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Development and Evaluation of a Trichoderma-Based Bioformulation for Enhancing Sustainable Potato Cultivation

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Abstract: The potato (*Solanum tuberosum*) is one of the most widespread and cultivated *Solanaceae* in the world. To overcome the increasing problems of intensive cultivation and climate change, finding new strategies to guarantee the needs of today and future generations has become mandatory. The use of biostimulants based on *Trichoderma* spp. can be an excellent alternative to reduce the use of pesticides, as well as to mitigate the effects of biotic and abiotic stresses. In this study we evaluated the effects of a new bioformulation containing two *Trichoderma* strains on potato growth and yield. *Trichoderma* strains were characterised morphologically and molecularly. Application of the new bioformulate was able to promote potato plant growth and caused a significant increase in plant fresh (+107%) and dry weight (+74%), and potato tuber fresh weight (+37%) and number (+41%), and it also improved potato yield (+36%). These findings suggest that the bioformulation is a viable alternative to reduce pesticide use and mitigate biotic and abiotic stress in potato cultivation.

Keywords: biostimulant; biocontrol agent (BCA); plant growth promoting microorganisms (PGPM); mycoparasite; fungicide; phenotyping

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1. Introduction

The potato (*Solanum tuberosum*) is one of the most widespread and cultivated *Solanaceae* in the world due to its nutritional properties and its wide adaptability to new growing environments as well as its easy affordability [1]. Although the potato is of South American origin, the continent with the highest potato production between 2012 and 2019 was Asia with 51.1% of world production, followed by Europe (29%) and the Americas (12.2%) [2]. As one of the most widespread intensive crops in the world, the effects and consequences of its intensive cultivation cannot be overlooked, such as loss of organic matter, soil erosion, pollution caused by using synthetic pesticides (such as insecticides fungicides or fertilisers), acidification or salinisation of soils, and loss of genetic biodiversity.

The damage caused by this type of intensive cultivation has already occurred in some places on the planet [3,4]. To contain these problems, in line with the European Green Deal, the sustainable management of environmental resources poses the basis for sustainable economic development, placing the agricultural sector at the centre of the ecological

transition of the European continent [5]. Therefore, preserving biodiversity, ecosystems and natural resources while at the same time ensuring food safety and sustainability has become mandatory today [6]. Furthermore, the potato is one of the most vulnerable crops in changing climates, with events such as long-lasting droughts, extreme heat, and unanticipated frosts [7,8]. Although crop management practices cause about 67% of the variations in potato yields, climate change is a significant challenge faced by the agricultural sector [9]. Potato development stages, such as sprouting, emergence, and leaf area development, are temperature sensitive. Temperature thresholds and photoperiod sensitivity are vital in determining the development of potatoes and initiating potato tuber induction, and vary with potato varieties. When the temperature exceeds 30 °C, it can cause slow tuber initiation and development and physical damage to the tubers [10]. Meanwhile, the elevation of CO_2 increases the rate of potato susceptibility to pests and diseases and yields phenology, causing interferences between implemented and natural biological processes [8,11]. Finding new strategies to guarantee the needs of today and future generations is now a key point of agricultural research [12]. The use of biostimulants based on a Biocontrol Agent (BCA) such as *Trichoderma* spp. can be an excellent alternative to reduce the use of pesticides, as well as to mitigate the effects of biotic and abiotic stress. Several *Trichoderma* spp. are registered as microbial biological control agents in Plant Protection Products commercialized for the control of a broad-spectrum plant diseases [13,14]. In nature, *Trichoderma* spp. are ubiquitous, and are capable of surviving in the rhizosphere as a skilled competitor towards other phytopathogens. Numerous studies have demonstrated a biocontrol activity of the *Trichoderma* genus against *Fusarium* spp. and *Rizoctonia solani* which can cause huge losses in potato cultivation [15]. The adaptive behaviour of the *Trichoderma* genus is due to its ability to modify the rhizosphere conditions in its favour producing siderophores for iron chelation, or competing for the main source of carbohydrates in soil, limiting factors for the growth of other microorganisms [16]. Competition is only one mode of survival implemented by the *Trichoderma* genus. In fact, its properties of producing antibiotic substances (antibiosis), such as metabolites of a volatile or non-volatile nature which limit the growth of other phytopathogens, are also known. The production of these secondary metabolites is specific to each species and includes compounds such as arzianic acid, alameticins, tricolines, antibiotic peptaibols, 6-penthyl-α-pyrone, massoylactone, viridine, gliovirine, glisoprenins, and heptelidic acid [15–17]. Mycoparasitisation occurs when *Trichoderma* spp. comes into contact with the host, after anchoring through the formation of appressoria on the surface of the target. In the anchoring sites of the appressoria there will be the formation of hyphae which produce hydrolytic enzymes (chitinases) that are necessary for the degradation of the host's cell wall [18]. Some species of *Trichoderma*, interacting with the host plant, influence the physiology, morphology, and metabolomics of the plants. Studies relating to the impact of *Trichoderma* interaction with plants have highlighted a significant effect on many horticultural crops such as melon, tomato, cucumber, eggplant, pea, lettuce, rocket, beans, and ornamental plants [18–24]. Also, *Trichoderma* spp. promotes the absorption of nitrogen and the solubilisation of phosphorus, increasing the yield even in the absence of fertilization [25], such as increasing the efficiency of use of water, reducing the stomatal opening of plants, and increasing chlorophyll photosynthesis [26]. Defined as an avirulent opportunistic plant symbiont, *Trichoderma* spp. manages to penetrate and colonize the roots, establishing a symbiotic relationship. Its ability to split sucrose through invertase and therefore to use the products deriving from hydrolysis as a carbon source favours its capacity as a colonizer [27]. One of the main effects due to the plant colonisation of *Trichoderma* spp. is the induction of Induced Systemic Resistance (ISR), an indirect defence mechanism activated by genes that encode the production of proteins linked to the production of hormones such as Salicylic Acid (SA) or Jasmonic Acid (JA) and Ethylene (ET) as a primary signal of host resistance following the production of antimicrobial metabolites [18,28]. In addition, an integrated approach combining BCAs with fungicides is put forward to reduce the fungicide doses to manage plant diseases and thereby their residue on harvested crops. However, to allow large-scale implementation of the use of

a new bioformulate, further knowledge is needed, comprising the timing, number, and interval of repeated BCA applications and their compatibility with fungicides [29].

The goal of the present study was to evaluate the effects of a mix of two new selected strains of *Trichoderma* in the sustainable management of potato cultivation evaluating the impact of the bioformulation on the quality and quantity of production.

2. Materials and Methods

2.1. Trichoderma spp. Evaluation and Bioformulation

Two strains of *Trichoderma* from the collection of CNR-IPSP were characterised at the morphological and molecular levels. Strains (1A and 1B) were grown on petri dishes with Potato Dextrose Agar (PDA) and then placed at 25 °C for about 7 days to allow mycelial development and sporulation.

2.1.1. Morphological Characterisation of Trichoderma Isolates

The morphological traits (mycelium structure, conidiophores, and conidia) were observed directly by a ZEISS Axiovert 5 digital inverted light microscope (ZEISS, Jena, Germany), equipped with fluorescence, brightfield, and phase-contrast vision. The structures, in both observation modes, were described by comparing them with those reported in appropriate taxonomic keys [30,31]. Conidia and conidiophores lengths were recorded using ImageJ v 1.53, a Sun-Java-based digital image processing computer program. The spores of each strain were collected in 50 mL tubes using distilled water. Counting was performed using Burker's chamber (depth 0.100 mm), and 10 µL of the spore suspension was loaded onto the counting chamber and visualised under a 40× magnification. Spore concentration was calculated by the following formula (specific to the counting chamber used):

$$\left(\frac{S_{tot}}{N}\right) \times 2.5 \times 10^5 \times \text{dilutionfactor}$$

N = number of cells observed; S_{tot} = total number of spores observed.

2.1.2. Molecular Analysis of Trichoderma Isolates

The genomic DNA of the two fungal isolates was obtained using Qiagen's DNeasy® Plant Pro kit (Qiagen, Hilden, Germany, EU) following the manufacturer's instructions. The DNA was quantified with a Thermo Fisher Scientific's Nanodrop 2000 spectrophotometer (ThermoFisher scientific, Waltham, MA, USA) and then amplified by PCR, using the Promega GoTaq® G2 DNA Polymerase kit (Promega, Madison, WI, USA) in a total volume of 25 µL. Reactions were subjected to an initial denaturation of 3 min at 95 °C, followed by 40 cycles of 45 s at 95 °C, 45 s at 58 °C, and 1 min at 72 °C, with a final extension of 10 min at 72 °C in a MiniAmp thermal cycler (ThermoFisher scientific, Waltham, MA, USA). For the molecular identification we used ITS, TEF1, and TRI-ACT genes. Primer sequences are reported in Table 1. The presence of the amplificated fragments was verified by electrophoresis on 1% agarose gel. Purification of the PCR products was performed using the Bioline ISOLATE II PCR and Gel Kit (Bioline, Memphis, TN, USA) following the supplier's protocol. The obtained DNA was quantified by both Qubit fluorometer and NanoDrop 2000 spectrophotometer (ThermoFisher scientific, Waltham, MA, USA). The samples were sequenced by the company Eurofins Genomics LLC (Ebersberg, Germany, EU) through Sanger sequencing. The GenBank BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the obtained sequences with those in the GenBank database.

Table 1. Nucleotide sequences of primer pairs used in this study.

Primer Name	Gene	Primer Direction	Sequence (5′-3′)
Internal Transcribed Spacer	ITS	ITS1 ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC
Translation elongation factor 1 alpha	Tef-1	EF1-728	CATCGAGAAGTTCGAGAAGG
		TEF1R	GCCATCCTTGGGAGATACCAGC
Beta-actin	β-actin	TRI-ACT1 TRI-ACT2	TGGCACCACACCTTCTACAATGA TCTCCTTCTGCATACGGTCGGA

2.1.3. Dual-Plate Bioassay

In vitro antagonism tests were set up to evaluate the mycoparasitic activity of the two *Trichoderma* strains against four different fungal pathogens (*Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Aspergillus* sp. and *Botrytis cinerea*). Biocontrol assays were established by inoculating 10 µL of spore suspension (1×10^5 spores·mL^{-1}) of the selected pathogen (except for *S. sclerotiorum* for which sclerotia were used) in the middle of the half-circle of the PDA petri dish. After 24 h, 10 µL of spore suspension (1×10^5 spores·mL^{-1}) of each *Trichoderma* strain were inoculated at diametrically opposite points of the same plates.

Biocontrol assays were followed according to the different growth requirements of the selected pathogen. The fungal growths were measured with a ruler (mm) every 24 h, up to the day of contact between the mycelium of the pathogen and the antagonist. The experiment was conducted in independent triplicates. Single cultures of the pathogens were used as control plates. The inhibitory effect of *Trichoderma* isolates was assessed by estimating the percentage (%) reduction on pathogen growth in the presence of the antagonist, according to the following formula: $(D - d)/D \times 100$, where D is the estimate of radial growth of a pathogen in the control sample and d is the estimate of radial growth of a pathogen in the bi-culture [32].

2.1.4. Trichoderma–Fungicide Interaction

In vitro studies were conducted to test the resistance of the two *Trichoderma* isolates against three commercial fungicides: Talendo® (Proquinazid, Corteva Agriscience™, Indianapolis, IN, USA); Lidal® (Tetraconazole, Corteva Agriscience™); and Biomic (Tannins, Nuovo Mondo Bio s.n.c., Soriano nel Cimino, Italy). Briefly, 10 µL of a spore suspension 1×10^5 spores mL^{-1} were inoculated in the centre of the petri dish containing PDA mixed with the single fungicide according to the label dose. Control samples were made, inoculating *Trichoderma* isolates on PDA plate. The experiment was conducted in independent triplicates. Following the inoculations, the plates were incubated at 25 °C and the radial growth of the various species was measured every 24 h for 7 days using a ruler (mm). The percentage of growth inhibition by the fungicide was calculated as previously reported for the dual-plate bioassay.

2.1.5. Bioformulation of Trichoderma Isolates

For the preparation of the new bioformulate (SOSTATA W), the two *Trichoderma* strains (1A and 1B) were inoculated into flasks containing Potato Dextrose Broth (PDB) and left to grow in a rotating incubator at 25 °C for 7 days at 150 rpm. The liquid suspension was used to inoculate rice (*Oryza* spp.), used as carrier previously autoclaved in a polypropylene zipper filter bag. The bags were incubated for at least one month at 25 °C in the dark. Rice completely covered with spores was distributed in the field as previously described in Section 2.1.

2.2. Experimental Field

During the seasons 2022 and 2023, three experimental Italian fields located in Cervinara (AV), Maddaloni (CE), and Avezzano (AQ) were cultivated with different potato cultivars.

Also, in 2023 two more fields located at Acerra (NA) and Airola (BN) were included in the trials (Table 2).

Table 2. Experimental fields conducted for two consecutive years in southern Italy. Table shows the site of cultivation with GPS coordinates, the extension of cultivated area in hectares, and the potato cultivar name.

Experimental Fields					
Site	Airola (41°03′10.2″ N 14°35′05.8″ E)	Cervinara (41°01′49.1″ N 14°37′05.0″ E)	Maddaloni (41°01′03.6″ N 14°21′42.4″ E)	Acerra (40°56′20.2″ N 14°24′25.8″ E)	Avezzano (42°03′53.5″ N 13°31′54.2″ E)
Cultivated area	4 ha	4 ha	2 ha	2 ha	2 ha
Cultivar	Agata	Agata	Inova	Colomba	Colomba & Cicero

The experimental study was designed to compare three treatments: (i) cultivation usually adopted by the farmer, Control (C); (ii) cultivation using a commercial *Trichoderma* product, Micosat (CCS Aosta srl) (O); and (iii) cultivation using a new *Trichoderma* formulation, SOSTATA (W). A repeated blocks design was used for the experiment. The new bioformulate was made using two *Trichoderma* strains selected from the collection of the Institute for Sustainable Plant Protection (IPSP) of the National Research Council (CNR). The bioformulate was distributed at 30 kg·ha^{-1} along the furrow near the tuber contemporary to the sowing, while Micosat was used following the instructions on the label. The potato cultivars evaluated for the purpose of the experimental design were: Inova, Colomba, Agata, and Cicero in ascending order of maturity or relative earliness of the ripening cycle. The experimental fields located in the Campania region (Airola, Cervinara, Maddaloni, and Acerra) had sandy loam soil, while the Avezzano field from the Abruzzo region was a clayey soil. The weather conditions were variable not only among the different sites but even more between the two years of the trial (Table 2).

2.3. Plant Response Evaluation

To evaluate the effectiveness of treatments, at the phenological stage of tuber swelling, five plants randomly collected from each thesis with their respective tubers were harvested to assess (i) plant fresh weight, (ii) potato tuber number, (iii) potato tuber fresh weight, and (iv) plant dry weight. Production was recorded at the end of the potato cultivation cycle.

Furthermore, in the experimental field of Cervinara, we assessed the plants' response to different treatments using a UAV multispectral flight (DJI Phantom multispectral, five bands: blue, green, red, red edge, and near-infrared (NIR)) conducted during the growing season on 20 June 2023. The images collected were processed with PIX4DMapper software v. 1.58.2 to calculate vegetative indices (such as NDVI and GNDVI) and to measure the crop elevation in each experimental plot.

The Normalized Difference Vegetation Index (NDVI) measures the amount of live green vegetation in an area, calculated from the visible and near-infrared light reflected by vegetation. The index ranges from −1 to 1, with higher values indicating denser and healthier vegetation, and it is calculated as follows:

$$\text{NDVI} = (\text{NIR} - \text{red})/(\text{NIR} + \text{red})$$

The Green Normalized Difference Vegetation Index (GNDVI) is a measure of a plant's "greenness" or photosynthetic activity. It utilises the near-infrared (NIR) and green bands of the electromagnetic spectrum. The index ranges from −1 to 1, with higher values indicating denser and healthier vegetation, and it is calculated as follows:

$$\text{GNDVI} = (\text{NIR} - \text{green})/(\text{NDVI} + \text{green})$$

2.4. Statistical Analysis

All data were subjected to statistical analysis. Statistical analysis was performed using the IBM® SPSS® Statistics software (SPSS 28.0). The significance of the bioformulation application (Control (C); Micosat (O); and SOSTATA (W)) over two consecutive field trials (2022 and 2023) was determined by comparing mean levels among treatments using a One-Way Analysis of Variance (ANOVA) with Tukey's HSD post hoc test at $p = 0.05$.

3. Results

3.1. Morphological and Molecular Characterisation of Trichoderma Isolates

A pure culture of the two *Trichoderma* isolates 1A and 1B was obtained on PDA petri dishes. The 1A isolate shows as white mycelium of spongy consistency and irregular growth with granule conidiation and scattered conidial masses (Figure 1a); the 1B isolate exhibits a concentric ring structure with a dark green pustular central zone bordering a light green ring, a light intermediate zone, and a light green peripheral ring (Figure 1d). The morphological analysis aligns with characteristics typical of the *Trichoderma* genus. Several *Trichoderma* isolates have exhibited abundant fluffy mycelium, with two to three distinct concentric rings of white mycelium and green conidia [33]. Other characteristics that define the genus *Trichoderma* are a fast growth in culture medium and development of conidia with a green-yellow colour [34]. These characteristics are similar to several *Trichoderma* species, so it was difficult to identify the isolates based only on morphological data [35]. The microscopic observation of isolate 1A showed branched and elongated conidiophores placed at different angles, on which 8 µm flask-like phialides were inserted, and ellipsoidal conidia (3.8 L–2.4 L µm) were highlighted (Figure 1b,c); meanwhile, the isolate 1B had compact, broad-branched conidiophores that assumed a pyramidal structure with 8 µm flask-like phialides. Conidia varied from globose (1.4 d µm) to sub-globose (1.9 D–1.3 d µm) (Figure 1e,f).

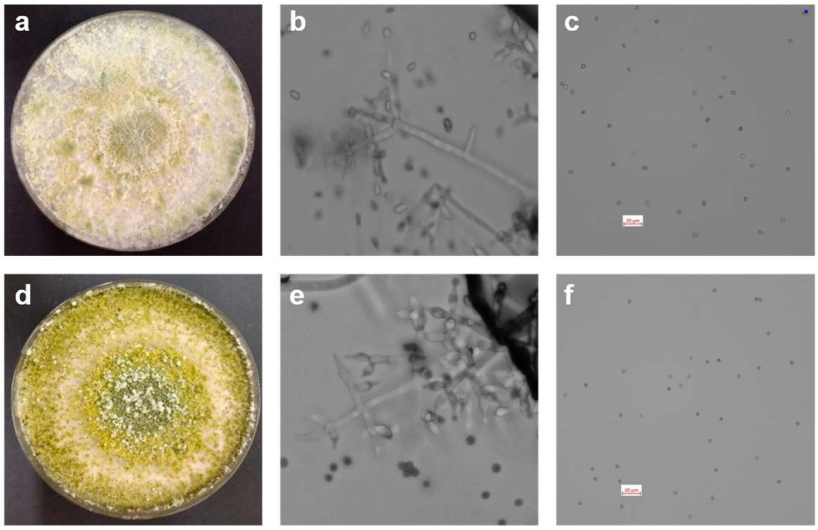

Figure 1. Morphological characterisation of *Trichoderma* isolates. (**a**) Micelium growth of the isolate 1A on PDA petri dish; (**b**) microscopic observation of conidiophores and (**c**) conidia of isolate 1A; (**d**) micelium growth of the isolate 1B on PDA petri dish; (**e**) microscopic observation of conidiophores and (**f**) conidia of isolate 1B.

As a result of the molecular analysis using the three gene markers (ITS; EF1; and ACT), we classified the two isolates at the species level as *Trichoderma asperelloides* (1A) and *Trichoderma harzianum* (1B). In Figure 2a neighbour-joining tree constructed with MEGA X software v. 10.2.4 is shown.

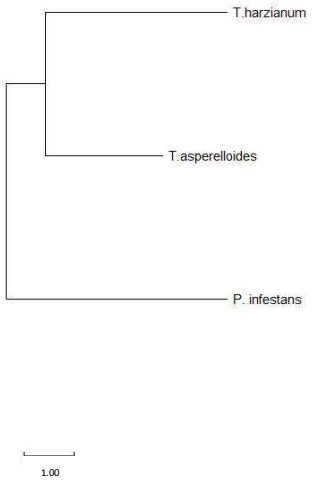

Figure 2. Phylogenetic neighbour-joining tree of two isolates of *Trichoderma* inferred by the analysis of ITS1, EF1, and ACT genes using MEGA 11 software. *Phytophthora infestans* was used as the outgroup.

3.2. Dual-Plate Bioassay

The mycoparasitic effect of *T. asperelloides* (1A) and *T. harzianum* (1B) was carried out with a dual-plate technique against agriculturally important plant pathogens such as *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Aspergillus* sp., and *Botrytis cinerea*. As shown in Figure 3, the pathogen inhibition effect of *T. asperelloides* isolate 1A ranged from 21% against *Asperigillus* sp. to a maximum of 36% when competing with *F. oxysporum*. The *T. harzianum* isolate 1B instead showed a more variable mycoparasitic behaviour with inhibition percentages ranging from a minimum of 0% in competition with *B. cinerea* to a maximum of 100% against *Aspergillus* sp.

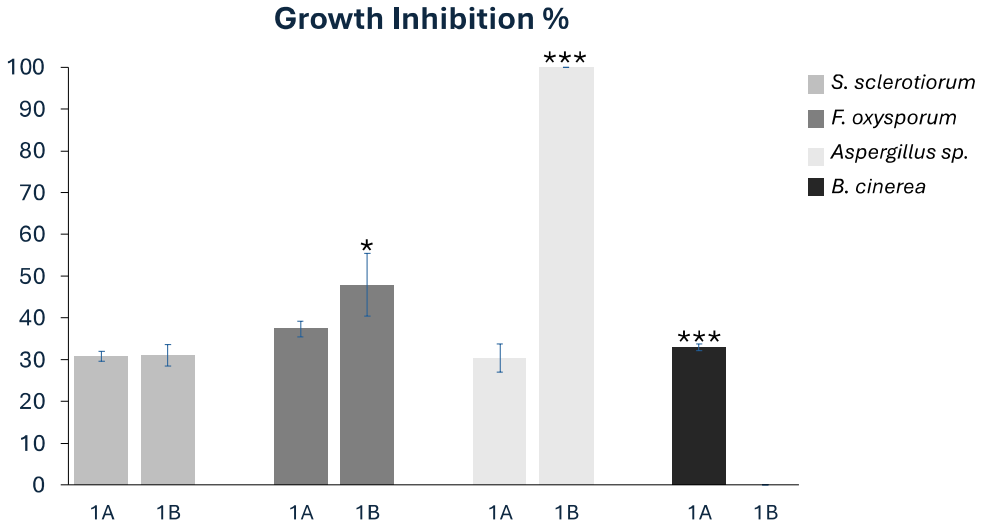

Figure 3. The mycoparasitic effect of *Trichoderma asperelloides* (1A) and *Trichoderma harzianum* (1B) in dual-plate assay against the plant pathogens *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Aspergillus* sp., and *Botrytis cinerea*. The graph shows the percentage of growth inhibition of individual pathogens by the two Trichoderma isolates. An asterisk indicates that differences are statistically significant for Tukey's HSD post hoc test at $p = 0.05$. *: $0.001 < p < 0.01$; ***: $p < 0.0001$.

3.3. Trichoderma–Fungicide Interaction

Of the three fungicides tested at label doses, Lidal (Tetraconazole) proved to be the most effective in inhibiting the growth of the two *Trichoderma* strains leading, after 7 days, to 100% and 54% of growth inhibition of *T. asperelloides* (1A) and *T. harzianum* (1B), respectively. Talendo (Proquinazid) and Biomic (Tannins) proved not to be effective in inhibiting growth

of the two *Trichoderma* strains, recording a percentage of inhibition equal to 0% for both (Table 3).

Table 3. Percentage of growth inhibition of *Trichoderma* strains 1A and 1B after 7 days on a PDA petri dish containing the three fungicides: Lidal (Tetraconazole) 1500 μL·L^{-1}; Talendo (Proquinazid) 250 μL·L^{-1}; and Biomic (Tannins) 4000 μL·L^{-1}.

% of Growth Inhibition						
	Lidal (Tetraconazole)		Talendo (Proquinazid)		Biomic (Tannins)	
	% inhib. 7 dpi	SD	% inhib. 7 dpi	SD	% inhib. 7 dpi	SD
Trichoderma asperelloides (1A)	100	0	0	0	0	0
Trichoderma harzianum (1B)	54.4	2.8	0	0	0	0

3.4. Evaluation of Morphological Plant Parameters and Yield

The parameters analysed were (i) plant fresh weight, (ii), potato tuber fresh weight (iii) plant dry weight, and (iv) potato tuber numbers. Significant differences among the treatments were found. Figure 4 reports the means of five plants *per* treatment in three different fields over two consecutive years of the experiment. In the 2023 season, two more fields located at Acerra (NA) and Airola (BN) were included in the trials. Comprehensively, our results showed that the treatment with the new bioformulate (SOSTATA W) had positively increased the parameters observed while the commercial biostimulant Micosat (O) had no significant impact on crop development. Field blocks treated with the new bioformulates had higher canopies and more vigorous plants.

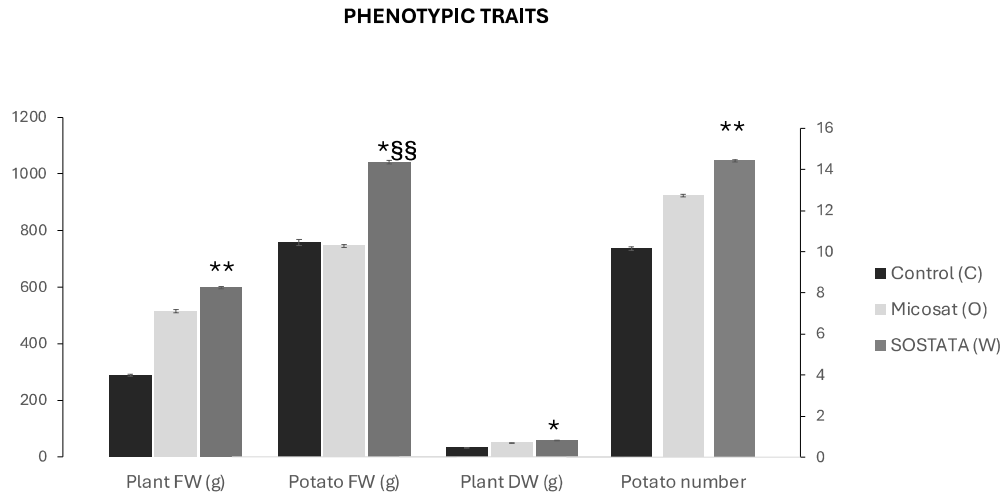

Figure 4. Mean values of five plants per treatment in three different fields over two consecutive years of experiment. The parameters observed were (i) plant fresh weight, (ii), potato fresh weight (iii) plant dry weight, and (iv) potato numbers. Differences are statistically significant for Tukey's HSD post hoc test at $p = 0.05$. *: $0.001 < p < 0.01$; **: $p < 0.001$; comparing SOSTATA (W) vs. Control (C); and §§: $p < 0.001$; comparing SOSTATA (W) vs. Micosat (O).

In particular, for plant fresh weight, the SOSTATA (W) treatment showed an average weight of 598.2 (±5.07) g/plant, significantly different from the farm Control (C) which was 288.4 (±2.68) g/plant. The potato tuber fresh weight of the SOSTATA (W) treatment (1059.5 ± 6.13) g/plant) was significantly greater than the Control (744.6 ± 10.75 g/plant) and interestingly also with respect to the Micosat (O) treatment (732.8 ± 4.9 g/plant).

Looking at the plant dry weight values, the difference between SOSTATA (W) treatment (57.3 ± 0.38 g/plant) and the farm Control (C) (32.8 ± 0.35 g/plant) was significant

as well as for the number of potatoes obtained, with an average of 14.4/plant and of 10.16/plant tubers for SOSTATA (W) and farm Control (C) treatment, respectively.

Figure 5 shows the collected data related to the two-year production of the three experimental fields. Also, in this case the SOSTATA (W) treatment was able to increase production by 20% and 36% compared to Micosat (O) and Control (C), respectively.

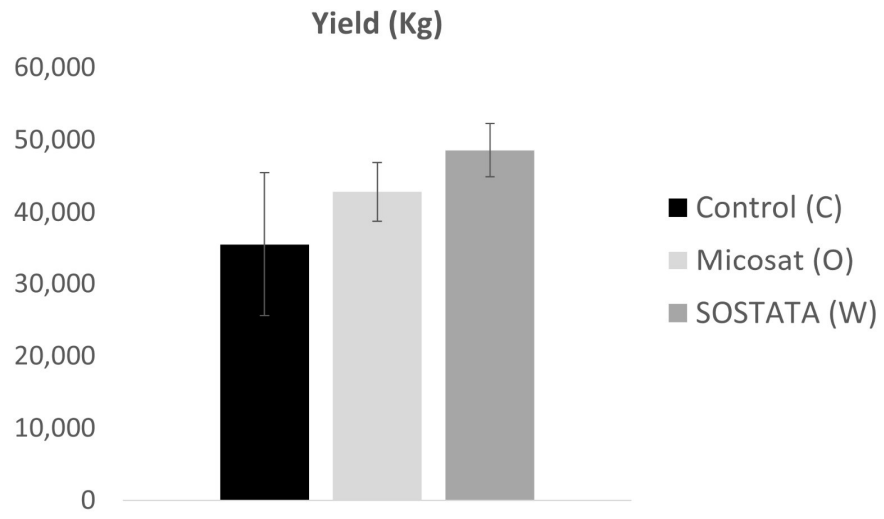

Figure 5. Average yield of five experimental fields over two consecutive years of the experiment. The graph bars represent three different treatments: Control = C; Micosat = O; and SOSTATA = W.

4. Discussion

To evaluate the potential of *Trichoderma* spp. as possible BCAs to promote plant growth and production in potato cultivation, two new *Trichoderma* strains were identified and used to treat experimental potato fields. The two *Trichoderma* strains were previously tested in vitro for their mycoparasitic activity and resistance to fungicides. The results of the in vitro competition assays against four selected pathogens showed different results in relation to the strains tested and the pathogen used. Even variation within one species was reported by Metz and Hausladen [36]; Consolo et al. [37]; and El-gamal et al. [38]. They tested the inhibitory effect of several *T. harzianum* strains against *Alternaria solani* isolates from potato and tomato, respectively. Also, the ability to inhibit the growth of pathogens such as *F. oxysporum*, *B. cinerea* and *Aspergillus* spp., which were used in our research, was reported by Bhale and colleagues [39] even with different fates, underlining the fact that the inhibition capacity is strictly dependent on the strain of *Trichoderma* tested. For example, Zhang et al. [40] reported 75% and 82% in vitro mycelial growth inhibition of *B. cinerea* and *R. solani* respectively, caused by *T. longibrachiatum*. Qualhato et al. [41] also found good results of mycelial growth inhibition of *S. sclerotiorum*, *R. solani*, and *Fusarium solani* when using different *Trichoderma* species. Additionally, Amin et al. [42] reported that *Tricoderma viride* highly inhibited the mycelial growth of *R. solani*, *Sclerotium rolfsii*, and *S. sclerotiorum* in comparison with *T. harzianum*. These findings underline the importance of a broad approach to evaluating the growth inhibition potential of different *Trichoderma* species on plates. In general, the results from the comparison vary with the *Trichoderma* isolate and with the phytopathogen accordingly, with results shown in this work.

To inhibit fungal pathogens, fungicides have been developed to target different components or mechanisms of the fungal cell, including respiration, nucleic acid metabolism, cell membrane integrity, protein synthesis, signal transduction, and cell mitosis [43]. However, most fungicides perform without distinguishing between harmful pathogens and nontarget organisms such as beneficial micro-organisms [44]. As such, fungicides could impact the growth of BCAs or reduce their population size, making biocontrol treatment ineffective. Previous studies have reported that fungicides combined with biocontrol agents can inhibit pathogens or improve the disease resistance of plants [45]. Myresiotis et al. [46] reported

that the combination of plant growth-promoting rhizobacteria and hymexazol could effectively control Fusarium crown and root rot on tomatoes. Our findings on the compatibility of the two *Trichoderma* strains (1A and 1B) with three fungicides showed a complete inhibition from Lidal (Tetraconazole), while Talendo (Proquinazid) and Biomic (Tannins) were ineffective. Selvakumar et al. [47] also demonstrated the limited inhibitory effects of tannins. They found that *T. virescens* and *T. reesii* were able to tolerate up to 3000 ppm of crude tannins extracted in water and acetone without experiencing any growth inhibition. Progressive growth inhibition of *T. virescens* and *T. reesii* occurred as the tannins themselves increased. Hence, the resistance of the strains employed in this study to specific fungicides could aid in comprehending the optimal utilization of the antagonistic capabilities of *T. harzianum* 1B and *T. asperelloides* 1A. However, new studies must be conducted in planta to better establish the limits of each strain and to enable their use in integrated disease management.

Trichoderma spp. can even exert positive effects on plants with an increase in plant growth (biofertilization) and the stimulation of plant-defence mechanisms [17]. The use of biostimulants based on *Trichoderma* spp. is widespread due to *Trichoderma*'s ability to promote plant growth in most agroecosystems with positive effects on the plant biomass, both epigeal and hypogeal [48]. For example, a study conducted on passion fruit showed that plants treated with *Trichoderma* had a greater leaf surface, greater biomass, and a greater commercial yield [49], as well as *Trichoderma* treatment having positive effects on tests carried out on wheat, in which a greater plant height, leaf length, root length, and tip length were recorded [50]. To assess the beneficial effects of *Trichoderma* treatment on plant growth and production, we collected data on morphological parameters such as plant fresh weight, plant dry weight, tuber dry weight, number of tubers per plant, and yield expressed in $kg \cdot ha^{-1}$. From the obtained results, the treatment with the SOSTATA (W) bioformulate showed significant differences compared to the control for all the morphological traits analysed. Application of the new bioformulate was able to promote potato plant growth, and caused a significant increase in plant fresh (+107%) and dry weight (+74%), potato tuber fresh weight (+37%) and number (+41%). In addition, for potato fresh weight, significant differences between the SOSTATA (W) treatment and Micosat (O) were found. Furthermore, the plants treated with SOSTATA (W) showed an increased production of 20% and 36% compared to Micosat (O) and Control (C) treatments, respectively. The data obtained are in line with other studies carried out both in a protected environment and in the open field. In fact, as reported by Contreras-Liza et al. [51], the inoculation of *Trichoderma* both in vitro and in the greenhouse promoted the growth of potato seedlings in vitro and significantly improved the height of the potato plant, increasing the size, calibre, and weight of seed tubers and plant biomass. Similar results were obtained by M. Rakibuzzaman et al. [48] where the ability to promote the growth of potato plants was evaluated in vivo, using different concentrations of *Trichoderma* compared to the control. The increase in production compared to untreated plants was 11.33% and 23.82% depending on the concentration of the treatment, and was due to an improvement in the dry matter content. The beneficial effects of the interaction of *T. harzianum* with *S. tuberosum* phureja was also evaluated in South America by Galindo et al. [52] where an increase in biomass of 30% to 38% compared to the control was highlighted. Application of a select microbial product composed of a consortium of *Bacillus subtilis* and *T. harzianum* effectively suppressed common scab disease and increased tuber yield by establishing a high relative abundance of beneficial bacteria in the rhizosphere as reported by Wang et al. [53]. An increase in potato tuber number and weight was also reported by J. Constantia and colleagues (2023) [54] using the commercial bioformulation TrichoPowder (*Trichoderma* sp.) and plant growth promoting rhizobacteria (PGPR). Ommati et al., [55] tested six species of *Trichoderma* inoculated together with *F. oxysporum* to evaluate the combined effect on the potato yield. The results showed that plants co-inoculated with *Trichoderma* and *Fusarium* had higher production compared to plants inoculated only with the pathogen. Lastly, the results obtained from vegetation indices (NDVI and GNDVI) computed from spectral UAV data show a spatial variability

that is in good agreement with the production variability observed with treatments. As shown in Figure 6, both average NDVI and GNDVI showed the highest values for the SOSTATA treatment (0.73 ± 0.09 and 0.57 ± 0.07 respectively), with similar but lower values for the Micosat (0.72 ± 0.09 and 0.56 ± 0.06 respectively) and Control (0.70 ± 0.09 and 0.54 ± 0.06 respectively). Regarding the crop elevation variability, we estimated 0.24 ± 0.07 m for the control, 0.27 ± 0.08 m for the Micosat treatment, and 0.24 ± 0.09 m for the SOSTATA treatment.

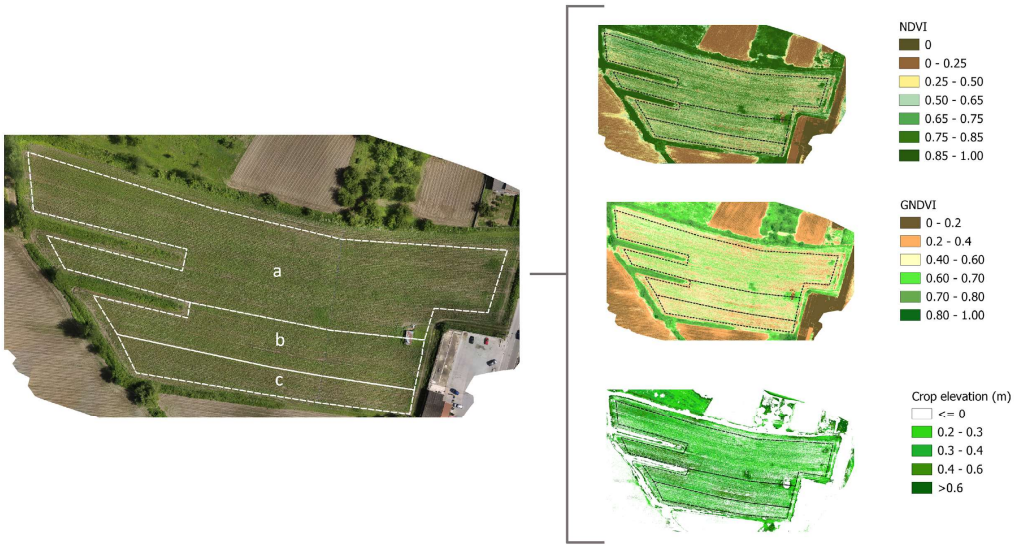

Figure 6. Vegetation indices NDVI and GNDVI and crop elevation for the study area, obtained by UAV DJI Phantom Multispectral flight. The letters in the area represent the different treatments: (**a**) SOSTATA, (**b**) Micosat, and (**c**) control.

Today, we can assert that there is sufficient scientific evidence confirmed with this research to prove that through the symbiosis between potato plants and microorganisms, it is possible to reduce the use of high energy consumption inputs with a negative environmental impact, such as fertilizers and agrochemicals, mitigating their harmful effects on the ecosystem [47]. A huge barrier in the study of potential biopesticides is the precise understanding and determination of their modes of action, i.e., their interactions in dynamic systems, composed of antagonistic organisms, pathogens, and plants [56–58]. In addition to climatic conditions, interactions with autochthonous microorganisms present in an environment may adversely affect the efficacy of microorganisms included in biopreparations [59]. According to Kubiak et al. [60], the main environmental factors affecting the activity and survival of beneficial microorganisms, including their sporulation processes, include temperature, humidity, acidification of the substrate, and availability of nutrients. The main challenge here is to select microbes that will show high tolerance to changing climatic conditions [58] and resistance to chemical pesticides.

5. Conclusions

The results of this study suggest the beneficial potential of two new *Trichoderma* strains in the sustainable management of potato cultivation. Through morphological and molecular analyses, the new *Trichoderma* isolates were identified as *T. asperelloides* (1A) and *T. harzianum* (1B). The in vitro trials both for antagonist capacity and fungicide resistance showed that these isolates can be good biocontrol agent candidates to be used in field application and also in integrated management. Moreover, the application of SOSTATA (W) bioformulate was able to promote potato plant growth and caused a significant increase in fresh and plant dry weight, and potato tuber fresh weight and number, improving overall potato yield. Our findings demonstrate how useful it is to exploit biodiversity in terms

of species and strains of beneficial microorganisms for providing new solutions with low environmental impact in intensive cultivation.

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