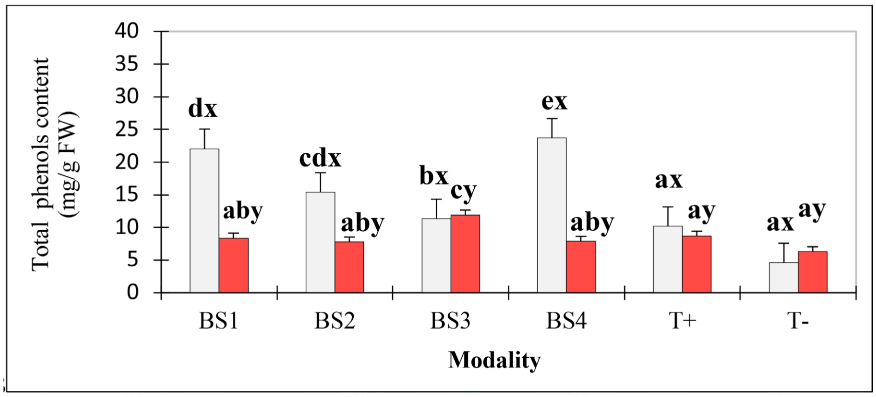
A		**Total chlorophylls (mg/g FW)**		**Total phenols (mg/g FW)**		**Total proteins (mg/g FW)**	
		R^2 = (97%)		R^2 = (100%)		R^2 = (89%)	
Source	df	F	*P*	F	*P*	F	*P*
Modality	5.000	52.425	**<0.000**	187.492	**<0.000**	5.815	<**0.000**
Variety	1.000	0.017	0.889	703.838	**<0.000**	54.111	<**0.000**
Repetition	2.000	0.3341	0.723	7.587	**<0.007**	0.645	0.542
Modality*Variety	5.000	17.537	**<0.000**	179.113	**<0.000**	1.926	0.163
Modality*Variety*Day	10.000	1.405	0.285	0.608	0.781	0.303	0.966
B		**PPO (µmol/min/ml)**		**POX (µmol/min/ml)**		**PAL (µmol/min/ml)**	
		R^2 = (99%)		R^2 = (90%)		R^2 = (94%)	
Source	df	F	*P*	F	*P*	F	*P*
Modality	5.000	382.850	<**0.000**	903.897	**<0.000**	406.507	**<0.000**
Variety	1.000	1042.904	<**0.000**	726.397	**<0.000**	544.510	**<0.000**
Repetition	2.000	2.706	0.107	0.033	0.967	0.002	0.998
Modality*Variety	5.000	150.575	<**0.000**	75.497	**<0.000**	125.280	**<0.000**
Modality*Variety*Day	10.000	1.817	0.163	0.054	1.000	0.469	0.880

Values in bold correspond to tests so the null hypothesis is rejected with a significant level alpha = 0.05. *DF is the degree of freedom*; *F is the F test value and P is the probability.*

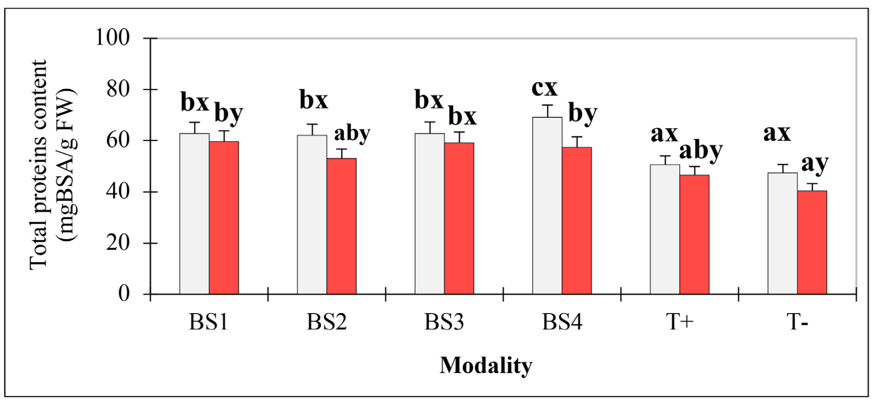
The biostimulant modalities (BS1, BS2, BS3 and BS4) improve very significantly overall the accumulation of total chlorophylls, total phenols, total proteins, polyphenol oxidases (PPO), peroxidases (POX) and phenylalanine ammonia-lyase (PAL) in plants compared to controls. The positive control modality (T+) improves the accumulation in these biomarkers compared to the negative control modality (T−) (**Figure 4** and **Figure 5**). The accumulation of these biomarkers varies from one variety to another. The best accumulation in these biomarkers was obtained with the BS4 modality for the white variety and the BS3 modality for the red variety. The total phenol content is about 5 times higher in the BS4 and BS1 modalities compared to the negative control (T−) of the white variety and 2 times higher in this modality compared to the positive control (T+) of the white variety (**Figure 4(B)**).



(A)



(B)



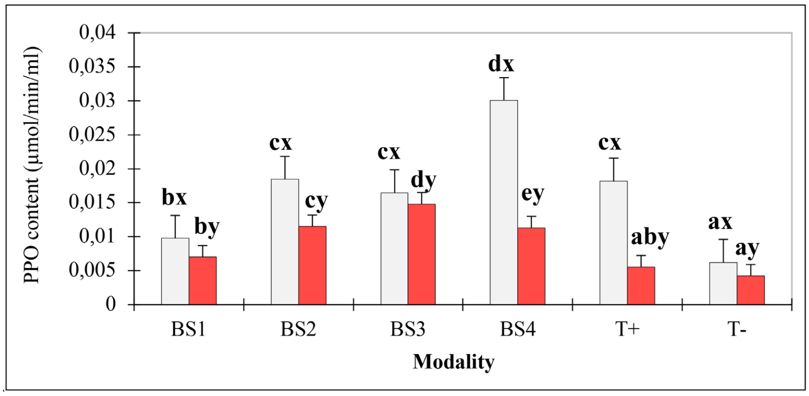
(C)

Each bar represents the standard deviations of three repetitions for each modality and the letters (a, b, et c) represent the means between statistical groups of different modalities and between varieties (x and y) according to the ANOVA test $P \leq 0.05$).

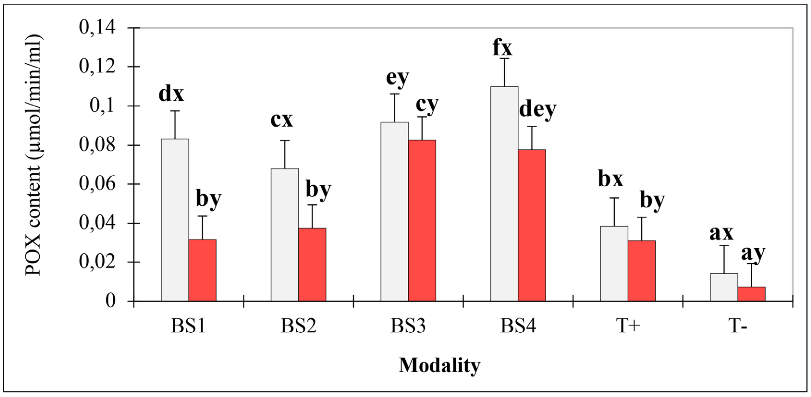
Figure 4. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of total chlorophyll (A), total phenols (B) and total proteins (C) in the leaves of three months old white bean (White) and red bean (Red) plants compared to the negative (T−) and positive (T+) controls modalities.

Overall, the PPO, POX and PAL contents were 2 to 3 times higher in the BS3 modality of the red variety compared to the controls (T+ and T−) and 3 to 4 times higher in the BS4 modality of the white variety compared to the controls

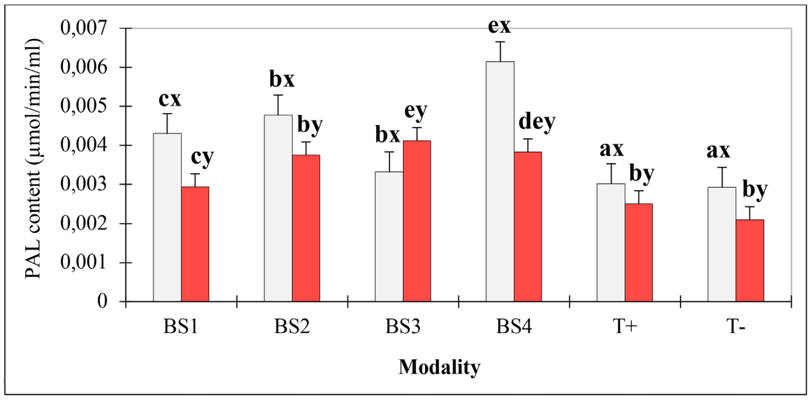
(T+ and T−) (**Figure 5(A)-(C)**). In the white bean, we can observe a better accumulation in total phenols, total proteins, PPO, POX and PAL compared to the red bean.



(A)



(B)



(C)

Each bar represents the standard deviations of three repetitions for each modality and the letters (a, b, c, d et e) represent the means between statistical groups of different modalities and between varieties (x and y) according to the ANOVA test $P \leq 0.05$.

Figure 5. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of polyphenol oxidase_PPO (D), peroxidase_POX (E) and polyphenol ammonia lyase_PAL (F) in the leaves of three months old white bean (White) and red bean (Red) compared to the negative (T−) and positive (T+) controls modalities.

3.4. Effect of Different Treatments on the Nutritional Value of Seeds

The effect of biostimulants on plant growth and seed filling was found to be very significant compared to controls. Indeed, the appearance of the seeds (size, shape and color) was different depending on the method of treatment applied during growth. The biostimulant BS2 and BS4 were those which presented the best seeds respectively for the white and red bean. The yield was also improved at the end of the culture with a better yield for the reds treated with BS4, while in the whites it was BS2 (**Table 4**).

Table 4. Total weight of red and white beans in different modalities in g after harvest.

Variety	T−	T+	BS3	BS4	BS2	BS1
Red	35.4 g	93.4 g	97.6 g	110.4 g	103.5 g	95.6g
White	52.7 g	86.3 g	91.5 g	97.7 g	105.2 g	88.3 g

The analysis of variance (ANOVA) of macronutrients, minerals, and antinutrients accumulation in bean seeds showed that the variables "modality" and "variety" each had a highly significant effect ($P < 0.0001$) on the content of dry matter, water, proteins, lipids, total sugars, iron, zinc, oxalates, phytates, tannins, and saponins. The coefficient of determination R^2 for all the variables was between 96% and 100%, indicating thus that the model used is reliable and reproducible (**Table 5**). Amongst these variables ("modality" and "variety"), the one that most influences the accumulation of macromolecules (proteins, lipids and total sugars), minerals (iron and zinc), phytates and saponins was the variable "modality", while that which most influences the accumulation of dry matter, water, oxalates and tannins was the variable "variety".

Table 5. Analysis of the variance of the effects of different biostimulants on the accumulation of dry matter, water, proteins, lipids, total sugars, iron, zinc, oxalates, phytates, tannins, and saponins in the white and red bean seeds.

		Dry matter content (g/100gFW)		Water content (g/100gFW)		Proteins content (g/100g DM)		Lipids content (g/100gDM)		Total sugars content (g/100g DM)	
		R^2 = (96%)		R^2 = (96%)		R^2 = (100%)		R^2 = (99%)		R^2 = (100%)	
Source	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Modality	5	9.286	**<0.001**	9.286	**<0.001**	1983.292	**<0.000**	162.267	**<0.000**	1983.292	**<0.000**
Variety (V)	1	219.538	**<0.000**	219.538	**<0.000**	3313.660	**<0.000**	204.308	**<0.000**	3313.660	**<0.000**
Repetition	2	0.320	0.732	0.320	0.732	2.182	**<0.003**	0.096	0.910	2.182	0.156
Modality*V	5	2.814	0.066	2.814	2.814	433.970	**<0.000**	19.920	**<0.000**	433.970	**<0.000**
Modality*V*Day	10	0.533	0.837	0.837	0.837	0.327	0.902	0.463	0.884	0.327	0.957

		Iron content (mg/100g DM)	Zn content (mg/100g DM)	Oxalates content (g/100g DM)	Phytates content (g/100g DM)	Tannins content (g/100g DM)	Saponins content (g/100g DM)
		R^2 = (100%)	R^2 = (100%)	R^2 = (100%)	R^2 = (99%)	R^2 = (100%)	R^2 = (99%)

Continued

Source	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Modality	5	2145.871	**<0.000**	719.414	**<0.000**	340.945	**<0.000**	280.290	**<0.000**	8210.839	**<0.000**	220.271	**<0.000**
Variety (V)	1	2776.663	**<0.000**	1200.491	**<0.000**	1484.087	**<0.000**	198.418	**<0.000**	216952.273	**<0.000**	434.772	**<0.000**
Repetition	2	0.022	0.978	0.379	0.692	1.934	0.187	3.493	0.064	4.685	**<0.031**	0.174	0.842
Modality*V	5	1571.638	**<0.000**	133.110	**<0.000**	85.558	**<0.000**	4.090	**<0.021**	4579.375	**<0.000**	9.490	**<0.001**
Modality*V*Day	10	0.132	0.998	0.557	0.819	0.862	0.587	0.414	0.914	1.142	0.405	2.140	0.106

Values in bold correspond to tests so the null hypothesis is rejected with a significant level alpha = 0.05. *DF is the degree of freedom*; *F is the F test value and P is the probability*.

The dry matter content in red bean seeds treated with BS3 and BS4 was higher compared to the controls (T+ and T−) and to the BS1 and BS2 treatments. However, regardless of the modality, the dry matter and water content were the same in white bean seeds. There was no significant difference between the positive control modality (T+) and the negative control modality (T−) (Table 6). Red bean presents a better dry matter accumulation compared to white bean, while water accumulation was higher in white bean compared to red bean regardless the modality.

Table 6. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of dry matter and water in bean seeds of three months old white bean (White) and red bean (Red) compare to the negative (T−) and positive (T+) controls modalities.

Variety	Content in	T−	T+	BS1	BS2	BS3	BS4
Whyte	Dry matter (g/100g FW)	92.431 ± 0.271^{ax}	92.762 ± 0.094^{ax}	92.838 ± 0.018^{ax}	92.739 ± 0.104^{ax}	92.762 ± 0.094^{ax}	92.829 ± 0.012^{ax}
Red		93.147 ± 0.094^{ay}	93.347 ± 0.094^{ay}	93.347 ± 0.094^{ay}	93.426 ± 0.011^{ay}	93.758 ± 0.094^{by}	93.833 ± 0.015^{by}
Whyte	Water (g/100gFW)	7.569 ± 0.271^{ax}	7.238 ± 0.094^{ax}	7.162 ± 0.018^{ax}	7.261 ± 0.104^{ax}	7.238 ± 0.094^{ax}	7.171 ± 0.012^{ax}
Red		6.853 ± 0.094^{by}	6.653 ± 0.094^{by}	6.653 ± 0.094^{by}	6.574 ± 0.011^{by}	6.24 ± 0.094^{ay}	6.167 ± 0.015^{ay}

The superscript values of different letters in the same line are significantly different ($P < 0.05$).

The accumulation of total proteins, lipids and sugars in bean seeds treated with biostimulants (BS1, BS2, BS3 and BS4) was greater compared to controls. The positive control modality (T+) improves the accumulation of these macromolecules more than the negative control modality (T−) (Table 7). Proteins content nearly doubled in BS3 and BS4 treated bean seeds compared to controls. While the lipids content was higher in bean seeds treated with BS1 compared to controls. The accumulation of total sugars was twice as high in the bean seeds treated with BS3 and BS4 compared to the controls (Table 7). The white bean has a higher content of total proteins and lipids in the seeds compared to the red bean. On the other hand, the total sugars content was higher in red beans compared to white beans (Table 7). Overall, BS3 and BS4 were the modalities that accumulated more macromolecules in bean seeds compared to BS1 and BS2 modalities and control modalities.

Table 7. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of macromolecules: (proteins, lipids and total sugars) in bean seeds of three months old white bean (White) and red bean (Red) compare to the negative (T−) and positive (T+) controls modalities.

Variety	Macromolecules content	T−	T+	BS1	BS2	BS3	BS4
White	Total Proteins (g/100g DM)	15.631 ± 0.257^{ax}	17.758 ± 0.270^{bx}	23.594 ± 0.243^{dx}	20.711 ± 0.163^{cx}	24.068 ± 0.129^{ex}	27.341 ± 0.140^{fx}
Red		14.267 ± 0.126^{ay}	15.488 ± 0.125^{by}	19.348 ± 0.121^{dy}	18.183 ± 0.281^{cy}	21.536 ± 0.153^{ey}	22.850 ± 0.145^{fy}
White	Total lipids (g/100g DM)	3.836 ± 0.010^{ax}	4.205 ± 0.014^{bx}	5.062 ± 0.091^{ex}	4.618 ± 0.009^{cx}	4.973 ± 0.008^{ex}	4.808 ± 0.013^{dx}
Red		3.651 ± 0.082^{ay}	4.086 ± 0.023^{by}	4.171 ± 0.053^{by}	4.280 ± 0.006^{by}	4.610 ± 0.082^{cy}	4.542 ± 0.079^{cy}
White	Total sugars (g/100g DM)	25.478 ± 0.321^{bx}	23.659 ± 0.001^{ax}	35.705 ± 0.321^{dx}	34.114 ± 0.321^{cx}	36.614 ± 0.001^{ex}	38.205 ± 0.321^{fx}
Red		27.296 ± 0.321^{ay}	30.478 ± 0.001^{by}	40.478 ± 0.321^{dy}	38.660 ± 0.001^{cy}	57.069 ± 0.557^{ey}	61.160 ± 0.964^{fy}

The superscript values of different letters in the same line are significantly different ($P < 0.05$).

Bean plants treated with biostimulants (BS1, BS2, BS3 and BS4) significantly improve minerals accumulation (iron and zinc) in bean seeds. However, the BS3 modality increases the accumulation of these minerals much more compared to the controls. The iron content in bean seeds treated with BS3 was approximately twice that of the negative control. The positive control modality (T+) improves the accumulation of these minerals more than the negative control modality (T−) (Table 8). The mineral content (iron and zinc) also varies from variety to variety. It was higher in white beans compared to red beans.

Table 8. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of Iron (A) and Zn (B) in bean seeds of three months old white bean (White) and red bean (Red) compare to the negative (T−) and positive (T+) controls modalities.

White	Mineral content	T−	T+	BS1	BS2	BS3	BS4
White	Iron (mg/100g DM)	9.457 ± 0.037^{dy}	7.887 ± 0.012^{ay}	8.327 ± 0.069^{by}	10.947 ± 0.029^{ey}	12.120 ± 0.106^{fy}	8.717 ± 0.029^{cy}
Red		10.640 ± 0.114^{cx}	10.680 ± 0.033^{cx}	13.897 ± 0.021^{dx}	7.860 ± 0.008^{ax}	14.737 ± 0.021^{ex}	9.067 ± 0.037^{bx}
White	Zn (mg/100g DM)	2.950 ± 0.049^{bcy}	3.513 ± 0.026^{dy}	3.027 ± 0.005^{cy}	2.510 ± 0.008^{ay}	3.623 ± 0.009^{ey}	2.907 ± 0.021^{by}
Red		3.007 ± 0.021^{ax}	3.667 ± 0.012^{cx}	3.553 ± 0.012^{bx}	3.067 ± 0.021^{ax}	3.727 ± 0.012^{cx}	3.673 ± 0.031^{cx}

The superscript values of different letters in the same line are significantly different ($P < 0.05$).

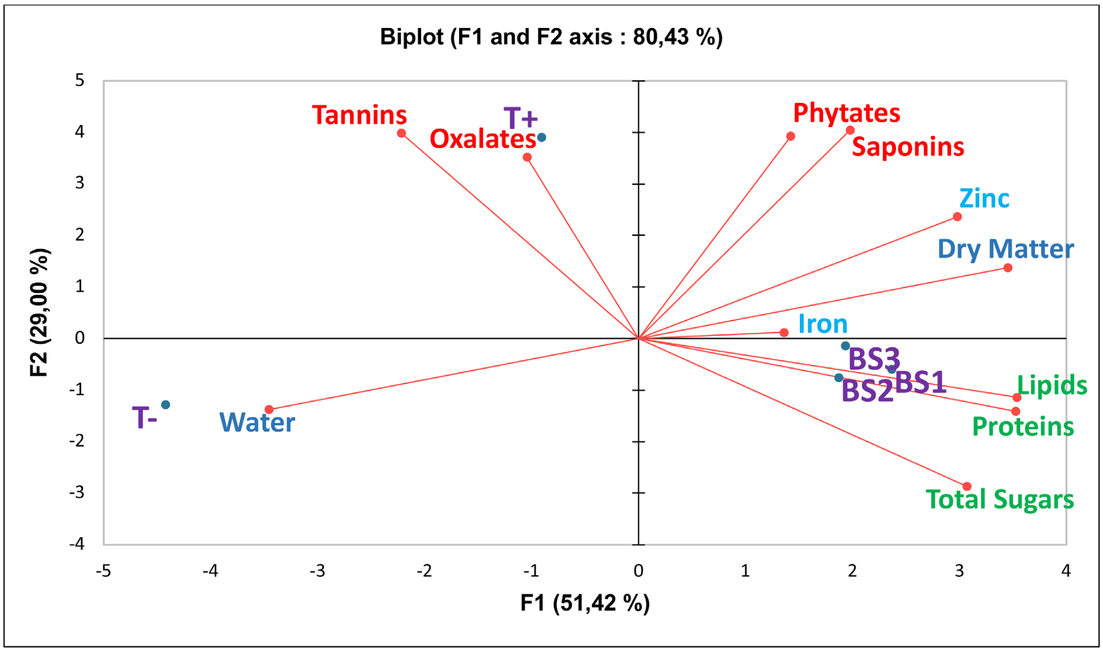
Globally, the positive control modality (T+) increases highly significantly accumulation of antinutrients (oxalates, phytates, tannins and saponins) in bean seeds compared to biostimulant modalities (BS1, BS2, BS3 and BS1). The accumulation of these antinutrients differs from variety to variety. The phytates content was approximately twice as high in the seeds of the positive control compared to the seeds of BS2, while the tannin content was three times higher in the positive control compared to BS4 and twice as high compared to BS3. The saponin content was half as high in the bean seeds treated with BS2 compared to the positive control. The positive control modality (T+) also increases highly significantly the accumulation in these antinutrients compared to the negative control modality (T−) (Table 9). The content of oxalates, tannins, and saponins was higher in red beans compared to white beans, while the phytates content was higher in white beans compared to red beans (**Table 9**). However, regardless the modality, the seeds of the positive control (T+) are those which present more accumulation in these antinutrients (oxalates, phytates, tannins and saponins).

Table 9. Effect of biostimulants (BS1, BS2, BS3 and BS4) on the accumulation of oxalates (A), phytates (B), tannins (C) et saponins (D) in bean seeds of three months old white bean (White) and red bean (Red) compare to the negative (T−) and positive (T+) controls modalities.

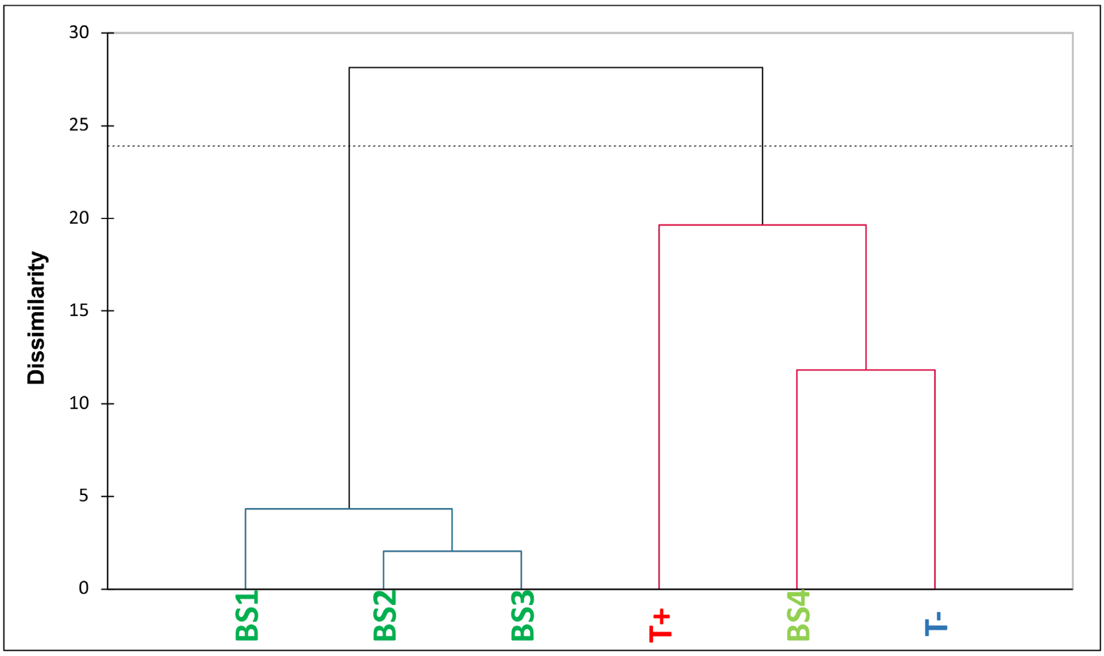
Variety	Antinutriments content	T−	T+	BS1	BS2	BS3	BS4
White	Oxalates (g/100g DM)	0.161 ± 0.000^{bx}	0.201 ± 0.001^{ex}	0.167 ± 0.003^{cx}	0.190 ± 0.001^{dx}	0.157 ± 0.000^{abx}	0.156 ± 0.000^{ax}
Red		0.188 ± 0.003^{ay}	0.205 ± 0.001^{cy}	0.197 ± 0.002^{by}	0.198 ± 0.001^{by}	0.195 ± 0.001^{by}	0.183 ± 0.000^{ay}
White	Phytates (g/100g DM)	1.616 ± 0.075^{bx}	2.669 ± 0.070^{ex}	1.853 ± 0.070^{cx}	1.463 ± 0.016^{ax}	2.253 ± 0.042^{dx}	2.197 ± 0.035^{dx}
Red		1.203 ± 0.063^{ay}	2.274 ± 0.055^{dy}	1.451 ± 0.034^{by}	1.257 ± 0.018^{ay}	1.994 ± 0.027^{cy}	2.025 ± 0.071^{cy}
White	Tannins (g/100g DM)	0.016 ± 0.001^{dx}	0.023 ± 0.001^{ex}	0.011 ± 0.000^{cx}	0.010 ± 0.000^{bcx}	0.009 ± 0.001^{abx}	0.007 ± 0.000^{ax}
Red		0.107 ± 0.001^{ey}	0.169 ± 0.000^{fy}	0.102 ± 0.001^{dy}	0.100 ± 0.000^{cy}	0.081 ± 0.001^{by}	0.057 ± 0.000^{ay}
White	Saponines (g/100 DM)	0.135 ± 0.033^{ax}	0.856 ± 0.032^{dx}	0.657 ± 0.066^{cx}	0.335 ± 0.065^{bx}	0.376 ± 0.033^{bx}	0.616 ± 0.033^{cx}
Red		0.538 ± 0.033^{ay}	1.063 ± 0.034^{dy}	1.023 ± 0.032^{cdy}	0.459 ± 0.033^{ay}	0.704 ± 0.033^{by}	0.934 ± 0.038^{cy}

The superscript values of different letters in the same line are significantly different ($P < 0.05$).

The white bean Principal component analysis (PCA) shows that the first two principal components explain respectively 51.42% and 29.00% of the total variance and define a plan allowing more than 80.43% of the variabilities to be taken into account (**Figure 6(A)**). Macromolecules (proteins, lipids and total sugars) are strongly positively correlated with each other as well as with BS1 and BS2. Iron is strongly positively correlated with BS1 and moderately with zinc and dry matter. Tannins and oxalates are strongly negatively correlated with the positive control (T+) and inversely negatively correlated with phytates and saponins. The water content is strongly negatively correlated with the negative control (T−). The positive control modality (T+) accumulates more antinutrients and the modality (BS4 and negative control T−) accumulates them less. The dissimilarity of nutrients and antinutrients of white beans in the different modalities shows that nutrients are accumulated more in BS2 and BS3 modalities, followed by BS1 (**Figure 6(B)**). The red beans PCA shows that the first two principal components explain respectively 53.26% and 26.09% of the total variance and define a plan allowing more than 79.34% of the variabilities to be taken into account (**Figure 7(A)**). There is a strong positive correlation between the contents of lipids, dry matter, proteins and total sugars as well as the BS1 and BS2 modalities. Iron is moderately positively correlated with macromolecules (lipids, proteins, total sugars) and dry matter. Tannins and oxalates are strongly negatively correlated with each other and with the positive control (T+). The water content is strongly negatively correlated with the negative control and inversely negatively correlated with the saponins, phytates and zinc contents. The control modalities (T+ and T−) accumulate them less compared to BS3 and BS4. The accumulation of antinutrients such as tannins and saponins is greater in the positive control modality. The dissimilarity of nutrients and antinutrients of red beans in the different modalities shows that nutrients are accumulated more in BS1 and BS2 modalities (Figure 7(B)).



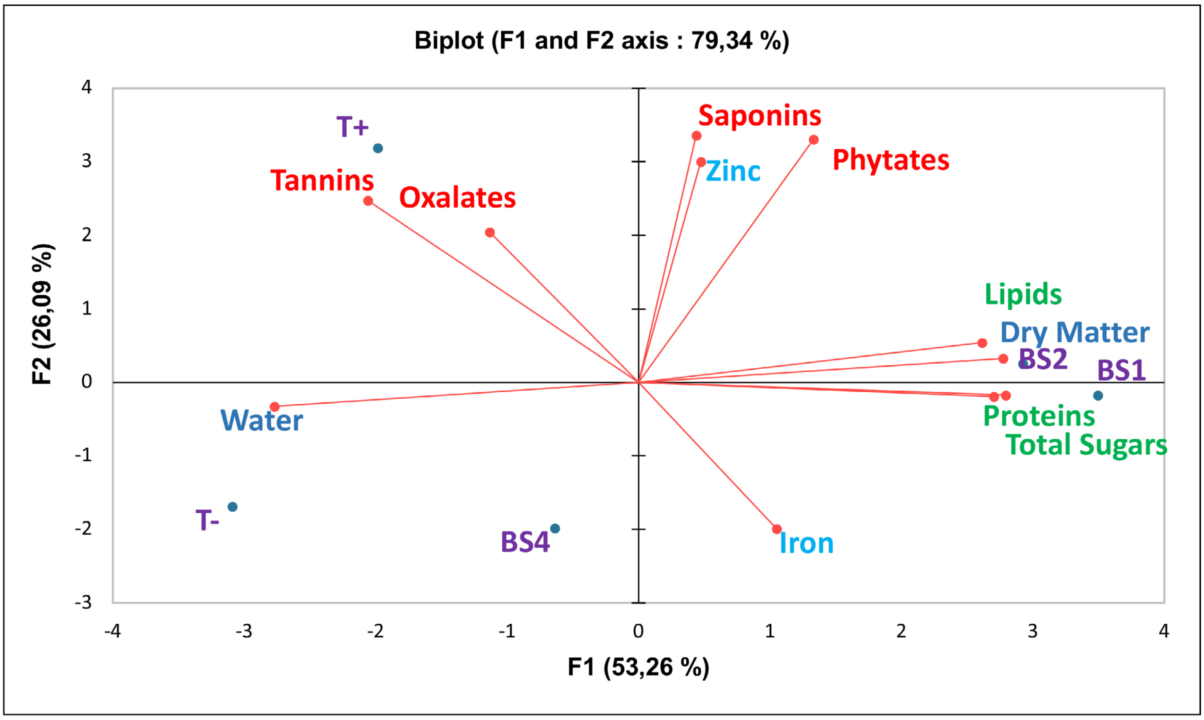
(A)



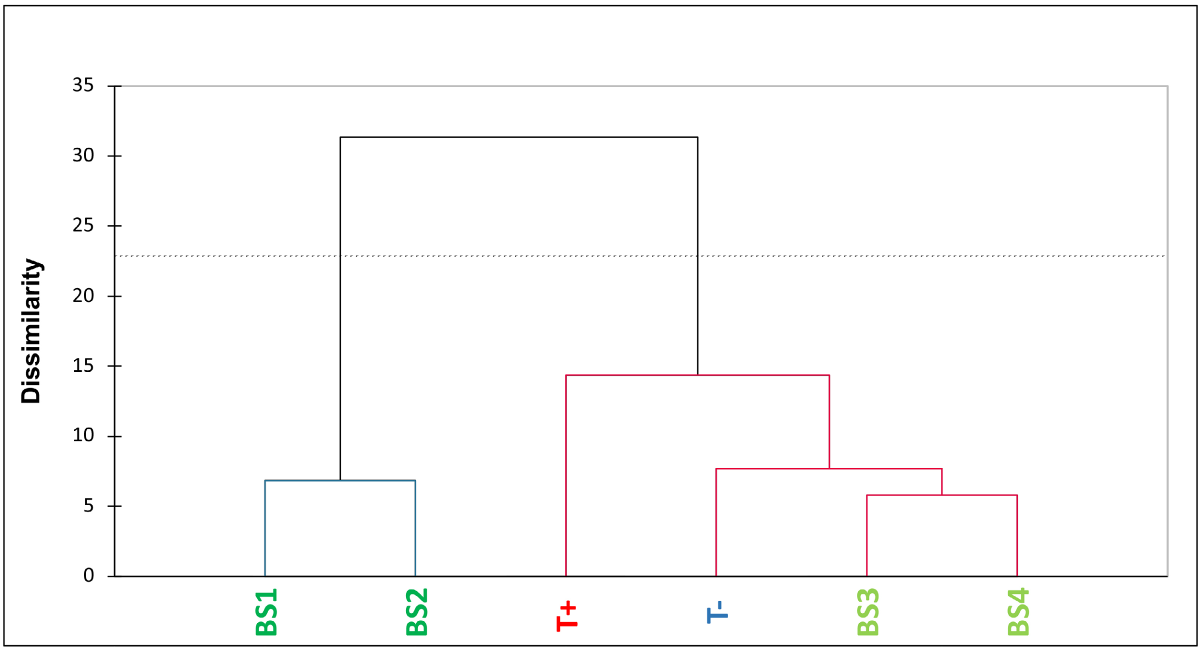
(B)

The PCA gives the positive or negative correlations, but also the strength of the correlation that exists between these variables at the 5% *threshold.*

Figure 6. Effect of biostimulants on the accumulation of macromolecules, minerals and antinutrients in the seeds of white bean treated with the different modalities: (A) Principal Component Analysis (PCA); (B) Dendrogram of distribution.



(A)



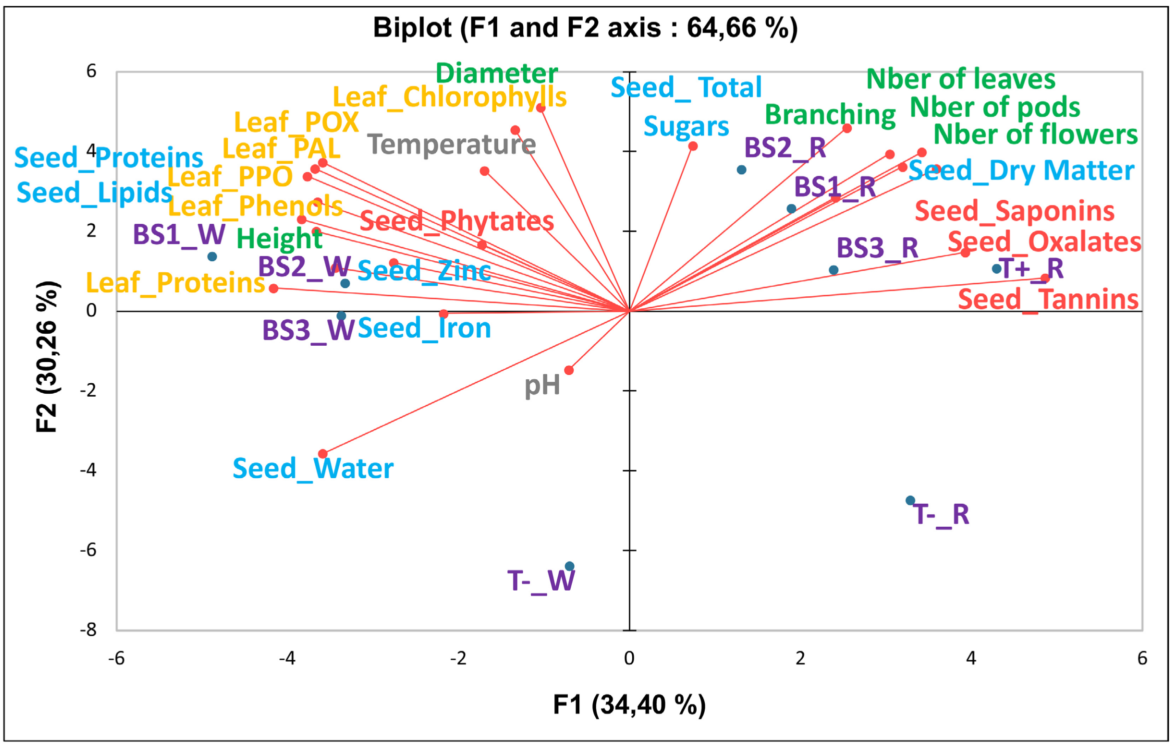
(B)

The PCA gives the positive or negative correlations, but also the strength of the correlation that exists between these variables at the 5% *threshold.*

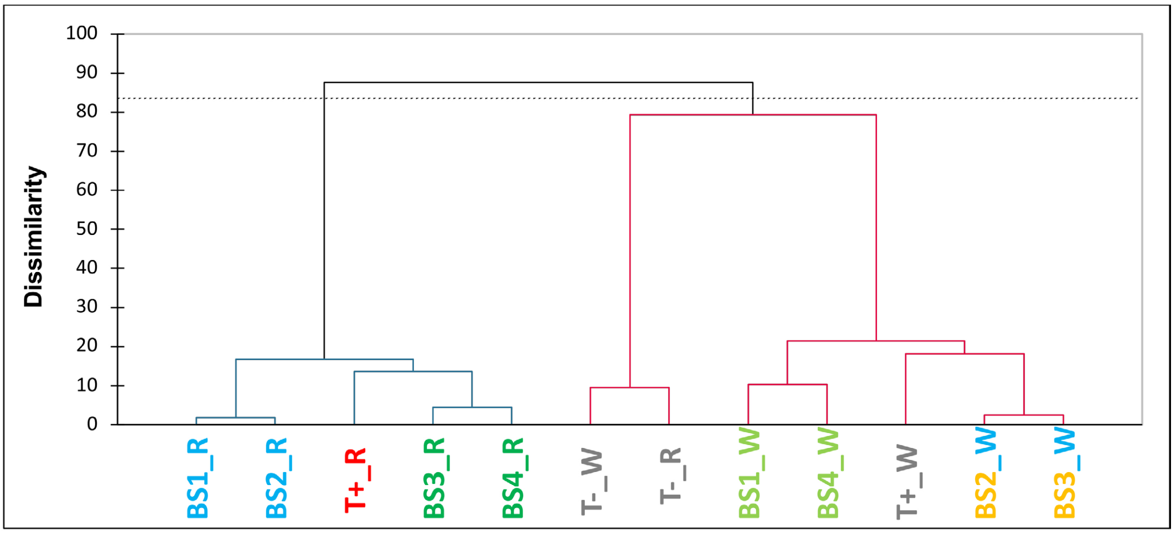
Figure 7. Effect of biostimulants on the accumulation of macromolecules, minerals and antinutrients in the seeds of red bean treated with the different modalities: (A) Principal Component Analysis (PCA); (B) Dendrogram of distribution.

Principal component analysis (PCA) of all white and red bean agromorphological responses (plant height, stem diameter, number of leaves, branches, number of flowers, number of pods, total chlorophylls, total phenols, total proteins, PPO, POX, PAL) as well as the physicochemical parameters of the substrates during plant growth (pH and temperature) and the content of macromolecules, minerals and antinutrients in the seeds of these plants revealed that there is a strong negative correlation between growth biomarkers (total chlorophylls, total phenols, total proteins) and defense biomarkers (POX, PAL, PPO) in white bean leaves (**Figure 8(A)**). Moreover, they are strongly negatively correlated with plant height, plant diameters and the content of minerals (iron and zinc), proteins, lipids, water in seeds and modalities BS1, BS2 and BS3. A strong positive correlation was observed between the contents of antinutrients (saponins, oxalates and tannins) and between agromorphological parameters (number of leaves, pods, branches, flowers). The total sugars content of the seeds is weakly positively correlated with the dry matter content and the BS2 and BS3 modalities of the white variety. Physicochemical parameters (pH and soil temperature) are weakly positively correlated with biomarkers of white bean growth and defense.

The dissimilarity of parameters (agromorphological and physicochemical), growth and defense biomarkers, as well as dry matter, water, minerals and antinutrient contents of white and red beans shows that in red beans, BS1 and BS2 are the best modalities followed by BS3, BS4 and finally the positive control (T+) which accumulates more antinutrients (**Figure 8(B)**). Whereas for white beans, BS2 and BS3 are the best modalities followed by BS1 and BS4 and finally the positive control (T+).



(A)



(B)

The PCA gives the positive or negative correlations, but also the strength of the correlation that exists between these variables at the 5% *threshold.*

Figure 8. Effect of biostimulants on agromorphological parameters as well as on the accumulation of macromolecules, minerals and antinutrients in the seeds of white and red bean treated with the different modalities: (A) Principal Component Analysis (PCA); (B) Dendrogram of distribution.

4. Discussion

The growth of bean plants in both varieties (white and red) was significantly improved by the biostimulants (BS1, BS2, BS3 and BS4) compared to the negative control. It results in an increase in agromorphological parameters such as the height of the plant, the diameter of the stem, the number of leaves, the branching, the number of flowers and pods. However, these agromorphological parameters are much more significant overall in the BS2 and BS4 modalities compared with the positive control having received the chemical fertilizer NPK. These results would be due to the stimulant action of biostimulants which have the capacity to improve the nutritional efficiency, the tolerance to abiotic stress and/or the quality characteristics of the crop, regardless its nutrient content [35]. Our results corroborate those of previous studies on the promotion of the growth of plantain PIF seeds [36].

The physicochemical characterization of the substrates (pH, temperature, insolation and relative humidity) in the field during the growth of the bean plants revealed that the different biostimulants varied significantly the pH of the soil and very significantly increased its temperature, with a low insolation (Low^-) and high relative humidity (Wet^+) of the soil. These results could be linked to the qualitative modification of soil microbial communities (new balances) resulting in an increase in their activity and the strengthening of plant-microorganism interactions [37].

The different biostimulants effectively influence the accumulation of significant amounts of total chlorophylls, total phenols, total proteins, as well as defense-related enzymes such as polyphenol oxidases, peroxidases, and phenylalanine ammonia-lyases in the leaves of plants beans compared to controls. These results could be due to the stimulating and fertilizing action provided by the constituents contained in the biostimulants. These results are in agreement with those of previous studies on the increase in the level of nutrients and defense metabolites, in particular proteins, total phenols, peroxidases and polyphenol oxidases, phenylalanine ammonia-lyases [12] [14]. The involvement of defense biomarkers has been demonstrated in the defense mechanisms of banana tissues [38]. These biomarkers seem to intervene at different levels in the physiological and biochemical processes of the plant. An increase production of chlorophylls is assimilated to a better growth of the plants because it allows thanks to photosynthesis, the production of the various basic molecules such as proteins. Proteins are part of the macroelements with various roles in plants (structural, reserve, energy). As a result, their accumulation would allow better development and better defense of plants. The pool high in total phenols is also comparable to better growth because they participate to many physiological processes such as cell growth, rhizogenesis, seed germination and fruit maturation [39] although they intervene also in the management of abiotic and biotic stress.

The different biostimulants BS3 and BS4 effectively improve the accumulation of dry matter in red bean seeds compared to controls. However, regardless of the modality, the dry matter content is the same in white bean seeds. These results could be related to nitrogen fertilization which increases the total dry biomass of plants at the fruiting stage. Indeed, when the nitrogen supply is low, the production of dry matter is lower, in particular in the leaves, which affects the production of photo-assimilates and their distribution towards the reproductive organs. Additionally, nitrogen is one of the most important nutrients for plant growth as it affects dry matter accumulation in many legume species [40].

Water content was significantly decreased in BS3 and BS4 treated red bean seeds compared to controls. However, this water content is the same in white bean seeds regardless of modality. These results could be due to the fact that, the water content varies mainly according to the variety, but also depends on the cultivation techniques, and the physiological age of the plant [41]. The low water content of the bean indicates that it is a food whose conservation can be done easily and for a long period [2].

The biostimulants (BS1, BS2, BS3 and BS4) globally significantly increase the accumulation of macromolecules (proteins, lipids and total sugars) in the bean seeds compared to the controls. This strong accumulation of macromolecules could result from a better bioavailability of the elements necessary for their synthesis in the plant thanks to the stimulating and fertilizing action provided by the constituents contained in the biostimulants [14]. These results are in agreement with those of previous studies on the increase in the nutritional value of tomato fruit [42].

White bean seeds had higher protein contents compared to red bean seeds. The biostimulants (BS1, BS2, BS3 and BS4) allow the biofortification of bean seeds compared to controls. The high protein contents obtained in our study show that beans (white and red) are a good source of proteins and would nevertheless constitute a good staple food to meet the protein needs of children and other groups of individuals. The low lipid content in beans (white and red) could be used in the management of cardiovascular diseases (obesity, overweight, high blood pressure). While the high values of total sugars would make beans an ideal energy supply food.

The different biostimulants significantly improve overall mineral accumulation (iron and zinc) in white and red bean seeds compared to controls. These results could be linked to the stimulation of microbial activity in the soil by biostimulants allowing an increase in the minerals content in the plant [37]. These results are in agreement with those of a previous study on the increase in the concentration of iron and zinc in red pepper treated with biostimulants [43].

The accumulation of antinutrients (oxalates, phytates, tannins and saponins) decreased considerably in bean seeds treated with biostimulants globally compared to the positive control. However, the phytate and saponin contents are higher in the BS1, BS3 and BS4 modalities compared to the negative control. These results could be explained by the pool high in total phenols in the plants assimilated to better growth because they participate to many physiological processes such as cell growth, rhizogenesis, seed germination and fruit maturation [39]. When consuming foods of plant origin, antinutrients interfere with the digestion of nutrients and reduce their nutritional intake, causing many adverse effects within an organism [44]. However, the reduction to low concentrations of these antinutrients improves the nutritional quality of the bean. Various processes applied to beans such as fermentation, sprouting, roasting, soaking and cooking improve the nutritional quality of beans while lowering their antinutrients content [45].

Regardless of the modality, the antinutrient contents in white and red bean seeds were below the toxicity limits although these levels were higher in the positive control bean seeds.

The oxalate levels in the seeds were well below the toxicity limit which is 2 to 5 g/day [46]. Those of phytates were also lower than the toxicity limit which is 2000 - 2600 mg/day for a vegetarian diet and 150-1400 mg/day for a mixed diet. As well as the tannin levels in the seeds below the toxicity limit which is 560 mg/day [47].

5. Conclusion

At the end of this study, it appears that these biostimulant products significantly improve the agromorphological parameters, the physicochemical properties of the soil, the accumulation of growth biomarkers and defense-related enzymes (BS2 and BS4), but also accumulation of macromolecules (total proteins, sugars and lipids), minerals (iron and zinc) and reduction of antinutrients (oxalates, phytates, tannins and saponins) in seeds (BS4). Because of this, they can be used in agriculture to increase plant biofortification, increase production at lower cost and reduce the use of synthetic products which are toxic and expensive for eco-responsible agriculture.

Conflicts of Interest

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

References

[1] Swamy, K.R.M. (2023) Origin, Domestication, Taxonomy, Botanical Description, Genetics and Cytogenetics, Genetc Diversity and Breeding of Beans (*Phaseolus vulgaris* L.). *International Journal of Current Research*, **15**, 24766-24795.

[2] Mananga, M., Noah Joseph Karrington, E., Taptue Charles, K., Ndjigoui Brice Didier, K. and Elie, F. (2022) Effect of Different Processing Methods on the Nutritional Value of Red and White Bean Cultivars (*Phaseolus vulgaris* L.). *Journal of Food and Nutrition Sciences*, **10**, 27-35. https://doi.org/10.11648/j.jfns.20221001.15

[3] Farinde, E., Olanipekum, O. and Olasupo, R. (2018) Nutritional Composition and Antinutrients Content of Raw and Processed Lima Bean (*Phaseolus lunatus*). *Annals Food Science and Technology*, **19**, 250-264.

[4] Dognon, S., Salifou, C., Dougnon, J., Dahouda, M., Scippo, M. and Youssao, A. (2018) Production, Import and Quality of Meat Consumed in Benin. *Journal of Applied Biosciences*, **124**, 12476-12487.

[5] Carbas, B., Machado, N., Oppolzer, D., Ferreira, L., Queiroz, M., Brites, C., *et al.* (2020) Nutrients, Antinutrients, Phenolic Composition, and Antioxidant Activity of Common Bean Cultivars and Their Potential for Food Applications. *Antioxidants*, **9**, Article 186. https://doi.org/10.3390/antiox9020186

[6] INS (2017) National Institute of Statistics. Cameroon Statistical Yearbook, 2017 Edition.

[7] IRAD (2013) Contribution of Research to the Improvement and Consumption of Food Legumes in Cameroon.

[8] Luan, H., Gao, W., Huang, S., Tang, J., Li, M., Zhang, H., *et al.* (2020) Substitution of Manure for Chemical Fertilizer Affects Soil Microbial Community Diversity, Structure and Function in Greenhouse Vegetable Production Systems. *PLOS ONE*, **15**, e0214041. https://doi.org/10.1371/journal.pone.0214041

[9] UNCTAD (United Nations Conference on Trade and Development) (2016) "Banana" a Commodity Profile by INFOCOMM; UNCTAD Agricultural Commodity Market Information Fund.

[10] TAAT (Technologies for African Agricultural Transformation) (2021) Common Bean Technology Toolkit Catalogue.

[11] Téné, T., Ewané, C., Effa, O. and Boudjeko, T. (2017) Effect of Chitosan and Oyster Shells on the Growth of Cocoa Plants and Resistance to Phytophthora Megakara, the Agent Responsible for Brown Rot in Cocoa Pods. *African Journal of Plant Science*, **11**, 331-340.

[12] Ewane, C.A., Ndongo, F., Ngoula, K., Tayo, P.M.T., Opiyo, S.O. and Boudjeko, T. (2019) Potential Biostimulant Effect of Clam Shells on Growth Promotion of Plantain PIF Seedlings (*var.* Big Ebanga & Batard) and Relation to Black Sigatoka Disease Susceptibility. *American Journal of Plant Sciences*, **10**, 1763-1788. https://doi.org/10.4236/ajps.2019.1010125

[13] Meshuneke, A., Ewané, C.A., Tatsegouock, R.N. and Boudjeko, T. (2020) *Tithonia diversifolia* Mulch Stimulates the Growth of Plantain PIF Seedlings and Induces a Less Susceptibility to *Mycosphaerella fijiensis* in the Nursery. *American Journal of Plant Sciences*, **11**, 672-692. https://doi.org/10.4236/ajps.2020.115050

[14] Ewané, C., Meshuneke, A., Tatsegouock, R. and Boudjeko, T. (2020) Vertical Layers of *Tithonia diversifolia* Flakes Amendment Improve Plantain Seedling Performance. *American Journal of Agricultural Research*, **5**, Article 95.

[15] Makeen, K., Suresh Babu, G., Lavanya, G.R. and Abraham, G. (2007) Studies of Chlorophyll Content by Different Methods in Black Gram (*Vigna mungo* L.). *International Journal of Agricultural Research*, **2**, 651-654. https://doi.org/10.3923/ijar.2007.651.654

[16] El Hadrami, I. and Baaziz, M. (1995) Somatic Embryogenesis and Analysis of Peroxidases *in Phoenix dactylifera* L. *Biologia Plantarum*, **37**, 197-203. https://doi.org/10.1007/bf02913210

[17] Marigot, G. (1973) Fractionation Method for Estimating Phenolic Compounds in Plants. *Analysis*, **2**, 106-110.

[18] Pirovani, C.P., Carvalho, H.A.S., Machado, R.C.R., Gomes, D.S., Alvim, F.C., Pomella, A.W.V., *et al*. (2008) Protein Extraction for Proteome Analysis from Cacao Leaves and Meristems, Organs Infected by *Moniliophthora perniciosa*, the Causal Agent of the Witches' Broom Disease. *Electrophoresis*, **29**, 2391-2401. https://doi.org/10.1002/elps.200700743

[19] Bradford, M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, **72**, 248-254. https://doi.org/10.1006/abio.1976.9999

[20] Ross, W. and Sederoff, R. (1992) Loblolly Pine Phenylalanine Ammonialyase: Enzyme Purification and Isolation of Complementary DNA Clones. *Plant Physiology*, **98**, 380-386.

[21] Baaziz, M., Aissam, F., Brakez, Z., Bendiab, K., El Hadrami, I. and Cheikh, R. (1994) Electrophoretic Patterns of Acid Soluble Proteins and Active Isoforms of Peroxidase and Polyphenoloxidase Typifying Calli and Somatic Embryos of Two Reputed Date Palm Cultivars in Morocco. *Euphytica*, **76**, 159-168. https://doi.org/10.1007/bf00022160

[22] Van Kammen, A. and Brouwer, D. (1964) Increase of Polyphenoloxidase Activity by a Local Virus Infection in Uninoculated Parts of Leaves. *Virology*, **22**, 9-14. https://doi.org/10.1016/0042-6822(64)90042-x

[23] AOAC (Association of Official Analytical Chemists) (1990) Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Edition, AOAC, 1105-1106.

[24] AFNOR (French Association for Standardization) (1984) Food Products: General Guidelines for the Determination of Nitrogen with Mineralization according to the Kjedahl Method. In: Godon, B. and Popineau Eds., *Practical Guide to Cereals*, Apria Frank, 263-266.

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

[26] FAO/WHO (1978) Human Protein Requirements: Interrelationships between Energy Intake and Nitrogen Balance in Men Consuming the Safe Level of Egg Protein.

[27] Bourely, J. (1982) Observation on the Dosage of Cottonseed Oil. *Cotton and Tropical Fibers*, **27**, 183-196.

[28] Fischer, E. and Stein, E. (1961) DNS Colorimetric Determination of Available Carbo-Hydrates in Foods. *Biochemical Preparation*, **8**, 30-37.

[29] Benton, J. and Vernon, W. (1990) Sampling, Handling and Analyzing Plant Tissue Samples. In: Westerman, R.L., Ed., *Soil Testing and Plant Analysis* (3*rd ed*), Soil Science Society of America, Inc, 784.

[30] Aina, V., Sambo, B., Zakari, A., Haruna, H., Umar, K. and Akinboboye, R. (2012) Determination of Nutritional and Antinutritional Content of Vitisvinifera (Grapes) Grown in Bomo (Area C) Zaira, Nigeria. *Advanced Journal of Food Sciences and Technology*, **4**, 225-228.

[31] Olayeye, L., Owolabi, B., Adesina, A. and Isiaka, A. (2013) Chemical Composition of Red and White Cocoyam (*Colocasia esculenta*) Leaves. *International Journal of Sciences and Research*, **11**, 121-125.

[32] Ndhlala, A.R., Kasiyamhuru, A., Mupure, C., Chitindingu, K., Benhura, M.A. and Muchuweti, M. (2007) Phenolic Composition of Flacourtia Indica, Opuntia Megacantha and Sclerocarya Birrea. *Food Chemistry*, **103**, 82-87. https://doi.org/10.1016/j.foodchem.2006.06.066

[33] Porter, L., Hrstich, L. and Chan, B. (1986) The Conversion of Procyanidins and Prodelphinidins to Cyanidin and Delphinidin. *Journal of Chemical Ecology*, **29**, 703-730.

[34] Obadoni, B.O. and Ochuko, P.O. (2002) Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Haemostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, **8**, 203-218. https://doi.org/10.4314/gjpas.v8i2.16033

[35] du Jardin, P. (2015) Plant Biostimulants: Definition, Concept, Main Categories and Regulation. *Scientia Horticulturae*, **196**, 3-14. https://doi.org/10.1016/j.scienta.2015.09.021

[36] Tatsegouock, R.N., Ewané, C.A., Meshuneke, A. and Boudjeko, T. (2020) Plantain Bananas PIF Seedlings Treatment with Liquid Extracts of *Tithonia diversifolia* Induces Resistance to Black Sigatoka Disease. *American Journal of Plant Sciences*, **11**, 653-671. https://doi.org/10.4236/ajps.2020.115049

[37] Faessel, L., Gomy, C., Nassr, N., Tostivint, C., Hipper, C. and Dechanteloup, A. (2014) Stimulation Products in Agriculture Aimed at Improving the Biological Functions of Soils and Plants. *Bio by Deloitte*, **94**, 115-144.

[38] Dhakshinamoorthy, S., Mariama, K., Elsen, A. and De Waele, D. (2014) Phenols and Lignin Are Involved in the Defence Response of Banana (Musa) Plants to Radopholus Similis Infection. *Nematology*, **16**, 565-576. https://doi.org/10.1163/15685411-00002788

[39] Lugasi, A., Hovari, J., Katalin, V. and Biro, L. (2003) The Role of Antioxidant Phyto-Nutrients in the Prevention of Diseases. *Acta Biologica Szegediensis*, **47**, 119-125.

[40] Guinet, M., Nicolardot, B., Revellin, C., Durey, V., Carlsson, G. and Voisin, A. (2018) Comparative Effect of Inorganic N on Plant Growth and N_2 Fixation of Ten Legume Crops: Towards a Better Understanding of the Differential Response among Species. *Plant and Soil*, **432**, 207-227. https://doi.org/10.1007/s11104-018-3788-1

[41] Mattila, P. and Hellström, J. (2007) Phenolic Acids in Potatoes, Vegetables, and Some of Their Products. *Journal of Food Composition and Analysis*, **20**, 152-160. https://doi.org/10.1016/j.jfca.2006.05.007

[42] Colla, G., Cardarelli, M., Bonini, P. and Rouphael, Y. (2017) Foliar Applications of Protein Hydrolysate, Plant and Seaweed Extracts Increase Yield but Differentially

Modulate Fruit Quality of Greenhouse Tomato. *HortScience*, **52**, 1214-1220. https://doi.org/10.21273/hortsci12200-17

[43] Turan, M., Ekinci, M., Yildirim, E., Güneş, A., Karagöz, K., Kotan, R., *et al*. (2014) Plant Growth-Promoting Rhizobacteria Improved Growth, Nutrient, and Hormone Content of Cabbage (*Brassica oleracea*) Seedlings. *Turkish Journal of Agriculture and Forestry*, **38**, 327-333. https://doi.org/10.3906/tar-1308-62

[44] Lo, D., HsinI, W., WanJen, W. and RayYu, Y. (2018) Anti-nutrient Components and Their Concentrations in Edible Parts in Vegetable Families. *CABI Reviews*, **13**, 1-30. https://doi.org/10.1079/pavsnnr201813015

[45] Duru, F., Ohaegbulam, P., Chukwudi, K. and Chukwu, J. (2020) Effect of Different Processing Methods on the Chemical, Functional and Phytochemical Characteristics of Velvet Beans (*Mucuna pruriens*). *International Journal of Agricultural Research and Food Production*, **5**, 55-73.

[46] Hassan, A. and Umar, K. (2004) Antinutritive Factor in African Locust Beans (*Parkia biglobosa*). *Proceedings of the* 27*th International Conference of the Chemical Society of Nigeria*, Benin City, 20-24 September 2004, 322-326.

[47] Emmanuel-Ikpeme, C., Eneji, C. and Igile, G. (2012) Nutritional and Organoleptic Properties of Wheat (*Triticum aestivum*) and Beniseed (*Sesame indicum*) Composite Flour Baked Foods. *Journal of Food Research*, **1**, 84-91. https://doi.org/10.5539/jfr.v1n3p84