Article

Phosphorus and Biofertilizer Application Effects on Growth Parameters, Yield and Chemical Constituents of Broccoli

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Abstract: Broccoli is a popular vegetable throughout the world and contains important nutritional features. This study was conducted to evaluate the effect the fertilization with different phosphorus sources (i.e., soil application of rock phosphate (RP) at 428 kg ha⁻¹, calcium superphosphate (CSP) at 670 kg ha⁻¹, phosphoric acid (PA) at 126 L ha⁻¹ and monoammonium phosphate (MAP) at 334 kg ha⁻¹) combined with biofertilizers (i.e., inoculation with phosphorin or mycorrhiza) on plant growth aspects, antioxidants enzyme activity, chemical constituents, yield and quality of broccoli leaves and heads (*Brassica oleracea* var. *italica* Plenck). The experiment was performed according to the randomized complete block design with three replications ($n = 3$), for two growing seasons (2018–2019) at the experimental farm of Benha University, Egypt. The obtained results showed that the highest values of plant height, leaf area, fresh and dry weight of leaves/plant, head weight and diameter, and the highest yield of heads ha⁻¹ were recorded in plants that received MAP fertilizer and were inoculated with mycorrhiza. On the other hand, the highest value of head length, total carbohydrate and total soluble solids (TSS) content were observed in plants fertilized with PA and inoculated with mycorrhiza. The highest leaf nitrogen % and head ascorbic acid content, as well as the lowest head total phenol content were recorded in plants supplemented with MAP fertilizer and inoculated with mycorrhiza. Moreover, broccoli plants fertilized with PA and inoculated with mycorrhiza showed promising results in terms of phosphorus, potassium and total carbohydrate content, antioxidant activity and dehydrogenase activity. In conclusion, phosphorus may affect plant growth, yield and chemical parameters in broccoli plants depending on the fertilizer source, while inoculation with mycorrhiza may also have a beneficial impact on these parameters.

Keywords: *Brassica oleracea* var. *italica*; phosphorus sources; total phenols; chemical composition; total carbohydrates

1. Introduction

Broccoli (*Brassica oleracea* var. *italica* Plenck) is widely consumed throughout the world due to its high nutritional value (e.g., a rich source of vitamins A, B₂, C, minerals [1,2], fibers and low number of calories [3]). It is also highly appreciated for its health beneficial

properties due to its high content in antioxidants and bioactive compounds such as α -tocopherol, β carotene and isothiocyanates [4]. Moreover, recent evidence has confirmed its anticancer properties associated with specific phytochemicals such as organosulfuric compounds and sulforaphane and glucosinolates, in particular [5–7].

The initiation and development of broccoli inflorescence (head) is highly affected by phosphorus fertilization [8,9]. Phosphorus is considered a key nutrient in different physiological and biochemical processes [10] and its addition may also improve yield parameters [11,12]. It activates nucleic acids and phospholipids formation, induces the metabolism of carbon and also promotes enzyme activities [13,14]. Phosphorus is involved in vital functions of photosynthesis, respiration, energy metabolism, organic compound biosynthesis and cell division; it is also a structural element of phospholipids and nucleic acids [15]. Although P is usually applied in high amounts, plants may only absorb it in small amounts, while it could become unavailable for plants' uptake [16] due to its conversion into fixed components with soil colloidal particles in soil of either acidic or alkaline pH [17].

There are two forms of P in the soil, namely organic or inorganic [18]. Inorganic phosphorous is the form commonly absorbed by plants [19]. In order to fulfill plant requirements, high amounts of P fertilizers must be applied [20], which apart from increasing cost of production, they also induce a detrimental impact on the environment and human health. Therefore, in order to reduce these negative impacts, it is important to replace inorganic P fertilizers with bio-fertilizers, within the context of sustainability and the application of environment-friendly cultivation methods.

Biofertilizers, including bacteria (*Bacillus megaterium*) and fungi (arbuscular mycorrhiza) that may solubilize phosphates, can improve absorption of P by plants. The useful functions of bio-fertilizers are associated with improving soil chemical and physical properties, enhancing root surface area, maintaining soil fertility in the long term, increasing nutrient uptake, yield and quality through the increase of health beneficial phytochemicals [21–24]. Bio-fertilizers play a major role in converting fixed phosphates into P available for plants through the production of organic acids and the modification of soil pH [25,26]. Microorganisms may also induce many beneficial traits, such as increased pathogen resistance and stress tolerance or higher survival rates after transplantation.

There are several reports of the effects of P fertilizers and biofertilizers application on broccoli's biochemical functions and yield, however the effect of various P fertilizer sources is not well examined. To determine the best source of P fertilization for broccoli cultivation, this study compared rock phosphate, calcium superphosphate, phosphoric acid and monoammonium phosphate combined with the application of phosphorin and mycorrhiza.

2. Materials and Methods

The experiment was performed for two successive growing seasons (2018 and 2019) at the experimental farm of Faculty of Agriculture, Moshtohor, Benha University, Egypt, aiming to evaluate the effect of phosphorus sources and bio-fertilizers on broccoli plants (*Brassica oleracea* var. *italica* Plenck cv. Waltham 29). The experimental area was located at an altitude of 45 m above mean sea level, 30.45 N latitude and 31.10 E longitude. Random soil samples were collected from the experimental soil at the depth of 0–30 cm before planting for the analysis of the physical and chemical properties according to the protocols described in the literature [27,28]. Physical and chemical characters of the used soil are shown in Tables 1 and 2, respectively. Additionally, Table 3 shows the metrological data for the experimental area during 2018 and 2019 seasons.

Table 1. Physical properties of the used soil.

Parameters	Unit	Seasons	
		2018/2019	2019/2020
Coarse Sand	%	5.38	4.93
Sand	%	15.68	16.32
Silt	%	21.35	22.12
Clay	%	57.59	56.63
Textural class		Clay	Clay

Table 2. Chemical properties of the soil used.

Parameters	Unit	Seasons	
		2018/2019	2019/2020
CaCO ₃	%	1.22	1.18
Organic matter	%	2.18	2.14
Available nitrogen	mg kg ⁻¹	44.35	42.87
Available phosphorus	mg kg ⁻¹	17.24	18.12
Available potassium	mg kg ⁻¹	131.9	128.2
Electrical Conductivity (EC)	dS·m ⁻¹	1.14	1.17

Table 3. Monthly meteorological data of the experimental site in 2018 and 2019 seasons.

Month	2018 Season				2019 Season			
	MaxT (°C)	MinT (°C)	RH (%)	DewT	MaxT (°C)	MinT (°C)	RH (%)	DewT
October	31.44	19.70	58.64	15.96	32.57	20.20	57.72	16.47
November	29.71	17.89	58.20	14.13	28.57	15.92	62.30	12.28
December	29.71	12.89	58.20	14.13	27.13	11.93	54.51	7.48
January	17.67	9.27	70.03	7.59	16.20	8.50	69.23	7.55
February	19.61	9.37	68.58	8.03	17.23	8.98	70.22	7.69
March	23.12	10.46	62.35	8.63	22.15	9.72	65.13	8.0

MaxT = Maximum temperature, MinT = Minimum temperature, RH (%) = Relative humidity %, DewT = Dew point temperature.

2.1. Plant Material and Experimental Conditions

Seeds of broccoli (*Brassica oleracea* var. *italica* Plenck cv. Waltham 29) were obtained from Modesto Seed Company (Modesto Seed Co. Inc., Modesto, CA, USA).

Each experimental plot occupied an area of 14 m², including five ridges of 4 m long and 70 cm in width. Broccoli seedlings were transplanted in the ridges at 30 cm apart on 2 October in both seasons of 2018 and 2019 when they reached the stage of 3–4 true leaves.

All plants received NK fertilizers at the recommended doses of the Egyptian Ministry of Agriculture i.e., 90 kg N ha⁻¹ as ammonium nitrate (33% N), and 96 kg K ha⁻¹ as potassium sulphate, (48% K₂O). All agricultural practices were performed according to the best practice guides for broccoli crops.

2.2. Experimental Treatments

The experimental treatments included four phosphorus sources, i.e., rock phosphate (RP), calcium superphosphate (CSP), phosphoric acid (PA) and monoammonium phosphate (MAP) applied in soil and their combination with the inoculation with phosphorin or mycorrhiza. The phosphorus fertilization treatments were as follows: (a) calcium superphosphate (16% P₂O₅) at 670 kg ha⁻¹, which is equivalent to the recommended dose of mineral P (108 kg ha⁻¹); (b) rock phosphate (25% P₂O₅) was used as a phosphatic source at 428 kg ha⁻¹, which is equivalent to the recommended dose of mineral P; (c) phosphoric acid (85% P₂O₅) at 126 L ha⁻¹, which is equivalent to the recommended dose of mineral P; (d) monoammonium phosphate (54% P₂O₅) at 334 kg ha⁻¹, which is equivalent to the recommended dose of mineral P.

The amounts of rock phosphate and calcium superphosphate were added during soil preparation as base dressing, whereas phosphoric acid and monoammonium phosphate were divided into five equal doses and were added just before irrigation.

The used bio-fertilizers were kindly provided by the microbiology department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt. They contained phosphate dissolving bacteria (phosphorin), such as *Bacillus megaterium* or phosphate mobilizing bio-fertilizer (Arbuscular mycorrhiza; AM). The AM inoculum was made up of *Glomus mosseae*-NRC31 and *Glomus fasciculata*-NRC15, which were initially isolated from Egyptian soils; the inoculum was allowed to grow on sterilized peat-vermiculite-perlite mixtures and then was added directly by dipping the roots of broccoli seedlings for approximately 10 min before transplantation in a liquid suspension (1 L) containing the mycorrhiza at concentration of 10^8 CFU mL⁻¹. For phosphorin treatments, *Bacillus megaterium* was included and prepared by growing the bacterial strains in nutrient broth medium with continuous shaking (140 rpm) at 30 °C up to optimum growth. Then the cells were harvested by centrifugation (4000 rpm) at 4 °C for 10 min. The cells were suspended using water peptone to prepare a suspension that contained 10^8 CFU mL⁻¹, as assessed by the optical density method, and then applied by directly dipping the roots of broccoli seedlings for approximately 10 min before transplantation in the liquid solution.

2.3. Data Collection

2.3.1. Plant Growth Parameters

Ten plants were sampled from each experimental plot ($n = 10$) at 60 days after transplanting to estimate the following growth parameters i.e., number of leaves, leaf area (cm²; the 5th true leaf from plant apex was measured by using a laser leaf area meter) and foliage of the fresh and dry weight (g) per plant.

2.3.2. Yield and Its Components

At the growth stage of marketable maturity (85 days after transplanting), total yield of broccoli heads (tons per hectare), average broccoli head weight (g), diameter (cm) and length (cm) were estimated in 10 plants for each replication.

2.3.3. Chemical Constituents of Plant Foliage

Total carbohydrate (%), nitrogen (%), phosphorus (%) and potassium (%) content of the leaves was determined in three replicates according to the methods previously described in the literature [29–32].

2.3.4. Chemical Composition of Heads

The following parameters were estimated in 10 broccoli heads from each plot:

a. Head total carbohydrate was determined in dry samples according to the method of Herbert et al. [29].

b. Total soluble solids (T.S.S.): A random sample of 10 heads from each experimental plot at marketable maturity stage was taken to determine the percentage of soluble solid content by using a hand refractometer (38–01 OPTi multiple scale digital handheld refractometer, Bellingham + Stanley, Xylem Analytics Germany Sales GmbH and Co. KG, WTW, Weilheim, Germany).

c. Total phenol content (mg/100 g fresh weight (F.W.)) was determined by the Folin–Ciocalteu protocol as described by Lu et al. [33], with minor adjustments. Polyphenols extraction was performed by adding 10 mL of methanol (85%) to 1 g of frozen head tissues. Sterile refined water (250 µL) was added to 250 µL of concentrate, and afterwards 2.5 mL of diluted Folin–Ciocalteu reagent (10%) and 2 mL of 7.5% sodium carbonate were added. Samples were shaken for 1.5–2 h and the absorbance of samples was estimated at 765 nm using a spectrophotometer (Model UV752/UV754-single beam UV/Vis spectrophotometer, YK Scientific, Shanghai, China). Gallic acid was used to obtain the calibration curve and results were expressed as mg g⁻¹ [33].

d. Ascorbic acid was determined using the indicator of 2,6 dichlorophenol indophenol by titration as the method mentioned by the Association of Official Analytical Chemists [34]. 5 g of head tissues and 5 mL 1.0% of hydrochloric acid (*w/v*) were mixed and the mixture was then subjected to centrifugation ($10,000 \times g$ for 10 min). The supernatant liquid was collected and ascorbic acid was determined via the absorbance of the concentrate at 243 nm (Model UV752/UV754-single beam UV/Vis spectrophotometer, YK Scientific, Shanghai, China).

e. Antioxidant activity ($\mu\text{mol g}^{-1} \text{ F} \cdot \text{W}$) was determined according to Lu et al. [33]. Radical scavenging activity was tested by using the free extreme 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) in the previously described methanol preparations (see Section 2.3.4.c). 5 g of ground frozen head tissues was mixed with 10 mL methanol and homogenized for two hours. Then, the measurement of radical scavenging activity was carried out using the DPPH solution. Briefly, 100 μL of concentrate and 3.9 mL of a $6 \times 10^{-5} \text{ mol L}^{-1}$ of DPPH solution were incubated for 30 min. The absorbance (A_n) at 515 nm was recorded at 0 and 30 min with a spectrophotometer (Model UV752/UV754-single beam UV/Vis spectrophotometer, YK Scientific, Shanghai, China). DPPH is diminished when it responds with a cell reinforcement aggravate that changes from profound violet to light-yellow color according to Sharma et al. [17], and antioxidant activity was estimated according to the following equation:

$$\text{Inhibition (\%)} = [(AB - AA)/AB] \times 100 \quad (1)$$

where: AB = absorption of blank sample after 0 min.

AA = absorbance of tested extract solution after 30 min.

Radical scavenging activity (RSA) was figured as % restrained by DPPH solutions for the indicated recipe.

f. Enzyme activity. The enzymes activity of dehydrogenase (DHA) was measured in the rhizosphere using the method of Schinner et al. [35]. Dehydrogenase activity can be measured using different tetrazolium salts, e.g., 2,3,5-triphenyl-tetrazolium chloride (TTC) as an artificial terminal hydrogen acceptor in the electron transport chain, reducing to red-colored triphenylformazan (TPF). TPF was extracted using organic solvents (e.g., methanol), and the color intensity of the extract was determined by spectroscopic methods. The intensity of the color was directly proportional to the concentration of the produced triphenylformazan.

2.4. Statistical Analysis

The experiment was designed according to a completely randomized block with two factors: the soil application of phosphorus and the application of bio-fertilizers. All data obtained in both seasons of the study were subjected to analysis of variance (ANOVA). The least significant difference (L.S.D.) method was used for means comparison according to Snedecor and Cochran [36].

3. Results

Different data presented by ANOVA in Table 4 showed that all parameters were affected by the application of phosphorus sources (Factor A), Biofertilizers (Factor B) and their interaction ($A \times B$) during 2018 and 2019 seasons. Factor A recorded a highly significant effect with vegetative, yield, chemical analysis, and head quality during both seasons. For factor B, data also indicted that biofertilizer application showed significant effect on most of the parameters during both seasons except for total phenol and ascorbic acid concentration during 2018 and 2019 seasons. For the interaction between phosphorus sources and biofertilizer application ($A \times B$), significant effects were observed on most of the tested parameters for both seasons during 2018 and 2019 seasons.

Table 4. The analysis of variance (ANOVA) results for the effect of phosphorus source (Factor A), bio-fertilizers application (Factor B) and their interaction (AB) on different parameters of broccoli plants during the 2018 (1st) and 2019 (2nd) seasons.

Source of Variance	DF	Number of Leaves/Plant		Leaf Area (cm ²)		Fresh Weight of Leaves/Plant (g)		Dry Weight of Leaves/Plant (g)	
		Mean Square		Mean Square		Mean Square		Mean Square	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Replicate	2	2.469	12.304	184.33	20.083	1090.08	924.250	131.490	37.586
Factor A	3	8.053 **	6.482 **	3373.33 **	2169.66 **	8707.0 **	242.917 **	316.545 **	272.544 **
Factor B	2	7.949 **	7.821 **	1252.75 **	3049.00 **	10,396.7 **	8365.75 **	499.621 **	396.311 **
AB	6	0.443 **	0.175 **	181.08 **	137.66 **	318.75 **	192.41 **	9.340 **	4.265 **
Error	22	4.503	4.271	134.33	118.720	146.81	175.52	54.365	41.975

Source of Variance	DF	Average Head Weight (g)		Total Yield (ton/ha)		Average Head Length (cm)		Average Head Diameter (cm)	
		Mean Square		Mean Square		Mean Square		Mean Square	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Replicate	2	1250.528	4757.58	0.140	48.565	0.943	3.326	10.253	62.230
Factor A	3	3711.583 **	14,104.91 **	8.526 **	21.996 **	9.449 **	13.362 **	27.177 **	32.497 **
Factor B	2	4535.194 **	10,647.00 **	10.494 **	13.519 **	28.068 **	34.230 **	22.327 **	15.707 **
AB	6	319.417 **	12,977.66 **	0.744 **	0.614 **	0.194 **	0.310 **	0.274 **	0.644 **
Error	22	436.801	6418.31	6.293	1.066	2.598	2.070	5.871	1.478

Source of Variance	DF	N%		P%		Total carbohydrates%		K%	
		Mean Square		Mean Square		Mean Square		Mean Square	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Replicate	2	0.001	0.008	0.000	0.000	0.180	1.726	0.009	0.019
Factor A	3	0.122 **	0.124 **	0.003 **	0.009 **	9.205 **	9.919 **	0.147 **	0.088 **
Factor B	2	0.117 **	0.335 **	0.005 *	0.012 **	28.27 **	21.638 **	0.104 **	0.126 **
AB	6	0.003 **	0.005 **	0.000 **	0.000 **	0.180 **	0.058 **	0.007 **	0.002 **
Error	22	0.021	0.012	0.000	0.002	1.041	1.896	0.004	0.001

Source of Variance	DF	TSS (Brix °)		Ascorbic Acid (mg/100 g fw)		Total Carbohydrates (%)		Total Phenols (mg/100 g fw)	
		Mean Square		Mean Square		Mean Square		Mean Square	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Replicate	2	1.726	4.996	5.88	6.032	4.996	62.230	3.784	3.987
Factor A	3	9.919 **	43.234 **	26.26 **	28.250 **	43.234 **	32.497 **	26.232 **	25.391 **
Factor B	2	21.638 **	20.208 **	8.2700	7.260	20.208 **	15.707 **	8.159	8.147
AB	6	0.058 **	0.143 **	0.182	0.187	0.143 **	0.644 **	0.278 **	0.256
Error	22	1.896	2.028	2.03	2.08	2.028	1.478	1.589	1.425

Source of Variance	DF	Antioxidant Activity %		Dehydrogenase Activity (µgTPF/g dw)	
		Mean Square		Mean Square	
		1st	2nd	1st	2nd
Replicate	2	0.012	0.028	0.135	0.145
Factor A	3	0.126 **	0.135 **	0.089 **	0.148 **
Factor B	2	0.338 **	0.235 **	0.019 **	0.109 **
AB	6	0.012	0.024	0.089	0.058
Error	22	0.029	0.028	0.039	0.018

DF: Degrees of freedom; Factor A: phosphorus source; Factor B: bio-fertilizers application; TSS: Total soluble solids; N: Nitrogen; P: Phosphorus; K: Potassium. Comparison between the means of the treatments was performed with the Least Significant Difference (LSD) test. * mean significant at 0.5%; ** Mean significant at 1%.

3.1. Vegetative Growth

Data regarding the vegetative growth parameters are presented in Table 5. The results showed that monoammonium phosphate (MAP) was most effective for all the studied parameters and for both growing seasons, although no significant differences from phosphoric acid (PA) were observed for the number of leaves/plant and the fresh and dry weight of leaves/plant. On the other hand, the application of mycorrhiza showed the highest values for all the tested vegetative parameters in both seasons, except for the case of leaf area in 2018 where no significant differences from phosphorin were detected, probably due to the slight differences in meteorological conditions between the two growing seasons. Moreover, phosphorin did not result in higher vegetative growth compared to the control treatment (no inoculum) for most of the tested parameters apart from the fresh weight of leaves per plant (both seasons) and leaf area (both season). Regarding the interaction effect between bio-fertilizer and phosphorus sources treatments, data in Table 1 indicate that the combination of MAP and mycorrhiza application resulted in the highest overall values for both growing seasons, while a varied effect was observed for the rest of the treatment combinations. In contrast, the lowest values of growth parameters were recorded in the case of non-inoculated plants that received rock or superphosphate as phosphorus source.

Table 5. The effect of phosphorus source and bio-fertilizers application on vegetative growth parameters of broccoli plants during the 2018 (1st) and 2019 (2nd) seasons.

Phosphorus Fertilizer Treatments		Number of Leaves/Plant		Leaf Area (cm ²)		Fresh Weight of Leaves/Plant (g)		Dry Weight of Leaves/Plant (g)	
Phosphorus Sources	Phosphorus Biofertilizer	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Rock phosphate (RP)		16.47	15.35	401	415	544	496	93.8	86.4
Superphosphate (SP)		17.1	15.37	410	415	559	504	97.1	88.0
Phosphoric acid (PA)		17.7	17.02	426	435	601	549	104.3	96.7
Monoammonium phosphate (MAP)		18.29	17.18	445	446	607	549	106.4	96.6
L.S.D. at 0.05 *		0.82	0.91	11	10	11	12	6.9	6.3
	Control (no inoculum)	16.8	15.5	409	411	555	501	95.2	86.6
	Phosphorin	16.96	16.08	422	431	567	520	98.3	91.2
	Mycorrhiza	18.4	17.11	430	442	611	553	107.6	98.0
		0.71	0.79	9	9	10	11	6.2	5.4
	Control (no inoculum)	15.29	14.51	389	406	512	473	87.1	81.3
	Phosphorin	16.26	15.36	402	416	534	489	91.8	85.0
	Mycorrhiza	17.87	16.19	413	425	587	528	102.7	92.9
	Control (no inoculum)	16.18	14.39	406	401	529	470	90.4	81.1
	Phosphorin	17.0	15.22	416	412	551	505	95.3	88.3
L.S.D. at 0.05	Mycorrhiza	18.13	16.51	408	432	598	538	105.2	94.6
	Control (no inoculum)	17.38	16.68	418	416	584	537	100.4	92.9
	Phosphorin	17.34	16.61	423	439	594	541	102.8	95.7
	Mycorrhiza	18.37	17.78	437	451	627	571	109.7	101.6
	Control (no inoculum)	18.37	16.43	426	421	598	524	103.1	91.1
	Phosphorin	17.25	17.14	448	457	590	547	103.3	95.7
	Mycorrhiza	19.24	17.96	462	462	634	576	112.8	103.1
		1.42	1.58	19	18	2	22	12.4	10.9

* Means comparison between treatments was performed with the least significant difference (LSD) test ($p = 0.05$).

3.2. Yield Parameters

The data related to yield parameters are presented in Table 6. The application of MAP as phosphorus source resulted in the highest head weight, total yield and head diameter in both seasons, without significant differences from PA treatment. Similarly, PA application resulted in the highest head length while no significant differences from MAP were recorded. The inoculation of broccoli plants with mycorrhiza showed the most beneficial effects on all the recorded yield parameters, while no significant differences from phosphorin were recorded in the case of head length and head diameter in the 1st growing season. Moreover, the combined application of MAP or PA and mycorrhiza resulted in the highest yield parameters. In particular, MAP $\times$ mycorrhiza application increased head weight and head diameter (in both seasons) and total yield (2nd season), whereas PA $\times$ mycorrhiza treatment was the most beneficial for total yield (1st season) and head length (in both seasons). The rest of the treatments combination showed a varied effect with no specific trends being observed, although non-inoculated plants that received rock or superphosphate recorded the lowest overall values (head weight, total yield and head diameter for rock phosphate and head length for superphosphate).

Table 6. The effect of phosphorus sources and bio-fertilizers application on yield parameters of broccoli plants during the 2018 (1st) and 2019 (2nd) seasons.

Phosphorus Fertilizer Treatments		Average Head Weight (g)		Total Yield ton/ha		Average Head Length (cm)		Average Head Diameter (cm)	
Phosphorus Sources	Phosphorus Biofertilizer	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Rock phosphate (RP)		529	517	25.09	24.50	17.3	17.8	20.1	20.6
Superphosphate (SP)		534	529	25.17	25.18	16.9	17.6	20.3	21.0
Phosphoric acid (PA)		563	573	26.86	27.31	19.1	20.0	22.8	23.9
Monoammonium phosphate (MAP)		569	581	26.87	27.67	18.6	19.6	23.5	24.3
	L.S.D. at 0.05 *	20	14	1.47	1.0	1.5	1.4	2.4	1.2
	Control (no inoculum)	530	521	24.96	25.07	16.3	16.9	20.3	21.5
	Phosphorin	548	550	26.18	26.23	18.4	19.0	21.5	22.2
	Mycorrhiza	569	571	26.79	27.19	19.2	20.3	23.0	23.7
	L.S.D. at 0.05	17	12	1.28	0.87	1.3	1.2	2.0	1.0
Rock phosphate (RP)	Control (no inoculum)	518	494	24.33	23.51	15.8	16.2	18.6	19.7
	Phosphorin	529	511	25.18	24.47	17.6	18.0	19.8	20.2
	Mycorrhiza	541	536	25.75	25.51	18.7	19.3	21.7	22.1
Superphosphate (SP)	Control (no inoculum)	526	518	24.54	24.66	15.5	16.1	18.9	20.2
	Phosphorin	521	527	25.13	25.08	17.2	17.8	20.1	20.1
	Mycorrhiza	557	542	25.84	25.80	18.1	19.0	21.9	22.8
Phosphoric acid (PA)	Control (no inoculum)	536	549	25.51	26.13	17.2	17.7	21.7	23.0
	Phosphorin	568	579	27.04	27.56	19.8	20.6	22.6	23.9
	Mycorrhiza	587	593	28.04	28.23	20.3	21.8	24.1	24.8
Monoammonium phosphate (MAP)	Control (no inoculum)	542	546	25.47	25.99	16.7	17.9	22.1	23.2
	Phosphorin	575	584	27.37	27.80	19.3	19.7	23.8	24.5
	Mycorrhiza	592	641	27.51	29.23	19.9	21.2	24.5	25.3
	L.S.D. at 0.05	35	25	2.56	1.74	2.7	2.4	4.1	2.0

* Means comparison between treatments was performed with the least significant difference (LSD) test ($p = 0.05$).

3.3. Chemical Composition of Foliage

The results in Table 7 indicated that macronutrient and total carbohydrate content in broccoli leaves were beneficially affected by either MAP or PA application. In particular, nitrogen (N) content (%) was the highest when plants were treated with MAP, without significant differences from the PA treatment being observed. On the other hand, phosphorus (P) and potassium (K) content was the highest for the PA treatment, while K content in the 1st growing season did not differ significantly between PA and MAP treatments. Finally, total carbohydrate content was beneficially affected by the application of either MAP or PA since no significant differences were observed between these treatments. Moreover, the inoculation of plants with mycorrhiza had a consistent beneficial effect on macronutrients and total carbohydrate content, being significantly different from the phosphorin and the control treatment in all the cases except for the N content in the 1st growing season where it did not differ from the phosphorin treatment. This finding could be partly attributed to the slight differences in meteorological conditions between the growing seasons, although no specific trends were recorded. Regarding the combined application of the tested phosphorus sources and biofertilizers, a varied effect was observed. In particular, N content was the highest when MAP was combined with mycorrhiza inoculation, the highest values for the rest of the tested parameter (K, P and total carbohydrate content) was recorded for the combination of PA and mycorrhiza inoculation. In contrast, the application of rock phosphate (RP) or superphosphate (SP) with no addition of biofertilizers resulted in the lowest overall values for N and K (only in the first season), P (in both seasons) and total carbohydrate content (only in the second season) in the case of RP, as well as for N and K (only in the second season) and total carbohydrate content (only in the second season) in the case of SP.

Table 7. The effect of phosphorus sources and bio-fertilizers application on the chemical composition of foliage of broccoli plants during the 2018 (1st) and 2019 (2nd) seasons.

Phosphorus Fertilizer Treatments		N%		P%		K%		Total Carbohydrates%	
Phosphorus Sources	Phosphorus Biofertilizer	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Rock phosphate (RP)		1.57	1.51	0.254	0.274	1.35	1.30	14.05	13.29
Superphosphate (SP)		1.60	1.54	0.262	0.282	1.32	1.26	13.73	13.18
Phosphoric acid (PA)		1.78	1.71	0.292	0.313	1.57	1.46	15.57	15.13
Monoammonium phosphate (MAP)		1.79	1.74	0.280	0.304	1.54	1.42	15.58	14.97
L.S.D. at 0.05 *		0.14	0.10	0.002	0.002	0.06	0.03	0.99	1.34
	Control (no inoculum)	1.59	1.44	0.250	0.273	1.36	1.26	13.25	12.86
	Phosphorin	1.69	1.65	0.237	0.289	1.44	1.36	14.70	14.02
	Mycorrhiza	1.78	1.77	0.292	0.317	1.54	1.46	16.32	15.54
		0.12	0.09	0.001	0.002	0.05	0.02	0.86	1.16
	Control (no inoculum)	1.46	1.35	0.238	0.252	1.27	1.21	12.35	11.82
Rock phosphate (RP)	Phosphorin	1.56	1.51	0.251	0.276	1.37	1.29	14.18	13.19
	Mycorrhiza	1.71	1.68	0.274	0.295	1.42	1.40	15.62	14.87
	Control (no inoculum)	1.51	1.32	0.241	0.262	1.29	1.18	12.14	11.91
Superphosphate (SP)	Phosphorin	1.62	1.58	0.264	0.274	1.31	1.24	13.94	13.02
	Mycorrhiza	1.68	1.72	0.281	0.312	1.38	1.36	15.12	14.61
	Control (no inoculum)	1.69	1.58	0.268	0.293	1.48	1.32	14.32	13.93
Phosphoric acid	Phosphorin	1.82	1.73	0.296	0.308	1.52	1.49	15.31	15.05
	Mycorrhiza	1.84	1.83	0.312	0.338	1.72	1.59	17.39	16.40
	Control (no inoculum)	1.70	1.54	0.254	0.286	1.41	1.34	14.21	13.80
MAP	Phosphorin	1.77	1.81	0.283	0.301	1.56	1.42	15.38	14.83
	Mycorrhiza	1.92	1.88	0.304	0.325	1.67	1.52	17.16	16.29
		0.24	0.18	0.003	0.004	0.10	0.05	1.72	2.33
L.S.D. at 0.05									

* Means comparison between treatments was performed with the least significant difference (LSD) test ($p = 0.05$).

3.4. Quality Parameters of Broccoli Heads

Quality parameters related to the chemical composition of broccoli heads are presented in Table 8. Our results showed that quality of broccoli heads is affected by phosphorus application depending on the selected phosphorus source. Therefore, total soluble solids content (TSS) was the highest when MAP or PA were applied in the first and second growing season, respectively, while ascorbic acid content was the highest when MAP was selected as the phosphorus source, regardless of the growing season. On the other hand, total carbohydrate content was beneficially affected by the application of PA, although no statistically significant differences were recorded from the treatment of MAP. Similarly, SP was the best performing treatment in terms of total phenol content, followed by the application of rock phosphate with no statistically significant differences between these two treatments. Regarding the tested biofertilizers, mycorrhiza inoculation resulted in the highest overall values for the tested parameters, except for the case of total phenols where the highest content was recorded when phosphorus was applied in the form of superphosphate, followed by the treatment of rock phosphate. The combined effect of phosphorus sources and biofertilizers showed a varied response depending on the evaluated parameter. Therefore, heads collected from plants treated with PA and mycorrhiza had the highest TSS and total carbohydrates content, while plants treated with MAP and mycorrhiza recorded the highest values of ascorbic acid content. Finally, total phenol content was recorded when plants were not inoculated with biofertilizers and the applied phosphorus source was superphosphate (second season) or rock phosphate (second season). On the other hand, the lowest overall values for most of the quality parameters tested were recorded for non-inoculated plants treated with rock (TSS and total carbohydrate content in the 1st season and ascorbic acid content in both seasons) or superphosphate (TSS and total carbohydrate content in the second season). Interestingly, the lowest total phenol content was observed in plants treated with the combination of MAP × mycorrhiza treatments.

Table 8. The effect of phosphorus sources and bio-fertilizers application on quality parameters of broccoli heads during the 2018 (1st) and 2019 (2nd) seasons.

Phosphorus Fertilizer Treatments		TSS (Brix °)		Ascorbic Acid (mg/100 g fw)		Total Carbohydrates (%)		Total Phenols (mg/100 g fw)	
Phosphorus Sources	Phosphorus Biofertilizer	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Rock phosphate (RP)		6.16	6.53	95.8	93.4	16.21	15.27	422	406
Superphosphate (SP)		6.20	6.54	99.9	98.7	16.40	15.57	427	414
Phosphoric acid (PA)		6.29	7.40	114.2	107.2	20.34	18.66	387	385
Monoammonium phosphate (MAP)		7.13	7.31	121.0	113.7	19.83	18.31	366	374
	L.S.D. at 0.05 *	0.18	0.11	4.2	3.4	1.39	1.03	10.9	28
	Control (no inoculum)	6.01	6.27	96.6	94.1	17.05	15.40	418	406
	Phosphorin	6.77	7.06	109.9	104.9	17.93	17.05	398	395
	Mycorrhiza	7.31	7.50	116.6	110.8	19.6	18.41	386	383
	L.S.D. at 0.05	0.16	0.10	3.6	3.0	1.20	0.89	9	24
	Control (no inoculum)	5.29	5.67	86.2	89.5	14.82	13.96	438	426
Rock phosphate (RP)	Phosphorin	6.37	6.84	98.7	92.8	16.17	15.28	421	402
	Mycorrhiza	6.84	7.08	102.6	98.1	17.64	16.58	408	391
	Control (no inoculum)	5.32	5.62	91.8	95.8	15.16	13.80	451	423
Superphosphate (SP)	Phosphorin	6.32	6.87	98.1	96.7	16.12	16.00	417	422
	Mycorrhiza	6.97	7.13	110.0	103.8	17.92	16.92	415	398
	Control (no inoculum)	6.82	6.94	102.4	92.7	19.29	16.91	401	396
Phosphoric acid (PA)	Phosphorin	7.24	7.32	118.3	112.2	20.12	18.74	398	382
	Mycorrhiza	7.83	7.96	121.8	116.7	21.60	20.34	369	378
	Control (no inoculum)	6.61	6.85	106.3	98.6	18.92	16.95	382	380
Monoammonium phosphate (MAP)	Phosphorin	7.18	7.24	124.6	118.0	19.31	18.16	364	374
	Mycorrhiza	7.62	7.85	132.0	124.5	21.25	19.82	352	368
	L.S.D. at 0.05	0.32	0.20	7.2	6.1	2.41	1.79	19	49

* Means comparison between treatments was performed with the least significant difference (LSD) test ($p = 0.05$).

3.5. Antioxidant and Enzymes Activity

The results of the antioxidant and enzyme activity are presented in Table 9. The application of MAP resulted in the highest antioxidant and dehydrogenase activity for both seasons, while similar results were recorded for the plants treated with mycorrhiza. Finally, the combination of these treatments resulted in the highest overall values for antioxidant and dehydrogenase activity, regardless of the growing season. In contrast, the lowest values were observed for the combination of RP × control (no inoculum) treatments.

Table 9. The effect of phosphorus sources and biofertilizer application on antioxidant and dehydrogenase activity of broccoli heads during the 2018 (1st) and 2019 (2nd) seasons.

Phosphorus Pertilizer Treatments		Antioxidant Activity %		Dehydrogenase Activity (µgTPF/g dw)	
Phosphorus Sources	Phosphorus Biofertilizer	1st Season	2nd Season	1st Season	2nd Season
Rock phosphate (RP)		0.58	0.54	40.9	37.8
Superphosphate (SP)		0.60	0.56	41.5	38.3
Phosphoric acid (PA)		0.68	0.63	48.2	43.6
Monoammonium phosphate (MAP)		0.70	0.66	50.5	46.7
L.S.D. at 0.05 *		0.04	0.03	1.7	1.0
	Control (no inoculum)	0.60	0.54	36.8	33.7
	Phosphorin	0.62	0.60	44.4	40.5
	Mycorrhiza	0.69	0.66	54.6	50.6
L.S.D. at 0.05		0.03	0.02	1.4	0.8
	Control (no inoculum)	0.52	0.48	32.7	29.5
	Phosphorin	0.59	0.54	41.8	38.7
	Mycorrhiza	0.64	0.61	48.3	45.2
Rock phosphate (RP)	Control (no inoculum)	0.56	0.46	34.2	31.0
	Phosphorin	0.57	0.58	40.3	38.0
	Mycorrhiza	0.68	0.64	50.2	46.1
	Control (no inoculum)	0.67	0.59	39.4	36.4
Superphosphate (SP)	Phosphorin	0.66	0.63	46.8	41.6
	Mycorrhiza	0.71	0.69	58.5	52.9
	Control (no inoculum)	0.68	0.63	41.2	38.2
	Phosphorin	0.69	0.65	49.0	43.7
Phosphoric acid (PA)	Mycorrhiza	0.74	0.72	61.4	58.4
	Control (no inoculum)	0.68	0.63	41.2	38.2
	Phosphorin	0.69	0.65	49.0	43.7
	Mycorrhiza	0.74	0.72	61.4	58.4
Monoammonium phosphate (MAP)		0.07	0.05	2.9	1.7
	Control (no inoculum)	0.68	0.63	41.2	38.2
	Phosphorin	0.69	0.65	49.0	43.7
	Mycorrhiza	0.74	0.72	61.4	58.4
L.S.D. at 0.05		0.07	0.05	2.9	1.7

* Means comparison between treatments was performed with the least significant difference (LSD) test ($p = 0.05$).

4. Discussion

Different sources of phosphorus are being used in crop production, mainly superphosphates and rock phosphate. However, additional phosphorus fertilizers have recently become available, such as phosphoric acid, monoammonium phosphate (MAP), monopotassium phosphate (MPK) and urea phosphate (UP), which can be used to manage P fertilization, especially in the cultivation of short growth cycle plants, such as vegetables. These P sources are popular due to their high phosphorus content and excellent physical properties [37,38]. Single and triple superphosphate precipitate when used for fertigation, thus blocking the emitters; therefore, it is necessary to utilize water soluble phosphorus sources, such as MAP and phosphoric acid to increase nutrient use efficiency and reduce production cost. Furthermore, the MAP fertilizer is composed of two fundamental components, NH_4^+ and H_2PO_4^- , which are essential for proper plant development [39].

On the other hand, biofertilizers may play an essential role in enhancing crop productivity and maintaining soil fertility in the long term, while reducing the environmental burden associated with fertilizer production and nutrients leaching to groundwater deposits. In addition, the microorganisms usually included in the biofertilizers may interact with the plants and boost their immunity, growth and development, and consequently increase the crop production [40–42].

In this study, the application of MAP and phosphoric acid as an alternative source of phosphorus enhanced the vegetative growth parameters, i.e., the number of leaves/plant, leaf area, and fresh and dry weight of leaves as compared to the other phosphorus sources tested. These findings could be attributed to the high solubility and availability of phosphorus in both fertilizers, especially MAP fertilizer, which recorded the highest values of the aforementioned parameters that could be linked to its high phosphorus content (61% as P_2O_5), high water solubility (365 g L^{-1} at $20\text{ }^{\circ}\text{C}$) and pH value (4.0 to 4.5). The pH of the solution surrounding its particles is moderately acidic, making MAP a preferable fertilizer in neutral and alkaline soils. Moreover, MAP contains about 12% of nitrogen in ammonium (NH_4^+) form, which is less prone to leaching and is also considered as a slower release form of nitrogen, compared to nitrate form. Additionally, the decrease of pH in the root zone by using ammonium nitrogen may increase the degeneration of calcium–phosphorus precipitated compounds and thus enhanced the phosphorus availability and plant uptake [39,43]. Moreover, the observed results could be attributed to the pivotal role of phosphorus in cell division [44], as well as to its use as a structural element in macromolecular structures (e.g., DNA and RNA), in phospholipids and in cell membranes. Moreover, phosphates are included in energy-rich ions [adenosine monophosphate (AMP), adenosine triphosphate (ATP) and adenosine diphosphate (ADP)], which are related to all energy metabolism processes of cells, i.e., photophosphorylation [10].

Concerning the effect of the biofertilization, the application with mycorrhiza significantly enhanced most of the vegetative attributes, i.e., fresh and dry weights of leaves/plant as compared to phosphorin and the control treatment. These results could be attributed to the role of arbuscular mycorrhizal fungi (AMF) in improving the soil structure [45], enhancing the water absorption [46], increasing the absorption and acquisition of nutrient elements from the soil by augmenting the root area and penetration of the growing media [47–49], as well as the activation and excretion of various enzymes from plant roots and/or mycorrhizal hyphae, and the increased concentrations of endogenous hormones, i.e., gibberellins and auxins, which promote plant growth [50]. According to Yildirim et al. [51], the application of plant growth promoting rhizobacteria and manure significantly increased the vegetative growth of broccoli plants and attributed this increase to the improved uptake of macro and micronutrients from plants. However, the interaction of plants and inoculants are highly complex and difficult to interpret; therefore, the involvement of mycorrhizal inoculants in phosphorus acquisition from plants and the corresponding mechanisms need further studies [25].

The results observed for the single factors (e.g., phosphorus source and biofertilizers) were also depicted in their combinatorial effect where the best combination was the application of MAP $\times$ mycorrhiza treatment. Similar to our study, Abd-Alrahman et al. [52] suggested that the combined application of mineral and biofertilizers that contained nitrogen fixing and phosphorus solubilizing microorganisms significantly increased the vegetative parameters of broccoli plants, while Abou El-Magd et al. [53] suggested that biofertilizers could substitute mineral fertilizers resulting in increased plant growth and reduced production costs. Moreover, the combination of MAP and mycorrhiza inoculation was beneficial to plant growth parameters of snap bean, suggesting a synergistic effect on nutrient use efficiency that improves plant development [43].

Regarding the yield and quality parameters, the MAP and PA treatments recorded significantly higher values than the rest of phosphorus sources for the tested parameters, i.e., head weight, total yield of heads, and the physical and chemical quality attributes of the head, i.e., head diameter, head length, and total carbohydrate, ascorbic acid and TSS

content. This could be explained by the high phosphorus content and water solubility of MAP and PA fertilizers. Hence, phosphorus enhances the number and mass of roots [43,44], the formation of lateral roots and root/shoot ratio of plants. Consequently, the uptake of nutrients from soil increased, leading to higher nutrient content, plant growth, total chlorophyll, total carbohydrates and carbon assimilation. According to Fihlo et al. [8], phosphorus availability regulated through the application of different doses of P_2O_5 (0–320 kg ha⁻¹) is essential for broccoli productivity since it may affect head weight and diameter. Moreover, Ouda et al. [54] suggested that the combined application of manure and inorganic fertilizers was beneficial to all the tested yield parameters in broccoli crops, while increasing soil organic matter content at the same time. In the same context, Tanwar et al. [22] suggested that supplementing superphosphate fertilizers with AMF inoculation significantly improved the yield parameters of broccoli.

On the contrary, the MAP treatment showed significantly lower values of total phenols, which could probably be attributed to the fact that plants grew under optimal nutrient status without suffering from any abiotic stress from nutrient deficiency. Such an argument can be justified by the yield parameters and nutrient status of plants, as indicated in Tables 4 and 5, where treatments RP and SP resulted to lower growth and macronutrient content. There were also substantial increases in N, P, and K percentages in plant leaves as well as total carbohydrates following MAP and/or PA treatments, which could result in enhanced root development and formation, nutrient uptake, as well as in increased vegetative growth parameters, photosynthesis, and carbon assimilation. The availability of phosphorus is associated with carbohydrates metabolism and several studies have reported that increasing phosphorus rates results in variable effects on sugars content related to a source/sink balance between roots and aerial tissues [14].

The opposite trend was observed for total phenol content detected in broccoli heads, which decreased considerably after the inoculation with mycorrhiza. This result is in contrast with previous reports where the total phenolic compound contents were not significantly affected by the arbuscular mycorrhizal fungi (AMF) inoculation as compared with non-inoculated basil plants [55], whilst the total phenol contents in mycorrhizal inoculated lettuce plants significantly increased as compared with the non-inoculated plants [56]. It is well evidenced that the application of AMF is usually associated with the alleviation of abiotic stressor effects on plant growth, which could also affect the induction of plant secondary metabolism and the biosynthesis of stress protectant compounds, such as polyphenols [20,23,24,46]. However, the effect of mycorrhizal fungi on secondary metabolites content varies significantly, depending on the crop [57]. Therefore, the total phenols and the antioxidant activity seem to be correlated [58] or not correlated depending on the antioxidant activity assay [59]. The effect of AMF inoculation on the antioxidant activity also differs depending on the crop, the obtained results revealed that AMF significantly increased the antioxidant activity as compared with the other treatments, as well as on onion plants, while it had no significant effect on garlic plants [60]. The enhancement in total phenolic contents and antioxidant activity could be explained that the plant considered the mycorrhizal inoculation as an invasion or stress by the endomycorrhiza fungi, thus the plants begin to synthesize secondary compounds in the early stages of colonization, such as polyphenols; flavonoids and isoflavones [61]. Notably, the ascorbic acid content in broccoli heads was significantly increased by the AMF treatment, which was reflected in the antioxidant activity.

Furthermore, the AMF treatment enhanced the mineral content in the treated plants as compared to phosphorus and non-inoculated plants. These findings can be attributed to the AMF merits, since AMF hyphae are thinner than roots, which make it easier to extend to soil particles and penetrate in soil pore spaces and cracks, thus allowing the plants to better absorb the nutrients through the fungal hyphae and transfer them to the root cortex through the arbuscules [23]. Furthermore, Bücking [49] highlighted the beneficial effects of mycorrhiza symbionts on nutrient uptake and transportation within plant tissues.

5. Conclusions

The substitution of mineral fertilizers with organic fertilizers and biofertilizers is a promising cultivation practice towards sustainable agriculture without compromising plant growth and crop yield. The results of this study showed that rock phosphate was the most effective source of phosphorus, followed by calcium superphosphate and phosphoric acid, especially when combined with the inoculation of mycorrhiza. Therefore, the use of biofertilizers and proper fertilizer sources is a viable cultivation practice since it may increase phosphorus use efficiency as indicated by higher crop productivity and quality. In conclusion, the results of our study provide useful information to improve broccoli yield parameters and maximize utilization efficiency of P fertilizer sources, while the substitution of inorganic fertilizers with biofertilizers could be useful towards the alleviation of negative impacts on the environment and the adoption of eco-friendly cultivation practices.

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