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Phosphate-Solubilizing Bacteria as a Panacea to Alleviate Stress Effects of High Soil $CaCO_3$ Content in *Phaseolus vulgaris* with Special Reference to P-Releasing Enzymes

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Abstract: The present study examines the role of leguminous compost (LC), humic acids (HA), and phosphate-solubilizing bacteria (P-SB) in alleviating the stress effects of high soil $CaCO_3$ content in *Phaseolus vulgaris*. Two pot trials for two consecutive seasons; fall 2019 and summer 2020 were implemented in an open greenhouse. A mixed three-way ANOVA, two independent factors (season and soil treatments) and one within factors (time) were used with four replicates. Residual maximum likelihood (REML) analysis was used for the mixed model of the studied traits. Inoculation of calcareous soil with P-SB (a 1:1 mixture of two *Pseudomonas* sp.; *Ps. mallei* and *Ps. cepaceae*) significantly exceeded LC, HA, or even LC+HA for the positive results obtained. P-SB facilitated nutrient solubility (e.g., N, K, Fe, and Mn), including conversion of insoluble phosphorous into a form available in the tested soil due to increased soil enzymatic activities (e.g., phosphatases and phytases). This mechanism, combined with a decrease in soil calcium carbonate content and an increase in cation exchange capacity (CEC) and organic matter (OM) content, increased the availability of various nutrients to plants, including P, in the soil, which contributed to the increased plant output. Adequate P content in plants led to a marked decrease in plant acid phosphatase activity under high content of $CaCO_3$. The study concluded that the use of P-SB promotes biological activities, nutrient availability, and thus the productivity of calcareous soils, enabling *Phaseolus vulgaris* plants to withstand stress produced by high $CaCO_3$ content through the development and/or adoption of potentially effective mechanisms. Strong highly significant interactions between the treatments and time were observed using the Wald's statistics test, which indicates a positive correlation.

Keywords: P-solubilizing bacteria; calcareous soil; P fixation; compost; humic acids; REML analysis

1. Introduction

The availability of essential nutrients for plant production, especially phosphorus (P), in defective agricultural lands such as calcareous soils, is very important due to the large global extent of these lands [1]. Frequent nutrient applications are required to achieve high crop yields if effective tools are not used to address soil problems, especially nutrient fixation. Like essential nutrients, P is a prime nutrient for plant performance, but unlike

N, there is no sizable atmospheric source that helps provide it biologically [2,3]. As a base nutrient with metabolic, structural, and functional properties, P in its available state is critical to the performance of the plant [4]. A great amount of total P is insoluble in the soil, and therefore cannot be absorbed by plant roots, which leads to a deficiency that limits crop productivity globally [5,6]. The availability of P, especially in calcareous soils, is largely controlled by rates of immobilization and mineralization as biological-mediated processes [7]. Unlike N, P supply is not easily replenished, so it is necessary to preferably utilize P reserves and rectify chemically bound P [8]. Thus, it is quickly restricted into unavailable forms resulting in lower P utilization efficiency regardless of the amount applied to soil [9]. In the absence of mechanisms leading to the release of P in the soil, bio-fixation and chemical precipitation would rapidly deplete every supply of available P, except for the very little P available for plant uptake [9]. Therefore, the release of bio-fixed and insoluble forms of P, which are dependent on soil pH, are key for elevating its availability [10].

Precipitation and adsorption of soil phosphorus usually depend on the soil pH [11]. Release of insoluble and fixed forms of soil P is an important aspect of increasing its availability [9]. Wang and Nancollas (2008) [12] stated that lower soil pH values (acidic) promote the solubility of calcium-complexed P. The lowering in pH of the medium suggests the secretion of organic acids by the P-solubilizing microorganisms [13,14].

Calcareous soil contains an evident quantity of free excess of $CaCO_3$ or $MgCO_3$, more than 7% active $CaCO_3$ concerning the soil's hydraulic properties [15]. It represents the predominant type of soil in the semi-arid and arid regions, which Egypt is part of [16–18], and it is widely spread in the Mediterranean regions [16]. The large quantity of $CaCO_3$ contained in the calcareous soil causes major problems for agricultural lands [19], as it restricts the availability of P and other nutrients [20] and controls the chemistry of these soils, causing alkaline reactions. Soils with a high content of $CaCO_3$ have a high pH value (around 7.5–9.0), causing most of the nutrients to be unavailable to plants, which negatively affects the physical properties of the soil, especially water availability, and adversely affects directly or indirectly, the chemical properties including nutrient availability (e.g., macro; N, P, K, Mg, etc., and micro; Mn, Zn, Cu, Fe, etc.) [21]. These soils with high $CaCO_3$ content and high pH are less productive due to lower organic matter (OM) content and enzyme activities, as well as lower available nutrients [1]. With the continuous application of P fertilizer in these soils, P is rapidly converted into insoluble forms [10]. Hence, phosphate-solubilizing microorganisms are needed to help convert the soil P into a form available to plants [22].

When the total biota decomposes in the environment, they turn into a useful product, although it is not a base source of P, it helps to mobilize it in the soil subsurface. This product is a humic substance (HS), including humic acids (HA). HS has been mentioned to boost soil fertility and reduce the detrimental influences of synthetic chemical fertilizers, which are positively reflected in the righteousness performance of plants [23,24]. HS affects plant performance directly and indirectly [25,26]. Directly through processes connected with the uptake and transport into plant tissues [25], and indirectly through the improvement of soil structure and properties, including aggregation, aeration, water-holding capacity, permeability, and nutrient availability leading to increased soil fertility [27]. HS also affects the solubility of nutrients by chelation or building complexes [28]. They also interact with P to lessen its bio-fixation and growing its uptake with other nutrients by plant roots [29] to improve plant performance under high carbonate content conditions [30–32]. HSs have remarkable positive influences on P retention and mobility in the soil, in addition to containing many nutrients that are added to the soil when applied.

The use of compost adds widely available nutrients like N, P, and K to the calcareous soil after planting, while the pH decreases slightly [33]. It has been found that the use of organic manures and biofertilizers for calcareous soil decreased the EC value of the soil paste extract and stimulated remarkable availability of N, P, and K for plants with the application of organic conditioner [34–37].

Studies aimed at selecting bacteria capable of dissolving and mineralizing soil P have been carried out to boost the sustainable development of agriculture. This can be achieved by striving to minimize the use of chemical fertilizers and favoring the development of ecologically balanced agricultural environments [11]. Many soil microorganisms can dissolve unavailable forms of P bound to Ca by organic acids excreted through metabolic activities. These organic acids either dissolve rock phosphate or chelate Ca ions to release P into soil solution [9]. There is strong evidence that many soil bacteria can convert P into a form available to plants [2]. Since the middle of the last century and possibly earlier, phosphate-solubilizing bacteria (P-SB) have been used as bio-fertilizers [37]. P-SB plays an important role in converting insoluble P into a form more available to plants [38]. A wide range of microbial species; bacteria, fungi, actinomycetes, and even algae play a base role in solubilizing P, but bacteria are the largest use because they are most effective at dissolving P. Microorganisms secrete organic acids to solubilize P complexes [38] and/or chelate cations, which bind to P ions (PO_4^{3-}) to release P [39]. Several bacteria can solubilize phosphate, among them the *Pseudomonas* sp. [40,41], which are found in a large number of biological environments and can solubilize the metallic P complexes and release the bioavailable form of P [1]. Mechanisms by which microorganisms act to solubilize P include the release of organic acid anions, siderophores, protons, hydroxyl anions, CO_2, and extracellular enzymes or biochemical P mineralization, and release of P during substrate degradation [42]. This promotes soil fertility and increases the availability of nutrients including P, thus shortening the period of repair of low-quality soil [43]. Extensive studies have been implemented to isolate P-SB from different plant rhizospheres [44–46].

Hence, the potential use of P-SB to increase phosphorous utilization efficiency through its application as bioinoculants has attracted the interest among the scientific community engaged in P acquisition and utilization [47]. P-SB improve plant growth by supplying macronutrient phosphorus and thus are thus very beneficial. They dissolve inorganic phosphates by secreting organic acids [48–51]. P-SB are able to mobilize insoluble inorganic phosphates to the soil solution, making them available for plant uptake [2]. These organic acids enhance phosphate solubility by ionizing protons to decrease the pH and to combine PO_4^{3-} to form HPO_4^{2-} or $H_2PO_4^{-}$. Organic acid anions can also form a complex with metal cations (Ca^{2+}, Al^{3+}, and Fe^{3+}) and consequently, release PO_4^{3-}. The main mechanisms of phosphate solubilization employed by soil microorganisms include the release of mineral-dissolving complexing agents and compounds including organic acid, protons, siderophores, hydroxyl ions, and CO_2 [52].

Compared with leguminous compost (LC), humic acids (HA), and humified compost (HA-LC), very little research has investigated the impact of P-SB on nutrient recycling, especially P, after their application to calcareous soils. The present study investigates the potential positive impact of inoculating calcareous soil (19.6% $CaCO_3$) with P-SB compared to the application of the tested soil with LC, HA, or HA-LC on *Phaseolus vulgaris* plant growth, yield, nutrient contents, including P, and acid phosphatase activity. Soil physicochemical properties, including soil nutrient contents and P-solubilizing enzyme activities, were also investigated. *Phaseolus vulgaris* is a crop sensitive to different stress types [44,45], including calcareous state stress [1], so it was selected for this study.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, and Experimental Design

Two pot experiments were conducted for two consecutive seasons; fall 2019 and summer 2020. Each trial for each season took 80 days using an open greenhouse located in the experimental farm (29°17′06″ N and 30°54′55″ E), Faculty of Agriculture, Fayoum University, Egypt. Climatic conditions were 22.2 ± 3.0 °C as average daily temperature and 66.8 ± 7.5% as average relative humidity, with an average of 12/12 h for light/darkness for both growing seasons.

Based on health, color, and size, the standard Bronco seed cultivar of common beans (*Phaseolus vulgaris* L.) was secured from the Agricultural Research Center (Horticulture

Research Institute), Egypt. Sodium hypochlorite solution (1%) was used to sterilize the seed surface for 5 min. Then, distilled water was used to wash the seeds thoroughly several times to exclude the residue of the sterilization solution. After drying in the air for 1 h, the seeds were prepared for sowing using plastic pots with a diameter of 36 cm and a depth of 30 cm. A weight of 12 kg calcareous soil with 19.6% $CaCO_3$ was allocated to each pot. Based on the methods detailed in [46,53], soil chemical and physical properties were analyzed and are shown in Table 1.

Table 1. Physical and chemical properties of the experimental soil.

Soil Properties	Values
Clay (%)	50.2 ± 2.4
Silt (%)	29.6 ± 1.6
Sand (%)	20.2 ± 1.4
Soil texture	Clay
pH	8.15 ± 0.41
EC (dS m^{-1})	2.30 ± 0.14
Organic matter (%)	0.54 ± 0.03
$CaCO_3$ (%)	19.6 ± 1.5
CEC ($cmol_c$ kg^{-1})	5.82 ± 0.34
Available macro- and micronutrients (mg kg^{-1} soil)	
Available N	8.42 ± 0.51
Available P	3.41 ± 0.16
Available K	14.7 ± 0.96
Available Fe	4.71 ± 0.28
Available Mn	3.34 ± 0.19
Available Zn	2.10 ± 0.13

dS m^{-1}—decisiemens per meter, CEC—cation exchange capacity, $cmol_c$ kg^{-1}—centimole of cation exchange capacity per kilogram soil, and mg kg^{-1}—milligram per kilogram.

For the fall season 2019, five treatments each with four replicates (5 pots for each replicate) for a total of 100 pots were assigned to this study. The calcareous soil of 20 pots was left without any supplementation and identified as a control. A mixture of *Pseudomonas cepaceae* and *P. mallei* identified as phosphate-solubilizing bacteria (P-SB) was used to inoculate the soil of another 20 pots. The leguminous compost (LC; 10 g kg^{-1} soil) and humic acids (90.3% net HA; 50 mg kg^{-1} soil) were added and mixed well with the calcareous soil, for 20 pots of each. A humified compost (HA-LC) was added at a rate of 5 g kg^{-1} to the soil of the remaining 20 pots. HA-LC was prepared by adding 50 g HA to 2.5 kg LC and mixing well. Before applying the investigated treatments, the soil of each pot (12 kg) was fertilized with 1.2, 2.4, and 3.6 g of potassium sulfate; 48% K_2O, calcium superphosphate; 15% P_2O_5 and ammonium sulfate; 20% N. These treatments were repeated for summer 2020 using the same soil as fall 2019.

Using a randomized complete plot design, the experimental treatments were arranged using 20 pots with four replicates each. Rotation (from place to place) was performed daily for pots of all treatments to ensure fairness in sunlight intensity and light distribution. Ten homogeneous seeds were planted in each pot. After full emergence, only three standard seedlings per pot were maintained by successful thinning. Plants of all treatments were watered daily; plus all necessary agricultural practices were applied as recommended to produce *Phaseolus vulgaris* commercially.

At 48 days after sowing (DAS) and after harvesting, soil samples were collected randomly from 3 pots in each treatment of each growing season to assess the changes in soil properties and soil enzymatic activities. At 48 DAS, plants (n = 9) were harvested for growth evaluation; weights of fresh and dry shoot for each plant. At harvesting, green pod yield and dry seed yield were assessed in the remaining pots.

2.2. Preparation of Leguminous Compost

Green faba bean shoots (2.50 kg) were mixed with different organic materials such as bulking agents (50 g), potassium humate (100 g), and N sources such as Egyptian clover plants (1.25 kg) and cattle manure (1.25 kg). The proportions specified for the mixtures of the compost were 48% for faba bean shoots, 25% for Egyptian clover, 25% for cattle manure, and 2% for potassium humate. All these mixtures were mixed well for composting in a pilot plant using the system of turning pile in trapezoidal piles (the base dimensions were 2 × 0.75 × 0.50 m in length, width, and height, respectively). From May to September, the piles were turned every 2 weeks during the bio-oxidative phase. Moisture and temperature were monitored during the composting process. While turning the piles, the moisture level was kept in the range of 40–60% by adding water. The analysis of the obtained compost was as follows: 19.6%, 7.5, 2.1 dS m^{-1}, 115 g kg^{-1}, 33 g kg^{-1}, and 152 g kg^{-1} for organic matter content, pH, EC, N, P, and K, respectively.

2.3. Phosphate-Solubilizing Bacteria (P-SB) Isolation and Identification

Pseudomonas cepaceae and *P. mallei* were obtained with the help of Nutrient Broth medium (NB). These bacteria were isolated from the plant rhizosphere and identified molecularly in the National Research Center, Egypt. The PCR technique was implemented to identify bacteria using the following oligonucleotide primers: Target species; *P. mallei* and *P. Cepaceae*, primers; CVP 23-2 and M 23-2, 23S rDNA helices containing target position; 78ab and 78ab, pequence; 5′-CAC CGA AAC TAG CG-3′ and 5′-CAC CGA AAC TAG CA-3′, size of PCR product (bp); 526 and 526, and annealing temperature; 47 and 47 °C, respectively. The bacteria (*Ps. cepaceae* and *Ps. mallei*) were tested for their capability of P solubilization and pH reduction. They were identified as P-SB and plant growth-promoting rhizobacteria. Besides, the two bacterial isolates had no anti-activity against one another.

2.4. Preparation and Application of P-SB

A mixture of a 1:1 ratio of compost and peat has functioned as a carrier for the P-SB inoculant. Using aluminum foil, this carrier was encapsulated and sterilized with an autoclave. Then, the carrier was provided with 10% P-SB inoculant, that is, each 10 kg carrier was enriched with 1 L of inoculant. The P-SB inoculant was used or was packed and stored in a dry place until use. For P-SB treatments, calcareous soil was inoculated with bacterial inoculant at 1 g (0.1 mL net P-SB) kg^{-1} of soil 48 h before sowing.

2.5. Soil Enzyme Activity Assay

Samples of the tested soil were collected 48 DAS, as well as at harvest (the end of the experiment), and then the replications were mixed well to clean by passing through a <2 mm sieve. Assaying the phosphatase activity was performed colorimetrically based on the procedures of [54]. Besides, phytase activity was assayed in suspensions and solutions of soil against a 20 mm acidified InsP6 substrate applying the procedures of George et al. [55] and Giaveno et al. [56]. Then, the concentration of P was determined by applying the procedures of Irving and McLaughlin [57]. As P released during 1 h assaying, calculation of phytase activity was performed as nKat g^{-1} soil using the following equation:

$$\text{Phytase activity} = [\text{P conc. (mg/L)} \times \text{divide ratio} \times \text{vol. (mL)} \times 16.67] / [\text{incubation time (1 h)} \times 31] \quad (1)$$

2.6. Assessments of Soil Properties

From each treatment, soil samples were collected 48 DAS, as well as at harvest from random three pots to assess organic matter (%), $CaCO_3$ (%), cation exchange capacity, and nutrient; P, N, K, Fe, and Mn content [46,53].

2.7. Growth and Yield Determinations

Plant shoots were sampled 48 DAS for the fresh weight (n = 9), as well as for dry weight after oven-drying at 70 °C until constant weights were obtained. In the green pods marketing stage (62-70 DAS), six plants were used for picking green pods to assess pod weight (g) and total green pods per plant (g). For the dry yield, the remaining 80-day-old plants were used, the pods were picked and left for air-drying for 3 d. Next, the dry pods were used to evaluate the dry seeds' weight per plant (g).

2.8. Determination of Leaf Contents of Nutrients

Powdered dry leaf samples from all investigated treatments were used to determine nutrient contents. Total N was assessed using procedures depending on the micro-Kjeldahl technique. P was assessed colorimetrically using stannous chloride-ammonium molybdate reagent [58], after its extraction by sodium bicarbonate [59]. K^+ was assessed using a flame photometer (ELE Flame Photometer, Leighton Buzzard, UK). Fe^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} contents were determined by atomic absorption spectrophotometry [60].

2.9. Acid Phosphatase Activity Assay

To extract the enzyme, sodium acetate-acetic acid buffer at 20 mL was used to grind 1.0 g of fresh material from plant leaves and roots. The extract centrifugation was practiced for 10 min (30,000$\times$ g, 2 °C). The acid phosphatase activity was assayed in the supernatant according to Basford's procedures [61]. Assaying the activity of acid phosphatase enzyme was guided by p-nitrophenol as a standard curve according to Clark [62].

2.10. Statistical Analysis

The procedures in [63,64] were used to test the homogeneity of error variances and the normality distribution, respectively. A mixed three-way ANOVA, two independent factors (season and soil treatments) and one within factors (time) were used with four replicates. The analysis of data was performed for the mixed model using residual maximum likelihood (REML) analysis with Wald's statistics test. The difference between every two means was significant at $p \leq 0.05$ with the use of the Bonferroni adjustment correction post hoc test [65]. The analysis was implemented statistically with the help of GenStat 17th Ed. (VSN Int. Ltd., Hemel Hempstead, UK).

3. Results

3.1. Soil Characterization

The chemical and physical characterization of the investigated soil data as presented in Table 1 indicates that the textural class is clay (clay percentage exceeds 50%, silt is about 29%, and sand is about 20%), pH is more than 8.15 which indicates that the soil is alkaline, salinity is somewhat low within the range of 2.3 dS m^{-1}, organic matter (OM) content is low (about 0.54%), calcium carbonate is high and exceeds 19% which indicate that the soil is calcareous as per Leytem and Mikkelsen [12], who defined calcareous soil as containing 14–17% or more calcium carbonate content. Cation exchange capacity (CEC) is low (about 5.82 $cmol_c$ kg^{-1}). The available N, P, and K as macronutrients and Fe, Mn, and Zn as micronutrients are also included. The soil is classified following the USDA norms and standards as Typic Haplotorrerts [66].

3.2. Effects of the Different Treatments on Soil and Plant Parameters

The resulted data of the effects of the growing season, sampling time, and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on soil and plant parameters including soil enzyme activities, soil properties (available P, OM, $CaCO_3$, CEC), soil and plant nutrient contents, plant growth and yield, and acid phosphatase activity in plant leaves and roots, are summarized below.

3.3. Soil Enzymatic Activities

For the growing season, phosphatase and phytase activities were significantly increased in soil samples taken in the summer season, 2020 by 83.2 and 73.0%, respectively, compared to their activities in soil samples gathered in the fall season, 2019 (Tables 2–4, Figures 1 and 2). Regarding sampling time, soil samples collected after plant harvesting awarded significant increases of 19.5 and 18.4% for phosphatase and phytase activities, respectively, in comparison with those of soil samples gathered at 45 days after sowing. Concerning soil treatments, all the soil applications; LC, HA, HA-LC, or P-SB significantly increased phosphatase and phytase activities compared to the control. The best soil treatment was P-SB, it significantly exceeded all the other treatments (e.g., LC, HA, and HA-LC) and conferred 256.9 and 221.6% increases, respectively, compared to control. As the main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, all interactions between/among the tested factors were significant ($p \leq 0.05$).

Table 2. Main effects of the growing season, sampling time, and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on the activity of soil enzymes.

Treatments	Parameters	
	Phosphatase ($mg\ P_2O_5\ 100\ g^{-1}\ h^{-1}$)	**Phytase ($nKat\ g^{-1}$ Soil)**
Season (S)	*	*
Fall season, 2019	1.67 ± 0.11b	13.44± 0.92b
Summer season, 2020	2.03 ± 0.14a	15.52 ± 0.99a
Sampling time (ST)	**	*
At 45 days after sowing	1.69 ± 0.11b	13.28 ± 0.87b
After plant harvesting	2.01 ± 0.14a	15.69 ± 1.02a
Soil reatments (STR)	*	*
Control	0.69 ± 0.01e	6.43 ± 0.42e
LC	1.80 ± 0.11d	12.34 ± 0.24d
HA	1.89 ± 0.08c	14.74 ± 0.56c
HA-LC	2.31 ± 0.08b	18.60 ± 0.63b
P-SB	2.56 ± 0.12a	20.30 ± 0.83a

Data presented are means ± SE. Means within the same column in each studied factor followed by the different letters indicate significant differences at $p \leq 0.05$ according to Bonferroni test. * means significant at $p \leq 0.05$ and ** means significant at $p \leq 0.01$. Different small letters (a, b, c, ...) in the same column indicate a significance.

Table 3. Wald's tests for fixed effects of the sampling time (T), growing season (S), soil treatments (STR), T × S, T × STR, S × STR and T × S × STR on phosphatase, phytase, available P, OM, and $CaCO_3$.

Fixed Term	Phosphatase ($mg\ P_2O_5\ 100\ g^{-1}\ h^{-1}$)		Phytase (nK at g^{-1} Soil)		Available P ($mg\ kg^{-1}$ Soil)		OM (%)		$CaCO_3$ (%)	
	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob
Time (T)	137.27	<0.001 **	109.94	<0.001 **	4087.62	<0.001 **	0.55	0.499 ns	125.35	<0.001 **
Season (S)	174.85	<0.001 **	102.83	<0.001 **	40.57	<0.001 **	1.66	0.206 ns	0.01	0.918 ns
Soil Treatments (STR)	2182.46	<0.001 **	2290.08	<0.001 **	34,501.51	<0.001 **	81.87	<0.001 **	1078.37	<0.001 **
T × S	0.64	0.427 ns	1.65	0.206 ns	7.22	0.01 **	0.77	0.387 ns	0.1	0.751
T × STR	53.31	<0.001 **	36.04	<0.001 **	376.29	<0.001 **	2.45	0.657 ns	30.07	<0.001 **
S × STR	42.8	<0.001 **	15.89	0.009 **	19.26	0.003 **	16.16	0.008 **	4.96	0.311 ns
T × S × STR	0.37	0.984 ns	6.52	0.188 ns	113.86	<0.001 **	1.21	0.875 ns	75.68	<0.001 **

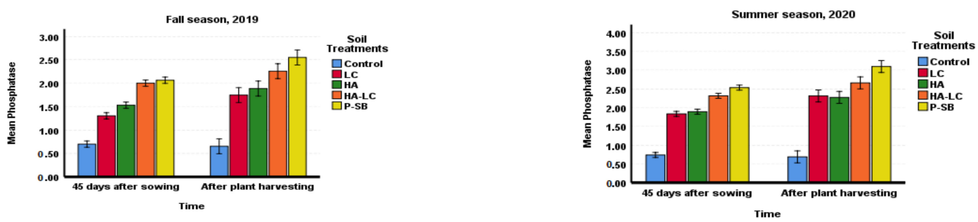
** significant at $p \leq 0.01$, * significant at $p \leq 0.05$, and ns not significant.

Table 4. Wald's tests for fixed effects of the sampling time (T), growing season (S), soil treatments (STR), T × S, T × STR, S × STR and T × S × STR on CEC, available N, available K, available Fe, and available Mn.

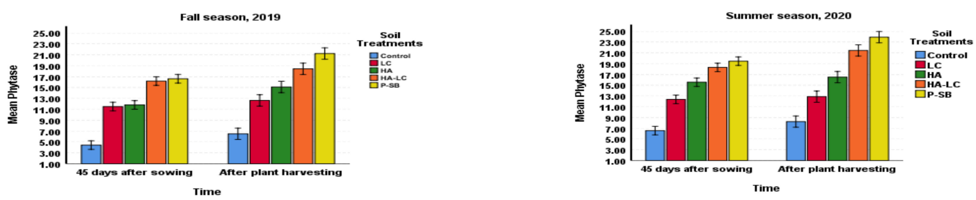
Fixed Term	CEC ($cmol_c$ kg^{-1})		Available N (mg kg^{-1} Soil)		Available K		Available Fe		Available Mn	
	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob	Walid Statistics	χ^2 Prob
Time (T)	1294.89	<0.001 **	2326.32	<0.001 **	680.91	<0.001 **	742.58	<0.001 **	3540.41	<0.001 **
Season (S)	782.11	<0.001 **	3850	<0.001 **	790.24	<0.001 **	826.81	<0.001 **	4558.12	<0.001 **
Soil Treatments (STR)	12,005.47	<0.001 **	338,970.44	<0.001 **	18,604.47	<0.001 **	14,046.5	<0.001**	18,728.89	<0.001 **
T × S	202.57	<0.001 **	0.01	0.939 ns	0.03	0.86 ns	0.55	0.464 ns	2.15	0.151 ns
T × STR	485.45	<0.001 **	78.4	<0.001 **	69.4	<0.001 **	116.61	<0.001 **	582.02	<0.001 **
S × STR	129.48	<0.001 **	60.88	<0.001 **	77.63	<0.001 **	87.67	<0.001 **	495.15	<0.001 **
T × S × STR	322.5	<0.001 **	5.96	0.226 ns	2.21	0.698	0.26	0.992ns	4.06	0.412 ns

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$, and ns not significant.

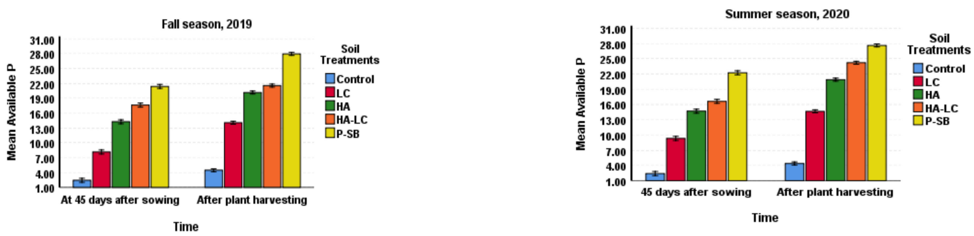
(a)



(b)



(c)



(d)

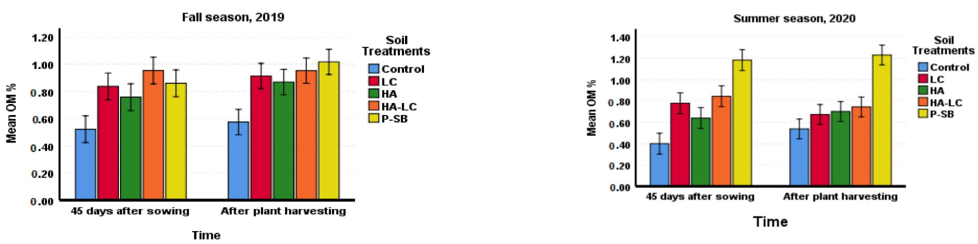


Figure 1. *Cont.*

(e)

Figure 1. Error bar showing the effects of the interaction between soil treatment and time under fall and summer seasons of (**a**) phosphatase, (**b**) phytase, (**c**) available P, (**d**) OM, and (**e**) $CaCO_3$. Blue bars indicate control values, dark red bars indicate leguminous compost treatment values, dark green bars indicate humic acid treatment values, light red bars indicate humified leguminous compost (humic acid + leguminous compost) treatment values, and dark yellow bars indicate phosphorus-solubilizing bacteria (P-SB) treatment values.

Results of the Wald's statistic to test the null hypothesis for a fixed model are presented in Table 3. Results indicated that the effects of main effects (time, season, and soil treatment), first order interaction (time × season, time × soil treatment and season × soil treatment), and second order interaction (time × season × soil treatment) were highly significant for most traits under the present study.

Results of the Wald's statistic to test the null hypothesis for a fixed model are presented in Table 4. Results indicated that the effects of main effects (time, season, and soil treatment), first order interaction (time × season, time × soil treatment and season × soil treatment), and second order interaction (time × season × soil treatment) were highly significant for most traits under the present study.

3.4. Soil Properties (Available P, OM, $CaCO_3$, and CEC)

For the growing season, the available phosphorous, organic matter, and CEC were significantly increased in soil samples collected in summer 2020 compared with those collected in fall 2019 by 122.92, 28.95, and 80.14%, respectively (Table 5). However, $CaCO_3$% decreased by 3.78%. With regards to sampling time, the available phosphorous and CEC were significantly increased in soil samples collected after plant harvesting compared with those collected at 45 days after sowing by 40.31 and 48.95% respectively.

Table 5. Main effects of the growing season, sampling time, and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on some properties (e.g., available P, organic matter (OM), calcium carbonate ($CaCO_3$), and cation exchange capacity (CEC)) of the tested calcareous soil.

Treatments	Parameters			
	Available P (mg kg^{-1} Soil)	**OM (%)**	**$CaCO_3$ (%)**	**CEC (cmol$_c$ kg^{-1})**
Season (S)				
Fall season, 2019	15.21 ± 1.44b	0.82 ± 0.04a	16.85 ± 0.36a	10.91 ± 0.67b
Summer season, 2020	15.72 ± 1.48a	0.77 ± 0.05a	16.86 ± 0.37b	12.73 ± 0.81a
Sampling time (ST)				
At 45 days after sowing	12.92 ± 1.26b	0.78 ± 0.05a	17.55 ± 0.32a	10.44 ± 0.62b
After plant harvesting	18.01 ± 1.50a	0.82 ± 0.06a	16.16 ± 0.35a	13.21 ± 0.80a
Soil treatments (STR)				
Control	3.41 ± 0.30a	0.51 ± 0.03a	19.36 ± 0.30a	5.82 ± 0.21a
LC	11.56 ± 0.86b	0.80 ± 0.04b	17.81 ± 0.23b	11.24 ± 0.43b
HA	17.51 ± 0.92c	0.74 ± 0.04b	17.04 ± 0.35c	11.21 ± 0.76b
HA-LC	10.02 ± 0.93d	0.87 ± 0.03b	15.51 ± 0.20d	14.76 ± 0.94c
P-SB	24.82 ± 0.91e	1.07 ± 0.09c	14.54 ± 0.31e	16.08 ± 0.34d

Data presented are means ± SE. Means within the same column in each studied factor followed by the different letters indicate significant differences at $p \leq 0.05$ according to Bonferroni test. Different small letters (a, b, c, ...) in the same column indicate a significance.

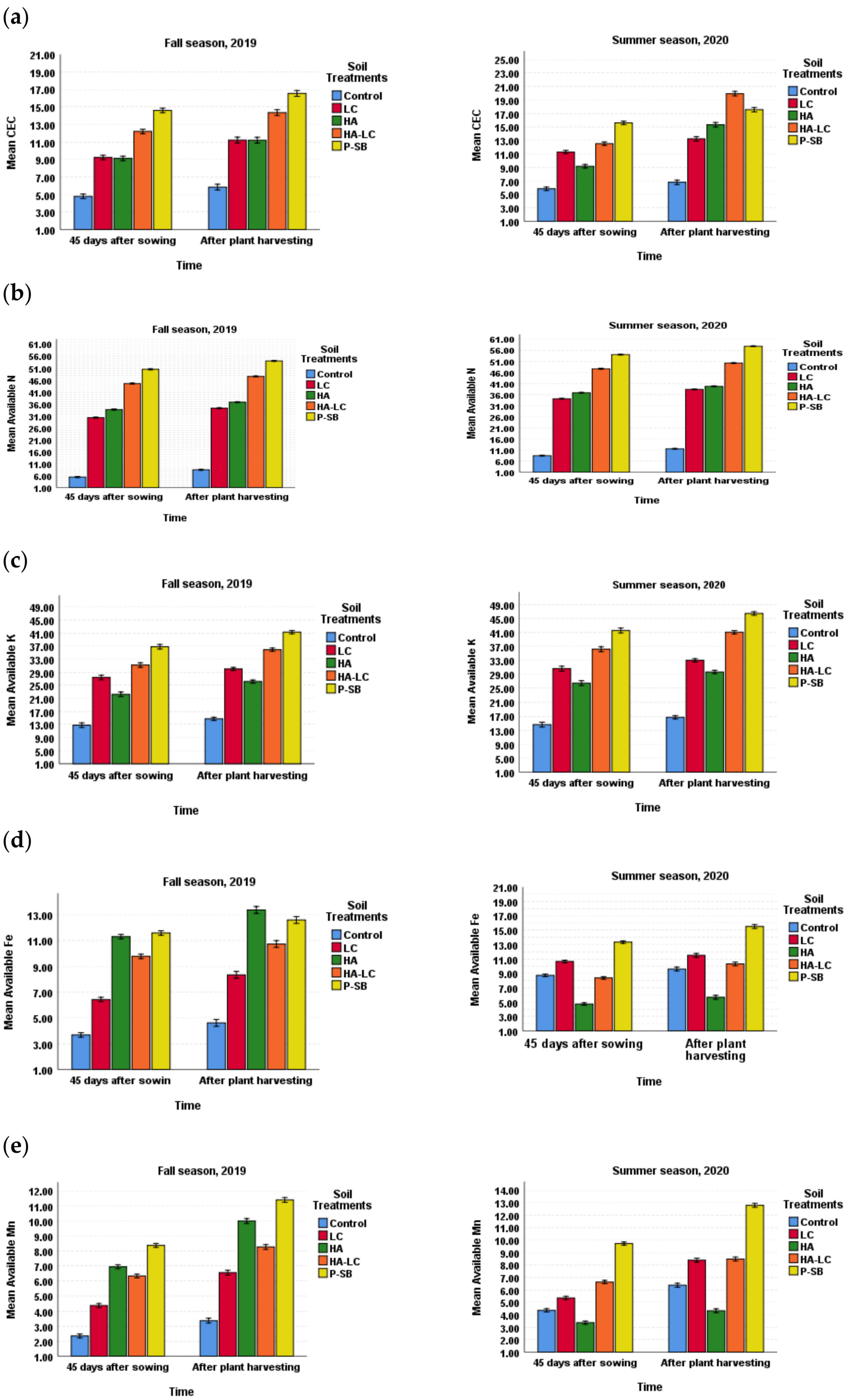


Figure 2. Error bar showing the effects of the interaction between soil treatment and time under fall and summer seasons of (**a**) CEC, (**b**) available N, (**c**) available K, (**d**) available Fe, and (**e**) available Mn. Blue bars indicate control values, dark red bars indicate leguminous compost treatment values, dark green bars indicate humic acid treatment values, light red bars indicate humified leguminous compost (humic acid + leguminous compost) treatment values, and dark yellow bars indicate phosphorus-solubilizing bacteria (P-SB) treatment values.

OM recorded a slight nonsignificant increase, 4.71%. However, $CaCO_3$ was decreased by 7.94%. Concerning soil treatments, all the soil applications including LC, HA, HA-LC, or P-SB significantly increased the available P, OM, and CEC, compared to the control. The best soil treatment was P-SB, it significantly exceeded all the other treatments (e.g., LC, HA, and HA-LC) and conferred 624.34, 88.89, and 182.82% increases for the available P, OM, and CEC respectively, compared to the control. As main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, growing season interaction with sampling time was highly significant for the available P ($p \leq 0.01$), but all other interactions between/among the tested factors were significant ($p \leq 0.05$), except for the interaction of growing season, sampling time, and soil treatments, which were not significant ($p \leq 0.05$ and $p \leq 0.01$).

3.5. Nutrient Contents (Available N, K, Fe, and Mn)

For the growing season, the available N, K, Fe, and Mn were significantly increased in soil samples collected in summer 2020 compared with the ones collected in fall 2019 by 124.15, 80.75, 80.59, and 86.32%, respectively (Table 6). With regards to sampling time, the available N, K, Fe, and Mn were significantly increased in soil samples collected after plant harvesting compared to the ones collected at 45 days after sowing by 40.57, 15.88, 18.45, and 44.6%, respectively. For soil treatments, all the soil applications including LC, HA, HA-LC, or P-SB significantly increased the available N, K, Fe, and Mn compared with the control.

Table 6. Main effects of the growing season, sampling time, and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on some nutrient contents of the tested calcareous soil.

Treatments	Parameters			
	Available N	Available K	Available Fe	Available Mn
		(mg kg^{-1} soil)		
Season (S)				
Fall season, 2019	34.51 ± 2.91b	27.87 ±1.64b	8.83 ± 0.55b	5.81 ± 0.41b
Summer season, 2020	37.84 ± 2.92a	31.64 ± 1.83a	10.27 ± 0.59a	7.97 ± 0.52a
Sampling time (ST)				
At 45 days after sowing	34.52 ± 2.91b	28.00 ± 1.65b	8.86 ± 0.55b	5.78 ± 0.40b
After plant harvesting	37.83 ± 2.92a	31.50 ± 1.83a	10.23 ± 0.59a	7.99 ± 0.52a
Soil treatments (STR)				
Control	8.46 ± 0.65a	14.73 ± 0.42a	4.68 ± 0.21a	3.36 ± 0.21a
LC	34.22 ± 0.86b	30.27 ± 0.6b	9.71 ± 0.22b	6.33 ± 0.42b
HA	36.72 ± 0.65c	26.15 ± 0.80c	8.36 ± 0.41c	6.51 ± 0.44b
HA-LC	47.44 ± 0.61d	36.11 ± 1.05d	11.59 ± 0.21d	8.38 ± 0.56d
P-SB	54.04 ± 0.78e	41.52 ± 1.30e	13.39 ± 0.45e	9.86 ± 0.62e

Data presented are means ± SE. Means within the same column in each studied factor followed by the different letters indicate significant differences at $p \leq 0.05$ according to Bonferroni test. Different small letters (a, b, c, ...) in the same column indicate a significance.

P-SB was the best soil treatment and significantly surpassed all the other treatments (e.g., LC, HA, and HA-LC) and conferred 541.33, 183, 184.93, and 195.21% increases for the available N, K, Fe, and Mn respectively, compared to the control (Table 6). As main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, all interactions between/among the tested factors were significant ($p \leq 0.05$), except for the interaction of growing season and soil treatments, which was highly significant ($p \leq 0.01$).

3.6. Growth and Yield

For the growing season, the weight of fresh and dry shoot for each plant, and the weight of green pods and dry seeds per plant increased significantly in plant samples collected in summer 2020 compared with the ones collected in fall 2019 by 25.64, 24.47, 14.22, and 17.79%, respectively (Table 7). Concerning soil treatments, all of the soil applications including LC, HA, HA-LC, or P-SB significantly increased the fresh and dry weights of plant shoots but recorded a highly significant increase for the weights of green pods and dry seeds per plant, compared with the control. P-SB recorded the best soil treatment and significantly exceeded all the other treatments (e.g., LC, HA, and HA-LC) and conferred increases of 134.01% for shoot fresh weight plant^{-1}, 158.33% for shoot dry weight plant^{-1}, 555.08% for green pods weight plant^{-1}, and 709.29% for dry seeds weight plant^{-1} compared to the control (Table 7). As the main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, all interactions between/among the tested factors were significant ($p \leq 0.05$).

Table 7. Main effects of the growing season and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on growth and yield of common bean (cv. Bronco) plants grown under calcareous soil conditions.

Treatments	Parameters			
	Growth Traits		Green and Dry Yield	
	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Green Pods Weight Plant^{-1} (G)	Dry Seeds Weight Plant^{-1} (g)
Season (S)	*	*	*	*
Fall season, 2019	23.4 ± 2.1b	3.31 ± 0.24b	40.8 ± 3.3b	9.05 ± 0.68b
Summer season, 2020	29.4 ± 2.5a	4.12 ± 0.29a	46.6 ± 3.9a	10.66 ± 0.85a
Soil treatments (STR)	*	*	**	**
Control	14.7 ± 1.3d	1.92 ± 0.14d	11.8 ± 1.0d	2.26 ± 0.21e
LC	25.8 ± 2.2c	3.59 ± 0.24c	32.3 ± 2.7c	7.09 ± 0.50d
HA	26.0 ± 2.3c	3.64 ± 0.26c	33.8 ± 2.9c	7.71 ± 0.60c
HA-LC	31.1 ± 2.7b	4.52 ± 0.33b	63.4 ± 5.4b	13.94 ± 1.04b
P-SB	34.4 ± 3.2a	4.94 ± 0.36a	77.3 ± 6.2a	18.29 ± 1.48a
		Significance		
S × STR	*	*	*	*

** significant at $p \leq 0.01$ and * significant at $p \leq 0.05$. Data presented are means ± SE (n = 9). Different letters next to mean values indicate significant differences at $p \leq 0.05$. Different small letters (a, b, c, ...) in the same column indicate a significance.

3.7. Activity of Acid Phosphatase Enzyme

For the growing season, the activity of the phosphatase enzyme of leaves and roots was significantly decreased in plant samples collected in summer 2020 compared with the ones collected in fall 2019 by 10.58 and 9.11%, respectively (Table 8). For soil treatments, all the soil applications including LC, HA, HA-LC, and P-SB markedly suppressed the activity of phosphatase enzyme of leaves and roots compared with the control. However, P-SB was the best soil treatment, and significantly surpassed all the other treatments (e.g., LC, HA, and HA-LC) and conferred decreases of 61.64% (of leaves) and 64.32% (of roots) for the phosphatase activity compared with the control (Table 6). As the main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, all interactions between/among the tested factors were significant ($p \leq 0.05$).

3.8. Leaf Macronutrient Contents

For the growing season, N, P, and K contents were markedly elevated in plant samples collected in summer 2020 compared with those collected in fall 2019 by 17.05, 16.22, and 17.73%, respectively (Table 9). Concerning soil treatments, all the soil applications including LC, HA, HA-LC, and P-SB markedly elevated N, P, and K contents compared with the control. However, P-SB was the best soil treatment and significantly surpassed all the other treatments (e.g., LC, HA, and HA-LC) and conferred increases of 87.25, 292.5, and 17.36% for the N, P, and K contents respectively, compared to the control (Table 9). As main factors showed significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, interactions between/among the growing season and soil treatments for P and K were significant ($p \leq 0.05$), but not significant for N.

Table 8. Main effects of growing season and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on the activity of acid phosphatase enzyme in leaves and roots of common bean (cv. Bronco) plants grown under calcareous soil conditions.

Treatments	Parameters	
	Phosphatase Activity in Leaves (μM P-Nitrophenol g^{-1} Leaf h^{-1})	**Phosphatase Activity in Roots (μM P-Nitrophenol g^{-1} Root h^{-1})**
Season (S)	*	*
Fall season, 2019	20.8 ± 0.68a	61.5 ± 1.3a
Summer season, 2020	18.6 ± 0.52b	55.9 ± 1.0b
Soil treatments (STR)	*	**
Control	31.8 ± 1.0a	95.0 ± 2.4a
LC	20.8 ± 0.7b	61.0 ± 1.2b
HA	19.4 ± 0.6b	56.8 ± 1.2b
HA-LC	14.4 ± 0.4c	46.9 ± 0.7c
P-SB	12.2 ± 0.4d	33.9 ± 0.5d
	Significance	
S × STR	*	*

** significant at $p \leq 0.01$ and * significant at $p \leq 0.05$. Data presented are means ± SE (n = 9). Different letters next to mean values indicate significant differences at $p \leq 0.05$. Different small letters (a, b, c, ...) in the same column indicate a significance.

Table 9. Main effects of growing season and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on leaf contents of macronutrients of common bean (cv. Bronco) plants grown under calcareous soil conditions.

Treatments	Parameters		
	N (mg g^{-1} DW)	**P (mg g^{-1} DW)**	**K (mg g^{-1} DW)**
Season (S)	*	*	*
Fall season, 2019	21.7 ± 0.5b	2.22 ± 0.11b	22.4 ± 0.5b
Summer season, 2020	25.4 ± 0.6a	2.58 ± 0.13a	26.3 ± 1.0a
Soil treatments (STR)	*	**	*
Control	14.9 ± 0.3d	0.80 ± 0.02e	17.4 ± 0.4d
LC	24.2 ± 0.5c	1.64 ± 0.12d	23.5 ± 0.6c
HA	24.7 ± 0.6c	2.45 ± 0.13c	23.7 ± 0.7c
HA-LC	26.2 ± 0.6b	2.97 ± 0.15b	26.5 ± 0.9b
P-SB	27.9 ± 0.8a	3.41 ± 0.17a	30.8 ± 1.1a
		Significance	
S × STR	Ns	*	*

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$, and ns non-significant. Data presented are means ± SE (n = 9). Different letters next to mean values indicate significant differences at $p \leq 0.05$. Different small letters (a, b, c, ...) in the same column indicate a significance.

3.9. Leaf Micro-Nutrient Contents

For the growing season, Cu, Zn, Mn, and Fe contents were markedly increased in plant samples collected in summer 2020 compared with those collected in fall 2019 by 13.19, 9.22, 9.77, and 11.11%, respectively (Table 10). For soil treatments, all the soil applications including LC, HA, HA-LC, and P-SB significantly increased the Cu, Zn, Mn, and Fe contents compared to the control. However, P-SB was the best soil treatment and significantly surpassed all the other treatments (e.g., LC, HA, and HA-LC) and conferred increases of 76.85% for Fe content, 111.03% for Mn content, 135.8% for Zn content, and 166.67% for Cu content compared to the control (Table 8). As the main factors showed either significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences, interactions between/among the growing season and soil treatments for Mn, Zn, and Cu, were significant ($p \leq 0.05$), but not significant for Fe.

Table 10. Main effects of the growing season and soil application with leguminous compost (LC), humic acids (HA), humified compost (HA-LC), or phosphate-solubilizing bacteria (P-SB) on leaf contents of micronutrients of common bean (cv. Bronco) plants grown under calcareous soil conditions.

Treatments	Parameters			
	Fe (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)
Season (S)	*	*	*	*
Fall season, 2019	288 ± 17b	217 ± 13b	133 ± 6b	90 ± 2b
Summer season, 2020	326 ± 19a	237 ± 14a	146 ± 7a	100 ± 3a
Soil treatments (STR)	*	*	*	*
Control	216 ± 12d	136 ± 7d	81 ± 2d	48 ± 1d
LC	292 ± 13c	216 ± 11c	130 ± 6c	94 ± 2c
HA	299 ± 16c	230 ± 12c	133 ± 7c	97 ± 3c
HA-LC	346 ± 22b	266 ± 17b	163 ± 9b	111 ± 4b
P-SB	382 ± 28a	287 ± 19a	191 ± 11a	128 ± 5a
		Significance		
S × STR	ns	*	*	*

** significant at $p \leq 0.01$, * significant at $p \leq 0.05$, and ns non-significant. Data presented are means ± SE (n = 9). Different letters next to mean values indicate significant differences at $p \leq 0.05$. Different small letters (a, b, c, ...) in the same column indicate a significance.

4. Discussion

There is an ongoing problem related to nutrients, especially P with calcareous soils [1]. The calcareous soil tested in the current study has undesirable properties, poor structure, low fertility, and nutritional imbalance. It also has a high $CaCO_3$ content and a high pH value, along with a low cation exchange capacity (CEC) and organic matter (OM) content, thus low available nutrient contents (Table 1). These unwanted characteristics always accompany less productive or unproductive soils [1,67]. Thus, *Phaseolus vulgaris*, as a crop sensitive to various stressors, becomes an unproductive crop when grown in such soils [44,45], including high $CaCO_3$ content [1]. Thus, effective tools must be applied to reform the harsh conditions of the soil tested in this study and make insoluble nutrients (including P) soluble, and available to plants.

The research strategy pursued in this study is to use four tools (e.g., humic acids; HA, leguminous compost; LC, humified compost; HA+LC, and phosphate-solubilizing bacteria; P-SB) to apply them to the tested calcareous soil (19.6% $CaCO_3$). They all succeeded in releasing the nutrients, especially P, to be available for uptake by the plant, but the treatment of inoculating the soil with P-SB was the best.

By adding OM such as HA or compost to defective (calcareous) soil, it tends to repair the soil [17,67,68] by improving its physical (e.g., soil water retention capacity, rate of infiltration, and particle aggregation), chemical (e.g., nutrients, $CaCO_3$, EC_e, pH, CEC, and OM), and biological (e.g., microorganisms) characteristics [16]. Many characteristics (e.g., nutrients (P, N, K, Fe, and Mn), CEC, OM, $CaCO_3$, and enzyme (phosphatase and phytase) activities) that were tested in this study were markedly improved with HA or LC application to the soil compared to those obtained with the control (Tables 2–6 and 11). As P solubilization has direct correlation with the pH of the medium [2], the production of P-SB results in a decrease in soil pH, which plays an important positive role in P solubilization [9]. These positive soil outcomes contributed to a marked decrease in leaf and root acid phosphatase activity (due to the increase in P content that meets the need of the plant), and a considerable increase in *Phaseolus vulgaris* plant growth, nutrient contents (e.g., N, K, Fe, Mn, Zn, and Cu), especially P and green pods and dry seed yields (Tables 4–8 and 11).

Table 11. Changes (%) in soil characteristics and plant performance in two seasons (fall, 2019 and summer, 2020) relative to the control in *Phaseolus vulgaris* plants under high $CaCO_3$ stress and soil treatments with LC, HA, LC-HA, and P-SB. Three color scale heatmap, yellow as the midpoint of control and parameters with insignificant values compared to control, red for changes below control values, and green for changes over control values.

Parameters	Control	Soil Treatments				Season		Sampling Date	
		LC	HA	LC-HA	P-SB	F-2019	S-2020	45 DAS	APH
Soil phosphatase act.	d	+150.0c	+163.9c	+218.1b	+256.9a	+81.9b	+233.3a	+134.7b	+180.6a
Soil phytase act.	e	+101.4d	+136.3c	+192.4b	+221.6a	+68.7b	+191.9a	+110.9b	+149.8a
Soil P content	e	+24.0d	+395.6c	+463.0b	+624.3a	+181.5b	+527.6a	+278.3b	+430.8a
Soil OM content	d	+59.3c	+48.1c	+70.4b	+88.9a	+40.7b	+81.5a	+57.4a	+64.8a
Soil $CaCO_3$ content	a	−9.7b	−12.2c	−20.9d	−25.5e	−5.6a	−9.2a	−3.6a	−11.2b
Soil CEC	d	+91.4c	+92.8c	+145.4b	+182.8a	+45.4b	+161.9a	+63.6b	+143.6a
Soil N content	d	+305.0c	+335.9c	+462.9b	+541.3a	+180.3b	+528.3a	+277.7b	+430.9a
Soil K content	e	+104.8c	+78.9d	+144.9b	+183.0a	+44.9b	+161.9a	+88.4b	+118.4a
Soil Fe content	e	+106.4c	+77.9d	+145.4b	+184.9a	+45.4b	+161.8a	+86.4b	+120.8a
Soil Mn content	d	+91.3c	+95.8c	+151.5b	+195.2a	+42.2b	+165.0a	+66.5b	+140.7a
Shoot fresh weight	d	+75.5c	+76.9c	+111.6b	+134.0a	+59.2b	+100.0a		
Shoot dry weight	d	+87.0c	+89.6c	+135.4b	+157.3a	+72.4b	+114.6a		
Pods weight plant^{-1}	d	+173.7c	+186.4c	+437.3b	+555.1a	+245.8b	+294.9a		
Seeds weight plant^{-1}	e	+213.7d	+241.2c	+516.8b	+709.3a	+300.4b	+371.7a		
Leaf phosphatase act.	a	−34.6b	−39.0b	−54.7c	−61.6d	−34.6a	−41.5b		
Root phosphatase act.	a	−35.8b	−40.2b	−50.6c	−64.3d	−35.3a	−41.2b		
Leaf N content	d	+62.4c	+65.8c	+75.8b	+87.2a	+45.6b	+70.5a		
Leaf P content	e	+105.0d	+206.3c	+271.3b	+326.3a	+177.5b	+222.5a		
Leaf K content	d	+35.1c	+36.2c	+52.3b	+77.0a	+28.7b	+51.1a		
Leaf Fe content	d	+35.2c	+38.4c	+60.2b	+76.9a	+33.3b	+50.9a		
Leaf Mn content	d	+58.8c	+69.1c	+95.6b	+111.0a	+59.6b	+74.3a		
Leaf Zn content	d	+60.5c	+64.2c	+101.2b	+135.8a	+64.2b	+80.2a		
Leaf Cu content	d	+95.8c	+102.1c	+131.3b	+166.7a	+87.5b	+108.3a		

LC—leguminous compost, HA—humic acids, LC-HA—humified leguminous compost, F-2019—fall season 2019, S-2020—summer season 2020, DAS—days after sowing, APH—after plant harvesting, act—activity, $CaCO_3$—calcium carbonate, OM—organic matter, and CEC—cation exchange capacity. The green color indicates the treatment-induced increase above the control level, where the steady increase in green concentration indicates a steady increase due to treatment compared to control. The red color indicates the treatment-induced decrease compared to the control level, where the steady decrease in red concentration towards purple to light purple (pink) indicates a steady decrease due to treatment compared to control. The yellow color indicates the control values, while the steady decrease in the yellow concentration indicates not significant decreases in contrast to the decreases highlighted by red color.

Increased yields under the stress of high soil $CaCO_3$ content may be due to the beneficial influence of HA on ameliorating growth and activation of biochemical processes (e.g., photosynthesis, chlorophyll content, and respiration) of plants [69], which contribute to all yields of *Phaseolus vulgaris* plants (Tables 3–7 and 11). These positive findings obtained with HA on defective soil are in parallel with those in [13,17]. Brady and Weil [13] stated, in general, that humus as colloid containing cations of the essential nutrients in a readily exchangeable form exemplifies 50–90% of the capacity to uptake cations in the mineral topsoil. Seyedbagheri [70], using calcareous soils, stated that HA improves the organic-clay complexes' reactions, which contribute to the formation of stable humus that ameliorates the physical, chemical, and biological functions of these soils. In the soil, HA helps cover clay domains with various active organic acids that have been liberated from HA. Then, these clay domains form coarse aqueous-stable aggregates segregated by a coarse pore procedure, which increases the permeability of the soil thus helping to easily leach the excess soluble salts to diminish the ECe value [17].

HA can increase soil biological activity (beneficial bacteria), which can efficiently contribute to restoring calcareous soils. The increase in bacterial activities by HA leads to produce certain organic acids and plant hormones (e.g., cytokinins and indole acetic acid). The hormones induce the roots and root hair proliferation to raise nutrient-absorbing surfaces. Additionally, the organic acids solubilize organic and inorganic forms of beneficial elements (especially P), thus increasing plant growth and different yields [17,71]. The perceived increase in the nutrient contents available in calcareous soil tested in this study with HA application may be due to the observed increase in soil CEC, $CaCO_3$, and OM contents, as well as enzyme (phosphatase and phytase) activities that help increase available P (Tables 2–5 and 11). When added to the calcareous soil, HA improves soil biology conditions, which encourage easy release and mobility of nutrients into the soil in forms more available to plants [72]. Belal et al. [17] attributed the improvement of biological activity of the calcareous soil with HA treatment to bioactive substances released to promote the nutrient solubility in the soil from both its native and additive sources and to keep these solubilized nutrients in forms more available to plants. The promoted impact of HA on phytonutrient contents (Tables 3–6 and 11) may be attributed to improved root system development [73] and boosted cell plasma membrane permeability [74]. The effect of a greater improvement of smaller molecular sizes of HA on uptake of plant nitrates [75] comes from their transfer into the cell plasma membrane, where they efficiently affect nutrient assimilations [76]. Khaled and Fawy [77] also reported that HA may interact with the structures of phospholipids in cell plasma membranes as a nutrient carrier, demonstrating anti-stress impacts under different conditions of abiotic stresses [78], such as the high $CaCO_3$ content (19.6%) under study.

Similar to our findings (Tables 3–6 and 9), Aboukila et al. [67] and Manirakiza and Şeker [68] indicated that calcareous soils treated with compost display a marked rise in the OM and nutrients; Zn, Mn, Fe, and K contents due to the compost's high content of these OM and nutrients, which are subsequently released into the soil through bacterial decomposition [79]. Manirakiza and Şeker [68] reported increased soil nutrient and OM contents, which they attributed to the richness of the compost in nutrients and organic carbon. Ghosh et al. [80] and Naeem et al. [81] showed a rise in the soil contents of OM and N compounds after composting because of the compost's richness in organic carbon and N and the acceleration of ammonification and nitrification rates after excretion of exudates from plant roots. The content of N compounds has also been reported to increase after adding compost to reduce leaching [82]. Like our data (Tables 2–11), the available P increases significantly after adding compost to the soil. The compost's richness in available P that is liberated from the compost into the soil through a process called "mineralization" could explain this finding [67,68]. Additionally, the sorption of Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, and K^+, especially acidic cations (Fe^{3+} and Al^{3+}), after adding the compost increases the available P in the soil solution [83]. This study presents an increase in available P attributable to low $CaCO_3$ content, and high OM content, CEC, and phosphatase and phytase activities in soil, which helped increase the solubility of P along with other nutrients after LC addition (Tables 2–11).

A synergistic affirmative influence on nutrient contents of plants has been reported after adding compost to soil [84]. Our findings (Tables 2–7 and 11) are supported by Manirakiza and Şeker [68] who reported enhanced plant growth traits due to soil treatment with compost, which can be explained by improved soil structure, fertility, and water retention after the release of nutrients from applied compost, and the synergism among nutrients and increase in their retention [85]. This study presents a higher pH (8.15 $\pm$ 0.41; Table 1) of the tested calcareous soil which falls outside the recommended range for optimal nutrient availability, and thus the nutrient availability for plants is very low [86]. However, in this study, compost use increased nutrient availability and uptake, which increased the nutrient contents (e.g., Cu, Zn, Mn, Fe, K, and N), especially P, in the plant (Tables 3–6 and 11) and was positively reflected in the *Phaseolus vulgaris* growth and yield components (Table 7). The findings of Manirakiza and Şeker [68] and Doan et al. [87] are similar to ours. As demonstrated by this study (Tables 3–6 and 11), the use of compost resulted in a marked rise in plant content of P and other nutrients (Cu, Zn, Mn, Fe, K, and N), which is due to improved soil fertility [68]. In calcareous soils, the significant binding of Ca-P decreased P availability and uptake, and thus decreased the P content in plants as demonstrated with the control in this study (Tables 3–7 and 9). However, the use of compost significantly increased the availability and uptake of P and P content and other nutrient contents in plants, which may be due to improved soil fertility (Tables 2–11). Jones and Jacobsen [86] indicated that the capacity of nutrient uptake depends on the density of the root system and the nutrient content in the soil solution. In calcareous soils, P is presented as a critical factor, like other essential nutrients, for plant performance. Compost increases the uptake of nutrients (Mn, Cu, Zn, Fe, Ca, K, P, and N) by crop plants grown in calcareous soil, and indicates that nutrient solubility is likely attributable to plant root-secreted organic compounds, which promote the availability of nutrients to plants [88,89].

Application of calcareous soil with HA+LC significantly exceeded both HA and LC applied alone for the investigated soil properties, growth and different yields of common bean plants and the plant content of different nutrients, especially P (Tables 2–11). These significant findings from HA+LC treatment compared to HA and LC separately applied are attributed to the synergistic and positive integrative effects of both HA and LC as elucidated above.

The treatment of soil inoculation with phosphate-solubilizing bacteria (P-SB; *Pseudomonas cepaceae* and *Pseudomonas mallei*) significantly exceeded all other treatments (HA, LC, and HA+LC) for the examined soil properties, growth, and different yields of *Phaseolus vulgaris* plants and the plant content of different nutrients, especially P (Tables 2–11).

In this study, P release in favor of plant roots could easily be achieved by inoculating the tested calcareous soil by P-SB, which effectively increased soil phytase and phosphatase activities, CEC, OM, available nutrients, and greatly reduced the soil pH value and $CaCO_3$ content. Thus, P-SB can make unwanted calcareous soils productive.

In the calcareous soil tested in this study, P-SB (a mixture from *Pseudomonas cepaceae* and *Ps. mallei*) simplified the conversion of insoluble P to be available to *Phaseolus vulgaris* plants, a mechanism that contributed to the increased P content in the plant, which in turn contributed to increasing plant productivity (Tables 4–7 and 11). The findings of Rady et al. [1] and Shi et al. [40] confirm the results of this study. This enhanced effect of P-SB strains was due to their effective phospholysis (P release) ability through the increased phytase and phosphatase enzyme activities in the soil as an efficient mechanism, resulting in increased availability of P to plant roots (Tables 4 and 11). The data of this study indicate that inoculation of calcareous soil with P-SB is a key determinant of its fertility. This positive finding can be elucidated based on higher available nutrients, including P, and OM, as well as lower $CaCO_3$ content obtained by P-SB treatment (Tables 5 and 11). These positive results were reflected in higher growth and different yield components of common bean plants (Tables 5 and 11).

Synergistically, *Pseudomonas* sp. work on the production of phosphatases (Tables 2–4 and 11) by some processes (e.g., immobilization and mineralization) to convert organic P into inorganic form throughout the plant life cycle, so that *Pseudomonas* sp. growth can be optimized continuously [90]. As another effective mechanism, various organic acids are both qualitatively and quantitatively secreted, mainly as a gene dependent, in soil by P-SB strains [1,91]. These organic acids compete with P ions for P adsorption sites, resulting in higher P release in favor of plants. P-SB enhance the calcareous soil productivity and increase its capacity for microorganisms, phytase, and phosphatase enzyme activities (biological activity), and nutrient contents including available P (biochemical activity) in this soil (Table 2, Table 3, Table 4, Table 5 and Table 11).

Alori et al. [92] reported some other conceivable mechanisms for P solubilization in calcareous soil including proton release after NH_4 assimilation by microbial cells, production of H_2SO_4 and HNO_3 (inorganic acids), and specific enzymes (Tables 2–4 and 11), which act on amphiphilic fatty substances. Along with the microbial solubility of P, microorganisms also mineralize the organo-P, playing a major role in cycling the P to be available to plants. Alori et al. [92] added that P-releasing enzymes (phytases and phosphatases) produced by P-SB broadly control the mineralization of P. Besides, other features deserve agricultural attention such as the production of plant hormones and antifungal compounds, and regulation of the main pathways included in plant metabolism to enhance the ability of plants to withstand environmental stresses [93].

The increased availability of nutrients, including P, through P-SB application to the soil enhanced the performance and nutrient content (including P) of *Phaseolus vulgaris* plants. This allowed *Phaseolus vulgaris* to possess the advantage of staying green (data are not shown), increasing the seed filling period under stress. This finding is obtained due to the plant's ability to efficiently uptake nutrients from calcareous soil (Tables 6 and 11). This allows plants to fulfill meristematic activities including cell expansions due to adequate provision of water against stress resulting from the increase in soil $CaCO_3$ content under study. The worthy increase in the content of K^+ ion (Tables 6 and 9) acted in its ionic state as a powerful osmoprotectant. Recently, Rady et al. [1] reported that increased solubilized P in calcareous soil due to inoculation with P-SB (Tables 2–4 and 11) is reflected positively in the P content of *Phaseolus vulgaris* plants (Tables 7 and 11). This report [1] added that the plants' high nutritional content-enabled them to have a potent antioxidant defense system against the harsh conditions of a high $CaCO_3$ state.

In this study, inoculation of calcareous soil with P-SB helps to provide plants with enough P, decreasing root, and leaf acid phosphatase activity (Tables 2–4 and 11). This finding can be attributed to the P content that was reached to meet plant needs. The findings of Rady et al. [1] confirm the findings of this study, indicating that increased plant content of P induces decreases in acid phosphatase activities in common bean leaves and roots. The authors attributed this finding to that when the soil contains sufficient available P (with P-SB) for uptake by plants, it restricts acid phosphatase activity in the plant and increases P mineralization in the soil. Additionally, phosphatase activity in a plant root system tends to increase along with a decrease in shoot P content, and under P deficiency, the activity of root and shoot phosphatase increases [94]. Eligible plants, with sufficient P through the application of P-SB, have several potential mechanisms to be developed and/or adopted to boost their tolerance to stress induced by high $CaCO_3$ content. For instance, the high plant K^+ ion content confers an osmoprotectant mechanism against water loss to keep sufficient leaf water content to help the plant perform well under the harsh conditions of high $CaCO_3$ stress.

The results obtained in the summer season of 2020 significantly exceeded the results obtained in the fall season of 2019 in terms of soil properties, growth and yields of *Phaseolus vulgaris* plants, and plant content of different nutrients, especially P (Tables 2–9). This may be attributed to the same soil used in the summer of 2020 for the fall season of 2019, which awarded an opportunity to release excess nutrients from the soil due to the increased decomposition of LC and HA added in the previous season, in addition to the greater solubility of P and other nutrients that occurred by P-SB. It is worth mentioning that the novelty of this research relies on the fact that very little research has investigated the impact of P-SB on nutrient recycling, especially P, and their application to calcareous soils and to compare its impact with leguminous compost and humic acids.

5. Conclusions

This study shows that inoculating the calcareous soil with phosphate-solubilizing bacteria (P-SB) (a 1:1 mixture of two *Pseudomonas* sp.; *Ps. mallei* and *Ps. cepaceae*) markedly exceeded the soil treatment with humic acids, leguminous compost, or humic acids+leguminous compost in enhancing the growth and productions of common bean plants under stress induced by high soil calcium carbonate ($CaCO_3$) content. P-SB facilitated the solubility of phosphorus (P) and other nutrients (e.g., Mn, Fe, K, and N) by increasing the enzymatic activities of the soil (e.g., phosphatase and phytase), along with an increase in the soil cation exchange capacity and organic matter content along with a lower $CaCO_3$ content, resulting in augmented nutrient availability in the soil for plant roots. This led to adequate P content in the *Phaseolus vulgaris* plant, leading to a marked decrease in acid phosphatase activity in plant leaves and roots. P-mediated growth promotion under high $CaCO_3$ stress was attributed to the improvement of soil biological activities, phytase and phosphatase activities, and available nutrient contents including P; mechanisms by which P-SB enabled *Phaseolus vulgaris* plants to boost their tolerance to the stress of high $CaCO_3$ content.

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