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Biological priming of potato cv. Spunta against Potato virus Y using Pseudomonas fluorescens and Trichoderma harzianum

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التهيئة الحيوية لصنف البطاطس "سبونتاً" ضد فيروس "واي" البطاطس باستخدام بكتيريا سيدموناس فلوريسنس وفطر ترايكودرما هارزيانوم"

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Abstract

Potato virus Y (PVY) is a major viral constraint to potato production. This study evaluated the response of potato cv. Spunta to PVY infection and assessed whether beneficial microbial treatments based on *Pseudomonas fluorescens* and *Trichoderma harzianum* could mitigate PVY-associated physiological injury under greenhouse conditions. Five treatments were compared: untreated control, PVY alone, preventive combined treatment with *P. fluorescens* + *T. harzianum* before PVY inoculation, preventive *Pseudomonas* treatment before PVY inoculation, and post-inoculation combined treatment. Biochemical traits were measured 3 and 12 days after the last treatment, and growth traits were recorded under greenhouse conditions. PVY-inoculated plants showed strong increases in catalase, peroxidase, polyphenol oxidase, and superoxide dismutase activities, together with higher H_2O_2 and MDA, and lower total protein, total phenolic compounds (TCP), biomass, length, and SPAD values. The preventive combined treatment produced the most favorable response, reducing H_2O_2 from 3200.50 to 980.40 mg l^{-1} at 3 days and from 1450.80 to 710.20 mg l^{-1} at 12 days, while lowering MDA from 45.60 to 20.10 mg l^{-1} and from 48.90 to 21.40 mg l^{-1}, respectively. At 12 days, the same treatment increased total protein from 410.50 to
520.10 mg l^{-1} and TCP from 1.10 to 2.70 mg l^{-1} relative to PVY alone. Growth suppression was also markedly alleviated, with shoot fresh weight restored from 4.20 to 7.20 g and root fresh weight from 5.10 to 11.50 g. Overall, the dataset indicates that preventive microbial application, especially the preventive combined treatment, substantially mitigated PVY-associated oxidative, biochemical, and growth-related damage in potato cv. Spunta.

Keywords: potato; Potato virus Y; Pseudomonas fluorescens; Trichoderma harzianum; oxidative stress; induced resistance; total phenolic compounds.

الملخص

يُعد فيروس البطاطس Y (PVY) أحد أهم العوامل الفيروسية المحددة لإنتاج البطاطس. قيّمت هذه الدراسة استجابة صنف البطاطس سبونتا للعدوى بفيروسPVY ، وبحثت ما إذا كانت المعالجات الميكروبية النافعة القائمة على سيدموناس فلوريسنس و ترايكودرما هارزيانوم يمكنها التخفيف من الإصابات الفسيولوجية المرتبطة بالفيروس تحت ظروف الصوبة الزراعية. تمت مقارنة خمس معاملات: تحكم غير معالج، وفيروس PVY وحده، ومعالجة وقائية مشتركة بسيدموناس فلوريسنس + ترايكودرما هارزيانوم قبل التلقيح بالفيروس، ومعالجة وقائية بسيدموناس وحده قبل التلقيح، ومعالجة مشتركة ما بعد التلقيح. قُدّرت الصفات الكيميائية الحيوية بعد 3 و12 يوماً من آخر معالجة، وسُجّلت صفات النمو تحت ظروف الصوبة. أظهرت النباتات الملقحة بالفيروس ارتفاعاً كبيراً في أنشطة الكاتلاز، والبيروكسيداز، وبولي فينول أوكسيديز، وديسموتاز الأكسيد الفائق، إلى جانب ارتفاع مستويات بيروكسيد الهيدروجين (H_2O_2) ومالدون ألدهيد (MDA) ، وانخفاض البروتين الكلي، والمركبات الفينولية الكلية (TCP) ، والكتلة الحيوية، والطول، وقيم الكلوروفيل (SPAD) . أعطت المعالجة الوقائية المشتركة الاستجابة الأفضل، حيث خفضت H_2O_2 من 3200.50 إلى 980.40 ملغم/لتر عند اليوم الثالث، ومن 1450.80 إلى 710.20 ملغم/لتر عند اليوم الثاني عشر، كما خفضت MDA من 45.60 إلى 20.10 ملغم/لتر ومن 48.90 إلى 21.40 ملغم/لتر على التوالي. وعند اليوم الثاني عشر، زادت نفس المعالجة البروتين الكلي من 410.50 إلى 520.10 ملغم/لتر، و TCP من 1.10 إلى 2.70 ملغم/لتر مقارنة بـ PVY وحده . كما خفّفت بشكل ملحوظ من كبت النمو، حيث استعاد الوزن الطازج للمجموع الخضري من 4.20 إلى 7.20 غرام، والجذور من 5.10 إلى 11.50 غرام. بشكل عام، تشير البيانات إلى أن التطبيق الميكروبي الوقائي، وخاصة المعالجة الوقائية المشتركة، خفّف بشكل كبير من الأضرار التأكسدية والكيميائية الحيوية والمتعلقة بالنمو المرتبطة بفيروس PVY في صنف البطاطس سبونتا.

الكلمات المفتاحية: البطاطس؛ فيروس البطاطس Y؛ سيدموناس فلوريسنس ؛ ترايكودرما هارزيانوم ؛ الإجهاد التأكسدي؛ المقاومة المُحثة؛ المركبات الفينولية الكلية.

Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most important food and cash crops, yet its productivity is strongly constrained by viral diseases. Among these pathogens, Potato virus Y (PVY; genus Potyvirus) is especially damaging because it reduces plant vigor, impairs photosynthetic performance, lowers tuber yield and quality, and spreads efficiently through infected propagative material and vectors. Physiologically, plant–virus interactions are often associated with oxidative imbalance, reactive oxygen species (ROS) accumulation, lipid peroxidation, and extensive reprogramming of antioxidant metabolism (García-Marcos *et al*., 2009; Hernández *et al*., 2016).

ROS are integral to defense signaling, but excessive ROS accumulation damages membranes and macromolecules, disrupts metabolism, and suppresses plant growth. In virus-infected plants, the antioxidant system commonly responds through altered activities of superoxide dismutase (SOD), catalase (CAT), and peroxidases, while hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) provide practical indicators of oxidative stress and membrane injury

(Hernández *et al*., 2016). In potato, PVY-associated stress has likewise been linked to oxidative disturbance and reduced physiological performance (Deja-Sikora *et al*., 2023).

Because direct curative control of plant viruses remains limited, biologically based strategies that enhance host resistance have attracted considerable interest. Plant growth-promoting rhizobacteria and beneficial fungi can induce systemic resistance, prime host defenses, and improve plant resilience under biotic stress (Pieterse *et al*., 2014; Yu *et al*., 2022). *Pseudomonas fluorescens* is among the best-characterized rhizobacteria in this regard and is known to modulate defense responses and ROS-related pathways (De Vleesschauwer *et al*., 2008). Likewise, *Trichoderma harzianum* is widely recognized for growth-promoting and defense-inducing effects, including responses relevant to virus-challenged plants (Gallou *et al*., 2009; Vitti *et al*., 2016).

For virus pathosystems, treatment timing is likely to be critical. Preventive application may enable priming before infection, whereas post-inoculation application may be less effective once viral establishment and downstream oxidative injury are already underway. Previous work has shown that biological agents, including *P. fluorescens*, can reduce PVY severity and improve potato growth under field conditions (Al-Ani *et al*., 2013). However, comparative physiological evidence for preventive and post-inoculation strategies in potato cv. Spunta remains limited.

The present study therefore evaluated the response of potato cv. Spunta to PVY under greenhouse conditions and examined whether foliar application of *P. fluorescens*, alone or combined with soil application of *T. harzianum*, could alleviate PVY-associated oxidative stress and growth suppression. The working hypothesis was that preventive application, particularly the preventive combined treatment, would be more effective than post-inoculation treatment in preserving biochemical stability and plant growth.

Materials and Methods

Plant Material and Experimental Design

Virus-free tubers of potato (*Solanum tuberosum* L. cv. Spunta) were planted in 15-cm plastic pots containing sterilized growth medium (a 1:1 v/v mixture of washed sand and peat moss) under controlled greenhouse conditions (25 ± 2 °C, 16 h photoperiod). The experiment was arranged as a Completely Randomized Design (CRD) with three replicates per treatment and three plants per replicate, yielding nine experimental units per treatment.

Preparation of Biocontrol Agents and Pathogens

The strain of *Trichoderma harzianum* and *Pseudomonas fluorescens* were obtained from the Microbial Vaccine Centre, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. *Trichoderma* inoculum was prepared by culturing the fungus on Potato Dextrose Agar (PDA) for seven days at 25 °C, after which the conidial suspension was adjusted to 1×10^7 conidia/mL. *Pseudomonas fluorescens* was cultivated on Luria-Bertani agar (LB agar) at 28 °C for 24 h, and the bacterial suspension was adjusted to 1×10^8 CFU/mL prior to application. "The viral isolate was obtained from infected plant tissues, which served as the primary source for preparing the infectious crude sap (inoculum). Mechanical inoculation was performed on the leaves of healthy indicator plants previously dusted with 600-mesh carborundum. Following inoculation, the leaves were thoroughly rinsed with distilled water to remove excess abrasive material and inoculum residue (Hull, 2002)."

Treatment Descriptions

Five treatments were established to assess the interactions between the viral pathogen, the bacterial soft rot pathogen, and the applied biocontrol agents:

•T1 (Control): Plants were treated with distilled water and remained free of any pathogen or biocontrol agent throughout the experiment.
•T2 (PVY): Plants were mechanically inoculated with Potato Virus Y (PVY).
•T3 (Bio + PVY): plants received foliar application of *P. fluorescens* and soil drenching with *T. harzianum*, followed 2 days later by mechanical inoculation with PVY.
•T4 (*P. fluorescens* + PVY): plants received foliar application of *P. fluorescens* 2 days before PVY inoculation.
•T5 (PVY + Bio): Plants were mechanically inoculated with PVY and, two days later, received foliar application of *Pseudomonas fluorescens* and soil irrigation with *Trichoderma harzianum*.

Biochemical Analyses

Leaf samples were harvested at two time points: 3 days (early response) and 12 days (late response) after the final treatment application. Fresh leaf tissue (0.5 g) was homogenized in 50 mM sodium phosphate buffer (pH 7.0) supplemented with 1% (w/v) polyvinylpyrrolidone. Homogenates were centrifuged at 15,000 × g for 20 min at 4 °C, and the resulting supernatants were retained for all enzymatic and biochemical assays. (Bradford, 1976 & و Aebi, 1984)

Antioxidant enzyme assays: SOD activity was quantified by monitoring the inhibition of photochemical nitro blue tetrazolium (NBT) reduction, according to the method of Giannopolitis and Ries (1977). CAT activity was determined by spectrophotometric measurement of H_2O_2 decomposition at 240 nm, following the protocol of Aebi (1984). POD activity was assessed using guaiacol as the hydrogen donor substrate, as described by Chance and Maehly (1955), while PPO activity was measured using catechol as substrate, according to Mayer and Harel (1991) . All enzyme activities are expressed as U/mg protein, based on the protein concentration determined by the method of Bradford (1976).

Oxidative stress markers: Lipid peroxidation was estimated by quantifying malondialdehyde (MDA) using the thiobarbituric acid (TBA) reactive substances assay, according to the method of Heath and Packer (1968). H_2O_2 content was determined spectrophotometrically following the protocol of Patterson *et al*. (1984). Both parameters are expressed as µmol/g fresh weight (FW).

Additional parameters: Total soluble protein concentration was determined by the Bradford method (Bradford, 1976) and expressed as mg/g FW. Total Phenolic Compounds (TCP) were extracted in methanol and quantified with the Folin-Ciocalteu reagent, with results expressed as mg gallic acid equivalents (GAE)/g FW, according to Singleton and Rossi (1965).

Growth Parameter Measurements

At the conclusion of the experiment, plants were carefully excavated and the following growth variables were recorded: fresh and dry weights (g) of shoots and roots; shoot and root lengths (cm) measured with a calibrated ruler; and leaf chlorophyll content index, determined non-destructively in fresh leaves using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan).

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA). Treatment means were separated using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Statistical analyses were conducted using CoStat statistical software (version 6.4).

Results

PVY induced marked oxidative and biochemical disruption

PVY-inoculated plants (T2) showed the strongest biochemical disturbance at both sampling times. At 3 days after the last treatment, PVY significantly increased CAT, POD, PPO, and SOD activities relative to the control, together with marked rises in MDA and H_2O_2, while total protein declined. The strongest change was observed for H_2O_2, which increased from 857.27 mg l^{-1} in the control to 3200.50 mg l^{-1} in PVY-inoculated plants. MDA increased from 18.20 to 45.60 mg l^{-1}, indicating pronounced membrane lipid peroxidation.

This stress pattern persisted at 12 days. PVY-inoculated plants again showed the highest CAT, POD, PPO, SOD, MDA, and H_2O_2 values, together with the lowest total protein and TCP values.

These data confirm that PVY imposed sustained oxidative stress and was associated with deterioration of both protein status and phenolic status in potato cv. Spunta.

The preventive combined treatment was most effective at 3 days

Among PVY-challenged plants, the preventive combined treatment (T3) produced the most favorable biochemical profile 3 days after the last treatment. Relative to PVY alone, T3 reduced CAT from 58.40 to 43.10 mg l^{-1}, POD from 95.20 to 65.40 mg l^{-1}, PPO from 0.52 to 0.36 mg l^{-1}, and SOD from 55.30 to 32.50 mg l^{-1}. Importantly, MDA decreased from 45.60 to 20.10 mg l^{-1} and H_2O_2 decreased from 3200.50 to 980.40 mg l^{-1}, whereas total protein increased from195.40 to 218.50 mg l^{-1}.

The preventive *Pseudomonas* treatment (T4) also improved the biochemical response relative to PVY alone, but its effect was consistently weaker than that of the preventive combined treatment. The post-inoculation combined treatment (T5) reduced injury-related markers compared with PVY alone, yet remained inferior to both preventive treatments. Overall, early microbial application before inoculation offered markedly stronger protection than post-inoculation treatment.

Table 1: Biochemical responses of potato cv. Spunta 3 days after the last treatment.

Treat	Catalase mg/ l	POD mg/ l	PPO mg/ l	SOD mg/ l	MDA mg/ l	Total Protein mg/ l	H2O2 mg/ l
T1 (Control)	41.50 ^{d} ± 0.15	60.16 ^{e} ± 0.21	0.32 ^{e} ± 0.01	27.46 ^{e} ± 0.61	18.20 ^{e} ± 0.45	221.36 ^{a} ± 0.00	857.27 ^{e} ± 1.15
T2 (PVY)	58.40 ^{a} ± 0.46	95.20 ^{a} ± 1.05	0.52 ^{a} ± 0.00	55.30 ^{a} ± 0.62	45.60 ^{a} ± 0.62	195.40 ^{e} ± 0.41	3200.50 ^{a} ± 1.15
T3 (*Pf*+*Tr*→PVY)	43.10 ^{c} ± 0.15	65.40 ^{d} ± 0.27	0.36 ^{d} ± 0.01	32.50 ^{d} ± 0.51	20.10 ^{d} ± 0.45	218.50 ^{b} ± 0.41	980.40 ^{d} ± 1.15
T4 (*Pf*→PVY)	46.20 ^{b} ± 0.15	72.30 ^{c} ± 0.32	0.40 ^{c} ± 0.01	38.40 ^{c} ± 0.58	28.50 ^{c} ± 0.45	210.20 ^{c} ± 0.41	1500.30 ^{b} ± 3.06
T5 (PVY→*Pf*+*Tr*)	48.50 ^{b} ± 0.09	78.10 ^{b} ± 0.53	0.44 ^{b}	45.20 ^{b} ± 0.68	32.40 ^{b} ± 0.45	205.10 ^{d} ± 0.00	1850.60 ^{c} ± 1.15

			± 0.01				
LSD (0.05)	**0.42**	**1.03**	**0.01**	**1.09**	**0.63**	**0.58**	**3.12**

Values are means ± standard deviation. Different letters within a column indicate significant differences at $P \leq 0.05$.

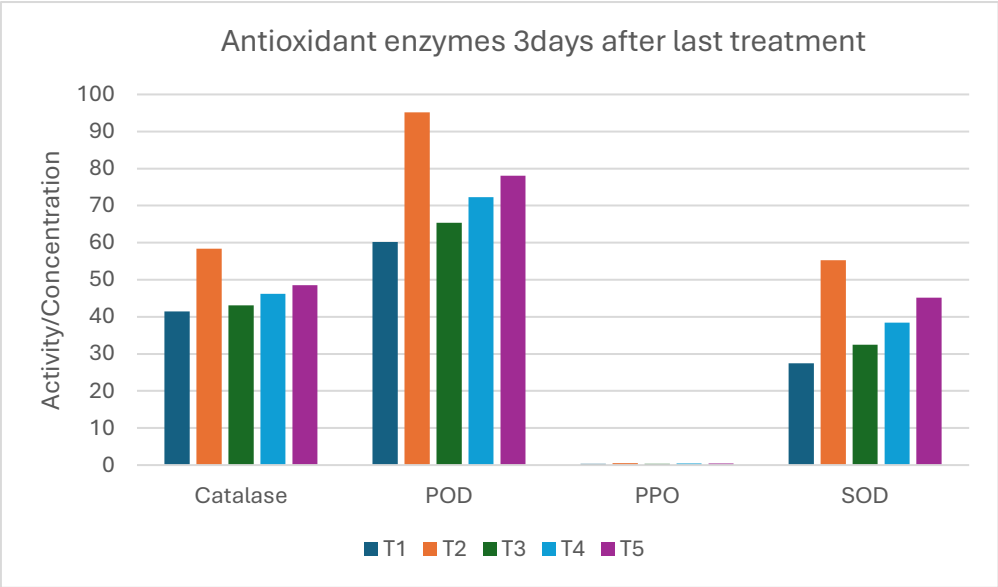

Figure 1. Oxidative damage, total protein, and hydrogen peroxide in potato cv. Spunta 3 days after the last treatment.

Interpretive note: The preventive combined treatment markedly reduced MDA and H_2O_2 accumulation while helping preserve total protein relative to PVY alone.

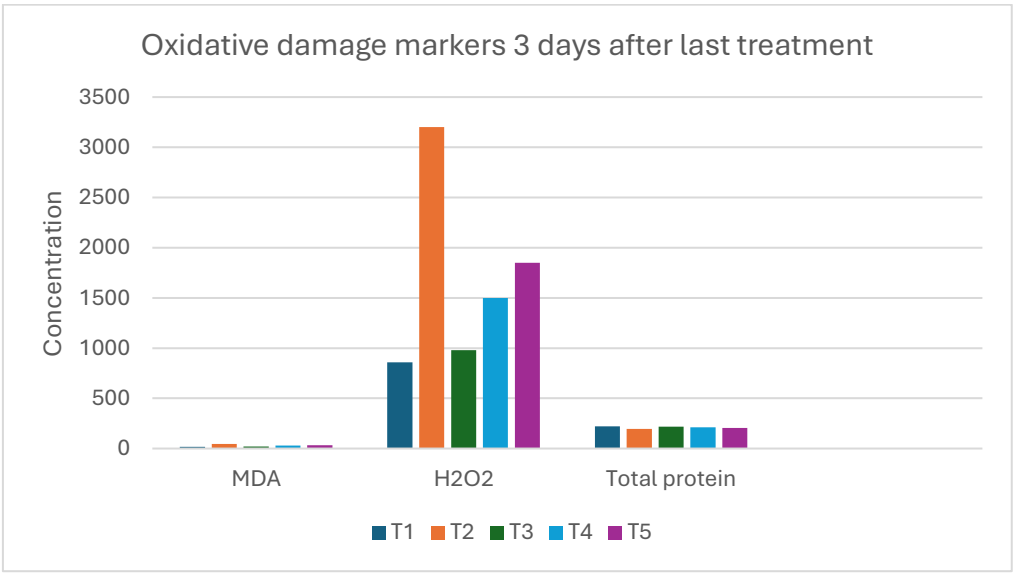

Figure 2. Oxidative damage, total protein, and hydrogen peroxide in potato cv. Spunta 3 days after the last treatment.

Interpretive note: The preventive combined treatment markedly reduced MDA and H_2O_2 accumulation while helping preserve total protein relative to PVY alone.

Protective effects remained evident at 12 days

Treatment differences remained significant 12 days after the last treatment. The preventive combined treatment again showed the best overall response among PVY-challenged plants. Relative to PVY alone, T3 lowered CAT by approximately 30%, SOD by approximately 43%, MDA by approximately 56%, and H_2O_2 by approximately 51%. At the same time, total protein increased from 410.50 to 520.10 mg l^{-1}, whereas TCP increased from 1.10 to 2.70 mg l^{-1}.

The preventive *Pseudomonas* treatment remained intermediate in effectiveness, whereas the post-inoculation combined treatment was beneficial but less protective than the preventive combined treatment and generally weaker than the preventive Pseudomonas treatment. The persistence of these differences indicates that preventive microbial application did more than delay injury; it substantially altered the physiological trajectory of PVY-inoculated plants over time.

Table 2: Biochemical responses of potato cv. Spunta 12 days after the last treatment.

Treat	Catalase mg/l	POD mg/l	PPO mg/l	SOD mg/l	MDA mg/l	Total Protein mg/l	H2O2 mg/l	TCP mg/l
T1 (Control)	41.96 ^{e} ± 0.15	66.82 ^{e} ± 0.05	0.38 ^{e} ± 0.00	27.20 ^{e} ± 0.25	19.50 ^{e} ± 0.00	533.02 ^{a} ± 3.52	634.60 ^{e} ± 2.00	2.85 ^{a} ± 0.01
T2 (PVY)	65.40 ^{a} ± 0.15	82.10 ^{a} ± 0.08	0.55 ^{a} ± 0.00	58.20 ^{a} ± 0.11	48.90 ^{a} ± 1.18	410.50 ^{e} ± 0.71	1450.80 ^{a} ± 0.00	1.10 ^{e} ± 0.01
T3 (*Pf*+*Tr*→PVY)	45.50 ^{d} ± 0.09	70.10 ^{d} ± 0.05	0.40 ^{d} ± 0.00	33.10 ^{d} ± 0.81	21.40 ^{d} ± 0.00	520.10 ^{b} ± 1.09	710.20 ^{d} ± 2.00	2.70 ^{b} ± 0.00
T4 (*Pf*→PVY)	49.20 ^{c} ± 0.00	74.50 ^{c} ± 0.21	0.44 ^{c} ± 0.00	40.50 ^{c} ± 0.27	29.80 ^{c} ± 0.00	495.30 ^{c} ± 0.71	920.40 ^{c} ± 1.15	2.10 ^{c} ± 0.01
T5 (PVY→*Pf*+*Tr*)	54.10 ^{b} ± 0.09	77.80 ^{b} ± 0.09	0.48 ^{b} ± 0.01	48.60 ^{b} ± 0.22	35.20 ^{b} ± 0.00	470.20 ^{d} ± 1.09	1050.50 ^{b} ± 0.00	1.80 ^{d} ± 0.01
LSD (0.05)	**0.20**	**0.20**	**0.01**	**0.74**	**0.96**	**3.24**	**2.49**	**0.02**

Values are means ± standard deviation. Different letters within a column indicate significant differences at $P \leq 0.05$.

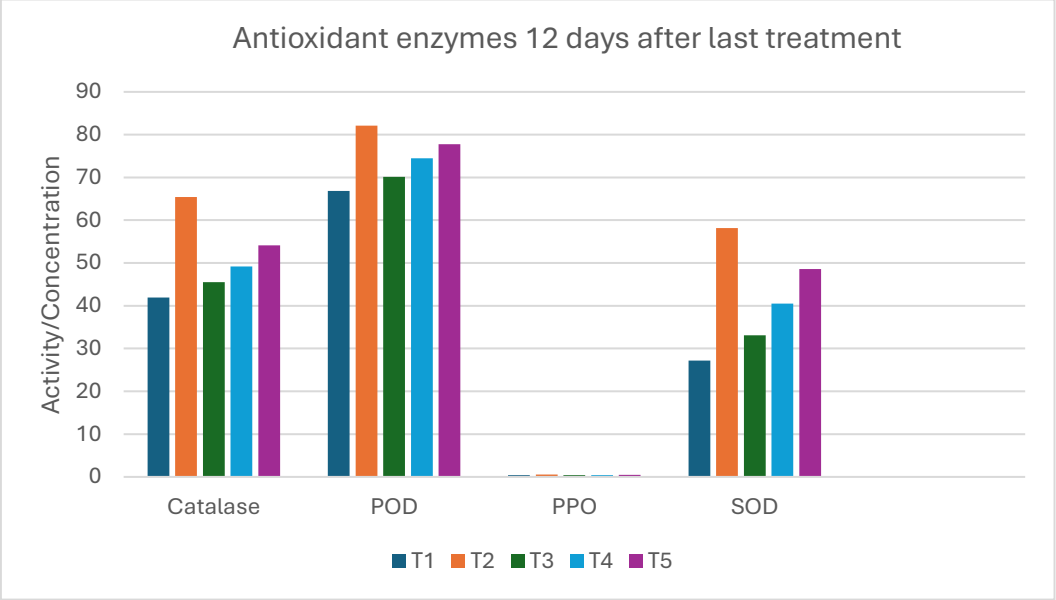

Figure 3. Antioxidant enzyme responses of potato cv. Spunta 12 days after the last treatment. Interpretive note: Treatment effects on antioxidant metabolism remained evident at 12 days, with the preventive combined treatment maintaining the least perturbed oxidative profile among PVY-challenged plants.

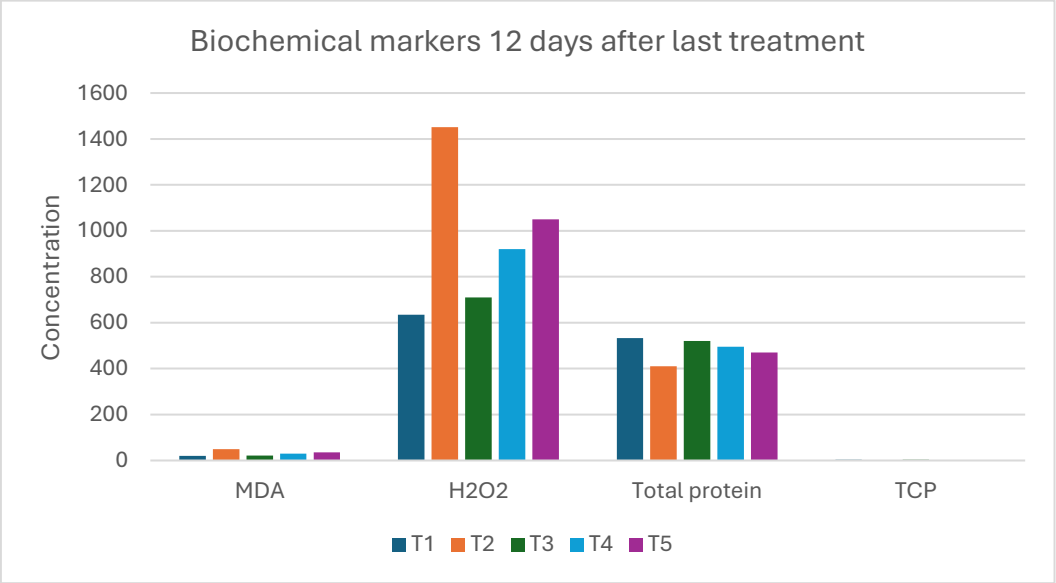

Figure 4. Oxidative damage, protein status, hydrogen peroxide, and total phenolic compounds in potato cv. Spunta 12 days after the last treatment.

Interpretive note: The preventive combined treatment remained the most favorable treatment for limiting oxidative injury while preserving both total protein and phenolic status.

Growth suppression by PVY was strongly alleviated by preventive treatment

PVY-inoculated plants showed clear reductions in all recorded growth traits. Shoot fresh weight declined from 7.50 g in the control to 4.20 g, whereas root fresh weight declined from 9.20 to 5.10 g. Similar reductions were observed for dry matter accumulation, shoot and root length, and SPAD value.

Table 3: Effect of different treatments on growth traits of potato cv. Spunta under greenhouse conditions

Treatment	Fresh Weight (g)		Dry Weight (g)		Length (cm)		SPAD unit
	Shoots	Roots	Shoots	Roots	Shoots	Roots	
T1 (Control)	7.50 ^{a} ± 0.50	9.20 ^{b} ± 0.80	0.65 ^{b} ± 0.03	1.10 ^{b} ± 0.10	28.00 ^{b} ± 2.00	22.00 ^{b} ± 3.00	42.50 ^{b} ± 1.50
T2 (PVY)	4.20 ^{d} ± 0.40	5.10 ^{d} ± 0.60	0.35 ^{d} ± 0.02	0.50 ^{d} ± 0.05	18.50 ^{d} ± 1.50	15.00 ^{d} ± 2.00	25.10 ^{d} ± 2.00
T3 (*Pf*+*Tr*→PVY)	7.20 ^{a} ± 0.45	11.50 ^{a} ± 0.90	0.68 ^{a} ± 0.03	1.35 ^{a} ± 0.12	27.50 ^{b} ± 1.80	26.00 ^{a} ± 2.50	41.80 ^{b} ± 1.20
T4 (*Pf*→PVY)	5.80 ^{c} ± 0.55	6.50 ^{c} ± 0.70	0.48 ^{c} ± 0.04	0.75 ^{c} ± 0.08	22.00 ^{c} ± 2.20	19.50 ^{c} ± 2.80	32.40 ^{c} ± 1.80
T5 (PVY→*Pf*+*Tr*)	5.10 ^{cd} ± 0.50	5.80 ^{cd} ± 0.65	0.42 ^{cd} ± 0.03	0.62 ^{cd} ± 0.07	20.50 ^{cd} ± 2.00	17.50 ^{cd} ± 2.50	29.50 ^{cd} ± 1.90
LSD (0.05)	**0.45**	**0.50**	**0.04**	**0.08**	**1.50**	**1.80**	**2.10**
Values are means ± standard deviation. Different letters within a column indicate significant differences at P ≤ 0.05							

The preventive combined treatment again gave the best response. Shoot fresh weight recovered to 7.20 g and root fresh weight increased to 11.50 g, exceeding the control value. Shoot dry weight and root dry weight rose to 0.68 and 1.35 g, respectively, root length reached 26.00 cm, and SPAD reached 41.80. Several of these values were statistically comparable to the control, and root-related traits exceeded the control. The preventive *Pseudomonas* treatment produced partial recovery, whereas the post-inoculation combined treatment produced only modest improvement.

Taken together, the growth data show that preventive microbial treatment, especially the preventive combined treatment, strongly buffered the negative effects of PVY on plant vigor and improved SPAD values relative to PVY-inoculated plants.

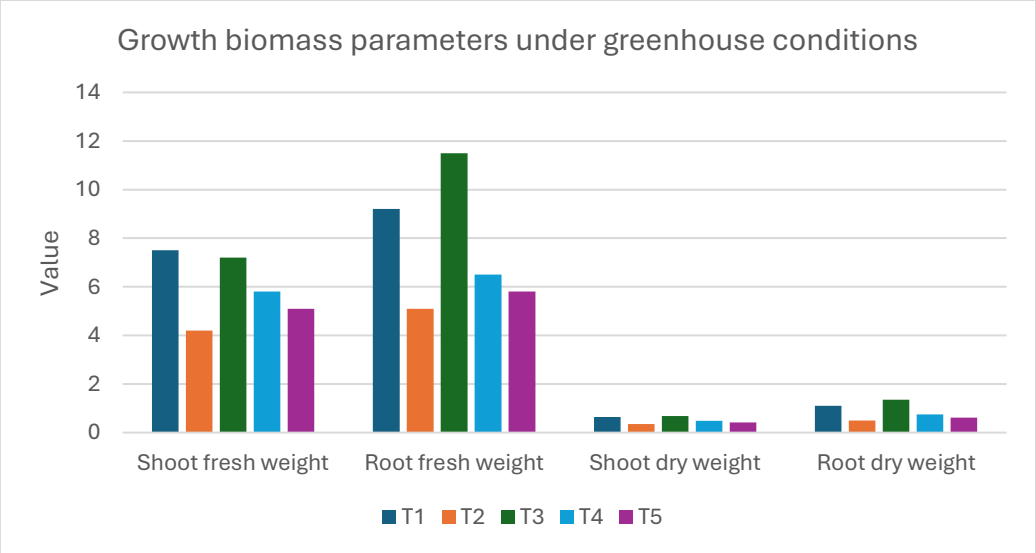

Figure 5. Biomass traits of potato cv. Spunta under the tested treatments.

Interpretive note: PVY reduced both fresh and dry biomass, whereas the preventive combined treatment most effectively restored shoot and root biomass.

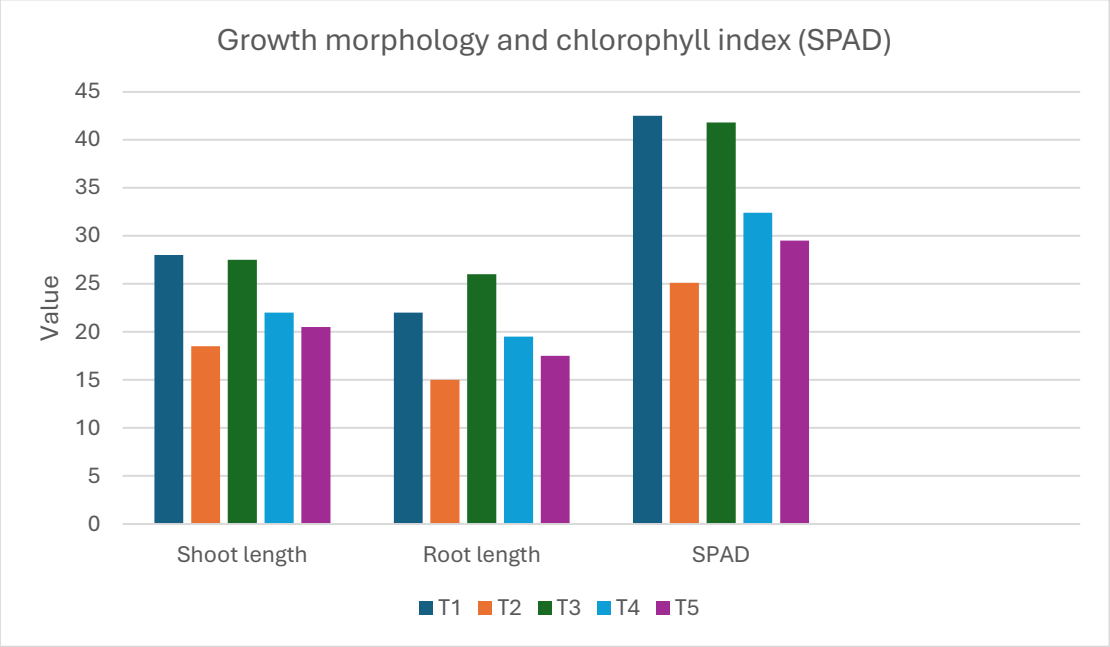

Figure 6. Morphological traits and SPAD values of potato cv. Spunta under the tested treatments.

Interpretive note: PVY-associated reductions in shoot length, root length, and SPAD were strongly alleviated by the preventive combined treatment.

Discussion

The present dataset demonstrates that PVY infection in potato cv. Spunta was associated with pronounced oxidative disturbance, as evidenced by the marked increases in H_2O_2 and MDA, together with enhanced activities of CAT, POD, PPO, and SOD. This pattern is consistent with the established view that viral infection stimulates ROS generation and triggers antioxidant reprogramming; however, excessive and sustained oxidative pressure ultimately contributes to cellular damage and symptom development (García-Marcos *et al*., 2009; Hernández *et al*., 2016). The strong increase in MDA indicates intensified membrane lipid peroxidation, whereas the reduction in total protein reflects broader metabolic disruption in infected plants.

One of the most important findings of this study is the decisive role of treatment timing. The preventive combined treatment with *Pseudomonas fluorescens* and *Trichoderma harzianum* consistently produced the most favorable outcomes at both 3 and 12 days after treatment. This trend supports the interpretation that microbial application prior to virus inoculation primed the host for a more efficient defensive response, thereby enabling the plant to moderate oxidative overreaction and maintain greater physiological stability following viral challenge. This explanation is in line with the current understanding of beneficial microbe-induced systemic resistance and defense priming (Pieterse *et al*., 2014; Yu *et al*., 2022).

The preventive *Pseudomonas* treatment alone also improved plant performance relative to PVY infection alone, although its effect remained consistently weaker than that of the preventive combined treatment. This finding suggests an additive or complementary contribution from the inclusion of *T. harzianum*. *P. fluorescens* is known to sensitize plant tissues for faster and stronger defense activation, often through jasmonate- and ethylene-related pathways, as well as through modulation of ROS-associated processes (De Vleesschauwer *et al*., 2008). In parallel, *Trichoderma* spp. are recognized for their ability to activate defense-related genes, improve nutrient acquisition, and enhance tolerance to biotic and abiotic stress (Gallou *et al*., 2009). The present results are consistent with such a synergistic protective framework.

From a biochemical perspective, the preventive combined treatment lowered H_2O_2 and MDA levels while maintaining higher total protein and total phenolic compounds than PVY-inoculated plants. This pattern suggests that the treatment did not merely intensify antioxidant activity, but rather promoted a more balanced physiological response in which oxidative damage was contained and defense-related metabolism was better preserved. The maintenance of phenolic status is particularly important, as phenolic compounds are closely linked to structural and biochemical defense mechanisms in plants.

The contrast between preventive and post-inoculation application was also biologically meaningful. Although the post-inoculation combined treatment improved plant status relative to PVY alone, it did not reach the level of protection achieved by the preventive treatments. This likely reflects the fact that oxidative imbalance and associated metabolic injury had already been initiated and progressed before microbial treatment was applied. From a practical standpoint, these findings strongly support preventive application as a more effective strategy than post-infection intervention for mitigating PVY-associated physiological damage.

The marked recovery of root growth under the preventive combined treatment deserves particular attention. Root fresh weight, root dry weight, and root length all increased substantially and, in some cases, exceeded the control values. This suggests that the microbial treatment not only mitigated virus-induced stress but also promoted plant growth directly. Enhanced root development may improve water and nutrient uptake efficiency, thereby indirectly strengthening plant tolerance to viral stress. This interpretation agrees with previous reports describing the beneficial effects of microbial agents on the performance of virus-challenged plants (Al-Ani *et al*., 2013; Deja-Sikora *et al*., 2023).

Nevertheless, the interpretation of these findings should remain appropriately cautious. The available data clearly document treatment effects on oxidative, biochemical, and growth-related parameters; however, they do not include direct measurements of viral accumulation in plant tissues. Therefore, the present study supports the conclusion that microbial treatments mitigated the physiological, biochemical, and growth-related damage associated with PVY infection, but it does not by itself demonstrate a reduction in virus titer or viral replication. Direct quantification of PVY using ELISA, RT-qPCR, or comparable methods would be necessary to substantiate that additional inference.

Conclusion

PVY infection caused severe oxidative, biochemical, and growth-related damage in potato cv. Spunta under greenhouse conditions. Across all comparisons, the preventive combined treatment consisting of foliar application of *Pseudomonas fluorescens* and soil drenching with *Trichoderma harzianum* before PVY inoculation was the most effective strategy. This treatment markedly reduced H_2O_2 and MDA, moderated antioxidant overactivation, preserved total protein and total phenolic compounds, and restored growth traits to near-control values.

The preventive *Pseudomonas* treatment was beneficial but less effective, whereas the post-inoculation combined treatment produced only partial recovery. Within the limits of the supplied dataset, these findings support the use of preventive beneficial microbial treatments as a sustainable component of PVY management in potato cv. Spunta.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.