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Azolla pinnata Extract as a Biostimulant Enhances Soil Fertility, Potato Yield, and Tuber Compositional Quality

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Abstract

Sustainable potato production requires eco-compatible biological inputs capable of improving soil fertility, crop productivity, and tuber nutritional quality while reducing dependence on synthetic agrochemicals. This study evaluated *Azolla pinnata* extract as a natural biostimulant for potato (*Solanum tuberosum* L. cv. Spunta) under field conditions. Dried *A. pinnata* biomass was first characterized for its biochemical composition, and aqueous extracts were applied at 25, 50, 75, and 100% and compared with the untreated control and a commercial seaweed-based biostimulant. Soil physicochemical properties, potato growth and yield traits, and tuber chemical composition were assessed after cultivation. *Azolla pinnata* biomass showed a protein-rich and phytochemically active profile, containing 28.36±2.44% protein, 6.23±0.60 mg g^{-1} DM chlorophyll, 2.80±0.55 mg g^{-1} DM carotenoids, 4.00±0.81 mg GAE g^{-1} DM total polyphenols, 3.20±0.73 mg QE g^{-1} DM flavonoids, and 1.20±0.18 mg CE g^{-1} DM tannins. Field application improved soil chemical indicators, particularly pH, electrical conductivity, total nitrogen, and organic matter. The strongest agronomic response was obtained with the 100% extract, which increased tuber weight to 974.0±10.82 g $plant^{-1}$ and estimated yield to 46.36±3.55 tons ha^{-1}, compared with 615.0±29.34 g $plant^{-1}$ and 32.60±1.20 tons ha^{-1} in the untreated control. Tuber composition was also enhanced at 100%, with higher total carbohydrates, protein, starch, total sugars, ash, and dietary fiber. These findings indicate that *A. pinnata* extract can enhance soil fertility, potato productivity, and tuber compositional quality, supporting its use as a sustainable input for quality-oriented potato production.

Keywords: biostimulant application; soil fertility improvement; Spunta potato; tuber nutritional composition; tuber yield

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INTRODUCTION

The search for sustainable crop production strategies has accelerated the development of biological inputs capable of improving crop productivity while reducing dependence on synthetic agrochemicals. Plant biostimulants have gained increasing attention because they can enhance nutrient-use efficiency, stimulate endogenous plant processes, improve tolerance to abiotic stress, and support crop performance through improved soil-plant interactions rather than acting only as conventional fertilizers. Recent studies describe biostimulants as multifunctional tools acting at the interface of plant metabolism, rhizosphere processes, and crop quality. This positions them as relevant for sustainable intensification and environmentally responsible food production (Boutahiri et al., 2024; Mashabela et al., 2025; López-Serrano et al., 2026).

Potato (*Solanum tuberosum* L.) is a major food crop whose productivity and quality are closely linked to soil fertility, nutrient availability, and sustainable crop management. Recent communications from the Food and Agriculture Organization have reaffirmed the importance of potatoes for food security, nutrition, rural livelihoods, and environmental sustainability (FAO, 2025). However, intensive potato production often relies heavily on mineral fertilization and synthetic inputs to maintain tuber yield and commercial quality. When applied excessively or inefficiently, these inputs may contribute to soil degradation, nutrient imbalances, and environmental pollution (Golin et al., 2024; FAO, 2025). Therefore, identifying biological amendments capable of improving soil properties, potato productivity, and tuber quality is an important priority for sustainable potato production.

Biostimulant responses are known to vary according to source material, formulation, dose, timing, cultivar, and environmental conditions. This variability is particularly relevant in potato, where recent field studies have shown that biostimulant efficacy cannot be generalized across production systems and requires crop-specific and product-specific validation (Golin et al., 2024). Such evidence highlights the need to evaluate novel biological resources using indicators directly related to soil fertility, agronomic performance, and tuber composition.

Within this context, *Azolla pinnata* represents a promising candidate for sustainable potato production. This aquatic fern is recognized for its symbiotic association with *Anabaena azollae*, which supports biological nitrogen fixation and contributes to nutrient cycling in agricultural soils. In addition to supplying organic nutrients, *Azolla*-based materials may improve soil organic matter, nutrient availability, microbial activity, water retention, and overall soil fertility. These properties suggest that *A. pinnata* may act as an organic amendment and biostimulant capable of improving soil conditions and supporting potato growth under sustainable production systems (Chandrababu et al., 2024; Consorti et al., 2025; Yang et al., 2025).

The value of *A. pinnata* application in potato production should therefore be assessed through parameters that reflect the title and scope of the study, namely soil physicochemical properties, potato productivity, and tuber chemical composition. This integrated approach is essential because sustainable potato production should be evaluated not only by tuber yield but also by soil preservation and nutritional quality. Despite the recognized agronomic potential of the genus *Azolla*, limited information is available on the combined effects of *A. pinnata* application on soil properties, potato growth, yield performance, and tuber chemical composition within the same experimental framework. Previous studies have often considered organic amendments, soil improvement, or crop productivity separately, whereas a unified evaluation of *A. pinnata* as a biostimulant for potato cultivation remains insufficiently documented (Golin et al., 2024; Yang et al., 2025).

The present study aimed to assess the potential of *A. pinnata* aqueous extract as a biological input for supporting sustainable potato cultivation. More specifically, the aims were to: (i) report on the overall differences in soil physicochemical status before and after the potato growing season; (ii) evaluate the effectiveness of the extract on potato growth and yield; and (iii) assess the alterations in tuber chemical composition following biostimulant treatments. By targeting yield and tuber quality attributes and considering soil parameters simply as general before-and-after variables, the current research cautiously assesses *A. pinnata* for an eco-compatible contribution towards a quality-based potato production system.

MATERIALS AND METHOD

Study site and climatic conditions

The field experiment was established in March 2024 at the experimental farm of Abdelhamid Ibn Badis University of Mostaganem, Mazagran, northwestern Algeria. The farm is located at 0°04'44.00" E, 35°53'35.00" N and 149 m above sea level. Climatic data were obtained from the nearby Mostaganem meteorological station. In March 2024, the mean air temperature was 17.1 °C, with mean minimum and maximum temperatures of 11.4 and 22.7 °C, respectively, and cumulative rainfall was 24.6 mm. Drip irrigation was applied uniformly to all treatments throughout the experiment (Lavanya et al., 2026; Mohamed et al., 2026).

Plant material

Azolla pinnata used in this study was collected from the experimental farm of Abdelhamid Ibn Badis University of Mostaganem, Algeria, during its active vegetative stage. The freshly harvested biomass was thoroughly washed with distilled water to remove adhering debris and impurities. It was then air-dried under shade at room temperature (25 to 30 °C) for 48 to 72 hours to minimize degradation of light-sensitive bioactive compounds, particularly phenolics and flavonoids. During this period, the biomass was turned regularly to ensure uniform dehydration and to prevent microbial deterioration. The partially dried material was subsequently oven-dried at 50 °C until a final moisture content of approximately 10% was reached, to improve storage stability and facilitate further processing. The dried biomass was then ground into a fine homogeneous powder and stored in clean, dry, airtight containers at room temperature, protected from light and moisture, until extract preparation. Seed tubers of *S. tuberosum* L. cv. Spunta were obtained from the Plant Protection Laboratory of the University of Mostaganem and used for the priming treatments and subsequent field experiment (Sadeghi et al., 2017).

Preparation of *Azolla* biostimulant extract

The biostimulant extract of *A. pinnata* was prepared according to the method of Bindhu (2013), with slight modifications to standardize the extraction procedure across all treatments. Briefly, 1 kg of dried and ground *A. pinnata* powder was mixed with 1,000 ml of distilled water to obtain the stock extract. The mixture was subjected to continuous agitation for 30 minutes at a boiling point of 100 °C to ensure efficient extraction of water-soluble constituents. After extraction, the mixture was filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was collected as the stock extract and stored at 4 °C in sterilized glass containers until use to preserve its stability. Different concentrations of the extract, namely 25, 50, 75, and 100% v/v, were subsequently prepared by dilution of the stock solution with distilled water (Bindhu, 2013; Shedeed, 2022).

Pre-treatment of potato seed tubers with *A. pinnata* biostimulant extract

Different concentrations of the *A. pinnata* stock extract were prepared for the priming treatment by diluting with sterile double-distilled water to obtain 25, 50, 75, and 100% (v/v) solutions. One day after extract preparation, seed tubers of *S. tuberosum* L. cv. Spunta, obtained from the Plant Protection Laboratory of the University of Mostaganem, were subjected to a priming treatment before planting. The tubers were soaked for 1 hour in the respective *A. pinnata* extract solutions, while the control treatment consisted of sterile double-distilled water only. This soaking period was selected based on previous reports indicating that short-duration priming promotes the uptake of bioactive compounds and supports early seedling establishment (Ashraf and Foolad, 2005; Paparella et al., 2015; Farooq et al., 2019).

Experimental design

A field experiment was conducted on a 500 m² plot to evaluate the effects of an *A. pinnata*-derived biostimulant on the growth and development of *S. tuberosum* L. cv. Spunta. The study was arranged in a randomized complete block design (RCBD) with 6 treatments and 6 blocks. Blocks were established along the main field gradient to minimize the effect of spatial heterogeneity across the experimental plot, particularly possible variations in soil fertility, soil moisture distribution, and slight microtopographical differences. Each block was therefore considered a relatively homogeneous experimental unit. Each block contained 6 rows, and each treatment was randomly assigned to 1 row within each block, resulting in 6 experimental replicates per treatment.

The treatments were as follows: T0, untreated control receiving water only; T1, commercial biostimulant based on marine algae (Altrak, Prosnel, Algeria); T2, *A. pinnata* extract at 25% v/v; T3, *A. pinnata* extract at 50% v/v;

T4, *A. pinnata* extract at 75% v/v; and T5, *A. pinnata* extract at 100% v/v. Each row measured 15 m in length. Plants were spaced 35 cm apart within rows and 70 cm between rows, corresponding to 43 plants per row and a total of 258 plants per treatment across the 6 blocks. For statistical purposes, each row was considered an experimental unit, whereas individual plants within a row were treated as subsamples. The graded concentrations of *A. pinnata* extract were included to allow both treatment comparisons and dose-response evaluations of potato growth, productivity, and tuber chemical composition.

Following the priming treatment, seed tubers were planted directly in their respective field plots. The *A. pinnata*-based biostimulant solutions were subsequently applied by manual soil drenching to ensure direct delivery to the root zone. Each plant received 30 ml of the corresponding solution per application, and treatments were applied once every 2 weeks throughout the experimental period. A drip irrigation system was used throughout the experiment to maintain adequate soil moisture. Emitters were spaced at 30 cm intervals along the drip lines and delivered water at a flow rate of 2 l $hour^{-1}$. Irrigation was scheduled twice daily, in the early morning and late afternoon, to reduce evaporative losses and improve water-use efficiency under field conditions.

Laboratory analysis

Biochemical and nutritional characterization of A. pinnata

The biochemical and nutritional composition of *A. pinnata* was determined using standard analytical procedures. Dry matter content was measured by oven-drying the samples at 105 °C for 24 hours and expressed as a percentage of the initial fresh weight. Total mineral matter was determined by dry ashing in a muffle furnace at 500 °C until constant weight and expressed as a percentage of dry weight. Crude protein content was quantified using the Kjeldahl method and calculated with a nitrogen-to-protein conversion factor of 6.25 (Noh et al., 2020; Aguirre, 2023). Total lipids were extracted according to the method of Folch et al. (1957) using a chloroform/ methanol mixture, 2:1 v/v, and lipid content was determined gravimetrically after solvent evaporation. Chlorophyll and carotenoid contents were measured after extraction in 80% v/v acetone, with absorbance recorded at 663, 645, and 470 nm, and pigment contents calculated according to Lichtenthaler and Wellburn (1983).

Antioxidant components and antioxidant activity of A. pinnata

Dried *A. pinnata* powder was extracted with methanol at a 1:10 (w/v) ratio under continuous stirring at 150 rpm for 24 hours at 25 °C. The mixture was then filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure at 40 °C using a rotary evaporator. The dried extract was reconstituted in distilled water and stored at 4 °C until further analysis. Total polyphenol content was determined using the Folin-Ciocalteu method, with gallic acid as the reference standard, and the results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM). Total flavonoid content was quantified using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents per gram of dry matter (mg QE g^{-1} DM). Total tannin content was determined using the vanillin-HCl assay, with catechin as the reference standard, and the results were expressed as mg catechin equivalents per gram of dry matter (mg CE g^{-1} DM) (Price et al., 1978; Singleton et al., 1999; Quettier-Deleu et al., 2000).

Antimicrobial activity of A. pinnata

The antimicrobial activity of the methanolic extract of *A. pinnata* was evaluated against selected phytopathogenic fungi and bacterial strains. The fungal strains included *Alternaria* sp. ATCC 20084, *Fusarium* sp. ATCC 60289, *Botrytis* sp. ATCC 11542, and *Phoma* sp. ATCC 12593. The antibacterial assay was performed against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Klebsiella pneumoniae* ATCC 27853, and *Proteus mirabilis* ATCC 10876. Dried *A. pinnata* powder was extracted with 80% (v/v) methanol at a 1:10 (w/v) ratio under continuous stirring at 150 rpm for 24 hours at 25 °C. The mixture was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure at 40 °C using a rotary evaporator. The dried extract was dissolved in dimethyl sulfoxide (DMSO) to prepare extract solutions at 10% and 20% w/v. Before use, these solutions were diluted with sterile distilled water so that the final DMSO concentration did not exceed 5% v/v under any assay condition. The reconstituted extracts were stored at 4 °C until analysis.

Antimicrobial activity was assessed by the agar well diffusion method. Bacterial strains were cultured on Mueller-Hinton agar and incubated at 37 °C for 18 to 24 hours, whereas fungal

strains were cultured on potato dextrose agar and incubated at 28 °C for 48 to 72 hours. Microbial suspensions were adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×108 CFU ml $^{-1}$ for bacteria and 105 spores ml^{-1} for fungi. Wells of 6 mm diameter were made in the inoculated agar plates, and 100 µl of each extract solution was introduced into the wells. After incubation, inhibition zones were measured in millimeters after 24 hours for bacteria and after 48 to 72 hours for fungi. A DMSO control containing ≤ 5% v/v solvent was used as the negative control, whereas streptomycin, 10 µl ml^{-1}, and fluconazole, 10 µl ml^{-1}, were used as positive controls for the antibacterial and antifungal assays, respectively (Qadi et al., 2023; Bouhalla et al., 2024).

Soil physicochemical analysis

Soil physicochemical properties were analyzed to evaluate soil status before and after potato cultivation. Soil samples were collected from the 0 to 20 cm layer. For each sampling point, 5 subsamples were taken randomly and thoroughly homogenized to obtain a composite sample for subsequent analysis. Soil samples were collected at the time of planting and at the end of the cropping season. However, no specific sampling points were included to provide individual results for any treatments. Therefore, the soil samples were representative of the prevailing conditions before, during, and after the growing season and were not used for the interpretation of treatments.

Soil pH was measured in a 1:2 soil-to-water suspension using deionized water. Soil texture was determined by the hydrometer method. Electrical conductivity was measured in a soil-water extract using a conductivity meter (Cond 3110, WTW, Weilheim, Germany). Cation exchange capacity was determined by the ammonium acetate method at pH 7. Total nitrogen content was analyzed using the Kjeldahl method. Total and active limestone contents were determined using the Bernard calcimeter method. Organic matter content was estimated by loss on ignition at 550 °C. Assimilable phosphorus was quantified using the Olsen extraction method with 0.5 M $NaHCO_3$ at pH 8.5, followed by colorimetric determination based on the molybdenum blue reaction. Absorbance was read at 882 nm using a UV-Vis spectrophotometer, Lambda 25, PerkinElmer, Waltham, MA, USA (Murphy and Riley, 1962; Bremner, 1996; Loeppert and Suarez, 1996; Nelson and Sommers, 1996; Rhoades, 1996; Sumner and Miller, 1996; Thomas, 1996; Gee and Or, 2002; FAO, 2022; Marzouk et al., 2024; Mosley et al., 2024; Dissanayake et al., 2026).

Potato growth, yield, and tuber biochemical analysis

The growth and yield performance of *S. tuberosum* L. cv. Spunta were evaluated using standard agronomic parameters, including tuber yield, plant height, number of tubers per plant, mean tuber weight, and biochemical composition of harvested tubers. Plant height was measured at the end of the growth cycle from the soil surface to the apex of the main stem using a ruler. Measurements were taken on 5 randomly selected plants within each plot, and the mean value was expressed in centimeters. The number of tubers per plant was determined at harvest by manually counting the tubers produced by the same 5 randomly selected plants within each plot, and the mean value was used as an estimate of individual plant productivity (Yayeh et al., 2025; Tueros et al., 2026).

Tuber yield was determined from the total fresh weight of harvested tubers obtained from each experimental plot and expressed in tons per hectare (tons ha^{-1}). Yield was calculated using the following corrected equation: Yield (tons ha^{-1}) = [tuber yield per plot (kg) × 10,000] / [plot area (m^2) × 1,000], where 10,000 is the area equivalent of 1 ha, and 1,000 is the conversion factor from kilograms to metric tons. Yield per plot was used as the experimental unit value for statistical analysis. Mean tuber weight was calculated by dividing the total fresh weight of tubers harvested from each plot by the total number of tubers collected from the same plot. The result was expressed in grams per tuber and used as an indicator of tuber biomass accumulation.

Biochemical composition analysis of potato tubers

The biochemical composition of potato tubers was analyzed to assess the effects of *A. pinnata*-based biostimulant treatments on tuber nutritional quality. Moisture content was determined by oven-drying fresh tuber samples at 105 °C to constant weight and expressed as a percentage of fresh weight. Ash content was determined by incineration in a muffle furnace at 550 °C to constant weight and expressed as a percentage of dry matter. Total protein content was quantified using the Kjeldahl method and calculated with a nitrogen-to-protein conversion factor of 6.25. Total lipids were extracted according to the method of Folch et al. (1957) using

chloroform/methanol, 2:1 v/v, and quantified gravimetrically after solvent evaporation.

Total carbohydrates were estimated using the phenol-sulfuric acid method with glucose as the reference standard. Starch content was determined enzymatically after hydrolysis with thermostable α-amylase and amyloglucosidase. Total sugars were quantified by the anthrone method, with absorbance measured at 620 nm. Dietary fiber was determined using a standard enzymatic-gravimetric procedure (DuBois et al., 1956; Folch et al., 1957; Hodge and Hofreiter, 1962; McCleary et al., 1997; Nielsen, 2017; Vershinina et al., 2025).

Statistical analysis

All data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Because the experiment followed a randomized complete block design (RCBD), data were analyzed using a linear mixed model with treatment as a fixed effect and block as a random effect. The model was $Yij = \mu + Ti + bj + \varepsilon ij$, where *Yij* is the observed value, μ is the overall mean, *Ti* is the fixed treatment effect, *bj* is the random block effect, and εij is the residual error. Residual normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene's tests, respectively. When treatment effects were significant, means were separated using Tukey's honestly significant difference (HSD) test at $p < 0.05$. Results are presented as mean±standard deviation (SD). Graphs and correlation analyses were generated using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Biochemical and bioactive composition of *A. pinnata*

The composition reported in Table 1 indicates that *A. pinnata* is a nutritionally and biochemically valuable biomass. The profile was dominated by crude protein (28.36±2.44%), whereas dry matter remained moderate (11.15±1.12%) and lipid content was low (4.50±1.71%). The biomass also contained measurable levels of chlorophyll, carotenoids, polyphenols, flavonoids, and tannins, showing that *A. pinnata* combines nutritional constituents with antioxidant-related metabolites. This pattern agrees with recent studies describing *Azolla* as a protein-rich and phytochemically active fern of interest for sustainable agricultural use (Hagag et al., 2025; Yang et al., 2025; Yuan et al., 2025).

The high crude protein content is the main compositional feature of the studied biomass and suggests that *A. pinnata* may provide organic nitrogenous compounds after decomposition, potentially supporting nutrient turnover in the rhizosphere. However, this interpretation should remain cautious because nitrogen mineralization, microbial activity, and nutrient release kinetics were not directly measured in the present study. The relatively low dry matter content is consistent with the aquatic nature of *Azolla* and mainly reflects its intrinsic hydration. In contrast, the low lipid level confirms that its functional value depends more on proteins, pigments, minerals, and phenolic compounds than on lipid accumulation (Hagag et al., 2025; Boukrouh, 2026).

The pigment and phenolic fractions further support the functional potential of the biomass. Chlorophyll and carotenoids reached 6.23±0.60 and 2.80±0.55 mg g^{-1} DM, respectively, while total polyphenols, flavonoids, and tannins reached 4.00±0.81 mg GAE g^{-1} DM, 3.20±0.73 mg QE g^{-1} DM, and 1.20±0.18 mg CE g^{-1} DM, respectively. These compounds are commonly associated with antioxidant and protective functions in plant-derived materials (Rahman et al., 2023; Bouattou et al., 2024; Yang et al., 2025). Thus, the biochemical profile of *A. pinnata* supports its potential as a multifunctional biomass for sustainable agricultural use, particularly through its protein, pigment, and phenolic fractions.

Nevertheless, these results should be interpreted primarily as a characterization of the biomass and methanolic extract potential, rather than as direct evidence explaining the potato responses, because the field-applied

Table 1. Estimated mean values and standard deviations of the biochemical and bioactive composition of *A. pinnata*

Variable	Means
Dry matter (%)	11.15±1.12
Mineral matter (%)	3.00±0.35
Crude protein (%)	28.36±2.44
Total lipids (%)	4.50±1.71
Chlorophyll (mg g^{-1} DM)	6.23±0.60
Tannins (mg CE g^{-1} DM)	1.20±0.18
Carotenoids (mg g^{-1} DM)	2.80±0.55
Total polyphenols (mg GAE g^{-1} DM)	4.00±0.81
Total flavonoids (mg QE g^{-1} DM)	3.20±0.73

biostimulant was prepared as an aqueous extract. This distinction is important because methanolic and aqueous extracts may differ in their phytochemical composition and concentration of bioactive compounds. Therefore, while Table 1 supports the biological interest of *A. pinnata*, the mechanisms underlying the observed field responses require further confirmation through direct characterization of the aqueous extract used for plant application.

Antimicrobial effect of *A. pinnata* extract

Figure 1 clearly shows that the methanolic *A. pinnata* extract has a concentration-dependent effect on antimicrobial inhibition against the tested bacteria and fungi. The inhibitory zones for 20% extract were higher compared to the 10% extract for all microorganisms tested. No zone of inhibition was observed in negative controls, meaning that the antimicrobial effect of the extract was responsible for inhibition, not the solvent system. As researchers had already expected, positive controls showed the largest zone of inhibition; this is because streptomycin and fluconazole are known antimicrobial pure products targeting specific structures, whereas *A. pinnata* extract is a natural complex mixture.

Bacteria were more susceptible to 20% methanolic extract of *A. pinnata*, including *E. coli* and *S. aureus*, followed by *Klebsiella pneumoniae* and *Proteus mirabilis*, which were slightly less susceptible. In the same way, for the fungal activity, this research found a stronger zone of inhibition in the case of *Alternaria* sp. and *Fusarium* sp., followed by *Phoma* sp. and *Botrytis* sp., with the same pattern of concentration-dependent effect. The differences in sensitivity among bacterial and fungal strains against the extract are commonly reported in similar studies using *Azolla* extracts (Bouattou et al., 2024; Aybey et al., 2025; Chanapanchai et al., 2025).

The higher activity against these organisms using the higher concentration could be partially attributed to increased accumulation of antimicrobial compounds like phenolics, flavonoids, tannins, and pigments, which are known to inhibit microbial growth either by disorganizing the microbial cell membrane or by inactivating enzymes, causing oxidative damage, and binding with proteins (Rahman et al., 2023; Bouattou et al., 2024). The variations in inhibitory zones between microorganisms suggest that their resistance mechanisms target specific compounds within the extract; their cellular components, as well as their susceptibilities, could contribute to these variations (Rahman et al., 2023; Bouattou et al., 2024). However, the mechanism explanation must be regarded with caution due to the absence of isolation and identification of the active principle. The results of the bioactivity test should be treated as supportive evidence of the biological significance of the methanolic extract of *A. pinnata*, rather than as a mechanistic explanation of potato field responses, as the aqueous extract was applied to plants.

Soil physicochemical properties

The soil data presented in Table 2 were obtained as pooled before-and-after values; therefore, they are interpreted as overall changes

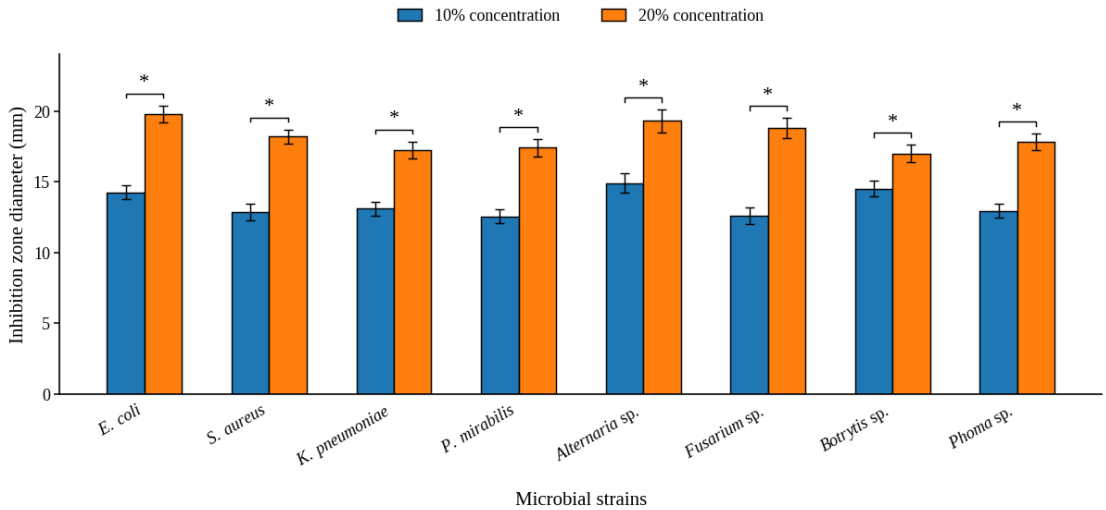

Figure 1. Inhibition zones produced by *A. pinnata* extract at 10% and 20% against selected bacterial and fungal strains

Note: Error bars indicate standard deviation. Asterisks indicate significant differences between concentrations within the same strain ($p < 0.05$)

Table 2. Physicochemical properties of the soil before and after *S. tuberosum* cultivation

Parameter	Before plantation	After plantation
Clay (%)	13.86±0.5	13.86±0.5^{ns}
Silt (%)	20.72±0.6	20.72±0.6^{ns}
Sand (%)	65.42±1.2	65.42±1.2^{ns}
Electrical conductivity (mS cm^{-1})	0.219±0.015^{b}	0.336±0.02^{a}
Cation exchange capacity (meq 100 g^{-1})	8.53±0.4	8.53±0.4^{ns}
Total nitrogen (%)	0.058±0.005^{b}	0.106±0.008^{a}
Total limestone (%)	11.03±0.8^{a}	5.94±0.7^{b}
Active limestone (%)	1.75±0.2^{a}	0.0±0.0^{b}
pH	7.4±0.1^{b}	7.82±0.12^{a}
Organic matter (%)	1.16±0.12^{b}	2.12±0.15^{a}
Assimilable phosphorus (ppm)	130.0±10.0^{a}	43.0±8.0^{b}

Note: Values are the mean±SD. Lowercase letters in the same row indicate significant differences between pooled soil samples before and after cultivation at $p < 0.05$; ns: not significant. As the soil samples were pooled and not treatment-resolved, these data should not be used to deduce treatment-specific impacts

in soil physicochemical status during the cultivation cycle, not as treatment-specific effects. Texture and cation exchange capacity remained unchanged, while electrical conductivity, pH, total nitrogen, organic matter, limestone fractions, and assimilable phosphorus changed between the beginning and end of cultivation.

Soil pH increased from 7.40±0.10 to 7.82±0.12, indicating a moderate shift toward greater alkalinity. Electrical conductivity also increased from 0.219±0.015 to 0.336±0.020 mS cm^{-1}, but the values remained within a low salinity range. This change may reflect increased ionic activity in the soil solution during cultivation rather than harmful salt accumulation. Because the samples were pooled, these changes cannot be attributed to a specific *A. pinnata* concentration or to the biostimulant (Altrak, Prosnel) treatment.

The increase in total nitrogen from 0.058±0.005% to 0.106±0.008% is agronomically relevant, which is probably an overall change in soil nitrogen status during the cropping cycle rather than as a treatment-resolved response. This trend is compatible with the known role of *Azolla* biomass in nitrogen enrichment through its association with *A. azollae* and subsequent mineralization of organic nitrogen compounds (Rashid et al., 2024; Yang et al., 2025; El-Beshbeshy et al., 2026). Organic matter also increased from 1.16±0.12% to 2.12±0.15%, suggesting an overall improvement in soil organic status during cultivation.

Assimilable phosphorus decreased from 130±10 to 43±8 ppm. This decline may reflect crop uptake, microbial immobilization, or redistribution into less labile phosphorus fractions during the growing period. Rather than indicating a simple deterioration of fertility, this result highlights the dynamic nature of phosphorus cycling under active crop growth. This overall analysis indicates changes in the chemical status of the soil during potato cultivation. However, unless treatment-resolved soil data are available, it is advisable to avoid treatment-specific conclusions.

Effect of *A. pinnata*-based biostimulant treatments on potato growth and yield

Table 3 shows that potato growth and yield responses varied according to treatment. The highest mean tuber weight and estimated yield were obtained with T5, 100% *A. pinnata* extract, followed by T4, 75% *A. pinnata* extract. These results suggest that higher extract concentrations were more effective in promoting tuber biomass accumulation under the present field conditions.

The response pattern was not strictly monotonic across all concentrations. T2 and T3 produced lower estimated yields than the untreated control, despite showing acceptable or higher tuber numbers. This suggests that low and intermediate extract concentrations may have stimulated tuber initiation without sufficiently supporting tuber bulking, leading to more or comparable tubers but lower final yield. This non-monotonic response may reflect a concentration threshold: below a certain level, the extract may be insufficient to enhance assimilate supply, nutrient uptake, or tuber filling, whereas higher concentrations may provide enough bioactive and nutritional compounds to support stronger sink development.

The yield pattern appears to be more closely related to tuber weight than to tuber number. T2 produced the highest mean number of tubers

Table 3. Effect of *A. pinnata*-based biostimulant treatments on potato growth, tuber weight, and estimated yield

Parameter	T0	T1	T2	T3	T4	T5
Tuber weight (g plant^{-1})	615.0±29.34^{e}	755.8±63.79^{c}	646.0±20.29^{e}	680.0±23.94^{d}	828.0±49.95^{b}	974.0±10.82^{a}
Estimated yield (ton ha^{-1})	32.60±1.20^{d}	35.97±2.05^{c}	24.56±1.84^{e}	25.70±3.61^{e}	39.41±2.44^{b}	46.36±3.55^{a}
Number of tubers (plant^{-1})	9.4±4.45^{c}	8.8±2.28^{d}	11.4±4.50^{a}	9.2±3.63^{c}	9.0±3.16^{c}	10.8±1.92^{b}
Number of stems (plant^{-1})	2.6±0.8^{c}	2.4±0.5^{c}	3.0±1.0^{b}	2.6±0.8^{c}	2.6±0.5^{c}	3.2±0.4^{a}

Note: Data are expressed as mean±SD (n = 6). Significant differences between treatments were revealed by a blocked RCBD analysis followed by Tukey's HSD post hoc test and were marked with lowercase letters within each row ($p < 0.05$). The presence of at least one shared letter between means denotes a lack of significant difference

per plant, but its yield remained low, whereas T5 produced the highest tuber weight and yield. This indicates that the main productive response was expressed through improved tuber bulking rather than increased tuber set. This interpretation is consistent with studies showing that potato yield response to biostimulants often depends more on assimilate allocation and tuber filling than on tuber initiation alone (Cortiello et al., 2024; Golin et al., 2024). The superior performance of T5 may be explained by a stronger stimulation of vegetative vigor, nutrient availability, and carbon allocation toward tuber filling.

However, because physiological parameters such as photosynthetic rate, enzyme activity, hormonal status, and nutrient uptake were not measured, these mechanisms remain plausible explanations rather than direct evidence. Compared with the commercial seaweed-based biostimulant, T5 produced higher mean productivity, suggesting that concentrated *A. pinnata* extract may represent a promising alternative biostimulant under the present conditions. Nevertheless, this result should be confirmed across seasons, cultivars, and soil types.

Chemical composition of potato tubers

The chemical composition of the potato tubers is given in Table 4. The majority of quality attributes, i.e., total carbohydrate, starch, total sugars, lipids, ash, and dietary fiber, yielded the highest mean values in the T5 (100% *A. pinnata* extract) treatment. This indicates that the response to *A. pinnata* extract was mediated not only by agronomic traits but also by changes in the compositional quality of the tubers, which is one of the most important indicators of both the nutritional and technological aspects of the potato crop.

Similar quality-oriented responses to the biostimulant effect have been confirmed in several potato experimental trials, specifically considering starch, protein, sugars, and some mineral content traits (Cortiello et al., 2024; Golin et al., 2024; Brążkiewicz et al., 2026). Total carbohydrates were increased from 16.35±0.50% in T0 to 17.57±0.30% in T5. Similarly, starch was increased from 15.08±0.60% to 16.28±0.30%. These results are indicative of a higher tendency of the potato to deposit carbon in the tuber as a whole rather than exclusively as fresh biomass under the highest *A. pinnata* concentration. This effect is highly significant considering

Table 4. Chemical composition of potato tubers grown with different concentrations of *A. pinnata* extract application

Parameter (%)	T0	T1	T2	T3	T4	T5
Moisture	78.08±1.20^{e}	79.00±1.00^{c}	78.03±1.20^{d}	79.11±1.10^{d}	79.20±1.00^{c}	79.58±0.90^{b}
Total carbohydrates	16.35±0.50^{d}	17.29±0.40^{b}	16.18±0.50^{c}	17.14±0.40^{c}	17.23±0.40^{b}	17.57±0.30^{a}
Protein	2.23±0.10^{c}	2.54±0.10^{a}	2.36±0.10^{b}	2.41±0.10^{b}	2.14±0.10^{d}	2.56±0.1^{a}
Total lipids	0.11±0.02^{d}	0.14±0.02^{b}	0.12±0.02^{c}	0.13±0.02^{c}	0.15±0.02^{b}	0.17±0.02^{a}
Ash	1.03±0.10^{d}	1.10±0.10^{b}	1.05±0.10^{c}	1.08±0.10^{c}	1.10±0.10^{b}	1.15±0.10^{a}
Starch	15.08±0.60^{d}	15.38±0.40^{b}	15.12±0.60^{c}	15.53±0.50^{c}	16.21±0.40^{b}	16.28±0.30^{a}
Total sugars	1.08±0.10^{d}	1.39±0.10^{b}	1.18±0.10^{c}	1.21±0.10^{c}	1.34±0.10^{b}	1.46±0.10^{a}
Dietary fiber	1.38±0.10^{d}	1.52±0.10^{b}	1.44±0.10^{c}	1.57±0.10^{c}	1.58±0.10^{b}	1.61±0.10^{a}

Note: Values are mean±SD (n = 6). Different lowercase letters within the same row are significantly different among treatments, as determined by Tukey's HSD test based on the blocked RCBD model ($p < 0.05$). Means with shared letters are not significantly different

starch is the primary storage component and heavily influences nutritional value, processing suitability, and textural properties. It can be hypothesized that a higher extract concentration can enhance plant vigor and the distribution of assimilates towards tubers. However, these conclusions must be drawn with extreme caution, given that mechanisms of photosynthesis, enzyme regulation, or carbon distribution were not directly measured.

Several recent experiments highlight that biostimulants can modify potato tuber composition and improve certain aspects, with starch-related traits among the most commonly affected by these treatments (Cortiello et al., 2024; Poberežny et al., 2025; Brążkiewicz et al., 2026). The content of total protein varied among treatments, with T1 and T5 showing higher mean values. Potato tubers cannot be considered as proteinaceous crops, but given their high biological value, even a slight improvement in protein content can be relevant for human nutrition. This could be associated with the effect of the extract on nitrogen availability, absorption, and assimilation, since *A. pinnata* is characterized by high protein content (Poberežny et al., 2025; Brążkiewicz et al., 2026).

Total sugars tended to increase as the extract concentration increased. It can be suggested that the use of higher concentrations of extract might have improved carbon metabolism by activating the biochemical pathways that lead to the accumulation of both starch and total sugars. Nonetheless, the accumulation of sugars in potato tubers depends on the processing purpose. A limited accumulation might be beneficial for the sensorial quality for direct consumption, while the concentration of sugars (especially reducing sugars) could cause unwanted browning reactions if potatoes are intended to be processed into chips or fries. Further studies on differentiation between reducing and non-reducing sugars would be necessary to establish the technological relevance of such changes.

Ash and dietary fiber also tended to be higher in the T5 treatments, suggesting a higher accumulation of minerals and structural fractions into tubers. Total lipids showed an increment in a numerically minor way, and their effect on the overall nutritional value can be considered limited given the naturally low-fat content of potatoes. Table 4 points out that, among the quality attributes measured, tubers obtained under T5 demonstrated the best overall pattern, particularly as far as carbohydrate composition (total

carbohydrate, starch, total sugars), ash, and dietary fiber were concerned. Considering the obtained results as a whole, they confirm that using concentrated *A. pinnata* extract could result in simultaneous improvement in yield and several tuber quality characteristics, but further *in vivo* analyses are required to verify these outcomes.

Correlation analysis of potato growth, yield, and tuber compositional traits

Figure 2 shows an exploratory analysis of correlations between potato growth, yield, and tuber compositional traits using a correlation heatmap. There was a positive correlation between tuber weight, yield, starch, ash, total sugars, and dietary fiber, suggesting that those treatments resulting in increased potato yield also promoted an increase in tuber quality traits. Similar trends were reported by studies using various biostimulants in potato, where yield improvement was associated with accumulation of starch and better-quality related traits (Cortiello et al., 2024; Golin et al., 2024; Wadas, 2026). Hence, this heatmap should be used in conjunction with treatment comparison rather than as an independent demonstration of any physiological process.

One interesting pattern is observed in tuber number versus tuber mass. There were generally weak correlations between tuber number and both final yield and most quality traits. In contrast, there were positive exploratory associations between tuber weight, yield, and several compositional variables. This indicates that the most responsive treatment to *A. pinnata* resulted in greater tuber mass and higher assimilate accumulation instead of increased tuber initiation.

This is consistent with the field results, which show that T2 resulted in higher tuber number, but the yield was relatively low compared to T5, where tuber mass was greater, leading to a higher estimated yield. Similar results on this matter are seen in previous research on biostimulants in potatoes, where the mechanism underlying the enhancement of tuber yield has been mainly attributed to increased assimilate partitioning to tubers rather than to an increase in the number of tubers (Golin et al., 2024; Salvage et al., 2024). The heatmap indicated a clear clustering among starch, total carbohydrates, ash, total sugars, and dietary fiber, suggesting that they may respond to biostimulation together in terms of improving potato quality.

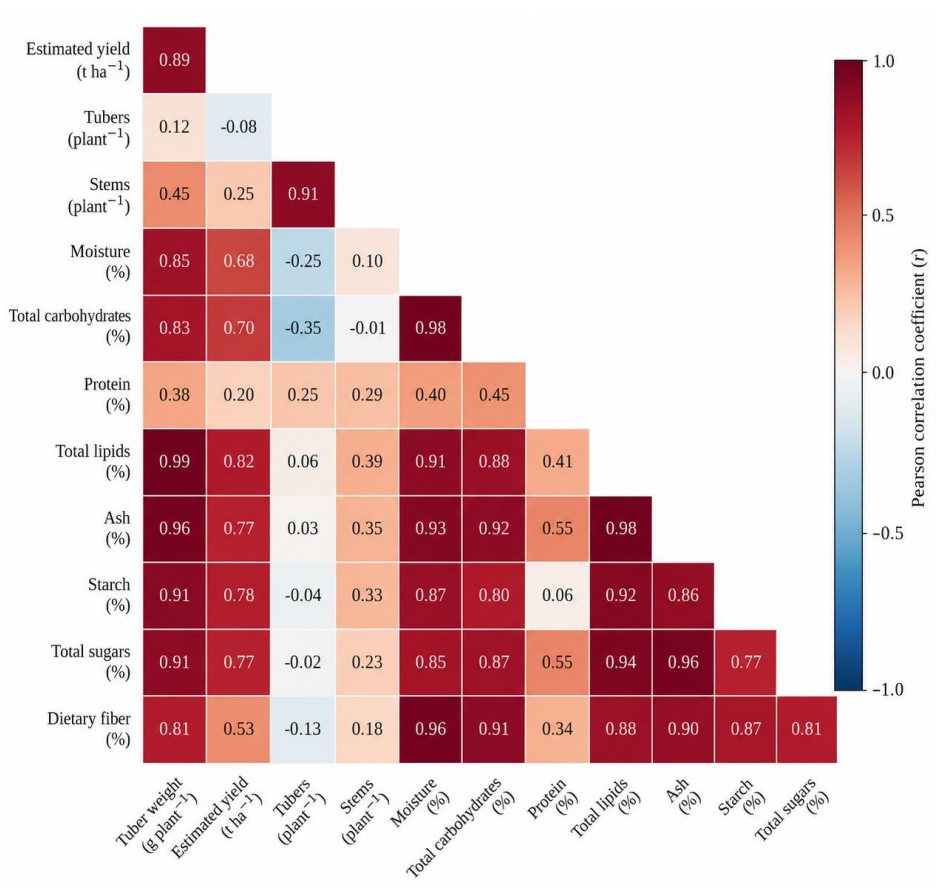

Figure 2. Pearson correlation heatmap of potato growth, yield, and tuber quality traits

The combined response pattern suggests potential broad effects on carbon allocation, mineral nutrient status, and the regulation of storage metabolism. Therefore, the observed coherence provides an exploratory basis for a common pattern response but not strong support for any causality among specific variables. A concerted change in carbohydrate storage and mineral contents associated with quality improvement in potato with biostimulant application has also been found in recent studies (Poberężny et al., 2025; Brążkiewicz et al., 2026; Wadas, 2026). The predominant pattern of association among traits observed in this study consisted of tuber weight and productivity enhancement coupled with improvements in starch, total sugars, ash, and dietary fiber, with relatively weak correlation with tuber number. Nevertheless, such correlations are suggestive of coordinated treatment responses but should be regarded as exploratory evidence, not as direct causation attributed to *A. pinnata* application.

Limitations of the study

Despite the finding that *A. pinnata*-based applications can increase potato yield and improve some tuber quality attributes, limitations of the current study should be noted. For example, the biostimulant used in the field study consisted of aqueous extracts, whereas biochemical analysis and antimicrobial tests were mainly performed on methanolic extracts. Therefore, the interpretation of the observed effects on biological processes and their potential mechanisms should be treated with caution. Additionally, the soils collected before and after the experiment were treated as whole samples rather than as samples assigned to specific treatments. Thus, alterations in soil properties could not be correlated with specific, treatment-induced effects.

Finally, as only 6 treatment mean values were used for the correlation analysis, the identified associations should be considered exploratory rather than confirmatory. Lastly, the study was performed in only one growing season, under a particular soil type, climate, and potato variety. Therefore, additional trials are needed over multiple seasons and in diverse environments, including those with varying potato cultivars and soil conditions. These trials should also involve a thorough characterization of the chemical composition of different *A. pinnata* extracts to further identify optimum application dosages for these biostimulants in the field and explore the underlying physiological mechanisms of their effects.

CONCLUSIONS

This study demonstrates that *A. pinnata* aqueous extract can improve potato productivity and tuber compositional quality under the field conditions tested. Overall, soil status changed during cultivation, but because soil samples were pooled, treatment-specific soil effects cannot be inferred. Among treatments, the 100% extract produced the highest tuber weight and estimated yield, mainly through enhanced tuber bulking and improved carbohydrates, starch, protein, ash, sugars, and dietary fiber. Thus, *A. pinnata* extract is a promising eco-compatible input for quality-oriented potato production. Future work should characterize the aqueous extract and validate rates across seasons, cultivars, and soils.

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DECLARATION OF GENERATIVE AI USE

No generative artificial intelligence (AI) tools were used in the preparation of this manuscript.

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