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Verification of the humic substances and PGPB biostimulants beneficial effects on the potato yield and bioactive substances content

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ABSTRACT

Potatoes are one of the most important sources of nutrients worldwide, but excessive doses of industrial fertilizers are usually used to achieve higher yields. Soil biostimulants are an increasingly used alternative for reducing fertilizer doses and growing healthy agricultural products. In this study, we examined the effects of humic substances (Agriful) and beneficial bacteria (Groundfix) based biostimulants applied by dripping irrigation on the yield and quality of potato tubers in comparison with the conventional N fertilization system. The small trail field experiment was founded in the growing season of 2020 in the Botanical Garden of the Slovak University of Agriculture in Nitra. The highest tubers yield had the combination of biostimulants and N fertilizer – 195.16% above to control. Simultaneously this combination reached an increase in refractometric dry matter content, starch content – 3.6%, and vitamin C content – 20% increase above to control. The Groundfix variant had the highest antioxidant activity with a 16.2% difference compared to the conventional nitrogen fertilization variant. These results show the positive effect of applied biostimulants on the yield and quality of cultivated potatoes.

Keywords: potatoes, yield, biostimulants, humic substances, beneficial bacteria

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) as one of the most important sources of antioxidants and energy (about 14%) in the human diet are together with rice and wheat the most important food crops for human consumption **[1]**, **[2]**, **[4]**. Tubers are a great source of carbohydrates, resistant starch, quality proteins, and vitamin C **[2]**, **[3]**. Plays an important role in the production of antioxidant defense systems by providing essential nutrient antioxidants, such as vitamins, β-carotene, polyphenols, and minerals (especially potassium) **[5]**. *Solanum tuberosum* L. is a typical plant that loves hummus in terms of soil and ecological conditions **[6]**. They prefer heated, deep, light to medium-heavy soils that are not too rocky and have an even water supply, however management of nutrients, especially nitrogen (N), is one of the most important factors in potato production. Within agronomic operations, soil compaction should be avoided as much as possible to achieve quality tuber yields **[7]**, **[8]**. Cropping systems and management practices that improve soil health may greatly enhance crop productivity **[9]**.

Since 1900, soil organic matter has declined drastically in farmlands around the world because of carbon turnover and cropping systems **[10]**. But it is only one of the problems farmers must face nowadays. Feeding the world's rising population is one of the biggest challenges, especially when the agriculture system is facing a multitude of complex problems arising from changing environments due to global climate change **[11]**. Current trends in agriculture are also focusing on enhancing the efficiency of fertilizer use since approximately 65% of applied mineral nitrogen is lost from the plant-soil system through gaseous emissions, runoff, erosion, and leaching **[12]**. Similarly, plant P uptake is inefficient due to poor soil P solubility, especially for potato (*Solanum tuberosum* L.) plants with relatively poor rooting efficiency **[13]**. To avoid excessive use of external inputs, without compromising potato crop performance, the increase of soil nutrient availability and nutrient use efficiency is fundamental in sustainable potato production **[19]**. Farmers start to understand that focusing on increasing fertilization rates may have a negative impact on food safety and the integrity of the ecosystem **[14]**. This is indicated by the fact that global demand for organic production is increasing by approx. 20% annually **[15]**. Plants are more and more affected by environmental stresses **[16]** while abiotic stresses strongly affect plant growth, development, and quality of production **[17]**. For these reasons plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy **[18]** and the reduction of environmental impact, ensuring both high and stable yield and high quality, is a primary goal in modern agriculture **[20]**. Various potato cultivation techniques have been developed for this purpose **[21]** but the increase of crop stress tolerance through genetic improvements requires long breeding programs and different cultivation environments for crop performance validation **[22]**. The horticultural sector is therefore searching for innovative and sustainable agronomic solutions **[23]** which simultaneously will bring repeated positive results, are easy to handle, and are reasonably priced **[24]**.

The use of plant biostimulants has gained substantial and significant heed worldwide as an environmentally friendly alternative to sustainable agricultural production **[11]**. Humic acid (HA) and plant growth-promoting rhizobacteria (PGPR) are among the most effective methods that utilize natural biologically active substances **[25]**. During evolution, plants have become associated with guilds of plant-growth-promoting rhizobacteria (PGPR), which raises the possibility that individual PGPR populations may have developed mechanisms to counteract one another on plant roots **[24]**. Currently, within industry and agricultural research, there is an increasing curiosity about microbial biostimulants, especially beneficial soil bacteria **[11]**. An increasing number of studies have illustrated the important role of microbiota in crop plant growth **[27]**, **[28]**. Increasing the presence of beneficial soil microorganisms in soil is considered a promising sustainable alternative to support conventional and organic fertilization and may help to improve crop health and productivity **[26]**. Different mechanisms are involved in bacteria-induced plant growth promotion, including biological nitrogen fixation (BNF), mineral solubilization, production of phytohormones, and pathogen biocontrol **[12]**. The claimed benefits of the tested microbial products include improving soil physical and biological health, stimulating beneficial microbial populations, increasing crop yield, root growth, plant establishment, drought tolerance, and reducing fertilizer requirements **[29]**. Each microorganism functions in coordination with the overall soil microbiome to influence plant health and crop productivity **[28]**. While there may not be one simple strategy that can effectively promote the growth of all plants under all conditions **[30]**, every new knowledge is very important. Humic substances (HS) are the major fraction of the soil organic matter which represent the final stage of a complex interaction between non-living organic matter and microbial communities and are among the most complex and biologically active compounds in the soil **[31]**, **[32]**. Their connection with humus quality plays dynamic roles in soil physical, chemical, and biological functions essential to soil health and plant growth **[33]**, **[10]**. Biostimulant effects of humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation, and distribution (nutrient use efficiency traits) **[34]**. It includes changes in root architecture and growth dynamics but is also extended to the major biochemical pathways since the driving force for most nutrient uptake is the electrochemical gradient across the plasma membrane **[32]**. Stimulatory effects of humic substances (HS) on plant growth have been observed and widely documented **[35]**, however recent research has shown that humic substances provide direct stimulation of plant growth under laboratory conditions **[36]**. They have many other supportive effects on plants **[34]**, **[37]** but the detailed nature of humic substances is still not very understood **[38]**. For example, the mitigating activity of HS can be defined as a phenomenon of lowering the adverse effects of contaminants toxicity and those of abiotic stress factors such as unfavorable temperature, pH, salinity, etc. As a rule, it is related to the detoxifying properties of HS or their beneficial effects on biota **[39]**.

If the application of single bio-effectors has shown satisfactory results, further improvements may arise by combining multiple beneficial soil microorganisms with natural bioactive molecules **[26]**. Enhanced knowledge of the effects on plants' physiology and biochemistry and interaction with rhizosphere and endophytic microbes should lead to achieving increased crop productivity through better use of HS inputs in Agriculture **[32]**. Combinations allow bacteria to interact with each other synergistically, providing nutrients and stimulating each other through physical and biochemical activities that may enhance some beneficial aspects of their physiology **[40]**. The stability and increased consistency of the potato plant response to bacterial inoculation in the presence of humic acid indicated should be a promising biotechnological tool to improve the growth, quality, and adaptation of potatoes to field conditions **[25]**. The challenge of this study was to verify the mutual effectiveness of two selected biostimulants, based on humic substances and PGPB.

Scientific hypothesis

The yield and quality of the potato tubers variety ´Spinela´ cultivated in the Botanical Garden of Slovak University of Agriculture will depend on the biostimulants and nitrogen application, while expected best results after the combined application of humic substances and beneficial bacteria due to their synergic effectivity.

MATERIAL AND METHODOLOGY

Samples

University of Agriculture (SUA) in Nitra, cultivated by the Institute of Horticulture of SUA (48°18'53" N, 18°5'15" E) in 2020. Within our research, we studied the effects of humic substances and beneficial bacteria biostimulant on potato tubers' total and marketable yield, together with their antioxidant activity and refractometric dry matter, starch, vitamin C, and total polyphenols content. Determination of refractometric dry matter, total polyphenols, vitamin C and antioxidant activity (AOA) in potatoes was carried out at the Institute of Horticulture of the Slovak University of Agriculture in Nitra. The analyses of total starch content took place in the laboratories of the Department of Storage and Processing of Plant Products of the FBP SPU in Nitra.

Chemicals

All chemicals were of analytical grade quality. For analyses of refractometric and gravimetric dry matter, we used distilled water. The following reagents were used for the tests of other qualitative parameters:
(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%, Acros Organics™, Denmark), 2,2-diphenyl-1-picrylhydrazyl (DPPH, ≤100%, Sigma Aldrich), Folin–Ciocalteu reagent (Merck Germany), gallic acid (GA, Sigma Aldrich), sodium carbonate (solution 20% w/w, Merck Germany), palladium nitrate ($Pd(NO3)_2$, palladium modifier, 0.1 $mol.l^{-1}$), ascorbic acid (AsA, solution 1%, w/w), methanol (pure pro analysis – purity grades of lab reagents, 70%, v/v, Fisher Scientific UK, Loughborough, UK). For starch content analyses HCl, 30% zinc sulfate solution and 15% potassium ferrocyanide solution was used. For Vitamin C analysis 2,6-dichlorophenolindophenol and 5% trichloroacetic acid solution was used.

Animals and Biological Material

Animal materials weren't used in this research. Groundfix is a bacterial biostimulant containing a rich spectrum of soil bacteria influencing plant growth and soil health condition: Bacillus subtilis, Bacillus megatherium, var. phosphaticum, Azotobacter chroococcum, Enterobacter, Paenibacillus polymyxa. The total number of living microorganisms is 0.5-1.5 x 109 CFU/cm^3. This biostimulant also contains other beneficial microorganisms (Lactobacilli, enzyme-producing bacteria), vitamins, phytohormones, amino acids, and other physiologically active substances. Agrigul is humic substances (HS) based biostimulant with a total content of humic substances 50% (humic acids 25% and fulvic acids 25%), nitrogen content (N) 4.5%, phosphorus content (P2O5) 1.0%, potassium content (K2O) 1.0%, remaining organic matter content 45.0%.

Instruments

The refractometric dry matter was measured by using a refractometer type CRUESS DR201-95.

Determination of antioxidant activity was performed with a spectrophotometer Jenway 6301 (Bibby Scientific Ltd., UK).

For determination of total polyphenols was used spectrophotometer Shimadzu UV/VIS-1240. The absorbance of the sample solution was measured at 765 nm of wavelength against blank.

Laboratory Methods

The tubers collected within each replicate and variant were weighed and subsequently sorted according to the applicable UN/ECE quality standards for tubers of late potato varieties for marketing and the proportion of marketable tubers was determined. From the measured data, we calculated the achieved total potato yield as well as the marketable yield of tubers. The refractometric dry matter was measured by measuring the average homogenized potato samples of the individual replications. The total dry matter content was determined by using the gravimetric weighing method. The average homogenized potato samples of the individual replications were dried at 105 °C up until the weight of the samples became stable. Determination of the starch content was carried out polarimetrically by the Ewers method. The polarimetric determination of starch content involves the conversion of starch by hydrolysis into an optically active substance. The analyses took place in the laboratories of the Faculty of Biotechnology and food science of the SUA in Nitra **[42]**. Determination of vitamin C content (ascorbic acid) was conducted by 2,6-Dichloroindophenol Titrimetric Method. Antioxidant activity (AOA) was determined by DPPH method and expressed as % inhibition of the DPPH radicals per g of sample and recalculated to Trolox equivalent (TE) **[43]**. The absorbance of the samples was measured at a wavelength of 517 nm (At_{30}).
Total polyphenol content (TPC) was estimated by using Folin–Ciocalteu method using gallic acid as a standard (GAE) spectrophotometrically at 765 nm according to **[44]** and calculated in milligram of GA equivalent (GAE) per kilogram dried weight (d.w.).

Figure 1 Solanum tuberosum L., variety ´Spinela´ (A), Experimental variant at the beginning of vegetation and (B), Sorting of tubers after harvesting.

Description of the Experiment

Sample preparation: Plant raw material (tubers) was weighed on laboratory weights and prepared in the labs at the Institute of Horticulture of SUA. Material for average samples was taken diagonally from the collected tubers and homogenized. 40 ml of methanol (70%, v/v) was added to 1 g of the homogenized mixture into 250 ml extraction flasks and left at room temperature for 20 h and then extracted with a horizontal shaker for 4 h **[80]**. We used 10 g of precisely weighed and homogenized mashed potatoes for individual starch content analyses and Vitamin C analyses.

Number of samples analyzed: We analysed 14 samples.

Number of repeated analyses: All biochemical procedures were duplicated – 2 qualitative analyses for each sample (variant) except for refractometric dry matter, where 3 analyses for each repetition were estimated.

Number of experiment replication: 7 reps. for each observed variant.

Design of the experiment: The small plot field experiment was established by the block method under open field conditions. The tubers were planted on 16 April 2020, with 13 tubers planted in each repetition in a single cultivation distance of 0.75 m x 0.3 m. The area of one experimental plot (repetition) was 3 m^2, and with four repetitions for variant, the area of one experimental variant was 12 m^2. Between the individual experimental variants was a protective line, the width of which was 1 m. The total area of the experiment was 108 m^2.

During the vegetation, additional sprinkler irrigation was applied as needed, based on climatic conditions and soil moisture status. Soil cultivation and weed control were carried out by manual hoeing. During the vegetation, the health status of the plants was monitored, and the plants were treated as necessary with authorized plant protection products. Potato harvesting took place once September 4, 2020.

In the experiment, we observed the differences in the biostimulating effect of selected biostimulants on selected quantitative and qualitative parameters of potato tubers. For these purposes, we have established 6 different variants:

- 1st variant (C) – control variant without applying N fertilizer and biostimulants.
- 2nd variant (N) – conventional variant with a full dose of N fertilizer, but without biostimulants
- 3rd variant (N + G) – conventional variant with a full dose of N fertilizer, with a combination of plant growth promoting bacteria biostimulant – Groudfix (G)
- 4th variant (N + G +A) – conventional variant with a full dose of N fertilizer, with a combination of plant growth promoting bacteria biostimulant – Groudfix (G) and biostimulant based on humic substances – Agriful (A)
- 5th variant (G +A) – variant without applying of N fertilizer, with a combination of plant growth promoting bacteria biostimulant (G) and biostimulant based on humic substances (A)
- 6th variant (G) – variant without applying of N fertilizer, with plant growth promoting bacteria biostimulant (G)

Before the establishment of the experiment, an agrochemical analysis of the soil was performed at the Department of Agrochemistry and Plant Nutrition, Faculty of Agrobiology and Food Resources, SUA in Nitra. The results are presented in Table 1. In the autumn, 2019 cow manure was applied at the dose of 30 $t.ha^{-1}$ and then plowed into the soil. Based on agrochemical analysis of the soil and the recommended standard for growing potatoes according to **[41]**, supplementary fertilization has been done in the form of nitrogen. N fertilizer was added in the form of urea (45.5% N), which is a nitrogen fertilizer with a slow-acting form of nitrogen at the dose of 130 $kg.ha^{-1}$ (100% of the recommended standard) two weeks before the planned planting of potatoes. Based on soil analysis, other macroelements were not applied to the soil.

For experimental purposes, potatoes (*Solanum tuberosum* L.) were selected as a model crop based on their importance to growers in the region. The variety "Spinela" was chosen as an available and recommended variety in an actual growing season. A ´Spinela´ is medium early red-skin variety intended for direct consumption designated based on processing characteristics as cooking type B with starch content in tubers 15-16%. This area is situated in a very warm agroclimatic region and a very dry sub-region. The data in Table 2 shows the weather condition of the experimental site during the growing season of 2020 including average rainfalls and average temperatures.

For starch content analyses was the homogenized sample quantitatively flushed with 100 cm^3 of HCl into a 200 cm^3 volumetric flask. The contents were thoroughly mixed, and the flask was placed in a pre-prepared boiling water bath for 15 minutes. We move the bank in the first three minutes. After 15 minutes, the flask was filled to about 180 cm^3 with distilled water and cooled to 20 °C. The contents of the flask are clarified by adding 2 cm^3 of a 30% zinc sulfate solution and 2 cm^3 of a 15% potassium ferrocyanide solution. After mixing, the clarifier is working for 5 minutes and then the flask is filled with distilled water exactly to the 200 cm^3 mark. After re-mixing, we filter the contents of the flask. The first portion of the filtrate is poured out and a 200 mm long polarimetric tube is filled with the next clear filtrate. AOA analyses of DPPH inhibition and spectrophotometric measurements were performed after a constant time of 30 min. Of note, 0.1 ml of the extract was pipetted into the spectrophotometer cuvette (depending on the nature of the sample) and supplemented with 70% methanol to 2.0 ml, and 4 ml of DPPH solution of about 25 $mg.l^{-1}$ concentration was added. Immediately after the DPPH solution was added, the absorbance of the mixture was measured at 517 nm (At_0). Thirty minutes later, the absorbance of each sample was measured at 517 nm (At_{30}). For the determination of total polyphenols content, the Folin–Ciocalteu phenol reagent was added to a volumetric flask containing 100 µl of extract. The content was mixed, and 5 ml of a sodium carbonate solution (20%, w/w) was added after 3 min. The volume was adjusted to 50 ml by adding distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at the wavelength of 765 nm against blank. Vitamin C: To prepare the titration reagent solution, 0.2 g of 2,6-dichlorophenolindophenol was weighed and diluted with a small amount of distilled water, the reason was to dissolve the reagent. Subsequently, we filtered the titrant through a blue filter and poured it into a measuring flask (500 ml). The reagent was made up to the mark with hot distilled water and cooled in a water bath. The cooled solution was kept in the cold. 10 g of the homogenized potato sample was placed in a volumetric flask, and we poured it with 30 ml of 5% trichloroacetic acid solution and let it macerate for 2 hours in the dark.

Table 1 Agrochemical characteristics of the soil before the experiment establishment in 2020.

pH/KCl	Humus %	Nutrients content in $mg.kg^{-1}$ of the soil			
		N_{an}	P	K	Mg
6.77 Neutral	3.89 Good	19.40 M	325.00 VH	650.00 VH	817 VH

Note: Nutrient content: M – medium content, H – high content, VH – very high content.

Table 2 Evaluation of the mean monthly air temperatures and total precipitations during growing season 2020 according to climatology normal 1961-1990.

Month	t [ºC]	Normal (1961-1990)	Characteristic	PRC [mm]	Normal (1961-1990)	Characteristic
V.	15.0	15.1	Normal	91	58	Extra Wet
VI.	20.3	18.0	Extra Warm	14	66	Extremely Dry
VII.	21.4	19.8	Warm	135	52	Extremely Dry
VIII.	19.5	19.3	Normal	35	61	Dry
IX.	17.5	15.6	Warm	37	40	Normal

Statistical Analysis

Differences between variants were tested using a one-way analysis of variance in Statgraphics Centurion XVIII (StatPoint Inc., USA). For the deriving, the relationship between the observed parameters, a Pearson's product-model correlation was determined. We used the LSD test ($p \leq 0.05$) and Microsoft Excel 2010 (USA) to test statistically significant differences in the calculated averages.

RESULTS AND DISCUSSION

Total and marketable yield

Over the past 17 years, many research papers were focused on the efficacy of different commercial humates products on potato production. Data from humic acid (HA) trials showed that different cropping systems responded differently to different products in relation to yield and quality **[10]**, which also supports our results. However, the efficacy of humic products would seem to depend on many factors, including solar radiation, weather damage, soil type, crop species, yield level, and the absence or presence of other yield constraints (disease, pests, weeds, water stress) **[75]**. Moreover, many soils are rich in naturally occurring humic substances and plants may not benefit from additional application in these soils to the expected extent **[13]** and the source of the HS have a strong impact on whether plant growth is significantly improved **[45]**.

In our experiments, the biostimulatory effect and conventional nitrogen fertilization effect on the yield of potato tubers were examined. The open-field trials showed that the potato marketable yield was significantly affected in all observed variants compared to the control variant without treatment. The average yields of cultivated potato tubers are stated in (Table 3) as control, nitrogen, and Groundfix variant for their combinations. The lowest yield was measured within the control variant where no biostimulants or fertilizers were applied. In comparison with the Control variant, the increased marketable yield after the application of conventional nitrogen fertilizer alone was 149.53% higher. Potato plants inoculated with biostimulants, alone or in combination, significantly increased the marketable yield of tubers compared to the control (C), however, only a combination of Nitrogen fertilizer with both biostimulants has a higher marketable yield than fertilized variant alone with 195.16% increase above control, 18.29% increase above conventional variant and with the marketable yield of 47.61 $t.ha^{-1}$. Other biostimulant treatments were also significantly effective and increases were, respectively 115.19% for a combination of nitrogen with Groundfix (PGPB), 71.17% for a combination of Groundfix (PGPB) with Agriful (HS) and 33.78% for Groundfix (PGPB) alone.

Within similar experiments, biostimulant based on humic substances have shown a yield of 50 $t.ha^{-1}$ with 37% increase compared to the control. Bacterial biostimulant had 14% increase in the yield of about 40 $t.ha^{-1}$ **[76]**. In the study **[25]**, PGPR and HA mixed culture increased total potato tuber yield by 140% while conventional single treatment of 100% NPK fertilizer only led to an increase in potato production of 111% compared to the untreated and unfertilized control. Treatment with humic substances increased potato yield in comparison to chemical fertilizers also in the experiments of **[77]**, **[78]** and authors of **[10]** stated that in many of their studies application of humic substances resulted in a yield of potatoes increased by 11.4% to a maximum of 20.3%. Similarly, the bio-fertilization of potatoes with PGPB significantly increased the total yield by 21% above the untreated plants in **[79]** and by 1.8% in **[47]**.

Table 3 Average yield of potato tubers ($t.ha^{-1}$) depending on the observed experimental variants *; Nitra, 2020

Variant	Total yield	Marketable yield	Difference (%)
Control	18.25 ±0.48 *a*	16.13 ±0.47 *a*	100.00
N	42.58 ±0.91 *d*	40.25 ±0.82 *d*	249.53
N + G	36.46 ±1.46 *c*	34.71 ±1.39 c	215.19
N + G + A	50.67 ±1.19 *e*	47.61 ±1.07 *e*	295.16
G + A	30.56 ±1.53 *b*	27.61 ±1.48 *b*	171.17
G	23.38 ±0.92 *a*	21.58 ±0.72 *a*	133.78

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($p < 0.05$).

Refractometric dry matter

The study showed (Table 4), that the refractometric dry matter content was significantly affected by N fertilization or biostimulant treatments in all observed variants except biostimulant combination without applied N. While the increase of concentrations of soluble solids in potato tubers was in all observed variants. The increase compared to the untreated variants was from 3% by the G + A variant to 34.5% by the N + G + A combined variant. These results are comparable to the results of the total and marketable yield of potato tubers as well as starch content.

Starch content

The Polarimetric Ewers method of starch content determination showed that used treatments of potato plants significantly increased (p <0.05) starch content in tubers in all observed variants (Table 4). The average tuber starch content after the application of biostimulants combination with N-fertilization was the highest, representing 15.85%, which means an increase of 13.6% compared to the control variant and an increase of 9.84% compared with the conventional cultivation without biostimulant. However, in contrast to these results, with the use of Groundfix alone the starch content in potato tubers was 13.83% which is less than both the control variant and the conventional variant.

Numerous studies reported a positive effect of biostimulants and an increase in vegetable quality after biostimulant application in many scientific papers. To compare our results, in **[40]** combined application of microbial inoculum and humic substances were the most effective variant, recording a 50% increase in shoot dry weight and a 43% increase in root dry weight compared to the control plants. In **[25]** tuber dry matter, starch and protein contents increased by 18.3%, 24.6%, and 48.6% respectively according to HS treatment. Similarly, in **[45]** shoot dry weight increases by 22 ±4%, and root dry weight increases by 21 ±6% in response to HS application. Plant growing pointers, plant nutritional status, and tubers excellence parameters (dry weight and other) responded positively to Humic acids application in experiments **[46]**. The results from hot pepper experiments indicate that fruit antioxidant activity, total phenolic, carbohydrate, capsaicin, carotenoids, total soluble solids, and titratable acidity significantly increased, but total flavonoid and ascorbic acid contents were not affected significantly by fulvic acid treatments applications **[55]** while similar results obtained also **[56]**. Fulvic acid improves fruit length, diameter, and yield of sweet pepper in **[76]** and vegetative growth, fruit yield, and quality of the three cultivars as compared with the control plants in the study **[58]**, moreover **[59]** confirmed increased maximum number of leaves plant, branches plant, plant height, stem diameter, number of fruits per plant, yield per plant and total yield. However, HA treatments had no significant effect on fruit firmness, fruit length, or diameter of pepper **[60]**. Cozzolino et al., **[26]** found a synergistic effect of the combination of HA with PGPB biostimulant, resulting in an improvement of potato parameters, especially P uptake. In **[71]** root application of PGPR strains significantly increased total soluble solids, total sugar, and reduced sugar, but decreased titratable acidity and had no important effect on the average strawberry fruit weight and pH. HA application had a significant effect on biological yield, grain yield, and harvest index of cereals in **[72]**, and PGPB and humic substances increased maize grain production by 65% under field conditions in **[12]**, similarly, root development of spring wheat inoculated with *A. brasilense* was significantly better that of noninoculated plants **[74]**.

Table 4 Average total dry matter, Refractometric dry matter and starch content depending on the observed experimental variants *, Nitra, Slovakia, 2020.

Variety/variant	Refractometric [°Bx]	Starch content [%]
Control	4.00 ±0.69 *a*	10.85 ±0.71 *a*
N	5.12 ±0.68 *bc*	14.43 ±0.47 *c*
N + G	4.86 ±0.23 *b*	13.89 ±0.83 *b*
N + G + A	5.38 ±0.18 *c*	15.85 ±0.78 *d*
G + A	4.12 ±0.38 *a*	14.09 ±0.53 *bc*
G	4.78 ±0.38 *b*	13.83 ±0.49 *b*

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties (p <0.05).

Vitamin C

The application of biostimulants (Agriful and Groudfix) had a statistically positive effect on vitamin C content in potato tubers within variants, where PGPB and HS biostimulants or N fertilizer alone was applied. G + A combination increased vitamin C content by 16% and G + A with N combination increased vitamin C by 20% in comparison to the untreated (control) variant, with the max value of 18.00 $mg.kg^{-1}$. On the contrary, applying PGPB alone or with N fertilizer did not positively affect the accumulation of vitamin C in tubers as (Table 5) shown. These results indicate the importance of organic material, the especially potentially synergic effect of humic and fulvic acids on soil microbiota.

Total polyphenols

Based on the results of this study, it can be assumed that the applied plant growth-promoting bacteria as well as humic substances have a positive effect on total polyphenols content in the potato tubers while in most cases these results were significant. All monitored variants accumulated a higher content of total polyphenols in the tubers than the control variant without fertilization and treatment of the stand in the range of 3-14.5% increase compared to the control variant as can be seen in (Table 5). The maximum total polyphenols promotion was obtained by PGPB alone inoculation with 2235.5 mg $GAE.kg^{-1}$ and a 14.5 % increase above control and a 9.4% increase compared to N fertilizer alone. PGPB an HS combination was second most fertile within results with 2150.50 mg $GAE.kg^{-1}$ of total polyphenols and 13.1% increase above control and 5.5% difference with nitrogen variant.

Antioxidant activity of potato tubers

Biostimulant treatment did not show statistically significant differences in the AOA (antioxidant activity) of potatoes except for the Groundfix variant where 38.00% of AOA was measured, which represents a 13.4% increase compared to the untreated variant. Similar results were obtained variant where N alone was fertilized. According to results obtained by the DPPH method, the antioxidant activity showed differences between control and observed variants from a 3% decrease in the N+G+A variant to an increase of 13.4% in the case of variants N and G, as is shown in (Table 5).

Table 5 Average vitamin C, Total polyphenols and antioxidant activity content depending on the observed experimental variants *, Nitra, Slovakia, 2020.

Variant	Vitamin C [$mg.kg^{-1}$]	TPC [mg $GAE.kg^{-1}$]	AOA [%]	AOA [mg $TE.kg^{-1}$]
Control	15.00 ±1.39 *b*	1901.00 ±1.90 *a*	33.50 ±2.12 *a*	179.00 ±8.49 *ab*
N	17.40 ±1.51 *c*	2044.00 ±8.49 *bc*	38.00 ±1.41 *b*	199.00 ±5.66 *c*
N + G	14.40 ±1.20 *a*	2113.50 ±44.55 *cd*	33.00 ±0.10 *a*	176.00 ±0.21 *ab*
N + G + A	18.00 ±1.25 *c*	1958.50 ±68.59 *ab*	32.50 ±0.71 *a*	173.00 ±4.24 *a*
G + A	17.40 ±1.40 *c*	2150.50 ±37.48 *cd*	35.50 ±0.71 *ab*	188.00 ±4.24 *bc*
G	15.00 ±1.20 *b*	2235.50 ±68.59 *d*	38.00 ±0.12 *b*	201.00 ± 1.41 *c*

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($p < 0.05$).

The increase in tuber yield due to the applied biostimulants did not have a negative effect on their quality in this case, on the contrary, in most cases, we could observe its increase. These results are supported by the study of **[19]**. In their case, applied HS plant biostimulants showed beneficial effects on both productive and qualitative potato parameters. Among quality features, tuber size, contents of protein, vitamin C, starch, and phenols are the principal parameters influenced by plant biostimulants, while reducing the normal dose of fertilizer without reducing yield. Within other similar scientific papers, compared to our study combined application of microbial consortium and humic substances was the most effective, recording a 50% increase in shoot dry weight and a 43% increase in root dry weight compared to the control plants **[41]**, or shoot dry weight increases of 22 ±4% and root dry weight increases of 21 ±6% in response to HS application **[45]**. Potato plant growing pointers, plant nutritional status, and tubers excellence parameters (dry weight and other) responded positively to humic acid application rates **[46]**. However different results were obtained **[37]**, where soil application of humic acid had no effect on tuber size, total yield, or other chemical compositions of tubers but increased mineral contents in the soil and tubers and substantially decreased incidence of hollow heart, which supports the results of **[47]**. Humic acids (HA) provide the formation of the organominerals in soil; thus, they improve the nutrient concentration of tomato leaves and agricultural production **[48]** and improved iron uptake under salinity stress **[49]**. This is also reflected in the better germination of tomato seeds **[50]** and the overall productivity and quality of plants **[51]**. The appropriate dose of HA treatment led to a significant increase in tomato plant height and fresh and dry weight together with marketable yield **[29]**, total production of fruits **[52]**, and vitamin C and total soluble solids (TSS) concentration as compared with control **[53]**. Ruiz and Salas **[54]** confirmed that significant increases in the yield and quality of the tomato fruit can be obtained by a combination of PGPB inoculation with appropriate organic materials used as fertilizers. Fulvic acid improves fruit length, diameter, and yield of sweet pepper in **[57]** and vegetative growth, fruit yield, and quality of the three cultivars as compared with the control plants in a study **[58]**, moreover **[59]** confirmed increased maximum number of leaves plant, branches plant, plant height, stem diameter, number of fruits per plant, yield per plant and total yield. The inoculated bell pepper plants always showed higher yield than the control ones in **[61]**, and in a study of **[62]** increased plant emergence, root and shoot length, and biomass and fruit yield but also caused an increase in available NPK content and sustain soil health. However, inoculation by beneficial bacteria did not affect marketable fruit yield or the pigments (chlorophylls, lycopene, and b-carotene) and carbohydrate contents in the pepper fruits, but flavonoids and anthocyanins were increased significantly by the addition of the bacteria **[63]**. Similar treatment had little effect on pod mineral content. chlorophylls, total carotenoid, and vitamin C contents in **[64]**. Data of **[65]** stated that all morphological characteristic parameters of eggplant plants (plant length, number of leaves and number of branches, and fresh and dry weight of leaves per plant) were improved by using HS biostimulator treatment compared to non-treated plants (control). Yield and its components of eggplant plants also followed the same trend with a similar result in the study **[66]**. Comparable great results were obtained from HS combination with NPK fertilizer **[67]**. Combining recommended rate and half the recommended rate of inorganic fertilizer with foliar fertilizer and PGPB improved the yield of eggplant **[68]**. Soil application of humic acid increased the dry weight of foliage, the number of tubers, yield per plant, and total yield of tubers with more average tuber weight and higher N, P, K, Total carbohydrates, and inulin contents also in **[69]** and similarly, inulin levels and yield were higher in the endophytic bacteria treatment under both conditions (normal and drought) but especially increased under drought stress in comparison to control **[70]**. In **[73]** results showed that the combinations of NPK and potassium humate increased onion bulb size, weight, and storage ability.

Pearson product-model correlation of observed parameters

The highest positive correlation was achieved between total polyphenols and antioxidant activity (+1.0), but also between total yield and total starch content of potato tubers (+0.77), total yield and vitamin C content (+0.62), and yield and refractometric dry matter (+0.63) (Figure 2**)**. These are important parameters for both the grower and the consumers, especially the fact, that despite the increase in yield, applied fertilizer or biostimulants did not influence the quality of tubers except in the case of the AOA and the total polyphenol content (-0.25). Based on our results and other studies in the discussion, the results are potentially attributed to the effect of nitrogen fertilization and biostimulating effect of applied PGPB and HS on the potato plants, especially synergic between those two.

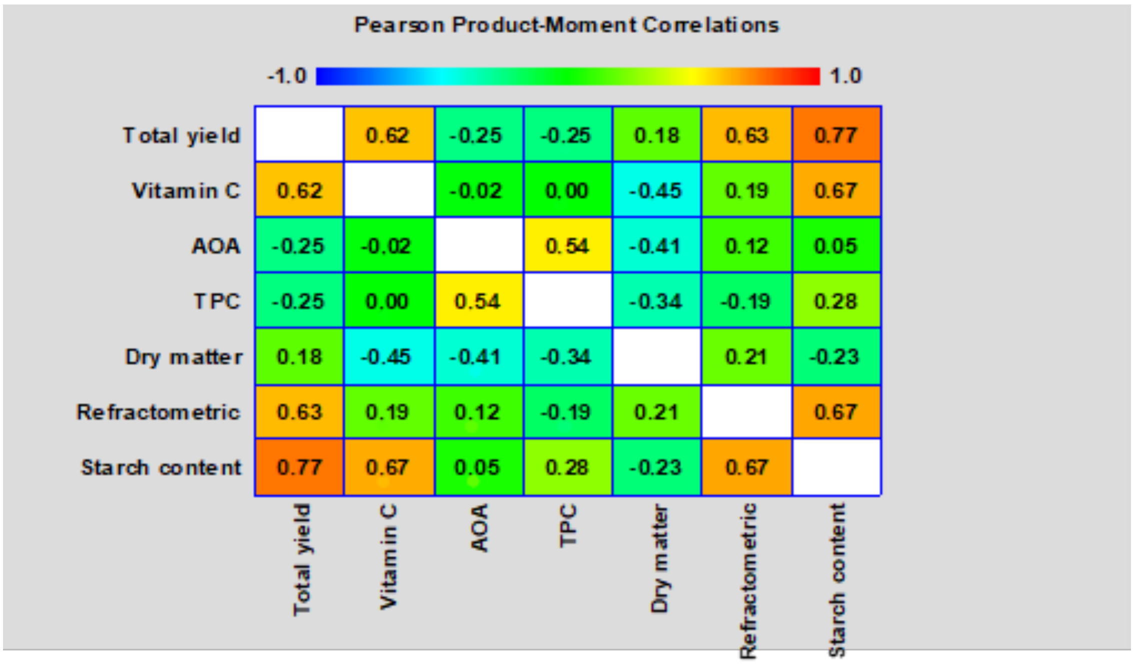

Figure 2 Pearson product-model correlation between observed parameters.

CONCLUSION

In recent years, there is a significant increase in the interest of farmers and scientific studies on biostimulant effects on cultivated plants' yield and quality. The presented results show that the observed biostimulants – Agriful and Groudfix had a significant effect on increasing the total yield of harvested potato tubers, but also a positive effect on the quality of harvested tubers was manifested. The total dry matter, refractometric dry matter, and starch content increased after biostimulant or nitrogen application, which can have a positive impact on the storability of the grown potatoes. In all observed variants total polyphenols and antioxidant activity increase compared to control, and a statistically significant increase in vitamin C content. We recorded the most positive changes in the case of variants with combined application of humic substances and PGPB, which we have substantiated with similar results in the extensive discussion. We believe that our similar results can lead to a better understanding of the biostimulating effect in different crops, soil, and climatic conditions, which can lead to more sustainable agriculture and healthy food production.

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