**Running title:** Improving organic potato production through microbial inoculates

**The Beneficial Effects of the Microbial Inoculates Application to Improve Organic Potato Production under Irrigated and Non- Irrigated Conditions**

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**Novelty statement**

The use of microbial inoculations in agriculture has recently received increasing attention. The results show that the arbuscular mycorrhiza successfully evolved and formed a symbiotic relationship with potato tubers. This can be observed in the measured parameters in both seasons. Based on the irrigated treatment, it can be concluded that the microbial inoculations achieved better results under non-irrigated conditions than under irrigated conditions. Furthermore, the microbial inoculations achieved better results under non-irrigated conditions than under irrigated conditions. We were also unable to demonstrate a positive effect with any of the inoculates. Even with the non-irrigated treatment, no significant benefit of inoculation was measurable.

**Abstract**

Green technologies such as microbial inoculation to either replace or reduce the use of agrochemicals and maintain productivity are good solutions to current agricultural challenges. Many microorganisms are known to have benefits for plants and can represent alternatives to chemical products that are suitable for environmental protection and plant value. These microorganisms include arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR) and *Trichoderma* spp. that can live in symbiosis with plants. Seven different treatments of microbial inoculum strains were used under irrigated and non-irrigated conditions in two cultivation periods to observe whether they have a beneficial effect on improving the potato yield and quality under irrigated and non-irrigated conditions. Several parameters were measured during the study, such as mycorrhizal parameters (F%, M% and A %), total phosphorus in potato tubers, total starch content and potato tuber yield. The results indicate that non-irrigated plots all performed better for AMF colonization, but no impact on yield or quality was seen. Our results could prove to be a practical addition to further research into the microbial inoculates on potato.

**Keywords:** Starch content. Phosphorus content. AMF. PGPR. Trichoderma. Tuber quality.

**Introduction**

Potato (*Solanum tuberosum* L.) is a perennial plant of the Solanaceae family (Muleta and Aga 2019), a short day, vegetatively propagated, C3 plant cultivated in temperate, subtropical, and tropical regions (Mallick et al. 2021). According to the Andean region, which is the most important center of potato diversity in the world (Quiro et al. 2018), 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 and Koudahe 2021; Albishi et al. 2013). It originated in Central and South America, and was introduced to India by Portuguese traders in the early 17th century (Saini et al. 2021). Potatoes are the second highest source of carbohydrates after cereals (Purwantisari et al. 2020). They are one of the most popular staple foods consumed around the world due to their high yield, relatively low cost of production, and adaptability to a wide variety of soil and climate types (Misra and Kulshrestha 2003). 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). Potato crop genotypic variation and relatively short growing season allows growers to find a suitable season for cultivation under a variety of weather patterns and less predictable climates (Kolch et al. 2015)**.**

The potato is a high-yielding crop, which therefore requires a variety of balanced plant nutrients for growth and development. Nitrogen (N), phosphorus (P) and potassium (K) are among the most important elements essential, also for potato productivity (Zelelew et al. 2016). Increasing chemical fertilizer requirements N-P-K for potato production is one of the growth problems that decreases the quality of the crop (Adavi and Tadayoun 2014). 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 AlMaliki 2019). Some plant-microbe interactions such as Plant Growth Promoting Rhizobacteria (PGPR), Arbuscular Mycorrhizal Fungi (AMF) and compost have been widely used to enhance plant growth through different mechanisms of action (Tahiri et al. 2022). Also, microbial inoculants are easy and inexpensive to manufacture compared to chemical pesticides (Elnahal et al.2022). The benefits of co-inoculating phosphate-solubilizing PGPR and/or nitrogen-fixing PGPR with mycorrhizae in plants have been demonstrated (Kumar et al. 2017).

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). 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). Farmers are advised to use less quantity of fertilizers than the previously recommended dosage along with some bio-inoculants to maintain soil fertility and attain yield goals, while reducing fertilizer expenses (Cruz-Cardenas et al. 2021). Sustainable agricultural systems currently use beneficial microbial products, including several types and species of living bacteria and fungi (Biro 2016).

The abundant AMF belong to the *Glomeromycota* phylum (Bonfante and Genre 2008). The AMF symbiosis supports plant growth by increasing the availability and translocation of numerous nutrients, mainly phosphorus (P) (Smith and Read 2010). The AMF form various structures like hyphae, arbuscules, hyphal and arbusculate coils, and vesicles in plant roots (Bharathy et al. 2021). AMF are biotrophic symbionts forming most extensive and oldest associations of about 80% of terrestrial plants (Lone et al. 2015). For AMF symbiosis is obligatory as they need carbohydrates and lipids from their host plant, for which they in turn provide necessary nutrients (De Gruyter et al. 2022). Many bacteria that colonize the rhizosphere, e.g., *pseudomonads*, typically produce substances that stimulate plant growth or prevent root pathogens (Srivastava et al. 2007). Numerous studies have described the association of potatoes with AMF or *Trichoderma* spp. under greenhouse or in vitro conditions, but few have been accomplished in the open field (Buysens et al. 2016). Potato plant growth response correlated with plant P uptake modified by biochar quality and quantity and AMF colonization (Yang et al. 2020). 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 (Chunjie 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).

Phosphate solubilizing microorganisms play an important role in plant nutrition by increasing P uptake by the plant, and plant growth promoting microbes also make an important contribution to bio fertilization of agricultural crops (Yadav et al. 2019). Rhizobacteria stimulate plant growth through a variety of known and yet unknown mechanisms (Velivelli et al. 2015). The search for PGPR and the understanding of its mode of action are increasing at a rapid pace as efforts are made to exploit them commercially as bio fertilizers (Vessy 2003). 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). It plays a significant role in soil, which is found to be beneficial for vegetable health and productivity (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 (Harmen 2006). Today, more than 50 different *Trichoderma*-based agricultural products can be found on the market, registered, and sold in many different countries (Woo et al. 2006). To date, *Trichoderma* spp. is 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 inoculate in *Trichoderma*-based products is attracting the attention of researchers to learn more about other potential benefits of *Trichoderma* spp. (Zin and Badaluddin 2020).

Keeping the previous studies in mind, our study attempts to answer key questions such as: Is there any beneficial effects of selected microbial inoculates to improve potato productivity with and without irrigation under field conditions? Which microbial inoculate combinations can give the highest performance and improve the production of potato under organic cultivation. The present study is based on field experiments conducted in two consecutive years where different combinations of microbial inoculum treatments (AMF, PGPR and Trichoderma) were applied with and without irrigation application.

**Materials and Methods**

**Study locations and field experiment**

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 by ÖMKi (Research Institute of Organic Agriculture, Hungary), 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 (H2O) 8,5, CaCO3 9% and humus 2,3%. Soil analysis study conducted in 2020 and 2021 gave the tabulated results in (Table1). The contents of nitrate, nitrite, and ammonium (mg/kg) (dry matter) in the soil samples were extracted with potassium chloride solution using automated method with segmented flow analysis. The size of the whole experimental plot was 864 m2 with inter spacing of 22.5 m2. A total of 64 experimental parcels were created, in which the irrigated area was 432 m2. One cultivar of potato ‘Desiree’ was used in the experiment. 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.

**Table 1:** Soil characteristics for experiment area in soil depth (0-30 cm)

|  |  |  |
| --- | --- | --- |
| Parameters | 2020 | 2021 |
|  | Irrigated | Control | Irrigated | Control |
|  |  |  |  |  |
| NO3-\_N mg/kg | n.d. | n.d. | 10.7 | 8.7 |
| NH4-N mg/kg | 6.8 | 7.3 | 6.6 | 7.2 |
| EDTA-P2O5 mg/kg | 528 | 476 | 524 | 475 |

n.d. no data

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.

**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 7mm of water /hour. Thus, 21mm of irrigation took 3 hours. 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’s 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 T (°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 |

**Microbial Inoculates Treatments**

Seven different treatments were used with AMF (Arbuscular Mycorrhizal Fungi), PGPR (Plant growth promoting Rhizobacteria) 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 years.

**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+ *Pseudomonas brassicacearum* 3Re2-7 | Rh+Ps | AMF+ Bac1 | 0.34567.20 | 6.00E-042.00E+08  | -1,60E+10 | 0.75 |
| *Rhizophagus irregularis* MUCL41833+ *Paraburkholderia phytofirmans* PsJN | Rh+Pa | AMF+Bac2 | 0.34566.40 | 6.00E-041.00E+08  | -9.00E+09 | 0.75 |
| *Rhizophagus irregularis* MUCL41833+ *Paraburkholderia phytofirmans* PsJN+ *Trichoderma asperelloides* A | Rh+Pa+Tr | AMF+Bac2+Fungi | 0.34566.400.86  | 6.00E-041.00E+081.50E+06  | -9.00E+091.00E+09 | 0.75 |
| Control treatment  | C (control) | Control | - | - | - | - |

**Potato Roots Sampling**

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).

 **Determination of mycorrhizal colonization**

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 (F%), Intensity of mycorrhizal colonization in root system (M%) and Arbuscular abundance in root system (A%) 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](http://www.starch.dk/isi/methods/starchct.htm)) 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 (HNO3 + H2O2) 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. 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 value >0.05). Means were separated using Tukey's test at a significance level of 0.05.

**Results**

**Mycorrhizal parameters**

In the first season (2020), the highest mycorrhizal colonization frequency (F%) and mycorrhizal colonization intensity (M%) were recorded on non-irrigated areas with Rh+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 *Rhizophagus Irregularis* MucL41833 (Rh), *Pseudomonas brassicacearum* 3Re2-7 (Ps), *Paraburkholderia phytofirmans* PSJN (Pa), *Trichoderma 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).

**Table 5:** Mycorrhizal parameters (F%, M%, A%,) 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 |

**Starch content by microbial inoculants treatments**

**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 irrigation2020 | Distribution of starch by inoculant and irrigation 2021 |
|  I |  C |  I | C |
| 1 | Ps | 16.30a± 0.542 | 15.73 a± 0.441 | 10.11 a± 0.448 | 11.61a±0.666 |
| 2 | Pa | 17.16 a± 0.331 | 16.37 a± 0.201 | 11.06 a± 0.611 | 11.55 a± 0.672 |
| 3 | Tr | 15.99 a± 1.308 | 16.09 a± 0.490 | 11.32 a± 1.037 | 10.83 a± 0.679 |
| 4 | Rh | 16.42 a± 0.570 | 15.13 a± 0.674 | 12.29 a± 0.372 | 11.60 a± 0.836 |
| 5 | Rh+Ps | 16.69 a± 0.826 | 16.33 a± 0.352 | 10.85 a± 0.540 | 11.49 a±0.494 |
| 6 | Rh+Pa | 16.73 a± 0.599 | 14.90 a± 1.134 | 11.31 a± 0.602 | 10.67 a± 0.757 |
| 7 | Rh+Pa+Tr | 15.86 a± 1.392 | 16.20 a± 0.804 | 11.48 a± 0.691 | 10.37 a± 0.362 |
| 8 | C (control) | 15.77 a± 0.832 | 16.32 a± 0.452 | 11.08 a± 0.712 | 11.04 a± 0.568 |

 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

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 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.

**Table 7:** Mean values of phosphorus content (mg P kg-1) in potato tubers sorted by microbial inoculantsunder 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 a±0.012 | 0.32 a±0.010 | 0.62 ab±0.044 | 0.56 ab±0.053 |
| 2 | Pa | 0.32 a±0.012 | 0.34 a±0.008 | 0.68 a±0.036 | 0.58 ab±0.022 |
| 3 | Tr | 0.33 a±0.010 | 0.32 a±0.006 | 0.64 ab±0.037 | 0.64 ab±0.045 |
| 4 | Rh | 0.31 a±0.014 | 0.32 a±0.013 | 0.54 ab±0.051 | 0.57 ab±0.034 |
| 5 | Rh+Ps | 0.31 a±0.007 | 0.30 a±0.016 | 0.63 ab±0.029 | 0.54 ab±0.018 |
| 6 | Rh+Pa | 0.31 a±0.012 | 0.32 a±0.005 | 0.68 a±0.053 | 0.66 ab±0.038 |
| 7 | Rh+Pa+Tr | 0.32 a±0.014 | 0.32 a±0.011 | 0.63 ab±0.014 | 0.54 ab±0.025 |
| 8 | C (control) | 0.32 a±0.018 | 0.35 a±0.010 | 0.69 a±0.031 | 0.50 b±0.030 |

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

**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.

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

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

**Discussion**

*Solanum tuberosum* L. is the most commonly grown tuberous crop. There are more than 4000 native potato varieties that form an important genetic pool for potato breeding. The variability between potato varieties is reflected in several physical aspects such as size, shape, flavor, texture, and color (Matteau et al. 2021).

With the risk of environmental and health damages, also with consumer demand, organic cultivation is becoming more and more popular around the world (Busnello et al.2019). Compared to conventional production, the yield in organic cultivation is lower (Maggio et al. 2008). However, it is claimed that the culinary properties are better for the organic potato (Lerna et al. 2022). According to some studies, the dry matter content is also higher in organic potatoes than in conventional cultivation (Dangour et al. 2009 ). In addition, the quality of the potato in organic cultivation depends highly on the climate and the varieties (Busnello et al. 2019).

Our current study showed that the arbuscular mycorrhiza could establish a symbiotic relationship with the potato tubers under irrigated and non-irrigated conditions for the two seasons. Bolin 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 (Zhu et al. 2022). 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 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.

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. A study by Berta et al (2014) showed that inoculation with PGPR 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. 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. 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 (Vazquez 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**

Microbial inoculates that improve nutrient uptake, protect plants from pests and diseases, and promote plant growth may replace agrochemicals in food production. In this experiment we examined the effect of different microbial inoculations applied with the combination of different treatments under irrigated and non-irrigated conditions in two seasons. Microbial inoculates that improve nutrient uptake, protect plants from pests and diseases, and promote plant growth may replace agrochemicals in food production. In this experiment we examined the effect of different microbial inoculations applied with the combination of different treatments under irrigated and non-irrigated conditions in two seasons. The results show that the arbuscular mycorrhiza successfully evolved and established a symbiotic relationship with potato tubers. This can be observed in the measured parameters in both seasons. According to the irrigation treatment, it can be concluded that the microbial inoculations achieved better results under non-irrigated conditions than under irrigated conditions. Also, we could not demonstrate any positive effect of any of the inoculates. Even with the non-irrigated treatment, no significant benefit of inoculates was measurable.

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**Author contributions**

Conceptualization and methodology ÖMKi -Research Institute of Organic Agriculture; writing-original draft preparation N.A., N.K., Z.P., validation, analysis, and visualization, N.A. and H.H; writing-review, and editing, N.K, Z.P, H.H. D.G, T.F. O.P, D.D; revision N.K, Z.P, H.H. D.G, F.T, O.P, D.D. All authors have read and agreed to the published version of the manuscript.

**Conflict 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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