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Comparative Analysis of Biofertilizers and Chemical Fertilizers on Yield Components and Nitrate Accumulation in Sante Potato Cultivar

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ABSTRACT

Background: In the context of sustainable agriculture, reducing chemical fertilizer use is crucial to protect environmental and human health. This study aimed to evaluate the effects of various fertilizer regimes and tuber inoculation with beneficial bacteria on the quality traits of the potato cultivar Santé.
Methods: A field experiment was conducted during the 2016-2017 growing seasons at the Agricultural Research Farm of Hamadan Islamic Azad University. Treatments included five fertilizer regimes: no fertilizer (control), 100% nitrogen (N), 66% N + 33% organic fertilizer, 33% N + 66% organic fertilizer, and 100% organic fertilizer. Further, tubers were inoculated with *Azospirillum*, *Pseudomonas*, or a combination of both.
Results: In 2017, the lowest nitrate content (16.67 mg/kg) was observed with 33% N + 66% organic fertilizer treatment combined with *Pseudomonas* inoculation. The highest protein content (7.05%) was recorded under the 100% organic fertilizer treatment with dual bacterial inoculation. The highest starch content (19.76%) and protein yield (1861 kg/ha) were obtained in 2016 under combined fertilizer and bacterial treatments. Yield components improved with partial nitrogen replacement by organic fertilizer, particularly when *Pseudomonas* was applied.
Conclusion: While biofertilizers alone may not fully replace chemical fertilizers, their integration with organic inputs can significantly enhance potato quality and yield while reducing nitrate accumulation. These findings support the adoption of integrated fertilization strategies as a viable approach toward more sustainable potato production systems.

1. Introduction

To enhance crop production per unit area, the widespread application of chemical fertilizers has become a common agricultural practice. However, in recent years, this approach has contributed significantly to environmental degradation, particularly through the contamination of water and soil resources. These pollutants can enter the human food chain, thereby posing substantial risks to public health. The intensification of agriculture to meet the demands for higher yields–especially in nutrient-demanding crops such as potatoes–often necessitates excessive use of chemical inputs. This not only elevates production costs but also exacerbates a range of environmental issues.

It is estimated that approximately 65% of the mineral nitrogen applied in agricultural systems is lost through gaseous emissions, surface runoff, erosion, and leaching (Bhattacharjee et al., 2008). In high-input agricultural systems, chemical fertilizers-primarily nitrogen, phosphorus, and potassium-are frequently overused to meet plant nutritional requirements and enhance productivity. However, due to low fertilizer use efficiency, only 30-40% of these nutrients are absorbed by plants, while the remainder accumulates in the soil, contributing to environmental

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degradation (Zhang et al., 2021). Research has shown that plant performance is influenced by both genetic and environmental factors such as temperature, rainfall, and elevation (Akbari et al., 2020; Dejahang et al., 2018). Nitrate is primarily used as a fertilizer in farming and, due to its high solubility in water, can cause environmental and biological issues. In the human stomach, nitrate is converted into nitrite and nitrosamine compounds, which can contribute to the development of bladder cancer (Chamandoos et al., 2016). Organic fertilizers not only supply essential nutrients but also significantly improve crop quality, enhance soil physical properties, and increase biological activity within the soil. The application of manure has been shown to enhance soil organic matter content, mineral nutrient availability, soil structure, and overall crop yield (Mohammadi et al., 2007). Organic amendments, such as manure, also contribute to increased soil organic carbon and influence a variety of soil properties and processes, both directly and indirectly (Prakash et al., 2007). The lowland areas used for agriculture had higher nitrate levels compared to other land uses, with the primary cause likely being the overuse of chemical fertilizers containing nitrates (Khosravi et al., 2017).

Biofertilizers refer to organic materials derived from animal manure, plant residues, green manure, and beneficial microbial populations such as bacteria and fungi, as well as the byproducts of their biological activity. Among these, plant growth-promoting rhizobacteria (PGPR) represent one of the most important categories of biofertilizers (Gupta et al., 2020). Biofertilizers have been proposed as sustainable alternatives to chemical fertilizers, aiming to improve soil fertility and support crop production (Singh, 2021; Younessi-Hamzekhanlu et al., 2021). Biofertilizers consist of various free-living microorganisms capable of transforming essential nutrients from unavailable to available forms through biological processes. This promotes better root development and seed germination. Global estimates suggest that biological nitrogen fixation can amount to approximately one ton per square meter annually, with about 80% facilitated by symbiotic bacteria and the remaining 20% by associative and free-living microorganisms. Species of the genus *Pseudomonas* are effective in suppressing pathogenic fungi. *Pseudomonas fluorescens*, in particular, promotes plant growth through mechanisms such as siderophore production, antibiotic synthesis, phytohormone production, improved phosphorus uptake, nitrogen fixation, and the synthesis of enzymes that regulate ethylene levels in plants (Abdul-Jaleel et al., 2007). The main PGPRs studied in recent years belong to the genera *Pseudomonas*, *Azotobacter*, and *Bacillus*. *Azospirillum* species, in addition to nitrogen fixation, produce plant growth-promoting substances that enhance root development, which in turn increases water and nutrient uptake and improves crop yield (Alsaady et al., 2020). The increased protein yield in the integrated system is attributed to better alignment between available nitrogen and the plant's needs, preservation of soil nutrients, prevention of nitrogen leaching, enhanced biological activity, and improved soil structure (Younessi Hamzekhanlu et al., 2020). Previous studies have shown that *Pseudomonas,* particularly *P. fluorescens*, are key rhizosphere organisms with proven positive effects on plant growth (Rasouli Sadaghiani et al., 2006). In general, increased microbial diversity and enhanced community interactions contribute to the production of various organic acids, which play a crucial role in the solubilization of insoluble phosphate compounds (Sharma, 2002). Compared to chemical inputs, biofertilizers offer several advantages, including non-toxicity, natural reproduction, improvement of soil health, cost-effectiveness, and environmental sustainability (Moalem & Eshghizadeh, 2007). Potato (*Solanum tuberosum* L.) is considered one of the priority crops for development and income generation due to its economic value and potential role in food security (Indrasti et al., 2021). Potatoes are used for various purposes, including as a staple food, animal feed, and propagation material (Wang et al., 2023). The tuber contains approximately 79% water, 2% protein, and 17% carbohydrates, of which 88% is starch (Ziachehreh et al., 2024). In addition to its high starch content, potato is rich in vitamins—especially vitamin C and B-group vitamins—minerals such as potassium and iron, and phenolic compounds that play an important role in human health. Furthermore, it contains negligible amounts of soluble sugars. Nitrogen deficiency in potato plants leads to yield reduction, increased susceptibility to diseases, and premature senescence. Conversely, excessive nitrogen stimulates vegetative growth, delays tuberization and maturity, and increases the proportion of oversized tubers. Importantly, nitrogen over-application can elevate nitrate concentrations in potato tubers, which is highly undesirable from a health perspective. The crop also contains bioactive compounds such as oxyphenolines and iron. High phosphorus availability has been shown to increase the number of tubers. In phosphorus-deficient soils, an application rate of 120-130 kg/ha is recommended, whereas in moderately fertile soils, 30-50 kg/ha is generally sufficient (Van Dingenen et al., 2019).

In many experiments on organic farming and sustainable agriculture systems, less attention has been paid to the quality traits of production (Framcis et al., 1990). Despite the known health risks associated with high nitrate concentrations in food, there are limited studies on nitrate accumulation in potato crops in Iran. Considering the widespread use of chemical fertilizers and the need to explore alternative methods for nutrient management, and given the economic significance and high input demand of potatoes in this region, there is a clear gap in research regarding the effect of different nitrogen sources on potato performance and nitrate content. Therefore, this study was conducted to investigate the effects of biofertilizers in combination with chemical and organic fertilizers on yield and selected quality traits of the Sante potato cultivar in Hamedan.

2. Materials and Methods

This study was carried out during the 2016 and 2017 growing seasons at the Research Farm of the Faculty of

Agriculture and Natural Resources, Islamic Azad University, Hamedan Branch, located at 34°47'N latitude and 48°31'E longitude, with an elevation of 1823 m above sea level. Field preparation began as soon as soil moisture conditions allowed, including autumn plowing on October 7, 2016. This was followed by two perpendicular passes with a disc harrow and land leveling on May 15, 2017. After soil preparation, soil samples were collected from two depths (0-30 cm and 30-60 cm) and analyzed in the Soil Science Laboratory. The results are presented in Table 1.

Table 1. Physicochemical properties of the experimental field soil (0-30 and 30-60 cm depth) before planting during the 2016-2017 and 2017-2018 growing seasons

Growing Season	2016	2017	2016	2017
Soil Depth (cm)	0-30	0-30	30-60	30-60
Total Nitrogen (%)	0.08	0.07	0.03	0.03
Available Phosphorus (ppm)	16.6	16.9	5.6	5.8
Available Potassium (ppm)	463	456	233	229
Electrical Conductivity (dS/m)	0.70	0.70	0.86	0.83
Organic Carbon (%)	0.79	0.80	0.32	0.30
pH	7.98	7.80	7.98	7.70
Saturation Percentage (%)	44	45	51	53
Soil Texture	Loam	Loam	Clay loam	Sandy clay loam
Sand (%)	42	48	48	36
Silt (%)	32	34	28	33
Clay (%)	26	28	30	31

ppm: parts per million; dS/m: decisiemens per meter

Manure was obtained from the Hamedan Agricultural and Industrial Complex, and a sample was analyzed for nutrient content (Table 2).

Table 2. Results of the manure fertilizer analysis used during the 2016-2017 growing season

Parameter	Value
Total Nitrogen (%)	2.27
Total Phosphorus (%)	0.78
Total Potassium (%)	1.31
Organic Carbon (%)	4.42
pH	7.9
Electrical Conductivity (dS/m)	1.17
C/N Ratio	18.6

Urea fertilizer containing 46% nitrogen was used as the nitrogen source. The potato cultivar used in the study was *Sante*, and certified seed tubers (SE class) were sourced from Peyvandak Company. The experimental design followed a factorial treatment structure with two factors. Factor A (Fertilizer application) included five levels: no fertilizer (control), 100% nitrogen fertilizer, 66% nitrogen + 33% manure, 33% nitrogen + 66% manure, and 100% manure. Factor B (biological inoculation) comprised four levels: no biofertilizer (control), *Azospirillum* inoculation, *Pseudomonas* inoculation, and co-inoculation with *Azospirillum* and *Pseudomonas*. The required biological fertilizers, including *Azospirillum irakense* and *Pseudomonas fluorescens*, were obtained from the microbiological collection of the Soil Biology Laboratory, Soil Science Department, Agricultural and Natural Resources Research Center of Hamadan. These strains, originally isolated from wheat plants in Golestan province, demonstrated high potential for auxin production, nitrogen fixation, and solubilization of low-solubility and insoluble phosphate compounds.

From the tubers treated with bacterial inoculants (*Azospirillum*, *Pseudomonas*, and *Azospirillum* + *Pseudomonas*), a random sample of 1 kg was selected to assess the bacterial colonization on the tuber surfaces. The bacterial population was quantified at the Agricultural and Natural Resources Research Center of Hamadan using the dilution plating method, followed by colony counting on specific media: *Azospirillum* on RC medium and *Pseudomonas* on King B medium (Table 3).

Table 3. Bacterial Population on Potato Seed Tubers

Bacterial Species	Bacterial Population on Seed Tubers (cells/ gram of seed)
Azospirillum irakense	3.3×10^8 (2016) / 3.3×10^8 (2017)
Pseudomonas fluorescens	2.4×10^7 (2016) / 2.4×10^7 (2017)
Azospirillum irakense + Pseudomonas fluorescens	4.1×10^8 (2016) / 4.1×10^8 (2017)

Before planting, part of the potato tubers required for the experiment were treated with a bacterial inoculant (*Azospirillum*, *Pseudomonas*, and *Azospirillum* + *Pseudomonas*) at a rate of 200 g of powdered inoculant dissolved in 20 L of water, sufficient to treat 0.5 hectares of seed.

After determining the experimental treatments and planting layout, the field was arranged according to the planting map. Each plot consisted of four rows, each 8 m long, with a fixed row spacing of 75 cm and an intra-low spacing of 25 cm. To prevent cross-contamination of bacterial treatments, plots were separated by a distance of 1.5 meters (equivalent to two unplanted buffer rows), and the distance between replicates was maintained at 3 meters to facilitate irrigation. Then, the tubers were planted at a depth of 12-15 cm in both growing seasons. Planting and initial irrigation were carried out on June 6, 2016, and June 3, 2017, respectively. In the second year, the same procedures were followed, with adjustments made to the planting map to avoid treatment interference. Additionally, the experimental field was moved 50 m from the original site to prevent the mixing of bacteria and fertilizers used in the first year's field with those in the second year's field. The manure (aged, powdered cow dung) was applied according to soil test recommendations and mixed uniformly in the soil to a depth of 30 cm in the relevant plots. Urea fertilizer was also applied based on soil analysis and treatment requirements. It was band-placed in furrows along the planting rows at a depth of 8-10 cm. The nitrogen fertilizer was applied in three equal splits: one-third at planting, one-third at hilling (July 6, 2016, and June 21, 2017), and the final third at the onset of flowering (August 5, 2016, and August 1, 2017). Drip irrigation was initiated immediately after planting, with separate water delivery systems for each replicate and plot to prevent inter-plot contamination. Irrigation was conducted at intervals of approximately 6-7 days, depending on prevailing climatic conditions. Weed control and other agronomic practices were performed manually. Throughout the growing season, a range of agronomic and quality traits were assessed. To determine total yield, a harvest area of 9 m² from the two central rows of each plot was used. Random tuber samples were collected from each plot to measure nitrate concentration, protein content, starch content, dry matter percentage, average yield per plant, average tuber weight per plant, tuber yield, and protein yield.

2.1 Nitrate Measurement

A 10-gram sample of potato tuber was homogenized, and 100 mL of water preheated to 80°C was added, along with 5 mL of Sodium tetraborate decahydrate (≥ 99.5%, Sigma-Aldrich, USA). The solution was heated in a boiling water bath for 15 min. Afterward, 2 mL of Potassium ferrocyanide solution (K_4 [Fe (CN)$_6$]. 3H_2O, Merck, Germany) and 2 mL of zinc acetate solution (Zn ($CH_3COO)_2$, Merck, Germany) were added. The solution was transferred to a 200 mL flask, and the volume was adjusted. After mixing, the solution was filtered, and 10 mL of the filtrate was analyzed using a spectrophotometer at a wavelength of 538 nm, with nitrate standards prepared for calibration.

2.2 Protein Measurement

A 2.5 g sample of potato tuber was ground with Liquid nitrogen (99.999%, Merck, Germany), then 10 µL of extraction buffer was added and mixed thoroughly. The mixture was centrifuged at 1300 rpm for 15 min at 4°C (Sigma 2-16K, Sigma Laborzentrifugen GmbH, Germany). The supernatant was transferred to a test tube containing 5 mL of Bradford reagent (Sigma-Aldrich, USA), and the absorbance at 595 nm was measured using a spectrophotometer (Schimatso, UV1601tc, Japan) (Bradford, 1976).

2.3 Dry Matter Measurement

A 200 g sample of tubers from each treatment was randomly selected, crushed, and placed in an oven (Memmert, model ULM 400, Memmert GmbH, Germany) at 75°C for 3-4 hours. After the weight stabilized, the samples were re-weighed, and the dry matter percentage was calculated based on the fresh-to-dry weight ratio (Ziachehreh et al., 2024).

2.4 Starch Measurement

Crushed tuber samples were mixed with distilled water at a ratio of 1:3 (w/v), and 0.1% sodium metabisulfite solution ($Na_2S_2O_5$, Merck, Germany) was added. The mixture was filtered through Whatman No. 1 filter paper (Whatman, UK), and the filtrate containing the starch was collected. The starch was then separated, dried at 30°C, and its content was quantified as a percentage of fresh tuber weight (Takahiro et al., 2004).

At the end of the growing season, he outer two rows and 0.5 meters from both ends of each plot were removed to eliminate edge effects. The remaining plants were harvested, and tubers were weighed to calculate tuber yield per hectare. Average plant yield was determined from the total tuber weight of 10 randomly selected plants using a precision balance (accuracy: 0.001 g). Average tuber weight per plant was calculated by dividing the total tuber weight of the 10 plants by the number of tubers per plant in each experimental plot. The homogeneity of variances was tested using Bartlett's test. The results from the experimental years were analyzed using a combined analysis of variance. Statistical analysis was performed using (Version 9.1 for Windows; SAS Institute, Cary, NC) software, and mean comparisons were conducted using the least significant difference (LSD) test at the 5% significance level.

3. Results and Discussion

3.1 Tuber Nitrate Levels

The results indicated that, during the first year, different sources of chemical, organic, and biological fertilizers did not significantly affect the nitrate levels in tubers. However, in the second year, the highest nitrate concentration (45.93 mg/kg) was found in the treatment combining 100% manure with *A. irakense* and *P. fluorescens*, which demonstrated a significant difference compared to the lowest nitrate accumulation (16.67 mg/ kg) recorded in the treatment of 33% nitrogen and 66% manure combined with *P. fluorescens* (Table 4). In this study, the application of beneficial bacteria in combination with nitrogen and manure fertilizers significantly reduced nitrate accumulation in plants. While nitrogen is an essential component of proteins, its synthesis can be compromised under suboptimal growth conditions, resulting in the accumulation of nitrogen in non-protein forms, such as nitrate. Excessive dietary intake of nitrates has been associated with potential toxicity in humans (Chamandoost et al., 2016). Elevated nitrate levels in plants are primarily associated with the inability to convert nitrate, which is mainly related to insufficient moisture, excessive soil nitrogen, and inappropriate harvest timing. Additional factors influencing nitrate accumulation include light intensity, moisture availability, genetic variations among plant species and cultivars, the application of chemical pesticides-particularly herbicides-the availability of nitrate in the root environment, and the supply of other nutrients such as phosphorus, sulfur, potassium, iron, molybdenum, calcium, manganese, and boron are all factors influencing nitrate accumulation (Ghobadi et al., 2013). Potatoes, belonging to the *Solanaceae* family, are considered nitrate accumulators. Overuse of nitrogen fertilizers leads to the accumulation of nitrate even in species that typically do not store significant amounts under normal conditions. Furthermore, deficiencies in other nutrients contribute significantly to nitrate accumulation (Peska et al., 2006; Kleinkopf et al., 1981). In this study, the nitrate levels detected were substantially below the permissible limits for nitrate (250–300 mg per kg) in tubers. In line with our results, seed inoculation and the use of biological fertilizers improved both the qualitative and quantitative traits of lettuce. Moreover, seed inoculation led to an increase in phenolic and flavonoid content and a reduction in leaf nitrate levels at all fertilization levels (Ashouri et al., 2024). Frydecka and Zegorska (1996) also noted that increased nitrogen fertilizer consumption directly leads to increased nitrate levels in tubers.

3.2 Protein Content in Tuber

The analysis of tuber protein percentages in the first year showed that the different fertilizer sources did not significantly impact the protein content of the tubers. However, in the second year, a significant effect was observed. The highest protein content (7.05%) was found in the interaction between the combined treatment of manure and *A. irakense* + *P. fluorescens*, while the lowest protein content (0.18%) was observed in the treatment where no fertilizer was applied (Table 4).

The combined treatment functions as both a nutrient source and a diverse microbial population enhancer. The protein content of tubers is a critical factor in determining potato quality, as increased protein levels enhance their edible quality. Furthermore, the protein content in plants is directly correlated with nitrogen levels. The application of biological fertilizers, in conjunction with other fertilizers, enhances mineral absorption from both soil and organic sources while simultaneously reducing the reliance on chemical fertilizers by mobilizing minerals present in soil and manure. Previous studies have shown that the combined application of biological fertilizers with other nitrogen sources significantly increases the protein content of potato tubers, which is consistent with our findings (Abdeldaym et al., 2019).

3.3 Protein Yield

Based on the comparison of the mean data for protein yield in the tubers, the highest yield (1861.6 kg per hectare) was observed in the combined treatment (66% nitrogen + 33% manure) with *P. fluorescens*, while the lowest yield (420.2 kg/ha) was found in the treatment with 100% manure (Table 4). In this experiment, the application of organic fertilizer alone led to a reduction in protein yield. This outcome may be attributed to the fact that organic materials contain nutrients in relatively low concentrations, which are released slowly through decomposition. As a result, they may be insufficient to significantly enhance yield or its components in the short term. On the other hand, the combination of different fertilizers increased protein yield. This is because the combined supply of nutrients using chemical and organic fertilizers reduces the use of chemical fertilizers, compensates for nutrient deficiencies, maintains soil fertility, and leads to sustainable crop production. Researchers attribute the increased yield in the integrated system to the better matching of available nitrogen with the plant′s needs. Other reasons for the increased yield in integrated systems include the preservation of soil nutrients, prevention of nitrogen leaching, increased biological activities, and improved soil structure through the use of manure (Singh et al., 2018; Wichrowska & Szczepanek, 2020). *Pseudomonas*, which is a siderophore-producing bacterium, was shown to increase protein yield due to its role in enhancing nutrient absorption (Sultana et al., 2021). In line with our results, researchers have found that combining biological, chemical, and organic fertilizers as a replacement for chemical fertilizers can create a balance between soil elements and improve rhizosphere conditions, such as reducing soil acidity and alleviating stress effects, which ultimately leads to increased tuber yield and protein content (Mohammady-Aria et al., 2010).

3.4 Tuber Starch Content

The results of the evaluation of tuber starch percentage showed that the interaction between the combined treatments of (urea + manure) × biological fertilizer had a significant effect on the starch content. The highest starch content (19.76%) was found in the treatment combining (33% nitrogen + 66% manure) with *A. irakense* + *P. fluorescens*, showing a significant difference compared to the lowest starch content (14.36%) found in the treatment with 100% nitrogen and *P. fluorescens* (Table 4). The increase in starch content observed under the combined fertilizer treatment may be attributed to the adequate nitrogen supply provided by the integration of biological inputs with urea and manure, which promotes starch accumulation. Nitrogen is a fundamental component in the biosynthesis of starch, and its availability enhances starch production in plants. Furthermore, the combined application of chemical, organic, and biological fertilizers improves overall growth conditions, enhances photosynthetic activity, and subsequently increases assimilate production and starch storage in the tubers (Gomma & Mohamed, 2007). *P. fluorescens*, by enhancing phosphorus uptake, indirectly increases nitrogen consumption, which, in turn, facilitates the conversion of sugars into starch (Cindy & Pieter, 2018). Studies in this area, particularly in potatoes, are limited, but varying phosphorus contents (from 308 to 1244 ppm) in different varieties have been observed. Phosphorus, in the form of phytates (a type of inositol phosphate), regulates starch synthesis during tuber growth (Noda et al., 2007). The effects of these biological fertilizers enhance plant growth and the synthesis of storage materials (especially carbohydrates and starch), leading to increased dry matter in the tubers (Ghobadi et al., 2013).

3.5 Tuber Dry Matter Content

The analysis of tuber dry matter content revealed that in the first year, the interaction between (urea + manure) × biological fertilizer was significant for dry matter content, but no significant effect was found in the second year. The treatment (33% nitrogen × 66% manure) × (*A. irakense + P. fluorescens*) had the highest dry matter content (28.39%), which was significantly different from the treatment (100% nitrogen × *A. irakense*) with 19.98% dry matter (Table 4). The highest dry matter content in potatoes was observed in the treatment with the combined use of all the bacteria along with chemical and manure fertilizers. Dry matter production is an indicator of the accumulation of photosynthetic materials in the plant and its capacity for nutrient absorption. Organic materials, such as manure, improve soil structure and increase its organic matter content, which promotes root growth, enhances the plant's ability to retain water, and increases the availability of absorbable nutrients. These processes ultimately enhance the plant's photosynthetic efficiency and contribute to increased dry matter production (Saeednejad et al., 2012). Dry matter content is an important factor in determining the quality of potato tubers. It should be noted that the dry matter percentage in a specific variety is not fixed but varies depending on factors such as soil, climate, and mineral content. The use of biological and nitrogen fertilizers, alongside mineral nitrogen, improves the quality of potatoes. Furthermore, growth-promoting bacteria increase the growth and the nutrient content of the crop, mainly through the production of plant growth regulators by the bacteria, which affect root growth and increase nutrient and water absorption from the soil (Hajiloo et al., 2010). The bacteria *A. irakense* and *P. fluorescens*, produce compounds that regulate plant growth and increase the plant's access to nutrients, enhancing photosynthesis and dry matter production.

3.6 Average Tuber Weight per Plant

The analysis of the average tuber weight per plant showed that in the first year, the interaction between (urea + manure) × biological fertilizer was significant for tuber weight, but in the second year, no significant effect was observed. The highest tuber weight per plant (183.65 g) was found in the combined treatment (66% nitrogen × 33% manure) with *P. fluorescens,* showing a significant difference compared to other treatments (Table 4).

The reason for this can be attributed to the role of *P. fluorescens* in increasing phosphorus absorption, which subsequently boosts nitrogen consumption, growth, and photosynthesis, ultimately leading to increased tuber weight per plant. This results in an increased yield and component yield. The similarity in average tuber weight among the other treatments is likely due to the plant's genetic potential for optimal utilization of environmental resources, especially biological fertilizers (Angelin-Bonnet et al., 2023). Salomon et al. (2014) identified *Bacillus licheniformis* and *P. fluorescens* as producers of phytohormones, such as abscisic acid, indole butyric acid, and gibberellin. *P. fluorescens* is known for producing indole butyric acid, which increases root hair length and root surface area, enhancing nutrient and water absorption by the plant. However, in high nitrogen treatments or combined treatments, the necessary symbiosis between the bacteria and the plant was not established. Nitrogen application in lower doses improves growth and increases photosynthesis efficiency, leading to larger tubers. Nitrogen has a lesser effect on tuber number but is more influential in determining tuber size and weight (Struik et al., 1990). However, if nitrogen levels exceed the optimal range, both tuber weight and number may decrease (Kleinhenz & Bennett, 1992).

3.7 Average Plant Yield

The results indicated that in the first year, the interaction effect of [(urea fertilizer + manure) × biological fertilizer] on the average plant performance was significant, but in the second year, it was not. The highest average plant performance (1309.50 g) was observed in the treatment of (66% nitrogen + 33% manure) combined with *Pseudomonas*, while the lowest (741.4 g) was seen in the non-fertilized control treatment. These results showed significant differences between the treatments (the second lowest value was in the treatment with no fertilizer and no bacteria, with 741.45 g). In this study, the simultaneous application of phosphate-degrading bacteria (*Pseudomonas*) and nitrogen-fixing bacteria (*Azospirillum*) not only contributed to nutritional roles but also promoted the tuber formation process in potatoes through hormone secretion, organic compound production, and the uptake of these materials by the roots (Kafi et al., 2019). Rhizosphere microorganisms promote plant growth through both direct and indirect mechanisms, including enhanced nutrient availability, production of plant hormones, suppression of diseases via biological control agents, mitigation of abiotic stress, and bioremediation of environmental pollutants. Collectively, these functions contribute to improved plant health and productivity (Glick & Gamalero, 2021). Phosphate-solubilizing bacteria are a group of microorganisms capable of converting insoluble phosphorus in the soil into soluble and plant-accessible forms. *Pseudomonas* is a significant genus in this group, including several species. These bacteria promote plant growth through various mechanisms, such as producing plant hormones, enhancing phosphorus uptake, nitrogen fixation, antibiotic and siderophore production, and releasing enzymes that regulate plant ethylene levels. Additionally, *Pseudomonas* plays an effective role in controlling pathogenic fungi (Tilak et al., 2005). It has been shown that microbial inoculants containing essential nutrients stimulate root and shoot growth, improve nutrient absorption, and increase yield under various agricultural and environmental conditions (Etesami et al., 2021). In line with our findings, the application of *Azospirillum brasilense* and *Rhizobium tropici* showed a synergistic effect, increasing plant biomass, nitrogen accumulation, seed weight, and the yield of common beans (Filipini et al., 2021). Moreover, the increased performance in the treatments that combined biological fertilizer and urea compared to the control group suggests that the bacteria in the biological fertilizer managed to stimulate plant roots through various natural mechanisms. This improvement enhanced water and nutrient uptake, which increased nitrate absorption, leading to higher plant performance (Zare et al., 2013).

3.8 Tuber Yield

The results showed that in the first year, the interaction effect of [(urea + manure) × biological fertilizer] on tuber yield was significant. The highest tuber yield (13.75 tons/ha) was observed in the treatment of (66% nitrogen + 33% manure) combined with *Pseudomonas*, while the lowest (16.40 tons/ha) was found in the 100% manure treatment with *Azospirillum* + *Pseudomonas*, which had a significant difference from other treatments. The use of nitrogen fertilizers along with manure and biological fertilizers led to increased tuber yield in this study. The reason for this is that in this treatment, equivalent to 216.5 kg of nitrogen and 7941 kg of manure/ha, combined with *Pseudomonas*, was used, which contributed to vegetative plant growth and tuber production.

It appears that a balanced combination of chemical, organic, and biological fertilizers in this treatment resulted in an increased tuber yield compared to other treatments. Previous studies have shown that the application of organic fertilizers to provide essential plant nutrients leads to enhanced forage production (Saeednejad et al., 2012). *Pseudomonas*, with its nitrogen-fixing and phosphate-solubilizing properties, promotes maize shoot development and significantly improves barley grain yield through major physiological changes in the plant. Moreover, *Pseudomonas* enhances plant nutrition and competes with pathogenic agents for root colonization, thus increasing plant resistance to disease attacks. These microorganisms colonize the root zone, rapidly expand their population, and prevent the growth of phytopathogens by producing secondary metabolites such as antibiotics and cyanohydrins, ultimately boosting plant growth (Leben et al., 1987). Additionally, Fontes et al. (2010) showed that nitrogen fertilization increases total and marketable potato yields. They concluded that the highest tuber yield occurs when 80 kg of nitrogen is applied. At this level, the percentage of large tubers reaches its maximum, and the average tuber weight is higher than at other levels. The reasons for reduced tuber yield in treatments with higher nitrogen percentages can be attributed to excessive nitrogen application and high plant density, which could lead to excessive vegetative growth. These conditions might delay tuber formation, reduce yield, and result in the premature harvest of immature tubers. Furthermore, these factors can negatively affect tuber quality (Aghighi Shahvardi, 2011).

Table 4. Comparison of Mean Values for Some Quality Traits of Potatoes Under the Influence of Biological, Organic, and Chemical Fertilizers

Treatment (Urea + Manure) × Biological Fertilizer		2016				2017			
		Average Plant Yield (kg/ha)	Average Tuber Weight (g)	Tuber Yield (ton/ha)	Tuber Protein Yield (kg/ha)	Starch (%)	Dry Matter (%)	Nitrate Concentration (mg/kg)	Tuber Protein (%)
(No Nitrogen and Manure Fertilizer)	No Bacteria	741.45c	110.06bc	42.65bc	1391ab	16.76ab	24.97b	21.9b	18.0b
	Azospirillum	1105.6abc	109.12bc	59.36abc	925.76b	16.01ab	22.42b	55.65ab	3.97b
	Pseudomonas	1256.36abc	118.97bc	70.06abc	1045.6b	16.86ab	22.42b	20.89b	2.98b
	Azospirillum + *Pseudomonas*	985.65abc	93.98bc	50.51abc	521.56d	15.23ab	22.38ab	38.96b	4.01b
(100% Nitrogen)	No Bacteria	1259.5abc	110.78bc	65.49abc	1037.7b	16.57ab	22.27b	22.93b	2.4b
	Azospirillum	1260.6abc	119.45bc	60.46abc	1029.4b	15.23b	19.98b	22.73b	3.03b
	Pseudomonas	1179.3abc	133.56abc	44.69bc	604.58b	14.36b	21.23b	21.97b	2.27b
	Azospirillum + *Pseudomonas*	1025.6abc	109.68bc	56.01abc	700.56cd	15.24ab	22.51b	28.31b	3.37b
Nitrogen + 33% Manure)	No Bacteria (66%	1110.9abc	106.12bc	58.09abc	946.54b	15.86ab	23.79b	20.42b	2.39b
	Azospirillum	1157.6abc	123.56bc	61.76abc	1234.3ab	16.01ab	21.09b	19.73b	2.41b
	Pseudomonas	1309.5a	183.65a	75.13a	1861a	18.01ab	23.79b	20.98b	3.03b
	Azospirillum + *Pseudomonas*	1061.6abc	97.65bc	62.29abc	871.56cd	17.76ab	22.61b	22.19b	2.51b
(33% Nitrogen + 66% Manure)	No Bacteria	1065.5ab	140.24ab	70.23abc	1476.25ab	15.67ab	22.43b	25.06b	2.71b
	Azospirillum	991.5abc	100.15bc	51.64abc	914.58b	15.81ab	23.43b	20.62b	2.49b
	Pseudomonas	1197.5ab	148.56ab	61.23abc	1071.06b	17.71ab	23.36b	21.67b	4.28b
	Azospirillum + *Pseudomonas*	1130.5abc	125.32bc	61.19abc	1144.06b	19.76a	28.93b	22.69b	2.51b
(100% Manure)	No Bacteria	912.2abc	103.74bc	48.56abc	420.25e	16.01ab	23.87b	23.58b	2.13b
	Azospirillum	850.5ab	108.34bc	43.91bc	634.25b	17.08ab	24.86b	20.42b	2.41b
	Pseudomonas	950.5abc	120.56bc	52.82abc	798.57b	17.71ab	23.42b	24.17b	2.27b
	Azospirillum + *Pseudomonas*	790.5c	78.82c	40.1c	605.36cd	14.04b	20.97b	93.45a	7.05a

* Letters within each column indicate statistical significance based on the least significant difference (LSD) test at the 5% level. Treatments with the same letter do not differ significantly.

4. Conclusion

This study evaluated the effects of chemical, organic, and biological fertilizers on potato quality. The results showed that combining reduced nitrogen use with manure and biological fertilizers improved crop yield and quality, while maintaining nitrate levels within safe limits. Notably, treatments involving *Pseudomonas* bacteria had the most positive effects on quality parameters. Overall, the findings suggest that integrated plant nutrition strategies can significantly enhance potato production and contribute to more sustainable agricultural practices.

Authors' Contributions

Saeed Piri Pireivatlou: Conceptualization; Supervision; Project administration. **Ali Akber Naghdi:** Investigation; Writing-original draft preparation; Data curation. **Ali Akber Naghdi, Ahmad Khaligi, Pejman Moradi:** Methodology; Investigation; Writing-original draft preparation; Writing-review and editing.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical considerations

There were no ethical considerations in this research. (Thesis code: 12348079347417516249075l).

Using artificial intelligence

The authors declare that they have not used any artificial intelligence techniques in this research.

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