INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0220/2024/31–1–57–64
DOI: 10.17957/IJAB/15.2115
http://www.fspublishers.org

Full Length Article

Beneficial Effects of Microbial Inoculation to Improve Organic Potato Production under Irrigated and Non-Irrigated Conditions

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Received 14 June 2023; Accepted 31 July 2023; Published 12 December 2023

Abstract

Many microorganisms such as arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR) and Trichoderma spp. can form a symbiotic association with host plants, which can have a significant impact on plant growth and yield. In two years experiment, we investigated the possible role of seven different treatments of microbial inoculates to improve the potato tubers production and quality under irrigated and non-irrigated conditions. Potato cultivar "Desiree" was used with seven treatments of mixture of microbial inoculates including AMF, PGPR and *Trichoderma* spp. in 64 experimental parcels. The results indicated that the mycorrhizal colonization intensity and mycorrhizal frequency were increased in non-irrigated treated and the control plants over the two years. Most of the treatments did not show arbuscular abundance in both the years except the mixture of *Rhizophagus irregularis* MucL41833+*Pseudomonas brassicacearum* (41%), *R. irregularis* MucL41833+ *Paraburkholderia phytofirmans* (24%) and *Trichoderma asperelloides* A (33%). Starch content was similar between different treatments in both the years, without significant differences with control plants. In 2020, the highest starch value was found in *P. phytofirmans* PSJN treatment with (17.16%) mean under irrigated and non-irrigated conditions. In 2021, the highest starch value among the irrigated parcels was found in *R. irregularis* MucL41833 treatment (12.29%). In terms of total phosphorus content, the control treatment in the second year (2021) gave the highest total phosphorus content in the irrigated conditions (0.69 mg kg^{-1}) followed by the mixture treatment of the microbial inoculates treated plants *R. irregularis* MucL41833 and *P. phytofirmans* (0.68 mg P kg^{-1}). For the tubers yield, the treated plants under irrigated conditions gave the highest mean. In the first year, *P. phytofirmans* PSJN gave the highest yield (15.21 kg/m^2) under irrigation conditions, while in the second year, *R. irregularis* MucL41833 showed the highest yield (16.72 kg/m^2) in the irrigated conditions as well, followed by the control treatment in both the years. Our findings could be a practical addition to further researches on the effects of microbial inoculates on organic potato production in field conditions, understanding the different interactions and effects of the applied microbial inoculates, which may differ according to the environmental conditions and the host plants. © 2024 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; PGPR; Phosphorus content; Starch content; *Trichoderma*; Tuber quality

Introduction

Potato (*Solanum tuberosum* L.) belongs to Solanaceae family (Muleta and Aga 2019), a short day, vegetatively propagated, C_3 plant cultivated in temperate, subtropical, and tropical regions (Mallick *et al.* 2021). Potato is considered the fourth largest crop in the world, after rice, wheat and corn, with a total annual production of 370 million tons (Djaman *et al.* 2021). The potato crop's exceptional adaptabilities, combined with its relative ease of cultivation and high nutritional value, has led to a steady increase in potato consumption in developing countries (Contreras-Liza 2021). Short vegetative duration and crop genetic variation allows growers to find a suitable season for cultivation under a variety of weather patterns and less predictable climates (Kolech *et al.* 2015).

To cite this paper: AlHadidi N, H Hasan, Z Pap, T Ferenc, O Papp, D Drexler, D Ganszky, N Kappel (2024). Beneficial effects of microbial inoculation to improve organic potato production under irrigated and non-irrigated conditions. *Intl J Agric Biol* 31:57–64

It requires a variety of balanced plant nutrients for growth and development. Nitrogen (N), phosphorus (P) and potassium (K) are among the most important essential elements for potato productivity (Zelelew *et al.* 2016). Overuse of chemical fertilizers leads to several environmental problems including groundwater pollution, soil degradation and their impact on crop growth (Savci 2012). To reduce these negative effects, alternative ways must be found, such as, the inoculation of beneficial microorganisms into the soil to raise potato productivity (Al-Zabee and Al-Maliki 2019). Some plant-microbes such as PGPR, AMF and compost have been widely used to enhance plant growth through different mechanisms of action (Javaid 2009; Sharf *et al*. 2021; Tahiri *et al*. 2022). Also, microbial inoculants are easy and inexpensive to manufacture compared to chemical pesticides (Elnahal *et al*. 2022).

Organic farming is becoming an important tool for maintaining soil quality, and therefore the use of bio-active ingredients as bio-fertilizers or bio-pesticides is an essential part of organic farming, especially in vegetable farming (Johri *et al.* 2002; Javaid and Bajwa 2011). Applying alternatives and environmentally friendly solutions is critical to substitute synthetic inputs with organic materials while ameliorating the chemical, physical and biological properties of soils (Papp *et al*. 2021). In sustainable agricultural systems, beneficial microbial products, including several types and species of living bacteria and fungi are being used (Biró 2016; Ali *et al.* 2020).

AMF are biotrophic symbionts forming most extensive and oldest associations of about 80% of terrestrial plants (Lone *et al*. 2015; Javaid and Khan 2019). Numerous studies have described the association of potatoes with AMF or *Trichoderma* spp. under greenhouse or *in vitro* conditions, but a few have been accomplished in the open field (Buysens *et al.* 2016). Arbuscular mycorrhiza can be recommended for high yield and quality crops; it will promote plant growth and yield by increasing N and P uptake and disease resistance (Li *et al*. 2022). Plants inoculated with mycorrhizae can easily adapt to greenhouse and field conditions (Altuntas 2021) but the role of field AMF inoculation on uptake of micronutrients such as Fe and Zn and accumulation in edible parts of plants has not yet been clarified (Pellegrino *et al.* 2020).

PGPRs show a clear potential to increase the nutrient use efficiency of potatoes, which could be developed as an important element in both low and high-input cropping systems (Oswald *et al*. 2007). They play a significant role in the soil, which is found to be beneficial for vegetable health and productivity (Shoaib *et al.* 2020; Mekonnen and Kibert 2021).

Fungi of the genus *Trichoderma* and rhizobacteria of the genera *Pseudomonas*, *Bacillus, Streptomyces* and others have evolved multiple mechanisms that lead to improvements in plant growth and productivity (Harman 2006; Khan and Javaid 2020). There are recently 355 *Trichoderma* products in the Indian market, mainly used in the field of fungal biopesticides (Chakraborty *et al*. 2023) To date, *Trichoderma* spp. are among the most studied fungi and are commercially marketed as bio pesticides, bio fertilizers and soil alternations (Vinale *et al*. 2008). The use of this microbial inoculant in *Trichoderma*-based products is attracting the attention of researchers to learn more about other potential benefits of *Trichoderma* spp. (Zin and Badaluddin 2020; Khan *et al*. 2021).

Due to the importance of producing economic crops, such as potatoes, which are considered a stable food all over the world. Nowadays, improving the quality and production of vegetables in ecological ways at a lower cost, which can be suitable in all climates, is becoming more hopeful for farmers. The objectives of this study were to determine if the selected microbial inoculates strains have a beneficial influence to improve the potato tubers quality in addition to explore out the difference within the applied microbial inoculates treatments on the tubers of potato.

Materials and Methods

Experimental details and treatments

Experimental material: The experiment was carried out at the Organic Educational Farm of the Hungarian University of Agriculture and Life Sciences (MATE) at Soroksár, Hungary (47.393077°N–19.147234°E) between 2020 and 2021 in frame of the SolACE Horizon 2020 research project. Organic farming methods have been applied on the experimental location for more than a decade, the pre-crop on the study site was rye in both years. The soil type on the experimental site is sandy soil with pH (H_2O) 8.5, $CaCO_3$ 9% and humus 2.3%. Soil analysis study conducted in 2020 and 2021 gave the tabulated results in (Table 1). The contents of nitrate, nitrite, and ammonium (mg. kg^{-1} dry matter) in the soil samples were extracted with potassium chloride solution using automated method with segmented flow analysis. A randomized complete block design was selected in the two years experiment. The size of the whole experimental plot was 864 m^2 with inter spacing of 22.5 m^2. A total of 64 experimental parcels were created, in which the irrigated area was 432 m^2. One cultivar of potato 'Desiree' was used in the experiment. The potato field was grown following the regulation of EU Regulation (EC No. 2018/848) common organic practices on the farm. Tubers were planted at 10 cm soil depth in April. After emergence, 20-30 cm tall ridges were prepared along the rows. Weed control was done manually and regular plant protection treatments were done against *Phytophthora infestans* and *Leptinotarsa decemlineata* using copper, *Bacillus thuringiensis* and Spinosad. Tubers were harvested at the beginning of September.

Treatments: Seven different treatments using AMF (Arbuscular Mycorrhizal Fungi), PGPR (Plant Growth Promoting Rhizobacteria), *Trichoderma* and a mixture of them were conducted under irrigated conditions, as well as without irrigation, each with four replicates with a total of 64 parcels. Each parcel was planted with 12 potato tubers. Parcels were separated and surrounded by a minimum of two buffer rows in every orientation.

Weather data during the study periods

Precipitation means per month (mm), temperature (°C), relative humidity (%), soil temperature (°C) and Leaf Wetness (%) of the experiment area during two years of growing season were recorded (Table 2 and 3). The total precipitation was 443.4 mm and 310 mm in 2020 and 2021, respectively. The meteorological station was set up by the University of Debrecen, using a plant production information system called Metagro, taking into account climatic conditions, plant water use, and meteorological forecasts.

The study site was irrigated. The amount of irrigation water was measured based on the irrigation system and its capability with 7 mm of water/hour. Thus, 21 mm of irrigation took 3 h. The amount of irrigation water depended on the amount of precipitation. Non-irrigated parcels were only irrigated in case the plant production was endangered by the draught, while irrigated parcels received optimal amount of water based on the plant production information system data.

Microbial inoculant treatments

Seven different treatments were used with AMF, PGPR and *Trichoderma*. The selected microorganisms were obtained from Université Catholique de Louvain – UCLouvain, Belgium. The isolates were selected for open-field testing in previous laboratory experiments conducted in frame of the SolACE project. The experiment was conducted for two seasons from April till August during 2020 and 2021. Three mixtures of inoculants were tested on potato compared to the untreated (no inoculation, water only) control (Table 4). The inoculants were formulated with Minigran technology (http://minigran.com/en). The inoculants were sensitive to heat and UV Light. Once the inoculants were applied onto tubers in the opened furrows at planting, they were manually covered as soon as possible. In accordance with organic production methods, the tubers were not treated with any chemicals (bactericide, fungicide). Inoculant treatments were done once at planting time for each growing season in both the years.

Potato roots sampling and determination of mycorrhizal colonization

Two potato plant roots were sampled from each treatment per replicate in the two years of the experiment after four months of transplanting. Ink based staining was carried out following the method suggested by Phillips and Hayman (1970). Colonization was determined using the method of Trouvelot *et al.* (1986). Slides were prepared to check hyphal and arbuscular development by light microscope. In the proposed equation, all colonization parameters which includes Frequency of mycorrhiza in root system, intensity of mycorrhizal colonization in root system and Arbuscular abundance in root system were calculated and expressed as a percentage using Mycocalc software (Zsombor 20XY). Frequency calculations were performed using a Windows Forms application written in C# and developed to facilitate the process, based on the equations of Trouvelot *et al.* (1986).

Starch content of harvested potato tubers

The measurement of the starch content was done according to EU-direction (International Starch Institute: Determination of Starch in Potatoes) in each year.

Total phosphorus content in the potato tubers

The total phosphorus content was determined according to the MSZ 21470-50:2006 Hungarian standard after digestion in a microwave-assisted (HNO_3 + H_2O_2) mixture, for which a CEM MARS 5 closed-chamber microwave oven was used with temperature and pressure sensors. The digested samples were filtered into 25 mL volumetric flasks and filled with Milli Q water. Then, the filtrates were analyzed on UV-Vis spectrophotometer (Spekol 221, Carl Zeiss Jena) for total phosphorus content determination.

Yield of potato tuber

For each parcel and for each treatment, just before harvest, the numbers of plants were counted. Yield was measured per row once the potato tubers were harvested; they were bagged and immediately measured.

Statistical analysis

Data analysis was carried out with SAS software version 9.4 (2013). Starch, total phosphorus content and yield were analyzed by one-way ANOVA model with three factors; year, microbial inoculate treatment and water treatment. Each treatment has four replicates. Before ANOVA, descriptive statistics for all the measurements was made in order to observe the distribution of the data and check the normality by general linear model ($P \leq 0.05$). Means were separated using Tukey's test at a significance level of $P \leq 0.05$.

Results

Mycorrhizal parameters

In the first season (2020), the highest mycorrhizal colonization frequency (F%) and mycorrhizal colonization intensity (M%) were recorded in non-irrigated areas with Rh

Table 1: Soil characteristics for experiment area

Parameters	2020		2021	
	Irrigated	Control	Irrigated	Control
NO_3^--N (mg kg^{-1})	nd	nd	10.7	8.7
NH_4-N (mg kg^{-1})	6.8	7.3	6.6	7.2
EDTA-P_2O_5 (mg kg^{-1})	528	476	524	475

nd: No data

Table 2: Means of monthly precipitation (mm), temperature (°C), relative humidity (%**)**, soil temperature (°C) and leaf wetness (%) in the experimental sites in 2020 (Soroksár, Hungary) (data source: Metagro system)

Month	T (°C)	RH (%)	Soil Temp (°C)	Precipitation (mm)	Leaf wetness (%)
April	11.09	54.35	14.13	13.8	21.83
May	14.94	62.98	18.58	16.2	31.26
June	20.58	74.14	23.26	77.6	40.24
July	21.92	69.93	24.56	58.0	45.81
August	23.12	68.28	24.75	42.8	42.93
September	17.78	72.46	20.082	30.4	48.57

Table 3: Means of monthly precipitation (mm), temperature (°C), relative humidity (%**)** and soil temperature (°C) and leaf wetness (%) in the experimental sites in 2021 (Soroksár, Hungary) (data source: Metagro system)

Month	T (°C)	RH (%)	Soil T (C)	Precipitation (mm)	Leaf wetness (%)
April	8.93	67.16	10.04	26.6	30.01
May	14.12	71.25	15.07	52.2	36.53
June	22.9	61.42	26.11	10.2	28.90
July	24.69	62.96	26.47	43.4	34.11
August	20.57	72.2	22.34	56.4	48.57
September	17.19	70.31	18.56	19.4	38.71

Table 4: Treatments and types of microorganisms of inoculum mixtures used in the potato field trial

Microbial inoculates strains	Inoculation treatments symbol	Microorganism type	Application rate/ biological material need in (g)	(CFU/tuber (for AMF: g/tuber)	Concentration of microbial product (CFU/g)	Quantity of granule per tuber (g)
Pseudomonas brassicacearum 3Re2-7	Ps	Bacteria1	7.20	2.00E + 08	1.60E + 10	0.75
Paraburkholderia phytofirmans PsJN	Pa	Bacteria2	6.40	1.00E + 08	9.00E + 09	0.75
Trichoderma asperelloides A	Tr	Fungi	0.86	1.50E + 06	1.00E + 09	0.75
Rhizophagus irregularis MUCL41833	Rh	AMF	0.3456	6.00E - 04	-	0.75
Rhizophagus irregularis MUCL41833	Rh + Ps	AMF + Bac1	0.3456	6.00E - 04	-	0.75
+ *Pseudomonas brassicacearum* 3Re2-7			7.20	2.00E + 08	1,60E + 10	
Rhizophagus irregularis MUCL41833	Rh + Pa	AMF + Bac2	0.3456	6.00E - 04	-	0.75
+ *Paraburkholderia phytofirmans* PsJN			6.40	1.00E + 08	9.00E + 09	
Rhizophagus irregularis MUCL41833	Rh + Pa + Tr	AMF + Bac2 +	0.3456	6.00E - 04	-	0.75
+ *Paraburkholderia phytofirmans* PsJN		Fungi	6.40	1.00E + 08	9.00E + 09	
+ *Trichoderma asperelloides* A			0.86	1.50E + 06	1.00E + 09	
Control treatment	C (control)	Control	-	-	-	-

+ Pa treatment with a combination of Rh and Pa, which was 96.67 and 28.56%. It was higher than F% and M% in the irrigation treatments but with no significant differences. With irrigation, F% was higher compared to no-irrigation treatments for *R. Irregularis* MucL41833 (Rh), *P. brassicacearum* 3Re2-7 (Ps), *P. phytofirmans* PSJN (Pa), *T. asperelloides* A (Tr) and Rh + Pa + Tr combination. Exposure to high moisture may be due to suppression of these microorganisms on other natural soil (AMF). This is confirmed by the second-year results, where all treatments under irrigated conditions gave lower F% than the control (no irrigation), which achieves a mycorrhizal colonization frequency (F%) of 100%. All these parameters during two study years are presented in Table 5.

Starch content by microbial inoculants treatments

The starch content was similar between different treatments in both years, without significant differences. In 2020, the highest starch value was found in *Pa*-treatment with (17.16%) and 16.37% mean under irrigated and non-irrigated conditions, respectively, followed by Rh + Pa-treatment with 16.73% mean under irrigated conditions. The lowest starch content was in the control treatment with irrigation, while the lowest starch content for the non-irrigated treatment was found in the Rh + Pa treatment (14.90%). Likewise, there were no significant differences in the second season (2021) with the highest starch content in Rh-treatment tubers under both irrigated and non-irrigated

Table 5: Mycorrhizal parameters in irrigated and non-irrigated treatments within two years of the experiment 2020 and 2021

Treatment code	Treatment	Mycorrhiza colonization Frequency				Mycorrhizal colonization Intensity				Arbuscular abundance			
		F%				M%				A%			
		2020		2021		2020		2021		2020		2021	
		I	C	I	C	I	C	I	C	I	C	I	C
C	Control	25.55	80	93.33	100	1.34	23.83	2.31	15.9	no arbuscular	no arbuscular	no arbuscular	no arbuscular
Rh	*Rhizophagus irregularis* MucL41833	55.55	32.22	100	100	3.02	1.63	7.22	25.65	no arbuscular	no arbuscular	no arbuscular	no arbuscular
PS	*Pseudomonas brassicacearum* 3Re2-7	88.89	56.67	96.66	100	13.35	5.27	6.23	22.06	no arbuscular	no arbuscular	no arbuscular	no arbuscular
Rh + Ps	*Rhizophagus irregularis* MucL41833+*Pseudomonas brassicacearum*	90	90.28	100	100	14.86	11.23	20.3	17.37	41	41	41	41
Pa	*Paraburkholderia phytofirmans* PSJN	89.99	36.67	94.44	100	16.19	3.12	3.09	22.35	no arbuscular	no arbuscular	no arbuscular	no arbuscular
Rh + Pa	*Rhizophagus irregularis* MucL41833+ *Paraburkholderia phytofirmans*	95.55	96.67	97.78	100	17.92	28.56	5.7	16.53	24	24	24	24
Tr	*Trichoderma asperelloides* A	100	48.89	100	100	26.11	1.03	4.13	16.03	33	33	33	33
Rh + Pa + Tr	*Rhizophagus irregularis* MucL41833+ *Paraburkholderia phytofirmans* PSJN+ *Trichoderma asperelloides* A	77.77	40	100	100	8.2	2.16	18.38	18.38	no arbuscular	no arbuscular	no arbuscular	no arbuscular

Table 6: Mean values of the starch content (%) sorted by microbial inoculant treatments under irrigated and non-irrigated conditions in 2020 and 2021

Microbial inoculants Treatment		Distribution of starch by inoculant and irrigation (2020)		Distribution of starch by inoculant and irrigation (2021)	
		I	C	I	C
1	Ps	16.30 ± 0.542a	15.73 ± 0.441a	10.11 ± 0.448a	11.61 ± 0.666a
2	Pa	17.16 ± 0.331a	16.37 ± 0.201a	11.06 ± 0.611a	11.55 ± 0.672a
3	Tr	15.99 ± 1.308a	16.09 ± 0.490a	11.32 ± 1.037a	10.83 ± 0.679a
4	Rh	16.42 ± 0.570a	15.13 ± 0.674a	12.29 ± 0.372a	11.60 ± 0.836a
5	Rh + Ps	16.69 ± 0.826a	16.33 ± 0.352a	10.85 ± 0.540a	11.49 ± 0.494a
6	Rh + Pa	16.73 ± 0.599a	14.90 ± 1.134a	11.31 ± 0.602a	10.67 ± 0.757a
7	Rh + Pa + Tr	15.86 ± 1.392a	16.20 ± 0.804a	11.48 ± 0.691a	10.37 ± 0.362a
8	C (control)	15.77 ± 0.832a	16.32 ± 0.452a	11.08 ± 0.712a	11.04 ± 0.568a

Symbol used ±= Standard error
Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$
The value has been calculated from 4 replications and represented as an average in the table

conditions. In 2021, the highest starch value among irrigated parcels was found in Rh treatment (12.29%), followed by Rh + Pa + Tr (11.48%). The lowest starch content was in the Ps treatment (10.11%). Under non-irrigated conditions, the highest starch content was found in Ps treatment (11.61%), followed by the Rh treatment (11.60%). The lowest starch content was apparent in the Rh + Pa + Tr treatment (10.37%). All results are presented in Table 6.

Total phosphorus in potato tubers

The total phosphorus content in the tubers is represented in Table 7. The results show non-significant differences in both years under both irrigated and non-irrigated conditions. The highest level of phosphorus was recorded for the Tr treatment under irrigated conditions, which was similar to that recorded for the control, and for the Rh + Pa + Tr combination with no irrigation treatment. There was an apparent increase in phosphorus levels in the second year (2021), but the highest amount was measured in the control treatment under irrigated conditions and in the Rh + Pa treatment without irrigation.

Potato tubers yield

As shown in Table 8, yield was not significantly affected by any of the treatments in the two test seasons. The yield of irrigated treatments was higher than that of non-irrigated treatments in both seasons. For inoculant effect, Pa gave the highest yield under irrigation in the first season, but Rh was highest in the second season. And among non-irrigated treatments, the control treatment was the highest, followed by Ps treatment in 2020, and in the second season, inoculation with Pa gave the highest yield, followed by Rh, while their combination showed a somewhat reduced yield.

Discussion

Our current study showed that arbuscular mycorrhiza could form a symbiotic relationship with potato tubers in both cultivation seasons under irrigated and non-irrigated conditions. Zhu *et al.* (2022) also showed that the combination of AMF with other compounds can further promote the establishment and growth of AMF, improve the nutrient utilization rate of the host plant, and thus strengthen the symbiotic link between plant and mycorrhizal fungi. Laranjeira *et al.* (2022) found that inoculation with beneficial microorganisms and additional irrigation at critical stages benefits chickpea growth and should be considered to increase plant productivity and promote agricultural sustainability. Our results show that mycorrhizal colonization

Table 7: Mean values of phosphorus content (mg P kg^{-1}) in potato tubers sorted by microbial inoculants under irrigated and non-irrigated conditions in 2020 and 2021

Microbial inoculants Treatment		Total phosphorus in the tubers 2020		Total phosphorus in the tubers 2021	
		I	C	I	C
1	Ps	0.32 ± 0.012a	0.32 ± 0.010a	0.62 ± 0.044ab	0.56 ± 0.053ab
2	Pa	0.32 ± 0.012a	0.34 ± 0.008a	0.68 ± 0.036a	0.58 ± 0.022ab
3	Tr	0.33 ± 0.010a	0.32 ± 0.006a	0.64 ± 0.037ab	0.64 ± 0.045ab
4	Rh	0.31 ± 0.014a	0.32 ± 0.013a	0.54 ± 0.051ab	0.57 ± 0.034ab
5	Rh + Ps	0.31 ± 0.007a	0.30 ± 0.016a	0.63 ± 0.029ab	0.54 ± 0.018ab
6	Rh + Pa	0.31 ± 0.012a	0.32 ± 0.005a	0.68 ± 0.053a	0.66 ± 0.038ab
7	Rh + Pa + Tr	0.32 ± 0.014a	0.32 ± 0.011a	0.63 ± 0.014ab	0.54 ± 0.025ab
8	C (control)	0.32 ± 0.018a	0.35 ± 0.010a	0.69 ± 0.031a	0.50 ± 0.030b

Symbol used ±= Standard error
Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$
The values have been calculated from 4 replications and are represented as an average in the table

Table 8: Mean values of potato tubers yield (kg/m^2) sorted by microbial inoculants under irrigated and non-irrigated conditions in 2020 and 2021

Microbial inoculants Treatment		Yield of potato 2020		Yield of potato 2021	
		I	C	I	C
1	Ps	12.81 ± 0.822ab	11.81 ± 0.629ab	16.24 ± 1.857ab	10.49 ± 0.489ab
2	Pa	15.21 ± 0.708a	12.02 ± 0.503ab	14.48 ± 1.729abc	10.11 ± 0.624c
3	Tr	14.05 ± 1.050ab	11.58 ± 1.251ab	15.18 ± 1.170abc	10.31 ± 0.839bc
4	Rh	13.37 ± 0.724ab	10.81 ± 0.563b	16.72 ± 0.861a	11.11 ± 1.035abc
5	Rh + Ps	14.00 ± 0.478ab	11.66 ± 0.560ab	14.20 ± 1.075abc	9.83 ± 0.968c
6	Rh + Pa	13.25 ± 1.078ab	11.54 ± 0.541ab	15.07 ± 1.183 abc	9.83 ± 0.997c
7	Rh + Pa + Tr	12.61 ± 1.202ab	10.78 ± 0.668b	16.66 ± 1.359a	10.43 ± 1.057bc
8	C (control)	14.45 ± 0.959ab	12.03 ± 0.552ab	15.09 ± 1.488abc	10.97 ± 0.849abc

Symbol used ±= Standard error
Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$
The values have been calculated from 4 replications and are represented as an average in the table

frequency and mycorrhizal intensity increased under non-irrigated conditions over the two years, demonstrating that the applied mycorrhizal inoculant was successful in establishing a symbiotic relationship with the treated potato tubers. This can be confirmed by Augé (2004) that AMF helps plants absorb water, and numerous mechanisms have been postulated to explain these effects. These include improved stomata regulation, higher root hydraulic conductivity and increased interaction with soil particles. Most treatments showed no arbuscular frequency in both years, with the exception of the mixture of *R. irregularis* MucL41833 + *P. brassicacearum* (41%), *R. irregularis* MucL41833 + *P. phytofirmans* (24%) and *T. asperelloides* A (33%)

In terms of the starch content, there was no significant difference in the two seasons using different treatments and irrigation conditions. However, the potato tubers treated with mycorrhizal inoculant and the microbial inoculant mixture yielded the highest starch content similarly. In 2020, the highest content of starch was observed in treatment with *P. phytofirmans* PSJN with a mean of (17.16%) under irrigated and non-irrigated conditions. while in 2021 the highest level of starch among the irrigated plots was found in the treatment with *R. irregularis* MucL41833 (12.29%). A study by Berta *et al.* (2014) showed that inoculation with PGPB and AMF increases starch content. Since the development of the AMF can also increase over time, the increase in the percentage of starch can be explained by the improvement in the development of the AMF over time. For total phosphorus content in potato tubers, there is an increase by time and by the applied microbial inoculates. Nevertheless, there is no significant difference between the treatments and irrigation conditions in our study. The second-year control treatment (2021) yielded the highest total phosphorus under irrigated conditions (0.69 mg kg^{-1}), followed by mixed treatment of microbial inoculated plants *R. irregularis* MucL41833 and *P. phytofirmans* (0.68 mg kg^{-1}). Results from research conducted by Adavi and Tadayoun (2014) concluded that tuber size, number of tubers per plant, tuber yield and starch yield were significantly affected by mycorrhizal inoculation as this biofertilizer can improve the uptake of phosphorus by the plant. As an overall result of the effect of different treatments on potato yield, an increase was also observed over time. In the first year, *P. phytofirmans* PSJN gave the highest yield (15.21 kg/m^2) under irrigated conditions, while in the second year, *R. irregularis* MucL41833 produced the highest yield (16.72 kg/m^2) also under irrigated conditions, followed by the control treatment in both years. This is demonstrated by a study of Szczałba *et al.* (2019) which shows that the combination of AMF and *Trichoderma* has a positive effect on plant yield.

The mixture of inoculation with different species could have an antagonistic effect or no effect according to studies. For the mixture of PGPR and AMF, inoculation of a mixture of the microorganism *Azospirillum* with

Pseudomonas showed no effect on plant growth (Vázquez *et al*. 2000). Also, inoculation of *Pseudomonas* and *Trichoderma* reduced the activity of other microorganisms that were inoculated. AMF colonization can eliminate the effect of *Trichoderma* on plant growth (Waschkies *et al*. 1994). Inoculation of only one microorganism in the plant can show a significant beneficial effect on the plant. However, during inoculation with other microorganisms, especially AMF, there may be a decrease in the effect of other inoculations. This could be explained by the qualitative change in root exudate caused by AMF colonization (Cox 1975). In our research, the results show that the treatments did not show a significant difference in most measurements in both study years. There was no significant difference between the results of 2020 and 2021 for either of the inoculation treatments. The non-irrigated plants showed better results regarding AMF colonization, a higher starch content, total phosphorus content in the non-irrigated samples compared to irrigated ones.

Conclusions

The microbial inoculations achieved better results under non-irrigated conditions than under irrigated conditions. Also, we could not demonstrate any positive effect of any inoculate. Even with the non-irrigated treatment, no significant benefits of the inoculates was obtained.

Acknowledgments

All co-authors are grateful and appreciative of European Union Horizon 2020 Research and Innovation funding made available to this project under Grant Agreement No. 727247 (SolACE). The authors highly appreciate and thank the Hungarian University of Agriculture and Life Sciences for their support.

Author Contributions

Original draft preparation by NA, NK and ZP; validation, analysis and visualization by NA and HH. Review and editing by NK, ZP, HH, DG., TF, OP and DD; revision by NK, ZP, HH, DG, FT, OP, DD. All the authors have read and agreed to the submitted version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this manuscript

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