sustainability

MDPI

Article

Integrated Use of Humic Acid and Plant Growth Promoting Rhizobacteria to Ensure Higher Potato Productivity in Sustainable Agriculture

Zehra Ekin

Faculty of Agriculture, Department of Field Crops, Van Yuzuncu Yil University, 65080 Van, Turkey; zehraekin@yyu.edu.tr

Received: 7 April 2019; Accepted: 19 June 2019; Published: 21 June 2019

check for updates

Abstract: In sustainable agriculture, seeking eco-friendly methods to promote plant growth and improve crop productivity is a priority. Humic acid (HA) and plant growth promoting rhizobacteria (PGPR) are among the most effective methods that utilize natural biologically-active substances. The aim of the present study was to analyze the effect of the presence of HA on potato (*Solanum tuberosum* L.) inoculation with PGPR (*Bacillus megatorium* and *Bacillus subtilis*) when compared to control and recommended doses of NPK. Seed tubers treated by humic acid (200, 400, and 600 kg ha^{-1}) and PGPR, separately or in combination, and NPK (50% and 100%) were planted into soil and untreated soil. Treatments were assessed for plant growth, classified tuber yields, quality, and mineral contents of potato tubers. There were highly significant increases in potato growth, tuber yields, and quality in PGPR and HA inoculated crops. Tuber size, weight, specific gravity, dry matter, starch, protein, and mineral contents (except Cu) were improved with PGPR treatments and further increased when administered with humic acids. Inoculation with PGPR mixed culture and 400 kg ha^{-1} HA increased total potato tuber yield by about 140% while conventional single treatment of 100% NPK fertilizer only led to an increase in potato production of 111% when compared to the control. The results demonstrated that this integrated approach has the potential to accelerate the transformation from conventional to sustainable potato production.

Keywords: Biostimulation; humic acid; PGPR; potato; sustainable agriculture

1. Introduction

The intensive input agricultural systems that ensure high yield and quality are one of the most disruptive practices for the planet's resources, but are justified by economic requirements, and the need to feed a growing population. Excessive use of non-renewable chemical fertilizers and pesticides risks agricultural sustainability through the deterioration of soil and water resources, environmental quality and health. Therefore, current trends in agriculture are focused on improving the efficiency of fertilizer use and reflect a revived an interest in transition from conventional to organic farming for basic vegetables consumed for human nutrition. Potatoes (*Solanum tuberosum* L.) are one of the most popular and nutritive vegetable crops world-wide with an annual production approaching 388.1 million tonnes cultivated in 19.3 million ha [1]. Potatoes are widely cultivated in more than 164 countries and consumed in fresh or processed form almost daily by more than a billion people. Organic production of this crop for human consumption promotes human health and enhances nutritional safety. Thus, the development of a vibrant, profitable, and sustainable organic potato sub-industry in potato growing countries depends mainly on improved nutrient management through organic matter mineralization and biological control of diseases and pests.

Sustainable agriculture requires using not only effective mineral fertilizers that contain macro and microelements, but also plant growth biostimulants, which are a rich source of biologically active compounds whose function is to stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality when applied to the rhizosphere or to plants [2–5]. This definition also entails diverse organic and inorganic substances and/or microorganisms such as humic acid, protein hydrolysates, seaweed extracts, chitosan, inorganic compounds, and beneficial bacteria and fungi.

Humic substances (HS), which include humic acid and fulvic acid, are among the most complex and biologically active organic matter compounds in the soil and are known to stimulate both plants and microbial activities through a number of mechanisms (e.g. through humic extracts of leonardite, compost or other organic fertilizer) [6]. HS do not only have a positive impact on soil physicochemical properties, and soil microbial community structure and activity, resulting in availability of higher nutrient content for plant growth, furthermore but it was also observed that they positively influence root growth, especially lateral root emergence and root hair initiation, involved in plant nutrient uptake [6,7]. Humic acid (HA) is also a naturally-available substance in the soil and a bio product of organic matter decomposition, which was successfully used in cultivation of various crops. In field experiments, direct effects of HA on plant growth were well described; these effects include enhanced macronutrient and micronutrient uptake and root growth [8], and the use of HA was successfully demonstrated in cultivation of several crops, such as potato [9], tomato [10], maize [7,11], Hungarian vetch [12], and blueberry [13].

Due to reduction of chemical inputs, improvement of diversity, changing fertilization management and to support integrated nutrient management, the use of beneficial soil microorganisms as well as HS use is one of the popular biological techniques that aim sustainable agriculture [14]. Plant growth promoting rhizobacteria (PGPR) formulated as bio-inoculants are an important part of the soil microbiota, known for their capacity to stimulate crop production through biofertilization mechanisms, such as biological nitrogen fixation, phosphate solubilization, production of phytohormones, and biocontrol processes [15]. Thus, PGPR are known as important biological inputs that, unlike fertilizers and pesticides, depend on a number of factors for their success in promoting crop yield. Previous reports have demonstrated that significant changes in root architecture in non-leguminous plants induced by humic substances may favor the fitness of the bacteria plant interaction due the enhancement of root attachment and infection sites [6–8], thus resulting in a significant increase in the bacteria attachment and survival on plant surface as well as endophytic colonization. On this mutual interaction between microorganisms and roots, Olivares et al. [16] reported that HS was a key factor in determining soil fertility as a candidate for suitable vehicle for PGPR, and co-inoculation could be an excellent application. Another investigation carried out by Olivares et al. [10] observed further benefit of the interaction between microorganism and organic matter through biological substrate enrichment.

The aim of the present study was to evaluate the integrated effects of two *Bacillus* strains OSU-142 (N_2-fixing) and M3 (P-solubilizing) and humic substances (HA: obtained from leonardite), separately or in combinations, on potato crop performance, quality, and tuber nutrient content under field conditions.

2. Materials and Methods

2.1. Study Site

Field experiments were conducted at a research farm located in Ahlat district (38° 46′N and 42°30′E with an altitude of 1722 m) in Eastern Anatolia region, Turkey during 2010 and 2011. The climate at this location is classified as continental with a total long-term average precipitation of 562.6 mm (1958–2017), mainly in winter. Annual mean air temperature is 9.3 °C, with an average temperature of −2.5 °C in January and 21.9 °C in July. Annual mean relative humidity is 63.8%. The study site climate variables were analyzed and averaged for each month (Table 1). In 2010 and 2011, total precipitation during the crop season (from May to October) was 165.6 mm, and 140.6 mm, respectively, and the long-term average for the same period was 181.5 mm (Table 1). The mean air temperature was 17.7 °C and 16.6 °C in 2010, 2011, respectively, and long-term average was 17.7 °C.

Table 1. Climate data: Monthly means of climate variables for the crop seasons of 2010, 2011, and long-term average (LTA: 1958-2017) in Ahlat, Turkey.

Month	Mean Air Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
	2010	2011	LTA	2010	2011	LTA	2010	2011	LTA
May	11.4	11.2	13.1	106.2	90.0	70.2	65.8	69.1	65.0
June	18.3	17.6	18.9	28.0	15.6	28.7	50.4	52.1	55.6
July	22.8	22.3	21.5	1.8	3.2	8.3	37.3	41.3	49.4
August	22.5	22.0	22.8	0.6	1.6	5.7	35.6	40.4	47.7
September	19.3	17.2	17.6	2.4	3.2	8.1	43.1	46.3	51.1
October	12.3	9.3	12.0	26.6	27.0	60.5	62.6	63.6	63.7
Season(M/T) *	17.7	16.6	17.7	165.6	140.6	181.5	49.1	52.1	55.4
Yearly(M/T)	10.9	8.6	9.3	399.0	566.6	562.6	59.6	56.4	63.8

* M: Mean, T: Total.

Prior to the experiments, soil samples were collected in the experiment site and analyzed for their physical and chemical properties using the methods described in Soil and Plant Analysis Laboratory Manual by Ryan et al. [17]. Soil samples were collected from soil cores at three different locations in the experiment site with an auger (2.0 cm in diameter and 15 cm high) before planting. All sample depths were the same: 0–30 cm. The soil samples were combined to form a single sample for analyzing the soil property. Soil texture [18], electrical conductivity (EC) [19], pH [20], total nitrogen (N) [21], plant-available phosphorus (P) [22], organic matter [23], available potassium (K) [24], available manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu) [25] were determined in the top 30 cm (<2 mm fraction) of soil. Soil properties are presented in Table 2.

Table 2. Physical and chemical properties of soil of the experimental site used for the field trial.

Feature	Units	Value
Clay	%	47.2
Silt	%	36.8
Sand	%	16.0
Electric conductivity	dS m^{-1}	1.16
pH (1:2 soil:water)		7.48
$CaCO_3$	%	6.8
Organic matter	%	1.59
Organic C	%	2.83
Total N	g kg^{-1}	0.15
Plant available P	mg kg^{-1}	7.95
Available K	mg kg^{-1}	196
Available Mn	mg kg^{-1}	3.30
Available Zn	mg kg^{-1}	1.44
Available Fe	mg kg^{-1}	5.85
Available Cu	mg kg^{-1}	0.59

2.2. *Plant Material and Experimental Design Approach*

Solanum tuberosum L. var. Caspar, which exhibits late maturity, high marketable yield, oval tuber shape with light yellow flesh and excellent long-term storability, was used as plant material. The experiment was constructed with randomized complete block (RCB) design with three replications. In the present study, eighteen treatments with different HA and PGPR combinations and inorganic fertilizer treatments were conducted and control was constructed with no HA, PGPR, and inorganic fertilizer application (Control), 100 + 50 + 50 kg ha^{-1} N, P and K fertilizer (NPK 50%), 200 + 100 + 100 kg ha^{-1} N, P, and K fertilizer (NPK 100%), *Bacillus megatorium* strain M3

(M3), *Bacillus subtilis* strain OSU-142 (OSU), PGPR's mixed culture (*Bacillus megatorium M3* and *Bacillus subtilis* OSU or M3OSU), 200 kg ha^{-1} HA (HA200), 400 kg ha^{-1} HA (HA400), 600 kg ha^{-1} HA (HA600), M3+200 kg ha^{-1} HA (M3H200), M3+400 kg ha^{-1} HA (M3H400), M3+600 kg ha^{-1} HA (M3H600), OSU+200 kg ha^{-1} HA (OSUH200), OSU+400 kg ha^{-1} HA (OSUH400), OSU+600 kg ha^{-1} HA (OSUH600), M3+OSU+200 kg ha^{-1} HA (M3OSUH200), M3+OSU+400 kg ha^{-1} HA (M3OSUH400), M3+OSU+600 kg ha^{-1} HA (M3OSUH600).

2.2.1. Fertilizer Treatments

In the present study, two inorganic fertilizer applications (NPK 50% and NPK 100%) were set up by adding 50% and 100% of recommended N, P and K (200 kg N ha^{-1} as ammonium sulfate, 21%; 100 kg P_2O_5 ha^{-1} as triple super phosphate, 45%; and 100 kg K_2O ha^{-1} as potassium phosphate, 50%) doses to the experiment soil. The NPK doses used in these experiments were commonly used doses by local potato farmers.

2.2.2. Humic Acid

Agro-Lig, a commercial product (Altintar Chemicals Company, Turkey) that includes crude humic acids (derived from leonardite), was used as humic acid treatment. Three HA doses (200, 400, and 600 kg ha^{-1}) were prepared by Agro-Lig (total humic acid 85%, total organic matter 75%, pH 3.5–5.5, max moisture 22%, silicon 0.5%, iron 0.5%, magnesium 0.5%, calcium 3.0%, sodium 0.3%, manganese 0.02%, copper 0.0003%, potassium 0.07%, titanium 0.02%, barium 0.03%, and boron 0.01%, cobalt 0.0002% dry matter basis) in granule form.

2.2.3. PGPR Strain Culture Conditions

The PGPR isolates (N_2-fixing *Bacillus subtilis* strain OSU-142 and phosphate solubilizing *Bacillus megatorium* strain M3) with plant growth promoting properties (IAA production, ACC-deaminase activity and potential bio fertilizer for agricultural crops) characterized by Çakmakçi et al. [26] and Orhan et al. [27] were kindly procured at Atatürk University, Department of Plant Protection, Turkey. Currently, these strains are protected in culture storage unit in the Department of Genetic and Bioengineering, Faculty of Engineering at Yeditepe University, İstanbul, Turkey. Both strains are indigenous, and were kept in nutrient broth (NB) with 15% glycerol at −80 °C for long-term storage, and grown on nutrient agar (NA) for routine use. Single colonies were transferred to 500 mL flasks that contained NB and incubated aerobically on a rotating shaker (150 rpm) overnight at 28′°C [26,28]. The bacterial suspension was then diluted in sterilized water to final concentration at cell densities of 10^9 colony forming units (CFU) ml^{-1} Seed tubers were inoculated with the liquid cultures of rhizobacteria [*Bacillus megatorium* M3, *Bacillus subtilis* OSU-142 and PGPR's consortium (*Bacillus megatorium* and *Bacillus subtilis)*] mixed with 10% sugar solution for 30 min, and were stored overnight to dry under room temperature.

2.3. Experimental Protocol

The tillage system was fall plow and spring cultivate. Different rates of humic acid (granule form) were applied to the soil in humic acid treated plots, according to the layout. Humic acid was applied once during planting and then mixed well with the soil. In chemical fertilizer applied plots, half of the N, and full P and K doses were applied during planting. The remaining half of the N dose was also applied during hilling when the plants were about 15 to 25 cm high. Finally, inoculated and non-inoculated well-sprouted tubers (50–60 g) were planted in 10 cm deep furrows with row plant spacing of 30 cm and between row spacing of 70 cm in late May in each year. Throughout the growth season, the production system was managed based on management practices recommended for the region, which included irrigation as required. The crops were harvested in early October, 140 days after sowingin each year.

2.4. Field and Laboratory Measurements

At the harvest time, growth and yield data were recorded for ten randomly selected potato plants in each plot. The observations on growth parameters such as plant height (cm) and number of stem plant^{-1} were also recorded on 50–55 days after planting. Yield and commercial tuber proportion were measured at maturity. In line with commercial practice to allow the tuber skins to set before the harvest, the tubers were sampled seven days after the aboveground harvest. Then, tubers were manually sorted into three categories (<35 mm: not for consumption or unmarketable; 35 to 50 mm: for direct consumption or marketable; >50 mm: for industrial use), counted and weighed. Analyses for physical and chemical quality parameters were conducted for the tubers larger than 35 mm determine the quality properties important for consumption and industrial processing. Tubers were processed with their skin due to the difficulty of uniformly peeling the potato tubers with irregular shapes.

2.4.1. Physical Quality Analysis

Physical quality properties were determined based on the tuber shape, tuber width (mm), tuber length (mm) and specific gravity (g/cm^3) [29]. Tuber shape was calculated with formula I.V. = L/W × 100, where, I.V. is the tuber index value; L is the tuber length and W is the tuber width. Tuber shape was determined based on the index value as round (<109), short-oval (110 to 129), oval (130 to 149), long-oval (150 to 169), and long (170 to 199), [30]. Tuber specific gravity was determined with the weight in air/ weight in water method with a 5 kg marketable potato sample [30,31].

2.4.2. Chemical Quality Analysis

Chemical quality analyses were determined based on dry matter, starch, protein and certain mineral element (P, K, Ca, Mg, Fe, Zn, Cu, and Mn) content. The starch and dry matter contents were determined as described by Esendal [30] using the specific gravity scale. Total N was analyzed with the Kjeldahl method and used for the calculation of the protein concentration by multiplication with a conversion factor of 6.25 [17]. For mineral content determination, tuber samples were wet-digested in HNO_3:$HClO_4$ (6:2 v/v) with the Advanced Microwave Digestion System, Ethos Easy and all sample extracts were analyzed with Inductively Coupled Plasma Optical Emission Spectrometry (iCAP 6000 SERIES, ICP Spectrometer).

2.5. Data Analysis

The two-year data were evaluated by analysis of variance (ANOVA) using SAS software (SAS Institute, Cary, NC, USA) statistical program and means were then compared with Duncan's multiple range tests (DMRT) at $p < 0.05$. Mean values are presented in tables.

3. Results

3.1. The Growth and Tuber Yield

The field trials demonstrated that the potato growth and tuber yield were significantly affected by both PGPR and HA treatment. The two-year mean potato growth and tuber yield values in response to PGPR and HA treatments compared with control and recommended doses of NPK are presented in Table 3. Highly significant increases were observed in potato growth, tuber yields, and quality after inoculation with both PGPR (*Bacillus* strains) and PGPR mixed culture (*Bacillus megatorium* M3 and *Bacillus subtilis* OSU-142), and further improved after combined application with humic acid. When compared to PGPR treatments both with and without HA, it was observed that PGPR mixed culture inoculation was more effective when compared to single inoculations. The tallest plant height and largest tuber weight values (63.2 cm and 132.8 g, respectively) were obtained with co-application of M3+OSU mixed culture inoculation with HA 400 kg ha^{-1} (71.7% and 118.8% increases in plant height and tuber weight relative to control, respectively), while the greatest stem and tuber number per plant

(5.2 and 14.3 per plant, respectively) were obtained with the recommended 100% NPK fertilizer dose (Table 3).

Table 3. The effects of humic acid (HA) and plant growth promoting rhizobacteria (PGPR) treatments on potato growth and tuber yield.

	Plant Height (cm)	No. of Stems (per plant)	No. of Tubers (per plant)	Tuber Weight (g)	Tuber Yield (t ha^{-1})			
					Unmarketable (<35 mm)	Marketable (35–50 mm)	Industrial (>50 mm)	Total
Control	36.8 k *	2.5 i	9.9 hi	60.7 j	2.2 gh	11.7 hg	14.2 l	28.3 k
NPK 50%	57.6 c	4.5 b	11.1 ef	80.0 h	3.2 bc	12.7 g	25.7 h	41.5 h
NPK 100%	60.5 b	5.2 a	14.3 a	100.3 e	2.7 ef	17.3 c	40.0 e	59.9 c
M3	42.6 j	3.0 h	9.8 i	82.6 gh	2.1 h	7.5 j	28.6 g	38.2 i
OSU	44.4 ij	3.3 gh	10.5 hg	95.7 f	2.2 gh	11.0 h	39.8 e	52.6 f
M3OSU	44.6 ij	3.4 efg	10.6 fg	106.9 d	2.1 h	9.2 i	42.8 d	54.7 e
HA200	47.0 h	3.3 gh	11.3 e	66.5 i	1.1 j	14.2 f	16.8 k	32.1 j
HA400	50.6 fg	3.7 def	11.9 cd	80.4 h	1.7 i	19.8 b	19.3 j	40.7 h
HA600	53.8 d	4.5 b	12.3 c	85.5 g	3.6 a	23.3 a	22.9 i	49.7 g
M3H200	44.8 i	3.4 efg	11.3 e	100.0 e	3.3 b	9.5 i	35.2 f	48.0 g
M3H400	48.8gh	3.7 def	13.7 b	95.5 f	3.6 a	16.9 c	35.5 f	56.0 de
M3H600	56.7 c	3.8 de	13.6 b	95.7 f	2.8 de	14.5 ef	38.4 e	57.3 d
OSUH200	51.3 ef	3.6 efg	12.0 cd	107.3 d	2.7 ef	12.8 g	42.6 d	58.0 cd
OSUH400	52.7 de	4.0 cd	13.5 b	117.3 c	2.5 gh	15.7 de	46.2 c	64.4 b
OSUH600	61.4 ab	4.6 b	13.2 b	115.7 c	2.9 de	12.8 g	50.8 b	66.5 a
M3OSUH200	52.6 de	3.7 de	11.9 cd	115.2 c	2.1 h	16.6 cd	44.1 d	62.8 b
M3OSUH400	63.2 a	4.6 b	11.4 de	132.8 a	3.0 cd	9.00 i	56.0 a	68.0 a
M3OSUH600	61.7 ab	4.3 bc	12.4 c	124.7 b	2.7 ef	14.9 ef	50.1 b	68.0 a

* $p < 0.05$ (Means followed by different letters are different by Duncan's multiple range tests (DMRT)).

Total and classified potato tuber yields were significantly affected by both PGPR and HA treatments, however co-application significantly increased the tuber yield when compared to single treatments (Table 3). The treatment combinations of M3 + OSU + 400 kg ha^{-1} HA, M3 + OSU + 600 kg ha^{-1} HA and OSU+ 600 kg ha^{-1} HA were the most efficient treatments in increasing total potato tuber yield 140% and 135% increases compared to the control, respectively). On the other hand, conventionally recommended 100% inorganic fertilizer treatment (NPK 100%) only led to an increase in total potato tuber yield by 111% when compared to the control. Furthermore, separate PGPR and HA applications significantly decreased unmarketable tuber yield when compared to the control plants. The lowest unmarketable potato tuber yield (1.1 t ha^{-1}) was obtained from the single HA 200 kg ha^{-1} treatment (50% decreases when compared to control), while the highest values were obtained with co-application of M3 inoculation with HA 400 kg ha^{-1} (3.6 t ha^{-1}) and the single HA 600 kg ha^{-1} treatment (3.6 t ha^{-1}). The single HA600 treatments was also the most efficient treatment especially in increasing marketable potato tuber yield (23.3 t ha^{-1}), and significantly increased marketable tuber yield by 99.1% and 34.7% when compared to the control and full NPK100% treatment, respectively. Furthermore, the effect of co-application of mono M3+OSU mixed culture inoculation and in the presence of HA 400 kg ha^{-1} and HA 600 kg ha^{-1}, which significantly promoted industrial potato tuber yield (66.5, 68.0 and 68.0 t ha^{-1}, respectively) was significant. Thus, a significant decrease was observed in marketable potato tuber yield with administration of both mono M3+OSU mixed culture and with 400 kg ha^{-1} HA dose.

3.2. The Tuber Quality and Mineral Contents

Tuber size and shape, the most important physical quality characteristics for potato, were significantly affected by PGPR and HA treatments based on the two- year average data (Table 4). Both PGPR and HA treatment significantly increased tuber size; however, the highest effect was observed with combined application of M3 + OSU + 400 kg ha^{-1} HA and M3 + OSU + 600 kg ha^{-1} HA, in particular in tuber length (83.5 and 83.3 mm, respectively). Similarly, the highest specific gravity, dry matter and starch contents were obtained with co-application of M3 + OSU mixed culture inoculation and HA 400 kg ha^{-1} (1.081 g cm^3, 20.0%, and 14.2%, respectively), the highest protein content was obtained with OSU bacterial treatment in HA 600 kg ha^{-1} with 11.4% (Table 4).

Table 4. The effects of HA and PGPR treatments on certain physical and chemical quality properties of potato tubers.

	Tuber Size		Tuber Shape		Specific Gravity (g cm^3)	Dry Matter (%)	Starch (%)	Protein (%)
	Width (mm)	Length (mm)	Index Value	Shape				
Control	49.1 j *	61.8 e	125.9	Short-Oval	1.067 l	16.9 l	11.4 k	7.4 i
NPK 50%	56.2 h	70.3 d	125.1	Short-Oval	1.069 k	17.3 k	11.8 j	8.8 gh
NPK 100%	57.0 gh	79.9 abc	140.2	Oval	1.078 d	19.2 cd	13.2 ef	9.7 e
M3	56.3 h	70.0 d	124.3	Short-Oval	1.071 i	17.8 i	12.2 i	9.0 f
OSU	58.5 fgh	78.1 abc	133.5	Oval	1.075 g	18.6 g	12.9 g	9.8 e
M3OSU	60.3 def	79.5 abc	131.8	Oval	1.076 f	18.9 f	13.1 f	10.1 d
HA200	49.7 ij	63.0 e	126.8	Short-Oval	1.069 k	17.4 j	11.9 j	8.7 h
HA400	51.6 i	64.3 e	124.6	Short-Oval	1.073 h	18.2 h	12.5 h	8.9 fg
HA600	51.8 i	64.4 e	124.3	Short-Oval	1.077 de	18.9 ef	13.2 ef	9.7 e
M3H200	67.7 a	75.0 cd	110.8	Short-Oval	1.073 h	18.2 h	12.5 h	9.8 e
M3H400	62.4 bcd	76.1 c	122.0	Short-Oval	1.075 g	18.6 g	12.9 g	10.7 c
M3H600	62.9 bc	77.1 bc	122.6	Short-Oval	1.078 d	19.2 cd	13.4 d	11.0 b
OSUH200	59.7 ef	78.1 abc	130.8	Oval	1.075 g	18.6 g	12.9 g	10.7 c
OSUH400	61.3 cde	82.3 ab	134.3	Oval	1.076 f	18.9 ef	13.5 cd	11.0 b
OSUH600	60.2 def	79.3 abc	131.7	Oval	1.079 c	19.3 c	13.6 c	11.4 a
M3OSUH200	63.9 b	77.9 abc	121.9	Short-Oval	1.077 de	19.1 de	13.3 de	10.7 c
M3OSUH400	59.7 ef	83.5 a	140.1	Oval	1.081 a	20.0 a	14.2 a	11.0 b
M3OSUH600	59.0 efg	83.3 a	141.5	Oval	1.080 b	19.6 b	13.8 b	11.1 b

* $p < 0.05$ (Means followed by different letters are different by DMRT).

The two years long field trials demonstrated that potato tuber mineral element content was significantly affected by both PGPR and HA treatments, however integrated administration significantly increased the mineral concentrations in tubers (except for Cu) when compared to mono treatments (Table 5). Similarly, it was also observed that PGPR's mixed culture inoculation was more effective on potato mineral content when compared to mono-inoculations. The highest P, K, Mg, Fe, Zn, and Mn levels were obtained in HA 600 kg ha^{-1} with M3 + OSU bacterial treatment (82.1, 51.1, 79.2, 90.2, 69.4, and 91.6% increases in P, K, Mg, Fe, Zn, and Mn when compared to control, respectively) and the highest Ca level was obtained with M3 bacterial treatment in HA 600 kg ha^{-1}. On the other hand, HA and PGPR treatments significantly decreased tuber Cu concentrations when compared to the controls (Table 5). Also, the highest Cu concentration was observed with 100% and 50% recommended inorganic fertilizer (NP100% and NP50%) treatments.

Table 5. The effects of HA and PGPR treatments on certain mineral compositions in potato tubers.

	P (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)
Control	1.62 j *	17.60 h	0.73 j	1.06 k	60.85 k	11.94 k	4.50 b	6.20 i
NPK 50%	1.91 i	18.91 g	0.88 i	1.34 i	64.45 k	16.32 fg	4.67 a	7.08 h
NPK 100%	2.76 c	22.52 c	1.25 d	1.70 c	98.35 d	16.25 fg	4.73 a	9.34 cd
M3	2.47 f	19.43 fg	1.09 f	1.32 i	76.29 i	14.60 ij	3.94 d	7.16 h
OSU	2.37 gh	20.25 ef	0.97 h	1.44 h	91.26 f	15.54 gh	3.70 f	7.68 fgh
M3OSU	2.65 de	22.37 c	0.97 h	1.68 d	99.91 d	17.02 ef	3.40 h	8.44 ef
HA200	1.92 i	17.60 h	0.89 i	1.30 j	71.40 j	15.20 hi	4.19 c	7.39 gh
HA400	2.36 h	21.03 de	1.10 f	1.35 i	81.40 g	14.41 j	3.87 de	7.98 efg
HA600	2.68 cde	22.52 c	1.17 e	1.65 e	91.16 f	13.99 j	3.58 g	8.36 ef
M3H200	2.69 cd	19.97 f	1.28 c	1.44 h	78.81 h	15.78 gh	3.80 e	7.30 gh
M3H400	2.76 c	21.03 de	1.31 c	1.52 g	92.97 f	16.95 ef	3.69 f	8.75 de
M3H600	2.85 b	21.78 cd	1.66 a	1.53 g	104.68 c	17.84 d	3.53 g	9.51 c
OSUH200	2.45 fg	21.70 cd	1.02 g	1.51 g	95.69 e	16.99 ef	3.57 g	8.58 e
OSUH400	2.52 f	22.05 c	1.19 e	1.59 f	104.27 c	18.25 cd	3.38 h	9.54 c
OSUH600	2.61 e	24.53 b	1.31 c	1.58 f	109.99 c	18.92 bc	3.31 hi	10.68 b
M3OSUH200	2.76 c	23.85 b	1.07 f	1.73 c	103.01 c	17.72 de	3.25 ij	9.61 c
M3OSUH400	2.86 b	25.90 a	1.18 e	1.79 b	112.02 b	19.27 b	3.24 ij	11.64 a
M3OSUH600	2.95 a	26.60 a	1.54 b	1.90 a	115.73 a	20.23 a	3.16 j	11.88 a

* $p < 0.05$ (Means followed by different letters are different by DMRT).

4. Discussion

In the present study, the integrated and individual effects of plant growth-promoting rhizobacteria (PGPR) *Bacillus subtilis* and *Bacillus megatorium* and humic substances, on potato crop performance, quality, and nutrient uptake were demonstrated under field conditions. These two strains in *Bacillus* genus are known for their effects that supported by the capacities to produce indole-3-acetic acid (IAA) and 1–aminocyclopropane–1-carboxylate (ACC) deaminase, to fix nitrogen or to solubilize phosphorus, and their biocontrol capacity against a wide range of bacterial and fungal pathogens that lead to significant economic losses in agricultural crops [26–28]. The literature reported the use of different bacterial genera as a feasible strategy to promote plant growth and nutrient uptake in different crops. These studies included wheat with *Azospirillum*, *Pseudomonas*, *Providencia*, and Anabaena [32–34]; sugar beet and barley with *Bacillus, Paenibacillus, Pseudomonas, and Rhodobacter* [26,28]; sugarcane with *Herbaspirillum, Gluconacetobacter* [35], sunflower with *Bacillus, Enterobacter* [36], rice with *Pseudomonas* and *Chryseobacterium* [37]; bean with *Trichoderma* [38], canola with *Azotobacter, Azospirillum,* and *Paenibacillus* [39]; maize with *Herbaspirillum,* Trichoderma, *Pseudomonas,* and *Bacillus* [11,40]; and soybean with *Azospirillum* [41]. However, only limited data were reported on PGPR colonization and growth promotion in potato plants. The literature on PGPR regarding potato included mostly *Pseudomonas, Bacillus*, and *Azosprillum* mono inoculation which were used to improve nitrogen and phosphorus uptakes [42–44], IAA production and biocontrol activity [45–47]. Recently, the co-administration of *Bacillus* genus and HA as important plant bio-stimulants was reported to be the most effective biologically active natural substances that contribute to e improving the growth, yield, and nutrient uptake in different crops [12,48], while reducing the dependency on chemical fertilizers. However, until now, no data were available on the integrated use of this genus with humic acid in potato, and, to our knowledge, this was the first study, where *B. megatorium and B. subtilis* strains and different HA concentrations, were used individually or in combination to improve potato production and quality in field conditions.

In the field experiments conducted in the present study it was observed that potato growth, quality and tuber nutrient content were affected at different levels by inoculation with both PGPR (*Bacillus* strains) and PGPR mixed culture (*Bacillus megatorium* M3 and *Bacillus subtilis* OSU-142), and further improved when applied in combination with humic acids. Although, significant differences were observed in treatments where inoculation was conducted with only PGPR strains without HA application, the PGPR mixed culture (M3OSU) was observed to be more effective when compared to mono-inoculations. Potato growth, tuber yield and quality were strongly improved by inoculation with mixed culture, resulting in an increased tuber weight by 76.1%, total tuber yield by 93.3%, tuber width and length by 22.8% and 28.6%, tuber dry matter, starch and protein contents by 11.8%, 14.9%, and 36.5% respectively, and tuber nutrient content such as P, K, Mg, Fe, and Zn increased by 63.6%, 27.1%, 58.5%, 64.2%, and 42.5% when compared to the control. These results were consistent with those reported by Orhan et al. [27], who reported that inoculation of mixed culture of two *Bacillus* strains (OSU-142+M3) significantly increased yield (74.9%), N (60%), P (%433), Fe (64.4%), Ca (%64), and Mn (117.0%) content in raspberry leaves when compared to the control under organic growth conditions. Previous microbial studies indicated that the N-fixing and phosphorus solubilizing bacterial strains had distinctive PGPR properties, providing nutrients and stimulating each other through physical and biochemical activities that may enhance certain beneficial physiological properties [49]. Thus, the innate PGPR potential of the strains may lead to differential growth in plants [47]. Depending on the strain combination, microbial interactions in these mixed cultures could have either positive or negative effects on inoculant establishment and may result or not lead to improved plant growth when compared to mono inoculation [16,27,49]. The largest and most consistent increases in shoot and root length, total biomass, total chlorophyll, and yield were reported with *Bacillus*, *Brevibacillus*, *Acinetobacter*, and *Micrococcus* when grown in mixed culture under field conditions [49]. Various studies also reported that PGPR inoculation generally led to a significant increase in N, P, K, Ca, Mg, Fe, Cu, Mn, and Zn contents in plant tissues [26–28,50]. Thus, it is important to note that plant responses to PGPR seem to be highly dependent on plant species, strain characteristics and mode of inoculation.

Humic substances constitute a major portion of the organic matter. The physiological effects of humic substances are widely reported [2–5], and summarized improvement nutrient efficiency, promoting assimilation of both macro and micronutrients and plant growth by induction of carbon, nitrogen, and through secondary metabolism [6]. Xu et al [8] and Suh et al. [9] treated potato with different concentrations of HA applied to the soil and demonstrated improvements in soil and tuber mineral contents, root elongation, and fresh tuber weight and size, and particularly in the weight of extra-large tubers. Similar results were observed in the present study for humic application. Such promotion of potato plant growth, tuber quality and nutrient content with HA was determined in HA 600 kg ha^{-1} dose, however not in the unmarketable tuber yield (50% decrease was observed when compared to the control). Similarly, HA applications especially favored the more marketable and industrial-grade tuber yields when compared to the unmarketable tuber yield. The lowest unmarketable tuber yield was obtained with HA 200 kg ha^{-1} treatment, while the highest marketable tuber yield was obtained with HA 600 kg ha^{-1}. Greater tuber weight production in proportion to tuber yield may be explained by the stimulation of growth and tuber mineral nutrition in potato plant, which may have been positively correlated with higher photosynthetic rates due to the availability of sufficient nutrient elements and increased water use efficiency. Selim et al. [51] observed that co-administration of HA and 100% NPK (the recommended fertilization rate) was more efficient when compared to 50%, 75%, and control (100% of the recommended mineral fertilizer without HA) applications on stimulating potato tuber growth and quality.

The roles of humic substances in basic plant physiology were extensively studied, however little is known about their effects on beneficial bacteria. Canellas et al. [11] and Olivares et al. [10] recently reviewed basic mechanisms and benefits of combined application of humic substances and plant growth-promoting bacteria to various crop fields, and demonstrated that the combined application of endophytic diazotrophic bacteria and humic substances increased maize grain production by 65% and tomato fruit production by 87.1% under field conditions. These findings were consistent with the present study findings. The combined HA and PGPR administration produced the best response, yielding the highest increases in growth, tuber yield, and nutrient content of potato under field conditions. Furthermore, the findings demonstrated that mixed culture (*Bacillus subtilis* OSU-142, *Bacillus megatorium* M3) inoculation with humic acid proved more effective on the increase in potato production when compared to mono-inoculation, which it might be linked to the ability of these strains to fix nitrogen and to solubilize phosphorus, as well as producing high IAA levels, thus improving root elongation and lateral root development [26–28]. In addition, previous studies reported that humic substances directly affected plant growth by inducing an increase in the absorptive surface area of roots, especially lateral root emergence [52]. Recently, Olivares et al. [16] reported that the most prominent morphological modification in plants was induced by both humic substances and PGPR, including the promotion of lateral roots emergence. Although there is no experimental data that demonstrated whether humic substances and PGPB affected leaf chlorophyll content and photosynthetic ability, apparently the mode of action of both might be partially attributed to the N-uptake/assimilation and IAA-like growth-regulating phytohormone activities. Canellas et al. [11] reported that a combination of bacteria and humic substances increased the net photosynthetic rate with the increase in humate concentration and *Herbaspirillum seropedicae* could in vitro produce IAA phytohormone. Chi et al. [53] reported that rice plants inoculated with various PGPR species showed increased photosynthetic rate, stomatal conductance, transpiration velocity, water utilization efficiency, flag leaf area, and accumulated higher levels of indoleacetic acid and gibberellins growth-regulating phytohormones. Thus, combining the benefits of humic substances and PGPR may provide higher plant performance and nutrient uptake, and this may ultimately lead to a well-established, vigorous, and healthy plant.

In the present study, the greatest changes in potato growth, tuber yield and quality were induced by co-administration of PGPR mixed culture (M3OSU) and HA 400 kg ha^{-1}, resulting in increased plant height by 71.7, tuber weight by 118.8%, industrial tuber yield by 294.3%, total tuber yield by 140.3%, tuber length by 35.1%, specific gravity by 1.31%, tuber dry matter, starch and protein contents by 18.3%, 24.6%, and 48.6% respectively. On the other hand, M3OSU inoculation with HA 600 kg ha^{-1} also proved to be the most effective application in improving the tuber nutrient contents such as P, K, Mg, Fe, Zn, and Mn when compared to control plants, while the increase in these nutrient contents reached 82.1%, 51.1%, 79.3%, 90.2%, 69.4%, and 91.6% respectively.

Various studies also reported synergistic effects induced by PGPR and humic substance on growth, nutrient uptake, and yield of various crops [10–12]. Baldatto et al. [54] demonstrated that pineapple growth was affected by *Burkholderia* strain inoculations, and further improvements were observed with combined administration with humic acids, leading to higher shoot and root biomass, as well as nutrient contents (N 132%, P 131%, K 80%) when compared to uninoculated plantlets. The present study findings were consistent with the studies mentioned above and those described by Schoebitz et al. [13], who reported that the combined administration of microbial consortium and HA increased N and K uptake and growth in blueberry plants, recording a 50% increase in shoot dry weight and a 43% increase in root dry weight when compared to the control plants.

5. Conclusions

In conclusion, given the promotion of the plant growth, improvement of tuber nutritive value and quality and improvement in tuber size and weight in potato plants co-inoculated with *Bacillus* strains and humic acid, a significant improvement was observed in potato production. Despite a low total tuber yield (28.3 t ha−1) in control treatment, combined inoculation increased potato production by around 140% which was with in the high range of the increase expected for both *Bacillus* strains and humic acid mono treatments. The stability and increased consistency of the potato plant response to bacterial inoculation in the presence of humic acid indicated a promising biotechnological tool to improve growth and adaptation of potatoes to field conditions.

Funding: This research received no external funding.

Acknowledgments: The author appreciates the valuable contributions by M. Figen Dönmez (who kindly provided the PGPR), Prof. Ismail Hakki Ekin (Department of Microbiology), Faruk Oguz (technical support), and Prof. Abdullah Yesilova (statistical analyses).

Conflicts of Interest: The author declares no conflict of interest.

References

1. Food and Agriculture Organization. *The State of Food Insecurity in the World 2019: Strengthening the Enabling Environment for Food Security and Nutrition*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2019. Available online: https://www.fao.org (accessed on 2 January 2019).
2. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in Plant Science: A Global Perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [CrossRef] [PubMed]
3. Du Jardin, P. Plant Biostimulants: Definition, Concept, Main Categories and Regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
4. Halpern, M.; Bar-Tal, A.; Ofek, M.; Minz, D.; Muller, T.; Yermiyahu, U. The Use of Biostimulants for Enhancing Nutrient Uptake. *Adv. Agron.* **2015**, *130*, 141–174. [CrossRef]
5. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural Uses of Plant Biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
6. Canellas, L.P.; Olivares, F.L. Physiological responses to humic substances as plant growth promoter. *Chem. Biol. Technol. Agric.* **2014**, 1–3. [CrossRef]
7. Puglisi, E.; Pascazio, S.; Suciu, N.; Cattani, I.; Fait, G.; Spaccini, R.; Crecchio, C.; Piccolo, A.; Trevisan, M. Rhizosphere microbial diversity as influenced by humic substance amendments and chemical composition of rhizodeposits. *J. Geochem. Explor.* **2013**, *129*, 82–94. [CrossRef]

8. Xu, S.; Zhang, L.; McLaughlin, N.B.; Mi, J.; Chen, Q.; Liu, J. Effect of synthetic and natural water absorbing soil amendment soil physical properties under potato production in a semi-arid region. *Soil Tillage Res.* **2015**, *148*, 31–39. [CrossRef]
9. Suh, H.Y.; Yoo, K.S.; Suh, S.G. Tuber growth and quality of potato (*Solanum tuberosum* L.) as affected by foliar or soil application of fulvic and humic acids. *Hortic. Environ. Biotechnol.* **2014**, *55*, 183–189. [CrossRef]
10. Olivares, F.L.; Aguiar, N.O.; Rosa, R.C.C.; Canellas, L.P. Substrate biofortification in combination with foliar sprays of plant growth promoting bacteria and humic substances boosts production of organic tomatoes. *Sci. Hortic.* **2015**, *183*, 100–108. [CrossRef]
11. Canellas, L.P.; Balmori, D.M.; Médici, L.O.; Aguiar, N.O.; Campostrini, E.; Rosa, R.C.C.; Façanha, A.R.; Olivares, F.L. A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (*Zea mays* L.). *Plant Soil* **2013**, *366*, 119–132. [CrossRef]
12. Esringü, A.; Kaynar, D.; Turan, M.; Ercisli, S. Ameliorative effect of humic acid and plant growth-promoting rhizobacteria (PGPR) on Hungarian vetch plants under salinity stress. *Commun. Soil Sci. Plant Anal.* **2016**, *47*, 602–618. [CrossRef]
13. Schoebitz, M.; López, M.D.; Serrí, H.; Martínez, O.; Zagal, E. Combined application of microbial consortium and humic substances to improve the growth performance of blueberry seedlings. *J. Soil Sci. Plant Nutr.* **2016**, *16*, 1010–1023. [CrossRef]
14. Shah, F.; Wu, W. Soil and crop management strategies to ensure higher crop productivity within sustainable environments. *Sustainability* **2019**, *11*, 1485. [CrossRef]
15. Bashan, Y.; De Bashan, L.E.; Prabhu, S.R.; Hernandez, J.P. Advances in plant growth promoting bacterial inoculant technology: Formulations and practical perspectives (1998–2013). *Plant Soil* **2014**, *378*, 1–33. [CrossRef]
16. Olivares, F.L.; Busato, J.G.; de Paula, A.M.; Lima, L.S.; Aguiar, N.O.; Canellas, L.P. Plant growth promoting bacteria and humic substances: Crop promotion and mechanisms of action. *Chem. Biol. Technol. Agric.* **2017**, *4*, 30. [CrossRef]
17. Ryan, J.; Estefan, G.; Rashid, A. *Soil and Plant Analysis Laboratory Manual*, 2nd ed.; ICARDA: Aleppo, Syria, 2012; ISBN1 10-8172337655, ISBN2 13-978-8172337650.
18. Bouyoucos, G.S. Recalibration of the hydrometer methods for making mechanical analysis of soil. *Agronomy J.* **1951**, *43*, 434–438. [CrossRef]
19. Richards, L.A. *Diagnosis and Improvement Saline and Alkaline Soils*; United States Department of Agriculture: Washington, DC, USA, 1954; p. 60.
20. McLean, E.O. Soil pH and lime requirement. In *Methods of Soil Analysis. Part II. Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Miller, R.H., KeeneyAgronomy, D.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1982; pp. 199–224.
21. Bremner, J.M. Nitrogen-total. In *Methods of Soil Analysis, Part III*; Bartels, J.M., Bigham, J.M., Eds.; American Society of Agronomy: Madison, WI, USA, 1996; pp. 1085–1121.
22. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; USDA, U.S. Government Printing Office: Washington, DC, USA, 1954.
23. Nelson, D.W.; Sommers, L.E. Organic matter. In *Methods of Soil Analysis Part II. Chemical and Microbiological Properties*, 2nd ed.; Norman, A.G., Ed.; Agronomy: Madison, WI, USA, 1982; pp. 574–579.
24. Thomas, G.W. Exchangable cations. In *Methods of Soil Analysis, Part II, Chemical and Microbiological Properties*, 2nd ed.; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; Agronomy Monograph: Madison, WI, USA, 1982; pp. 159–165.
25. Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [CrossRef]
26. Çakmakçi, R.; Kantar, F.; Sahin, F. Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. *J. Plant Nutr. Soil Sci.* **2001**, *164*, 527–531. [CrossRef]
27. Orhan, E.; Esitken, A.; Ercisli, S.; Turan, M.; Fikrettin, S. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Sci. Hort.* **2006**, *111*, 38–43. [CrossRef]
28. Çakmakçi, R.; Dönmez, F.; Aydin, A.; Sahin, F. Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem.* **2006**, *38*, 1482–1487. [CrossRef]

29. Högy, P.; Fangmeier, A. Atmospheric CO_2 enrichment affects potatoes: 2. Tuber quality traits. *Eur. J. Agron.* **2009**, *30*, 85–94. [CrossRef]
30. Esendal, E. *Potato*; University of Ondokuz Mayis, Faculty of Agriculture: Samsun, Turkey, 1990; p. 221, ISBN 975-7636-06-1.
31. Edgar, A.D. Determining the specific gravity of individual potatoes. *Am. Potato J.* **1951**, *28*, 729–731. [CrossRef]
32. Rana, A.; Joshi, M.; Prasanna, R.; Shivay, Y.S.; Nain, L. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *Eur. J. Soil Biol.* **2012**, *50*, 118–126. [CrossRef]
33. Rosas, S.B.; Avanzin, G.; Carlier, E.; Pasluosta, C.; Pastor, N.; Rovera, M. Root colonization and growth promotion of wheat and maize by *Pseudomonas aurantiaca* SR1. *Soil Biol. Biochem.* **2009**, *41*, 1802–1806. [CrossRef]
34. Hungria, M.; Campo, R.J.; Souza, E.M.; Pedrosa, F.O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil* **2010**, *331*, 413–425. [CrossRef]
35. Silva, S.F.; Olivares, F.L.; Canellas, L.P. The biostimulant manufactured using diazotrophic endophytic bacteria and humates is effective to increase sugarcane yield. *Chem. Biol. Technol. Agric.* **2017**, *4*, 24. [CrossRef]
36. Shahid, M.; Hameed, S.; Imran, A.; Ali, S.; Elsas, J.D. Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. *World J. Microbiol. Biotechnol.* **2012**, *28*, 2749–2758. [CrossRef]
37. Lucas, J.A.; Ramos Solano, B.; Montes, F.; Ojeda, J.; Megias, M.; Gutierrez Mañero, F.J. Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa* L.) in Southern Spain. *Field Crop. Res.* **2009**, *114*, 404–410. [CrossRef]
38. Hoyos-Carvajal, L.; Orduz, S.; Bissett, J. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biol. Control* **2009**, *51*, 409–416. [CrossRef]
39. El-Howeity, M.A.; Asfour, M.M. Response of some varieties of canola plant (*Brassica napus* L.) cultivated in a newly reclaimed desert to plant growth promoting rhizobacteria and mineral nitrogen fertilizer. *Ann. Agric. Sci.* **2012**, *57*, 129–136. [CrossRef]
40. Thonar, C.; Lekfeldt, J.D.S.; Cozzolino, V.; Kundel, D.; Kulhánek, M.; Mosimann, C.; Neumann, G.; Piccolo, A.; Symanczik, S.; Walder, F. Potential of three microbial bio-effectors to promote maize growth and nutrient acquisition from alternative phosphorous fertilizers in contrasting soils. *Chem. Biol. Technol. Agric.* **2017**, *4*, 7. [CrossRef]
41. Cassán, F.; Perrig, D.; Sgroy, V.; Masciarelli, O.; Penna, C.; Luna, V. *Azospirillum brasilense* Az39 and Bradyrhizobium japonicum E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). *Eur. J. Soil Biol.* **2009**, *45*, 28–35. [CrossRef]
42. Hanif, M.K.; Hameed, S.; Imran, A.; Naqqash, T.; Shahid, M.; Van Elsas, J.D. Isolation and characterization of a b-propeller gene containing phosphobacterium *Bacillus subtilis* strain KPS-11 for growth promotion of potato (*Solanum tuberosum* L.). *Front. Microbiol.* **2015**, *6*, 583. [CrossRef]
43. Naqqash, T.; Hameed, S.; Imran, A.; Hanif, M.K.; Majeed, A.; Van Elsas, J.K. Differential Response of Potato Toward Inoculation with Taxonomically Diverse Plant Growth Promoting Rhizobacteria. *Front. Plant Sci.* **2016**, *7*, 144. [CrossRef]
44. Abbas, M.T.; Hamza, M.A.; Youssef, H.H.; Fayez, M.; Monib, M.; Heqazi, N.A. Bio-preparates support the productivity of potato plants grown under desert farming conditions of north Sinai: Five years of field trials. *J. Adv. Res.* **2013**, *5*, 41–48. [CrossRef]
45. Andreote, F.D.; De Araújo, W.L.; De Azevedo, J.L.; Van Elsas, J.D.; Da Rocha, U.N.; Van Overbeek, L.S. Endophytic colonization of potato (*Solanum tuberosum* L.) by a novel competent bacterial endophyte, *Pseudomonas putida* strain P9, and its effect on associated bacterial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 3396–3406. [CrossRef]
46. Calvo, P.; Ormeño-Orrillo, E.; Martínez-Romero, E.; Zúñiga, D. Characterization of *Bacillus* isolates of potato rhizosphere from Andean soils of Peru and their potential PGPR characteristics. *Braz. J. Microbiol.* **2010**, *41*, 899–906. [CrossRef]

47. Ghyselinck, J.; Velivelli, S.L.; Heylen, K.; O'Herlihy, E.; Franco, J.; Rojas, M.; De Vos, P.; Prestwich, B.D. Bioprospecting in potato fields in the central Andean highlands: Screening of Rhizobacteria for plant growth promoting properties. *Syst. Appl. Microbiol.* **2013**, *36*, 116–127. [CrossRef]
48. Pishchik, V.N.; Vorobyov, N.I.; Walsh, O.S.; Surin, V.G.; Khomyakov, Y.V. Estimation of synergistic effect of humic fertilizer and *Bacillus subtilis* on lettuce plants by reflectance measurements. *J. Plant Nutr.* **2016**, *39*, 1074–1086. [CrossRef]
49. Jha, C.K.; Saraf, M. Evaluation of multispecies plant-growth promoting consortia for the growth promotion of *Jatropha curcas* L. *J. Plant Growth Regul.* **2012**, *31*, 588–598. [CrossRef]
50. Yolcu, H.; Gunes, A.; Gullap, M.K.; Cakmakci, R. Effects of plant growth-promoting rhizobacteria on some morphologic characteristics yield and quality contents of Hungarian Vetch. *Turk. J. Field Crop.* **2012**, *17*, 208–214.
51. Selim, E.M.; El-Neklawy, S.A.S.; El-Ashry, M. Beneficial effects of humic substances fertigation on soil fertility to potato grown on sandy soil. *Aust. J. Basic Appl. Sci.* **2009**, *3*, 4351–4358.
52. Schmidt, W.; Santi, S.; Pinton, R.; Varanini, Z. Water-extractable humic substances alter root development and epidermal cell pattern in *Arabidopsis*. *Plant Soil* **2007**, *300*, 259–267. [CrossRef]
53. Chi, F.; Shen, S.H.; Cheng, H.P.; Jing, Y.X.; Yanni, Y.; Dazzo, F.B. Ascending migration of endophytic rhizobia from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Appl. Environ. Microbiol.* **2005**, *71*, 7271–7277. [CrossRef]
54. Baldotto, L.E.B.; Baldotto, M.A.; Canellas, L.P.; Bressan-Smith, R.; Olivares, F.L. Growth promotion of pineapple 'vitória' by humic acids and *Burkholderia* spp. during acclimatization. *Rev. Bras. Cienc. Solo* **2010**, *34*, 1593–1600. [CrossRef]